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Research article

Comparative phenolic compound profiles and antioxidative activity of the fruit, leaves, and roots of Korean ginseng (*Panax ginseng* Meyer) according to cultivation years

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ABSTRACT

Background: The study of phenolic compounds profiles and antioxidative activity in ginseng fruit, leaves, and roots with respect to cultivation years, and has been little reported to date. Hence, this study examined the phenolic compounds profiles and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free-radical-scavenging activities in the fruit, leaves, and roots of Korean ginseng (*Panax ginseng* Meyer) as a function of cultivation year.

Methods: Profiling of 23 phenolic compounds in ginseng fruit, leaves, and roots was investigated using ultra-high performance liquid chromatography with the external calibration method. Antioxidative activity of ginseng fruit, leaves, and roots were evaluated using the method of DPPH free-radical-scavenging activity.

Results: The total phenol content in ginseng fruit and leaves was higher than in ginseng roots ($p < 0.05$), and the phenol content in the ginseng samples was significantly correlated to the DPPH free-radical-scavenging activity ($r = 0.928^{****}$). In particular, *p*-coumaric acid ($r = 0.847^{****}$) and ferulic acid ($r = 0.742^{****}$) greatly affected the DPPH activity. Among the 23 phenolic compounds studied, phenolic acids were more abundant in ginseng fruit, leaves, and roots than the flavonoids and other compounds ($p < 0.05$). In particular, chlorogenic acid, gentisic acid, *p*- and *m*-coumaric acid, and rutin were the major phenolic compounds in 3–6-yr-old ginseng fruit, leaves, and roots.

Conclusion: This study provides basic information about the antioxidative activity and phenolic compounds profiles in fruit, leaves, and roots of Korean ginseng with cultivation years. This information is potentially useful to ginseng growers and industries involved in the production of high-quality and nutritional ginseng products.

1. Introduction

Ginseng (*Panax ginseng* Meyer) is a perennial plant belonging to the *Araliaceae* family and has been used as a medicinal plant or as a natural tonic in many Asian countries for more than 2,000 years [1]. Although ginseng is now distributed in 35 countries, only four countries, China, Korea, Canada, and the USA, are responsible for >99% of the global ginseng production. The global ginseng market is estimated to be worth $2,084 million; in particular, the Korean market is estimated to be worth $1,140 million, which is the biggest market worldwide [2]. Ginseng production in Korea in 2012 was estimated to be 26,057 ton, and fresh ginseng accounted for 50% of this production. A further 44% of the ginseng produced was used for making red ginseng and processed products such as dietary supplements, medicines, drinks, soups, and jellies [2,3].

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Ginseng is known to possess various biological properties and pharmacological properties, such as immunostimulant, anticancer, antiemetic, antioxidant, and antiproliferative properties, as well as other health benefits [4–8]. These biological and pharmacological properties are strongly related to the phytochemicals present in ginseng, including saponins, alkaloids, polyacetylenes, polysaccharides, free amino acids, polyphenolics, and volatile compounds such as limonene [9–11]. In particular, Korean ginseng is known to possess better biological and pharmacological properties than other ginseng species [12].

Recent studies have reported the biological and pharmacological activities of ginseng, especially those of the ginseng root. Furthermore, a variation in the chemical constituents (especially ginsenosides) of the ginseng roots with respect to the processing conditions used in ginseng production has also been reported [13–17]. However, only a limited number of studies have reported the chemical constituents or biological activity of ginseng flowers, fruit (berry), and/or leaves [18–21].

The phenolic compounds present in ginseng possess various biological properties such as antioxidant and anticancer properties; however, these compounds are relatively less well known to consumers compared with the ginsenosides that are mostly found in ginseng roots. More than 10 phenolic compounds, including caffeic acid, ferulic acid, vanillic acid, *p*-hydroxybenzoic acid, gentisic acid, and syringic acid, have previously been reported in fresh and/or processed ginseng [10,13,22]. To the best of our knowledge, there is a lack of information on how the composition and content of the phenolic compounds found in ginseng fruit, leaves, and roots depend on the cultivation years. Hence, this study reports the total phenol content and profile of 23 phenolic compounds present in the fruit, leaves, and roots of 3–6-yr-old Korean ginseng. Furthermore, we determined the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical-scavenging activity of the fruit, leaves, and roots of 3–6-yr-old Korean ginseng, and conducted a correlation analysis between the phenolic profile and the DPPH antioxidant activity observed in ginseng fruit, leaves, and roots. This study extends current knowledge of the profile of phenolic compounds in ginseng fruit, leaves, and roots with respect to the cultivation years, and provides useful information to industries interested in the production of ginseng products.

2. Materials and methods

2.1. Ginseng materials

Three-to-six-yr-old ginseng fruit, leaves, and roots were obtained from the Ginseng and Medicinal Plants Experiment Station (N38°15′133″/E127°23′375″), Gangwondo Agricultural Research and Extension Services in Korea. The fruit and leaves were randomly collected in August 2012, and the roots were randomly collected between August 2012 and October 2012. Ginseng samples (fruit, leaves, and roots) were collected and stored at -70°C until required for analysis. All land management, including chemical pesticide and herbicide treatments, was carried out using local recommendations during the ginseng cultivation period [13].

