



Effect of environmental factors on the polysaccharide content of *Dendrobium officinale*

Daopeng TAN¹ , Yao ZENG¹, Jiangmei WANG¹, Yanliu LU^{1,2}, Shudong SUN³, Lin QIN^{1,2*} , Yuqi HE^{1,2*}

Abstract

Dendrobium officinale is a traditional Chinese medicine and nourishing food in China. Polysaccharide content was used as a criterion for evaluating the quality of *D. officinale*. At present, epiphytic cultivation is the main cultivation method of *D. officinale* in China. In the present study, *D. officinale* was collected under various growing conditions to compare its polysaccharide content in an attempt to discover the most favorable growing conditions for *D. officinale*, which can be used to guide the artificial cultivation production of *D. officinale*.

Keywords: *Dendrobium officinale*; polysaccharide; quality control.

Practical Application: This investigation provides valuable guidance for the cultivation production of *D. officinale*.

1 Introduction

In recent years, the R&D of natural functional products from herbs has attracted more and more attention in the world (Ruiz-Cisneros et al., 2022; Wang et al., 2022a, b; Yin et al., 2022). The genus *Dendrobium* is one of the largest genera in the orchid family, containing 1500-2000 species (Hou et al., 2017). Among them, *Dendrobium officinale* Kimura et Migo is the main source of the traditional Chinese medicine *Dendrobii caulis*, which is widely distributed throughout the world, such as the United States, Australia, and Japan (Tang et al., 2017). In particular, *D. officinale* is widely grown in various regions of China, including Zhejiang, Anhui, Hunan, Guizhou, Fujian, Guangxi, and Yunnan provinces (Liu et al., 2020; Yan et al., 2015; Yang et al., 2020).

D. officinale is firstly recorded in the “Shen Nong’s Herbal Classic” (Dong Han Dynasty, A.D.25–220) and used as traditional Chinese medicine or functional food in China (Yin et al., 2021). In traditional medicine, *D. officinale* was as a tonic to nourish Yin, clear heat, nourish stomach, and replenish body fluid (Cakova et al., 2017; Shin et al., 2017) and used for various diseases or as beverages (Cakova et al., 2017; Tan et al., 2023). In term of modern pharmacological effects, *D. officinale* exhibits various pharmacological effects such as enhancing immunity, anti-fatigue, antioxidant, hypoglycemia, hypotension, and others (He et al., 2022; Huang et al., 2019; Lv et al., 2020).

According to current phytochemical investigations, more than 190 compounds have been isolated from *D. officinale*, including polysaccharides, alkaloids, amino acids, flavonoids and other nutritional components (He et al., 2022). Among them, polysaccharides have been shown to regulate intestinal homeostasis and protect against carbon tetrachloride-induced liver injury in mice, and was considered to be its most important active components. In the *Chinese Pharmacopoeia* (Ch.P., 2020 edition) (*Chinese Pharmacopoeia Commission*, 2020),

polysaccharides have been selected as the only quality markers of *D. officinale*, and polysaccharide content was used as a criterion for evaluating the quality of *D. officinale*.

To obtain a large amount of green and organic *D. officinale* herbs, it is now common to grow *D. officinale* in China by attaching trees (Figure 1) (Tan & He, 2020). There have been several reports in the literature that the cultivation sites and cultivation techniques of *D. officinale* affect its polysaccharide content (Tan et al., 2020; Zeng et al., 2020). However, these reported samples are limited in number and do not accurately reflect the main growing factors of polysaccharide content of *D. officinale*. Therefore, in this study, *D. officinale* was collected under various growing conditions to compare its polysaccharide content in an attempt to discover the most favorable growing conditions for *D. officinale*, which can be used to guide the artificial cultivation production of *D. officinale*.

2 Materials and methods

2.1 Chemicals

The reagents required for the polysaccharide assay including phenol, anhydrous ethanol, concentrated sulfuric acid, and glucose standards were purchased from Merck (Darmstadt, Germany), Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), and Kelong Chemical Reagent Factory (Chengdu, China), respectively.

