Research Article

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The Extraction of DAM Enhance Antioxidant Capacity of Mice *In-vivo*

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ABSTRACT

A mixture of functional complex extraction from *Dendrobium Candidum* and other Chinese herbs enhances. Plant polysaccharides are one of the most bioactive substances present in plants. Due to the broad-spectrum therapeutic properties and biosafety, natural polysaccharides isolated from plants have recently been attracted attention by more and more researcher. The present study was designed to investigate the possible anti-aging and intestinal flora effect of *Dendrobium Candidum* and *Astragalus* mongholicus (DAM) on the aging model mouse. In the experiment, 48 aging female Institute of Cancer Research mice (ICR mice) were randomly divided into the control group and low-medium-high dose groups (200,400,800 mg/kg/day). The results showed that gradually recovered the body weight, the activity of daily living, and organ indices of mice. To be specific, DAM obviously improved the activities of antioxidant enzymes in liver and spleen of ICR mice, including catalase (CAT) and superoxide dismutase (SOD), as well as decreased malondialdehyde (MDA) levels when compared with those in control group mice. Furthermore, the DAM can improve the weight-bearing swimming time of the mice, increase the tolerance of the aging body, shorten the mouse's adaptation to the labyrinth, increase the number of individuals entering the open end and prolong their stay time on the basis of not affecting the mobility of the mice and has a certain anti-anxiety effects. The abundance of pathogenic bacteria of *Bilophila* and *Oceanobacillus* in the intestinal tract of aging mice was higher than that of the compound compound intragaemia group. The abundance of intestinal probiotics such as *Akkermansia, Bacteroides* and *Bifidobacterium* in the low-dose group was higher than that of the aging control group, and also higher than that of the medium-dose and high-dose groups. These results demonstrate that DAM preparation had an effect on improving the antioxidant capacity of organs in aging mice, delaying the aging of organs, and enhancing the resistance of aging individuals.

KEYWORDS: *Dendrobium Candidum; Astragalus* mongholicus; Aging mice; Antioxidant

INTRODUCTION

Aging, in other words "senescence", is a physiological process which may induce deleterious changes of the structural integrity and physiological function, and is always accompanied with the senile change and the occurrence of progressive memory loss, degradation of cognitive function, even lead to Parkinson's and Alzheimer's diseases and so on [1]. Therefore, how to maintain the health of the elderly and delay the occurrence of aging and aging-related diseases has already become a research hotspot for researchers. The free radical theory of aging is well-accepted that the most significant determinant of aging is oxidative damage caused by overproduction of reactive oxygen species (ROS) [2]. Free radicals, produced from normal biochemical reactions in metabolic processes of the body, play important role in the human

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body and become harmful only when they are produced in high amounts. The formation of excess ROS directly and indirectly results in oxidative stress and leading to protein oxidation, lipid peroxidation, DNA damage and the endogenous antioxidant system damage, even causing diseases, such as cancer, atherosclerosis, immunosuppression, diabetes and so on. Antioxidants, just like scavenger, can clean up free radicals and alleviating oxidative stress to prevent the physiological damage on organism [3]. So, it is received more and more academic attentions currently. However, the widely used synthetic antioxidants are suspected to have potential damage to liver, stomach, even causing cancer. Recently, more and more researchers take attention to find the natural and effective antioxidants to replace the synthetic antioxidants. Reduction of oxidative stress has thus been found to contribute to the maintenance of healthy living. Many natural plant secondary metabolites are good botanical antioxidants. Natural oxidants are bioactive phytoconstituents with the ability to scavenge reactive oxygen and nitrogen species, which are the major causes of oxidative stress in the body. The use of natural antioxidant in the management of disorders associated with oxidative stress is becoming an attractive alternative in recent time. At present, polysaccharides, extracted from plants, have been widely exploited and used in health products such as *ganodorma lucidum*. Meanwhile, the antiaging activities of polysaccharide are also worthy of further researches.

