Comparative Analysis of Ginsenoside Profiles: Antioxidant, Antiproliferative, and Antigenotoxic Activities of Ginseng Extracts of Fine and Main Roots

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ABSTRACT: The aim of this study was to compare ginsenosides profiles, and antioxidant, antiproliferative, and antigenotoxic activities of ginseng extract derived from fine and main roots. The result of the analysis showed a higher total content of ginsenoside in fine roots than in main roots; differences in levels between the different extracts were also confirmed. The oxygen radical absorbance capacity (ORAC) assay showed that H_2O main root extract had a significantly higher activity than that from fine roots. MeOH and H_2O extracts from the fine and main roots also exhibited stronger cellular antioxidant capacity 2,2'-azobis(2-amidinopropane) dihydrochloride-induced oxidative stress in HepG2 cells compared with the positive control. Through calculating the half-maximal inhibitory concentration values, the cytotoxicity of the main root extracts were ranked as follows: MeOH (6.1±1.2 μg/mL)> H_2O (6.6±0.1 μg/mL)> ethanol (10.4±0.6 μg/mL); however, the cytotoxicity of all fine root extracts did not significantly differ. All the fine root extracts showed an inhibitory capacity against 4-hydroxynonenal-induced DNA damage, however only the MeOH extract of the main root showed a decrease in DNA damage. All three solvent extracts from the fine roots reduced DNA damage more in the H_2O_2-treated group, whereas only the MeOH and H_2O extracts of the main roots produced a significant reduction. Levels of Rg3 ginsenoside were positively correlated with indices of the ORAC value, and total ginsenoside contents showed a negative correlation with DNA damage induced by H_2O_2. This study suggests that ginseng and the extraction solvent both affect levels of ginsenoside. Furthermore, the antioxidant potency of ginseng can be attributed to the content of some ginsenosides.

Keywords: ginseng (*Panax ginseng* C.A. Meyer) root, ginsenoside, antioxidant activity, DNA damage, antiproliferative activity

INTRODUCTION

Oxidative stress is initiated by reactive oxygen species (ROS) that are stabilized through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells and, thereby, cause protein and DNA damage and lipid peroxidation (Ames et al., 1993). Oxidative reactions can produce free radicals, including hydroxyl, peroxyl, and superoxide radicals, which can initiate chain reactions that damage cells (Oyinloye et al., 2015). These free radicals have been implicated as potential contributors to the pathogenesis of cancer, diabetes, atherosclerosis, cardiovascular diseases, aging, and inflammatory diseases (Braca et al., 2002; Maxwell, 1995). Consumption of diets that contain foods rich in antioxidants, such as fruits and vegetables, can significantly reduce the risk of many cancers, indicating that such antioxidants could be effective agents for the suppression of carcinogenesis (Fresco et al., 2006).

In Asian countries, ginseng (*Panax ginseng* C.A. Meyer) preparations are renowned as effective tonics that promote a better quality of health and may even prolong life (Yun, 2001). According to many studies, 29 ginsenosides, including Rb1, Rb2, Rc, Rd, Rg1, Re, Rf, and Ro, can be used as reliable biomarkers for ginseng, and are considered in the evaluation of ginseng quality (Christensen et al., 2006; Shi et al., 2013; Sun et al., 2011). Six-year-old ginseng roots have hard necks (rhizome), a body (main root) length of 7 to 10 cm, and a diameter of approximately 3 cm. The roots also have many side branches (lateral roots and fine roots) (Choi, 2008). Based on physical shape, roots of ginseng can be divided into three types:

main, lateral, and fine roots (Christensen, 2008).

The content of ginsenoside is known to vary depending on the variety, growth conditions, and age of the ginseng source. In particular, ginsenoside has been reported in high quantities in the periderm of roots, with a higher content in roots with smaller diameters (Zhang et al., 2014).

