Research Article

An UPLC-Q-TOF/MS-Based Analysis of the Differential Composition of Dendrobium officinale in Different Regions

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1.Introduction

The genus Dendrobium is one of the largest genera of the Orchidaceae, which contains 1500–2000 species [1]. Among them, Dendrobium officinale Kimura et Migo is the major source of Dendrobii caulis, a traditional Chinese medicine, which is widely distributed worldwide, such as the United States, Australia and Japan [2]. Especially, D. officinale is widely cultivated in various regions of China, including Anhui, Zhejiang, Hunan, Fujian, Guangxi, Sichuan, and Yunnan provinces [3–5].

D. officinale is a traditional Chinese medicine first recorded in the “Shen Nong’s Herbal Classic” and used alone or as a prescription [6]. According to the practitioner of Chinese medicine’s consensuses, the fresh or dried stems of D. officinale are considered to be a drug of nourishing Yin, which comprises the efficacy of nourishing the stomach for promoting the production of fluid, nourishing Yin and clearing heat, brightening the eyes, strengthening the waist and tonifying lung and kidney [7]. In addition, modern pharmacological studies have revealed that D. officinale has multiple promising bioactivities, including immunomodulation, antitumor, antioxidant, antifatigue, glycolipid regulation, hepatoprotection, etc [8–11]. According to current phytochemical investigations, more than 190 compounds have been isolated from D. officinale, including polysaccharides, alkaloids, amino acids, flavonoids, trace elements, and other nutrients, and its medicinal components are mainly composed of polysaccharides, bibenzyl, phenanthrene, flavonoids, and alkaloids [12–15]. Among them, polysaccharide was selected as the only quality marker in the current edition of the Ch.P., the relationship between polysaccharide and other small molecular components content is not yet known.
Due to the overexploitation and depletion of wild plant resources, D. officinale has become one of the rarest and most endangered Chinese herbal medicines in China, step by step [16,17]. In addition, with the current quality standards, the quality of D. officinale varies greatly from different region and growing environment [18]. Fortunately, the manual cultivation of D. officinale has made a great breakthrough, and currently D. officinale was mainly grown in greenhouses with the advantages of fast growth, high yield, and stable production, and became the most important source of the traditional Chinese medicine Dendrobii caulis. The components in Chinese herbal medicine are very complex. Thus, the quality of D. officinale cannot yet be accurately evaluated by its polysaccharides content alone. Therefore, the present study proposal explores the relationship between small molecular components and polysaccharides in D. officinale from greenhouse cultivation in different regions (AH, GX, GZ, and YN). To provide a theoretical basis for the collection, conservation, and utilization of germplasm resources of D. officinale in different regions.

2. Materials and Methods

2.1. Chemicals. LC-MS grade acetonitrile, methanol, formic acid, and water were purchased from Merck (Darmstadt, Germany), CNW Technologies GmbH (Duesseldorf, Germany), and Watsons (Hong Kong, China), respectively, for the LC-MS analyze. The reagents required for the polysaccharide assay including anhydrous ethanol, phenol, concentrated sulfuric acid, and glucose standards were purchased from Kelong Chemical Reagent Factory (Chengdu, China), Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), and Merck (Darmstadt, Germany), respectively.

2.2. Sample Preparation. A total of 71 samples of D. officinale were collected from four regions, including D. officinale from Huoshan County of Anhui province (AH), D. officinale from Rong County of Guangxi province (GX), D. officinale from Danzhai County of Guizhou province (GZ) and D. officinale from Menglian County of Yunnan province (YN), in 2019 (see Table 1). The fresh stems of the 3-year-old samples were dried at 60°C, ground into fine powder and stored at −80°C for the assay.