Polysaccharides are extensively distributed in the cell membranes of animals, higher plants, algae, bacteria and fungi. Chemically, they are macromolecules composed of more than ten monosaccharide units linked by glycosidic bonds [4]. Plant polysaccharides are compounds that are common in nature and consist of many identical or different monosaccharide alpha-or betaglyglycoside bonds. Natural plant polysaccharides widely exist in plants, is an extremely important biological macromolecule in plants, and is also one of the basic substances to maintain and ensure the normal operation of biological activities. Plant polysaccharides have relatively extensive sources and no cytotoxicity. Therefore, the study of plant polysaccharides has been paid more and more attention. The 21st century is even called the century of polysaccharides. Scientific experimental studies have shown that many plant polysaccharides have biological activities, including immune regulation, anti-tumor, reduce blood glucose and lipid, anti-radiation, antioxidant activities, inhibition of lipid peroxidation, anti-aging [4], antibacterial antiviral, liver protection and other health effects.

Dendrobium candidum Wall. ex Lindl is a kind of traditional Chinese medicine plants and food for many years. It grows within 1600 meters mountain with wet rock. It is rare medicinal herbs owing to its widely medicinal use [5-7]. The medicinal part of *Dendrobium* is fresh or dry stem, beneficial to stomach and fluid, nourishing Yin and clearing heat, immune regulation, anti-aging and other effects; Used for Yin wound in deficient, dry mouth irritated thirst, food less dry vomiting, disease after virtual heat, dark eyes.

In traditional Chinese medicine, *D. candidum* is often combined with other traditional Chinese medicine to prepare compound preparations for disease treatment. The composition analysis of *D. candidum* shows that the main containing polysaccharide it is beneficial to human body [8], such as on the medicinal function research, *D. candidum* able to resist the body fatigue [9], strengthen immunity [10], activity of oxidase [11], promote digest, inhibit tumor growth [12] and radiation protection, etc [13,14]. Gao [15] study *D. candidum* compound preparations of antioxidant and anti-aging effect, by the Caenorhabditis elegans oxidative stress model, to confirm this compound mixture antioxidant activity of the body, at the same time under the condition of compound mixture of subjects for a health model the influence of life, it is concluded that compound mixture with antioxidant activity, can improve the survival rate of *C. Elegans* oxidative stress model and extend the life of the conclusion.

In the present study, aging activity of the extractive DAM were investigated in mice *in-vivo*.

MATERIALS AND METHODS

Materials and Chemicals

D. candidum was purchased from nanjing. Commercially available kits for SOD, MDA and CAT were provided by Nanjin Jiancheng Bioengineering Institute (Nanjing, China) [16].

Animals

Healthy six-month-old female ICR mice, weighing approximately 42g, were purchased from the Center of Laboratory Animal Science of Shanxi Medical University (certificated number: SCXK (Jin) 2015-0001). Mice were maintained in cages at an ambient temperature of 23±2°C with 55 ± 10% relative humidity in a 12 h light/dark automatic lighting cycle during the experimental period of four weeks. They could access to the standard pellet diet and drink water libitum throughout the study period.

Preparation of Aqueous Extract of DAM

There is 50 g DAM was weighed, mixed with water at a ratio of 1: 10 (w/v). Then them were placed in 37 °C 250W ultrasonic cleaning instrument for 30 min, centrifugation was 6000r/min for 6 min, repeated extraction was performed once, supernatant obtained from two extracts was combined, and rotary evaporation was used for later use.

Preparation of Aging Mouse Model and DAM Treatment

Table 1: Treatment schedule of the study.

Groups	DAM		
	200(mg/kg/day)	400(mg/kg/day)	800(mg/kg/day)
Control	-	-	-
HD	-	-	+
MD	-	+	-
LD	+	-	-

Note: += With treatment, −= Without treatment

After adaptation for one week, mice were randomly divided into four groups of eight mice each according to Table 1. The model mice chose natural aging mice at 6 months of age. Mice in HD, MD, and LD groups were treated with the sample solutions (three different doses of DAM) per day by gavage, respectively, which were administered daily as the treatment schedule displayed in Table 1. Each experimental mouse was gavaged at 0.1 ml/10 g per day, while the control group was given normal saline at the same dose for 31 days.

Daily Observation of Mice

During the experimental period, the body weight of mice was measured by an electronic balance once a week. In addition, the appetite, appearance, mental condition, and behavioral activity of mice in each group were observed and recorded per week, respectively [17].

Table 2: Anti-oxidation activity of liver of mice fed with DAM.

