

## ***In Vitro* and *In Vivo* Antioxidant Activity of Aged Ginseng (*Panax ginseng*)**

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**ABSTRACT:** Fresh ginseng roots were aged in an oven at 80°C for 14 d. The *in vitro* and *in vivo* antioxidant activities of this aged ginseng, in comparison with those of the white and red ginsengs, were evaluated. In *in vitro* antioxidant assays, the ethanolic extracts from aged ginseng showed significantly higher free radical scavenging activity and reducing power than those of the white and red ginsengs. In *in vivo* antioxidant assays, mice were fed a high fat diet supplemented with white, red, or aged ginseng powders. High fat feeding resulted in a significant increase in lipid peroxidation and a substantial decrease in antioxidant enzymes activities in the animals. However, diet supplementation of ginseng powders, particularly aged ginseng, markedly reduced lipid peroxidation and enhanced the antioxidant enzymes activities. The results illustrate that the aged ginseng has greater *in vitro* and *in vivo* antioxidant capacity than the white and red ginsengs. The aged ginseng also showed considerably higher total saponin, phenolic, and flavonoid contents, indicating that its antioxidant capacity may have been partly due to its high levels of antioxidant compounds. This new ginseng product may be useful as a functional food with strong antioxidant potential.

**Keywords:** aged ginseng, red ginseng, antioxidant capacity, antioxidant enzymes, phenolics

### **INTRODUCTION**

Known for its ability to boost the immune system and improve health, ginseng (*Panax ginseng*) has been used for thousands of years in traditional Asian medicine (1). It is a slow-growing perennial herb with fleshy roots that are usually harvested after 4 to 6 years of cultivation. Previous investigations revealed that the triterpene saponins or ginsenosides, the major bioactive compounds of ginseng, increased with the age of the plant, attaining a maximum yield at the age of 5 (2,3). Recent studies have shown that extracts from ginseng roots exhibited anti-diabetic and anti-obesity effects in mice (4,5). Furthermore, orally administered ginseng extracts significantly improved the lipid profile and increased the antioxidant activity in rats (6-8).

The ginseng roots are sold in the market as unprocessed raw roots or processed ones such as dried white and red ginsengs, powder, extract, and tea. White ginseng is prepared by air-drying the peeled ginseng roots, while the red ginseng is produced by steaming the unpeeled ginseng root for 2~3 h and then sun-dried (4,9). The red ginseng has been shown to have greater biologi-

cal activity than the white ginseng due to its higher ginsenoside and phenolic contents (9,10). In our previous study, we found that aging could increase the amount of bioactive compounds and enhance the antioxidant activity of ginseng (11). The aging process involves heat treatment of ginseng roots in an oven for a specific period of time. Compared with the red ginseng, the aged ginseng is easier and cheaper to produce. Studies in the past have shown that heat treatment of ginseng for an extended time increases its ginsenoside content and pharmacological activity (12,13).

Nowadays, the incidence of various metabolic disorders is rapidly increasing worldwide. With the rising cost of health care services, there is a growing public interest on natural food products that have enhanced functional properties and strong antioxidant activity. This study was conducted to evaluate the *in vitro* and *in vivo* antioxidant activity of aged ginseng, in comparison with those of the white and red ginsengs. Moreover, the aged ginseng was prepared using 4- and 5-year-old ginseng roots in order to determine if the plant age affects the antioxidant capacity of aged ginseng.

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## MATERIALS AND METHODS

### Materials

Fresh ginseng roots (4 and 5 years old) were obtained from the Punggi Ginseng Cooperative Association (Yeongju, Korea). They were washed, placed in a plastic food container with a lid (15×20×10 cm) to keep them from drying out, and aged in an oven (SW 90D, Sang Woo Scientific Co., Bucheon, Korea) at 80°C with 70% relative humidity for 14 d. The aging temperature and time were selected based on the results of our previous study on the optimum aging processing conditions (11). The white and red ginseng samples (4 years old) were purchased from a local market in Daegu, Korea. All ginseng samples were ground into powder and passed through a 100-mesh sieve prior to vacuum freeze-drying (FreeZone 6 Liter Benchtop Freeze Dry Systems, Labconco Corp., Kansas City, MO, USA). The chemicals used were of analytical grade and procured from Sigma-Aldrich Co. (St. Louis, MO, USA).