2.3. Determination of Polysaccharides. The quantification of polysaccharides in D. officinale follows the guidance of the Ch.P. (2020 edition) [6]. Firstly, a calibration curve was prepared by each UV absorbances(y) against concentrations (x, μg/mL) of glucose standard. The linear regression equation was showed as y = ax + b. And then, the D. officinale powder sample (0.06 g) was heated and refluxed in a tested round bottom flask for 2h. After cooling, it was precipitated with anhydrous ethanol for 1 h, then washed with 80% ethanol and the precipitate was dissolved with hot water to obtain the sample to be tested. Then, the phenol-sulfuric acid reaction was performed: 5% phenol solution (1.0 mL) and concentrated sulfuric acid (5.0 mL) were added sequentially to the sample to be tested (1.0 mL), rapidly shaken, heated in boiling water for 20 min, immediately removed and ice bathed for 5 min. The absorbance of the reaction solution was measured at 488 nm and the polysaccharide content was calculated according to the glucose standard calibration curve.

2.4. Metabolite Extraction. Dried and crushed D. officinale powder (75 mg) was weighed precisely, dispersed in 1 mL of 70% methanol, extracted by ultrasonication (400 W, 50 kHz) for 30 min, cooled, centrifuged at 12000 rpm for 5 min, and the supernatant was taken to analyze the secondary metabolites of D. officinale by UPLC-Q-TOF/MS.

2.5. UPLC-Q-TOF/MS Conditions. Samples were evaluated using a Waters 1290 Infinity II UPLC liquid chromatograph system (Waters Corp., Milford, MA, USA) equipped with a binary pump, in-line degasser, autosampler, and thermally controlled column chamber. The separation of an aliquot of 1 μL sample solutions was performed on a Waters CORTECS UPLC C18 (100 mm × 2.1 mm, 1.6 μm) maintained at 40°C. The mobile phase was solvent consisting of solvent A (0.1% formic acid in water, v/v) and solvent B (0.1% formic acid in acetonitrile, v/v) with a flow rate of 0.4 mL/min and a separation time of 10 min. Gradient elution conditions: 0–0.5 min, 5% B; 0.5–4 min, 40% B; 4–5 min, 75% B; 5–5.1 min, 95% B; 5.1–6.5 min, 95% B; 6.5–6.6 min, 5% B; 6.6–10 min, 5% B. The injection volume was 1 μL.

Mass spectrometry was performed using an Agilent QTOF/MS system (Agilent, MA, USA), with separate acquisitions in positive and negative ion mode. Ion source parameters: mass scan ranged m/z 50 to 1200; gas temperature, 350°C; dry gas flow rate, 10 L/min; nebulizer, 45 psig; sheath gas temperature, 350°C; sheath gas flow rate, 11 L/min; vcap voltage, 4000 V; nozzle voltage, 1000 V; fragmentor, 175 V. Secondary mass spectrometry information was acquired using Auto MS/MS mode with CE of 20, 30, and 40 V, respectively. Data acquisition and processing were performed by Agilent Mass Hunter Profnder analysis software.

2.6. Data Processing and Analysis of Secondary Metabolites. Peak matching, peak alignment, ion fusion, and deconvolution were performed on the raw data using Agilent Mass Hunter Profnder software (version 10.0). Fragmented peaks with false positives were excluded based on peak area, retention time, and molecular weight. The analysis was performed by unsupervised pattern recognition principal component analysis (PCA) and projections on the latent structure-discrimination analysis (PLS-DA) models of SIMCA 14.1 software, with variable importance in the projection (VIP) value greater than 3 was used as a threshold to screen differential compositions. The ggplot2, pheatmap, and other packages were applied in the R program (version 4.1.1) for other visualizations. p < 0.05 was considered statistically significant.
3. Results

3.1. Comparison of Polysaccharide Content in D. officinale in Different Regions. The polysaccharide contents of 71 samples collected from four regions were determined. The Ch.P. stipulates that D. officinale polysaccharide content of not less than 25% is qualified. The results showed (see Figure 1) that the average polysaccharide contents in D. officinale of the four regions AH, GX, GZ, and YN were 33.51%, 40.11%, 28.39%, and 26.26%, and their passing rates were 83%, 94%, 72%, and 50%, respectively. The polysaccharide content of D. officinale in GX was significantly higher than that of other places.

