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Quantitative Comparison and Metabolite Profiling of Saponins in Different Parts of the Root of *Panax notoginseng*

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Supporting Information

ABSTRACT: Although both rhizome and root of *Panax notoginseng* are officially utilized as notoginseng in "Chinese Pharmacopoeia", individual parts of the root were differently used in practice. To provide chemical evidence for the differentiated usage, quantitative comparison and metabolite profiling of different portions derived from the whole root, as well as commercial samples, were carried out, showing an overall higher content of saponins in rhizome, followed by main root, branch root, and fibrous root. Ginsenoside Rb_2 was proposed as a potential marker with a content of 0.5 mg/g as a threshold value for differentiating rhizome from other parts. Multivariate analysis of the metabolite profile further suggested 32 saponins as potential markers for the discrimination of different parts of notoginseng. Collectively, the study provided comprehensive chemical evidence for the distinct usage of different parts of notoginseng and, hence, is of great importance for the rational application and exploitation of individual parts of notoginseng.

KEYWORDS: *notoginseng, ginsenosides, LC−MS, metabolomics, root*

INTRODUCTION

The root of *Panax notoginseng* (Burk.) F.H. Chen, commonly referred to as notoginseng and "*Sanqi*", is a well-known botanic material that has been historically used as both medicinal herb and food. It has been widely used as a tonic and hemostatic agent for the treatment of cardiovascular diseases, inflammation, different body pains, trauma, and internal or external bleeding caused by injury. Substantial studies have demonstrated hemeostatic,[1−3] antioxidant,[4,5] hypolipidemic,[6] hepatoprotective,[6−8] renoprotective, and estrogen-like activities of notoginseng.[6,9] Moreover, chemopreventive and antitumor activities of this herb and its major constituents have been increasingly reported.[10−14]

Owing to the time-honored benefits for health, notoginseng is also widely used as a distinguished functional food beyond the medicinal application. In China, 47 health foods with notoginseng as a single or major ingredient, including a variety of tablets, capsule, and medicinal liquors, have been approved by the China Food and Drug Administration for manufacturing. These health foods are claimed to benefit people with low immunity and hyperlipidemia because of their health functions such as liver protection, immunomodulation, blood lipid regulation, antifatigue effect, and antihypoxia activity. In addition, notoginseng is also widely consumed in the form of tea, powder, and raw materials of Chinese soup in China and other Asian countries. In the United States, a variety of notoginseng products are available as dietary supplements in the health food market.[5,15]

Although both rhizome and root (Notoginseng Radix et Rhizoma) are officially described as notoginseng in "Chinese Pharmacopoeia" since 1985, individual parts of the whole root, that is, rhizome, main root, branch root, and fibrous root, were separately used, and their market values are greatly different. Rhizome, commonly known as "*Jiankou*", is usually used for the manufacturing of ginsenosides and related preparations.[16] The main root is generally utilized in Chinese medical clinics and traditional Chinese proprietary medicines, whereas branch root (known as "*Jintiao*") and fibrous root are often ground to yield powder as health food. The market prices of the different parts rank in the order main root > rhizome and branch root > fibrous root.

Due to the different utilizations of individual parts of notoginseng, chemical differences among them are of great significance for both authentication and medicinal usage of different parts of this herb. Although numerous studies have been carried out to assess the chemical differences among notoginseng, American ginseng (the root of *Panax quinquefolius* L.), and Asian ginseng (the root of *Panax ginseng* C.A. Mey.),[17,18] only limited studies have been performed to assess the chemical characteristics of individual parts of notoginseng.[19−24] Consequently, the following limitations still existed: (1) More than 80 dammarane-type saponins with various bioactivities have been isolated from notoginseng and steamed notoginseng,[7,25−33] but only major ginsenosides, that is, notoginsenoside R_1, ginsenosides Rg_1, Re, Rb_1, and Rd, have been examined in most of the previous studies.[21,23,34−37] Several recent studies quantitatively determined both high- and low-abundance ginsenosides,[24,38] but the total

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Table 1. Validation Data of UPLC-MS Quantitative Method for Eight Saponins

				precision (RSD%) (n = 6)					
marker	regression eq	linearity (μg/mL)	r^2	intraday	interday	repeatability	recovery rate (%)	LOD (μg/mL)	LOQ (μg/mL)
R_1	$y = 132995x + 4969.8$	0.125−10	0.9998	1.99	1.25	2.25	98.66 (1.81)[a]	0.005	0.021
Rg_1	$y = 125416x - 6776.7$	0.125−10	0.9998	1.28	1.99	1.08	99.10 (2.90)	0.004	0.024
Re	$y = 148540x + 15054$	0.125−10	0.9995	2.67	2.18	2.11	103.03 (2.22)	0.005	0.017
Rb_1	$y = 64969x - 4164.7$	0.125−10	0.9997	2.31	2.60	1.72	99.65 (3.29)	0.015	0.054
Rb_2	$y = 116356x - 5883$	0.125−10	0.9997	2.87	1.68	3.84	99.06 (3.43)	0.011	0.029
Rb_3	$y = 134365x - 851$	0.05−4	0.9999	3.00	3.44	1.61	97.7 (1.26)	0.008	0.025
Rc	$y = 122449x - 500.33$	0.05−4	0.9999	2.18	2.21	2.79	99.62 (2.70)	0.008	0.026
Rd	$y = 152720x - 6790.6$	0.125−10	0.9999	2.41	1.22	2.97	101.65 (1.60)	0.006	0.021

[a]The values in the parentheses are the RSDs of six replicated determinations of recovery rate.

