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Article

Determination of Dencichine in *Panax notoginseng* in the Forest and Field Using High Performance Liquid Chromatography

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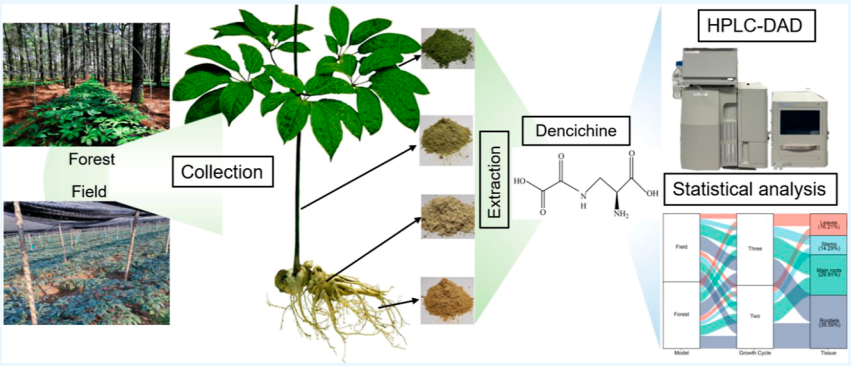

ABSTRACT: Dencichine is a nonprotein amino acid, an effective ingredient in *Panax notoginseng* with hemostatic and anti-inflammatory effects. There are few studies on the effects of regions and cultivation models on the accumulation of dencichine. In the current study, the content of dencichine in *P. notoginseng* collected from its global cultivation and trading center Yunnan, China, (>640 samples) was determined using an optimized high-performance liquid chromatography method coupled with a diode array detector but without derivatization. The recovery rate of this method was 80−110%, the relative standard deviation was <10%, and the limits of detection and quantification were 0.003% (w/w) and 0.01% (w/w), respectively. The content of dencichine in each part of *P. notoginseng* was as follows: rootlets (39.59%) > main roots (29.91%) > leaves (16.21%) > stems (14.29%). For leaves, *P. notoginseng* in the forest (5.52 ± 2.26 mg/g) was significantly higher than that in the field (3.93 ± 1.72 mg/g) but opposite for main roots. The origins and altitudes made different contributions to the accumulation of dencichine in *P. notoginseng*. This study provides an effective analytical method to determine dencichines in various parts of *P. notoginseng* from different origins and altitudes and supports quality control and product development of *P. notoginseng*.

INTRODUCTION

Panax notoginseng (Burk.) F. H. Chen, commonly known as Sanqi or Tianqi, is a perennial herb belonging to the genus *Panax*, and is widely used in medicine, health care, cosmetics and other industries,[1,2] with its global cultivation and trading center in Yunnan, China.[3] The main cultivation models of *P. notoginseng* are in-field cultivation and in-forest cultivation. In-field cultivation imitates the conventional crop planting model.[4] By supplying a suitable growing environment and having a high level of biodiversity, in-forest cultivation uses allelopathy to stimulate plant growth and prevent pests and diseases.[5−7] The in-forest cultivation is a developing planting model that is being used to improve the quality of medicinal herbs.[5,8−10]

P. notoginseng has a wide range of pharmacological activities,[11−14] and is especially effective in improving the cardiovascular system. Therefore, it is an essential component of many Chinese medicines.[15] Previous studies focused on identification and characterization of numerous bioactive saponins, such as ginsenosides and notoginsenosides, present in these *Panax* species.[16,17] With the refinement of market demand and the advancement of detection technology, it is not sufficient to use a single component (e.g., saponins) as the quality evaluation standard to reflect the overall quality and efficacy of *P. notoginseng*.[18] Recent studies indicated that dencichines are one of the main functional ingredients in *P. notoginseng*. It mainly has the functions of hemostasis,[19] increasing the number of platelets,[20,21] inhibiting the formation of osteoclasts,[22] as well as reducing the damage by diabetic nephropathy,[23] anti-inflammation,[24] and antioxidation.[25] In

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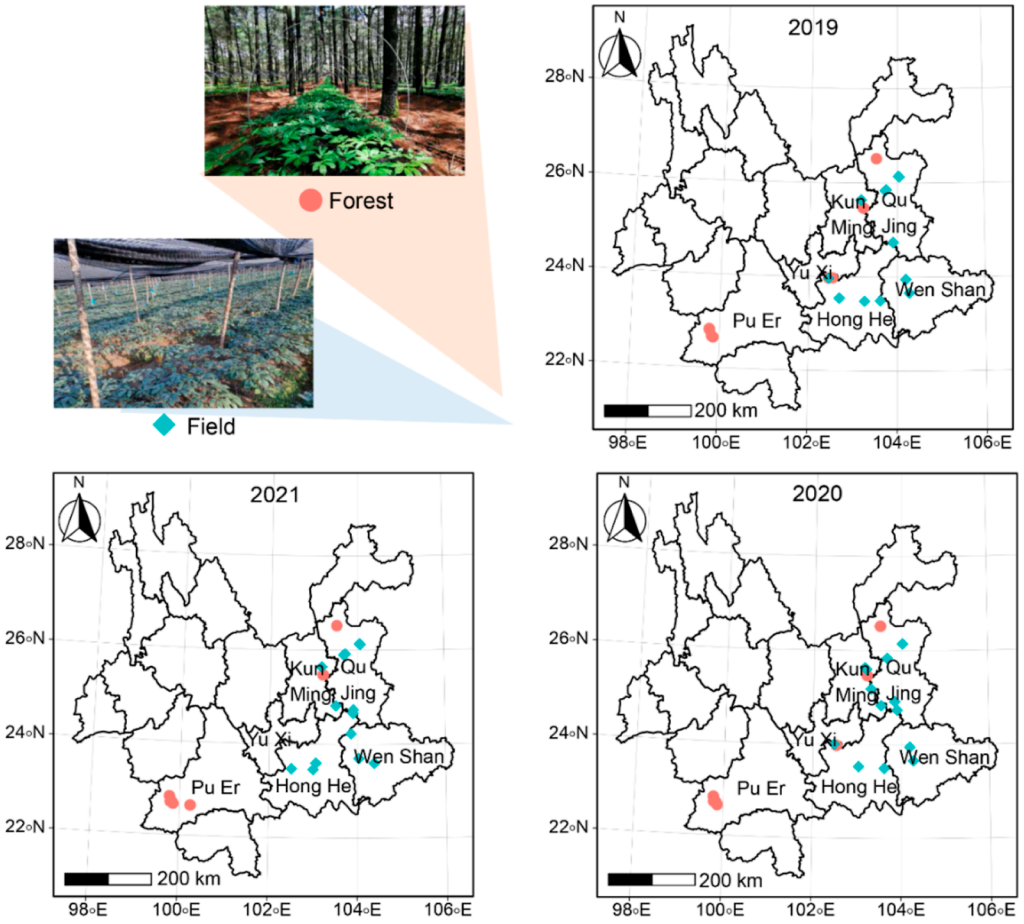

