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.

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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].
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 caffic 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, forominetin, and biochanin A) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). DPPH radical, Folin–Ciocalteu reagent, and sodium carbonate (Na₂CO₃) were also purchased from Sigma–Aldrich. Hydrochloric acid (HCl, 0.1 N) was purchased from Daejun 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 (Freeezxone 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 then filtered 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 (r² = 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, C18, 2.1 × 100 mm, 2.6 μm). Previously reported analytical conditions [13] were slightly modified for our UHPLC analysis. The mobile phase 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: $\text{LOD} = 3 \times SD/S$ and $\text{LOQ} = 10 \times SD/S$, 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 (%) = ($A_0 - A_1$)/$A_0$ × 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.

### Table 1

| 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                 | 9,745.7          | 27.7       | 0.008          | 0.028        |              |
|           | Proocatechic acid | PA         | 0.01–10               | 11,112           | 102.7      | 0.027          | 0.092        |              |
|           | Gentisic acid  | GT          | 0.01–50               | 3,837.9          | 58.9       | 0.056          | 0.185        |              |
|           | p-Hydroxybenzoic acid | pH      | 0.01–10               | 15,681           | 2,074.9    | 0.396          | 1.323        |              |
|           | Syringic acid  | SA          | 0.01–25               | 27,748           | 2,371.1    | 0.209          | 0.999        |              |
|           | Chlorogenic acid | CA         | 0.01–50               | 5,469.2          | 278.2      | 0.078          | 0.260        |              |
|           | p-Coumaric acid | PC          | 0.01–25               | 7,268.2          | 27.5       | 0.008          | 0.026        |              |
|           | Ferulic acid  | FA          | 0.01–5                | 11,944           | 876.5      | 0.22           | 0.733        |              |
|           | m-Coumaric acid | mC         | 0.01–100              | 48,157           | 226.7      | 0.013          | 0.044        |              |
|           | o-Coumaric acid | oC         | 0.01–1                | 30,379           | 143.9      | 0.014          | 0.047        |              |
| Flavonoid | Naringin     | NA          | 0.01–1                | 12,082           | 22.9       | 0.005          | 0.019        |              |
|           | Catchein      | CN          | 0.01–1                | 2,335.2          | 27         | 0.003          | 0.011        |              |
|           | Naringenin    | NG          | 0.01–10               | 23,238           | 1,321.1    | 0.170          | 0.568        |              |
|           | Hesperetin    | HN          | 0.01–1                | 27,844           | 146.1      | 0.015          | 0.052        |              |
|           | Rutin         | RN          | 0.01–25               | 4,857.6          | 461.7      | 0.285          | 0.950        |              |
|           | Myricetin     | MY          | 0.01–1                | 117.79           | 5.6        | 0.142          | 0.475        |              |
|           | Quecitin      | QN          | 0.01–1                | 5,995.9          | 128.1      | 0.064          | 0.213        |              |
|           | Formononetin  | FN          | 0.01–1                | 25,179           | 104.8      | 0.012          | 0.043        |              |
|           | Kaempferol    | KA          | 0.01–5                | 10,117           | 1,094.9    | 0.324          | 1.082        |              |
|           | Biochanin A   | BA          | 0.01–1                | 15,953           | 116.5      | 0.021          | 0.073        |              |
|           | Resveratrol   | RE          | 0.01–1                | 31,260           | 54.2       | 0.005          | 0.017        |              |
|           | Vanillin      | VN          | 0.01–1                | 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 quantitation (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. hesperitin, 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.
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, queretin, 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 cultivation years \((r > 0.874^{* * *})\). 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 \((\leq 3.5\%)\) 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 2

| Cultivation y | Root | Fruit | Leaf | µg/g, dry weight base |
|---------------|------|-------|------|----------------------|
| 3 yr old      | 260.97 ± 27.62^a | 2,276.17 ± 459.34^bv | 2,476.25 ± 421.59^bx | 2,820.69 ± 396.10^ay |
| 4 yr old      | 304.94 ± 27.85^c | 2,286.67 ± 185.50^ay | 2,640.68 ± 289.62^ca | 2,215.14 ± 238.19^ky |
| 5 yr old      | 248.47 ± 50.30^k | 2,820.69 ± 396.10^ay | 2,215.14 ± 238.19^ky | 2,215.14 ± 238.19^ky |
| 6 yr old      | 334.58 ± 59.19^m | 2,676.94 ± 122.73^ay | 1,270.00 ± 309.56^my | 1,270.00 ± 309.56^my |

1) Results are expressed as mean ± SD \((n = 3)\). Values highlighted with different letters differed statistically with cultivation years \(* * * (p < 0.05)\) or ginseng parts \(* * (p < 0.05)\).
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].