3.2. Secondary Metabolomics of D. officinale in Different Regions. The metabolic information of D. officinale from four regions was investigated by nontargeted metabolomics techniques. The base peak chromatogram (BPC) of quality control samples showed retention times mainly within one to eight min in positive ion (e.g., in Figure 2(a)) and negative ion (e.g., in Figure 2(b)) modes. A total of 82 metabolites were identified (see Table 2). In the BPC plots of typical samples from each region, there were differences in metabolic composition between them in both positive ion (e.g., in Figure 2(c)) and negative ion (e.g., in Figure 2(d)) modes. In the PCA plot (e.g., in Figure 2(e)), the overall profile of the distribution of D. officinale samples from different regions could be observed.

3.3. Discovery of Differential Metabolites of D. officinale in Different Regions. To further reveal the differences in the chemical composition of D. officinale in different regions, multivariate statistical analysis was used for the analysis. The PLS-DA model developed had good predictive power ($R^2_X (cum) = 0.698$, $R^2_Y (cum) = 0.668$) and confidence ($Q^2 (cum) = 0.504$) (e.g., in Figure 3(a)). The results showed that YN and GX clustered separately by region, while the metabolite profiles of the two regions AH and GZ crossed obviously, indicating that the metabolites of D. officinale in the two regions have a similar expression. 32 differential metabolites were screened according to the variable importance for the projection (VIP) value $>1$ (e.g., in Figure 3(b)). Further cluster heatmap analysis was done for these differential metabolites (e.g., in Figure 3(c)), and overall, the region with more differential metabolites was YN, followed by GZ, AH, and GX in that order.

3.4. Identification of Differential Compositions in D. officinale from Different Regions. Based on the analysis of the extracted ion chromatograms and the results of VIP $>1$, 22
Table 2: Secondary metabolites of *D. officinale* from four regions.