Figure 1. Geographical distribution map of sampling *P. notoginseng*.

addition to the *Panax* genus,[26] the same structure has also been identified in *cycas* L.,[27] *Acacia* Mill.,[28] and *Lathyrus* L.[29]

Dencichine, also known as *β*-*N*-oxalyl-L-*α*, *β*-diaminopropionic acid (*β*-ODAP),[29] is a nonprotein free amino acid and is soluble in water. It was first isolated from the seeds of *Lathyrus sativus* (grass pea seeds) with hemostatic and anti-inflammatory effects.[30] Several methods have been developed to determine dencichine, including electrophoresis,[31] mass spectrometry,[20,32,33] enzyme-based sensors,[34] and high-performance liquid chromatography (HPLC).[35,36] HPLC is one of the most widely applied methods to detect dencichines due to its high sensitivity and accuracy. Because of its strong polarity, weak ultraviolet absorption, and little retention on the C_{18} column,[37,38] precolumn derivatization and postcolumn derivatization[39] were used to solve this challenge. However, derivatization is time-consuming and laborious, complicated to operate, and has poor derivative stability and intensive reagent interference. Therefore, an accurate, sensitive, rapid, and simple analytical method based on chromatography should be developed to determine dencichine.

This study aims to establish an HPLC method for accurately determining dencichine in leaves, stems, main roots, and rootlets of *P. notoginseng* without derivatization and further quantifying dencichine in *P. notoginseng* of five origins and under two cultivation modes.

MATERIALS AND METHODS

Materials and Reagents. A total of 648 *P. notoginseng* samples were collected from the main planting origins in Yunnan Province, China, during 2019−2021. A total of 73 sampling bases are summarized in Figure 1 and Table S1. The five main planting origins are WenShan, QuJing, HongHe, KunMing, and PuEr. Besides, *P. notoginseng* under in-field and in-forest cultivation models was randomly sampled at three points in each planting base. Eight to ten *P. notoginseng* samples were collected at each point as mixed samples.

Dencichine (CAS: 5302-45-4, $C_5H_8N_2O_5$, purity of 98%) was purchased from Guizhou Dida Co., Ltd. (Guizhou, China). HPLC-grade acetonitrile, methanol, and ammonium formate were purchased from Merck (Darmstadt, Germany). Ultrapure deionized water (ddH_2O) was prepared using a Millipore Milli-Q water system (Millipore Corporation, Bedford, MA, USA).

Sample Extraction and Standard Preparation. The collected leaves, stems, main roots, and rootlets of *P. notoginseng* were washed with clean water, dried at 60 °C, coarsely crushed, ground to an ultrafine powder using a Planetary Mono Mill (PULVERISETTE 6, Fritsch, Idar-Oberstein, Germany), and then passed through 80 mesh sieves. To dissolve the samples, 0.1 g of *P. notoginseng* powder was accurately weighed in a 1.5 mL centrifuge tube, and then 1.0 mL of ddH_2O was added and mixed. The mixed solution was then placed in an ultrasonic cleaner (37 kHz) for 45 min, followed by centrifugation at 9168*g* for 3 min. A total of 300 *μ*L of the supernatant was added to 700 *μ*L of acetonitrile, followed by sonication for 10 min. After centrifugation at 13,201*g* for 5 min, the supernatant extract was filled into liquid chromatography vials and stored at 4 °C until use.

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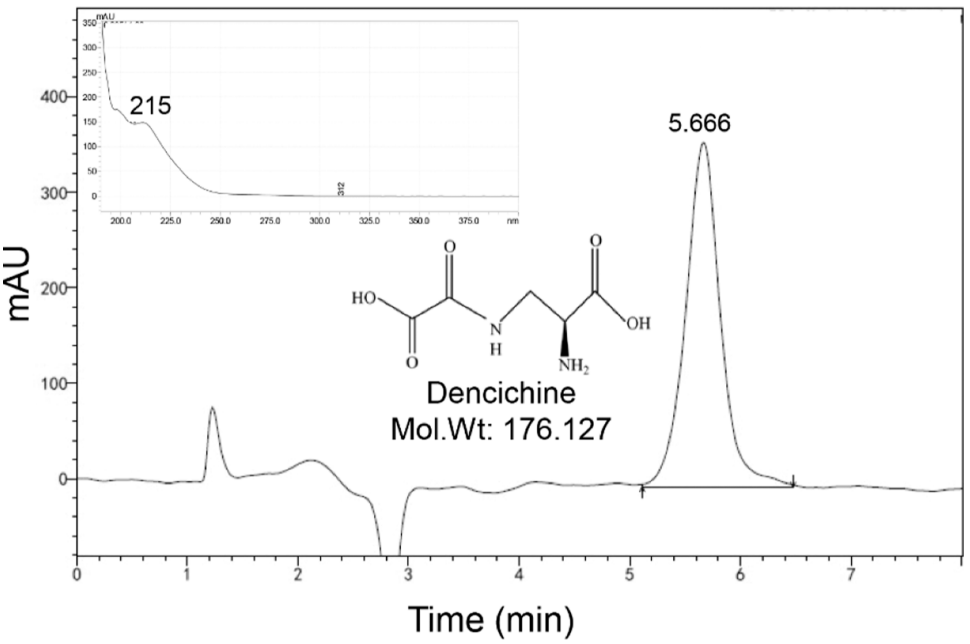

Figure 2. Chromatogram and ultraviolet absorption spectra of dencichine at 100 μg/mL.

