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Effect of superfine-grinding on the physicochemical and antioxidant properties of *Dendrobium nobile* powders

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Abstract

In herbal markets, an overwhelming majority the *Dendrobium nobile* are sold in dried strips form, and this product type would lead to the incomplete release of the active ingredient. To increase the bioaccessibility of *Dendrobium nobile*, we obtained powders of different particle sizes by shear-breaking and superfine-grinding to further compare its sensory properties, physical properties, the leaching rate of active ingredients and antioxidant activity in vitro. The results showed that the superfine-grinding changed the sensory properties and physical properties of *Dendrobium nobile*, with an increase in bulk density, a decrease in swelling capacity and water-holding capacity and an increase in water solubility index. The method also contributed to the release of total polysaccharides, dendrobine, total polyphenols and total flavonoids components, thereby increasing ABTS, DPPH and nitrite ion scavenging activity. In addition, scanning electron microscopy and infrared spectroscopy analyses showed that superfine-grinding had no effect on the primary structure of the fractions as with decreasing particle size. This study suggested that superfine-grinding could be a promising method for producing *Dendrobium nobile* powders with higher contents of bioactive compounds and bioactivity.

Keywords: *Dendrobium nobile*; superfine-grinding; physical properties; bioactive compounds; antioxidant activity.

Practical Application: Superfine-grinding could be a promising method for producing *Dendrobium nobile* powders with higher contents of bioactive compounds and bioactivity.

1 Introduction

Dendrobium nobile Lindl. is a perennial epiphytic herb of the genus Dendrobium in the family Orchidaceae, and is one of the common species of Dendrobium in Chinese medicine, as well as the primary source of Dendrobium herbs in the 2020 edition of the Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2020; Zhang et al., 2020a). It is native to China and is also distributed in India, Myanmar, Thailand, Laos and Vietnam (Wang et al., 2010a; Wang et al., 2010b), and is widespread in southwest China (Wang et al., 2017). The Divine Husbandman's Classic of the Materia Medica classifies it as a "superior product" (Yang & Shun, 2012) and records that it is the most important tonic for deficiencies, mainly treating injuries in the middle, strengthening yin and benefiting essence; tonic for internal deficiencies; tonifying the kidneys and benefiting strength, strengthening tendons and bones, warming water and organs, lightening the body and prolonging life, etc (Zhang et al., 2018; Xie et al., 2018). Modern medical and TCM research shows that the main active ingredients of *Dendrobium nobile* are dendrobine, polysaccharides, flavonoids, sesquiterpenoids, phenols and coumarins (Wang et al., 2022; Xv, 2015), which have pharmacological effects such as immune enhancement (Fan et al., 2020), anti-tumour (Wang et al., 2010c), anti-depression (Xiong et al., 2021), anti-aging (Nie et al., 2020; Lv et al., 2020), retinal damage protection (Hsu et al., 2022) and liver protection (Zhang et al., 2021; Li et al., 2019). At present, many functional foods of *Dendrobium nobile* have been developed, such as *Dendrobium nobile - Panax quiquefolium* capsule (China Health Food approval number G20080243), *Dendrobium nobile* capsule (China Health Food approval number G20120229), and *Dendrobium nobile* lozenge tablet (China Health Food approval number G20060563) (Li et al., 2014).

The current sales situation in the Chinese herbal medicine market is mainly based on traditional Chinese herbal beverage tablets, however, this product type can lead to incomplete release of active ingredients. In recent years, many new types of Chinese herbal tablets, such as Chinese herbal superfine powder, have stood the test of the market and overcome the shortcomings of traditional Chinese herbal tablets, making them the future direction of Chinese herbal tablets. Superfine-grinding refers to the crushing of material particles to the micron or even nano level under the action of mechanical or fluid power, and its application to Chinese herbal medicines is mainly reflected in its ability to improve the processing performance of Chinese herbal medicines. Chinese herbal ingredients are mostly stored in the cells, and the use of superfine-grinding technology can break the cell walls of the plant herbs, so that the active ingredients inside the cells can be fully released, thus improving the bioavailability of the herbs, reducing the dosage of Chinese herbs, saving costs and maximising the utilisation of Chinese herbal

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resources (Chen et al., 2002). It has been applied to *Astragalus membranaceus* (Zhao et al., 2010), *Turkish galls* (Lu et al., 2018), *Dendrobium officinale* (Meng et al., 2017; Meng et al., 2018), *Oenanthe javanica* (He et al., 2019), yam (Wu et al., 2022), black tea (Xiao et al., 2017), *Lycium ruthenicum* (Zhang et al., 2020b), etc. However, so far, no systematic studies have been reported on the effects of superfine-grinding on the properties of *Dendrobium nobile* powders.

The aim of this study is to apply superfine-grinding to *Dendrobium nobile*, firstly, to study its physical properties such as rheological properties and hydration properties, and then to compare the leaching rate of the active ingredients Dendrobine, polysaccharides, total flavonoids and total polyphenols, as well as the differences in the scavenging rates of ABTS radicals, DPPH and nitrite ions radicals. In addition, scanning electron microscopy, Fourier infrared spectroscopy and sensory properties were used to systematically investigate the differences in the intrinsic quality characteristics of *Dendrobium nobile* powders of different particle sizes.