However, previous studies on ginseng are limited to investigations such as ginsenoside content analysis (Samukawa et al., 1995; Shi et al., 2013), quality standardization methods (Ahn et al., 2008), and analysis of bioactivity by variety or country of origin (Dong et al., 2011; Zhang et al., 2014). Therefore, in this study we designed a three-stage systematic study to explore the physiological activity of ginseng. First, we analyzed ginsenoside content, which is the typical active ingredient of fine and main roots of ginseng extracts, using three solvents. Different solvent were used since the contents and components of extracts differ according to the solvent polarity (Akowuah et al., 2005). Second, we analyzed the physiological activity of each ginseng sample using the oxygen radical absorbance capacity (ORAC) assay. Moreover, we measure the actual antioxidant activity of each sample in HepG2 cells using the cellular antioxidant capacity (CAC) assay. Finally, we analyzed the antiproliferative activity and ability of ginseng to inhibit DNA damage induced by oxidative stress.

MATERIALS AND METHODS

Chemicals and reagents
Standards of the ginsenosides Rg1, Re, Rf, Rb1, Rg2, Rc, Rh1, Rb2, Rb3, Rd, Rg3, and Rh2 were purchased from Ambo Institute (Seoul, Korea). Acetonitrile and high-performance liquid chromatography (HPLC)-grade water were purchased from J.T. Baker (Phillipsburg, NJ, USA). A solid-phase extraction (SPE) column (Bond Elut Plexa Cartridge 6 mL/200 mg, Agilent, Santa Clara, CA, USA) was used to purify and concentrate the samples. All other chemicals used were of analytical reagent grade.

Plant material and extraction
Six-year-old ginseng plants were obtained from the Gaeseong Ginseng Cooperative Association (Pocheon, Korea). The average annual temperature and precipitation of the Gyeonggi-do region is 10.5°C and 1,300 mL, respectively. Gingseng roots are usually separated into fine and main roots (often based on some chosen root, 2 mm). The ginseng was divided into main and fine roots, which were freeze-dried and stored at −70°C. Solvent extracts of the freeze-dried main and fine roots were prepared as follows. Powdered ginseng (5.0 g) was suspended and extracted with 100 mL of distilled water (H_2O), ethanol

(EtOH), or methanol (MeOH) at 80°C for 2 h. The extracts were filtered through Whatman no. 2 filter paper (Whatman International Ltd., Kent, UK) and rinsed with 50 mL of each extraction solvent. The residue was repeatedly extracted under the same conditions. The resulting two filtrates of the MeOH and EtOH extracts were combined, separated, and evaporated using a vacuum evaporator at 40°C. The water filtrates were frozen and lyophilized. Sample solutions were prepared by resuspending 100 mg dried extract in 1 mL methanol and filtering through a 0.45 μm filter.

Analysis of ginsenosides
SPE column purification: SPE was performed using a Varian Vac Elute SPS 24 vacuum manifold (Varian, Inc., Walnut Grove, CA, USA). The SPE cartridge (Bond Elut Plexa Cartridge 6 mL/200 mg, Agilent) was conditioned with 6 mL methanol and 6 mL water before introduction of the sample solution. A 1.0 mL aliquot of the sample solution was introduced into the SPE cartridge at a flow rate of 0.1 mL/min. Then, a washing step was performed with 1.0 mL ultra-pure water at the same flow rate. The retained analytes were eluted with 1.0 mL MeOH at a flow rate of 0.08 mL/min. The eluate solution was filtered through a 0.22 μm filter prior to analysis.

HPLC determination of ginsenosides: Ginsenosides were determined using the method of Dong et al. (2011) with slight modifications. Analyses were performed using an Agilent 1260 liquid chromatography system (Hewlett Packard, Wilmington, NC, USA) equipped with a quaternary gradient pump and a multiple wavelength detector operating at 203 nm. The samples were separated using a Zorbax Eclipse XDB-C18 column (4.6 mm×150 mm, 5 μm; Agilent) at 35°C with a sample injection volume of 30 μL. The mobile phase consisted of water (A) and acetonitrile (B), and was run on the following gradient: 20% B (0 min), 20% (0~10 min), 32% (10~40 min), 50% (40~55 min), 65% (55~70 min), 90% (70~82 min), and 80% (82~90 min). Data analysis was performed using the Chemstation software (Hewlett Packard, Palo Alto, CA, USA). The flow rate of the mobile phase was 0.9 mL/min.