### Determination of the total saponin content

Following the method of Kim et al. (14), 2 g of freeze-dried ginseng powder was extracted with 100 mL of 80% methanol for 3 h using a reflux condenser. The extract was concentrated in a rotary vacuum evaporator (Eyela N-1000, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The concentrate was mixed with distilled water, washed with ethyl ether, and extracted with water-saturated *n*-butanol. The sample extract was then washed with distilled water, and the *n*-butanol layer was concentrated in a rotary evaporator. The concentrate was weighed to determine the saponin content.

### Preparation of ethanolic extracts from ginseng samples

The ginseng powder was extracted 3 times with 70% ethanol, with a solvent to solid material ratio of 3:1 (15 mL ethanol : 5 g ginseng). The extracts were filtered using a Whatman no. 2 filter paper to remove any debris. The filtrates were concentrated using a rotary evaporator (Eyela N-1000, Tokyo Rikakikai Co., Ltd.), then lyophilized, and stored at -80°C in a deep-freezer. The extracts were dissolved in 0.1 g/mL dimethyl sulfoxide prior to use.

### Determination of total phenolic content

The total phenolic content of the ginseng extracts was determined using the Folin-Ciocalteu colorimetric method (15). Briefly, the extract was mixed 2% Na<sub>2</sub>CO<sub>3</sub> and 50% Folin-Ciocalteu's reagent. The absorbance was measured at 750 nm, and the results were expressed as gallic acid equivalents (mg of gallic acid/g of ginseng).

### Determination of total flavonoid content

The total flavonoid content of the ginseng extracts was measured using a colorimetric method (16). Briefly, the sample extract was mixed with distilled water and 5% NaNO<sub>2</sub> solution. The mixture was then added to 10% AlCl<sub>3</sub>·6H<sub>2</sub>O and 1 M NaOH. The absorbance was measured at 510 nm, and the results were expressed as (-)-epicatechin equivalents (mg of epicatechin/g of ginseng).

### Analysis of antioxidant activity *in vitro*

**Free radical scavenging activity assay:** The 1,1-diphenyl-2-picrylhydrazyl (DPPH)-radical scavenging activity of the ginseng extracts was determined using the method of Hatano et al. (17). Briefly, the sample was mixed with the DPPH solution and shaken vigorously and left to stand for 30 min in the dark at room temperature. The absorbance of the mixture was measured at 517 nm, and the scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{1 - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

**Reducing power assay:** The reducing power of the sample extracts was measured according to the method described by Oyaizu (18). The sample extract was mixed with 2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, 10% trichloroacetic acid was added, and the mixture was centrifuged at 650 g for 10 min. The supernatant was mixed with 0.1% ferric chloride and distilled water. The absorbance was measured at 700 nm. Higher absorbance indicates higher reducing power.

### Analysis of antioxidant activity *in vivo*

**Animals and diet:** Forty-eight male C57BL/6N mice (4-week-old, approximately 12 g each) were purchased from Orient Inc. (Seoul, Korea). Each animal was housed in a stainless steel cage in a room maintained at 25±2°C with 50% relative humidity and 12-h light/dark cycle. They were fed a pelletized chow diet for a week upon arrival and randomly divided into 6 dietary groups (*n*=8). The first and second groups were fed a normal control diet and a high fat diet, respectively. The other 4 groups were fed a high fat diet supplemented with white ginseng (WG), red ginseng (RG), aged 4-year-old ginseng (AG4), or aged 5-year-old ginseng (AG5) powder. The composition of the experimental diets (Table 1) was based on the AIN-76 semisynthetic diet (19). The animals were fed the experimental diets for 8 weeks and allowed free access to food and water. At the end of the experimental period, the mice were anaesthetized with ketamine-HCl following a 12-h fast. The blood samples were drawn from the inferior vena cava into a heparin-coated tube and centrifuged at 1,000 g for 15 min at

