# Comparative Analysis of Nutritional Quality of *Dendrobium Officinale Kimura Et Migo* After Transplantation of Artificially Cultivated Plants From South to North China During Different Growing Years

**Xiaohui Guo**
China Agricultural University
**Shijing Zhang**
China Agricultural University
**Ting Yan**
China Agricultural University
**Guoqiang Yuan**
China Agricultural University
**Yafeng Dai**
Jiuxianzun Huoshan Dendrobium Co., Ltd. Anhui
**Lanying Dong**
Beijing Jiawancheng Biological Technology Co., Ltd
**Zengli Wang** ( wangzengli@cau.edu.cn )
China Agricultural University

**Research Article**

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# Abstract

**Background:** *Dendrobium officinale Kimura et Migo* is a traditional functional food and medicinal plant in China. Due to low natural regeneration rates, habitat destruction, excessive collection and commercial trade, *D. officinale* is severely threatened, and commercial artificial-sheltered cultivation has been massively used to meet the needs of the market.

**Aim:** To comprehensively compare the accumulation of nutritional compounds during 3-5 years of introduced, artificial-sheltered cultivation from southern to northern China.

**Methods:** *D. officinale* of the same species were artificially cultivated in the southern traditional cultivation area, Anhui and the new northern cultivation area, Beijing. First, samples were collected in the third, fourth, and fifth years of growth, and nutritional quality indexes, including polysaccharides, alkaloids, flavonoids and total phenolic content, were determined. Second, an untargeted metabolomics method was used to investigate metabolic variations in *D. officinale* stems between Anhui and Beijing cultures in the fifth year.

**Results:** After comparing the nutrient accumulation in different growing years, the idea harvest time was found in the third growing year in both cultivation areas. Of them, the contents of polysaccharides, flavonoids and total polyphenol were higher in cultivation in Anhui than Beijing, but the accumulation of alkaloid content was much lower in Anhui. The highest amount of polysaccharides of *Dendrobium officinale* was found in the three-year cultivation in Anhui, which reached 515.75 mg/g. When metabolites were analysed, a total of 272 metabolites were detected in the current study, including 27 up-regulated and 73 down-regulated metabolites in *D. officinale* cultivated in Beijing compared with samples from Anhui.

**Conclusion:** *D. officinale* artificially and transplanted cultivated from southern to northern China showed some significant differences in the accumulation of nutrient compounds. Planting in northern China has some specific advantages, but the overall nutritional value is not as good as planting in southern China. Our study contributes to a better understanding of the nutrient profiles of *D. officinale* through artificial cultivation in different areas.


# 1. Background

*Dendrobium officinale Kimura et Migo (D. officinale)* is a traditional functional food and medicine herbal, which is widely planted in southern China such as Anhui, Fujian, Zhejiang, Guizhou and Yunnan provinces[1, 2]. Healthy effects are found in the prevention and treatments of diabetes, hypertension, obesity, protection against gastric ulcers and hypertension, and immune enhancement in animal models[3–9]. The widely recognised functional properties are mainly due to the various phytochemicals in the D. officinale, which mainly include polysaccharides, phenanthrenes, bibenzyls, saccharides and glycosides, essential oils, alkaloids and polyphenols[1, 10].

Due to the demanding growing conditions, low natural fecundity, slow growth and destructive collection, the source of wild D. officinale are threatened with extinction[11]. To meet the market demand, artificial cultivation is gradually displacing wild D. officinale. Thus, from 2014 to 2016, a rapid expansion of cultivated areas can be observed in the south and Southwest China, except for the traditional wild cultivated areas in Yunnan and Zhejiang[12]. However, the development of Dendrobium industry has been inhibited by the lack of product standards and low product level. In addition, D. officinale has been rarely cultivated in northern China, although the integration of cultivation technology has solved the technical bottleneck in the conversion from wild to artificial-sheltered cultivation[13].

The objective of the current study was to comprehensively compare the accumulation of nutritional compounds during 3–5 years of artificial-sheltered cultivation in southern and northern China.

## 2. Results

## 2.1 Comparisons of phytochemicals in different growing years.

Comparisons of major nutrients, including polysaccharide, flavonoid, polyphenol, and alkaloid contents are shown in Fig. 2. When phytochemicals were compared between different growing years, significant differences were found for both growing regions. There was a decrease in the contents of polysaccharides, flavonoids and polyphenols when comparing *D. officinale* grown in Beijing with *D. officinale* grown in Anhui after 3 years, while in contrast, alkaloid contents increased significantly after the introduction of cultivation in Beijing.

In particular, the highest amount of *D. officinale* polysaccharides was found in three years cultivation in Anhui, which reached 515.75mg/g. With the expansion of cultivation, *D. officinale* polysaccharides decreased significantly in both Beijing and Anhui. For *D. officinale* flavonoids, the highest value was 27.85mg/g, which was found in Anhui area at three years cultivation. In addition, we found a total polyphenol content of 15.99mg/g after three years of cultivation in Anhui. The highest content of alkaloids was found in Beijing region with 0.24 mg/g.