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 [38,40].

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

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**Table 4**

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

| Cultivation yr | Root | Fruit | Leaf | % inhibition |
|----------------|------|-------|------|-------------|
| 3 yr           | 18.08 ± 0.972 <sup>a</sup> | 89.64 ± 0.64 <sup>a</sup> | 85.17 ± 0.95 <sup>y</sup> | 62 <sup>a</sup> |
| 4 yr           | 19.26 ± 1.14 <sup>b</sup> | 92.42 ± 0.33 <sup>b</sup> | 83.52 ± 1.73 <sup>y</sup> | 67 <sup>b</sup> |
| 5 yr           | 20.01 ± 2.48 <sup>b</sup> | 88.28 ± 0.52 <sup>b</sup> | 84.37 ± 3.10 <sup>y</sup> | 70 <sup>b</sup> |
| 6 yr           | 25.61 ± 1.34 <sup>b</sup> | 91.05 ± 1.70 <sup>b</sup> | 79.43 ± 8.01 <sup>y</sup> | 73 <sup>b</sup> |

<sup>1</sup> Results are expressed as mean ± SD (n = 3). Values highlighted with different letters differed statistically with cultivation years <sup>a</sup> or ginseng parts <sup>y</sup> (p < 0.05)
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 cultivation years ($r = 0.888$), 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 isoforms (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 phenolic profile 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 ($r = 0.742**$) were greatly affected by 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.