To prepare the standard solution, 1.0 mg of dencichine was accurately weighed in a 1.5 mL centrifuge tube, and 1.0 mL of ddH_2O was then added and mixed until the solution was completely dissolved. The standard working solutions were prepared in the range of 6.25, 12.5, 25, 50, 100, 200, and 400 μg/mL in 70% acetonitrile, and then stored at 4 °C for further use.

HPLC-DAD Analysis of Dencichine. HPLC analysis was performed on a Prominence LC-20A HPLC system with a diode array detector (DAD; Shimadzu, Kyoto, Japan). A Syncronis HILIC HPLC column (2.1 × 150 mm, 5 μm, Thermo Scientific, San Jose, CA, USA) was used for sample separation at 30 °C with a flow rate of 0.4 mL/min. An aqueous solution of 80 mM ammonium formate (phase A) and acetonitrile (phase B) served as the mobile phase, which was prepared by vacuum filtration before use. The isocratic elution program was 25:75 (A/B) for 9 min. The sample injection volume was 10 μL and the detection wavelength was set at 215 nm. LabSolutions software was used for data processing.

Method Validation. This method was verified according to ICH guide ICH guidelines (https://www.ich.org/page/ich-guidelines) for linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). The linear range was tested using seven standard solutions of known concentrations ranging from 6.25 to 400 μg/mL. The experiment was performed at least in triplicate. The LOD and LOQ were determined according to the definition of the signal-to-noise ratio of 3 and 10, respectively.[40] The accuracy and precision were evaluated by using the recovery rate and relative standard deviation (RSD) via addition of the standard solutions to leaves, stems, main roots, and rootlets of *P. notoginseng*. Furthermore, the blank solution (ddH_2O/acetonitrile = 3:7) and the quality control standard solution (100 μg/mL dencichine standard) were also run between samples to verify the stability of the instrument.

Statistical Analysis. All statistical analyses were conducted in R (v4.2.2; http://www.r-project.org/). Most results were visualized using the "ggplot2" package unless otherwise indicated. Data were expressed as the mean ± standard deviation. Significance analysis was performed using the "agricolae" package and "ggpubr" package for Duncan's test and the *t*-test, respectively. The Sankey diagram was obtained using the "ggalluvial" package.

RESULTS AND DISCUSSION

Optimization of HPLC-DAD for Detecting Dencichine. Prior to determination of dencichine using a HPLC-DAD, the ODS, ACQUITY UPLC BEH HILIC (2.1 × 50 mm), and Syncronis HILIC (2.1 × 150 mm) columns were compared to obtain the best extraction performance for dencichine. The HILIC columns were reported to be used for highly polar compounds[24] and determination of β-ODAP,[38] and therefore, it was selected to determine dencichine. According to our preliminary study, separation efficiency was increased along with the increase in column length (data not shown); therefore, the Syncronis column was selected for further studies.

The chromatographic conditions were then optimized. Specifically, 75% acetonitrile and 25% ammonium formate were selected as the mobile phases to separate dencichine. In addition, 10 μL was selected as the injection volume, and 215 nm was selected as the working wavelength. The representative chromatogram is shown in Figure 2.

Optimization of Conditions for Extracting Dencichine in *P. notoginseng*. There are three methods for the extraction of dencichine, namely immersion,[41] the ultrasonic method[42] and oscillation.[26] In the current study, the ultrasonic method was selected because of the best efficiency of extraction (10.95 ± 0.12 mg/g), which was significantly higher than that of the other two methods (Figure S1a). Then, different reagents were used and compared for the extraction of dencichine. Among 75% ethanol,[33] 75% methanol,[20,42] 0.2 M perchloric acid[39] and ultrapure deionized water,[33] ultrapure deionized water had the highest efficiency of extraction (8.89 ± 0.10 mg/g), which was significantly higher than that by 0.2 M perchloric acid (4.70 ± 0.21 mg/g), 75% methanol (4.01 ± 0.31 mg/g) and 75%

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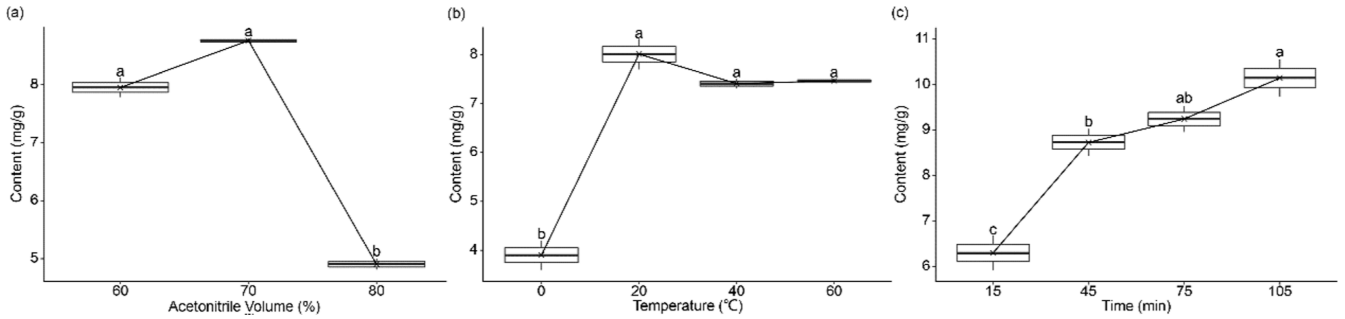

Figure 3. Results of the single factor test. (a) Content of extracted dencichine in *P. notoginseng* in different acetonitrile volumes; (b) content of extracted dencichine in *P. notoginseng* at different ultrasonic temperatures; (c) content of extracted dencichine in *P. notoginseng* at different ultrasonic times; each treatment was repeated at least in triplicate. Different lowercase letters indicate significant differences between treatments by Duncan's test ($P < 0.05$).

ethanol (2.99 ± 0.22 mg/g), as shown in Figure S1b. It might be related to the fact that dencichine is a strong polar and water-soluble substance.[37]