## 2.2 Comparisons of qualitative metabolic profiling of *D. officinale*.

Table 1 shows the metabolic profiling classification of the stem samples from Anhui and Beijing, where the data were analyzed using the widely targeted metabolomics approach. The diverse molecules detected could be roughly classified into 10 major classes, mainly alkaloids, amino acids and derivatives, flavonoids, lignans and coumarins, lipids, nucleotides and derivatives, organic acids, phenolic acids and terpenoids. Of them, we found a total of 267 and 266 subclassification species in Anhui and Beijing, respectively. In addition, for the average ion abundance of each major classification, significant differences were found between Anhui and Beijing for amino acids and derivatives, nucleotides and

derivatives, organic acids, phenolic acids, terpenoids and others in the current study. The detailed information of ion abundance of *D. officinale* in Anhui and Beijing are shown in Supplementary Table 1 and Supplementary Table 2.

Table 1.

Classification of the detected metabolites in *D. officinale* stem in Anhui and Beijing.

| **Class Organic** | **Number of Compounds** | | ***P*** |
|---|---|---|---|
| | **Anhui** | **Beijing** | |
| Alkaloids | 32 | 32 | 0.717 |
| Amino acids and derivatives | 48 | 49 | 0.014 |
| Flavonoids | 65 | 64 | 0.587 |
| Lignans and Coumarins | 2 | 2 | 0.481 |
| Lipids | 44 | 43 | 0.831 |
| Nucleotides and derivatives | 32 | 32 | < 0.001 |
| Organic acids | 8 | 8 | < 0.001 |
| Phenolic acids | 9 | 9 | 0.010 |
| Terpenoids | 1 | 1 | 0.033 |
| Others | 26 | 26 | < 0.001 |
| *P* shows the difference between total contents of each classification, and were analyzed by pared t test. | | | |

# 2.3 Comparisons of quantitative metabolic profiling of *D. officinale.*

Figure 3 shows a heatmap hierarchical clustering of detected metabolites and a heatmap divided into different primary metabolic categories in the *D. officinale* stem in Anhui and Beijing. Both heatmaps differed in the hierarchical clustering of the detected metabolites of *D. officinale* from Anhui and Beijing, suggesting that although the original *D. officinale* belongs to the same species, the metabolites may differ significantly according to years of plant environment. In addition, the classified heat map (Fig. 3B) showed that some metabolites were more accumulated in the samples from Anhui than Beijing.

The scores plot obtained from PCA analysis and the PLS-DA scores plot of *D. officinale* stem in Anhui and Beijing were shown in Fig. 4. PCA showed that the percentages of the values in the metabolite analysis of PC1 and PC2 were 39.96% and 12.25%, respectively. Partial least squares discriminant analysis (PLS-DA) is widely used as an effective method for screening different metabolites between groups. In the current

study, we found a clear separation between the samples from Anhui and Beijing, which also suggests that the planting area and planting environment might contribute significantly to the metabolite composition, again indicating potentially different health benefits of the same species.

## 2.4 Differentially Accumulated Metabolites between Anhui and Beijing

The differences in metabolites between the two growing regions were shown in Fig. 5. In the current study, a total of 272 metabolites were detected, including 27 up-regulated and 73 down-regulated metabolites in *D. officinale* grown in Beijing compared to samples from Anhui. Among these metabolites, the top 10 up-regulated metabolites are 6 flavonoids, 2 amino acids and derivatives, 1 nucleotide and derivatives, and 1 polysaccharide, while the top 10 down-regulated metabolites include 6 flavonoids, 2 nucleotides and derivatives, 1 amino acid and derivatives, and 1 lipid, as the data shown in Fig. 5B.

Figure 6 showed the KEGG enrichment maps of the differential metabolites. After annotating all metabolites to the Kyoto Encyclopedia of Genes and Genomes (KEGG) compound database, 48 metabolites were found, among which there were 3 metabolites with significant differences. Of these, four other differential metabolites that were detected in the samples but were not annotated on the KEGG pathway were added to KEGG pathways according to their similar properties. Among them, pathways including pyrimidine metabolism, ABC transporter, purine metabolism changed significantly ($p < 0.01$).

# 3. Discussion

*D. officinale*, a traditional Chinese functional food and perennial medicinal herb, has shown great pharmacological activity and economic value in recent years[1, 14]. Due to low natural regeneration rates, habitat destruction, excessive collection and commercial trade, wild resources are on the verge of extinction and cannot meet the growing market demand, so massive commercial artificially protected cultivation has been used to expand production[12]. However, most consumers believe that the quality and efficacy of *D. officinale* is safer and more effective among the wild collections[12].. There are a number of factors that affect the overall quality of *D. officinale*, including temperature, moisture, minerals, and pathogenic bacteria[15]. In the current study, in order to make a comprehensive comparison of *D. officinale* cultivated by the same species in different environments, we compared the main phytochemical contents between wild planting in Anhui and artificial-sheltered cultivation in Beijing during 3–5 years growing, and we explore the characteristics of nutrient components in a circle of 12 months. In addition, we compared the metabolite characteristics of both cultivated samples at their 5-year planting.