2.2. Chemicals

The 23 phenolic standards (STDs) used in this study (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, gentisic acid, chlorogenic acid, catechin, syringic acid, vanillin, ferulic acid, *o*-coumaric acid, *m*-coumaric acid, *p*-coumaric acid, rutin, naringin, myricetin, resveratrol, *trans*-cinnamic acid, quercetin, naringenin, kaempferol, hesperetin, formononetin, and biochanin A) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). DPPH radical, Folin-Ciocalteu reagent, and sodium carbonate (Na_2CO_3) were also purchased from Sigma-Aldrich. Hydrochloric acid (HCl, 0.1 N) was purchased from Daejung Chemical & Materials Co. Ltd. (Gyeonggi-Do, Korea). Water and methanol (MeOH) were obtained from Fisher Scientific Korea Ltd. (Seoul, Korea). Acetonitrile (ACN) was purchased from Merck (Darmstadt, Germany). Glacial acetic acid was obtained from J. T. Baker (Phillipsburg, NJ, USA). All the solvents used for sample extraction and instrumental analyses were of high performance liquid chromatography (HPLC) analytical grade.

2.3. Sample extraction

Analysis of phenolic compounds in the ginseng samples was performed using a modification of a prior method [23]. Briefly, each ginseng sample was lyophilized at a temperature below −40°C (Freexeone 4.5, Labconco, Kansas City, MO, USA) and then pulverized prior to sample extraction. The pulverized ginseng sample (1 g) was added to 10 mL of ACN and 2 mL of 0.1 N HCl, and the resulting mixture was extracted using a shaker (Green-Seriker, Vision Scientific Co. Ltd., Gyeonggi-Do, Korea) at 200 rpm for 2 h at room temperature. The crude ginseng extract was filtered through No. 42 Whatman filter paper (Maidstone, UK); the filtrate was concentrated *in vacuo* at <35°C using a vacuum evaporator (SB-1200, EYELA, Tokyo Rikakikai Co. Ltd., Japan). The residue was reconstituted with 80% aqueous MeOH (5 mL), and then filtered through a 0.2 μm syringe filter (17 mm, TITAN, Rockwood, TN, USA). This filtrate was used for the analysis of phenolic compounds present in the ginseng sample and the measurement of the DPPH free-radical-scavenging activity.

2.4. Determination of total phenol content using the Folin-Ciocalteu method

The total phenol content of the ginseng samples was measured using the Folin-Ciocalteu method [24]. In brief, an aliquot (20 μL) of the ginseng sample or a phenolic STD (i.e., gallic acid) was mixed with water (1.58 mL) and the Folin-Ciocalteu reagent (100 μL). After 8.5 min, a saturated solution of sodium carbonate (300 μL) was added to the sample mixture, which was then mixed and stored at room temperature for 2 h. The total phenol content of the ginseng samples was measured using an OPTIZEN POP UV-spectrophotometer (Mecasys Co., Daejeon, Korea) at 765 nm. In this study, the total phenol content of the ginseng samples was expressed as the gallic acid equivalent (GAE, μg/g, dry weight basis). An external calibration curve was obtained using 10–1,000 μg/mL of gallic acid, and good linearity ($r^2 = 0.9954$) was observed in this range.

2.5. Profile of 23 phenolic compounds in ginseng by ultra-HPLC

The presence of 23 phenolic compounds in the ginseng samples was measured using ultra-HPLC (UHPLC, ACCELA UHPLC system, Thermo Fisher Scientific Inc., USA) with a reverse phase column (Thermo, C_{18}, 2.1 × 100 mm, 2.6 μm). Previously reported analytical conditions [13] were slightly modified for our UHPLC analysis. The mobile phase used was composed of 0.1% glacial acetic acid in distilled water (Solvent A) and 0.1% glacial acetic acid in ACN (Solvent B). The linear gradient of the mobile phase consisted of: 0 min: 98% (A) -2% (B); 0.50 min: 95% (A) -5% (B); 2.20 min: 90% (A) -10% (B); 5.00 min: 85% (A) -15% (B); 7.50 min: 84.3% (A) -15.7% (B); 8.00 min: 83.4% (A) -16.6% (B); 9.00 min: 82.2% (A) -17.8% (B); 9.50 min: 76.1% (A) -23.9% (B); 14.00 min: 55.0% (A) -45.0% (B); 15.00 min: 0% (A) -100% (B); 15.50 min: 0% (A) -100% (B); 16.00 min: 98% (A) -2% (B); 25.00 min: 98% (A) -2% (B). The flow rate of the mobile phase was 0.5 mL/min and the injection volume

was 4 μL. The absorbance of the phenolic compounds of the ginseng samples was measured at 280 nm.

2.6. Quantitation of phenolic compounds

An external calibration curve method was used for the quantitation of the 23 phenolic compounds present in the ginseng fruit, leaves, and roots. The 23 phenolic STDs were prepared in either MeOH or dimethyl sulfoxide (DMSO) as a 100 ppm stock solution. Calibration curves (3–7 points) with appropriate dilutions of each STD stock solution were used for the quantitation, and the concentration ranges used for the 23 phenolic STDs are shown in Table 1. The phenolic compounds were identified by comparing the retention times of the authentic phenolic STDs and the peaks observed in the sample aliquot (Fig. 1). Furthermore, each phenolic STD was also added (fortified) to the sample aliquot (sample aliquot + phenolic STD is indicated by the red solid line in Fig. 1B–D) to confirm the correct peak assignments in the ginseng samples. All the calibration curves showed good linearity ($r^2 > 0.99$) over the concentration ranges investigated in this study. The limit of detection (LOD) and limit of quantitation (LOQ) of the 23 phenolic compounds were determined using each calibration curve as follows: LOD = 3 × SD/S and LOQ = 10 × SD/S, where SD is the standard deviation of a response, and S is the slope of the calibration curve [25]. In this study, the LOD ranged from 0.003 ppm to 0.396 ppm (μg/mL) and the LOQ ranged from 0.011 ppm to 1.323 ppm (Table 1).