2.2 Plant materials

Under the premise of ensuring sustainable utilization and representativeness, the *D. officinale* samples were collected from the Good Agricultural Practices (GAP) bases located in Guizhou and Zhejiang Province of China in 2019. All samples

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¹Guizhou Engineering Research Center of Industrial Key-technology for *Dendrobium Nobile*, School of Pharmacy, Zunyi Medical University, Zunyi, China

²Joint International Research Laboratory of Ethnomedicine of Ministry of Education, Zunyi Medical University, Zunyi, Guizhou, China

³Anlong County Xichengxiushu Agriculture and Forestry Co., Ltd., Anlong, Guizhou, China

*Corresponding author: 505181163@qq.com; yqhe.pharm@foxmail.com



Figure 1. Growing environment of *D. officinale*.

were authenticated by Associate Professor Daopeng Tan (pharmacognosy, Zunyi medical university).

2.3 Polysaccharides content determination

Sample preparation

Samples of *D. officinale* was dried at 60 °C, crushed, and sieved through No.3. Take about 0.06 g of sample, weighed precisely, and refluxed with 40 mL of water, heated for 2 h. Cooled, transferred into a 50 mL volumetric flask, and fixed with water to the scale line. 4000 rpm, centrifuged for 15 min. 2 mL of supernatant is taken, 10 mL of anhydrous ethanol is added, mixed well, and left for 1 h at 4 °C. Centrifuge at 4000 rpm for 20 min and discard the supernatant. Add 8 mL of 80% ethanol solution, centrifuge at 4000 rpm for 20 min, discard the supernatant, and repeat once. Dissolve the precipitate with heated water, allow to cool, transfer to a 10 mL volumetric flask, add water to the scale to obtain the sample solution to be tested.

Polysaccharide assay

Take 1.0 mL of the sample to be measured into a 10 mL stoppered test tube, add 1.0 mL of 5% phenol solution (ready to use) and 5 mL of concentrated sulfuric acid, shake well rapidly, heat in boiling water for 20 min, then ice bath for 5 min. 200 μ L of the reaction solution was taken into a 96-well plate, and the absorbance was measured at 488 nm with an enzyme marker, and the polysaccharide content was calculated by substituting into the standard curve.

Preparation of glucose standard curve

The glucose standard was weighed precisely and prepared into 180 μ g/mL of glucose solution, i.e. the reserve solution. The reserve solution was diluted and prepared as 30.47, 60.93, 91.40, 121.87, 152.33 and 182.80 μ g/mL, and the phenol-sulfuric acid color development reaction was performed according to the method of polysaccharide assay, and the absorbance was determined together with the samples.

Method validation

The linearity, precision, repeatability, stability, and recovery were checked for method validation.

2.4 Statistical methods

SPSS18.0 was used for statistical analysis, and t-test was used to calculate the comparison between two groups, One-way ANOVA was used to calculate the comparison between more than two groups, and the measurement data were expressed as Mean \pm SD, and $P < 0.05$ was statistically different.

3 Results and discussion

3.1 Sample collection

The samples were collected at five *Dendrobium* cultivation sites in Dushan county, Danzhai county, and Xingyi city of Guizhou province, and Taizhou and Yueqing city of Zhejiang

province (Figure S1). *Dendrobium* samples were collected according to different altitudes, different epiphytic species and different illuminance. Eight to ten samples of *D. officinale* were collected for each factor, 380 samples were collected in total. The information in detail is listed in Table 1. The fresh stems were dried, ground into powder, passed through a sieve with 300 mesh, and stored at -80 °C for subsequent analysis.

3.2 Validation of the method

Linear regression equations (e.g., $y = ax + b$) were constructed by absorbance (x) of each analyte against analyte concentrations (y; $\mu\text{g/mL}$). The results are shown as Figure 2, and the linear

equation of the standard curve was $y = 215.18x - 0.9978$, $r = 0.9999$. The results indicated that there was a good linear relationship between the absorbance and concentration of glucose in the range of 30.47~182.80 $\mu\text{g/mL}$.

Precision was evaluated by using variability assessed with six replicates within one day. The variation (RSD%) for precision was shown in Table 2. The repeatability was conducted using six replicates of the same sample, and the variations of repeatability was 0.63% (Table 3). The stability of the sample solution was investigated at 0, 0.5, 1, 2, 4, 6, and 12 h. The RSD% of absorbances of the analyzed sample was 2.39% (Table 4), indicating that the samples were stable at least 12 h.

Table 1. Sample information of *D. officinale*.