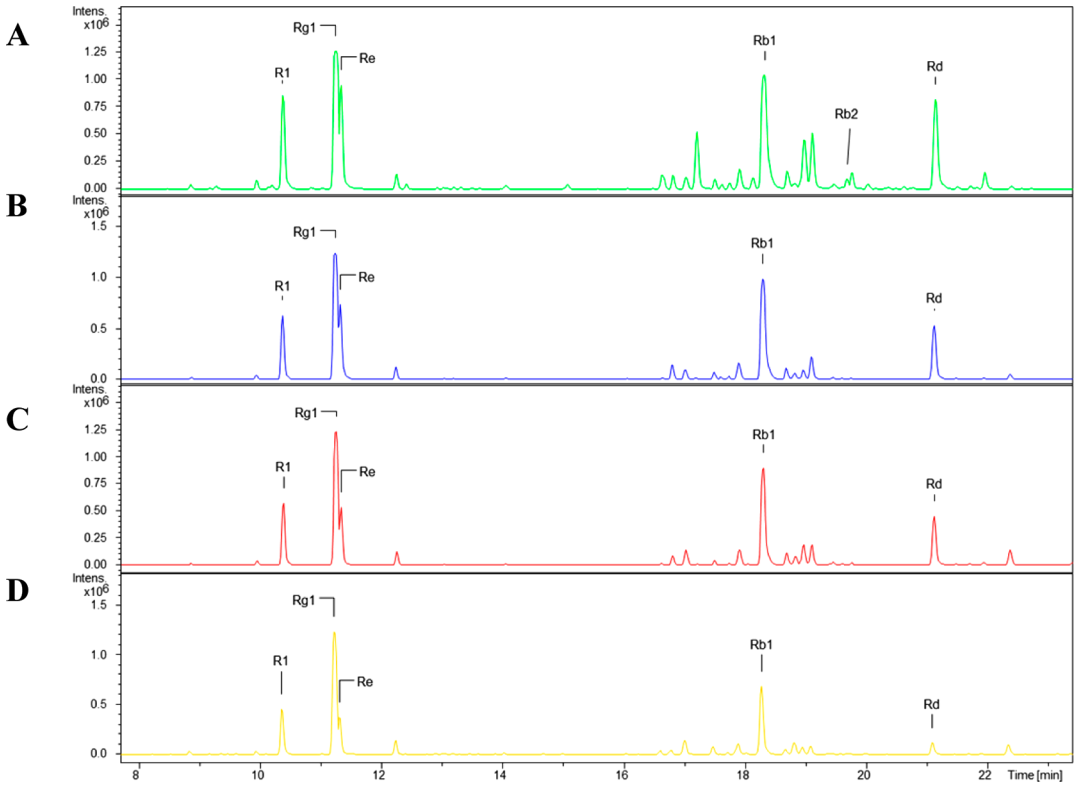

Figure 1. Total ion chromatogram (TIC) of rhizome (A), main root, (B) branch root (C), and fibrous root (D) of *Panax notoginseng*.

number of quantified saponins is still quite limited. (2) Metabolite profiling by using UPLC-ESI-MS has been applied for evaluating the chemical characteristics of different parts of *P. notoginseng*, that is, flower, leaf, seed, rhizome, and main root,[19,39,40] or raw notoginseng and steamed notoginseng.[40] However, this approach is still not well applied for the examination of different underground parts of *P. notoginseng*. Especially, comparison among the four parts of notoginseng by using a metabolomics approach is absent. (3) Comparison was often made among the different parts sampled from roots of diverse origin (e.g, roots collected from different regions or collected at different seasons). However, the different origin itself might introduce significant variation in the content of chemical constituents. As such, the "real" variation resulting from biosynthesis in different portions of the root might be compromised by the origin-derived variation.

Therefore, we herein conducted a quantitative comparison on the different parts derived from the whole root of *P. notoginseng* collected from the major production region of notoginseng, Wenshan city, Yunnan province. Eight dammarane-type saponins were quantitatively compared by using the validated UPLC-Q-TOF MS method. Commercial samples of individual parts of notoginseng were also analyzed to verify the chemical difference. A nontargeted metabolomics approach was subsequently applied for discrimination of different parts of the root. This combinative comparison approach facilitated an overall evaluation of chemical difference among these closely related samples.

MATERIALS AND METHODS

Plant Materials. Twelve batches of the whole root of *P. notoginseng* (3 years) were collected from the wholesale markets of Wenshan, Yunnan province, China, in December 2008. For each batch of the root, three to five of the whole roots were divided into rhizome, main root, branch root, and fibrous root. The obtained individual parts were then mixed to yield the representative portion of each batch. The yield of each part from the whole root was measured to be 15% (rhizome), 66% (main root), 18% branch root, and 2% (fibrous root) on average. Commercial samples of individual parts of notoginseng were also collected from the markets of Wenshan during 2006−2008, including

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Table 2. Potential Marker Compounds Responsible for Differentiation of Rhizome, Main Root, Branch Root, and Fibrous Root of *Panax notoginseng*