2 Materials and methods

2.1 Materials

Dendrobium nobile was collected in Chishui, Guizhou Province, China. Aluminium nitrate, nine-hydrate, sodium hydroxide, DPPH (2,2-biphenylphenyl-1-picrylhydrazyl), ABTS (2,2’-homo-bis (3-ethylbenzothiazoline-6-sulphonic acid) diamine salt), potassium persulphate, p-aminobenzenesulphonic acid, naphthylenediamine hydrochloride, L-ascorbic acid were purchased from Macklin (Shanghai, China). Glucose standards, acetonitrile and methanol (LC-MS) were purchased from Sigma-Aldrich. All other chemicals and reagents were of analytical grade or better.

2.2 Sample preparation

Dendrobium nobile was purified and cut into sections, and then freeze-dried using an LGJ-10 vacuum freeze-dryer (Beijing, China). The water content was determined to be 3.01 ± 0.41% after freeze-drying, which complied with the standard of the Chinese Pharmacopoeia. The freeze-dried *Dendrobium nobile* was randomly divided into three groups and then ground by different methods. In the first method, *Dendrobium nobile* was shear crushed using a Xingsheng XS-10 pulveriser (Shanghai, China) and then passed through an 80 mesh sieve, the sample prepared was named D80. It is worth noting that the high temperature caused by the pulveriser may damage the phytochemical composition, therefore intermittent crushing was used in the process to reduce potential loss of phytochemicals. In the second method, *Dendrobium nobile* freeze-dried samples were first pre-crushed as described above, then transferred to a 1.2 L stainless steel jar and superfine-grinding in a Billion® WZJ-6J vibratory drug ultra-micronizer (Shandong, China), where the samples were passed through 300 mesh as well as 500 mesh pharmacopoeia sieves and the resulting powders were named D300 and D500 respectively.

2.3 Particle size distribution

The particle size distribution of *Dendrobium nobile* powder was determined by a dry method using a FBS2020-L laser particle size tester (Fujian Province, China). The particle size distribution was characterised by D_{10}, D_{50} and D_{90} values, respectively, indicating the equivalent volume diameter at 10%, 50% and 90% of the cumulative volume. In addition, span values (referring to the width of the particle size distribution) and cell breakage rates (Φ) were also calculated by the following Equations 1-2:

$$\text{Span} = \frac{\left(D_{90} - D_{10}\right)}{D_{50}} \tag{1}$$

$$\phi = 1 - \left(1 - \frac{10}{D_{50}}\right)^3 \tag{2}$$

2.4 Sensory properties

Fifteen trained panelists assessed the sensory characteristics of *Dendrobium nobile* powder samples of different particle sizes, including colour, texture, reconstituability, mouthfeel and flavour. The best colour is uniform and bright. The texture is best when there are no large particles, no grit to the touch and excellent flow. The reconstituability is best when dissolved without clots or precipitating particles. The mouthfeel is best when it is soft and smooth, with no sand grains. And the best flavor is the distinctive light flavour of *Dendrobium nobile*. The group members evaluated them with the 9-point hedonic scale (1 = extremely disliked, 9 = extremely liked).

2.5 Scanning Electron Microscope (SEM)

An SEM instrument (HITACHI Regulus 8220, Japan) was used to observe the microstructure of different particle sizes of *Dendrobium nobile* powder. For the specific method, refer to Xu et al. (2020).

2.6 Physical properties

Bulk density

The determination of bulk density was based on the method of Meng et al. (2017), with minor modifications. A certain amount of sample (*M*) was weighed into a 10 mL measuring cylinder and the cylinder was repeatedly tapped on the surface of the cylinder every 2 s from a height of 2.5 cm until the volume of powder in the cylinder was essentially constant, at which point the volume of powder *V* was recorded and the bulk density of the sample was calculated as follows (Equation 3):

$$\rho_{bulk}\left(\text{g}/\text{cm}^3\right) = \frac{M}{V} \tag{3}$$

Swelling capacity

The samples of different particle sizes of *Dendrobium nobile* powder (*M*) were accurately weighed and put into a measuring cylinder respectively, the volume *V1* was recorded and 10 mL of distilled water was added. Stir well with a glass rod. Let stand at room temperature for 24 h. Read out the volume *V2* at this time, and the swelling capacity was calculated as follows (Equation 4):

$$SC = \frac{(V2 - V1)}{M} \quad (4)$$

Water holding capacity

The WHC was determined by reference to the method of Zhang et al. (2020b) with modifications. The sample powder (*M1*) was weighed and dispersed uniformly in an aqueous solution and kept in a constant temperature water bath at 60 °C for 60 min. The mixture was centrifuged at 4000 r/min for 15 min, the supernatant was discarded and the wet weight of the sample (*M2*) was weighed. the water holding capacity index (WHC) was calculated as follows (Equation 5):

$$WHC(\%) = \frac{(M2 - M1)}{M1} \times 100\% \quad (5)$$

Water solubility index

The water solubility of different particle sizes of *Dendrobium nobile* powder was measured according to the method of Lu et al. (2018) with minor modifications. The sample powder (*M1*) was weighed, distilled water (1:250, w/v) was added, mixed well and then water bath at 80 °C for 60 min. The mixture was centrifuged at 4000 r/min for 15 min, the supernatant was dried and weighed (*M2*). The water solubility index (WSI) was calculated as follows (Equation 6):

$$WSI(\%) = \frac{M2}{M1} \times 100\% \quad (6)$$

2.7 Active ingredient leaching rate analysis

Total polysaccharides

The polysaccharides were extracted according to the method specified in the Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2020). Samples of *Dendrobium nobile* of different particle sizes were extracted by heating at reflux for 2 h. After centrifugation, the supernatant was aspirated and added to anhydrous ethanol and left for 3 h at 4 °C to precipitate polysaccharides. The precipitation was washed with 80% ethanol and then centrifuged, and repeated twice. The final precipitate was dissolved in double-distilled water and transferred to a measuring flask for volume determination, and shaken well to obtain the sample solution.