2.7. Measurement of DPPH free-radical-scavenging activity

The DPPH free-radical-scavenging activity was measured using a prior method [26] with some modifications. A 0.4 mM solution of DPPH was prepared in MeOH, and 2.8 mL of this solution was mixed with a 0.2 mL aliquot of each ginseng sample (see Sample extraction). The mixture was placed in a dark room for 10 min and the absorbance was then measured using an OPTIZEN POP UV-spectrophotometer at 517 nm. The DPPH free-radical-scavenging activity was calculated as an inhibition percentage based on the following equation: Inhibition (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the ginseng sample aliquot.

2.8. Statistical analysis

Statistical analysis was conducted using a general linear model procedure and the correlation analysis of the statistical analysis program (SAS, Version 9.3, SAS Institute Inc. Cary, NC, USA). The experimental design, including sample extraction and all instrumental measurements, was a completely randomized design in triplicate. The least significant different test was based on a 0.05 probability level.

3. Results

Table 2 shows the total phenol contents of the ginseng fruit, leaves, and roots with respect to the cultivation years. The total phenol contents of the ginseng fruit, leaves, and roots were affected by the cultivation year ($p < 0.05$). The total phenol contents in the 3–6-yr-old ginseng fruit and leaves were 4–9-fold higher than that in the 3–6-yr-old ginseng roots ($p < 0.05$). Moreover, the total phenol contents in the 3–6-yr-old ginseng fruit, leaves, and roots were approximately 0.03–0.3% of each ginseng sample (dry weight basis). The younger ginseng leaves (3 yr and 4 yr old) had higher total phenol contents than the older ginseng leaves (5- and 6-yr-old). The total phenol content significantly decreased in the 6-yr-old ginseng leaves. In contrast, the older ginseng fruit had a higher total phenol content than the younger ginseng fruit ($p < 0.05$).

Table 3 shows the content and composition of the 23 phenolic compounds found in the ginseng fruit, leaves, and roots by cultivation years. The total amount of the 23 phenolic compounds was significantly higher in the 3–6-yr-old ginseng fruit than in the 3–6-yr-old ginseng roots and leaves ($p < 0.05$). In general, the total amount of the 23 phenolic compounds in the ginseng roots and fruit increased by 20–48% with the increase in the cultivation year. In contrast, the total amount of the 23 phenolic compounds decreased by 40–50% in 6-yr-old ginseng leaves compared with the amount found in the 3-yr-old ginseng leaves ($p < 0.05$). These 23 phenolic compounds accounted for 30–50% (ginseng roots), 80–

Table 1
Concentration range, linearity, limit of detection, and limit of quantification of 23 phenolic standards examined in this study

Groups	Chemicals	Abbreviation	Concentration[1)] μg/mL	Linearity (r^2)	Slope (S)	SD of response	LOD[2)] μg/mL	LOQ[2)] μg/mL
Phenolic acid	Gallic acid	GA	0.01–1	1	9,745.7	27.7	0.008	0.028
	Protocatechuic acid	PA	0.01–10	1	11,112	102.7	0.027	0.092
	Gentisic acid	GT	0.01–50	0.9986	3,837.9	58.9	0.056	0.185
	p-Hydroxybenzoic acid	*p*H	0.01–10	0.9995	15,681	2,074.9	0.396	1.323
	Syringic acid	SA	0.01–25	0.9949	27,748	2,371.1	0.299	0.999
	Chlorogenic acid	CA	0.01–50	0.9901	5,469.2	278.2	0.078	0.260
	p-Coumaric acid	*p*C	0.01–25	0.9852	7,268.2	27.5	0.008	0.026
	Ferulic acid	FA	0.01–5	0.9994	11,944	876.5	0.22	0.733
	m-Coumaric acid	*m*C	0.01–100	0.9966	48,157	226.7	0.013	0.044
	o-Coumaric acid	*o*C	0.01–1	1	30,379	143.9	0.014	0.047
	t-Cinnamic acid	*t*C	0.01–1	1	79,059	130.4	0.004	0.016
Flavonoid	Naringin	NA	0.01–1	1	12,082	22.9	0.005	0.019
	Catechin	CN	0.01–1	1	2,335.2	2.7	0.003	0.011
	Naringenin	NG	0.01–10	0.9999	23,238	1,321.1	0.170	0.568
	Hesperetin	HN	0.01–1	1	27,844	146.1	0.015	0.052
	Rutin	RN	0.01–25	0.9999	4,857.6	461.7	0.285	0.950
	Myricetin	MY	0.01–1	0.9962	117.79	5.6	0.142	0.475
	Quercetin	QN	0.01–1	0.9992	5,995.9	128.1	0.064	0.213
	Formononetin	FN	0.01–1	1	25,179	108.4	0.012	0.043
	Kaempferol	KA	0.01–5	0.9986	10,117	1,094.9	0.324	1.082
	Biochanin A	BA	0.01–1	0.9999	15,953	116.5	0.021	0.073
Other	Resveratrol	RE	0.01–1	0.9999	31,260	54.2	0.005	0.017
	Vanillin	VN	0.01–1	0.997	39,731	5,077.3	0.383	1.277