Location	Epiphytic trees	Longitude and Latitude	Altitude(m)	Illuminance	Sample size
Dushan	<i>Pinus</i>	E107°31'2"N5°56'5"	852	sunny	10
Dushan	<i>Pinus</i>	E107°31'2"N5°56'5"	852	shady	10
Dushan	<i>Pinus</i>	E107°31'2"N25°55'47"	918	sunny	10
Dushan	<i>Pinus</i>	E107°31'2"N25°55'47"	918	shady	9
Dushan	<i>Cunninghamia lanceolata</i>	E107°31'2"N5°56'5"	852	sunny	8
Dushan	<i>Cunninghamia lanceolata</i>	E107°31'2"N5°56'5"	852	shady	9
Dushan	<i>Cunninghamia lanceolata</i>	E107°31'2"N25°55'47"	918	sunny	9
Dushan	<i>Cunninghamia lanceolata</i>	E107°31'2"N25°55'47"	918	shady	10
Dushan	<i>Cyclobalanopsis glauca</i>	E107°31'2"N5°56'5"	852	sunny	8
Dushan	<i>Cyclobalanopsis glauca</i>	E107°31'2"N5°56'5"	852	shady	9
Dushan	<i>Quercus fabri</i>	E107°31'2"N25°55'47"	918	sunny	9
Dushan	<i>Quercus fabri</i>	E107°31'2"N25°55'47"	918	shady	9
Dushan	<i>Quercus fabri</i>	E107°31'2"N5°56'5"	852	sunny	9
Dushan	<i>Quercus fabri</i>	E107°31'2"N5°56'5"	852	shady	9
Danzhai	<i>Camellia sinensis</i>	E107°79'90"N26°25'23"	891	shady	28
Xingyi	<i>Cyclobalanopsis glauca</i>	E105°24'54"N24°58'37"	835	sunny	9
Xingyi	<i>Cyclobalanopsis glauca</i>	E105°24'54"N24°58'37"	835	shady	8
Xingyi	<i>Cyclobalanopsis glauca</i>	E105°27'8"N24°59'12"	978.5	sunny	10
Xingyi	<i>Cyclobalanopsis glauca</i>	E105°27'8"N24°59'12"	978.5	shady	9
Xingyi	<i>Cyclobalanopsis glauca</i>	E105°27'57" N24°54'3"	1122	sunny	8
Xingyi	<i>Cyclobalanopsis glauca</i>	E105°27'57" N24°54'3"	1122	shady	9
Xingyi	<i>Albizia julibrissin</i>	E105°24'54"N24°58'37"	835	sunny	9
Xingyi	<i>Albizia julibrissin</i>	E105°24'54"N24°58'37"	835	shady	10
Xingyi	<i>Albizia julibrissin</i>	E105°27'8"N24°59'12"	978.5	sunny	9
Xingyi	<i>Albizia julibrissin</i>	E105°27'8"N24°59'12"	978.5	shady	9
Xingyi	<i>Albizia julibrissin</i>	E105°27'57" N24°54'3"	1122	sunny	9
Xingyi	<i>Albizia julibrissin</i>	E105°27'57" N24°54'3"	1122	shady	9
Xingyi	<i>Cyclobalanopsis glauca</i>	E105°24'54"N24°58'37"	835	sunny	8
Xingyi	<i>Cyclobalanopsis glauca</i>	E105°24'54"N24°58'37"	835	shady	9
Xingyi	<i>Cyclobalanopsis glauca</i>	E105°27'8"N24°59'12"	978.5	sunny	9
Xingyi	<i>Cyclobalanopsis glauca</i>	E105°27'8"N24°59'12"	978.5	shady	9
Xingyi	<i>Cyclobalanopsis glauca</i>	E105°27'57" N24°54'3"	1122	sunny	9
Xingyi	<i>Cyclobalanopsis glauca</i>	E105°27'57" N24°54'3"	1122	shady	9
Taizhou	<i>Amygdalus persica</i>	E121°6'57"N28°26'26"	34	sunny	9
Taizhou	<i>Cinnamomum camphora</i>	E121°6'57"N28°26'26"	34	shady	10
Yueqing	<i>Myrica rubra</i>	E121°9'53"N28°22'7"	166	sunny	9
Yueqing	<i>Myrica rubra</i>	E121°9'53"N28°22'7"	166	shady	8
Yueqing	<i>Ziziphus jujuba</i>	E121°7'14"N28°26'16"	28	sunny	9
Yueqing	<i>Ziziphus jujuba</i>	E121°7'14"N28°26'16"	28	shady	8
Yueqing	wooden pile	E121°7'14"N28°26'16"	28	sunny	10

Table 2. Precision of polysaccharide content in *D. officinale*.