Groups	MDA (U/mgprot)	Increase Rate (%)	SOD (U/mgprot)	Increase Rate (%)	CAT (U/mgprot)	Increase Rate (%)
Control	97.50±11.50	-	254.12±17.86	+	171.91±10.20	+
HD	50.89±15.67*	-47.81	259.67±0.55	2.2	200.05±6.12	16.37
MD	49.15±5.55*	-49.56	268.20±13.35	5.54	238.38±18.98**	38.67
LD	39.23±12.47*	-59.77	278.12±15.12	9.44	212.16±3.60*	23.41

Note: *p<0.05,**p<0.01, ***p<0.001(All significance analyses were compared with controls).

Preparation of Mice and Sample Collection

After 31 days, 24 h after the last drug administration, the mice were fasted overnight, and their blood samples were taken from the retrobulbar venous plexus. The whole blood of mice was centrifuged at 3000 r/min for 30 min, and the upper serum was collected. After packaging, it was stored at -40 °C for later use. Subsequently, the spleens and livers of each mouse were dissected out, washed with cold sterile physiological saline and weighed. After that, the liver and spleens were stored immediately at -80 °C for the sequent biochemical analysis.

Measurement of the Activities of SOD, CAT, and MDA in Mice Liver, Spleens and Serum

The activities of SOD, CAT, and MDA in liver, spleens and serum were determined by the assay kits.

Measurement of the Weight-Loaded Swimming Capacity

On the 31st day of the experiment, the weight-loaded swimming test was employed in our study to evaluate the effects of DAM on the endurance capacity of mice. The swimming time to exhaustion was used as the index of the degree of fatigue. The mice were assessed to be exhausted when they cannot keep their nose out of the water within a 10 s period.

Elevated Plus Maze

Affective behavior was assessed in the elevated plus maze. The apparatus consisted of two open arms and two closed arms and was located 50 cm above the floor. Mice were placed in the center of the maze and the duration open (OT) and closed arm (CT) entries was measured. In the meantime, the number of entries to both the open (OE) and closed arms (CE) was recorded. The time spent in the two open or closed arms was recorded for 4 min. The number of downward inquiries (HD) and upright legs (RE) were recorded.

Statistical Analysis

Graph Pad Prism 6 software was used for statistical analysis of all experimental data, and the results were expressed as x±s, and one-way analysis of variance was used for differences among groups.

RESULTS

Observation of Mice Behavior

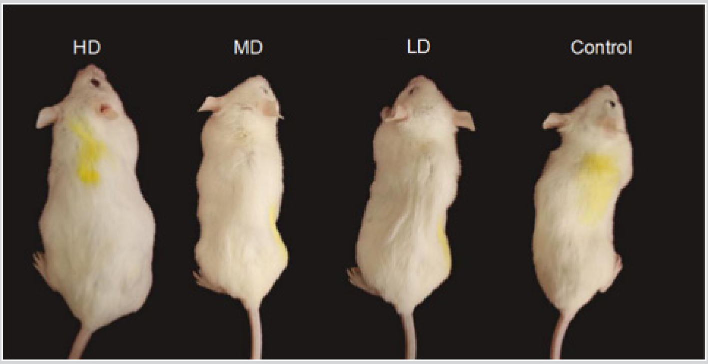

Figure 1: Individual size and spine bending in each group.

After adaptive feeding, the body weight intake of experimental animals tended to be stable, and there was basically no difference in the body weight of experimental mice in each group. At three weeks after the experiment, all experimental animals in each group showed signs of aging, such as reduced activity, curled up in piles, lethargy, hair thinning on the top of the head, and spinal curvature, indicating that the natural aging model could be used for further experiments (Figure1).

The Effect of DAM on Antioxidant Indices of Live and Spleen

SOD plays an important role in cell antioxidant process, can eliminate oxygen free radical (O^2 -), plays the role of scavenger in the cell, can eliminate lead to human aging free radical, let the cell avoid being attacked. MDA is a product of lipid peroxidation. Its activity level reflects the severity of free radical attack on cells. Therefore, the measurement of SOD and MDA content can indirectly reflect the antioxidant capacity of body machinery. Hydrogen peroxide is a waste product of the metabolic process that can cause damage to the body. To avoid this damage, hydrogen peroxide must be quickly converted to other harmless or less toxic substances. CAT is a tool often used by cells to catalyze the decomposition of hydrogen peroxide.

