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FULL PAPER

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Simultaneous Determination and Analysis of Major Ginsenosides in Wild American Ginseng Grown in Tennessee

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Ginsenosides are the major constituent that is responsible for the health effects of American ginseng. The ginsenoside profile of wild American ginseng is ultimately the result of germplasm, climate, geography, vegetation species, water, and soil conditions. This is the first report to address the ginsenoside profile of wild American ginseng grown in Tennessee (TN), the third leading state for production of wild American ginseng. In the present study, ten major ginsenosides in wild American ginseng roots grown in TN, including Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2, and Rg3, were determined simultaneously. The chemotypic differences among TN wild ginseng, cultivated American ginseng, and Asian ginseng were assessed based on the widely used markers of ginsenoside profiling, including the top three ginsenosides, ratios of PPD/PPT, Rg1/Rb1, Rg1/Re, and Rb2/Rc. Our findings showed marked variation in ginsenoside profile for TN wild ginseng populations. Nevertheless, TN wild ginseng has significant higher ginsenoside content and more ginsenoside diversity than the cultivated ginseng. The total ginsenoside content in TN wild ginseng, as well as ginsenosides Rg1 and Re, increases with the age of the roots. Marked chemotypic differences between TN wild ginseng and cultivated American ginseng were observed based on the chemotypic markers. Surprisingly, we found that TN wild ginseng is close to Asian ginseng with regard to these characteristics in chemical composition. This study verified an accessible method to scientifically elucidate the difference in chemical constituents to distinguish wild from the cultivated American ginseng. This work is critical for the ecological and biological assessments of wild American ginseng so as to facilitate long-term sustainability of the wild population.

Keywords: American ginseng, wild population, simultaneous determination, ginsenoside.

Introduction

Plants of the genus *Panax* have historically been among the most intensively used medicinal herbs worldwide.[1] The most commonly used species are *Panax ginseng* C.A.Mey. (Asian ginseng) and *P. quinquefolius* L. (American ginseng), which are native to Asia and North America, respectively.[2] Their roots are used as dietary health supplements and additives to food and beverages[3] and for the treatment of many human ailments, such as fatigue, neurodegradation, cardiovascular diseases, stress, and cancer.[4–6]

The growth of *P. quinquefolius* L. is greatly influenced by many factors, including germplasm, geographical origin, population, age, soil conditions, climate, and water source.[7–10] These factors appear to produce distinct chemical composition in the roots of

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American ginseng and correspondingly, diversity in their medicinal efficacy.[11] The state of Tennessee was one of the first states where wild American ginseng was discovered and exported, and it remains the third leading state for production of wild American ginseng. Tennessee's location in the southeast portion of the Appalachian mountain range has a unique natural climate, vegetation, and soil structure, which drives a distinct composition in the ginseng found there. Therefore, the impact of the geographical origin on American ginseng in Tennessee and the chemical composition of authentic wild Tennessee ginseng are worthy of further research.

Pharmacological studies indicate that the major constituent, ginsenosides, are responsible for the health effects of ginseng.[12] Phytochemistry studies showed that ginsenosides are mainly triterpenoid saponin glycosides.[13] Ginsenosides isolated from the roots, leaves, stems, and flower buds of American ginseng can be categorized into two classes based on the number, type, and position of attached sugar moieties: protopanaxadiol (PPD), which includes Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, and protopanaxatriol (PPT), which includes Re, Rf, Rg1, Rg2, Rh1.[12,14–16] For American ginseng root, the ginsenosides fingerprint was reported as Rb1 > Re > Rg1 ≈ Rc > Rd according to a number of studies.[17–19]

Wild American ginseng has been listed in Appendix II of the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES) since 1974, and its harvest and trade is strictly regulated by the U.S. Fish and Wildlife Service (USFWS). Due to the scarcity of wild harvested ginseng, there was limited research on the wild populations of American ginseng. The phytochemistry studies have shown chemical analysis of wild American ginseng collected in Ontario,[19] Quebec,[19] Maine,[19] Vermont,[19] Wisconsin,[19] Maryland,[13] New York State,[8] and North Carolina.[20] Assinewe et al. were the first ones to conduct a phytochemistry study of wild American ginseng from ten populations in both USA and Canada using high-performance liquid chromatography (HPLC) in 2003.[19] The natural contents of ginsenosides in roots varied from 1 to 16% and there was no statistical difference in ginsenoside content between 4-year old wild and cultivated American ginseng roots. Schlag et al.[13] determined the contents of five ginsenosides including Rg1, Re, Rb1, Rc, and Rd in wild and cultivated American ginseng roots grown in Maryland and found that the most abundant ginsenoside in roots was either Rg1 or Re, followed by Rb1. The wild ginseng from the Eastern USA has high Rg1 and low Re, which is distinctive from that of cultivated ginseng as well as from wild ginseng from the Northern USA and Canada. Qin et al. developed a microchip electrophoresis method to authenticate wild and cultivated American ginseng[21] and found that wild and cultivated American ginseng could be distinguished on the basis of allele sizing. In another report by Wang et al.,[22] the authors did not reveal the state origin of the wild American ginseng, but they found that wild American ginseng has a significantly higher Rg1/Rd ratio than cultivated ginseng and the Rg1/Rd ratio could be a characteristic marker to differentiate these two groups. Similarly, the ratio of (Rg1 + Re)/Rd and the ratio of PPT-type to PPD-type ginsenosides also exhibited a significant difference between wild and cultivated ginseng. Despite of the long history and production of wild American ginseng in Tennessee, there was little report on the chemical composition of authentic wild Tennessee ginseng. As the chemical profile of wild ginseng is ultimately the result of climate, geography, vegetation species, water and soil conditions, the ginsenoside profile of wild ginseng in Tennessee may be unique and should be identified.