Preparation of standard solutions: Stock solutions containing the ginsenosides Rg1, Re, Rf, Rb1, Rg2, Rc, Rh1, Rb2, Rb3, Rd, Rg3, and Rh2 were prepared with MeOH. A series of standard working solutions of different concentrations were obtained by diluting the standard stock solution.

Antioxidant activities
A peroxyl radical scavenging capacity ($ORAC_{ROO}$) assay for ginseng fine root extracts prepared using different solvents was carried out using the method described by Ou et al. (2001). The ORAC assay was performed using a

Tecan GENios multi-functional plate reader (Tecan Trading AG, Salzburg, Austria) equipped with fluorescent filters (excitation and emission wavelength: 485 and 535 nm, respectively). The final results were calculated based on the difference in the area under the fluorescence decay curve between the blank and each sample. ORAC values were expressed as micromoles of 1 mM Trolox equivalents (TE).

To determine the effects of the root extracts on intracellular ROS, the peroxyl-radical oxidative stress generated 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was measured spectrofluorometrically using the 2',7'-dichlorofluorescein diacetate (DCFH-DA) method described by Lautraite et al. (2003). HepG2 cells were initially cultured in 96-well plates (5×10^5 cells/mL) for 24 h. After the cells had been incubated with different concentration of ginseng fine and main root extracts for 30 min, the media were discarded, and the wells were gently washed twice with phosphate-buffered saline (PBS), which does not affect the fluorescence. Dichlorofluorescein fluorescence intensity was measured at excitation and emission wavelengths of 485 and 535 nm, respectively, using a Tecan GENios fluorometric plate reader (Tecan Trading AG). DCFH-DA, which is commonly used to detect ROS generated in cells, is taken up by cells and hydrolyzed to DCFH by cellular esterases.

Antiproliferative activity against human colon cancer cells
The cytotoxicity of samples were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, according to the method described by Mosmann (1983). HT-29 cells were preincubated in 96-well plates at a density of 5×10^4 cells/mL for 24 h. Cells were treated with different concentrations of the extracts (0.1, 1.0, and 10.0 μg/mL) and 20 mM glutamate for 24 h. For the MTT assay, the optical density (OD) of each well was measured at a wavelength of 540 nm using an enzyme-linked immunosorbent assay reader (Sunrise™, Tecan Co., Ltd., Grödig, Austria). The OD values of the untreated control cells were designated as 100% (the standard).

Cytotoxicity was determined by measuring the release of lactate dehydrogenase (LDH) using an LDH release assay kit (Wako Pure Chemical Industries, Osaka, Japan). HT-29 cells were seeded in 96-well plates (5×10^4 cells/mL) and cultured overnight (24 h) in the presence of different concentrations of ginseng fine root extracts. The LDH assay reaction was initiated by mixing 50 μL of the cell-free supernatant with potassium phosphate buffer containing nicotinamide adenine dinucleotide and sodium pyruvate to a final volume of 100 μL. The amount of formazan salt produced was proportional to the level of LDH activity in the sample, and the intensity of the resultant red color (measured at 540 nm) was proportional to the activity of LDH.

Antigenotoxic capacity in leukocytes
An alkaline comet assay was conducted according to the procedure described by Singh et al. (1998), with minor modifications. Leukocytes were isolated as a fraction of mononuclear cells (containing lymphocytes and monocytes) derived from two healthy male volunteers through gradient centrifugation using Histopaque®-1077 (Sigma, St. Louis, MO, USA). This study was approved by the Kyungnam University Institutional Bioethics Committee (KUIRB 2013-15). Leukocytes (2×10^4 cell/mL) were incubated with 10 μg/mL extract for 30 min at 37°C in a dark incubator. Leukocytes were then resuspended in PBS and treated with 200 μM 4-hydroxynonenal (HNE) and hydrogen peroxide (H_2O_2) for 5 and 30 min, respectively. Measurements were performed based on image analysis (Komet version 5.0; Kinetic Imaging, Liverpool, UK) and fluorescence microscopy (Leica DMLB; Leica, Wetzlar, Germany), determining the percentage of fluorescence in the tail (% tail intensity) of 50 cells from each of two replicate slides.