Antioxidant activity in mice liver

Table 3: Antioxidant activity of spleen in mice fed with DAM.

Groups	MDA (U/mgprot)	Increase Rate (%)	SOD (U/mgprot)	Increase Rate (%)	CAT (U/mgprot)	Increase Rate (%)
Control	99.36±6.82	-	158.04±4.15	+	37.88±5.85	+
HD	66.72±14.28	-32.85	170.84±1.00*	8.1	89.99±27.03	137.58
MD	83.19±7.85	-16.27	197.83±3.44**	25.18	149.34±4.07**	294.29
LD	85.97±38.28	-13.45	187.46±3.08**	18.61	109.48±3.20*	189.04

Note: *p<0.05, **p<0.01, ***p<0.001 (All significance analyses were compared with controls).

As shown in Tables 2 that the SOD, CAT activity and MDA content of all the experimental groups of DAM were better than the aging control group. The MDA content in the liver of mice in the low-dose group decreased 59.77% compared with the control group, and the effect of the medium-dose group was better than the high-dose group. For SOD activity, the low dose group had the best effect, and SOD activity in low dose group was 9.44% higher than that in the control group. The CAT activity of the medium dose group was the best, which was 38.66% higher than the aging control group. It can be clearly seen from the horizontal comparison of enzyme activity in each dose group that all indexes in the high-dose group are the lowest in the experimental group, which may be related to the role of liver in the body, as well as the concentration of gavage in the experiment. Liver is an important metabolic organ of the body. The feeding concentration we designed is relatively large for normal feeding of mice, which may affect the metabolism of mice liver and thus affect enzyme activity.

Mice spleen antioxidant activity

The SOD and CAT activities indicate the ability to scavenge oxygen free radicals. The MDA contents reflect the current degree of lipid peroxidation. The effect of DAM on spleen enzyme activity in mice was shown in Table 3. The results showed that the average value and relative growth rate of enzyme activity of each group showed that SOD and CAT enzyme activity of mice after gavage were better than the aging group. For spleen, the indexes of the medium-dose group were the best, followed by the low-dose group, and were the worst in the high-dose group. Comparing the optimal indexes with the aging group, the SOD activity increased by 25.18%, and the CAT activity increased by 294.29%. MDA content in the high-dose group decreased the most, 32.84% compared with the control group. Based on all the results, the DAM has an effect on improving the antioxidant capacity of spleen in senescent mice, which can delay the senescence of spleen and enhance the resistance of senescent individuals. However, the gavage dose may be too high, the experimental group with low dose has an obvious effect, and the edible concentration needs to be further explored.

Mice Behavior Experiment

Mice weight-bearing swimming experiment

Table 4: Exhaustive swimming time of DAM.

Groups	Repeats (n)	Weight-Bearing Swimming Time(s)
Control	6	307.3867±45.3697
HD	6	1080.1167±207.7109***
MD	6	659.8017±84.4867*
LD	6	502.605±73.3369

Note: *p<0.05, **p<0.01, ***p<0.001 (All significance analyses were compared with controls).

The effect of DAM on the fatigue time of mice swimming under load is shown in Table 4. The mean exhaustion time of all experimental mice with oral and gastric compound preparation increased compared with the aging model group, indicating that DAM could increase the exercise endurance and delay the exercise fatigue of animals. Longitudinal comparison of the results of each group showed that the experimental mice fed high-dose compound preparation showed excellent anti-fatigue effect, which was significantly different from the model control group and the low-dose group ($p < 0.001$).

Overall mobility and anti-anxiety test of mice

Table 5: Statistics of elevated plus mazes of mice fed with DAM.

Groups	Action-Ability (OE+CE)	Enter Open Arm Times (s)	Percentage of Open Arms (OE%)	Stay Open Arm Time(s)	Percentage of Retained Open Arm (OT%)
Control	7.60±2.15	0.80±0.4	10.23±5.74	14.63±16.42	6.98±7.52
HD	11.20±3.66	2.60±0.8*	26.31±11.52*	51.00±7.29**	38.51±2.41***
MD	12.80±2.79*	3.00±1.33**	25.71±10.23*	53.31±1.31**	23.84±2.29**
LD	10.40±2.32	2.00±0.89	18.31±4.82	36.50±12.16	21.71±2.13

Note: *p<0.05, **p<0.01, ***p<0.001 (All significance analyses were compared with controls).