The aim of the present study was to investigate the individual and total ginsenoside contents in the roots of wild American ginseng grown in Tennessee. To our knowledge, this is the first report to address the chemical properties of wild American ginseng grown in Tennessee. This work is needed for the ecological and biological assessments of wild American ginseng so as to facilitate long-term sustainability of the wild population. Furthermore, this study also verified a simple and accessible method to scientifically elucidate the difference in chemical constituents to distinguish wild from the cultivated American ginseng.

Results

Processing and Extraction of Fresh Wild Roots

The detailed information such as location, collection date, and age is summarized in *Table 1*. We obtained an average drying rate of 30.0%, ranging from 18.5% to 44.6%. The average drying rate is consistent with the previous report that the fresh ginseng roots have high moisture content of approximately 70%.[23] It was noted that the moist content in wild fresh roots varies significantly, from 55.4% to 81.5%. The average yield of extract from the dried roots was 34.9% (*Table 2*). In addition to the significant morphological difference in wild ginseng roots compared with the cultivated roots,

Table 1. Production type, location grown, collection date and age of American ginseng root collected in Tennessee.

Sample	Production	Origin	Collection date	Age* [years]
W1	Wild, fresh	Unicoi county, East TN	Oct-17	7
W2	Wild, fresh	Cocke County, East TN	Oct-17	9
W3	Wild, fresh	Cocke County, East TN	Oct-17	8
W4	Wild, fresh	Cocke County, East TN	Oct-17	10
W5	Wild, fresh	Cocke County, East TN	Oct-17	8
W6	Wild, fresh	Cocke County, East TN	Oct-17	9
W7	Wild, fresh	Unknown county, Middle TN	Oct-17	15
W8	Wild, fresh	Unknown county, Middle TN	Oct-17	10
W9	Wild, fresh	Unknown county, Middle TN	Oct-17	12
W10	Wild, fresh	Unknown county, Middle TN	Oct-17	10
W11	Wild, fresh	Unknown county, Middle TN	Oct-17	19
W12	Wild, fresh	Unknown county, Middle TN	Oct-17	13
W13	Wild, fresh	Unknown county, Middle TN	Oct-17	14
W14	Wild, fresh	Unknown county, Middle TN	Oct-17	15
W15	Wild, fresh	Unknown county, Middle TN	Oct-17	18
W16	Wild, fresh	Unknown county, Middle TN	Oct-17	20
W17	Wild, fresh	Unknown county, Middle TN	Oct-17	19
W18	Wild, fresh	Unknown county, Middle TN	Oct-17	14
W19	Wild, fresh	Unknown county, Middle TN	Oct-17	17
W20	Wild, fresh	Unknown county, Middle TN	Oct-17	12
W21	Wild, fresh	Unknown county, Middle TN	Oct-17	11
W22	Wild, fresh	Unknown county, Middle TN	Oct-17	16
W23	Wild, fresh	Unknown county, Middle TN	Oct-17	13
W24	Wild, fresh	Unknown county, Middle TN	Oct-17	22
W25	Wild, fresh	Unknown county, Middle TN	Oct-17	10
W26	Wild, fresh	Unknown county, Middle TN	Oct-17	7
W27	Wild, fresh	Unknown county, Middle TN	Oct-17	8
W28	Wild, fresh	Unknown county, Middle TN	Oct-17	19
C1	Cultivated, dried	Marathon County, WI	Oct-17	4
C2	Cultivated, dried	Marathon County, WI	Oct-17	4
C3	Cultivated, dried	Marathon County, WI	Oct-17	4

the texture of wild roots is more compact and stiff than cultivated roots.

Simultaneous Determination of Ten Ginsenosides

The chromatogram of mixed reference standards and a typical chromatogram of the sample are presented in *Figure 1*. The equations and r^2 of all analytes are listed in the supplementary *Table 1*. All coefficients of determination (r^2) for each of the tested ginsenoside are higher than 0.998, suggesting a good linearity over certain concentration ranges. Contents of major ginsenosides in roots, including Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2, and Rg3, were quantitatively determined by HPLC (*Table 3*). According to previous studies, these ten ginsenosides account for more than 90% of the total ginsenoside content existing in ginseng roots.[24]

The wild ginseng roots exhibited a wide range of total ginsenoside content, from 1.30 to 22.89 mg/g. In contrast, the range of total ginsenoside content for cultivated ginseng was from 2.03 to 3.43 mg/g. In average, the most abundant ginsenoside in tested ginseng roots was Rb1 for both wild and cultivated ginseng, following by Rg1 for the wild ginseng and Re for cultivated ginseng.