The total water-soluble polysaccharide content of different particle sizes of *Dendrobium nobile* powder was determined based on the colour development reaction of polysaccharides and their derivatives with phenol and concentrated sulphuric acid (Meng et al., 2017). 1 mL of the sample solution was transferred to a test tube and then 1 mL of 5% phenol solution was added. The solution was mixed thoroughly, 4 mL of concentrated sulphuric acid was added, shaken well and placed in a water bath at 80 °C for 20 minutes. The solution was then removed, cooled in an ice bath and the absorbance was subsequently measured at 488 nm. A calibration curve was prepared using glucose as the reference and the regression equation was: Y = 211.67X - 017.82, $R^2 = 0.9995$, where Y is the absorbance and X is the concentration. The polysaccharides were quantified according to a linear calibration plot of absorbance versus the corresponding concentration.

Dendrobine

The samples were extracted by refluxing with 0.05% formic acid A at a solid-liquid ratio of 1:150 for 3 h. The samples were filtered through a 0.22 um microporous membrane and stored in the injection bottle for LC-MS analysis. The analytical conditions were as follows.

The *Dendrobium nobile* extract was separated using SCIEX liquid chromatography. The mobile phase consisted of ultra-pure water (with 0.1% formic acid) in phase A and acetonitrile in phase B; elution gradient: 0.00-1.00 min, 10% B, 1.00 min-3.00 min, 10%-25% B, 3.00-4.10 min. The gradient was composed of: 0.00-1.00 min, 10% B, 1.00 min-3.00 min, 10%-25% B, 3.00-4.10 min, 25% B, 4 min-5 min, 25%-10% B, 5 min-7 min, 10% B, 7 min-7.5 min, 40%-10% B, 7.5 min-9 min, 10% B; flow rate 0.30 mL/min; column temperature 30 °C; injection volume 2 μL.

MRM was performed on *Dendrobium nobile* extract using an API4000TM triple quadrupole mass spectrometry system in positive ion mode with the following ion source parameters: source temperature 450 °C; ion spray voltage 5500 V, ion source gas I, gas II and curtain gas set at 40, 45 and 25 psi respectively, and collision gas at 8 psi.

Total flavonoids and total polyphenols

The samples of *Dendrobium nobile* with different particle sizes were extracted by adding 70% ethanol at a solid-liquid ratio of 1:100, and the extraction time lasted for 2 h. The sample solution was obtained by filtration.

Add 70% ethanol to 10 mL, add 0.5 mL of 5% sodium nitrite solution, mix well and leave for 6 minutes, then add 0.5 mL of 10% aluminium nitrate solution, shake well and leave for 6 minutes. Add 4 mL of 1 mol/L sodium hydroxide solution, shake well and leave for 15 minutes, use the corresponding reagent as blank, measure the absorbance at 508 nm and plot the standard curve with absorbance as the vertical coordinate and concentration as the horizontal coordinate. The concentration of rutin in the test solution was read out from the standard curve and calculated to obtain the content of total flavonoids in *Dendrobium nobile* samples of different particle sizes.

Add 1.5 mL of 20% sodium carbonate solution, add water to fix the volume to 10 mL, shake thoroughly, leave for 20 min at room temperature, measure the absorbance at 760 nm, and plot the standard curve with absorbance as the vertical coordinate and concentration as the horizontal coordinate. The concentration of gallic acid in the test solution was read out from the standard curve and calculated to obtain the content of total polyphenols in *Dendrobium nobile* samples of different particle sizes.

2.8 Fourier Transform Infrared Spectroscopy (FTIR)

Powder of *Dendrobium nobile* of different particle sizes were ground with KBr powder at a ratio of 1:100 (w/w) and pressed into 1 mm thick pellets, which were then examined by FTIR spectroscopy (Nicolet iS50, Thermo Scientific, Waltham, USA). The IR spectra were obtained in transmission mode and all spectra were scanned 32 times in the 4000-400 cm^{-1} range with a resolution of 4 cm^{-1}.

2.9 Antioxidant capacity evaluation

Preparation of extracts

As described in the determination of polysaccharide content, powder extracts of *Dendrobium nobile* with different particle sizes were prepared, and the absorbance value was measured by MULTISKAN GO full-wavelength microplate reader (Thermo Company, USA).

DPPH radical scavenging assay

The 2,2-diphenyl-1-pyridylhydrazyl (DPPH) radical scavenging activity of different particle sizes of *Dendrobium nobile* powder was determined by the method reported by Shen et al. (2022) with a simple modification. Briefly, 1 mL of sample solution at different concentrations (1, 5, 10, 15, 20 and 25 mg mL^{-1}) was taken in a test tube, 3.5 mL of 1.9×10^{-4} mol L^{-1} DPPH solution was added, mixed immediately, left in a shaded place for 30 min and the absorbance value was measured at 517 nm. The positive control group replaced the sample solution with ascorbic acid. The results were expressed as the percentage of DPPH radicals scavenged and calculated using the following formula (Equation 7):

$$\text{DPPH scavenging activity}(\%) = \frac{A0-(A1-A2)}{A0} \times 100\% \quad (7)$$

Where *A0*, *A1* and *A2* are the absorbance of the control (DPPH solution without sample), the sample and the sample without DPPH solution respectively.