no.	identificaton	VIP	t_R (min)	calcd mass	measured mass	error (ppm)	ion species	molecular formula	fragment (m/z)
1	ginsenoside Rb_1	22.67	18.26	1153.6011	1153.6003	−0.7	$[M + HCOO]^-$	$C_{54}H_{92}O_{23}$	1107.5952 $[M - H]^-$, 945.5421 $[M - H - Glc]^-$, 783.4901 $[M - H - 2Glc]^-$, 621.4370 $[M - H - 3Glc]^-$, 459.3851 $[M - H - 4Glc]^-$
2	malonyl-ginsenoside Rb_1	17.71	18.64	1193.5961	1193.6004	3.6	$[M - H]^-$	$C_{57}H_{94}O_{26}$	1149.6009 $[M - H - CO_2]^-$, 1107.5986 $[M - H - malonyl]^-$, 1089.5857 $[M - H - malonyl - H_2O]^-$, 945.5532 $[M - H - malonyl - Glc]^-$, 927.5230 $[M - H - malonyl - Glc - H_2O]^-$, 825.4972 $[M - H - CO_2 - 2Glc]^-$, 783.4871 $[M - H - malonyl - 2Glc]^-$, 621.4402 $[M - H - malonyl - 3Glc]^-$
3	ginsenoside Rd	16.77	21.20	991.5483	991.5482	−0.1	$[M + HCOO]^-$	$C_{48}H_{82}O_{18}$	945.5423 $[M - H]^-$, 783.4901 $[M - H - Glc]^-$, 765.4788 $[M - H - Glc - H_2O]^-$, 621.4374 $[M - H - 2Glc]^-$, 603.4284 $[M - H - 2Glc - H_2O]^-$, 459.3844[M-H-3Glc]$^-$
4	ginsenoside Rg_1	14.03	11.22	845.4904	845.4913	1	$[M + HCOO]^-$	$C_{42}H_{72}O_{14}$	799.4825 $[M - H]^-$, 637.4316 $[M - H - Glc]^-$, 619.4183 $[M - H - Glc\text{-}H_2O]^-$, 475.3796 $[M - H - 2Glc]^-$, 457.3689 $[M - H - 2Glc - H_2O]^-$
5	notoginsenoside K	11.94	22.36	991.5483	991.5509	2.6	$[M + HCOO]^-$	$C_{48}H_{82}O_{18}$	945.5419 $[M - H]^-$, 783.4870 $[M - H - Glc]^-$, 765.4788 $[M - H - Glc - H_2O]^-$, 621.4356 $[M - H - 2Glc]^-$, 603.4214 $[M - H - 2Glc - H_2O]^-$, 459.3840 $[M - H - 3Glc]^-$
6	notoginsenoside R_1	11.89	10.34	977.5327	977.5291	−3.7	$[M + HCOO]^-$	$C_{47}H_{80}O_{18}$	931.5278 $[M - H]^-$, 799.4845 $[M - H - Xyl]^-$, 781.4743 $[M - H - Xyl - H_2O]^-$, 769.4744 $[M - H - Glc]^-$, 751.4629 $[M - H - Glc - H_2O]^-$, 637.4324 $[M - H - Xyl - Glc]^-$, 619.4220 $[M - H - Xyl - Glc - H_2O]^-$, 475.3797 $[M - H - Xyl - 2Glc]^-$
7	notoginsenoside Rw_1	11.69	17.17	947.5221	947.5199	−2.3	$[M + HCOO]^-$	$C_{46}H_{78}O_{17}$	901.5160 $[M - H]^-$, 769.4766 $[M - H - Xyl]^-$, 637.4311 $[M - H - 2Xyl]^-$, 475.3794 $[M - H - 2Xyl - Glc]^-$
8	ginsenoside Rb_2	9.43	19.60	1123.5906	1123.5945	3.4	$[M + HCOO]^-$	$C_{53}H_{90}O_{22}$	1077.5861 $[M - H]^-$, 945.5278 $[M - H - Xyl]^-$, 915.5379 $[M - H - Glc]^-$, 783.4869 $[M - H - Xyl - Glc]^-$, 765.4677 $[M - H - Xyl - Glc - H_2O]^-$, 621.4399 $[M - H - Xyl - 2Glc]^-$, 603.4351 $[M - H - Xyl - 2Glc - \text{-}H_2O]^-$, 459.3876 $[M - H - Xyl - 3Glc]^-$
9	malonyl-ginsenoside Rd	9.07	21.91	1031.5432	1031.5465	3.2	$[M - H]^-$	$C_{51}H_{84}O_{21}$	987.5508 $[M - H - CO_2]^-$, 945.5399 $[M - H - malonyl]^-$, 927.5307 $[M - H - malonyl - H_2O]^-$, 783.5049 $[M - H - malonyl - Glc]^-$
10	ginsenoside Re	8.65	11.30	991.5483	991.5509	2.6	$[M + HCOO]^-$	$C_{48}H_{82}O_{18}$	945.5430 $[M - H]^-$, 799.4834 $[M - H - Rha]^-$, 783.4900 $[M - H - Glc]^-$, 765.4793 $[M - H - Glc - H_2O]^-$, 637.4323 $[M - H - Rha - Glc]^-$, 619.4214 $[M - H - Rha - Glc - H_2O]^-$, 475.3792 $[M - H - Rha - 2Glc]^-$
11	ginsenoside Rg_3	8.47	24.84	829.4955	829.4989	4.1	$[M + HCOO]^-$	$C_{42}H_{72}O_{13}$	783.4896 $[M - H]^-$, 621.4426 $[M - H - Glc]^-$, 603.4216 $[M - H - Glc - H_2O]^-$, 459.3803 $[M - H - 2Glc]^-$
12	notoginsenoside R_4/ ginsenoside Ra_3	6.04	17.45	1239.6374	1239.6379	−2.46	$[M - H]^-$	$C_{59}H_{100}O_{27}$	1107.5934 $[M - H - Xyl]^-$, 1089.5813 $[M - H - Xyl - H_2O]^-$, 1077.5904 $[M - H - Glc]^-$, 945.5438 $[M - H - Xyl - Glc]^-$, 915.5320 $[M - H - 2Glc]^-$, 783.4890 $[M - H - Xyl - 2Glc]^-$, 765.4824 $[M - H - Xyl - 2Glc - \text{-}H_2O]^-$, 621.4360 $[M - H - Xyl - 3Glc]^-$
13	notoginsenoside R_2/ ginsenoside F_5/ ginsenoside La	5.88	18.09	815.4798	815.4792	0.95	$[M + HCOO]^-$	$C_{41}H_{70}O_{13}$	769.4728 $[M - H]^-$, 637.4318 $[M - H - Xyl]^-$, 475.3783 $[M - H - Xyl - Glc]^-$
14	notoginsenoside Fa/ notoginsenoside R_4/ ginsenoside Ra_3	5.59	16.77	1239.6374	1239.6399	1.19	$[M - H]^-$	$C_{59}H_{100}O_{27}$	1107.6014 $[M - H - Xyl]^-$, 1077.5904 $[M - H - Glc]^-$, 945.5473 $[M - H - Xyl - Glc]^-$, 927.5299 $[M - H - Xyl - Glc - H_2O]^-$, 783.489 $[M - H - Xyl - 2Glc]^-$, 765.4788 $[M - H - Xyl - 2Glc - \text{-}H_2O]^-$
15	floralginsenoside E/ floralginsenoside F	5.51	4.92	861.4853	861.4848	0.59	$[M + HCOO]^-$	$C_{42}H_{72}O_{15}$	815.4779 $[M - H]^-$, 653.4239 $[M - H - Glc]^-$, 635.4188 $[M - H - Glc - H_2O]^-$, 491.3739 $[M - H - 2Glc]^-$
16	ginsenoside Rc	5.36	18.97	1077.5852	1077.5851	0.68	$[M - H]^-$	$C_{53}H_{90}O_{22}$	945.5278 $[M - H - Xyl]^-$, 915.5383 $[M - H - Glc]^-$, 783.4916 $[M - H - Xyl - Glc]^-$, 765.4830 $[M - H - Xyl - Glc - H_2O]^-$, 621.4399 $[M - H - Xyl - 2Glc]^-$, 603.4351 $[M - H - Xyl - 2Glc\text{-}H_2O]^-$, 459.3876 $[M - H - Xyl - 3Glc]^-$
17	notoginsenoside I	4.76	17.58	568.2989	568.2970	−3.3	$[M - H + HCOO]^{2-}$	$C_{54}H_{92}O_{22}$	1091.5981 $[M - H]^-$, 929.5497 $[M - H - Glc]^-$, 767.4939 $[M - H - 2Glc]^-$, 605.4420 $[M - 2H - 3Glc]^-$
18	10-hydroxy-4,6-decadiy-noic acid	4.74	3.56	503.1770	503.1764	1.19	$[M - H]^-$	$C_{22}H_{32}O_{13}$	503.1769 $[M - H]^-$, 341.1234 $[M - H - Glc]^-$
19	ginsenoside Rf/ginseno-side la/ginsenoside lb/ notoginsenoside U	4.62	16.60	845.4904	845.4898	0.91	$[M + HCOO]^-$	$C_{42}H_{72}O_{14}$	799.4825 $[M - H]^-$, 637.4316 $[M - H - Glc]^-$, 475.3796 $[M - H - 2Glc]^-$
20	notoginsenoside R_2/ ginsenoside F_5/ ginsenoside La	3.98	16.98	815.4798	815.482	2.7	$[M + HCOO]^-$	$C_{41}H_{70}O_{13}$	769.4728 $[M - H]^-$, 637.4318 $[M - H - Xyl]^-$, 619.4202 $[M - H - Xyl - H_2O]^-$, 475.3783 $[M - H - Xyl - Glc]^-$, 457.643 $[M - H - Xyl - Glc - H_2O]^-$