The ginsenoside Rf is considered as a marker to distinguish the two ginseng species, Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius*).[8,13,19,22,25] Strikingly, we detected Rf in two wild American ginseng roots (W12 and W16), although in very low concentration. Rf was not detected in cultivated American ginseng roots.

Additionally, Re was not detected in one cultivated ginseng root and eight wild ginseng roots. Among the total 31 samples, Rg2 was only detected in W15 and W21; and Rg3 was only detected in W10 and W21; the contents of these ginsenosides were less than 0.11 mg/g.

Table 2. Yield of drying and extraction processes.

Sample	Fresh weight [mg]	Dry weight [mg]	Dry ratio [%]	Extract [mg]	Extraction yield [%]
W1	6320	1167	18.5	246	21.1
W2	2830	761	26.9	190	25.0
W3	2420	601	24.8	220	36.7
W4	1550	473	30.5	138	29.1
W5	2220	554	25.0	148	26.7
W6	3120	869	27.9	242	27.9
W7	1722	593	34.4	189	31.9
W8	2268	781	34.5	282	36.1
W9	2608	950	36.4	377	39.7
W10	2662	1186	44.6	406	34.3
W11	2668	830	31.1	311	37.5
W12	2706	778	28.8	369	47.5
W13	2355	856	36.3	331	38.7
W14	2319	736	31.7	247	33.5
W15	2187	707	32.3	253	35.7
W16	2719	545	20.0	194	35.6
W17	2232	709	31.8	240	33.8
W18	2291	606	26.5	299	49.3
W19	2600	814	31.3	302	37.1
W20	2284	651	28.5	258	39.7
W21	2001	606	30.3	157	25.9
W22	3200	896	28.0	329	36.7
W23	2510	871	34.7	374	42.9
W24	2258	734	32.5	276	37.6
W25	1890	622	32.9	190	30.6
W26	2644	508	19.2	212	41.8
W27	1935	586	30.3	237	40.5
W28	1841	536	29.1	193	36.1
C1	N/A	1112	N/A	279	25.1
C2	N/A	1016	N/A	334	32.9
C3	N/A	1707	N/A	601	35.2

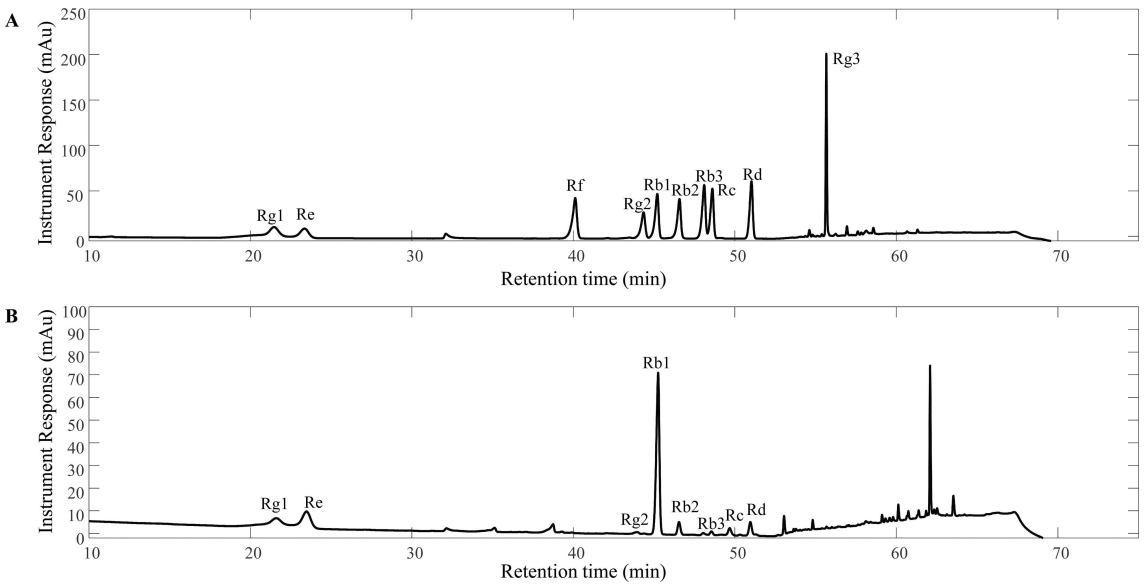

Figure 1. Typical chromatograms of mixed reference standards (upper) and the sample (bottom).

Table 3. Ginsenoside content of wild and cultivated populations of American ginseng (mg/g in dry root).