<table>
<thead>
<tr>
<th>No.</th>
<th>Identification</th>
<th>RT (min)</th>
<th>Add ion</th>
<th>m/z</th>
<th>ppm</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sucrose</td>
<td>0.587</td>
<td>[M-H]</td>
<td>341.1092</td>
<td>0.7</td>
<td>C$<em>{13}$H$</em>{22}$O$_{11}$</td>
</tr>
<tr>
<td>2</td>
<td>Succinylcarnitine</td>
<td>0.628</td>
<td>[M+H]$^+$</td>
<td>262.1292</td>
<td>2.6</td>
<td>C$<em>{11}$H$</em>{19}$O$_{6}$</td>
</tr>
<tr>
<td>3</td>
<td>O-methylmalonyl-L-carnitine</td>
<td>0.755</td>
<td>[M+H]$^+$</td>
<td>262.1291</td>
<td>2.4</td>
<td>C$<em>{11}$H$</em>{19}$O$_{7}$</td>
</tr>
<tr>
<td>4</td>
<td>Guanosine</td>
<td>0.807</td>
<td>[M-H]</td>
<td>282.0838</td>
<td>2.0</td>
<td>C$<em>{10}$H$</em>{22}$N$<em>{4}$O$</em>{5}$</td>
</tr>
<tr>
<td>5</td>
<td>O-glutaroyl-L-carnitine</td>
<td>0.898</td>
<td>[M+H]$^+$</td>
<td>276.1453</td>
<td>4.0</td>
<td>C$<em>{10}$H$</em>{17}$N$<em>{4}$O$</em>{5}$</td>
</tr>
<tr>
<td>6</td>
<td>2-Methyl-1H-indol-7-yl-β-D-mannopyranoside or isomer</td>
<td>1.416</td>
<td>[M+H]$^+$</td>
<td>310.1289</td>
<td>1.4</td>
<td>C$<em>{14}$H$</em>{22}$O$_{6}$</td>
</tr>
<tr>
<td>7</td>
<td>Protocatechic acid-3-O-glucoside</td>
<td>1.452</td>
<td>[M-H]</td>
<td>315.0716</td>
<td>1.7</td>
<td>C$<em>{13}$H$</em>{16}$O$_{5}$</td>
</tr>
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<td>8</td>
<td>di-O-methylcrenatin</td>
<td>1.741</td>
<td>[M-H]</td>
<td>347.1313</td>
<td>6.9</td>
<td>C$<em>{13}$H$</em>{22}$O$_{6}$</td>
</tr>
<tr>
<td>9</td>
<td>2,6-Dihydroxybenzoic acid 2-O-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside</td>
<td>1.915</td>
<td>[M-H]</td>
<td>447.1136</td>
<td>1.8</td>
<td>C$<em>{18}$H$</em>{24}$O$_{13}$</td>
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<td>10</td>
<td>Benzyl-β-gentiobioside</td>
<td>1.992</td>
<td>[M-H]</td>
<td>431.1549</td>
<td>2.3</td>
<td>C$<em>{13}$H$</em>{22}$O$_{11}$</td>
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<td>11</td>
<td>Dendromoniliside C</td>
<td>2.251</td>
<td>[M-H]</td>
<td>443.1912</td>
<td>2.4</td>
<td>C$<em>{13}$H$</em>{24}$O$_{12}$</td>
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<td>12</td>
<td>2,6-Dihydroxybenzoic acid 2-O-β-D-apiofuranosyl-(1→2)-β-D-xylopyranoside</td>
<td>2.306</td>
<td>[M-H]</td>
<td>417.1031</td>
<td>1.8</td>
<td>C$<em>{17}$H$</em>{25}$O$_{12}$</td>
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<td>13</td>
<td>Syringin</td>
<td>2.408</td>
<td>[M+Na]$^+$</td>
<td>395.1310</td>
<td>0.5</td>
<td>C$<em>{13}$H$</em>{22}$O$_{8}$</td>
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<tr>
<td>14</td>
<td>Dihydroxyacin</td>
<td>2.480</td>
<td>[M+H]$^+$</td>
<td>397.1470</td>
<td>0.3</td>
<td>C$<em>{15}$H$</em>{26}$O$_{8}$</td>
</tr>
<tr>
<td>15</td>
<td>Khaephuoside A</td>
<td>2.526</td>
<td>[M+Na]$^+$</td>
<td>501.1575</td>
<td>0.7</td>
<td>C$<em>{13}$H$</em>{24}$O$_{9}$</td>
</tr>
<tr>
<td>16</td>
<td>Gastrodin</td>
<td>2.612</td>
<td>[M-H]</td>
<td>309.0946</td>
<td>0.3</td>
<td>C$<em>{13}$H$</em>{24}$O$_{7}$</td>
</tr>
<tr>
<td>17</td>
<td>2-Methoxyphenyl-1-O-β-D-apoliofuromosyl-(1→2)-β-D-glucopyranoside</td>
<td>2.637</td>
<td>[M-H]</td>
<td>417.1393</td>
<td>2.4</td>
<td>C$<em>{14}$H$</em>{22}$O$_{11}$</td>
</tr>
<tr>
<td>18</td>
<td>Viccin 2</td>
<td>2.654</td>
<td>[M-H]</td>
<td>593.1503</td>
<td>1.5</td>
<td>C$<em>{15}$H$</em>{26}$O$_{15}$</td>
</tr>
<tr>
<td>19</td>
<td>2-methylphenyl-O-β-D-xylopyranosyl-(1→6)-O-β-d-glucopyranoside</td>
<td>2.709</td>
<td>[M-H]</td>
<td>401.1446</td>
<td>1.7</td>
<td>C$<em>{15}$H$</em>{26}$O$_{10}$</td>
</tr>
<tr>
<td>20</td>
<td>Viccin 1</td>
<td>2.797</td>
<td>[M-H]</td>
<td>563.1399</td>