Statistical analysis
All data are expressed as the mean of three experimental repeats, and were analyzed using the statistical package for the social sciences (SPSS) package for Windows (version 14.0; SPSS Inc., Chicago, IL, USA). Mean values of all treatments were compared using a one-way analysis of variance (ANOVA) followed by Tukey's b test. A $P<0.05$ was considered statistically significant. Evaluation of the associations between parameters was carried out using Pearson's correlation.

RESULTS AND DISCUSSION

Ginsenosides in extracts
Twelve ginsenosides were detected in the three solvent extracts of ginseng fine and main roots (Table 1). For the fine roots, the total ginsenoside contents in the MeOH, EtOH, and H_2O extracts were 478.5, 214.3, and 294.2 mg/100 g extract, respectively. The differences in the content of ginsenosides between extracts was caused by different polarities affecting extraction (Akowuah et al., 2005). The corresponding total ginsenoside contents in the main roots were 153.3, 98.9, and 262.1 mg/100 g, respectively; these contents were determined to all significantly differ. The 20(S)-protopanaxadiol (PPD) group of ginsenosides were higher in the fine root extracts than all three of the main root extracts, whereas the 20(S)-protopanaxatriol (PPT) group of ginsenosides was only detected in the fine root MeOH extract. Lee et al. (2009) found that the protopanaxadiol : protopanaxatriol ratio

Table 1. Concentration of ginsenosides in different solvent extracts of ginseng fine and main roots   (unit: mg/100 g extract)

	Fine root			Main root		
	MeOH	EtOH	H_2O	MeOH	EtOH	H_2O
20(S)-protopanaxadiol (PPD) groups						
Rb1	79.0 ± 2.5^b	28.2 ± 0.9^a	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Rb2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Rb3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Rc	105.6 ± 3.0^b	38.6 ± 1.6^a	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Rd	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Rg3	103.5 ± 2.8^c	72.7 ± 1.5^b	251.2 ± 7.0^f	124.1 ± 1.7^d	63.6 ± 2.5^a	228.2 ± 4.0^e
Rh2	45.7 ± 2.6^c	74.8 ± 1.6^b	42.9 ± 1.5^c	29.2 ± 1.1^a	35.4 ± 1.5^b	33.8 ± 1.9^b
Sum of PPD group	333.7 ± 9.6^f	214.3 ± 1.0^c	294.2 ± 8.4^e	153.3 ± 2.4^b	98.9 ± 3.9^a	262.1 ± 2.2^d
20(S)-protopanaxatriol (PPT) groups						
Rg1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Re	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Rf	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Rg2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Rh1	144.8 ± 10.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sum of PPT group	144.8 ± 10.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total content	478.5 ± 14.0^f	214.3 ± 1.0^c	294.2 ± 8.4^e	153.3 ± 2.4^b	98.9 ± 3.9^a	262.1 ± 2.2^d

Values are the means with standard deviation (n=3).
Means with different letters (a-f) indicate significant differences at $P<0.05$, as determined by Tukey's b test.

was lower in the main body of the ginseng plant root than in the lateral and fine roots. This is consistent with the results of this study. In addition, when Samukawa et al. (1995) reported the distribution of ginsenosides throughout *P. ginseng* cultivated in Nagano, Japan, they found the contents of ginsenosides were higher in the fine roots than in the main roots. Analysis of individual ginsenosides indicated that Rg3 and Rh2 were present in all fine and main root extracts, whereas Rb1 and Rc were only detected in the fine root MeOH and EtOH extracts, and Rh1 was only detected in the fine root MeOH extract. A comparison of the ginsenosides profiles in fine and main roots indicated that in addition to having a higher total ginsenoside content, fine roots contain a greater diversity of ginsenosides. During ginseng growth, the ginsenoside composition of the different parts of the plant is thought to changes due to migration from sprouts to root hairs, and subsequently to the leaves and main roots (Kim et al., 2014). This is consistent with the findings of Kang and Kim (2016), who analysed the content of ginsenosides in the leaves, main roots, and root hairs of ginseng.