Sample	PPD Type						PPT Type				Total
	Rb1	Rb2	Rb3	Rc	Rd	Rg3	Re	Rf	Rg1	Rg2	
W1	0.53	0.21	0	0.06	0.09	0	0	0	0.41	0	1.30
W2	2.16	0.51	0.06	0.21	0.21	0	0	0	1.18	0	4.33
W3	0.95	0.32	0.03	0.09	0.07	0	0	0	0.84	0	2.29
W4	2.22	0.31	0.03	0.09	0.08	0	0	0	3.04	0	5.77
W5	1.94	0.49	0.05	0.11	0.16	0	0.12	0	2.59	0	5.45
W6	3.91	0.74	0.06	0.17	0.25	0	0.13	0	2.14	0	7.39
W7	2.50	0.51	0.06	0.15	0.19	0	1.23	0	0.66	0	5.30
W8	3.74	0.55	0.07	0.20	0.15	0	0.70	0	2.56	0	7.96
W9	4.46	0.50	0.06	0.14	0.68	0	2.51	0	2.45	0	10.8
W10	4.41	0.27	0	0.05	0.21	0.02	1.18	0	1.32	0	7.47
W11	1.77	0.29	0	0.05	0.03	0	0	0	1.18	0	3.32
W12	10.05	1.45	0.14	0.27	0.41	0	5.00	0.06	5.35	0.11	22.85
W13	4.73	0.45	0.06	0.10	0.19	0	1.92	0	0.89	0	8.35
W14	4.15	0.35	0.05	0.10	0.26	0	2.45	0	2.64	0	10.00
W15	5.36	0.32	0.06	0.13	0.12	0	3.45	0	5.26	0.09	14.82
W16	6.38	1.07	0.14	0.28	0	0	0	0.04	9.09	0	17.00
W17	5.04	0.66	0.08	0.19	0.11	0	0.34	0	5.27	0	11.69
W18	11.65	1.20	0.15	0.26	0.77	0	4.88	0	3.98	0	22.89
W19	4.11	0.46	0.07	0.15	0.13	0	2.95	0	4.26	0	12.13
W20	5.42	0.69	0.08	0.15	0.50	0	0.31	0	5.00	0	12.16
W21	3.22	0.32	0.05	0.10	0.41	0.03	2.2	0	4.23	0.09	10.65
W22	3.58	0.78	0.08	0.17	0.10	0	0	0	5.94	0	10.66
W23	3.99	0.54	0.06	0.12	0.17	0	2.06	0	0	0	6.93
W24	6.78	0.55	0.08	0.15	0.36	0	3.95	0	6.44	0	18.30
W25	3.90	0.44	0.07	0.14	0.21	0	1.68	0	2.09	0	8.53
W26	8.04	1.50	0.16	0.32	1.08	0	0.23	0	4.30	0	15.64
W27	4.34	0.48	0.06	0.13	0.28	0	0.30	0	3.20	0	8.78
W28	1.19	0.53	0.05	0.14	0.06	0	0	0	1.30	0	3.27
C1	2.10	0.20	0	0.03	0.19	0	0.58	0	0	0	3.09
C2	1.14	0.13	0	0	0.25	0	0.51	0	0	0	2.03
C3	2.72	0.36	0	0.10	0.25	0	0	0	0	0	3.43

Comparison of Ginsenosides Contents in Dried Roots of TN Wild Ginseng with Cultivated Ginseng

In this study, the average of total ginsenosides in dry roots of TN wild ginseng was more than 3.5 times higher than that of the cultivated American ginseng (*Figure 2*). Ginsenoside content of most wild ginseng roots was much higher than those of cultivated ginseng, except W1. Among all the wild roots, W18 and W12 had the highest content of total ginsenosides, which were more than ten times of that of W1. This work also showed that ten individual ginsenosides have greater content in the wild roots than the cultivated roots (*Figure 2*). The most significant difference came from Rg1, which was an average of 3.13 mg/g in wild roots but none was detected in cultivated roots. In comparison with previous reports on Rg1 content in wild population found in MD, NY, and NC, the Rg1 content detected in the wild ginseng grown in Tennessee is similar to wild population in Maryland (2.3–7.8 mg/g),[13] higher than wild population in New York State (0.1–2 mg/g),[8] but lower than wild population in North Carolina (5.76 mg/g).[8] Additionally, Rb3 and Rc also showed significantly higher concentration in the wild roots compared with cultivated roots. Further, five major ginsenosides Rb3, Rg3, Rg1, Rg2, Rf were only detected in the wild roots but not the cultivated roots.

Correlation between Age and Ginsenoside Content

The cultivated ginsengs are all four years old according to the product information. The ages of the wild roots were carefully determined by counting the number of neck scars and the age ranges from seven to 22 years old. Despite the large variation, a noticeable trend was observed in *Figure 3A*, that the content of total ginsenosides increases with the age of the

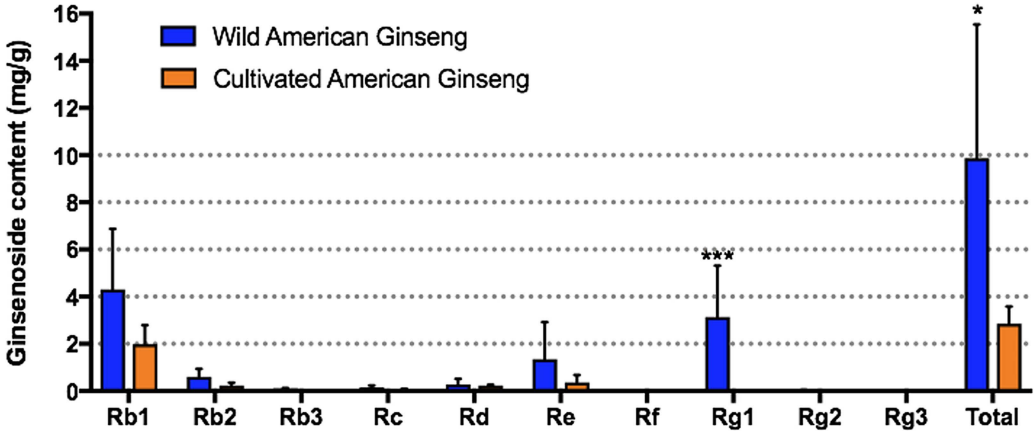

Figure 2. Comparison of ginsenoside content (mg/g) in dry root between wild ginseng in Tennessee and cultivated ginseng. Multiple *t*-tests were performed and individual *p* value was calculated. Data are represented as mean ± standard deviation ($n = 28$ for wild American ginseng and $n = 3$ for cultivated American ginseng).