ABTS radical scavenging assay

The method used to test the ABTS radical cation scavenging activity of *Dendrobium nobile* powders of different particle sizes was modified according to Zhu et al. (2022). Briefly, an ABTS radical solution ($ABTS^+$) was prepared by mixing 7 mM ABTS solution with 2.45 mM potassium persulphate solution 1:1 and left to stand at room temperature and protected from light for 12-16 h. For use, the $ABTS^+$ solution was diluted with PBS (pH = 7.4) to give an absorbance at 734 nm was 0.70 (± 0.02). 0.5 mL of the sample at different concentrations (1, 5, 10, 15, 20, 25 mg mL^{-1}) was added to 2.5 mL of ABTS radical solution, mixed thoroughly and left to stand for 6 min at room temperature and the absorbance at 734 nm was recorded immediately. Using ascorbic acid as a positive control, the ABTS radical cation scavenging activity was calculated according to the following Equation 8:

$$\text{ABTS scavenging activity}(\%) = \frac{A0-(A1-A2)}{A0} \times 100\% \quad (8)$$

Where *A0*, *A1* and *A2* are the absorbance of the control (ABTS solution without sample), the sample and the sample without ABTS solution respectively.

Nitrite ion scavenging assay

The nitrite ion scavenging activity of different particle sizes was experimented by referring to the method of Kuo et al. (2015) with modifications. The determination was carried out by the colourimetric method using the nitroso system sulphonamide. 1 mL of sample solution at different concentrations (1, 5, 10, 15, 20 and 25 mg/mL) was taken in a test tube, 0.5 mL of $NaNO_2$ standard solution at 5 μg/mL was added to a constant temperature water bath at 37 °C for 30 min, 1 mL of 0.4% p-aminobenzenesulphonic acid solution was added immediately after removal, shaken and left for 5 min. Add 0.5 mL of 0.2% naphthylenediamine hydrochloride solution, add distilled water to the scale of 10 mL, shake well, leave for 15 min, and determine the absorbance value at 538 nm. Using Vc as a positive control, calculate the ABTS radical cation scavenging activity according to the following Equation 9:

$$NO_2^- \text{ scavenging activity}(\%) = \frac{A0-(A1-A2)}{A0} \times 100\% \quad (9)$$

Where *A0*, *A1* and *A2* are the absorbance of the control (NO_2- solution without sample), the sample and the sample without NO_2- solution respectively.

2.10 Statistical analysis

All experiments were repeated at least three times and data are reported as means (± SD). Significance was defined as $p < 0.05$ by one-way ANOVA.

3 Results and discussion

3.1 Superfine-grinding reduces the particle size of Dendrobium nobile

Table 1 gives the D_{10}, D_{50}, D_{90}, Asf, Span values and the breakage rate (Φ) of *Dendrobium nobile* powder. The results show that the D_{10}, D_{50} and D_{90} of the samples were significantly reduced after superfine-grinding, where D_{50} represents the mean median diameter, as shown in Table 1, the D_{50} value of decreased from 93.16 μm to 18.07 μm and 11.75 μm when Asf values increased by 4.6 and 6.6 times respectively. Span represents the width of the particle size distribution, with smaller span values indicating a narrower and more uniform particle size distribution. Our results show that the D500 sample has the largest span value, indicating the widest particle size distribution, which is consistent with

Table 1. Particle size distribution of *Dendrobium nobile* powder with different particle sizes.

Samples	D80	D300	D500
D_{10} (μm)	24.87 ± 0.27^a	4.59 ± 0.02^b	3.17 ± 0.02^c
D_{50} (μm)	93.16 ± 1.07^a	18.07 ± 0.18^b	11.75 ± 0.18^c
D_{90} (μm)	213.36 ± 6.30^a	50.57 ± 1.29^b	35.12 ± 0.91^c
Asf	124.4 ± 1.12^c	573.23 ± 2.76^b	813.35 ± 6.01^a
Span	2.02 ± 0.04^c	2.55 ± 0.05^b	2.72 ± 0.04^a
Φ (%)	28.87 ± 0.29^c	91.10 ± 0.33^b	99.67 ± 0.09^a

Data in the same column and labeled with different superscripts are significantly different (P <0.05).

the particle size distribution curve in Figure 1A. In addition, the Φ for D300 and D500 was 91.1% and 99.67% respectively, compared to only 28.87% for D80, thus the superfine-grinding facilitated the breaking of the cell wall of the powder, which would help to release the intracellular material by reducing the mass transfer resistance.

Each value represents mean ± standard deviation of three replicates; different letters in the same column mean significant difference ($P < 0.05$).

3.2 Superfine-grinding changes the sensory evaluation of Dendrobium nobile

Fifteen testers were invited to conduct sensory evaluation of *Dendrobium nobile* powder with different particle sizes, and the results are shown in Figure 1B. After superfine-grinding, the colour, texture, reconstituability, mouthfeel and flavour of *Dendrobium nobile* showed varying degrees of improvement, with the sensory indicators of colour, texture, reconstituability and mouthfeel all showing significant improvements with decreasing particle size, with more uniform colour, finer texture and no graininess, and no clumps or precipitated particles at all after brewing, with excellent mouthfeel. However, in terms of flavour, we found that the differences between the three were not as great as for colour, texture, reconstituability and mouthfeel, and there were no significant differences between D80 and D300. We deduce that the superfine-grinding does not improve the flavour of *Dendrobium nobile* to a great extent, but significantly enhances its colour, texture, reconstituability and mouthfeel, improving the sensory properties of *Dendrobium nobile* in general, so as to promote the development of its functional food.