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Table 2. continued

no.	identificaton	VIP	t_R (min)	calcd mass	measured mass	error (ppm)	ion species	molecular formula	fragment (m/z)
21	notoginsenoside Fa/ notoginsenoside R_4/ ginsenoside Ra_3	3.69	18.24	1239.6374	1239.6375	0.84	$[M - H]^-$	$C_{59}H_{100}O_{27}$	1107.5901 $[M - H - Xyl]^-$, 1089.5897 $[M - H - Xyl - H_2O]^-$, 945.5394 $[M - H - Xyl - Glc]^-$, 783.4790 $[M - H - Xyl - 2Glc]^-$, 765.4824 $[M - H - Xyl - 2Glc - -H_2O]^-$, 621.4331 $[M - H - Xyl - 3Glc]^-$, 459 $[M - H - Xyl - 4Glc]^-$
22	ginsenoside Ra_1/ ginsenoside Ra_2/ notoginsenoside Fc /notoginsenoside FP_2	3.32	19.02	1209.6274	1209.629	0.69	$[M - H]^-$	$C_{58}H_{98}O_{26}$	1077.5815 $[M - H - Xyl]^-$, 945.5171 $[M - H - Xyl - Ara]^-$, 915.5203 $[M - H - Xyl - Glc]^-$, 783.4702 $[M - H - Xyl - Ara - Glc]^-$, 621.4456 $[M - H - Xyl - Ara - 2Glc]^-$, 459.4373 $[M - H - Xyl - Ara - 3Glc]^-$
23	notoginsenoside A/ koryoginsenoside Rg_2	3.22	12.88	584.2938	584.2926	−2.2	$[M - H + HCOO]^{2-}$	$C_{54}H_{92}O_{24}$	1123.5821 $[M - H]^-$, 961.5508 $[M - H - Glc]^-$, 799.4908 $[M - H - 2Glc]^-$, 561.2922 $[M - 2H]^{2-}$
24	ginsenoside Rg_2	2.61	17.86	829.4955	829.4969	1.7	$[M + HCOO]^-$	$C_{42}H_{72}O_{13}$	783.4896 $[M - H]^-$, 637.4303 $[M - H - Rha]^-$, 619.4279 $[M - H - Rha - H_2O]^-$, 475.3790 $[M - H - Rha - Glc]^-$, 457.3690 $[M - H - Rha - Glc - H_2O]^-$
25	ginsenoside Rh_1	2.47	17.85	683.4376	683.4345	4.5	$[M + HCOO]^-$	$C_{36}H_{62}O_9$	637.4318 $[M - H]^-$, 475.3770 $[M - H - Glc]^-$
26	panaxoside C	2.30	20.73	975.5534	975.5544	0.81	$[M + HCOO]^-$	$C_{48}H_{82}O_{17}$	929.5470 $[M - H]^-$, 767.4978 $[M - H - Glc]^-$
27	20-glucoginsenoside Rf/ floralginsenoside La/ floralginsenoside Lb	2.28	23.66	961.5378	961.5380	0.03	$[M - H]^-$	$C_{48}H_{82}O_{19}$	799.4854 $[M - H - Glc]^-$, 781.4751 $[M - H - Glc - H_2O]^-$, 637.4334 $[M - H - 2Glc]^-$, 619.4242 $[M - H - 2Glc - H_2O]^-$, 475.3794 $[M - H - 3Glc]^-$
28	6,20-di-*O*-*β*-D-glucopyranoside	2.28	11.23	843.4748	843.4740	2.25	$[M + HCOO]^-$	$C_{42}H_{70}O_{14}$	797.4710 $[M - H]^-$, 635.4210 $[M - H - Glc]^-$
29	notoginsenoside J	2.17	2.85	879.4959	897.4953	1.07	$[M + HCOO]^-$	$C_{42}H_{74}O_{16}$	833.5020 $[M - H]^-$, 671.4385 $[M - H - Glc]^-$
30	notoginsenoside D/ notoginsenoside T	2.06	18.27	1371.6802	1371.6806	1.05	$[M - H]^-$	$C_{64}H_{108}O_{31}$	685.3421 $[M - 2H]^{2-}$, 619.3171 $[M - 2H - Xyl]^{2-}$, 553.7970 $[M - 2H - 2Xyl]^{2-}$
31	vinaginsenoside R_{20}/ ginsenoside III	1.89	13.28	1005.5276	1005.5266	1.45	$[M + HCOO]^-$	$C_{48}H_{80}O_{19}$	959.5205 $[M - H]^-$, 797.4775 $[M - H - Glc]^-$, 635.4040 $[M - H - 2Glc]^-$, 473.3665 $[M - H - 3Glc]^-$
32	notoginsenoside H	1.87	4.55	993.5276	993.5285	0.93	$[M - H]^-$	$C_{48}H_{82}O_{21}$	947.5253 $[M - H]^-$, 785.4690 $[M - H - Glc]^-$, 767.4594 $[M - H - Glc - H_2O]^-$, 653.4277 $[M - H - Xyl - Glc]^-$, 635.4162 $[M - H - Xyl - Glc - H_2O]^-$, 491.3763 $[M - H - Xyl - 2Glc]^-$

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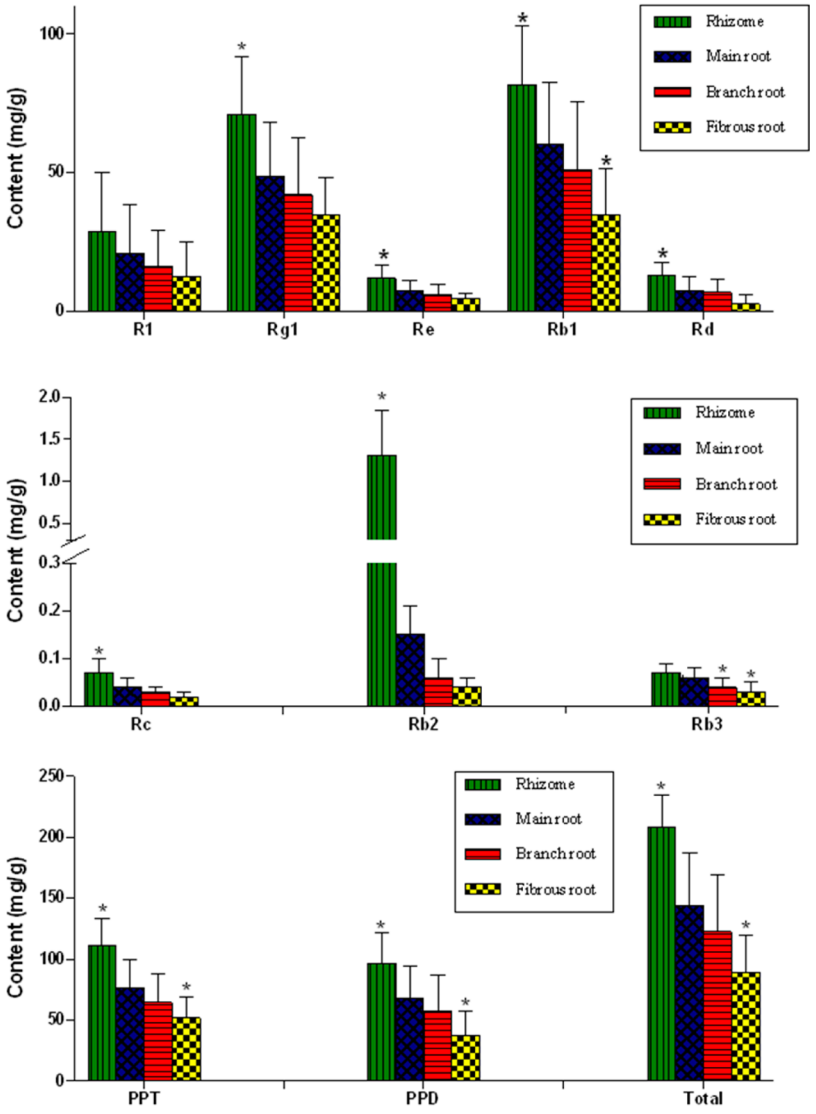