<td>1.3</td>
<td>C$<em>{14}$H$</em>{24}$O$_{14}$</td>
</tr>
<tr>
<td>21</td>
<td>Schaftoside</td>
<td>2.858</td>
<td>[M-H]</td>
<td>563.1398</td>
<td>1.5</td>
<td>C$<em>{14}$H$</em>{24}$O$_{14}$</td>
</tr>
<tr>
<td>22</td>
<td>Dihydroxymethyloside</td>
<td>2.880</td>
<td>[M-H]</td>
<td>327.1079</td>
<td>1.9</td>
<td>C$<em>{13}$H$</em>{22}$O$_{8}$</td>
</tr>
<tr>
<td>23</td>
<td>Isochaftoside</td>
<td>2.951</td>
<td>[M-H]</td>
<td>563.1398</td>
<td>1.4</td>
<td>C$<em>{14}$H$</em>{24}$O$_{14}$</td>
</tr>
<tr>
<td>24</td>
<td>Syringaresinol 4,4′-di-O-β-D-glucopyranoside</td>
<td>2.970</td>
<td>[M+Na]$^+$</td>
<td>765.2566</td>
<td>1.4</td>
<td>C$<em>{15}$H$</em>{26}$O$_{18}$</td>
</tr>
<tr>
<td>25</td>
<td>Apigenin 6-C-β-D-xyllopyranosyl-8-C-α-L-arabinopyranoside</td>
<td>3.012</td>
<td>[M-H]</td>
<td>533.1292</td>
<td>1.6</td>
<td>C$<em>{14}$H$</em>{26}$O$_{13}$</td>
</tr>
<tr>
<td>26</td>
<td>Dendromoniliside E</td>
<td>3.026</td>
<td>[M+Na]$^+$</td>
<td>621.2147</td>
<td>1.1</td>
<td>C$<em>{15}$H$</em>{26}$O$_{14}$</td>
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<tr>
<td>27</td>
<td>Shashenoside 1</td>
<td>3.048</td>
<td>[M+Na]$^+$</td>
<td>527.1734</td>
<td>0.2</td>
<td>C$<em>{14}$H$</em>{26}$O$_{13}$</td>
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<tr>
<td>28</td>
<td>Violanthin</td>
<td>3.084</td>
<td>[M-H]</td>
<td>577.1555</td>
<td>1.3</td>
<td>C$<em>{15}$H$</em>{26}$O$_{14}$</td>
</tr>
<tr>
<td>29</td>
<td>Dendrobine</td>
<td>3.114</td>
<td>[M+H]$^+$</td>
<td>264.1957</td>
<td>0.4</td>
<td>C$<em>{8}$H$</em>{12}$N$<em>{2}$O$</em>{3}$</td>
</tr>
<tr>
<td>30</td>
<td>Apigenin 6,8-di-C-arabinoside</td>
<td>3.106</td>
<td>[M-H]</td>
<td>533.1291</td>
<td>1.8</td>
<td>C$<em>{13}$H$</em>{26}$O$_{13}$</td>
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<tr>
<td>31</td>
<td>Viccin 3</td>
<td>3.111</td>
<td>[M-H]</td>
<td>563.1394</td>
<td>2.2</td>
<td>C$<em>{14}$H$</em>{26}$O$_{14}$</td>
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<tr>
<td>32</td>
<td>Rutin</td>
<td>3.111</td>
<td>[M-H]</td>
<td>609.1455</td>
<td>1.1</td>
<td>C$<em>{17}$H$</em>{26}$O$_{16}$</td>
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<td>Apigenin-7-O-β-D-glucoside</td>
<td>3.161</td>
<td>[M-H]</td>
<td>431.0972</td>
<td>2.6</td>
<td>C$<em>{21}$H$</em>{26}$O$_{16}$</td>
</tr>
</tbody>
</table>
compounds with clear chemical structures were tentative identified (see Figure 4), including 1 amides (cis-N-feruloyltyramine: A1), 3 benzylics (dendrocaninolide E: B1, dendrocanin U: B2, dendrocanin B: B3), 1 disaccharide (sucrose: D1), 9 flavonoids (vicenin 2: F1, vicenin 1: F2, dendrocanin D: B2, dendrocanin B: B3), 1 disaccharide (see Figure 4), including 1 amides (cis-N-feruloyltyramine: E1), 3 benzylics (dendrocaninolide E: B1, dendrocanin U: B2, dendrocanin B: B3), 1 disaccharide (sucrose: D1), 9 flavonoids (vicenin 2: F1, vicenin 1: F2, schaftoside: F3, isoschaftoside: F4, apigenin 6,8-di-C-arabinobiside: F5, vicenin 3: F6, rutin: F7, apigenin 6-C-α-L-arabinopyranosyl-2"-O-β-D-glucopyranoside: F8, apigenin 6-C-α-L-arabinopyranosyl-2"-O-β-D-glucopyranoside: F9), 3 organic nitrogenous compounds (succinylcarnitine: O1, O-methylmalonyl-L-carnitine: O2, O-glutaroyl-L-carnitine: O3), 5 phenolic glycosides (di-O-methylecrratin: P1, 2,6-dihydroxybenzoic acid 2-O-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside: P2, 2,6-dihydroxybenzoic acid 2-O-β-D-glucopyranoside: P3, 2,6-dihydroxybenzoic acid 2-O-β-D-glucopyranoside: P4, 2,6-dihydroxybenzoic acid 2-O-β-D-glucopyranoside: P5, 2,6-dihydroxybenzoic acid 2-O-β-D-glucopyranoside: P6, 2,6-dihydroxybenzoic acid 2-O-β-D-glucopyranoside: P7).