3.3 Superfine-grinding changes the morphology of Dendrobium nobile

SEM micrographs of different particle sizes of Dendrobium nobile powder are shown in Figure 2, it can be seen that D80 presents irregular shape, rough surface and large flakes, after superfine-grinding, the particle size decreases due to mechanical force, it can be seen that long fragments transform into short fragments until very small parts and fragments are obtained. Both D300 and D500 present smooth surface, round and irregular shape. Most of the particle cells were broken, which may have contributed to improved powder properties, such as dissolution and dispersion properties. These results indicate that the superfine-grinding greatly disrupted the structure and

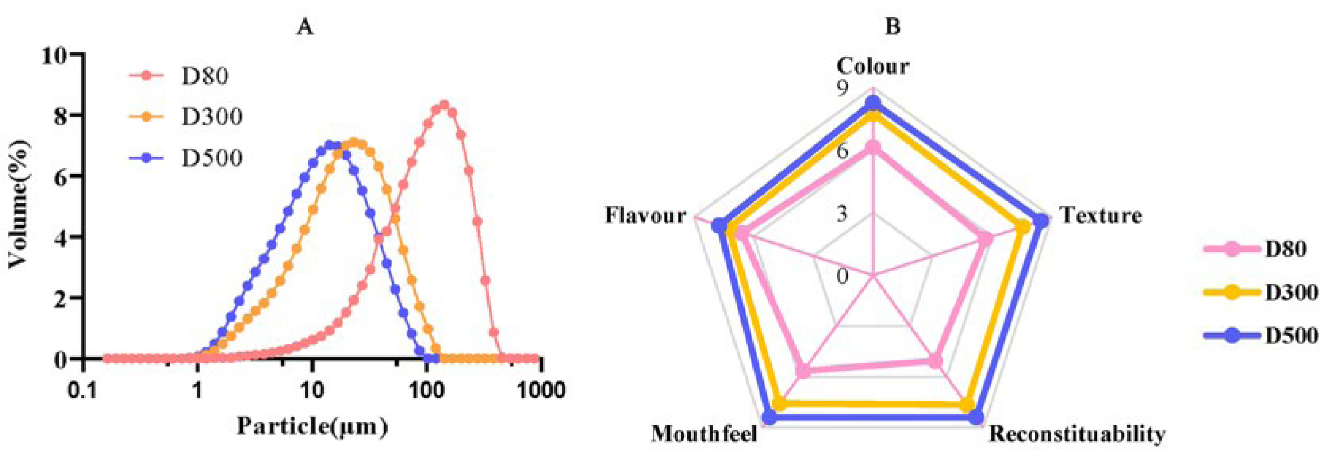

Figure 1. Particle size distribution by % channel and sensory evaluation of *Dendrobium nobile* Powders. A: Particle size distribution; B: Sensory evaluation.

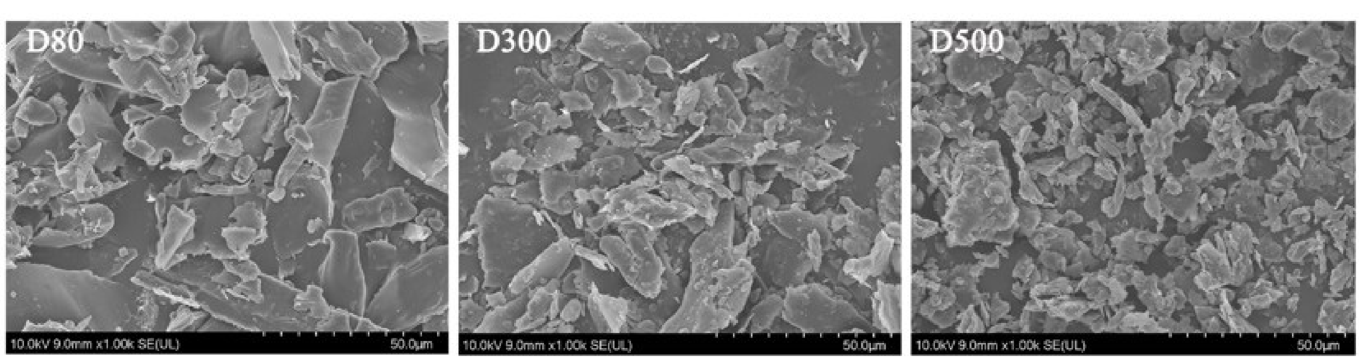

Figure 2. SEM images of *Dendrobium nobile* powder with different particle sizes. Data in the same column and labeled with different superscripts are significantly different ($P <0.05$).

reduced the particle size, which verifies that the superfine-grinding in Table 1 has a disruptive effect on the cell walls of *Dendrobium nobile*, which in turn exposes and releases the chemical components. In addition more agglomerates appeared on the surface after superfine-grinding, which may be the result of interactions between molecules through electrostatic interactions due to the polar groups exposed after grinding.

3.4 Superfine-grinding improves the physical properties of Dendrobium nobile

Since inter-particle interactions affect the bulk properties of powders and can also interfere with the flow of powders, comparing the bulk density provides a measure of the relative importance of these interactions in a given powder. In this study, the bulk density of the three *Dendrobium nobile* powders was analysed and the results showed that the bulk density of the superfine-grinded powder was significantly higher than that of the shear crushed powder ($p<0.05$), which may be due to the fact that the smaller particle size also has smaller pores between the superfine powder particles, resulting in an increased bulk density (Zhao et al., 2009). High bulk density powders are often required in the preparation of tablet or capsule products, as high compaction density facilitates product formation, e.g. okra particles with higher bulk density facilitate the filling of tablet or capsule products (Chen et al., 2015). This was also confirmed by morphological observations that the shear crushed samples presented a rough surface, but the superfine-grinding powder presented a smooth surface, a result that is consistent with previous studies. Therefore, in the pharmaceutical industry, superfine-grinding is a better option for the production of high quality drugs.