Three main parameters, namely, acetonitrile volume (60, 70 and 80%), ultrasonic temperature (0, 20, 40 and 60 °C) and ultrasonic time (15, 45, 75, and 105 min), were further optimized. The optimized result of acetonitrile volume is shown in Figure 3a, indicating that 70% acetonitrile had the highest efficiency of extraction (8.76 ± 0.04 mg/g). As shown in Figure 3b, the extraction efficiency of ultrasonic treatment for 105 min was the highest (10.14 ± 0.41 mg/g), which was not significantly different from that for 75 min (9.24 ± 0.29 mg/g). Besides, the extraction efficiency was the highest when the ultrasonic temperature was set at 20 °C (8.01 ± 0.32 mg/g), as shown in Figure 3c. According to the results of the single factor test and the principle of Box−Behnken experimental design, the optimized extraction method was determined by three-factor and three-level response surface methodology (RSM). The factor level of RSM is shown in Table S2. Theoretically optimized design for extracting dencichine in *P. notoginseng* was as follows: the ultrasonic temperature was 17.716 °C, the ultrasonic time was 45.539 min, and the fraction of acetonitrile volume was 66.384%. Considering the convenience of actual operation, the parameters of extraction were modified as an ultrasonic temperature of 20 °C, an ultrasonic time of 45 min, and a fraction of acetonitrile volume of 70%. The results under optimized extraction condition are shown in Table 1 and Table S3. The *P* value of the model was <0.0001 and the lack of fit was not significant ($P = 0.0851$). According to a previous study, for gas chromatography−mass spectrometry with ethyl chloroformate derivatization,[20] methanol and water were selected as the first and second extraction solvents to separate saponins and then extract dencichine, with a total extraction time of up to 6 h. Compared to that of this developed method, the extraction time used in the current study was significantly less (i.e., 45 min).

Validation of HPLC-DAD for Determining Dencichine in *P. notoginseng*. Calibration curves were obtained using a series of standard solutions containing dencichines at seven different concentrations diluted in acetonitrile. The linearity was evaluated by using the least-squares method. As shown in Table 2, the curve was linear over a wide concentration range from 6.25 to 400 μg/mL, and the coefficient of determination (R^2) was 0.9993. The LOD and LOQ of dencichine were 0.10 and 0.33 mg/g, respectively. Then, the accuracy of this analytical method was evaluated by analytical recovery. Table S4 shows the recovery rate and RSD of dencichine in main roots, rootlets, stems, and leaves of *P. notoginseng*. The average recoveries of dencichine in *P. notoginseng* ranged from 82.94 to 94.19%, and the RSDs were <10% (ranging from 1.83 to 4.10%). Thus, this method had high accuracy and could meet the requirement of determining dencichine in *P. notoginseng*. Derivatization was applied in most previous studies to solve the challenge of the abundance of weak ions from underivatized dencichine, but this could introduce some impurities. For example, HCl residue in sample solution generated interference signals.[43] In the current study, we established and optimized the method without derivatization for the quantification of dencichine.

Table 1. ANOVA for the Response Surface Quadratic Model

source	sun of squares	df	mean square	*F*-value	*P*-value	
model	155.23	9	17.25	51.49	<0.0001	significant
A	6.90	1	6.90	20.60	0.0027	
B	2.84	1	2.84	8.49	0.0225	
C	52.94	1	52.94	158.05	<0.0001	
AB	0.38	1	0.38	1.15	0.3199	
AC	1.05	1	1.05	3.14	0.1199	
BC	1.50	1	1.50	4.48	0.0721	
A^2	17.04	1	17.04	50.86	0.0002	
B^2	3.16	1	3.16	9.44	0.0180	
C^2	62.87	1	62.87	187.67	<0.0001	
residual	2.34	7	0.33			
lack of fit	1.82	3	0.61	4.68	0.0851	not significant
pure error	0.52	4	0.13			
Cor total	157.57	16				

Distribution of Dencichine in *P. notoginseng*. A previous study reported that the main pharmacological components of *P. notoginseng* were concentrated in the main roots.[44] In the current study, the content of dencichine in the rootlets (39.59%, 10.51 ± 0.48 mg/g) was determined to be significantly higher than that in the other three tissues (i.e., roots, stems, and leaves). The content of dencichine was also detectable in leaves (16.21%, 4.73 ± 1.12 mg/g) and stems (14.29%, 3.72 ± 0.12 mg/g) of *P. notoginseng* (Figure 4 and Table S5), which was consistent with a previous study on the gene level of dencichine in *P. notoginseng*.[45]

Effects of Different Cultivation Models on Accumulation of Dencichine in *P. notoginseng*. *P. notoginseng* was sampled and collected from a total of 51 planting bases, including 17 in-forest and 34 in-field, for 3 consecutive years

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Table 2. Linear Range, Regression Equation, Correlation Coefficient (R^2), LOD and LOQ of HPLC-DAD to Determine Dencichine in *P. notoginseng*

compound	linear range (μg/mL)	linear equation	correlation coefficient (R^2)	LOD (mg/g)	LOQ (mg/g)
dencichine	6.25−400	$Y = 17750X + 128{,}360$	0.9993	0.1	0.33

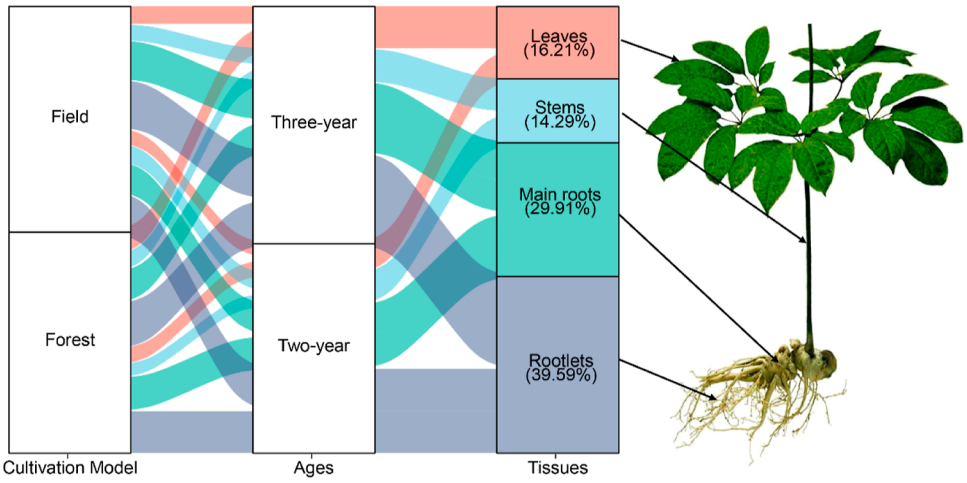

Figure 4. Sankey diagram for the distribution of dencichines in *P. notoginseng*.