[1)] Calibration curve was made by using 3–7 different concentrations of each phenolic standard solution
[2)] Limit of detection (LOD) and limit of quantification (LOQ) was determined using each calibration curve as follows: LOD = 3 × SD/S and LOQ = 10 × SD/S, where SD is a standard deviation of response, S is a slope of each calibration curve

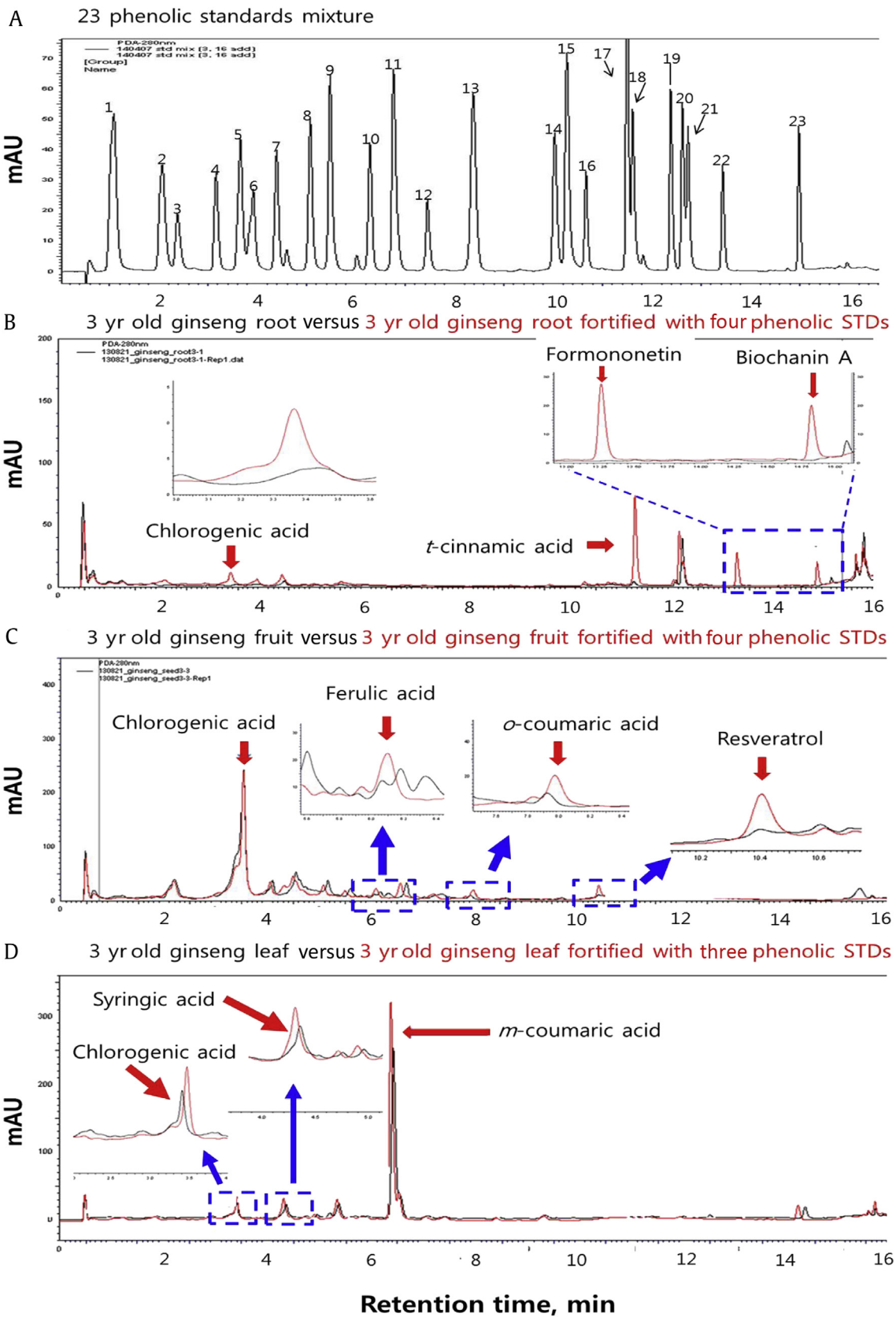

Fig. 1. Representative ultra-high-performance liquid chromatography (UHPLC) chromatograms of a mixture of the 23 phenolic standard compounds (A) and ginseng samples (B–D; the black solid line indicates the ginseng sample and the red solid line indicates the same ginseng sample fortified with some phenolic standards). (A) 1. gallic acid, 2. protocatechuic acid, 3. gentisic acid, 4. *p*-hydroxybenzoic acid, 5. chlorogenic acid, 6. catechin, 7. syringic acid, 8. vanillin acid, 9. *p*-coumaric acid, 10. ferulic acid, 11. *m*-coumaric acid, 12. rutin, 13. *o*-coumaric acid, 14. naringin, 15. myricetin, 16. resveratrol, 17. *trans*-cinnamic acid, 18. quercetin, 19. naringenin, 20. kaempferol, 21. hesperetin, 22. formononetin, and 23. biochanin A; (B) four phenolic standards (chlorogenic acid, *trans*-cinnamic acid, formononetin, and biochanin A) were spiked into the 3-yr-old ginseng root sample; (C) four phenolic standards (chlorogenic acid, ferulic acid, *o*-coumaric acid, and resveratrol) were spiked into the 3-yr-old ginseng fruit sample; (D) three phenolic standards (chlorogenic acid, syringic acid, and *m*-coumaric acid) were spiked into the 3-yr-old ginseng leaf sample.