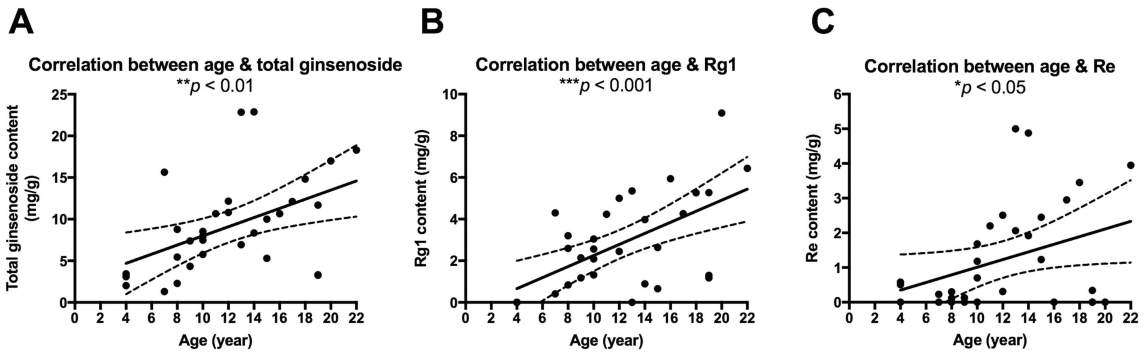

Figure 3. A) Correlation between age and content of total ginsenoside of TN wild ginseng. B) Correlation between age and Rg1 content of TN wild ginseng. C) Correlation between age and Re content of TN wild ginseng. Pearson correlation coefficient test was performed and a two-tailed *p* value with 95% confidence interval was calculated for each correlation. Each dot represents a sample.

ginseng roots. The correlation between the age of the roots and the total ginsenoside content has a *p* value < 0.01. The same trend was shared with ginsenosides Rg1 and Re (*Figure 3B* and *3 C*). However, ginsenoside Rb1, Rb2, Rc do not exhibit such correlation.

Nevertheless, there were a few outliners worth mentioning. For example, W11 and W28, which are both 19-year old roots, have only 3.3 mg/g of total ginsenosides compared with an average of 9.7 mg/g of all samples. While W12 and W18, which are 13 and 14 years old, respectively, have the highest total ginsenoside content among all wild roots.

Rg1/Rb1 Ratio

There are several differences between American and Asian ginseng with regard to chemical constituents. The ratio of Rg1/Rb1 has been widely used to distinguish these two ginseng species. An Rg1/Rb1 ratio of less than 0.4 is considered to be an indicator of American ginseng, whereas the ratio of greater than 0.4 are considered as a characteristic of Asian ginseng.[26,27] However, our study showed that while all cultivated American ginseng (C1–C3) have an average Rg1/Rb1 ratio of less than 0.4, the average Rg1/Rb1 ratio in TN wild ginseng is 0.78. Only five out of 28 TN wild ginseng (W7, W10, W13, W18, and W23) demonstrates Rg1/Rb1 ratios of less than 0.4; most TN wild ginseng have an Rg1/Rb1 ratio of greater than 0.4 (*Figure 4*).

Rg1/Re Ratio

Similar to the Rg1/Rb1 ratio, a review on ginsenosides in American ginseng addressed that Rg1/Re is considered to be an indicator for differentiation of American ginseng with Asian ginseng.[12] Although rare cases of

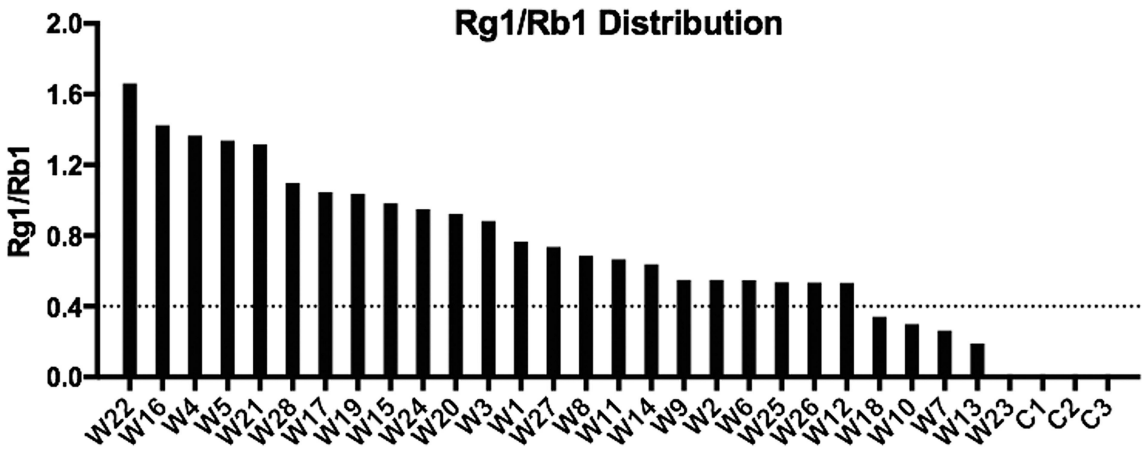

Figure 4. Rg1/Rb1 ratios of TN wild ginseng and cultivated ginseng.