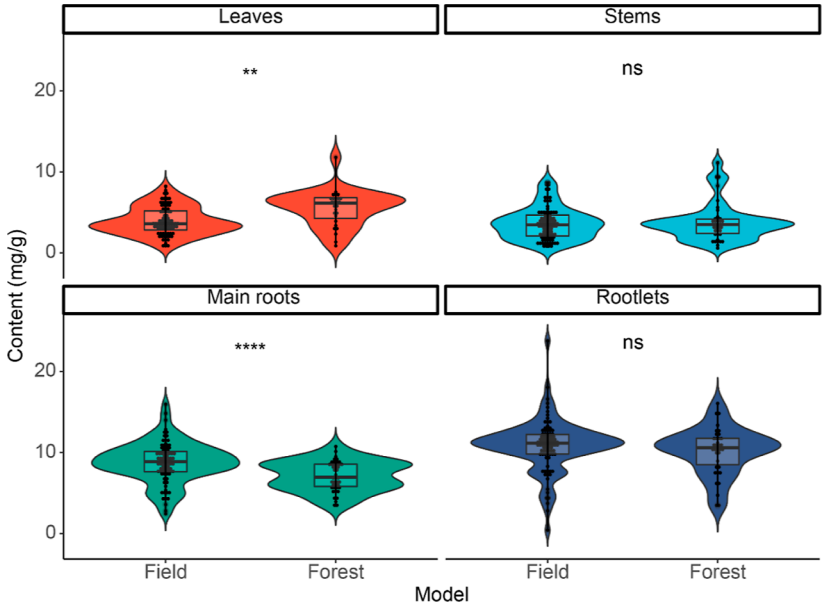

Figure 5. Accumulation of dencichine in leaves, stems, main roots, and rootlets of *P. notoginseng* under different cultivation models. The *t*-test was used to determine the significance, ****($P < 0.0001$), **($P < 0.01$), and ns ($P > 0.05$).

during the harvest period (Table S1). The content of dencichine in main roots, rootlets, stems and leaves of *P. notoginseng* was determined. As shown in Figure 5, the content of dencichine in leaves in the forest (5.52 ± 2.26 mg/g) was significantly higher than that of a monoculture in the field (3.93 ± 1.72 mg/g), whereas dencichine in the main roots in the forest (7.09 ± 1.84 mg/g) was significantly lower than that of a monoculture in the field (8.79 ± 2.51 mg/g). To reduce the limitation of soil sickness and improve the quality of *P. notoginseng*, in-forest cultivation was developed. In a previous study, *P. notoginseng* was successfully cultivated in the forest.[5] However, the forested cultivation model did not significantly increase the accumulation of dencichine in *P. notoginseng*. Furthermore, a recent study reported that selenium in soil stimulated the accumulation of dendritic acid in *P. notoginseng*. Dencichine and selenium contents in soil were positively correlated.[46] Therefore, a forest with sufficient selenium might significantly increase the dencichine content in *P. notoginseng*, promoting the sustainable growth of *P. notoginseng* and reducing the impact of continuous cropping on soil organic matter degradation and structural degradation under in-field planting.

Effects of Different Origins and Altitudes on Accumulation of Dencichine in *P. notoginseng*. *P. notoginseng* from 5 main planting origins, namely, WenShan, QuJing, HongHe, KunMing and PuEr was sampled and collected for 3 consecutive years during the harvest period. The content of dencichine in main roots, rootlets, stems and

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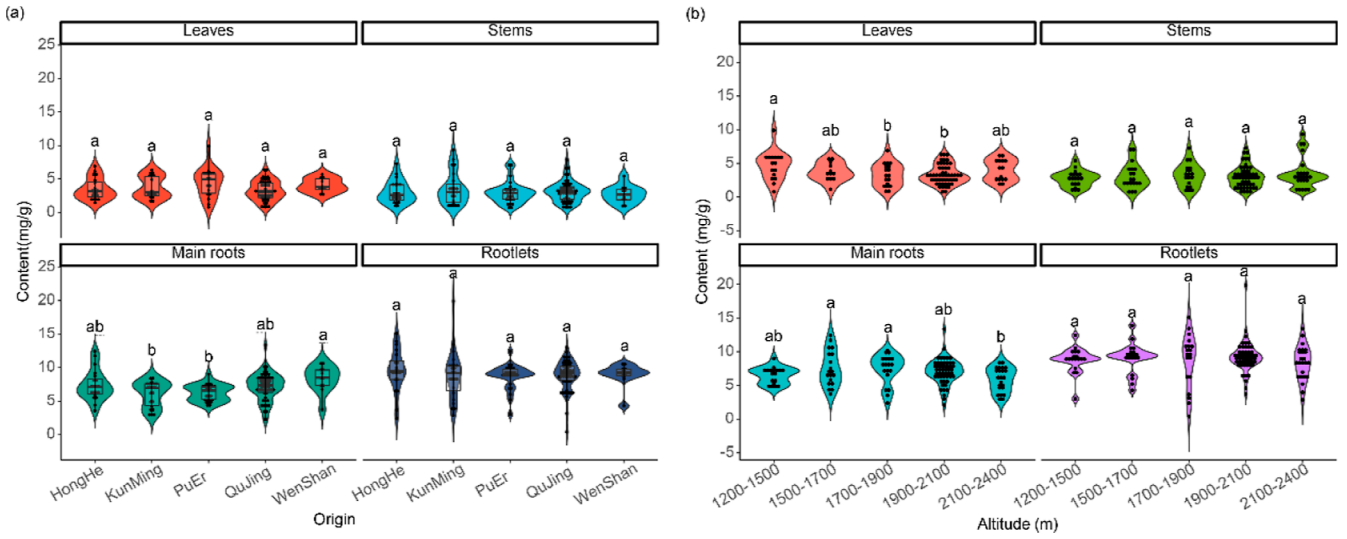