Antioxidant activities

No single *in vitro* assay can be used to determine the total antioxidant properties of samples. Therefore, it is necessary to use a range of different methods to account for the various modes of antioxidant action (Huang et al., 2005; Prior and Cao, 1999). In this study, antioxidant activity was analyzed using extracellular and intracellular systems.

The ORAC assay system has been successfully used to determine inhibition of damage caused by hydroxyl radi-
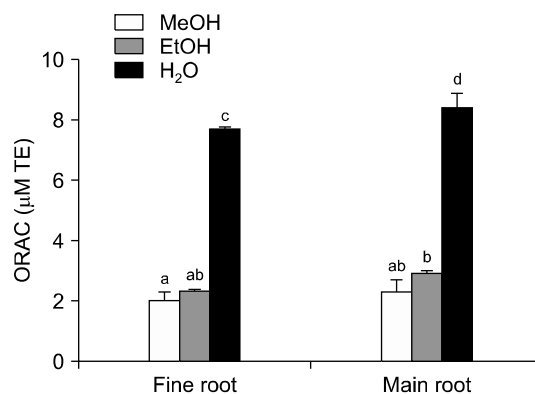

Fig. 1. Peroxyl radical scavenging capacity of different solvent extracts of ginseng fine and main roots. Oxygen radical absorbance capacity (ORAC) values expressed as μM Trolox equivalents (μM TE) obtained in response to treatment with to a 10 μg/mL concentration of ginseng fine and main root extracts. Values represent means±standard deviation (n=3). Bars with different letters (a-d) are significantly different at $P<0.05$, as determined using Tukey's b test.

cals, which are among the most harmful ROS in biological systems. Hydroxyl radicals are generated by reactions involving H_2O_2 (Kim and Jang, 2011). The results of the ORAC assay performed in the present study are presented in Fig. 1. For both fine and main roots, extract activity was found to increase in the order of MeOH, EtOH, and H_2O; H_2O extracts showed significantly higher (approximately four-fold) activity than the other two extracts. ORAC activity exhibited a significant correlation with Rg3 [20(S)] ginsenoside (r=0.515, $P<0.05$). This result indicates that ginsenoside is directly related to the physiological activity of ginseng.

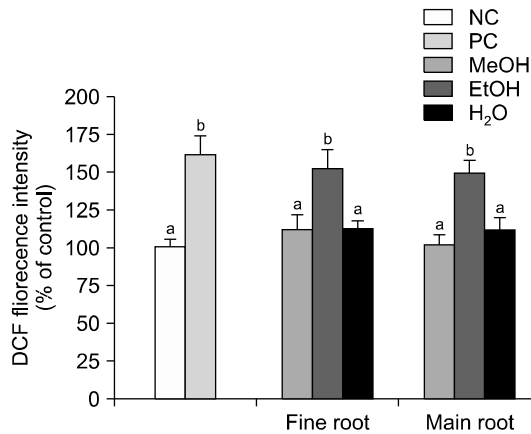Kim (2011) showed that ORAC activity is high in gin-

Fig. 2. Cellular antioxidant capacity of 10 μg/mL concentrations of different fine and main root extracts against oxidative stress induced by 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH) in HepG2 cells. Values represent means±standard deviation (n= 3). Bars with different letters (a,b) are significantly different at $P<0.05$, as determined using Tukey's b test. DCF, dichlorofluorescein; NC, Hank's balanced salt solution–treated negative control; PC, 80 μM AAPH–treated positive control.

seng fractions with high saponin content, which supports the results of our study. Fig. 2 shows the CAC results of ginseng root extracted in HepG2 cells. Cells were pre-incubated with each for 30 min, after which the effects on AAPH-induced oxidative stress were measured. We found that the MeOH and H_2O extracts of both fine and main roots showed the highest CAC against AAPH-induced oxidative stress. The activities of fine and main root EtOH extracts were 150.7% and 147.8%, respectively, indicative of almost no active oxygen removal ability compared with the positive control (PC). Whereas, MeOH and H_2O extracts had more potent peroxyl radical scavengers compared with EtOH extracts.