No.	Absorbance	Mean	RSD
1	0.5422	0.5372	0.63%
2	0.5397		
3	0.5377		
4	0.5361		
5	0.5344		
6	0.5331		

Table 3. Repeatability of polysaccharide content of *D. officinale*.

No.	Content	Mean	RSD
1	39.50%		
2	38.18%		
3	37.53%	38.35%	2.05%
4	38.73%		
5	38.68%		
6	37.45%		

Table 4. Stability of polysaccharide content in *D. officinale*.

Time (h)	Absorbance	Mean	RSD
0	0.5353	0.5266	2.39%
0.5	0.5241		
1	0.5359		
2	0.5089		
4	0.5371		
6	0.5097		
12	0.5353		

The recovery test was used to evaluate the accuracy of the method. Precisely weighed six powders of *D. officinale* in parallel, and about 23 mg of dextran was added to each of them. The test samples were prepared according to the method of polysaccharide assay, and the absorbance was determined according to the method of preparation of glucose standard curve. The recovery of each spiked reference standards was calculated by the formula $\text{recovery}\% = \frac{\text{found amount} - \text{original amount}}{\text{spiked amount}} \times 100\%$. The results are shown in Table 5. The recoveries of polysaccharides ranged from 95.27% to 100.73% with the RSD value of 2.00%, indicating that the method is accurate.

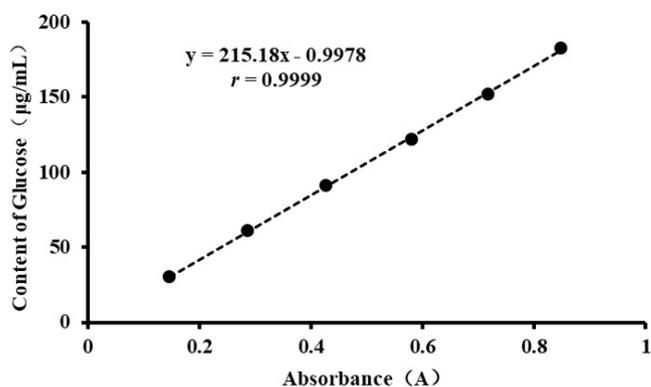
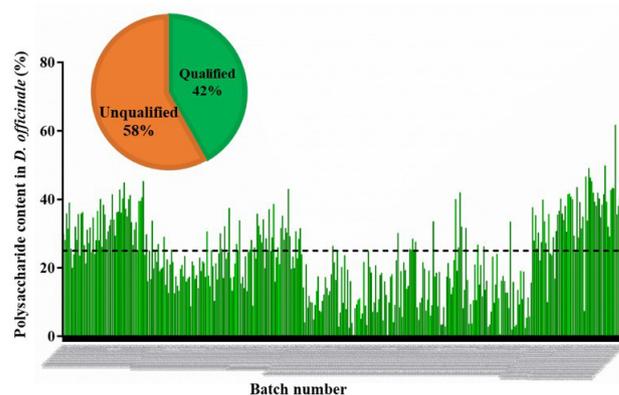
3.3 Analysis of polysaccharide content of *D. officinale*

Overall profile of polysaccharide content of *D. officinale*

A total of 380 *D. officinale* samples were collected according to different regions, altitudes and illuminance (Figure S1). The overall profile of polysaccharide content in 380 *D. officinale* samples was analyzed to determine the polysaccharide content. The horizontal coordinate in the figure is the sample number, the vertical coordinate is the percentage content of polysaccharide in *D. officinale*, and the dotted line is the standard limit of 25.00% of polysaccharide content in *D. officinale* as stipulated in the Chinese Pharmacopoeia 2020 edition, a part of herbs and beverages, and higher than this line means that the *D. officinale*

Table 5. Accuracy of polysaccharide content in *D. officinale*.

No.	Recovery	RSD
1	98.62%	
2	100.30%	
3	100.73%	2.00%
4	98.05%	
5	95.27%	
6	99.52%	

**Figure 2.** Standard curve of glucose solution.**Figure 3.** Overall profile of polysaccharide content in *D. officinale* of 380 batch.

samples are qualified. As shown in Figure 3, the polysaccharide content in 380 *D. officinale* samples was significantly different, and the highest polysaccharide content was 61.69%. 42% of the 380 samples were qualified. Among them, the polysaccharide content of *D. officinale* was mainly concentrated between 20% and 35%.