DAM gastric mice anti-anxiety test results are shown in Table 5, the table shows that through the mouth for all groups, 9 mice action ability, into the open arms number and retention time were increased, in does not affect the test based on individual ability to act (there was no significant difference of total mobility), compound high dose group and the middle dose group of OE percentage and OT percentage have significantly increased, high dose group has the optimal effect, was 26.31% and 38.51% (Figure 2).

The influence of DAM on the number of mice elevated cross maze and the number of hind legs was shown in Table 6. According to the data analysis in the table, the number of mice fed with DAM in the open end of the maze and the number of closed end hind legs increased compared with the aging control group, but there was no significant difference between the groups of different doses.

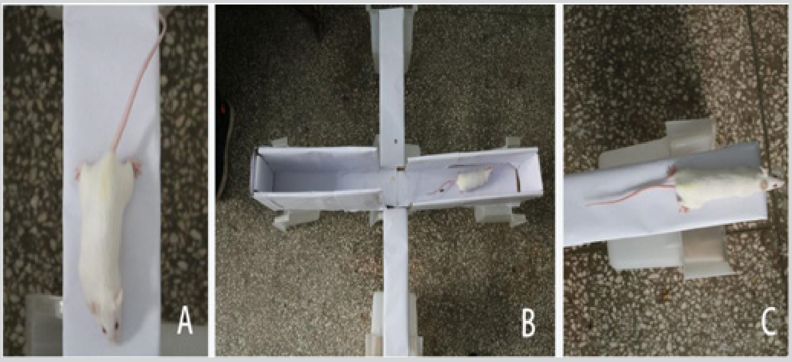

Figure 2: Elevated plus maze experiment.

Table 6: DAM intragastric mice inquire-avoidance test.

Groups	Repeats (n)	HD(n)	RE(n)
Control	6	2.00±2.28	16.80±4.26
HD	6	5.80±1.33**	16.40±3.88
MD	6	4.20±1.60	27.20±6.04*
LD	6	4.60±1.49	23.00±5.83

Note: *p<0.05, **p<0.01, ***p<0.001 (All significance analyses were compared with controls).

Effect of DAM on Intestinal Flora of Mice

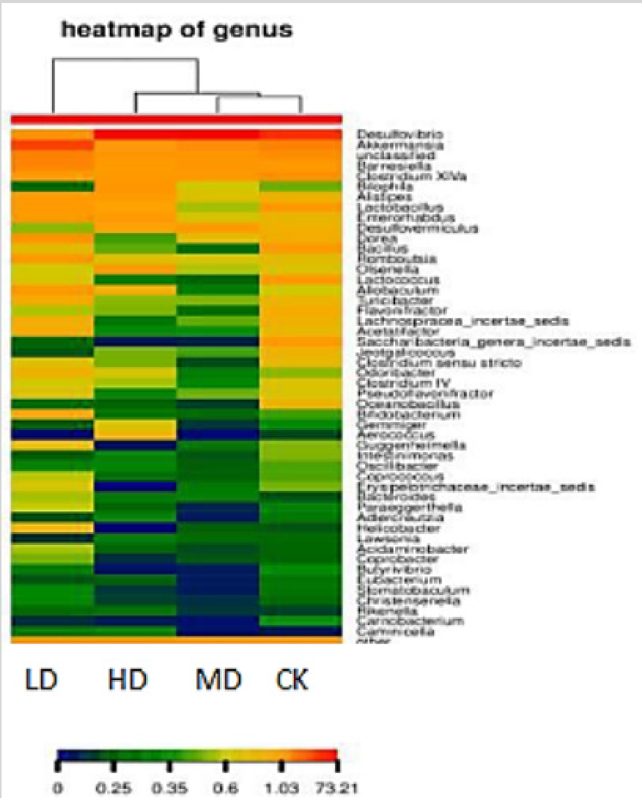

Figure 3: Heat map of genus level species abundance.