**Table 1.** Composition of the experimental diets

(%)

	NC <sup>1)</sup>	HF	HF-WG	HF-RG	HF-AG4	HF-AG5
Casein	20.00	23.31	23.31	23.31	23.31	23.31
Sucrose		20.14	20.14	20.14	20.14	20.14
Dextrose	50.00	11.65	7.65	7.65	7.65	7.65
Corn starch	15.00	8.48	8.48	8.48	8.48	8.48
Cellulose	5.00	5.83	5.83	5.83	5.83	5.83
Soybean oil	5.00	2.91	2.91	2.91	2.91	2.91
Lard		20.69	20.69	20.69	20.69	20.69
Mineral mixture <sup>2)</sup>	3.50	5.24	5.24	5.24	5.24	5.24
Vitamin mixture <sup>3)</sup>	1.00	1.17	1.17	1.17	1.17	1.17
L-Cystine	0.30	0.35	0.35	0.35	0.35	0.35
Choline bitartrate	0.20	0.23	0.23	0.23	0.23	0.23
WG			4.00			
RG				4.00		
AG4					4.00	
AG5						4.00
Total (%)	100.00	100.00	100.00	100.00	100.00	100.00
Calorie (kcal/g)	385.00	466.72	466.72	466.72	466.72	466.72

<sup>1)</sup>NC, normal control diet; HF, high fat diet; HF-WG, high fat diet + white ginseng; HF-RG, high fat diet + red ginseng; HF-AG4, high fat diet + aged 4-year-old ginseng; HF-AG5, high fat diet + aged 5-year-old ginseng.

<sup>2)</sup>AIN-76 mineral mixture.

<sup>3)</sup>AIN-vitamin mixture.

4°C to obtain the plasma and erythrocytes. The hemoglobin concentration was measured using a commercial assay kit (Asan Pharmaceutical, Seoul, Korea). The current study protocol was approved by the Ethics Committee of Kyungpook National University for animal studies (KNU2011-80).

**Lipid peroxidation:** The plasma and erythrocyte thiobarbituric acid reactive substances (TBARS) were analyzed according to the method described by Ohkawa et al. (20). The plasma or red blood cells were mixed with 5% trichloroacetic acid and 0.06 M thiobarbituric and incubated at 80°C for 90 min. After cooling, the mixture was centrifuged at 2,000 g for 25 min. The absorbance of the supernatant was measured at 535 nm. A malondialdehyde solution was used as the standard, and the results were expressed as nmol/mL or g Hb.

**Antioxidant enzyme activities:** The hepatic enzyme source was prepared following the method of Hulcher and Oleson (21). Briefly, the liver (0.3 g) was homogenized in a buffer solution containing 0.1 M triethanolamine, 0.2 M ethylenediaminetetraacetic acid (EDTA), and 0.002 M dithiothreitol and centrifuged at 1,000 g for 15 min at 4°C. The supernatant was centrifuged at 10,000 g for 15 min at 4°C and the resulting precipitate served as the mitochondrial fraction, while the supernatant was further centrifuged at 105,000 g for 1 h at 4°C. The resulting supernatant and precipitate were the cytosol and microsome fractions, respectively. The protein content was measured using the Bradford protein assay (22).

The activity of superoxide dismutase (SOD) enzyme was spectrophotometrically measured based on the method of Marklund and Marklund (23). The reaction mix-