Effect of different origins on polysaccharide content of *D. officinale*

For the polysaccharide content of *D. officinale* from five regions, including Dushan county, Danzhai county, and Xingyi city of Guizhou province, and Taizhou and Yueqing city of Zhejiang

province were analyzed as shown in Figure 4. The polysaccharide content of *D. officinale* in Yueqing city was the highest among the five regions, and the polysaccharide content of *D. officinale* in Xingyi city was the lowest. The polysaccharide contents of *D. officinale* in Yueqing city were compared with those of Dushan, Denzhai, Xingyi and Taizhou respectively, and $P < 0.05$, which was statistically different, and the polysaccharide contents of *D. officinale* in Yueqing city were significantly different from those of Dushan, Denzhai, Xingyi and Taizhou. The polysaccharide contents of Xingyi city was statistically different from those of Dushan, Denzhai, Taizhou and Yueqing by two-by-two comparison with $P < 0.05$, and the polysaccharide contents of *D. officinale* in Xingyi city was significantly lower than those of the other four origins.

Effect of different altitudes on polysaccharide content of D. officinale

Analysis was conducted to compare the polysaccharide content of *D. officinale* at different altitudes (Figure 5.). The samples collected in Guizhou province ranged from 835 m to 1122 m in altitude, and the highest polysaccharide content of *D. officinale* was found at 918 m in altitude. The samples collected in Zhejiang Province ranged from 28 to 166 m above sea level, and the highest

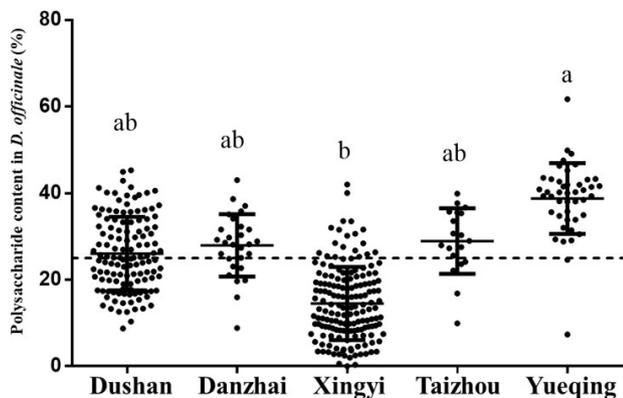


Figure 4. The effect of origin on polysaccharide content of *D. officinale* ($\bar{x} \pm SD$, $n=19-162$, a compared to Xingyi, $*P < 0.05$, b compared to Yueqing, $*P < 0.05$).

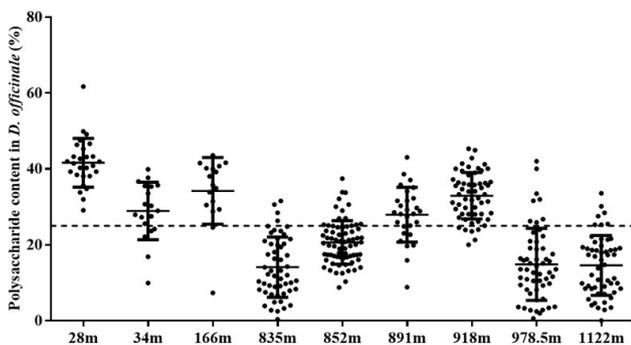


Figure 5. The effect of altitudes on polysaccharide content of *D. officinale* ($\bar{x} \pm SD$, $n=17-71$).

polysaccharide content of *D. officinale* was found at 28 m above sea level. Altitude was found to be the main factor affecting the polysaccharide content of *D. officinale*.

Effect of different tree species on polysaccharide content of D. officinale

The areas with more epiphytic tree species were selected to analyze the effects of different tree species on the polysaccharide contents of *D. officinale* in Dushan county of Guizhou province. As shown in Figure 6, there was no significant difference in the polysaccharide contents of *D. officinale* attached to different tree species such as *Cyclobalanopsis glauca*, *Quercus fabri*, *Cunninghamia lanceolata*, and *Pinus*, however, the polysaccharide content of *D. officinale* at an altitude of 918 m was significantly higher than that of the sample at an altitude of 852 m. Those results indicated that the main influencing factor is related to the altitude, but not to the epiphytic tree species.