Table 2
Total phenolic content in ginseng root, fruit, and leaf with cultivation years[1)]

Cultivation y	Root	Fruit	Leaf
	μg/g, dry weight base		
3 yr old	260.97 ± 27.62^{b,z}	2,274.17 ± 459.34^{b,y}	2,476.25 ± 421.55^{ab,x}
4 yr old	301.94 ± 27.85^{a,z}	2,286.67 ± 185.50^{b,y}	2,640.83 ± 289.62^{a,x}
5 yr old	248.47 ± 50.30^{b,z}	2,820.69 ± 396.10^{a,x}	2,215.14 ± 238.19^{b,y}
6 yr old	334.58 ± 50.19^{a,z}	2,676.94 ± 122.73^{a,x}	1,270.00 ± 305.56^{c,y}

[1)] Results are expressed as mean ± SD ($n = 3$). Values highlighted with different letters differed statistically with cultivation years^{a-c} or ginseng parts^{x-z} ($p < 0.05$)

90% (ginseng fruit), and ~20% (ginseng leaves) of the total phenol content measured in this study using the Folin-Ciocalteu method (Table 2). This result indicates that other phenolic compounds are likely to be present in the ginseng leaves and roots, whereas the total phenol content in the ginseng fruit is mostly composed of the 23 phenolic compounds measured in this study (Tables 2 and 3).

The 23 phenolic compounds used in this study could be classified as 11 phenolic acids, 10 flavonoids, and two other types of phenolic compounds. Thus, phenolic acids were more abundant than the flavonoids and other compounds. Moreover, the ginseng fruit contained more phenolic compounds than the ginseng roots and leaves in all the cultivation years (Fig. 2). The ginseng leaves were also found to contain more phenolic acids than the ginseng roots, whereas the ginseng roots contained more flavonoids than the ginseng leaves in all the cultivation years ($p < 0.05$).

Amongst the 23 phenolic compounds, gallic acid, myricetin, and biochanin A were not found in the 3–6-yr-old ginseng fruit, leaves, and roots. Furthermore, syringic acid, catechin, quercetin, kaempferol, and resveratrol were not detected in one of the 3–6-yr-old ginseng samples. In the ginseng roots, naringenin was the major phenolic compound and accounted for ~20–35% of the total amount of the 23 phenolic compounds in all cultivation years. Gentisic acid, chlorogenic acid, and catechin in the ginseng roots significantly increased with increasing cultivation years ($r \geq 0.874^{****}$). In contrast, ferulic acid, ($r = -0.780^{****}$), *p*-coumaric acid ($r = -0.698^{****}$), and formononetin ($r = -0.645^{****}$) decreased with increasing cultivation year, although their contents in the ginseng roots were small portion (≤ 3.5%) to the total 23 phenolic compounds. However, the content of other phenolic compounds did not correlate or very weakly correlate with the cultivation years in this study (Table 3). Furthermore, chlorogenic acid was the predominant phenolic compound in the ginseng fruit and accounted for ~50% of the total amount of the 23 phenolic compounds. Gentisic acid and rutin were the next most predominant phenolic compounds found in the ginseng fruit. Chlorogenic acid and *m*- and *p*-coumaric acids (10–40%) were the predominant phenolic compounds found in the ginseng leaves. The amounts of the 23 phenolic compounds in the ginseng leaves generally decreased with increasing cultivation year, whereas the amounts of the 23 phenolic compounds found in older ginseng roots and fruit was higher than those found in younger ginseng roots and fruit (Table 3).

Table 4 shows the DPPH free-radical-scavenging activity (DPPH activity) of the 3–6-yr-old ginseng fruit, leaves, and roots. In general, the DPPH activity in all the cultivation years was ranked as follows: fruit > leaves > roots. The DPPH activity was 3–5-fold higher in the ginseng fruit than in the ginseng roots; the DPPH activity was also slightly higher (5–14%) in the ginseng fruit than in the ginseng leaves ($p < 0.05$). The DPPH activity of the ginseng roots increased with increasing cultivation year, whereas that of the ginseng leaves decreased with increasing cultivation year ($p < 0.05$). These results are consistent with those reported in a prior study which showed that DPPH activity was higher in ginseng fruit and leaves than in ginseng roots [27].

Table 3
Profiling of 23 phenolic compounds in ginseng root, fruit, and leaf with cultivation years[1)]