ture containing 50 mM Tris-HCl buffer (pH 8.5), 10 mM EDTA, 0.1 mL cytosol, and 7.2 mM pyrogallol was incubated at 25°C for 10 min and then mixed with 50 µL of 1 N HCl. The absorbance was measured at 420 nm. The catalase (CAT) activity was determined using the method of Aebi (24). A mixture of 50 mM potassium phosphate buffer (pH 7.4) and 10 µL of mitochondrial fraction was pre-incubated at 25°C for 5 min and then mixed with 0.1 mL of 30 mM H<sub>2</sub>O<sub>2</sub>. The disappearance of H<sub>2</sub>O<sub>2</sub> was monitored spectrophotometrically at 240 nm for 5 min. A molar extinction coefficient of 0.041/mM/cm was used to calculate the CAT activity, which was expressed as nmol decreased H<sub>2</sub>O<sub>2</sub>/min/mg protein. The glutathione peroxidase (GPx) activity was determined using the method of Paglia and Valentine (25) with slight modifications. A 0.1 mL cytosol was added to the reaction mixture (6 mM glutathione, 1.2 mM NADPH, and 1.25 µM H<sub>2</sub>O<sub>2</sub> in 20 mM Tris-HCl, pH 7.0) that was pre-incubated at 25°C for 5 min. The mixture was further incubated at 25°C for 5 min, and its absorbance was measured at 340 nm. A molar extinction coefficient of 6.22/mM/cm was used to calculate the activity, which was expressed as nmol oxidized NADPH/min/mg protein. The glutathione reductase (GR) activity was determined following the method of Mize and Langdon (26). A 10 µL cytosol was added to the reaction mixture (1 mM EDTA and 1 mM glutathione disulfide in a 0.1 M potassium phosphate buffer, pH 7.4), and the oxidation of NADPH was monitored at 340 nm. The activity was expressed as nmol oxidized NADPH/min/mg protein. The paraoxonase (PON) activity was measured using the method of Mackness et al. (27). The microsome (50 µL) was added

**Table 2.** Total saponin, polyphenol, and flavonoid contents of the ethanolic extracts from ginseng samples

Group <sup>1)</sup>	Total saponin (mg/g dry weight)	Total polyphenol (mg/g)		Total flavonoid (mg/g)	
		1 mg/mL	10 mg/mL	1 mg/mL	10 mg/mL
WG	9.22±0.06 <sup>a</sup>	2.04±0.00 <sup>a</sup>	7.40±0.05 <sup>a</sup>	1.04±0.00 <sup>a</sup>	1.55±0.02 <sup>a</sup>
RG	18.79±0.09 <sup>b</sup>	3.20±0.03 <sup>b</sup>	9.44±0.05 <sup>b</sup>	1.00±0.02 <sup>a</sup>	1.95±0.16 <sup>a</sup>
AG4	25.24±0.05 <sup>c</sup>	7.05±0.05 <sup>d</sup>	10.30±0.00 <sup>c</sup>	1.82±0.02 <sup>c</sup>	8.00±0.12 <sup>c</sup>
AG5	20.17±0.08 <sup>b</sup>	6.25±0.22 <sup>c</sup>	10.31±0.08 <sup>c</sup>	1.63±0.02 <sup>b</sup>	6.65±0.26 <sup>b</sup>

<sup>1)</sup>WG, white ginseng; RG, red ginseng; AG4, aged 4-year-old ginseng; AG5, aged 5-year-old ginseng.

Values are means±SD (*n*=3). Means in the same column followed by different letters (a-d) are significantly different at *P*<0.05.

to 1 mL Tris/HCl buffer (100 mM, pH 8.0) containing 2 mM CaCl<sub>2</sub> and 5.5 mM paraoxon. The absorbance of the mixture was measured at 412 nm at 25°C to determine the generation rate of 4-nitrophenol. A molar extinction coefficient of 17100/M/cm was used in calculating the PON activity.

### Statistical analysis

All data are presented as the mean±SE. The data were evaluated by one-way ANOVA using a Statistical Package for Social Sciences software program (SPSS Inc., Chicago, IL, USA), and the differences between the means were assessed using Tukey's test. Statistical significance was considered at *P*<0.05. Correlations between the bioactive compounds and antioxidant activities were determined using Pearson's correlation analysis.