In terms of the distribution of expression of these differential compositions (see Figure 5), the highest expression of differential compositions was still YN, followed by GZ,
AH, and GX in that order. Among flavonoids, the higher expression regions were YN and AH. It is also evident that this differential composition was almost absent in GX samples. Among the phenolic glycosides, the higher expression regions were YN and GX. In the organic nitrogenous compounds and amides, the highest expression region was YN. In the bibenzyls and disaccharide, the higher expression region was GZ.

4. Discussion

*D. officinale*, as a widely used valuable Chinese herbal medicine, adopts polysaccharides as its main active ingredient and quality control standard in the Ch.P. In the present study, we selected *D. officinale* from different origins of greenhouse cultivation with relatively stable quality as the research object, and firstly analyzed their polysaccharide content according to the Ch.P. method. The results showed that in terms of polysaccharide content, the order was GX > AH > GZ > YN. It has been observed that the production of polysaccharides in wolfberry and lingonberries decreases gradually, with the increase in altitude and decrease in temperature [19,20]. This has similarity with our results, in that GX (average altitude 97 m) and AH (average altitude 80 m) were at lower altitude and they both had high polysaccharide content, followed by GZ (average altitude 895 m), YN (average altitude 1116 m), respectively.

Previous literature reported that *D. officinale* is rich in flavonoids, phenanthrenes, bibenzyl, and other small molecule chemical components in addition to polysaccharides [21–24]. Therefore, it is unsystematic and incomplete to use polysaccharide content alone as a criterion for the medicinal value and quality evaluation of *D. officinale*. Herein, we used metabolomic analysis, combined with heat map and hierarchical clustering analysis, to reveal the differences in small molecule chemical composition among *D. officinale* from four different origins. The results elucidated that 22 compounds, including 1 amides (Cis-N-Feruloyltyramine), 3 bibenzyls (Dendromoniliside E, Dendrocandin U, Dendrocandin B), 1 disaccharide (Sucrose), 9 flavonoids (Vicenin 1, Vicenin 2, Vicenin 3, Schaftoside, Isoschaftoside, Rutin, Apigenin 6,8-di-C-arabinoside, Apigenin 6-C-α-L-
arabinopyranosyl-2"-O-β-D-glucopyranoside, apigenin 8-C-α-L-arabinopyranosyl-2"-O-β-D-glucopyranoside), 3 organic nitrogenous compounds (Succinylcarnitine, O-Methylmalonyl-L-carnitine, O-Glutaroyl-L-carnitine), 5 phenolic glycosides (Di-O-methylcrenatin, Paeonolide, 2,6-Dihydroxybenzoic acid 2-O-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside, 2,6-Dihydroxybenzoic acid 2-O-β-D-apiofuranosyl-(1→2)-β-D-xylopyranoside, 2-Methylphenyl-O-β-D-xylopyranosyl-(1→6)-O-β-D-glucopyranoside) were the main differential chemical constituents in *D. ofcinale* from different origins. The growth environment of *D. officinale* cultivated in greenhouses is relatively controlled, and thus the differences in the chemical composition of *D. officinale* from these different regions are more from the influence of origin.