Figure 6. Effects of different origins and altitudes on accumulation of dencichine in *P. notoginseng*. (a) Accumulation of dencichine in leaves, stems, main roots, and rootlets of *P. notoginseng* in different origins. (b) Accumulation of dencichine in leaves, stems, main roots, and rootlets of *P. notoginseng* at different altitudes. Different lowercase letters indicate significant differences between groups by Duncan's test ($P < 0.05$).

leaves of *P. notoginseng* was determined. As shown in Figure 6a, the contents of dencichine in PuEr leaves (4.45 ± 2.24 mg/g) and KunMing stems (3.53 ± 2.41 mg/g) were significantly higher than those from other origins, whereas the content of dencichine in WenShan main roots (8.33 ± 2.01 mg/g) was significantly higher than that in KunMing and PuEr (6.05 ± 1.90 and 6.22 ± 1.23 mg/g). Moreover, HongHe rootlets had the highest content of dencichine (9.48 ± 3.18 mg/g).

We then determined the content of dencichine in three-year *P. notoginseng* grown at different altitudes of 1500, 1500−1700, 1700−1900, 1900−2100, and 2100−2400 m. The content of dencichine in leaves of *P. notoginseng* in the low-altitude area (1200−1500 m, 4.65 ± 2.14 mg/g) was significantly higher than that in the middle altitude area (1700−1900 and 1900−2100 m, 3.29 ± 1.69 and 3.31 ± 1.45 mg/g). The content of dencichine in the main roots of *P. notoginseng* in the high-altitude area (2100−2500 m, 5.90 ± 1.78 mg/g) was significantly lower than that in the middle altitude area (1500−1700 and 1700−1900 m, 7.53 ± 2.60 and 7.42 ± 2.27 mg/g). The content of dencichine in stems of *P. notoginseng* at an altitude of 2100−2400 m (3.51 ± 2.29 mg/g) was significantly higher than that at other altitudes. The content of dencichine in rootlets of *P. notoginseng* at altitudes of 1900−2100 m (9.27 ± 2.33 mg/g) was significantly higher than that at other altitudes (Figure 6b). Taken together, origins and altitudes had little effect on the accumulation of dencichines in *P. notoginseng*.

CONCLUSIONS

In the current study, we established an HPLC-DAD method for determining dencichines in leaves, stems, main roots, and rootlets of *P. notoginseng* without derivatization. The average recovery rate was between 82.94 and 94.19%, and the RSD was <10%. It was validated to have great accuracy and sensitivity, with an LOD of 0.10 mg/g and LOQ of 0.33 mg/g. This method had a relatively short detection time (<8 min) and extraction time (<45 min). The content of dencichine in rootlets (39.59%) was higher than that in main roots (29.91%), followed by leaves (16.21%) and then stems (14.29%). The content of dencichine in leaves under in-forest planting (5.52 ± 2.26 mg/g) was significantly higher than that under traditional in-field planting (3.93 ± 1.72 mg/g), while the content of dencichine in main roots under in-forest planting (7.09 ± 1.84 mg/g) was lower than that under in-field planting (8.79 ± 2.51 mg/g). Altitudes and origins had little effect on the accumulation of dencichines in *P. notoginseng*.

ASSOCIATED CONTENT

Supporting Information

This information can be found online. The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02962.

Comparison of different methods of extraction and reagents, sampling areas, factor level of response surface analysis, experimental design and results, recovery rate and RSD, and distribution of dencichine (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Li, G.; Chen, J.; Li, Z.-K.; Wu, W.; Yang, H.-J. Analysis of patent data of Panax notoginseng and enlightenment of industrial development. *China J. Chin. Mater. Med.* **2020**, *45*, 1011−1022.

(2) Wang, T.; Guo, R.; Zhou, G.; Zhou, X.; Kou, Z.; Sui, F.; Li, C.; Tang, L.; Wang, Z. Traditional uses, botany, phytochemistry, pharmacology and toxicology of Panax notoginseng (Burk.) F.H. Chen: A review. *J. Ethnopharmacol.* **2016**, *188*, 234−258.

(3) Yue, J.; Zuo, Z.; Huang, H.; Wang, Y. Application of Identification and Evaluation Techniques for Ethnobotanical Medicinal Plant of Genus Panax: A Review. *Crit. Rev. Anal. Chem.* **2021**, *51*, 373−398.

(4) Wang, Y.-l.; Cui, X.-m.; Lan, L.; Chen, W.-d.; Li, R.-b.; Wang, C.-x.; Yang, X.-y.; Liu, D. -h.; Yang, Y. Light and temperature and their effects on photosynthesis characteristics of stereoscopic cultivation in Panax notoginseng. *China J. Chin. Mater. Med.* **2015**, *40*, 2921−2929.

(5) Ye, C.; Fang, H. Y.; Liu, H. J.; Yang, M.; Zhu, S. S. Current status of soil sickness research on Panax notoginseng in Yunnan, China. *Allelopathy J.* **2019**, *47*, 1−14.

(6) Wu, H.; Xia, J.; Qin, X.; Wu, H.; Zhang, S.; Zhao, Y.; Rensing, C.; Lin, W. Underlying Mechanism of Wild Radix pseudostellariae in Tolerance to Disease Under the Natural Forest Cover. *Front. Microbiol.* **2020**, *11*, 1142.

(7) Donald, P. F. Biodiversity Impacts of Some Agricultural Commodity Production Systems. *Conserv. Biol.* **2004**, *18*, 17−38.

(8) Chen, W.; Balan, P.; Popovich, D. G. Ginsenosides analysis of New Zealand-grown forest Panax ginseng by LC-QTOF-MS/MS. *J. Ginseng Res.* **2020**, *44*, 552−562.

(9) Suh, H.; Seo, S. M.; Lee, D.-S.; Woo, S. Y. Ginsenosides contents of forest cultivated Ginseng (Panax ginseng) in Korea. *For. Ind. Sci. Technol.* **2010**, *6*, 118−121.

(10) Zhu, L.; Xu, L.; Dou, D.; Huang, L. The distinct of chemical profiles of mountainous forest cultivated ginseng and garden ginseng based on ginsenosides and oligosaccharides. *J. Food Compos. Anal.* **2021**, *104*, 104165.