Figure 2. Contents of eight dammarane-type saponins and total saponins in rhizome, main root, branch root, and fibrous root of *P. notoginseng*. Each bar represents the mean ± SD (n = 12). (∗) $p < 0.05$, one-way ANOVA (compared with main root).

four batches of rhizome; main roots with growing years of 3 years (four batches), 4 years (two batches), and 5 years (two batches); four batches of main root without growing year information; four batches of branch root; and six batches of fibrous root.

Chemicals. Notoginsenoside R_1 and ginsenoside Rg_1 (purity > 98%) were purchased from Kunming Zhongzheng Biotech Co., Ltd. (Kunming, China); ginsenosides Re, Rb_1, Rb_2, Rb_3, Rc, and Rd (each purity > 98%) were purchased from Chengdu Scholar Biotech Co., Ltd. (Chengdu, China). HPLC grade methanol and acetonitrile were purchased from Anaqua Chemicals Supply Inc., Ltd. (Houston, TA, USA), and distilled water was purified by a Milli-Q system (Millipore).

Preparation of Standard and Sample Solution. Stock solutions of notoginsenoside R_1 and ginsenosides Rg_1, Re, Rb_1, Rb_2 and Rd in 70% methanol were diluted to a series of working solutions (0.125, 0.625, 1.25, 2.5, 5, and 10 μg/mL), whereas those of ginsenosides Rb_3 and Rc were diluted to yield working solutions of 0.05, 0.25, 0.5, 1, 2, and 4 μg/mL.

Sample solutions were prepared following the procedures developed in our previous studies.[41,42] Briefly, 200 mg of powdered samples of each representative part was accurately weighed into a PTFE-stopped tube and sonicated with 10 mL of 70% methanol for 1 h. The mixture was centrifuged, and the supernatant was collected and then diluted by 200- and 4-fold for the quantitation of major ginsenosides (R_1, Rg_1, Re, Rb_1, Rd, and Rb_2) and minor ginsenosides (Rb_3 and Rc), respectively. All samples were filtered through a 0.2 μm filter before analysis.

LC-MS Analysis. LC-MS was performed using a Waters Acquity UPLC system (Waters, Milford, MA, USA) coupled to a Bruker MicroTOF mass spectrometer with an ESI source (Bruker, Bremen, Germany). All of the operation, acquisition, and data analysis were operated by Hystar software (Bruker). MS/MS experiments were carried out on an Agilent 1290 infinity UHPLC system (Santa, Clara, CA, USA) coupled with an Agilent 6550 QTOF.

Chromatographic separation was performed on an Acquity UPLC BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), with a flow rate of 0.35 mL/min. The following gradient was used: 0−23 min, 10−40% B; 23−27 min, 40−85% B; 27−29 min, 85−100% B; 29−32 min, 100% B; 32−36 min, 10% B. The column was maintained at 40 °C, and the injection volume of each sample was 1 μL.

The mass spectrometer was operated in the negative mode with a scanning range of m/z 100−3000. The optimized mass conditions were as follows: drying gas (N_2) flow rate, 8 L/min; drying gas temperature, 180 °C; capillary voltage, 4500 V; nebulizing gas (N_2) pressure, 2.5 bar; end plate offset, −500 V. The following ions were extracted for the quantitative analysis of different compounds: m/z 977.5 ($[M + HCOO]^-$, notoginsenoside R_1), m/z 845.5 ($[M + HCOO]^-$, ginsenoside Rg_1), m/z 991.5 ($[M + HCOO]^-$, ginsenosides

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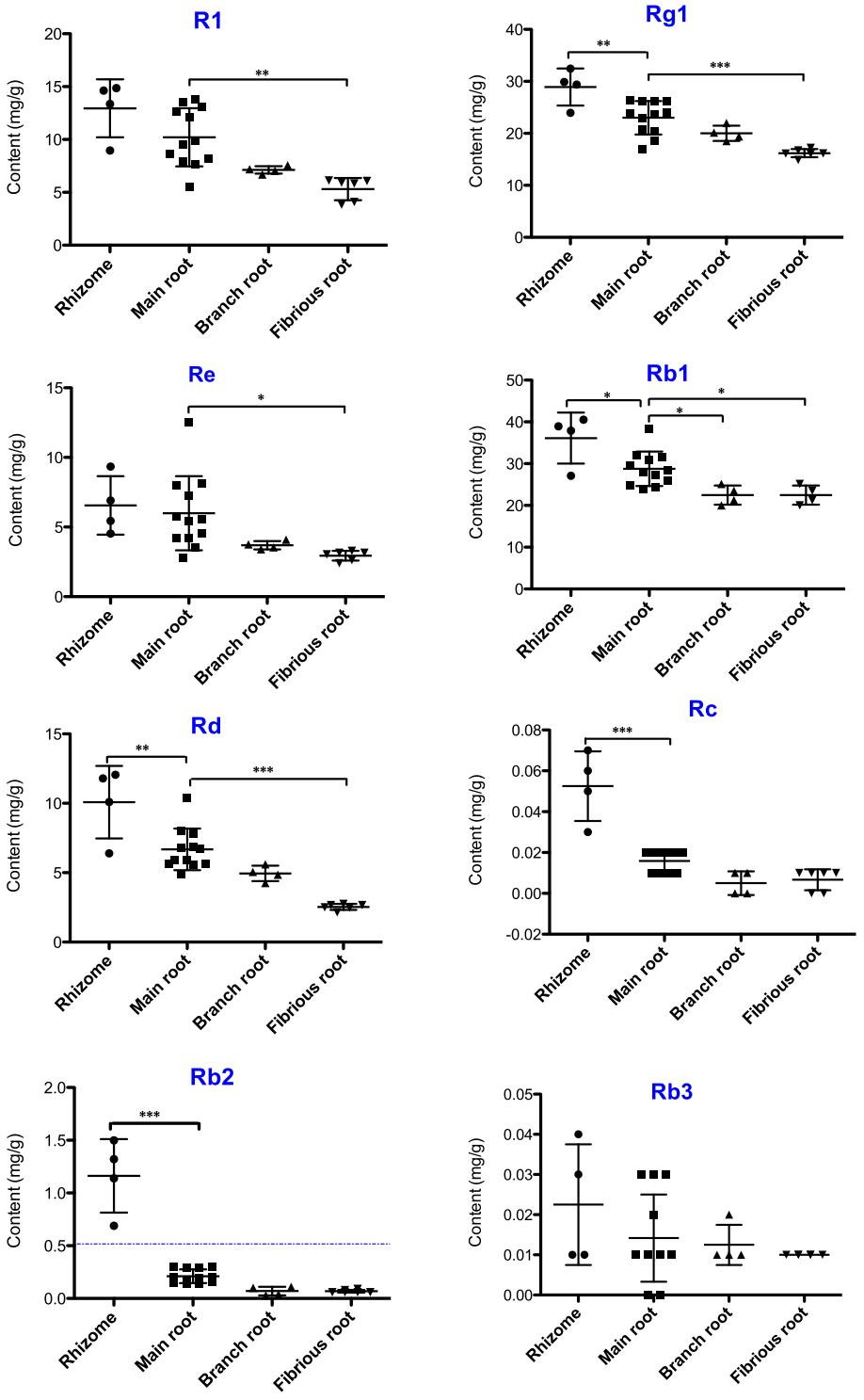