SC, WHC and WSI may reflect the effect of particle size on the hydration properties. SC provides information on the amount of water is able to absorb, with the swelling capacity decreasing from 6.47% to 4.33% as the size of *Dendrobium nobile* particles decreases, possibly due to superfine-grinding, where many short cellulose chains may pile up and therefore are unable to form large swelling spaces (Hong & Zhang, 2005). WHC is the ability of a moist material to retain moisture when subjected to external centrifugal forces or compression. Kirwan et al. (1974) reported that WHC depends on the roughness or fineness of the material, with coarse powders having more space than fine powders. Our study similarly confirms this, as shown in Figure 3, where the WHC significantly decreased with decreasing particle size, due to the comminution process, especially the superfine-grinding process, disrupting the polysaccharide chains of insoluble dietary fibres that can retain a certain amount of water through hydrogen bonds. Also, the decrease in WHC values may be due to the destruction of polar amino acid residues exposed on the surface of in *Dendrobium nobile* powder during the milling process. In addition as the particle size decreased, the WSI increased, especially the solubility of D500 increased significantly. This may be attributed to the increased surface area and surface energy after superfine crushing, which improves the diffusion of water-soluble molecules in the particles and thus promotes the release of large amounts of soluble substances in close association (Liu et al., 2011). In addition, hydrophilic groups in *Dendrobium nobile* may have been exposed, which allowed for easy binding to water, increased dispersion and solubility, and ultimately increased WSI.

3.5 Superfine-grinding improves the leaching rate of active ingredients of Dendrobium nobile

Studies have shown that *Dendrobium nobile* mainly contains compounds such as dendrobine, polysaccharides, flavonoids and phenols, and dendrobine is the signature bioactive component,

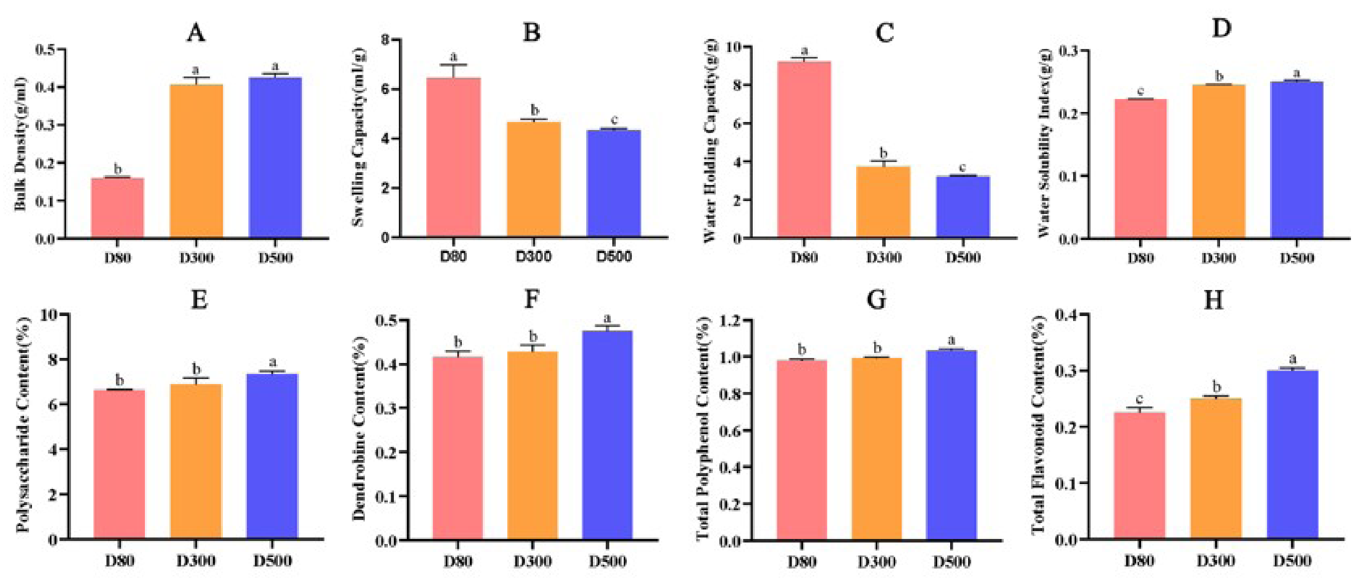

Figure 3. Physical properties and leaching rate of active ingredients of different particle sizes of *Dendrobium nobile* powder. A: Bulk density; B: Swelling capacity; C: Water holding capacity; D: Water solubility index; E: polysaccharide content; F: dendrobine content; G: total polyphenol content; H: total flavonoid content. Each value represents mean ± standard deviation of three replicates; different letters in the same column mean significant difference ($P < 0.05$).