A number of in vivo and in vitro studies have shown that *D. officinale* has many health benefits, including antioxidant, anti-inflammatory, anti-diabetic, immune enhancing, antibacterial, and others. The proven benefits are attributed to its functional constituents, which mainly include polysaccharides, alkaloids and polyphenols[16–19]. Since *D. officinale* is a perennial herb, it usually takes 2–3 years for nutrient accumulation to reach the maximum level[12]. Previous studies have shown that the optimum harvesting

time is in the third year[20, 21], but few studies have further investigated the properties after 3 years of growth. In this study, we also found that the best harvesting time is in the third year and with the extension of the growth year, the accumulation of nutrients in the stem decreased significantly. Among the major nutrients detected, the contents of polysaccharides, flavonoids and total polyphenol were higher cultivated in Anhui than in Beijing, but the accumulation of alkaloids was much lower in Anhui than in Beijing. This might be due to the difference in hydrogen potential of irrigation water between the two locations. Polysaccharides are the main biological compounds in stems, we found that both collected samples could fully meet the specified requirements of more than 25.0% in the 2015 edition of Chinese Pharmacopoeia. Moreover, our results showed higher polysaccharide contents than previous studies[21], indicating that artificial cultivation can meet the basic quality requirements in polysaccharide contents. Nutrient accumulation may be closely related to the growing environment. The environmental differences between Anhui and Beijing were shown in Supplementary Table 3, the main difference between the two growing areas are the regulation of temperature and humidity.

Untargeted metabolic analysis has been widely used for a comprehensive comparison of detailed metabolite identifications under different conditions[11, 22, 23]. In our study, a total of 272 metabolites were detected in the current study, and the number of detections was greater than some studies. For example, a work aimed to compare metabolite characteristics between two medicinal Dendrobium found 139 major metabolites from *D. officinale*[20]. Others found more metabolites after different treatments, for example, one study shows a total number of 529 metabolites based on different cultivation substrates[23], another found 3,655 annotated metabolites with Ultraviolet-B radiation treatments, indicating that ultraviolet-B radiation helps to the accumulation of active compounds in *D. officinale*. Although different studies showed significant differences in the number of metabolites detected, this suggests that environmental factors play an important role in the types and amounts of metabolites.

With different metabolites, there may be potential differences in biological efficacy, and the application value may also be different, so specific metabolite analysis could be an important basis for quality control of *D. officinale*. In the current study, we found 6 significant differences among 10 nutritional classifications when we compared samples from Anhui with Beijing. Among them, the two most up-regulated flavonoids are isorhamnetin-o-gallate and cyanidin chloride, the former is a flavonol and belongs to the group of plant secondary metabolites known as flavonoids, which have been reported for many biological activities such as antioxidant, anticancer, antimicrobial and anti-inflammatory effects[24]. The latter is one of the most abundant types of anthocyanins and has been shown to have antioxidant properties inhibiting nitric oxide production in many studies[25, 26]. Moreover, sinapine was significantly upregulated in Peking samples, which led to significant improvements in energy, lipid and glucose metabolism in animal models[27–30], indicating that sinapine may be an important alkaloid in *D. officinale* cultivated in Beijing. In future research, the separation and purification of the discovered up-regulated substances and their health effects will help to explore new pharmacological mechanisms from the introduced cultivated *D. officinale.*

The main advantages of the current study include the following: First, the comparison of the accumulation of nutrients under different growing years (3-year, 4-year, and 5-year) between cultivation in Anhui and Beijing was conducted, and the data support that the best harvest time is in the third growing year. Second, the nutritional properties were fully compared in a one-year cycle, and the results show that the accumulation of nutrients is different in different months, and indicate that harvesting must be done according to specific circumstances and nutritional requirements. Finally, an in-depth comparison of the metabolite characteristics of Dendrobium in the two planting areas was conducted, which may provide further support for an in-depth study of biological effects in the future. Moreover, we have some limitations in this study, for example, the environmental factors affecting the accumulation of nutrients should be fully captured and the correlation needs to be analyzed in the future.

# 4. Conclusion

This study comprehensively compared the characteristics of the nutritional components of *D. officinale* before and after the introduced artificial cultivation during its 3- to 5-year growth. This study confirmed that the ideal harvest time of *D. officinale* is the third year of growth. The contents of major biological constituents, including polysaccharides, polyphenols and flavonoids, accumulated in Anhui were significantly higher than those cultivated in Beijing. However, the alkaloid content is higher than that cultivated in Beijing. The nutrient accumulation of *D. officinale* collected in different months differs greatly, and the metabolites are also significantly different. In conclusion, *D. officinale* artificially cultivated and transplanted from Anhui to Beijing cultivated have some significant differences in the accumulation of nutrient compounds. Planting in northern China has some specific advantages, but the overall nutritional value is not as good as planting in southern China. Our study contributes to a better understanding of the nutritional profiles of *D. officinale* through artificial cultivation in different areas.

# 5. Material And Methods

# 5.1 Materials and reagents

The seedlings used in the current study were of the same species from Lu'an, Anhui. Briefly, the stems of *D. officinale* collected from Juxianzun Huoshan Dendrobium Co, Ltd in Anhui and samples were tissue cultured, seeded in the bottle and then artificially cultivated in a multi-span greenhouse both in Anhui and Beijing (Beijing Jiawancheng Biological Technology Co, Ltd). No approvals were necessary during the course of this sample collection. The formal identification of the samples was undertaken by Anhui Province Non-major Crop Variety Appraisal and Registration Committee in the current study. The flowchart was shown in Fig. 1.