(11) Wu, S.; Yang, T.; Cen, K.; Zou, Y.; Shi, X.; Zhou, D.; Gao, Y.; Chai, L.; Zhao, Y.; Sun, Y.; Zhu, L. In Vitro Evaluation of the Neuroprotective Effect of Panax notoginseng Saponins by Activating the EGFR/PI3K/AKT Pathway. *Evid.-based Complement. Altern. Med.* **2020**, *2020*, 1−11.

(12) Chen, S.; Wu, Y.; Qin, X.; Wen, P.; Liu, J.; Yang, M. Global gene expression analysis using RNA-seq reveals the new roles of Panax notoginseng Saponins in ischemic cardiomyocytes. *J. Ethnopharmacol.* **2021**, *268*, 113639.

(13) Liu, Y.-H.; Qin, H.-Y.; Zhong, Y.-Y.; Li, S.; Wang, H.-J.; Wang, H.; Chen, L.-L.; Tang, X.; Li, Y.-L.; Qian, Z.-Y.; Li, H.-Y.; Zhang, L.; Chen, T. Neutral polysaccharide from Panax notoginseng enhanced cyclophosphamide antitumor efficacy in hepatoma H22-bearing mice. *BMC Cancer* **2021**, *21*, 37.

(14) Tan, M.-M.; Chen, M.-H.; Han, F.; Wang, J.-W.; Tu, Y.-X. Role of Bioactive Constituents of Panax notoginseng in the Modulation of Tumorigenesis: A Potential Review for the Treatment of Cancer. *Front. Pharmacol.* **2021**, *12*, 738914.

(15) Liao, P.; Liu, P.; Wang, Y.; Huang, C.; Lan, L.; Yang, Y.; Cui, X. Stereoscopic cultivation of Panax notoginseng: A new approach to overcome the continuous cropping obstacle. *Ind. Crops Prod.* **2018**, *126*, 38−47.

(16) Chen, W.; Balan, P.; Popovich, D. G. Analysis of Ginsenoside Content (Panax ginseng) from Different Regions. *Molecules* **2019**, *24*, 3491.

(17) Li, Y.; Yang, B.; Guo, W.; Zhang, P.; Zhang, J.; Zhao, J.; Wang, Q.; Zhang, W.; Zhang, X.; Kong, D. A novel strategy to interpret the differences among white ginseng, red ginseng and American ginseng