which is considered as one of the criteria for evaluating the quality and has the effects of improving gestational diabetes (Feng et al., 2021), antiviral (Li et al., 2017), neuroprotection (Li et al., 2022) and cancer inhibition (Kim et al., 2021). Polysaccharides isolated from Dendrobium have been found to have various biological functions such as immune modulation (Wang et al., 2010d) improvement of cerebral ischemia (Liu et al., 2021), anti-tumour (Luo & Fan, 2011), and hypoglycaemia (Pan et al., 2014). In addition to this, polyphenols and flavonoids are closely related to antioxidant activity and are natural antioxidants (Solichah et al., 2022; Kurek-Górecka et al., 2013). Figure 3E-3H summarizes the leaching rate of total polysaccharides, dendrobine, total polyphenols and total flavonoids in different particle sizes of *Dendrobium nobile* powder, it can be seen that the leaching rate of total polyphenols, total flavonoids, total polysaccharides and dendrobine in the extract increased as the particle size decreased. When the particle size was reduced to 11.75 μm, there was a significant increase. This is due to the fact that superfine-grinding breaks large structures into smaller particles, increasing the surface area of the powder, providing a large contact surface with the solvent and improving the dissolution of the functional components. Superfine-grinding also alters or disrupts the molecular matrix structure, thereby exposing and releasing compounds, a result that favours the solubilisation, and these findings are consistent with other studies (Lu et al., 2018; He et al., 2019).

3.6 Superfine-grinding does not change the main structure of the components of Dendrobium nobile

Figure 4 shows the FTIR spectral characteristics of different particle sizes. It can be seen that the overall spectral profiles of the different particle sizes of *Dendrobium nobile* powders are similar, with no new spectral peaks generated and no spectral peaks disappearing, indicating that no new chemical groups were generated during the superfine-grinding process. We attributed some characteristic peaks, as shown in Table 2, listing the detailed peak positions and assignments for the three samples. As can be seen in Figure 4 and Table 2, the spectra are very complex and contain many bands assigned to the main components, with some small peaks at and near 3418 cm^{-1} associated with stretching vibrations of -OH and N-H, and wave numbers at 2923 cm^{-1} and 2853 cm^{-1} are associated with stretching vibrations of CH and -CH_2; wave numbers at 1739 cm^{-1} are associated with C=O stretching vibrations; wave numbers near 1604 cm-1 are associated with COO stretching vibrations; wave numbers near 1375 cm^{-1} are associated with symmetric bending of C-H and CH_2. Wave numbers around 1245 cm^{-1} and 1054 cm^{-1} both correspond to CO stretching vibrations; wave numbers around 607 cm^{-1} are attributed to OH stretching vibrations.

However, the exact peak intensities of *Dendrobium nobile* powders differed between particle sizes, specifically, wave numbers around 1604, 1507, 1425, 1245, 1160, 1054 and 607 cm^{-1} could be seen to decrease with decreasing particle size. In contrast, wave numbers near 3418, 2923, 2853, 1739, 1458 and 1375 cm^{-1} increase with decreasing particle size, with the detailed displacements likely due to the disruption of intramolecular hydrogen bonds in cellulose and hemicellulose and the formation of soluble sugars and new amorphous cellulose due to mechanical forces generated by superfine-grinding (Zhao et al., 2013). In addition, the exposure and conversion of hydrophilic groups could effectively increase the hydration capacity of the powder, which is the material basis for the high antioxidant activity of the superfine-grinding powder (Ramachandraiah & Chin, 2016).

3.7 Superfine-grinding enhances the in vitro antioxidant activity of Dendrobium nobile

Compounds with high antioxidant capacity can act as reducing agents, transition metal chelators and inhibitors of oxidative stress-related enzymes in foods, pharmaceuticals and chemicals. However, there are differences in the chemical properties and antioxidant mechanisms of different biological antioxidants, so it is necessary to comprehensively evaluate the antioxidant activity through various methods. In this study, we measured the scavenging capacity of DPPH radicals, ABTS radicals and nitrite ions to compare the antioxidant activity of different particle sizes of. Figure 5A-5C show the antioxidant activity of *Dendrobium nobile* powders of different particle sizes.

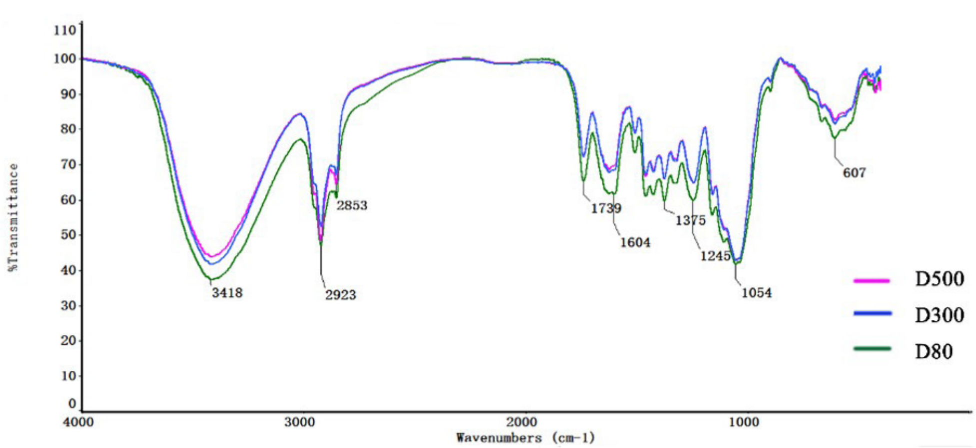

Figure 4. FTIR spectra of *Dendrobium nobile* powders of different particle sizes.

Table 2. The main absorption peaks of the infrared spectra of *Dendrobium nobile* powders of different particle sizes.