Among the flavonoids, most of them were flavone-C-glycosides. In plants, flavonoids have a variety of biological functions, such as regulating cell growth, enhancing nutrient recycling, and resisting biotic and abiotic stresses [25,26]. A flavonoids-rich diet not only helps prevent some chronic diseases, but it also has biological activities such as anti-inflammatory, anticancer, cardiovascular protection, and blood lipid regulation [27–29]. It has been reported that *Astragalus* polysaccharides significantly improve the

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**Figure 5**: Expression of differential compositions in *D. officinale* from four regions.
solubility, stability, and solubilizing effect of flavonoids [30]. Synergistic effects of polysaccharides with flavonoids have been shown, which may contribute to better pharmacological effects. However, almost all the differential flavonoids were higher in the YN sample with the lowest polysaccharide content, while the opposite result was observed in the GX sample with the highest polysaccharide content. And, it was corroborated in the YN samples from the samples with qualified and unqualified polysaccharide contents. This also reinforces that the evaluation of the quality of *D. officinale* by polysaccharide content alone is imperfect, and the content of small molecule components needs to be examined systematically.

Bibenzyls and disaccharide were mainly highly expressed in GZ. The bibenzyls is one of the active ingredients in *Dendrobium* genus. The main pharmacological activities identified in the compounds are antitumor, antidiabetic, antiplatelet aggregation, anti-inflammatory, etc [31–33]. It is expressed that the bibenzyls had a wide range of medicinal effects in *Dendrobium* genus. Sucrose, a disaccharide, is the main product of photosynthesis and can act as a signaling molecule for a wide range of plant growth processes [34]. It has specific functions in plant metabolism, growth, and development. Phenolic glycosides, amides, and organic nitrogenous compounds were the main sign component of YN. Of these, phenolic glycosides are widely distributed in plants and are phenylalanine and tyrosine metabolites with antimalarial, antineuroinflammatory, antiobesity and antioxidant, and other activities, mainly [35–38]. It has lower biological activity compared to the corresponding glycosides, some of which may later be used as nutritional agents or adjuvants [35]. Some studies have shown that the accumulation of total phenols may be positively correlated with altitude gradient [39]. It has been observed that succinylcarnitine (O1) correlates with total cholesterol or LDL, activated partial thromboplastin time [40]. These studies also predicted that *D. officinale* containing different components is suitable for different disease treatment.

5. Conclusion

In summary, this study identified the differential compositions of *D. officinale* cultivated in greenhouses in four regions of China (AH, GX, GZ, YN). The results showed that the polysaccharide content in *D. officinale* was strongly related to its growing altitude, with the highest average polysaccharide content in the GX sample (altitude: 97 m) and the lowest average polysaccharide content in the YN sample (altitude: 1116 m). There was also a significant difference in the small molecule chemical composition in *D. officinale*, where the content of flavonoids showed an opposite trend to that of polysaccharides. These results indicated that the current quality standard for evaluating the quality of *D. officinale* by polysaccharide content alone is imperfect, and small molecule compounds need to be included as quality markers.

Data Availability

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors’ Contributions

Qianqian He: wrote the manuscripts and analyzed the data. Qianqian He and Zhou Yang: conducted experiments and assisted in the writing of the article. Anjing Lu: provided help in the experiment and provided suggestions for the writing of the article. Qianru Zhang: provided help in the data analyzing. Lin Qin: provided help in the experiment and provided suggestions for the writing of the article. Daopeng Tan: reviewed and revised the paper. Yanliu Lu and Daopeng Tan: provided the design of the experiment. Yuqi He: provided the direct design of the experiment and funding acquisition.

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Supplementary Materials

The description of the supplementary materials is as follows: This is the detailed information of secondary metabolites of *D. officinale* from four regions, supplemented with molecular weight and MS/MS information. (Supplementary Materials)

References