Samples were collected at each September after the 3rd, 4th, and 5th growing year. The 3-year-old *D. officinale* refers to harvest at the first September after the flowering period in the third year; the 4-year-old *D. officinale* refers to harvest at the second September after the first harvest at 3-year-old; and the 5-year-old *D. officinale* refers to harvest at the third September after the second harvest at the 4-year-old. After

sample collection, roots and leaves were removed and stems were washed with pure water, then dried to constant weight with hot-air drying under 60 ℃, and then samples were frozen using liquid nitrogen and finally stored at − 80 ℃ before determination.

Analytical-grade sodium hydroxide, concentrated hydrochloric acid, concentrated sulfuric acid, glucose, aluminum nitrate, sodium nitrite, sodium carbonate from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents used in the metabolites analyzed were HPLC-grade. Methanol, acetonitrile, and ethanol were purchased from Sigma (American). Rutin, gallic acid and Folin-Ciocalteu reagents were purchased from Sigma (USA). Deionized water was prepared by a MilliporeMilli-Q Plus system (Millipore, Bedford, USA).

# 5.2 Chemical testing

The total sugar content of the dried *D. officinale* stem was determined using the phenol–sulfuric acid method and D-glucose as the reference substance[31]. The total flavonoid content was determined through sodium nitrite–aluminum nitrate colorimetry and rutin as a standard product[32]. The total polyphenol content was determined by the Folin–Ciocalteu assay by using gallic acid as the reference[33]. The total alkaloid contents were measured by acid dye colorimetry, and dendrobine was used as the standard[34].

# 5.3 Metabolomics analysis

# 5.3.1 Sample Preparation and Extraction

The freeze-dried stem was crushed using a mixer mill (MM 400, Retsch) with a zirconia bead for 1.5 mins at 30 Hz. 100mg powder was weighted and extracted overnight at 4℃ with 0.6 ml 70% aqueous methanol. Following centrifugation at 10, 000g for 10 mins, the extracts were absorbed (CNWBOND Carbon-GCB SPE Cartridge, 250mg, 3ml; ANPEL, Shanghai, China, www.anpel.com.cn/cnw) and filtrated (SCAA-104, 0.22μm pore size; ANPEL, Shanghai, China, http://www.anpel.com.cn/) before UPLC-MS/MS analysis.

# 5.3.2 HPLC Conditions

The sample extracts were analyzed using an UPLC-ESI-MS/MS system (UPLC, Shim-pack UFLC SHIMADZU CBM30A system, www.shimadzu.com.cn/; MS, Applied Biosystems 4500 Q TRAP, www.appliedbiosystems.com.cn/). The analytical conditions were as follows, UPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm*100 mm); The mobile phase was consisted of solvent A, pure water with 0.04% acetic acid, and solvent B, acetonitrile with 0.04% acetic acid. Sample measurements were performed with a gradient program that employed the starting conditions of 95% A, 5 % B. within 10min, a linear gradient to 5% A, 95% B was programmed, and a composition of 5% A, 95% B was kept for 1min. Subsequently, a composition of 95% A,5.0 % B was adjusted within 0.10 min and kept

for 2.9 min. The column oven was set to 40°C; The injection volume was 4μl. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS.

## 5.3.3 ESI-Q TRAP-MS/MS Conditions

LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (Q TRAP), API 4500 Q TRAP UPLC/MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in positive and negative ion mode and controlled by Analyst 1.6.3 software (AB Sciex). The ESI source operation parameters were as follows: ion source, turbo spray; source temperature 550°C; ion spray voltage (IS) 5500 V (positive ion mode)/-4500 V (negative ion mode); ion source gas I (GSI), gas II(GSII), curtain gas (CUR) was set at 50, 60, and 30.0 psi, respectively; the collision gas (CAD) was high. Instrument tuning and mass calibration were performed with 10 and 100 μmol/L polypropylene glycol solutions in QQQ and LIT modes, respectively. QQQ scans were acquired as MRM experiments with collision gas (nitrogen) set to 5 psi. DP and CE for individual MRM transitions was done with further DP and CE optimization. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.

## 5.3.4 Bioinformatic analysis of the untargeted metabolomic dataset

Three softwares, were used to process the LC-MS/MS raw data (Smith et al., 2006). For MS data pretreatment, a series of operations, including peak and second peak grouping, peak picking, retention time (RT) correction, and annotation of isotopes and adducts, were carried out. Each ion was recognized by combining RT and m/z values. Intensity of each peak were calculated and a 3D matrix containing arbitrarily assigned peak indices (retention time-m/z pairs), sample names and ion intensity information was constructed. The peak features that were detected in less than 50% of quality control (QC) samples or less than 80% of experimental samples were disregarded, the remaining peaks were processed using the K-nearest neighbor algorithm to improve their quality. Principal component analysis was carried out for outlier detection and batch effects evaluation. In addition, the relative standard deviations of the peak features were recorded across all the QC samples, and those more than 30% were deleted.

## 5.3.5 PCA analysis

Unsupervised PCA (principal component analysis) was performed by statistics function prcomp within R (www.r-project.org). The data was unit variance scaled before unsupervised PCA.

## 5.3.6 Identification of differentially accumulated metabolites (DAMs) between groups.

Wilcoxon tests were used to detect the differences in metabolite levels between two sample groups. The *P* values were adjusted for multiple tests using an FDR with Benjamini– Hochberg method. Supervised partial least squares-discriminant analysis was applied through meta X software to discriminate the variables between two groups. A VIP cut-off value of 1.0 was used to screen key features. The

differentially accumulated metabolites (DAMs) were selected with |fold change|>2 and with statistical significance ($P < 0.05$).