Part	Cultivation yr	Phenolic acid										Flavonoid								Other		Sum
		GA	PA	GT	*p*H	CA	SA	*p*C	FA	*m*C	*o*C	*t*C	CN	RN	NA	MY	QN	NG	KA	HN	FN	BA	VN	RE	
		μg/g, dry weight base																							
Root	3	nd	6.93^{ab}	7.65^{c}	1.99^{c}	7.42^{c}	nd	3.18^{a}	2.87^{a}	5.04^{b}	0.38^{c}	1.09^{b}	2.82^{c}	8.28^{a}	2.54^{b}	nd	nd	30.48^{b}	nd	0.96^{a}	0.25^{a}	nd	1.06^{a}	0.58^{a}	83.54^{a}
	4	nd	7.53^{a}	17.07^{b}	3.66^{a}	10.79^{b}	nd	2.25^{b}	1.71^{b}	5.07^{b}	0.60^{b}	1.24^{a}	1.73^{c}	6.74^{ab}	2.73^{b}	nd	nd	29.46^{b}	nd	0.80^{ab}	0.12^{b}	nd	1.08^{a}	0.30^{bc}	92.90^{b}
	5	nd	5.93^{c}	19.76^{b}	2.26^{c}	11.55b	nd	2.08^{b}	1.16^{c}	5.05^{b}	0.46^{c}	1.12^{ab}	23.16^{b}	2.77^{c}	2.73^{b}	nd	nd	20.95^{c}	nd	0.60^{b}	0.14^{b}	nd	0.87^{c}	0.24^{c}	100.85^{b}
	6	nd	6.61^{bc}	61.72^{a}	3.09^{b}	13.61^{a}	nd	2.10^{b}	1.33^{c}	5.17^{a}	0.70^{a}	1.17^{ab}	33.00^{a}	4.76^{bc}	3.03^{a}	nd	nd	34.97^{a}	nd	0.87^{a}	0.13^{b}	nd	0.96^{b}	0.47^{ab}	173.72^{a}
Fruit	3	nd	5.46^{b}	614.38^{c}	3.55^{d}	1,075.54^{c}	34.30^{d}	53.76^{c}	12.74^{b}	20.35^{c}	0.19^{c}	0.22^{c}	nd	126.74^{d}	8.29^{b}	nd	nd	1.87^{d}	nd	0.46^{c}	0.46^{a}	nd	15.97^{d}	nd	1,974.31^{b}
	4	nd	4.90^{c}	580.11^{d}	11.88^{a}	929.51^{d}	56.58^{b}	59.29^{b}	11.87^{b}	28.64^{a}	0.57^{a}	0.64^{a}	nd	170.70^{a}	10.37^{a}	nd	nd	3.13^{c}	nd	1.67^{b}	0.25^{b}	nd	29.42^{a}	nd	1,899.55^{c}
	5	nd	5.89^{b}	787.95^{a}	5.68^{c}	1,377.28^{a}	53.54^{c}	74.34^{a}	16.49^{a}	23.89^{b}	0.38^{b}	0.25^{c}	nd	137.20^{c}	5.15^{d}	nd	nd	3.43^{b}	nd	2.26^{a}	0.50^{a}	nd	23.88^{c}	nd	2,518.13^{a}
	6	nd	7.32^{a}	749.00^{b}	8.38^{b}	1,323.59^{a}	65.65^{a}	59.49^{b}	15.05^{a}	19.98^{c}	0.43^{b}	0.30^{b}	nd	156.93^{b}	6.31^{c}	nd	nd	3.85^{a}	nd	2.37^{a}	0.48^{a}	nd	27.20^{b}	nd	2,446.35^{a}
Leaf	3	nd	30.41^{a}	17.97^{c}	1.37^{c}	126.21^{a}	9.82^{a}	121.88^{a}	3.37^{c}	155.24^{b}	0.57^{a}	0.93^{a}	4.96^{a}	38.60^{a}	1.30^{ab}	nd	0.91^{a}	nd	nd	0.14^{c}	0.34^{a}	nd	3.29^{a}	0.22^{c}	517.58^{b}
	4	nd	28.84^{a}	91.95^{a}	13.66^{a}	69.52^{b}	nd	90.75^{c}	3.95^{c}	261.13^{a}	0.37^{b}	0.98^{a}	5.28^{a}	24.39^{b}	1.79^{a}	nd	1.12^{a}	nd	nd	0.28^{ab}	0.14^{b}	nd	3.21^{ab}	0.79^{b}	596.96^{a}
	5	nd	19.61^{b}	108.54^{a}	11.38^{a}	125.41^{a}	nd	109.13^{b}	11.24^{a}	156.88^{b}	0.60^{a}	0.97^{a}	5.69^{a}	21.73^{b}	1.02^{b}	nd	1.60^{a}	nd	0.67^{a}	0.34^{a}	0.05^{c}	nd	2.78^{bc}	1.60^{a}	577.91^{a}
	6	nd	12.13^{c}	54.31^{b}	5.72^{b}	61.93^{b}	nd	63.68^{d}	6.53^{b}	62.05^{c}	0.28^{b}	0.56^{b}	2.97^{b}	19.77^{b}	1.13^{b}	nd	1.47^{a}	nd	0.50^{b}	0.23^{b}	0.07^{c}	nd	2.65^{c}	0.21^{c}	296.20^{c}

nd = non-detect
[1)] Results are expressed as a mean value ± SD ($n = 3$). Values highlighted with different letters differed statistically with cultivation year^{a-d} ($p < 0.05$)