Figure 3. Contents of eight dammarane-type saponins in the commercial sample of rhizome (n = 4), main root (n = 12), branch root (n = 4), and fibrous root (n = 6) of *P. notoginseng*. (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, one-way ANOVA (compared with main root).

Re and Rd), m/z 1153.5 ($[M + HCOO]^-$, ginsenoside Rb_1), m/z 1123.5 ($[M + HCOO]^-$, ginsenosides Rb_2, Rb_3, and Rc).

Method Validation for Quantitation. The calibration curve ($y = a + bx$) for each ginsenoside was constructed by plotting the peak area against concentration, and the linearity was verified by correlation coefficients (r^2). The lower limit of detection (LOD) and lower limit of quantification (LOQ) were determined on the basis of response at signal-to-noise ratios (S/N) of 3 and 10, respectively. The precision was determined by intra- and interday variations. The intraday precision was performed by six replicate analyses of a mixed standard solution within a day, and the interday precision was carried out by analyzing the same mixed standard solution on five consecutive days. The repeatability was evaluated by carrying out six replicate analyses of the same sample. The recovery test was performed by spiking a sample with the mixed standards, and the recovery rate was calculated by using the following formula: recovery rate (%) = (observed amount − original amount)/spiked amount × 100%. Variations were expressed as relative standard deviations (RSD%).

Multivariate Analysis. The UPLC-Q-TOF MS data of different parts of notoginseng were first exported as cdf format. The peak

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finding, alignment, and filtering of raw data were preprocessed by MassLynx V4.1 (Waters, Milford, MA, USA). The parameters were set as the following conditions: retention time of 2−27 min, mass range of 100−1500 Da, mass tolerance of 0.05 Da. The resulting three-dimensional matrix using retention time, mass to charge ratio, and intensities of all detected peaks were tabulated and exported for subsequent analysis. Principal component analysis (PCA), partial least squares discriminate analysis (PLS-DA), and orthogonal partial least squares discriminate analysis (OPLS-DA) were performed by using SIMCA 13.0 software (Umetrics, Umea, Sweden). Components that played important roles in differentiation were picked out according to the variable importance in the projection (VIP) value.

RESULTS AND DISCUSSION

Method Validation for Quantitation. All calibration curves showed good linearity (r^2 > 0.999). The LOD ranged from 0.0038 to 0.0146 μg/mL, and the LOQ ranged from 0.0171 to 0.0541 μg/mL. The calibration curves, correlation coefficients, LOD, and LOQ of each compound are given in Table 1. The UPLC-Q-TOF MS method showed good precision for the quantitation of the eight saponins with intra- and interday variations of 1.28−3.00 and 1.22−3.44% and repeatability variations of 1.08−3.84%. The overall recoveries ranged from 97.70 to 103.03%, with variations of 1.60−3.46% (Table 1). These results demonstrated that the established UPLC-Q-TOF MS method was sensitive, accurate (with recovery accuracy within the criteria of 95−105%), and precise (with precision of <5%) and thereby appropriate for the quantitative analyses of notoginsenoside R_1 and ginsenosides Rg_1, Re, Rb_1, Rb_2, Rb_3, Rc, and Rd in different parts of notoginseng.

Quantitative Comparison of Different Parts of Notoginseng. The contents of chemical constituents are always influenced by various factors such as the place of origin, the growing year, and the harvesting season. To evaluate the variation of ginsenosides inherently associated with the different parts of the root, we specially collected the whole root for the comparative study. The qualitative results revealed quite similar content distribution of eight dammarane-type saponins in different parts of the root (Figure 1). As can be seen in Table 1, in all parts of the root, ginsenosides Rb_1 and Rg_1 were shown to be the most abundant ginsenosides, both accounting for >20% of the total saponins (Table 2). Notoginsenoside R_1 was less abundant than Rg_1 and Rb_1, but with great variation among individual samples in both absolute content and relative composition of the total amount of saponins. Content of ginsenosides Re and Rd also varied greatly among individual samples, but their percentage in the total amount of saponins remained at about 1−10% in most portion samples of the root, whereas ginsenosides Rb_2, Rc, and Rb_3 were minor constituents in all parts of the root (Supporting Information, Table S1). In commercial samples, similar patterns were observed for all individual parts of notoginseng (Figure 3).

Among different parts of the root, the content of each saponin was always the highest in rhizome, followed by main root and branch root, and then fibrous root. This observation was consistent with previous studies.[24,39,43] Individually, except for notoginsenoside R_1, contents of all ginsenosides in rhizome were significantly higher than those in main root (Figure 2), among which Rb_2 was the most important ginsenoside as featured by up to 8-fold content in rhizome of that in main root (Figure 2). The higher content of each saponin in rhizome accumulatively led to the significantly higher content of subtotal PPD-type and PPT-type ginsenosides, as well as the content of total saponins than that in main root (Figure 2). On average,