Table 4. Comparison summary of ginsenoside composition of American ginseng (cultivated and wild), Asian ginseng, and TN wild ginseng.

Chemical composition	American ginseng (cultivated)	Asian ginseng	American ginseng (wild)[a]	TN wild American ginseng: this study
Major ginsenosides	Rb1, Re, Rd[12,22]; Rb1, Re, Rg1[27]	Rb1, Rg1, Rb2[12]; Rg1, Rb2, Rc[28]	Rb1, Rg1, Re[13,19]; Rb1, Rg1, Rc[20,22]	Rb1, Rg1, Re
Rf	None[12]	1–2 mg/g[12]	N/A	0–0.06 mg/g
PPD-type/PPT-type	>2.0[12] (2.27[22]; 2.44[29])	<2.0[12] (0.8[29])	1.22–1.42[22]	1.45
Rg1/Rb1	<0.2[12] or <0.4[26,27] (0.06[22,30])	>0.2[12] or >0.4[26,27]	1.45[13]; 0.33[19]; 0.67[20]; 0.27[22]; 0.99[22]	0.78
Rg1/Re	<1.0[12,29] (0.11[22]; 0.12[29])	>1.0[12,31] (0.94[29])	>1.0[8]; 0.2[13]; 4.1[13]; 0.66[19]; 4.97[20]; 0.5[22]; 64.66[22]	>5.82
Rb2/Rc	<0.4[12] (0.16[22]; 0.25[29]; 0.26[31])	>0.4[12] (1.3[29]; 0.8[31])	0.21[19]; 0.42[20]; 0.19–0.21[22]	3.86

[a] Wild American ginseng populations: New York state;[8] Maryland;[13] Ontario, Quebec, Maine, Vermont, and Wisconsin;[19] North Carolina;[20] or purchased under compliance but wild source not stated.[22]

high Rg1/low Re chemotype were observed in wild American ginseng,[13,22] low Rg1/high Re (<1.0) was reported in most populations of American ginseng;[12] whereas a high Rg1/low Re ratio (>1.0) was constantly observed in Asian ginseng (*Table 4*). In this study, both chemotypes, high Rg1/low Re and low Rg1/high Re, were observed. Among 28 TN wild ginseng samples, only five of them (W7, W9, W13, W18, and W23) demonstrate chemotype of low Rg1/high Re ($Rg1/Re<1.0$); most TN wild ginseng samples have a chemotype of high Rg1/low Re ($Rg1/Re>1.0$). The average ratio of Rg1/Re is greater than 5.82 as Re was not detected in eight of the total 28 wild ginseng samples. It is worth mentioning that among the five samples in which Rg1/Rb1 ratios <0.4, four out of them (W7, W13, W18, W23) also have an Rg1/Re ratio <1.0.

Rb2/Rc Ratio

The previous report also suggested that Rb2/Rc ratio <0.4 has been shown to be indicative of American ginseng and conversely the opposite is true for Asian ginseng.[12] Our study showed that the average Rb2/Rc ratio was 3.86 with the lowest of 2.3 in all TN wild American ginseng roots (*Table 4*).

Discussion

Although over 100 ginsenosides have been identified in the plants belong to the genus *Panax*, the six major ginsenosides (Rb1, Rb3, Rc, Rd, Re, and Rg1) account for more than 70% of total ginsenosides in American

ginseng,[32] while ten major ginsenosides (Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2, and Rg3) account for more than 90% of the total ginsenosides.[24] The current work simultaneously determined for the first time the ten major ginsenosides in American ginseng roots collected from various wild populations grown in the state of Tennessee. Besides the significant variation in the moist content (from 55.4% to 81.5%), the wild ginseng roots also exhibited a wide range of total ginsenoside content, from 1.30 to 22.89 mg/g. This variation observed in ginsenoside content is consistent with other studies of wild American ginseng[19] and could be easily explained by both environmental and genetic factors: Influences from the environment, including soil nutrients, composition, humidity, light conditions, temperature, growth length, etc., can affect the chemical profile of wild populations. In addition, we showed that ginsenosides Rb1, Rg1, and Re are the most abundant ginsenosides in the wild roots and also are most susceptible to varying environmental conditions.

Previous studies showed that the age of the plant also affected ginsenoside contents. Court et al.[28] showed that ginsenoside contents increase with the age of the root, especially Rb1 and Re. Zhang et al.[33] reported that the total content of ginsenosides increased with plant age in root, but not the leaf and stem. Lim et al. reported that Rb1, Rc, Rd and total ginsenoside contents in wild NY populations were affected by root age.[8] Conversely, Schlag et al. observed no obvious relationship between age and total ginsenoside content in age 5–10 years old although lower total ginsenoside content in wild Maryland population of age 3–4 years old.[30] In the present study, we observed that the content of total ginsenosides as well as Rg1 and Re increase with the age of the ginseng roots from 7–22 years old. However, ginsenosides Rb1, Rb2, Rc do not exhibit such correlation with age.