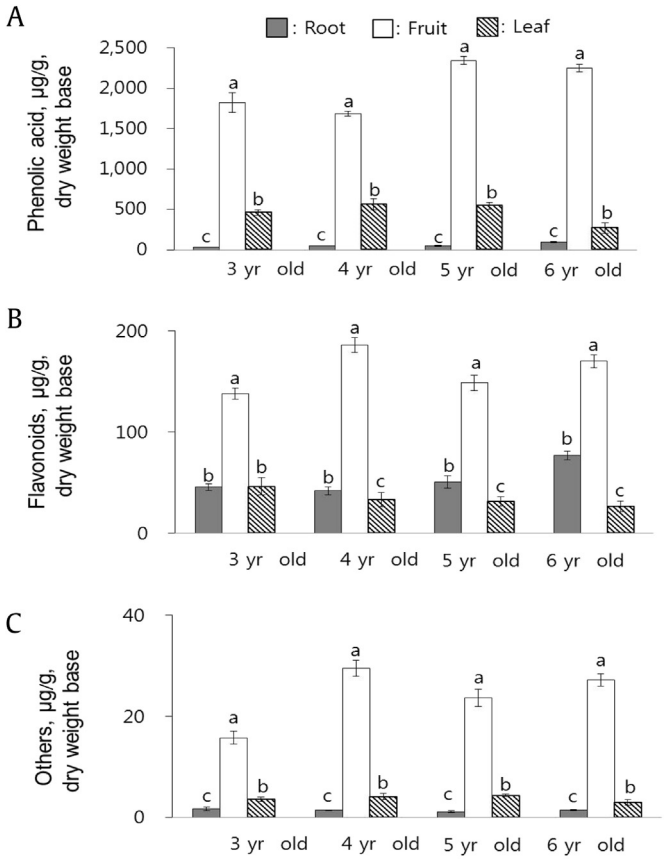

Fig. 2. Contents of the three types of phenolic compounds in ginseng fruit, leaf, and root with cultivation years. The results are expressed as mean ± SD ($n = 3$). Values highlighted with different letters are statistically different depending on the ginseng parts[a–c] ($p < 0.05$).

4. Discussion

Ginseng has been used as a popular medicinal plant or food for more than 2,000 yrs because of its various health benefits. Ginseng is mostly used as a 4–6-yr-old root. Because of the growth characteristics of ginseng, its cultivation requires a specific climate and specific soil conditions [28]. Ginseng consists of 60% carbohydrate, 8–15% crude protein, 1–3% lipid, 4–6% ash, 3–7% crude saponin, and other chemicals, including phenolic and volatile compounds [29].

In general, ginsenosides are known as the principle phytochemicals of ginseng. Interest in the phenolic compounds of ginseng has increased recently because of their various biological and pharmacological properties, such as antioxidant, anticancer, and whitening properties, or because of their ability to reduce hypertension [30,31]. More than 10 phenolic compounds in fresh and/or processed ginseng have previously been reported as follows: salicylic acid, vanillic acid, ascorbic acid, *p*-coumaric acid, ferulic acid, caffeic acid, gentisic acid, *p*-hydroxybenzoic acid, maltol, cinnamic acid, protocatechuic acid, syringic acid, and quercetin [22]. Among these phenolic compounds, ferulic acid is considered a phenolic compound with anticancer properties. Maltol, which is found in processed ginseng, shows strong scavenging activity against reactive oxygen species [32,33]. Korean ginseng usually contains more phenolic compounds than Chinese ginseng; therefore, Korean ginseng has more health benefits than other ginseng species [34].

Table 4
2,2-Diphenyl-1-picrylhydrazyl (DPPH) free-radical-scavenging activity of ginseng root, fruit, and leaf with cultivation years[1)]

Cultivation yr	Root	Fruit	Leaf
		% inhibition	
3 yr	$18.08 \pm 0.972^{c,z}$	$89.64 \pm 0.64^{c,x}$	$85.17 \pm 0.95^{a,y}$
4 yr	$19.26 \pm 1.14^{bc,z}$	$92.42 \pm 0.33^{a,x}$	$83.52 \pm 1.73^{ab,y}$
5 yr	$20.01 \pm 2.48^{b,z}$	$88.28 \pm 0.52^{d,x}$	$84.37 \pm 3.10^{a,y}$
6 yr	$25.61 \pm 1.34^{a,z}$	$91.05 \pm 1.79^{b,x}$	$79.43 \pm 8.03^{b,y}$

[1)] Results are expressed as mean ± SD ($n = 3$). Values highlighted with different letters differed statistically with cultivation years[a–c] or ginseng parts[x–z] ($p < 0.05$)

In the present study, the maximum value of the total phenol content in 3–6-yr-old ginseng roots was ~0.03% (dry weight basis), with gentisic acid and naringenin as the major phenolic compounds. According to previous studies, the total phenol content was 0.42% (dry weight basis) in 5-yr-old ginseng roots and mostly consisted of esterified and insoluble phenolic forms rather than free phenolic forms. Vanillic acid, ferulic acid, and gentisic acid were the most abundant phenolic acids in the free, esterified, and insoluble phenolic forms, respectively [35]. Salicylic acid, vanillic acid, and *p*-coumaric acid were the major phenolic compounds in fresh ginseng roots despite different ginseng cultivars [36]. In addition, using preparative TLC, a polyphenol compound (MW 578) was found to occur exclusively in fresh Korean ginseng; this compound was not found in American ginseng [37]. The differences in the composition and content of phenolic compounds between this study and prior studies probably arose because of the use of different extraction methods, ginseng cultivars, and others factors (i.e., cultivation conditions, including soil and climate).

In general, ginseng fruit and leaves are less attractive to consumers compared with the ginseng roots; in fact, the fruit and leaves are usually discarded [28]. Therefore, information on the phenolic compounds present in the ginseng fruit or leaves is very limited to date. Traditionally, ginseng leaves have been used as a form of tea. In addition, the leaves of hydroponically grown ginseng have been recently used as salad [28]. Furthermore, American ginseng berries have been reported to inhibit the growth of colorectal cancer both *in vitro* and *in vivo* [38].

The leaves of soil-cultivated ginseng have been reported to contain more cinnamic acid than *p*-coumaric acid [39]. In contrast, the leaves of hydroponically cultivated ginseng contain higher amounts of *p*-coumaric acid. In addition, the amount of cinnamic acid was higher only in the fruit of hydroponically cultivated ginseng. Furthermore, maltol was not found in either ginseng fruit or leaves [28]. In particular, *p*-coumaric acid is known as an effective antioxidant, which is responsible for ABTS (2, 2′-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid) radical-scavenging activity. The different amount of this compound in different ginseng parts can be used to explain the higher ABTS activity of leaves compared with the fruit and roots of hydroponically cultivated ginseng [28,40].