Dendrobium nobile Lindl. particle size (μm)			Assignments
D_{80}	D_{300}	D_{500}	
Wave number (cm^{-1})			
3418.96	3419.36	3419.54	O-H and N-H group stretching
2923.85	2924.08	2924.38	CH and CH_2 stretching
2853.83	2854.17	2854.32	CH and CH_2 stretching
1739.96	1740.17	1740.32	C=O stretching
1604.76	1623.50	1623.78	COO- stretching
1507.99	1507.69	1507.54	Aromatic skeletal stretching
1458.50	1459.76	1460.23	CH and Aromatic skeletal stretching
1425.68	1424.91	1424.72	CH_2 and CH symmetric bending
1375.62	1375.69	1375.91	CH_2 and CH symmetric bending
1245.26	1244.12	1243.74	C-O stretching
1160.48	1158.68	1158.00	C-O-C stretching
1054.38	1053.80	1051.86	C-O stretching
607.51	606.81	604.99	O-H bending

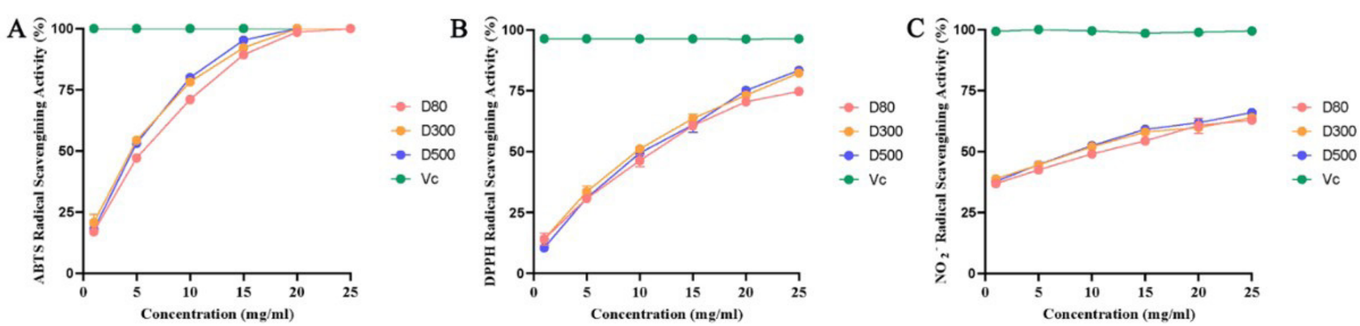

Figure 5. Antioxidant activity of different particle sizes of Dendrobium nobile powders. A: ABTS radical scavenging activity; B: DPPH radical scavenging activity; C: NO2-radical scavenging activity.

ABTS analysis is commonly used to assess the total antioxidant capacity of single compounds and complex mixtures of various plants (Fan et al., 2009). ABTS radical scavenging assays are based on the transfer of electrons from antioxidants to ABTS radicals, based on the principle that when blue-green $ABTS^+$ reacts with antioxidants, the solution fades to colourless (Liu et al., 2019). In the present study, the scavenging activity of *Dendrobium nobile* powder with different particle sizes against ABTS radicals was shown in Figure 5A, with the decrease in particle size of *Dendrobium nobile* after superfine crushing, ABTS scavenging activity increased.

The DPPH radical scavenging ability assay is widely used to estimate the radical scavenging activity of natural compounds due to its rapidity and sensitivity (Le et al., 2022; Wang et al., 2018). The method is based on the reduction of the original purple-red DPPH radical to yellow (non-radical form, DPPH-H) by providing hydrogen and electrons, with the maximum absorption value of the original purple-red colour at 517 nm being reduced (Shen et al., 2018). In Figure 5B, the DPPH radical scavenging activity was compared for different particle sizes and it can be seen that the DPPH scavenging activity of *Dendrobium nobile* powder extract showed a similar trend to the ABTS radical scavenging activity, i.e. as the particle size of *Dendrobium nobile* decreased, the DPPH scavenging activity increased.

Excessive consumption of nitrite can lead to negative effects such as oxidation of haemoglobin, leading to methaemoglobinaemia (Choi et al., 1989), and is highly mutagenic and carcinogenic (Lin et al., 2012). Nitrite (NO_2-) is a precursor to the formation of nitrite, which is also potentially harmful to human health. Therefore, if a substance has the ability to scavenge nitrite or its precursors, then it may have a cancer-preventive effect (Yan et al., 2003). The principle is that nitrite is diazotized with p-aminobenzenesulphonic acid under weakly acidic conditions and then coupled with naphthylenediamine hydrochloride to produce a red compound. We found that the nitrite ion scavenging ability of *Dendrobium nobile* samples increased with decreasing particle size, showing the same trend as the scavenging ability of ABTS and DPPH.

4 Conclusion

In this study, we obtained *Dendrobium nobile* powders of different particle sizes by shear crushing and superfine-grinding, and further investigated the effects of different particle sizes on the sensory properties, physical properties, chemical composition and antioxidant properties. The results showed that superfine-grinding could effectively reduce the particle size and change the sensory properties and physical properties, as evidenced by an increase in bulk density and Water solubility index, and a decrease in swelling capacity and water holding capacity. In addition, the method facilitated the release of total polysaccharides, dendrobine, total polyphenols and total flavonoid components, thereby increasing ABTS, DPPH and nitrite ion scavenging activity. FTIR and SEM analyses showed no effect of superfine-grinding on the primary structure of the fractions with decreasing particle size. These results provide a useful basis for improving the quality and clinical application of *Dendrobium nobile* and demonstrate that it is an effective grinding method. This study is expected to provide new insights into the effects of superfine-grinding on the properties of *Dendrobium nobile* powders and provide theoretical support for its application in the pharmaceutical industry and development of functional food.

Conflict of interest

There are no conflicts to declare.

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