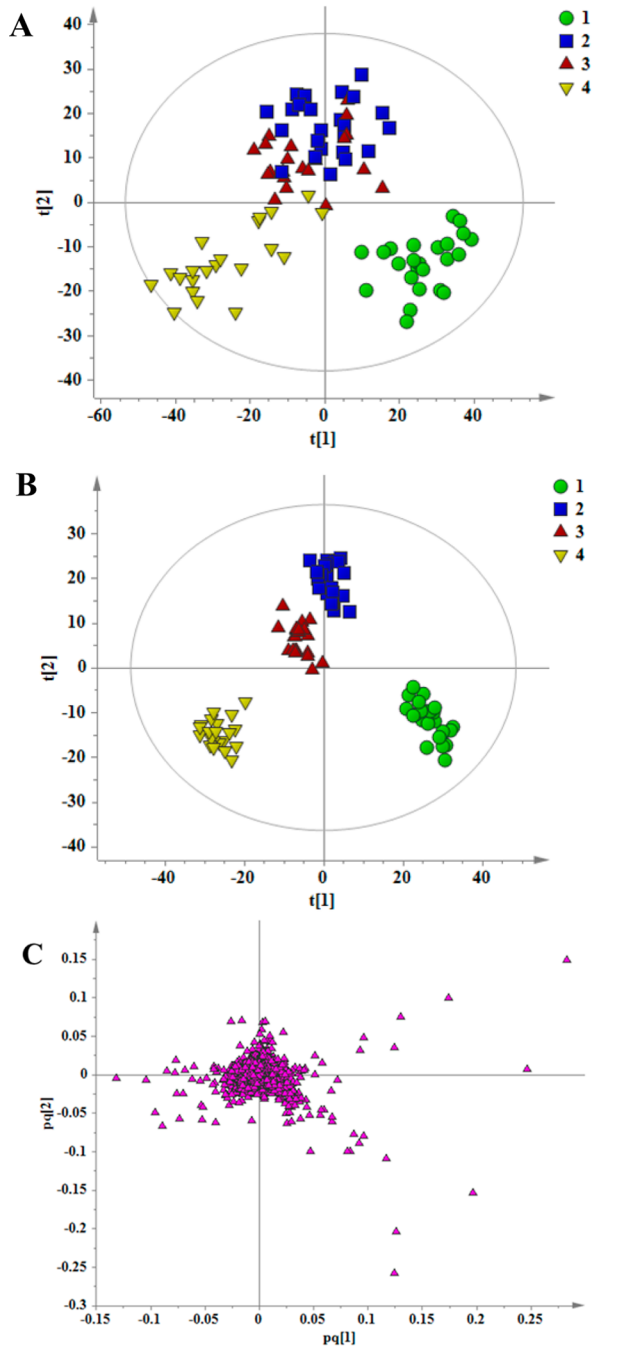

Figure 4. PLS-DA scores plot (A), OPLS-DA scores plot (B), and loading plot (C) of UPLC-Q-TOF MS data of rhizome (1), main root (2), branch root (3), and fibrous root (4) of *P. notoginseng*.

the content of total ginsenosides in rhizome is about 20−60% higher than that in main root and about 50−90% higher than that in branch root. The higher saponin content, together with the considerable biomass of the rhizome (about 15% of the whole root), supported the utilization of rhizome as the raw material for manufacturing ginsenoside-based products.

The contents of all eight ginsenosides in branch root appear to be lower than that in main root, but statistical significance for such difference was not observed (p value > 0.05). Moreover, in PLS-DA scores plot (Figure 4A), branch root is almost superimposed with main root but separated from rhizome and fibrous root. In OPLS-DA scores plot, branch root is separated

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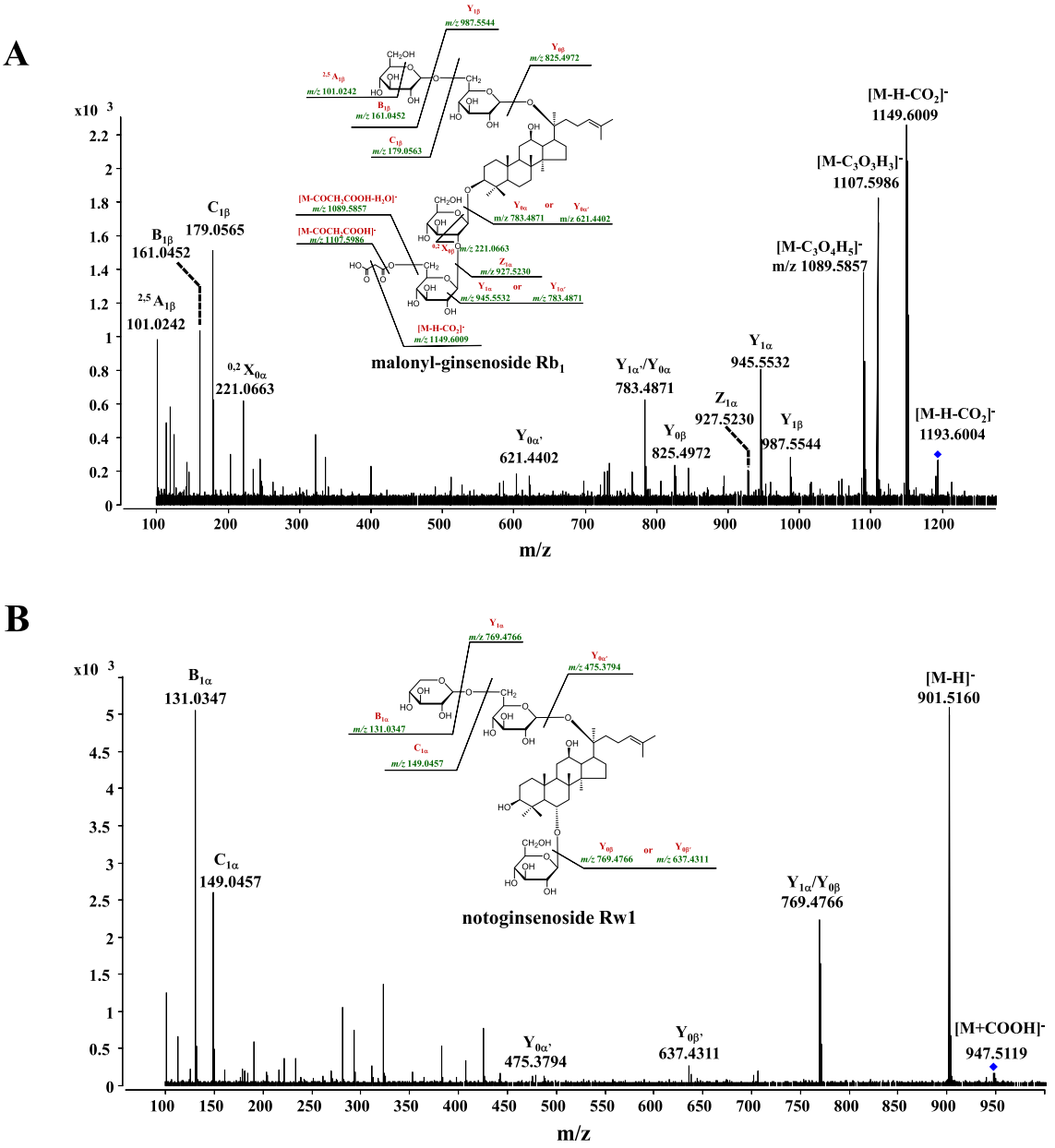

Figure 5. Targeted MS/MS of representative markers (A, malonyl ginsenoside Rb_1; B, notginsenoside Rw_1) contributing to the differentiation of different parts of notoginseng. Nomenclature of the fragments was assigned according to a systematic nomenclature for carbohydrate fragmentations.[46]

from main root but still very close to the main root in the space. These results indicated an overall similar chemical profile of branch root with main root, therefore supporting the equivalent usage of branch root and main root as indicated in "Chinese Pharmacopeia".