## 5.4 Statistical analysis

SPSS 26.0 software (SPSS, Inc., Chicago, IL, U.S.A.) was performed to process all data presented as mean ± standard deviation (S.D.). Statistical analyses of the data were performed by one-way ANOVA using post hoc multiple comparisons; $P < 0.05$ was considered a significant difference.

# 6. Declarations

### 6.1 Ethics approval and consent to participate

Not applicable.

### 6.2 Consent for publication

Not applicable.

### 6.3 Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

### 6.4 Competing interests

The authors declare that they have no competing interests.


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### 6.6 Authors' contributions

“Conceptualization, X.H, G.; methodology, S.J, Z.; software, T, Y.; validation, S.J, Z., T, Y., and G.Q, Y.; formal analysis, X.H, G.; resources, Y.F, D., and L.Y, D.; writing-original draft preparation, X.H, G.; writing-review and editing, Z.L, W.; project administration, S.J, Z. all authors have read and approved the manuscript.


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# Figures

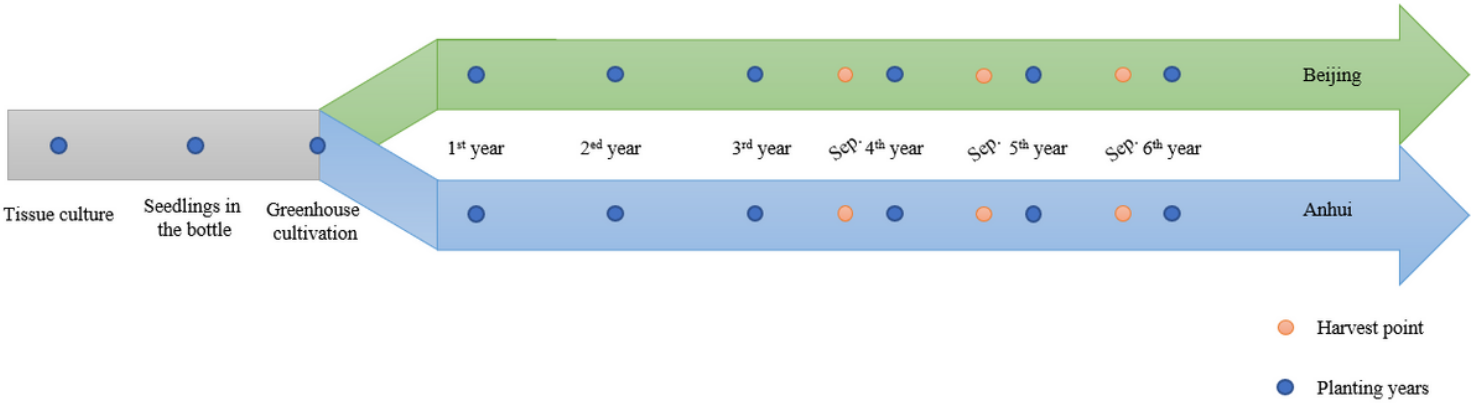


**Figure 1**

Flow chart of samples harvest The 3-year-old D. officinale refers to harvesting on the first of September after flowering in the third year; The 4-year-old D. officinale refers to harvest on the second of September after the first harvest in the 3-year-old year; The 5-year-old D. officinale refers to harvest on the third of September after the second harvest in the 4-year-old.