## RESULTS AND DISCUSSION

### Total saponin, phenolic, and flavonoid contents

The aged ginseng exhibited markedly higher total saponin, phenolic, and flavonoid contents than the white and red ginsengs (Table 2). The amounts of phenolics and flavonoids increased with increased concentration of the sample extracts. The red ginseng showed higher saponin and phenolic contents than the white ginseng. It was previously reported that the steaming process during red ginseng production could cause gelatinization of the ginseng starch, resulting in the increase in saponin content (12). A study conducted by Chung et al. (9) also revealed that red ginseng contained higher amount of phenolics than white ginseng. The change in the phenolic content of red ginseng is believed to be caused by the browning reaction during the steaming process of ginseng (9,28). In the present study, it appears that the aging process resulted in a higher saponin and phenolic contents of aged ginseng than the steaming process of red ginseng. While red ginseng is widely known for its high amount of bioactive compounds, its production is complicated and time-consuming, which involves a series of steaming and drying process (29). The production of aged ginseng, on the other hand, only involves a sim-

ple process of heat treatment in an oven, which makes aged ginseng easier and cheaper to produce than red ginseng. Between the two aged ginseng samples, the AG4 exhibited generally higher levels of saponin, phenolics, and flavonoids than the AG5, suggesting that plant age affects the amount of bioactive compounds in the ginseng root and that 4-year-old ginseng may be better for the production of aged ginseng than the 5-year-old ginseng roots.

### *In vitro* antioxidant activity of ethanolic extracts from ginseng

The *in vitro* antioxidant capacity of the ginseng extracts was evaluated based on their DPPH radical scavenging activity and ferricyanide reducing power. The DPPH radical scavenging activity assay, a simple and accurate method in the determination of *in vitro* antioxidant activity of plant extracts, measures the ability of the sample extract to donate an electron to the stable free radical DPPH resulting in the discoloration of the DPPH solution (30, 31). The reducing power, on the other hand, measures the ability of the extract to donate an electron to the Fe<sup>3+</sup>/ferricyanide complex, transforming it to its ferrous form (32). Higher reducing power indicates greater antioxidant activity. The free radical scavenging ability was highest in the aged ginseng among the samples, both at higher and lower concentrations of the extract (Table 3).

**Table 3.** Free radical scavenging activity and reducing power of the ethanolic extracts from ginseng samples

Group <sup>1)</sup>	Free radical scavenging activity (%)		Reducing power (OD at 700 nm)
	5.0 mg/mL	0.5 mg/mL	100 mg/mL
Ascorbic acid	87.52±0.43 <sup>d</sup>	35.01±1.80 <sup>d</sup>	0.69±0.00 <sup>c</sup>
WG	26.67±0.61 <sup>a</sup>	4.72±0.61 <sup>a</sup>	0.28±0.00 <sup>a</sup>
RG	42.06±0.76 <sup>b</sup>	7.10±0.74 <sup>b</sup>	0.33±0.03 <sup>a</sup>
AG-4	83.53±0.29 <sup>c</sup>	10.00±0.83 <sup>c</sup>	0.47±0.02 <sup>b</sup>
AG-5	83.47±0.10 <sup>c</sup>	11.19±1.45 <sup>c</sup>	0.49±0.00 <sup>b</sup>

<sup>1)</sup>WG, white ginseng; RG, red ginseng; AG4, aged 4-year-old ginseng; AG5, aged 5-year-old ginseng.

Values are means±SD (*n*=3). Means in the same column followed by different letters (a-d) are significantly different at *P*<0.05.

The white ginseng extract showed the lowest scavenging activity. Studies in the past have shown that heat processing of ginseng could increase its free radical scavenging activity (31,33). The reducing power was also significantly higher in the aged ginseng compared with that of the white and red ginsengs. The AG4 and AG5 exhibited similar free radical scavenging activity and reducing power. These findings suggest that both the aged ginseng samples have strong antioxidant potential and that their *in vitro* antioxidant capacity is greater than that of the white and red ginseng extracts.