Effect of illuminance on polysaccharide content of D. officinale

For the analysis of the effect of illuminance on the polysaccharide content of *D. officinale*, the polysaccharide content of *D. officinale* with shade and sunny growth was compared. It was found that the polysaccharide content of sunny-grown *D. officinale* was generally higher than that of the shady-grown. The passing rate of sunny-grown *D. officinale* was generally high, and all samples of sunny-grown *D. officinale* at an altitude of 1122 m passed in this sampling. The mean values of the polysaccharide content of *D. officinale* at different elevations of sunny and shady grown *D. officinale* were subjected to paired t-test, and the results showed that the polysaccharide content of sunny-grown *D. officinale* was significantly higher than that of the shady-grown (Figure 7).

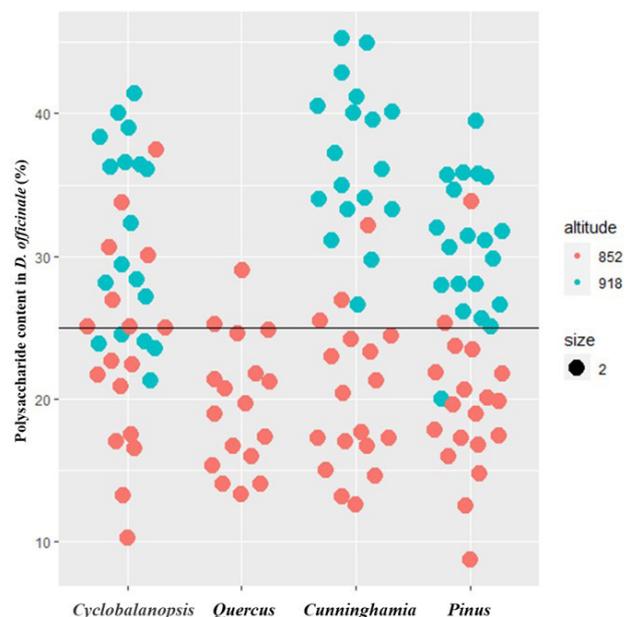


Figure 6. The effect of tree on polysaccharide content of *D. officinale*.

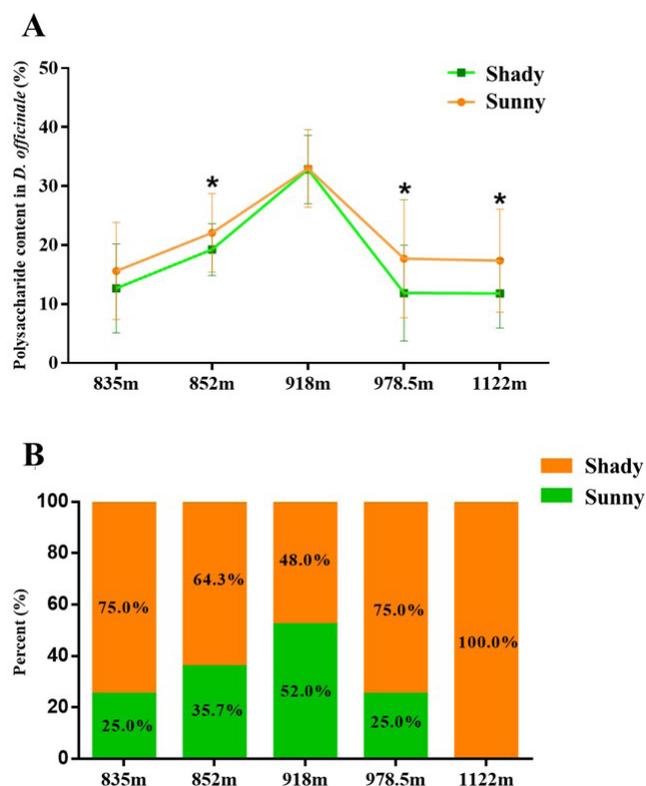


Figure 7. The effects of illuminance on polysaccharide content of *D. officinale* (A) illuminance on polysaccharide content of *D. officinale* (B) the proportion of qualified sunny and shady grown *D. officinale* $\bar{x} \pm SD$, $n=19-81$, * $P < 0.05$).

4 Conclusion

At present, epiphytic cultivation is the main cultivation method of *D. officinale* in China. In the present work, the results of our study showed that different epiphytic tree species did not have significant effects on the polysaccharide content of *D. officinale*. The environmental factors affecting the polysaccharide content of *D. officinale* mainly include the origin and altitude. Those results will provide valuable guidance for the cultivation of *D. officinale*.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Material

Supplementary material accompanies this paper.

Figure S1. Sample collection of *D. officinale*

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