In our study, the total phenol content was 0.22–0.28% in the ginseng fruit and 0.13–0.26% in the ginseng leaves. Chlorogenic acid and gentisic acid were the most abundant phenolic compounds in the ginseng fruit, followed by rutin, *p*-coumaric acid, and salicylic acid. In addition, chlorogenic acid and *m*- and *p*-coumaric acids were also major phenolic compounds in the ginseng leaves. The phenolic compositions and contents determined in this study were slightly different from those previously reported [28,39]. The phenolic profiles of the ginseng fruit and leaves were affected by several factors such as ginseng cultivar, cultivation conditions, extraction method, and/or harvesting period. In particular, the harvesting period of the ginseng fruit and leaves was critical for the composition and content of the phenolic compounds, because the various metabolites (including the phenolic compounds described in this study) were greatly affected by the ginseng growth and development stages. In the present study, ginseng fruit and leaves were collected during a limited period (August 2012 for ginseng

fruit and leaves; August–October 2012 for ginseng roots) with multiple replicates to minimize any variation in the phenolic compounds found in the ginseng fruit and leaves. This methodology could be one of the critical reasons why the phenolic compound profiles in this study differ from those previously reported.

The correlation analyses showed that the total phenol content increased in the ginseng roots ($r = 0.365^{*}$) and fruit ($r = 0.501^{**}$) and decreased in the ginseng leaves ($r = -0.740^{****}$) with increasing cultivation years. In addition, the total amount of the 23 phenolic compounds increased in the ginseng roots ($r = 0.847^{****}$) and fruit ($r = 0.801^{****}$) and decreased in the ginseng leaves ($r = -0.581^{***}$) with increasing cultivation years. It was also found that the DPPH activity increased in the ginseng roots ($r = 0.799^{****}$) and decreased in the ginseng leaves ($r = -0.389^{*}$) with increasing cultivation years. The DPPH activity of the ginseng fruit was not statistically correlated with the cultivation years.

In addition, the phenolic acids, but not the flavonoids, significantly affected the total amount of the 23 phenolic compounds ($r = 0.999^{***}$) and the total phenol content ($r = 0.734^{***}$) in the ginseng samples; this result shows that the phenolic acids are the predominant type of phenolic compound in the ginseng fruit, leaves, and roots. In addition, the phenolic acids in the ginseng samples also increased with increasing flavonoids ($r = 0.888^{***}$) and other compounds ($r = 0.939^{***}$). In particular, chlorogenic acid ($r = 0.639^{****}$), *p*-coumaric acid ($r = 0.831^{****}$), and ferulic acid ($r = 0.699^{****}$) were positively correlated and naringenin ($r = -0.857^{****}$) was negatively correlated with the total phenol content in ginseng. This result means that the total phenol content increased with increasing chlorogenic acid, *p*-coumaric acid, and ferulic acid in ginseng, as well as with the decreasing amount of naringenin in ginseng. Furthermore, the content of coumaric acid isomers (*o*-, *m*-, and *p*-) in ginseng was not statistically correlated with the isomer type ($p > 0.637$).

DPPH activity was strongly correlated with the total phenol content in ginseng fruit, leaves, and roots ($r = 0.928^{****}$); DPPH activity was also significantly correlated with the total phenolic acids content ($r = 0.707^{***}$). In particular, the content of phenolic acids, such as *p*-coumaric acid ($r = 0.847^{****}$), ferulic acid ($r = 0.742^{****}$), and chlorogenic acid ($r = 0.612^{****}$) greatly affected the DPPH activity. However, the content of catechin ($r = -0.770^{****}$) or naringenin ($r = -0.939^{****}$) in ginseng was negatively correlated with the DPPH activity.

In conclusion, the present study reported the profile of phenolic compounds and the antioxidant activity of the fruit, leaves, and roots of Korean ginseng with respect to the cultivation year. The total phenol contents in 3–6-yr-old ginseng fruit, leaves, and roots were 0.03–0.3% (dry weight basis) of each ginseng sample and the phenol content was usually found to be higher in ginseng fruit and leaves than in ginseng roots ($p < 0.05$). The total phenol content of ginseng roots ($r = 0.365^{*}$) and fruit ($r = 0.501^{**}$) increased with increasing cultivation year, whereas that of ginseng leaves ($r = -0.740^{****}$) decreased. Among the 23 phenolic compounds studied, the phenolic acids were more abundant in ginseng fruit, leaves, and roots than the flavonoids and other compounds ($p < 0.05$). This study showed that chlorogenic acid, gentisic acid, *p*- and *m*-coumaric acid, and rutin were the main phenolic compounds in 3–6-yr-old ginseng fruit, leaves, and roots. In contrast, gallic acid, myricetin, and biochanin A were not found in 3–6-yr-old ginseng fruit, leaves, and roots. In addition, the DPPH activity was significantly correlated with the total phenol content in the ginseng samples ($r = 0.928^{****}$). In particular, *p*-coumaric acid ($r = 0.847^{****}$) and ferulic acid ($r = 0.742^{****}$) greatly affected the DPPH activity. This study provides basic information about the composition and content of phenolic compounds in ginseng fruit, leaves, and roots with respect to the cultivation years. This information is potentially useful to ginseng growers and industries involved in the production of high-quality and nutritional ginseng products.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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