#### ***In vivo* antioxidant activity of ginseng powder**

A high fat diet has been shown to induce the formation of free radicals and reactive oxygen species, resulting in lipid peroxidation and oxidative stress (35). The TBARS concentration is commonly used as an indicator of lipid peroxidation and oxidative stress in laboratory animals. To regulate the destructive potential of free radicals and control oxidative stress, the cells have developed a com-

plex antioxidant defense system that includes antioxidant enzymes. These enzymes catalyze free radicals-quenching reactions. In the present study, the TBARS levels in plasma and erythrocytes and the activities of various antioxidant enzymes in the liver and erythrocytes in mice fed a high fat diet supplemented with ginseng powder were examined. High fat feeding resulted in a significant increase in the TBARS levels in mice (Table 4). On the other hand, addition of ginseng powder in the high fat diet markedly decreased lipid peroxidation. The mice fed the aged ginseng showed lower erythrocyte and plasma TBARS levels than those fed the white and red ginsengs. A marked decrease in the activities of hepatic and erythrocyte SOD, GPx, CAT, GR, and PON enzymes was observed in the high fat-fed mice relative to the control group (Table 5). However, diet supplementation of the ginseng samples significantly increased the activities of these enzymes. In particular, the mice fed the aged ginseng showed higher CAT, GR, and PON activities than those fed the white and red ginsengs. The enzyme SOD protects cells from oxidative damage by transforming superoxide radicals into H<sub>2</sub>O<sub>2</sub>, which are then degraded into non-toxic products by the enzymes CAT and GPx (36). The enzyme GR converts oxidized glutathione into antioxidant reduced glutathione, while the enzyme PON hydrolyzes oxidized phospholipids and destroys lipid H<sub>2</sub>O<sub>2</sub> (37,38). The substantial decrease in the TBARS concentration and enhanced activities of the antioxidant enzymes in the aged ginseng-fed groups indicate a marked improvement in the *in vivo* antioxidant defense status of the animals. In general, the AG4 and AG5 exhibited similar *in vivo* antioxidant capacity.

The current study showed, for the first time, that aging of ginseng could enhance its *in vitro* and *in vivo* antioxidant capacity. Compared with the white and red ginsengs, the aged ginseng had superior antioxidative properties. This may have been partly due to the high levels

**Table 4.** Plasma and erythrocyte TBARS levels in mice fed a high fat diet supplemented with ginseng powder (unit: nmol/mL)

Group <sup>1)</sup>	Erythrocyte TBARS	Plasma TBARS
NC	6.01±0.23 <sup>b</sup>	14.21±0.57 <sup>a</sup>
HF	7.23±0.35 <sup>c</sup>	17.27±0.59 <sup>d</sup>
HF-WG	6.04±0.55 <sup>b</sup>	16.19±0.42 <sup>c</sup>
HF-RG	6.12±0.52 <sup>b</sup>	15.98±0.23 <sup>c</sup>
HF-AG4	5.84±0.28 <sup>b</sup>	15.43±0.43 <sup>b</sup>
HF-AG5	5.37±0.24 <sup>a</sup>	15.27±0.55 <sup>b</sup>

<sup>1)</sup>NC, normal control diet; HF, high fat diet; HF-WG, high fat diet + white ginseng; HF-RG, high fat diet + red ginseng; HF-AG4, high fat diet + aged 4-year-old ginseng; HF-AG5, high fat diet + aged 5-year-old ginseng.

Values are means±SD (*n*=8). Means in the same column followed by different letters (a-d) are significantly different at *P*<0.05.