based on normalized ginsenoside profiles; *Research Square*, 2022. [Preprint].
(18) Nie, L.-X.; Dai, Z.; Ma, S.-C. Guideline principle and technical requirement for preparing traditional Chinese medicine reference drug. *China J. Chin. Mater. Med.* **2017**, *42*, 3672−3675.
(19) Huang, L.-F.; Shi, H.-L.; Gao, B.; Wu, H.; Yang, L.; Wu, X.-J.; Wang, Z.-T. Decichine enhances hemostasis of activated platelets via AMPA receptors. *Thromb. Res.* **2014**, *133*, 848−854.
(20) Xie, G.-X.; Qiu, Y.-P.; Qiu, M.-F.; Gao, X.-F.; Liu, Y.-M.; Jia, W. Analysis of dencichine in Panax notoginseng by gas chromatography-mass spectrometry with ethyl chloroformate derivatization. *J. Pharm. Biomed. Anal.* **2007**, *43*, 920−925.
(21) Jie, L.; Pengcheng, Q.; Qiaoyan, H.; Linlin, B.; Meng, Z.; Fang, W.; Min, J.; Li, Y.; Ya, Z.; Qian, Y.; Siwang, W. Dencichine ameliorates kidney injury in induced type II diabetic nephropathy via the TGF-β/Smad signalling pathway. *Eur. J. Pharmacol.* **2017**, *812*, 196−205.
(22) Cang, D.; Zou, G.; Yang, C.; Shen, X.; Li, F.; Wu, Y.; Ji, B. Dencichine prevents ovariectomy-induced bone loss and inhibits osteoclastogenesis by inhibiting RANKL-associated NF-κB and MAPK signaling pathways. *J. Pharmacol. Sci.* **2021**, *146*, 206−215.
(23) Li, J.; Qiu, P.; Wang, S.; Wu, J.; He, Q.; Li, K.; Xu, L. β-N-Oxalyl-L-α,β-diaminopropionic acid from Panax notoginseng plays a major role in the treatment of type 2 diabetic nephropathy. *Biomed. Pharmacother.* **2019**, *114*, 108801.
(24) Yang, Y.; Nian, H.; Tang, X.; Wang, X.; Liu, R. Effects of the combined Herba Epimedii and Fructus Ligustri Lucidi on bone turnover and TGF-β1/Smads pathway in GIOP rats. *J. Ethnopharmacol.* **2017**, *201*, 91−99.
(25) Huang, C.; Cheng, L.; Feng, X.; Li, X.; Wang, L. Dencichine ameliorates renal injury by improving oxidative stress, apoptosis and fibrosis in diabetic rats. *Life Sci.* **2020**, *258*, 118146.
(26) Long, Y.-C.; Ye, Y.-H.; Xing, Q.-Y. Studies on the neuro-excitotoxin β-N-oxalo-L-α, β-diaminopropionic acid and its isomer α-N-oxalo-L-α, βdiaminopropionic acid from the root of Panax species. *Int. J. Pept. Protein Res.* **1996**, *47*, 42−46.
(27) Pan, M.; Mabry, T. J.; Cao, P.; Moini, M. Identification of nonprotein amino acids from cycad seeds as N-ethoxycarbonyl ethyl ester derivatives by positive chemical-ionization gas chromatography-mass spectrometry. *J. Chromatogr. A* **1997**, *787*, 288−294.
(28) Quereshi, M.; Pilbeam, D. J.; Evans, C. S.; Bell, E. The neurolathyrogen, α-amino-β-oxalylaminopropionic acid in legume seeds. *Phytochemistry* **1977**, *16*, 477−479.
(29) Kosuge, T.; Yokota, M.; Ochiai, A. Studies on Antihemorrhagic Principles in the Crude Drugs for Hemostatics. II. On Antihemorrhagic Principle in Sanchi Ginseng Radix. *J. Pharm. Soc. Jpn.* **1981**, *101*, 629−632.
(30) Yan, Z. Y.; Spencer, P. S.; Li, Z. X.; Liang, Y. M.; Wang, Y. F.; Wang, C. Y.; Li, F. M. Lathyrus sativus (grass pea) and its neurotoxin ODAP. *Phytochemistry* **2006**, *67*, 107−121.
(31) Zhao, L.; Chen, X.; Hu, Z.; Li, Q.; Chen, Q.; Li, Z. Analysis of beta-N-oxalyl-L-alpha,beta-diaminopropionic acid and homoarginine in Lathyrus sativus by capillary zone electrophoresis. *J. Chromatogr. A* **1999**, *857*, 295−302.
(32) Kuo, Y.-H.; Ikegami, F.; Lambein, F. Neuroactive and other free amino acids in seed and young plants of Panax ginseng. *Phytochemistry* **2003**, *62*, 1087−1091.
(33) Emmrich, P. M. F.; Rejzek, M.; Hill, L.; Brett, P.; Edwards, A.; Sarkar, A.; Field, R. A.; Martin, C.; Wang, T. L. Linking a rapid throughput plate-assay with high-sensitivity stable-isotope label LCMS quantification permits the identification and characterisation of low β-L-ODAP grass pea lines. *BMC Plant Biol.* **2019**, *19*, 489.
(34) Marichamy, S.; Yigzaw, Y.; Gorton, Lo; Mattiasson, B. Isolation of obligate anaerobic rumen bacteria capable of degrading the neurotoxin β-ODAP (β-N-oxalyl-L-α,β-diaminopropionic acid) as evaluated by a liquid chromatography/biosensor analysis system. *J. Sci. Food Agric.* **2005**, *85*, 2027−2032.
(35) Wang, F.; Chen, X.; Chen, Q.; Qin, X.; Li, Z. Determination of neurotoxin 3-N-oxalyl-2,3-diaminopropionic acid and non-protein amino acids in Lathyrus sativus by precolumn derivatization with 1-fluoro-2,4-dinitrobenzene. *J. Chromatogr. A* **2000**, *883*, 113−118.
(36) Yan, Z. Y.; Jiao, C. J.; Li, F. M.; Liang, Y. M.; Li, Z. X. Analysis of Toxin β-N-Oxalyl-α, β-diaminopropionic Acid (β-ODAP), its Isomer α-ODAP and Other Free Amino Acids in Lathyrus sativus. *Chin. Chem. Lett.* **2005**, *16*, 627−630.
(37) Koh, H.-L.; Lau, A.-J.; Chan, E. C.-Y. Hydrophilic interaction liquid chromatography with tandem mass spectrometry for the determination of underivatized dencichine (β-N-oxalyl-L-α,β-diaminopropionic acid) inPanax medicinal plant species. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 1237−1244.
(38) Bento-Silva, A.; Gonçalves, L.; Mecha, E.; Pereira, F.; Vaz Patto, M. C.; Bronze, M. d. R. An Improved HILIC HPLC-MS/MS Method for the Determination of β-ODAP and Its α Isomer in Lathyrus sativus. *Molecules* **2019**, *24*, 3043.
(39) Yan, Z. Y.; Jiao, C. J.; Wang, Y. p.; Li, F. m.; Liang, Y. m.; Li, Z. x. A method for the simultaneous determination of β-ODAP, α-ODAP, homoarginine and polyamines in Lathyrus sativus by liquid chromatography using a new extraction procedure. *Anal. Chim. Acta* **2005**, *534*, 199−205.
(40) Nagar, P. S.; Rane, S.; Dwivedi, M. LC-MS/MS standardization and validation of glycyrrhizin from the roots of Taverniera cuneifolia: A potential alternative source of Glycyrrhiza glabra. *Heliyon* **2022**, *8*, No. e10234.
(41) Gresta, F.; Rocco, C.; Lombardo, G. M.; Avola, G.; Ruberto, G. Agronomic Characterization and α- and β-ODAP Determination through the Adoption of New Analytical Strategies (HPLC-ELSD and NMR) of Ten Sicilian Accessions of Grass Pea. *J. Agric. Food Chem.* **2014**, *62*, 2436−2442.
(42) Arslan, M.; Oten, M.; Erkaymaz, T.; Tongur, T.; Kilic, M.; Elmasulu, S.; Cinar, A. β-N-oxalyl-L-2,3-diaminopropionic acid, L-homoarginine, and asparagine contents in the seeds of different genotypes Lathyrus sativus L. as determined by UHPLC-MS/MS. *Int. J. Food Prop.* **2017**, *20*, S108−S118.
(43) Zhang, Y.; Chen, X.; Li, X.; Zhong, D. Development of a liquid chromatographic−tandem mass spectrometric method with precolumn derivatization for the determination of dencichine in rat plasma. *Anal. Chim. Acta* **2006**, *566*, 200−206.
(44) Dong, T. T. X.; Cui, X. M.; Song, Z. H.; Zhao, K. J.; Ji, Z. N.; Lo, C. K.; Tsim, K. W. K. Chemical assessment of roots of Panax notoginseng in China: regional and seasonal variations in its active constituents. *J. Agric. Food Chem.* **2003**, *51*, 4617−4623.
(45) Yang, Z.; Liu, G.; Zhang, G.; Yan, J.; Dong, Y.; Lu, Y.; Fan, W.; Hao, B.; Lin, Y.; Li, Y.; Li, X.; Tang, Q.; Xiang, G.; He, S.; Chen, J.; Chen, W.; Xu, Z.; Mao, Z.; Duan, S.; Jin, S.; Yang, S. The chromosome-scale high-quality genome assembly of Panax notoginseng provides insight into dencichine biosynthesis. *Plant Biotechnol. J.* **2021**, *19*, 869−871.
(46) Dai, C.; Huang, J.; Cui, X.; Qiu, L.; Liu, C.; Guan, H.; Qu, Y.; Yang, Y. Selenium enhances antioxidant capacity by improving dencichine content in Panax notoginseng. *Ind. Crop. Prod.* **2019**, *131*, 250−256.

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