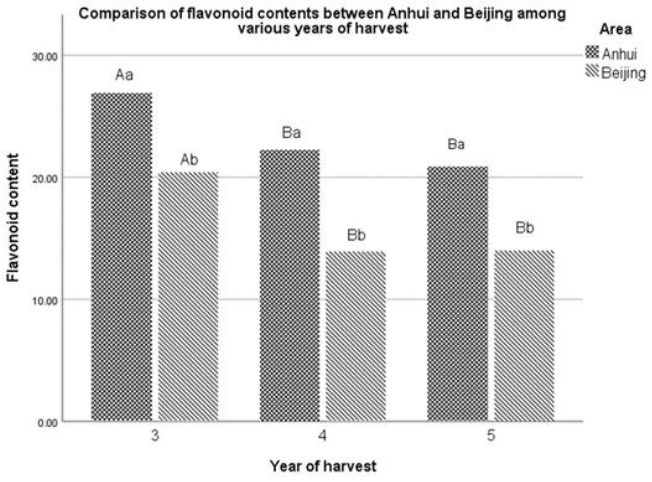

Figure2 A

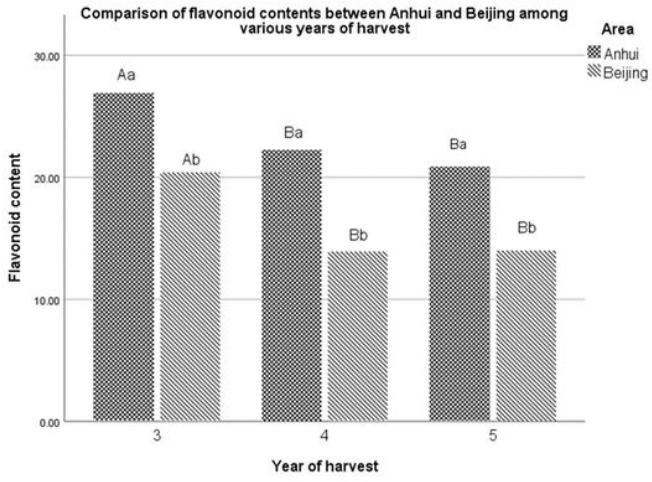

Figure2 B

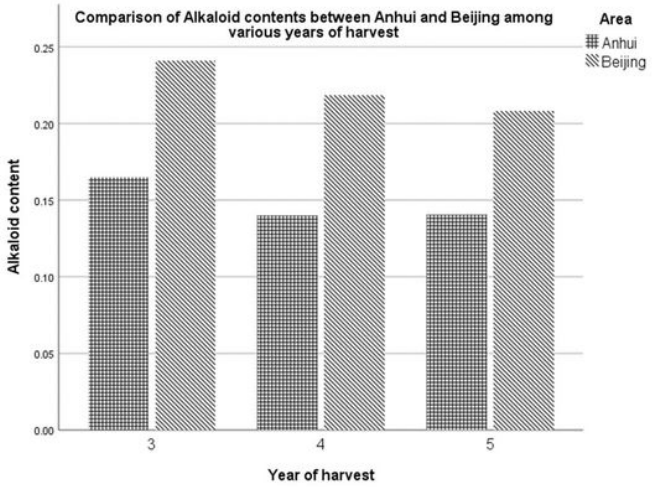

Figure2 C

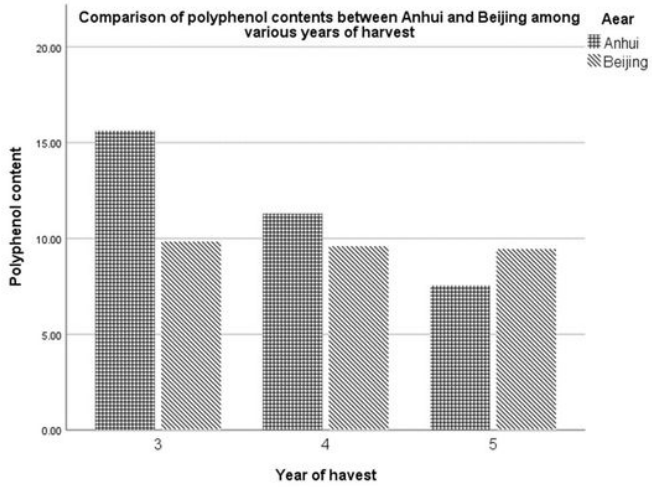

Figure2 D

## Figure 2

Comparisons of main nutritional compounds between Anhui and Beijing among various years of harvest. Figure2 A, comparison of polysaccharides contents between Anhui and Beijing; Figure2 B, comparison of flavonoids contents between Anhui and Beijing; Figure2 C, comparison of polyphenol contents between Anhui and Beijing; Figure2 D, comparison of alkaloids contents between Anhui and Beijing. Different capital letters above the same cultivation area indicate significant differences among different growth years. Different lowercase letters above the same growth year indicate significant differences between cultivation area.

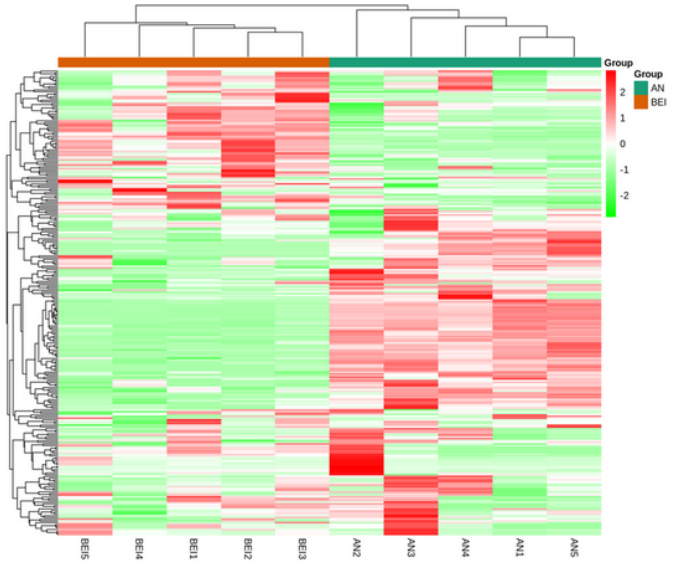


Figure3a. A heatmap hierarchical clustering of the detected metabolites

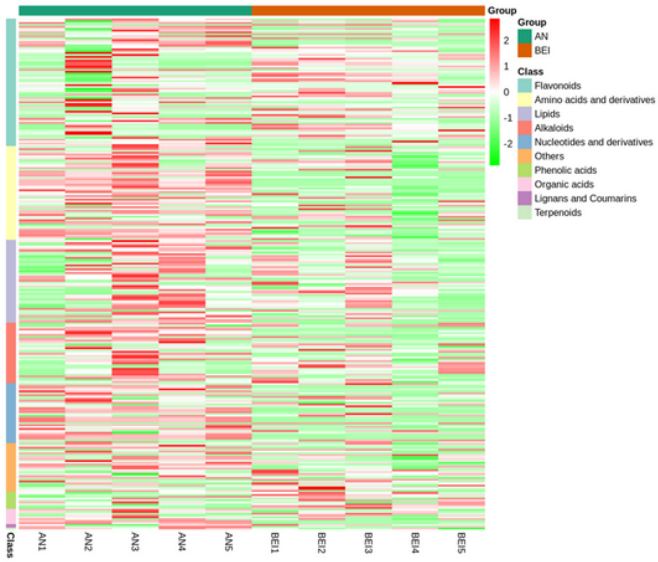


Figure3b. A heatmap of the metabolites classed into various major primary metabolic categories.