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**References**

[1] Jung MY, Jeon BS, Bock JY. Free, esterified, and insoluble-bound phenolic acids in white and red Korean ginsengs (Panax ginseng C. A. Meyer). Food Chem 2002;79:105–11.
[2] Baeg IH, So SH. The world ginseng market and the ginseng (Korea). J Ginseng Res 2013;37:1–7.
[3] Ministry of Agriculture FaRA, 2012 Ginseng Statistical Data (No. 11-15430000-000004-10). In: Ministry of Agriculture FaRA; 2013. p. 2–7.
[4] Xiaoming S, Jian C, Kedsirin S, Ruili L, Songhua H. Enhancement of immune responses to influenza vaccine(H1N1) by ginsenoside Re. Int Immunopharmacol 2010;10:351–6.
[5] Kwok HH, Ng WY, Yang MS, Mak NK, Wong RN, Yue FY. The ginsenoside protopanaxatriol protects endothelial cells from hydrogen peroxide-induced cell injury and cell death by modulating intracellular redox status. Free Radical Bio Med 2010;48:437–45.
[6] Naval MV, Gomez-Serranillos MP, Carretero ME, Villar AM. Neuroprotective effect of a ginseng (Panax ginseng) root extract on astrocytes primary culture. J Ethnopharmacol 2007;112:262–70.
[7] Hu JN, Lee JH, Shin JA, Choi JE, Lee KT. Determination of ginsenosides content in 6-yr-old ginseng fruit, leaves, and roots. J Ginseng Res 2009;33:194.
[8] Yuan C-S, Wang C-Z, Mehdendale SR. Commonly used antioxidant botanicals: active constituents and their potential role in cardiovascular illness. Am J Chin Med 2007;35:543–58.
[9] Lee KS, Kim GH, Kim HJ, Chang YL, Lee GH. Volatile components of Panax ginseng C. A. Meyer cultured with different cultivation methods. J Food Sci 2012;77:C805–10.
[10] Lee JW, Do JH, Lee SK, Yang JW. Determination of total phenolic compounds from Korean red ginseng, and their extraction conditions. J Ginseng Res 2006;24:64–7.
[11] Park JW, Jeon BS, Yang JW. The chemical components of Korean ginseng. Ind Med Plants Res 2012;6:3030–6.
[12] Jung MY, Jeon BS, Bock JY. Free, esterified, and insoluble-bound phenolic acids in white and red Korean ginsengs (Panax ginseng C. A. Meyer). Food Chem 2002;79:105–11.
[13] Chung IM, Kim JW, Seguin P, Jun YM, Kim SH. Ginsenosides and phenolics in fresh and processed Korean ginseng (Panax ginseng C. A. Meyer): Effects of cultivation location, year, and storage period. Food Chem 2012;130:73–83.
[14] Kong YH, Rho JH, Cho CW, Kim MH, Lee YC, Kim SS, Lee PJ, Choi SY. Variation of phenolic ingredient and ginsenoside content in red ginseng extract by acid treatment. J Ginseng Res 2009;33:194–8.
[15] Hwang EY, Yong YH, Lee YC, Kim YC, Yoo KM, Jo YQ, Choi SY. Comparison of phenolic compounds contents between white and red ginseng and their inhibitory effect on melanin biosynthesis. J Ginseng Res 2006;30:82–7.
[16] Lee JW, Kim YS, Kyung JS, Song YB, Do JH, Kim DC, Lee SD. Identification of anticoagulant components in Korean red ginseng. J Ginseng Res 2010;34:355–62.
[17] Cho CH, Kim GN, Lee SH, Lee JS, Jang HD. Effects of heat processing time on total phenolic content and antioxidant capacity of ginseng jang kwa. J Ginseng Res 2010;34:198–204.
[18] Lee NR, Han JS, Kim JS, Choi JE. Effects of extraction temperature and time on ginsenoside content and quality in ginseng (Panax ginseng) extract. Kor J Med Crop Sci 2011;19:271–5.
[19] Chang HK. Effect of processing methods on the saponin contents of Panax ginseng leaf-tea. J Food Sci Nut 2003;16:46–53.
[20] Hu JN, Lee JH, Shin JA, Choi JE, Lee KT. Determination of ginsenosides content in Korean ginseng seeds and roots by high performance liquid chromatography. Food Sci Biotech 2008;17:430–3.
[21] Yang SO, Lee SW, Kim YO, Sohn SH, Kim YC, Hyun DY, Hong YP, Shin YS. HPLC-based metabolic profiling and quality control of leaves of different Panax species. J Ginseng Res 2013;37:248–53.
Kong YH, Lee YC, Choi SY. Neuroprotective and anti-inflammatory effects of phenolic compounds in *Panax ginseng* C.A. Meyer. J Ginseng Res 2009;33:111–4.

Kim EH, Ro HM, Kim SL, Kim HS, Chung IM. Analysis of isoflavone, phenolic, soyasapogenol, and tocopherol compounds in soybean (*Glycine max* (L.) Merrill) germplas of different seed weights and origins. J Agric Food Chem 2012;60:6045–55.

Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. Am J Enol Viticul 1977:28:49–55.

Kim EH, Ro HM, Kim SL, Kim HS, Chung IM. Analysis of isoflavone, phenolic, soyasapogenol, and tocopherol compounds in soybean (*Glycine max* (L.) Merrill) germplas of different seed weights and origins. J Agric Food Chem 2012;60:6045–55.

Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. Am J Enol Viticul 1977:28:49–55.

Shrivastava A, Gupta V. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chronic Young Sci 2011;2:21–5.

Blosis MS. Antioxidant determination by the use of a stable free radical. Nature 1958;181:1199–200.

Chon SU, Kim YM. Differential physiological activity in different ages of *Panax ginseng*. Kor J Crop Sci 2011;56:80–7.

Choi SY, Cho CW, Lee Y, Kim SS, Lee SH, Kim KT. Comparison of ginsenoside and phenolic ingredient contents in hydroponically-cultivated ginseng leaves, fruits, and roots. J Ginseng Res 2012;36:425–9.

Kim KY, Shin JK, Lee SW, Yoon SR, Chung HS, Jeong YJ, Choi MS, Lee CM, Moon XD, Kwon JH. Quality and functional properties of red ginseng prepared with different steaming time and drying methods. Kor J Food Sci Technol 2007;39:494–9.

Hong HD, Kim YC, Rho JH, Kim KT, Lee YC. Changes on physicochemical properties of *Panax ginseng* C. A. Meyer during repeated steaming process. J Ginseng Res 2007;31:222–9.