In commercial unassorted samples, the content of individual saponins in the rank order of rhizome > main root ≥ branch root > fibrous root was also observed, demonstrating this is an overall pattern of saponin distribution in different parts of notoginseng. Consistent with that revealed in whole root-derived samples, significant differences in the content of ginsenosides Rg_1, Rb_1, Rd, Rc, and Rb_2 between rhizome and main root were observed, despite the great intragroup variation (Figure 3). In addition, significant difference in the contents of R_1, Rg_1, Re, and Rb_1 between main root and fibrous root were observed. Most notable is the significantly different contents of Rb_2 in rhizome and other parts (Figure 3). It has been found that the content of Rb_2 in rhizome was >0.5 mg/g in all samples, whereas the contents in other parts were all <0.3 mg/g and mostly <0.2 mg/g. This evidence facilitated 0.5 mg/g of Rb_2 as a threshold value for the discrimination of rhizome and other parts of notoginseng.

Multivariate Analysis of Different Parts of Notoginseng. Untargeted metabolomics has the ability to profile diverse classes of metabolites and thereby has been widely used for comparing the overall metabolic composition between samples.[44] Among the techniques used for untargeted metabolomics, UPLC-Q-TOF MS has been especially widely utilized for profiling metabolites due to its superiority in high-resolution mass, precision, and sensitivity. In the current study, >15000 ions were achieved in the UPLC-Q-TOF MS data of notoginseng. Analysis results of these ions were exported for subsequent multivariate analysis by using SIMCA 13.0. As

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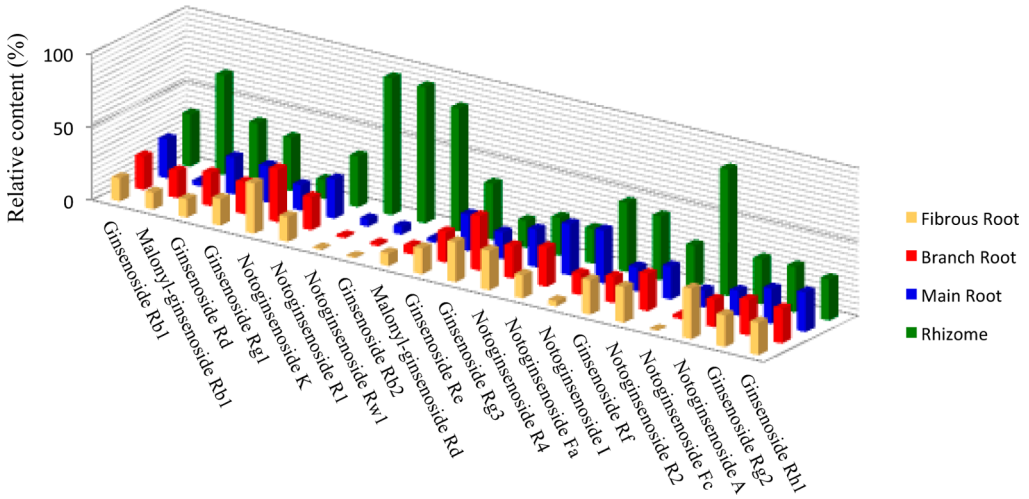

Figure 6. Relative contents of 20 marker compounds in different parts of notoginseng. To compare the level of individual markers between different parts, the content of each marker compound was normalized as a relative content, which was calculated by using the following equation: relative content = content in individual part/sum of the contents in four parts × 100%.

shown in Figure 4A, separation of rhizome, fibrous root, and main/branch root can be observed in the PLS-DA plots. Q^2, the parameter that estimates how well the model predicts Y, was calculated to be 63.8% (five significant components, R^2Y = 91.2%) for this PLS-DA model, suggesting the good predictive capacity of the model. To minimize any effects of nonrelevant metabolite variability, OPLS-DA analyses were also carried out to identify the differences between groups (Figure 4B). Consistent with the results obtained from quantitative analysis, branch root and main root were very close in the OPLS-DA plot, indicating high similarity of the two parts. In a loading plot, the ions farthest away from the main cluster of ions possess the higher VIP value and play more important roles in the differentiation. As shown in Figure 4C, a number of ions were distant from the main cluster and were identified as the marker ions. These marker ions were identified according to the accurate mass, retention time, and targeted MS/MS data. Figure 5 represents examples for the identification of potential markers by MS/MS data. A list of the identified marker ions is shown in Table 2, which included both major and minor constituents in notoginseng. This result suggested ginsenosides Rf, Rg_1, Re, Rg_2, Rh_1, Rb_1, Rb_2, Rd, and Rg_3 and notoginsenosides A, Fa, Fc, I, K, R_1, R_2, R_4, and Rw_1, as well as malonyl-ginsenosides Rb_1 and Rd were the major potential marker compounds responsible for differentiation of rhizome, main root, branch root, and fibrous root of *P. notoginseng*.

The relative contents of the first 20 potential marker compounds in each part of notoginseng were then compared on the basis of their peak areas. As shown in Figure 6, notoginsenosides Fc and Rw_1, malonyl-ginsenoside Rd, and ginsenoside Rb_2 were found to present in rhizome at significantly higher content than in other parts. It is worth pointing out that both quantitative and multivariate analyses showed that the content of ginsenoside Rb_2 was much higher in rhizome than in other parts of notoginseng. This implied the potential marker compounds identified from the multivariate analysis were instructive.

In the current study, quantitative comparison by using a validated UPLC-Q-TOF MS method and metabolite profiling approach was combinatively employed for evaluating the similarities and differences among rhizome, main root, branch root, and fibrous root of *P. notoginseng*. Both quantitative analysis and metabolite profiling study suggested that rhizome was significantly different from the other parts. It contained the highest contents of individual saponin and total saponins, clearly suggesting the suitability of this part as the raw material for the manufacture of ginsenoside-based products. The chemical compositions of the main root and branch root are quite similar and thereby can be utilized equivalently. The fibrous root also showed a clear separation from the other parts and contained the lowest content of saponins. The multivariate study suggested that notoginsenosides Fc and Rw_1, malonyl-ginsenoside Rd, and ginsenoside Rb_2 could be potential chemical markers for discrimination of different parts of notoginseng. Because processing (e.g., steaming) and transformation in the gastrointestinal tract would further introduce chemical diversity of saponins,[32,45] chemical differences among individual parts of notoginseng might be amplified or become more complicated after processing or oral consumption. Overall, our work represents the first comprehensive study dealing with four parts of notoginseng by using an approach integrating quantitative and qualitative comparison. The result is of importance for the appraisal of the chemical characteristics of different parts of notoginseng and provides chemical evidence for the rational application of each part of notoginseng.

■ ASSOCIATED CONTENT

Supporting Information

Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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