## Figure 3

Heatmap of untargeted metabolite profiling identifies the metabolites of D. officinale between Beijing and Anhui. The Log2 of the metabolite quantification was used. The columns correspond to the organs while the rows represent the different metabolites. Red means high content, green means low content.

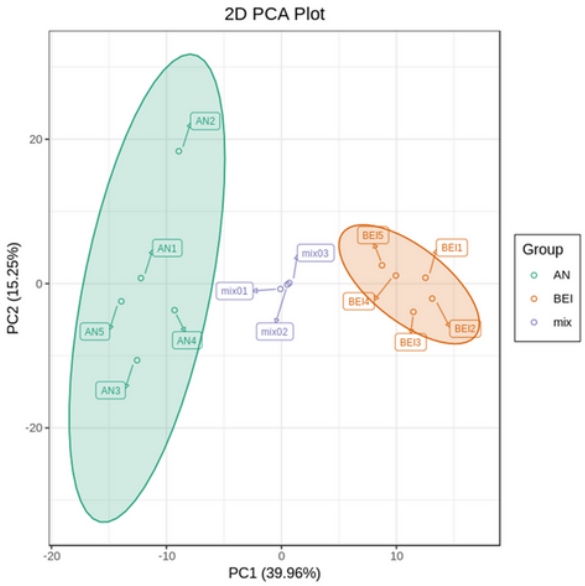


**Figure 4a.** Score plot of principal component analysis (PCA) for the stem of *D. officinale* cultivated in Beijing and Anhui.

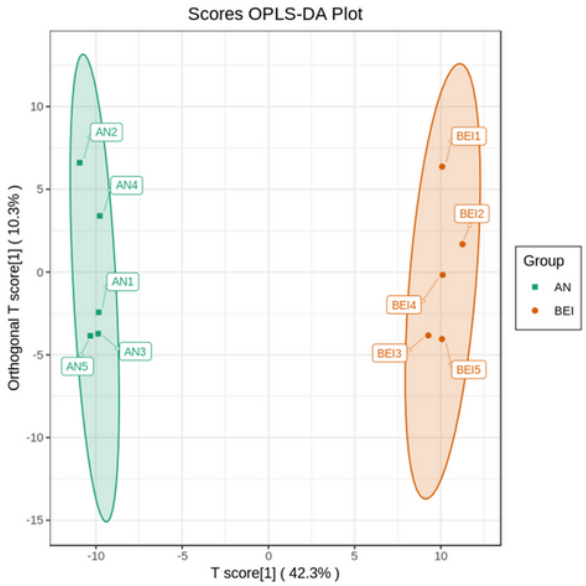


Figure 4b. Score plots of orthogonal partial least squares discriminant analysis (PLS-DA) from the stem of *D. officinale* cultivated in Beijing and Anhui.

## Figure 4

PCA analysis and the PLS-DA scores plot of D. officinale stem in Anhui and Beijing. PC1 represents the first principal component and PC2 represents the second principal component. The X-axis is the predictive principal component, and the Y-axis is the orthogonal principal component.

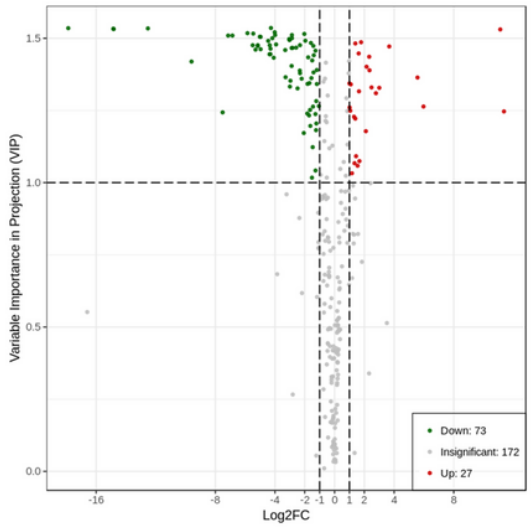


**Figure 5a.** Volcano plot showing the increased and decreased metabolites between the samples cultivated in Beijing and Anhui.

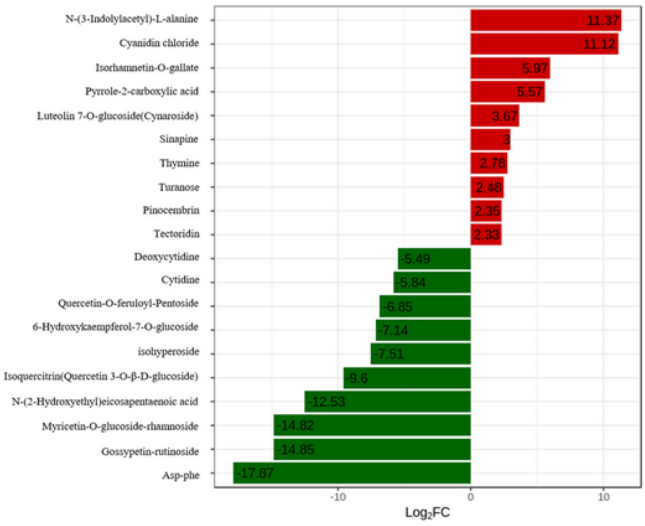


**Figure 5b.** Top 10 most up- and down- regulated metabolites under the comparison between the samples collected from Beijing to Anhui.