**Table 5.** Activities of antioxidant enzymes in mice fed a high fat diet supplemented with ginseng powder

	NC <sup>1)</sup>	HF	HF-WG	HF-RG	HF-AG4	HF-AG5
Hepatic enzyme activity (nmol/min/mg protein)						
SOD <sup>2)</sup>	2.88±0.23 <sup>b</sup>	1.42±0.31 <sup>a</sup>	4.92±0.43 <sup>c</sup>	5.23±0.17 <sup>c</sup>	2.62±0.28 <sup>b</sup>	2.40±0.40 <sup>b</sup>
GPx	2.83±0.07 <sup>b</sup>	0.94±0.05 <sup>a</sup>	3.27±0.17 <sup>bc</sup>	3.56±0.38 <sup>cd</sup>	3.84±0.26 <sup>d</sup>	3.71±0.21 <sup>cd</sup>
CAT	0.15±0.02 <sup>b</sup>	0.11±0.00 <sup>a</sup>	0.13±0.00 <sup>ab</sup>	0.14±0.00 <sup>b</sup>	0.19±0.02 <sup>c</sup>	0.19±0.02 <sup>c</sup>
GR	8.03±0.24 <sup>b</sup>	4.21±0.21 <sup>a</sup>	11.20±0.26 <sup>c</sup>	11.39±0.57 <sup>c</sup>	15.64±0.33 <sup>d</sup>	15.09±0.40 <sup>d</sup>
PON	0.97±0.08 <sup>b</sup>	0.54±0.05 <sup>a</sup>	1.02±0.07 <sup>b</sup>	1.17±0.02 <sup>c</sup>	1.38±0.03 <sup>d</sup>	1.22±0.05 <sup>c</sup>
Erythrocyte enzyme activity (nmol/min/mg Hb)						
SOD	7.37±0.59 <sup>b</sup>	5.40±0.55 <sup>a</sup>	7.82±0.61 <sup>b</sup>	8.42±0.50 <sup>b</sup>	7.70±0.47 <sup>b</sup>	8.16±0.42 <sup>b</sup>
GPx	0.16±0.02 <sup>ab</sup>	0.12±0.00 <sup>a</sup>	0.17±0.02 <sup>b</sup>	0.20±0.03 <sup>b</sup>	0.18±0.02 <sup>b</sup>	0.19±0.03 <sup>b</sup>
CAT	0.03±0.00 <sup>b</sup>	0.02±0.00 <sup>a</sup>	0.04±0.00 <sup>c</sup>	0.05±0.00 <sup>d</sup>	0.06±0.00 <sup>e</sup>	0.06±0.00 <sup>e</sup>
GR	0.28±0.02 <sup>c</sup>	0.14±0.02 <sup>a</sup>	0.23±0.00 <sup>b</sup>	0.23±0.02 <sup>bc</sup>	0.39±0.02 <sup>d</sup>	0.35±0.05 <sup>d</sup>

<sup>1)</sup>NC, normal control diet; HF, high fat diet; HF-WG, high fat diet + white ginseng; HF-RG, high fat diet + red ginseng; HF-AG4, high fat diet + aged 4-year-old ginseng; HF-AG5, high fat diet + aged 5-year-old ginseng.

<sup>2)</sup>SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; GR, glutathione reductase; PON, paraoxonase activity. Values are means±SD (*n*=8). Means in the same row followed by different letters (a-e) are significantly different at *P*<0.05.

**Table 6.** Correlation coefficients between the bioactive compounds and antioxidant activities

	Relevant characters		
	Total saponin content	Total polyphenol content	Total flavonoid content
DPPH radical	0.859	0.991**	0.972*
Reducing power	0.824	0.896	0.958*
CAT <sup>1)</sup>	0.910	0.988*	0.983*
PON	0.976*	0.883	0.880

<sup>1)</sup>CAT, catalase activity; PON, paraoxonase activity. Significantly different at \* $P < 0.05$  and \*\* $P < 0.01$ .

of total phenolic and total flavonoid in the aged ginseng. Significant positive correlations were found between the phenolic content and the DPPH radical scavenging activity and CAT enzyme activity (Table 6). Similarly, the flavonoid contents were positively correlated with the DPPH scavenging activity, reducing power, and CAT activity. The saponin content also showed significant positive correlations with PON enzymes activities. The phenolics and flavonoids are natural antioxidant compounds that have the ability to scavenge free radicals and inhibit the formation of reactive oxygen species (32). The aged ginseng may be useful as a functional food with strong antioxidant potential. However, further investigations on the specific bioactive compounds and changes in the composition responsible for the antioxidant activities of aged ginseng are needed.

Results of the present study demonstrated that the aged ginseng has greater *in vitro* and *in vivo* antioxidant activity than the white and red ginsengs. The aged 4-year-old and 5-year-old ginsengs have generally similar antioxidant capacity. The total saponin, phenolic, and flavonoid contents were also substantially higher in the aged ginseng, indicating that the antioxidant capacity of this new ginseng product may be due to its high levels of antioxidant compounds.

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## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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