The intestinal flora of each group of mice was analyzed by the method of macro genome sequencing. The number of microbial groups in each group of mice was similar at the genus level. The top 50 species with the highest species richness were selected for analysis, and as shown in Figure 3, the redder the color block, the higher the abundance, while the bluer the lower the abundance. Different color depth indicates different microbial abundance. According to the figure, *Bilophila* is a gram-negative bacterium, which is common in patients with appendicitis. *Oceanobacillus* is a kind of pathogenic bacteria common in skin infection, which can cause infection and diseases. The abundance expression of several pathogenic bacteria in the intestinal tract of aged mice was higher than that in the group of compounds. *Lactobacillus* is widely found in the intestinal tract of the body and can increase gastrointestinal peristalsis. *Bifidobacterium* is an important intestinal probiotic, which has the functions of enhancing nutrition, enhancing immunity, anti-tumor, improving gastrointestinal function and anti-aging. *Bacteroides* are bacteria that can break down polysaccharides and cellulose that the human body cannot digest. The discovery of this kind of bacteria is closely related to human health, such as regulating obesity, diabetes and heart disease. *Akkermansia*, a bacterium found to degrade mucin, which keeps the lining of the gut intact, is one of the hottest bacteria associated with weight loss. As can be seen from the figure, the abundance of these intestinal probiotics in mice in the low-dose group was higher than that in the aging control group, and also higher than that in the medium-dose and high-dose groups. This result indicates that the compound preparation has the function of regulating intestinal flora, and the effect is the best when taking low-dose group.

DISCUSSION

The internal mechanism of the body consists of many enzymes and non-protein compounds that protect the body from free radicals and reactive oxygen species produced during normal metabolism, which are also produced by external stimulation. These include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione, which al so play an important role in detoxification and coordinating the body's antioxidant defense [18]. Superoxide dismutase is a metal protein that removes superoxide anions[19] .Catalase is a heme protein, located in the peroxidosome or microperoxidosome, which catalyzes the decomposition of H_2O_2 into water and oxygen, thus protecting cells from oxidative damage caused by H_2O_2 [20].Malondialdehyde content is one of the important parameters reflect the body's antioxidant potential ability, lipid oxidation end-products of malondialdehyde in vitro influence mitochondrial respiratory chain complexes and key enzyme activity in the mitochondria, it can also aggravate the damage of membrane, can reflect the body lipid peroxidation rate and intensity, can indirectly reflect the organization peroxidation damage degree [21].

In this study, the self-formulated DAM was used to feed ICR aging female mice for 31 days. The behavior and weight changes of the mice were recorded and the activity of antioxidant enzymes in the homogenate of organ tissues was measured. The compound preparation can obviously increase the spleen and liver coefficient of aging body and may play an important role in improving immunity [22]. The loading swimming time and open-end retention time of mice in the high-dose group were significantly higher than those in the control group, indicating that the preparation could improve the exercise endurance of the organism and change the anxiety of the organism. The experiment provided the basis for determining the optimal dosage of DAM, and found its effect on anti-anxiety, anti-depression and improving intestinal flora, which provided the theoretical basis for derivative products of *D. Candidum* [23].

CONCLUSION

Dendrobium Candidum is rich in active ingredients, among which polysaccharides are the most important. In this study, polysaccharides of DAM were extracted to gavage aging model mice, and its anti-oxidative aging effect was studied. During the experiment, the behavior of the mice was observed, and the levels of SOD, CAT and MDA in the spleen and liver of the mice were measured. The changes of the intestinal flora of the mice were studied by the load-bearing swimming experiment and the elevated cross maze experiment. The results showed that the activity of antioxidant enzyme in each group increased, among which the middle dose group was the best. The activity of CAT enzyme in spleen increased by 294.29% and SOD increased by 25.18% compared with the control group. However, the experiments of weight-bearing swimming and viaduct maze in mice showed that the time of weight-bearing swimming and the time of open-end detention in mice were significantly increased in the high dose group. The intestinal probiotics abundance of the low dose mice was higher than that of the aging control group. The above results indicated that the DAM had the effect of scavenging oxygen free radicals, improving the exercise endurance of the organism and changing the anxiety of the organism. The experiment provided a basis for determining the optimal dosage of DAM, and found its effects on anti-depression, anti-anxiety and improvement of intestinal flora, which provided a theoretical basis for *Dendrobium Candidum* derivatives.

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