## Figure 5

The variations in the metabolites between two treatment groups. The green dots in the plots illustrated that the differential metabolites were significant and down-regulated, while the red dots illustrated that the differential metabolites were significant but up-regulated, and the black dots illustrated that the metabolites could be detected in samples but did not have any significant difference. The green color refers to the most up-regulated metabolites and the red color refers to the most down-regulated metabolites.

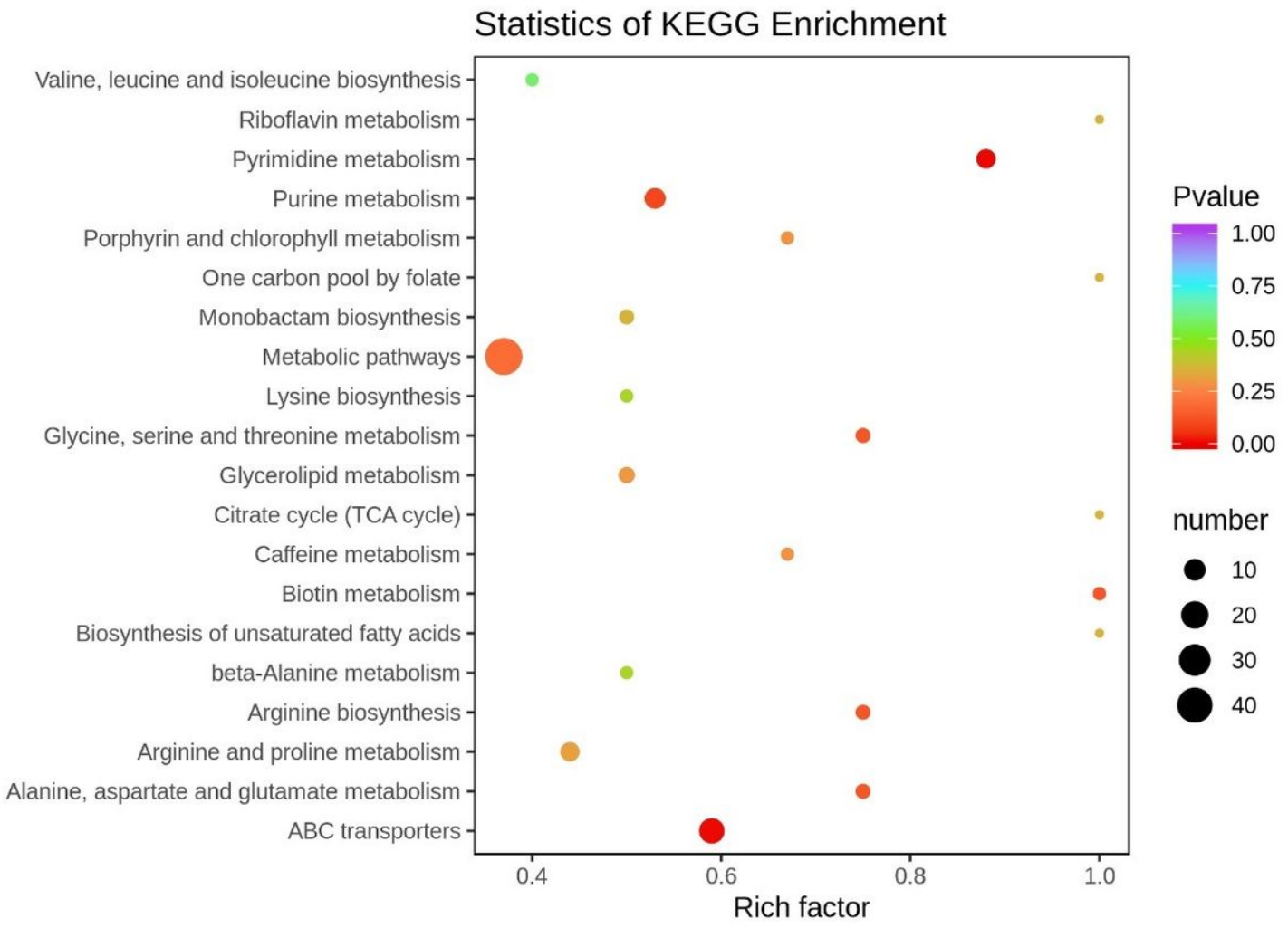


**Figure 6**

KEGG enrichment maps of differential metabolites. The abscissa represents the enrichment factor of the pathway and the ordinate shows the names of pathways. The color of the dot represents the p-value, and the deeper the red of the dot, the stronger the enrichment effects. The size of points represents the number of metabolites enriched in the pathways.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx
- SupplementaryTable2.metabolitesinformationbetweenBeijingandAnhui..xlsx
- Supplementarytable3.EnvironmentaldifferencebetweenAnhuiandBeijing.docx