Asian consumers believe that wild ginseng is more potent and effective than the cultivated ginseng,[34] and this preference results in a large difference in market value between wild and cultivated ginseng. Wild American ginseng roots are mainly exported overseas, especially to Asia, at a price of HK$66,138/kg, whereas cultivated ginseng were only HK$952/kg in 2013.[35] Wild American ginseng can be easily distinguished from cultivated ginseng by the visible traits such as root form, size, and color. However, limited sampling size of wild population present significant obstacles to obtaining sufficient scientific data to assess the chemical variability between wild and cultivated ginseng, which may be responsible for difference in their pharmacological activities. There were very few attempts to compare the chemical profile between the wild and cultivated.[13,19] Two studies failed to discover significant differences in the total ginsenosides between wild and cultivated plants;[13,19] while another two studies found that total ginsenoside contents in wild or wild-simulated roots were significantly higher than cultivated roots.[8] In the present study, for the first time we analyzed the ginsenoside composition in the wild TN ginseng population. The average of total ginsenosides in all except one tested TN wild ginseng roots showed higher ginsenoside content than the cultivated ones, with the average total ginsenoside content of more than 3.5 times higher than that of the cultivated ginseng ($*p < 0.05$). Besides, five major ginsenosides Rb3, Rg3, Rg1, Rg2, Rf were only detected in the wild roots but not the cultivated roots, and ginsenoside Rc showed significantly higher concentration in wild population ($**p < 0.01$).

It is particular interesting that, consistent with the previous reports, the wild American ginseng showed distinctively different chemotypes from those reported for cultivated American ginseng (*Table 4*). We have compared the most widely used chemotypic markers of ginsenoside profiling, including the top three ginsenosides; ratio of PPD-type to PPT-type ginsenoside; ratio of Rg1 to Rb1; ratio of Rg1 to Re; and ratio of Rb2 to Rc. We found dramatic differences in these characteristics between wild and cultivated ginseng according to all available literature on chemical studies of wild American ginseng as well as the present study. Our findings are: 1) The three leading ginsenosides are Rb1, Re, Rd in cultivated American ginseng and are Rb1, Rg1, Re or Rc in wild populations;[12,13,19,20,22,27,28] 2) PPD-type/PPT-type is greater than 2.0 in the cultivated ginseng but less than 2.0 in wild ginseng;[12,22] 3) Rg1/Rb1 is less than 0.2 in the cultivated ginseng while greater than 0.2 in the wild ginseng;[12,13,19,20,22,26,27,30] 4) Only chemotype low Rg1/ high Re was reported in cultivated ginseng, but two chemotypes (both low Rg1/ high Re and high Rg1/low Re) were reported in wild ginseng, with high Rg1/ low Re being most common chemotype in wild ginseng;[12,13,19,20,22,29,31] 5) Only chemotype $Rb2/Rc < 0.4$ was reported in cultivated ginseng, but two chemotypes (both $Rb2/Rc < 0.4$ and $Rb2/Rc > 0.4$) were found in wild ginseng.[12,19,20,22,29,31] The chemotype $Rb2/Rc > 0.4$ was only identified in wild American ginseng populations in TN (this study) and NC.[20] Based on our present study and the previous studies from various research groups, the common reported chemical markers to distinguish American ginseng and Asian ginseng, are not applicable to the wild American ginseng.

Although deriving from the same genus *Panax* and alike in appearance, the American ginseng (*P. quinquefolius*) and Asian ginseng (*P. ginseng*) differ in traditional medicinal application and pharmacological effects. The former is considered to be calming and nourishing yin (cooling effects), while the latter is believed that it is capable to enhance and invigorate yang (heating effects).[36] Recent studies have also confirmed that Asian and American ginseng had different bioactivities at cellular and molecular levels.[27] The differences in pharmacological and biological activities between these main types of ginseng were considered to be a result of the bioactive compound, ginsenosides, attributed to each type.[27] American ginseng and Asia ginseng have markedly different ginsenoside profiles and the above-mentioned chemical characteristics are widely used to distinguish these two plant species (major ginsenosides; ratios of PPD/PPT, Rg1/Rb1, Rg1/Re, and Rb2/Rc) (*Table 4*). Surprisingly, our study found that TN wild ginseng is close to Asian ginseng with regard to these characteristics in chemical composition. Most strikingly, ginsenoside Rf, which was claimed as a marker to distinguish Asian ginseng and American ginseng,[8,13,19,22,25] was identified at low level in two wild TN ginseng samples. It may need further identification because ginsenoside F11, which is a marker for American ginseng, has the same molecular weight and therefore similar retention times as Rf and may not be distinguished under most LC conditions.[31] Overall, these results suggest the unique chemotype of TN wild ginseng that is distinctive from cultivated ginseng as well as from wild ginseng from the Northern USA and Canada. This also implies that in addition to chemotypic markers, alternative method such as genetic markers need to be used to differentiate between Asian and American ginseng. Given TN wild ginseng comprises approximately one third of all wild American trade worldwide and the unique chemotype may be associated with pharmacological effects, it is therefore important to investigate the pharmacological activities of TN wild ginseng in the future.

Conclusions

For the first time, we investigated the chemical profile of wild populations of American ginseng in Tennessee. Our findings showed marked variation in ginsenoside profile for TN wild ginseng populations. Nevertheless, overall, TN wild ginseng has significant higher ginsenoside contents and more ginsenoside diversity than the cultivated ginseng. Marked chemotypic differences between the wild and cultivated ginseng were also reported in the present study, such as ratios of PPD/PPT, Rg1/Rb1, Rg1/Re, and Rb2/Rc. TN wild ginseng exhibited distinctive ginsenoside profile from cultivated ginseng as well as from wild ginseng from the Northern USA and Canada. The results from this study can be used to further identify the correlation between the genetic variability and chemotypic variability. Further studies sampling wider ranges of wild populations in Tennessee should be carried out to have a more holistic analysis of ginsenoside diversity in the region. Due to the uniqueness of the chemotypes of TN wild ginseng, it will be interesting to compare the bioactivities of TN wild ginseng with cultivated ginseng and Asian ginseng.

Experimental Section

Sample Collection and Processing

The 28 wild ginseng roots were collected from various locations in the State of Tennessee and identified by Michael Boring in October 2017 during the legal harvesting season. The voucher specimens (Registration numbers of 2017GQ-W1 to 2017GQ-W28) were deposited with the Ginseng Herbarium at the International Ginseng Institute at Middle Tennessee State University. The age of the root was determined by counting the number of neck scars.[13] After collection, the fresh roots were rinsed in running tap water and gently brushed to remove any soil. Each root was divided into two portions, one portion was stored in −80 °C for future references, and the other portion was cut into 1–2 mm thin slices and dried naturally till achieving a constant weight (2–4 weeks). The weight of each sample was recorded prior to and after drying, and the drying rate was calculated for each sample. Three types of cultivated ginseng roots (catalog numbers 101–4, 103–4, and 113–4) were purchased from Hsu's Ginseng Enterprises Inc. (WI, USA).

Chemicals and Reagents

Reference standards of ten ginsenosides (HPLC grade) were purchased from Sigma–Aldrich (MO, USA). Acetonitrile (LC/MS grade), methanol (HPLC grade), DMSO, and ethanol were purchased from Fisher Scientific (MA, USA). Deionized water was prepared by A10-Synthesis water polishing system (Merck KGaA, Darmstadt, Germany).

Ginsenoside Extraction

Ginseng root slices were ground into granulated powder. Subsequently, an accurately weighed powder (1 g) was extracted twice with 30 mL of 80% ethanol (v/v) by reflux in a water bath at 85 °C for 5 h each. The extract was filtered through a paper filter and ethanol was dried up using a water bath. The residue was accurately weighed and transferred to a 2 mL centrifuge tube. Our trial test suggested that the extract was not dissolved completely in small amounts of methanol, thus a mixed solution of DMSO and methanol (1 : 1, v/v) was added to the extract, followed by sonication for 20 min. The extract solution was then vortexed for 30 s and centrifuged at 5000 rpm for 10 min. Finally, the solution was re-filtered through a 0.22 μm filter and 20 μL were injected in the UHPLC system immediately.

Determination of Ginsenoside Profile by HPLC

Analytical HPLC was performed using a Thermo Ultimate 3000 Ultra High-performance liquid chromatography (UHPLC) system (Thermo Fisher Scientific, Waltham, MA, USA) with gradient elution and a reversed-phase C_{18} column (ZORBAX Eclipse XDB, 3.0× 250 mm, 5 μm). The mobile phases consisted of water (A) and acetonitrile (B). The binary gradient employed a program with a flow rate of 0.8 mL/min as followed: 0–20 min, 19% B; 20–50 min, 19%–35% B; 50–60 min, 35%–95% B; 60–65 min, 95% B; 65–67 min, 95%–19% B; 67–75 min, 19% B. The Diode array detector was set at 203 nm. Column oven was set at 25 °C. The ginsenoside stock solutions of 1.0 mg/mL were prepared in methanol and they were then diluted to concentrations of 20.0, 10.0, 5.0, 2.5, 1.25, and 0.625 μg/mL as calibration solutions. An injection volume of 20 μL was used for all calibration standards and sample analyses. The concentration of individual ginsenoside in each sample was calculated using standard curves based on the reference standards. Sample order was randomized.

Data Analysis

The data collected in the present study were analyzed with Graphpad Prism version 8 (GraphPad Software, San Diego, California USA). For comparison of ginsenoside content between wild and cultivated roots, multiple *t*-tests were performed and individual *p* value was calculated. For analysis of the correlation between age and ginsenoside content, Pearson correlation coefficient test was performed and a two-tailed *p* value with 95% confidence interval was calculated for each correlation. *P* value <0.05 was considered to be statistically significant.

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Author Contribution Statement

L. C., Y. G. and J. L. performed the experiments. M. Z. analyzed the data. Y. G. conceived and designed the experiments and wrote the article.

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