These results differed from those obtained from the ORAC activity, indicating that the activity of the extract differed from that following intracellular uptake. The CAC assay is complementary to conventional bioactivity assays

used to measure antioxidant levels. HepG2 cells are useful cell models widely used to analyze important biological activities such as cellular uptake, metabolism, and the physiological activity of components (Liu and Finley, 2005). In the present study, we demonstrated that a variety of antioxidant compounds in ginseng extracts, including ginsenoside, may readily permeate the cell membrane and act as antioxidants to scavenge peroxyl radicals.

Jo et al. (2011) investigated the antioxidant activity of different parts of the roots of 6-year-old ginseng and reported that the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and ferric reducing antioxidant power were highest in fine roots, followed by lateral, and then main roots. These results contrast with those obtained in the present study, in which we detected no significant difference in the activities of extracts obtained from different parts of ginseng roots. This discrepancy may be attributable to differences in sample treatment and extraction methods.

Antiproliferative activity in human colon cancer cells
Table 2 shows the results of the analysis of the inhibitory activity of the ginseng extract against human-derived cancer cells. Cell viability was measured using the MTT assay after 24-h treatments with 0.1, 1.0, and 10.0 μg/mL concentrations of the different solvent extracts. The inhibitory effect of all extracts on colon cancer cell growth increased in a concentration-dependent manner. In particular, proliferation of HT-29 cancer cells was reduced over 50% by all extracts at a concentration of 10.0 μg/mL. The half-maximal inhibitory concentration (IC_{50}) values of fine root MeOH, EtOH, and H_2O extracts were 8.9, 7.2, and 7.8 μg/mL, respectively, all of which did not differ significantly. In contrast, IC_{50} values of the main root MeOH, EtOH, and H_2O extracts were 6.1, 10.4, and 6.6 μg/mL, respectively, and the activities of the MeOH and

Table 2. Inhibitory effect of different solvent extracts of ginseng fine and main roots determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and lactate dehydrogenase (LDH) release   (unit: %)

		Fine roots			Main root		
		MeOH	EtOH	H_2O	MeOH	EtOH	H_2O
MTT reduction	NC	100.0±4.5[c]	100.0±4.5[c]	100.0±4.5[c]	100.0±9.6[c]	100.0±9.6[c]	100.0±9.0[c]
	0.1	66.8±4.9[b]	60.0±6.6[b]	52.0±7.0[b]	52.7±3.8[b]	55.4±9.2[b]	56.6±5.8[b]
	1.0	63.2±5.3[b]	51.3±1.9[ab]	44.2±3.5[a]	48.1±9.6[a]	55.5±2.2[b]	44.5±3.7[ab]
	10.0	47.6±0.8[a]	42.4±5.9[a]	42.9±1.5[a]	46.9±1.9[a]	37.3±3.0[a]	39.8±1.1[a]
	IC_{50} (μg/mL)	8.9±1.0[AB]	7.2±1.8[AB]	7.8±2.2[AB]	6.1±1.2[A]	10.4±0.6[B]	6.6±0.1[A]
LDH release	NC	8.7±0.2[a]	8.7±0.2[a]	8.7±0.0[a]	8.7±0.2[a]	8.7±0.2[a]	8.7±0.2[a]
	0.1	9.6±0.4[b]	8.9±0.9[a]	8.9±0.2[a]	8.8±0.7[a]	9.9±0.3[a]	9.2±0.9[a]
	1.0	10.5±0.5[c]	10.5±0.4[b]	10.6±0.9[b]	10.0±0.7[b]	10.4±0.7[b]	11.0±0.4[b]
	10.0	12.0±0.2[d]	11.8±0.6[c]	12.6±0.6[c]	12.2±0.5[c]	11.0±0.9[c]	12.3±0.5[c]

Values are the means with standard deviation (n=3).
Means with different small letters (a–c) within same column and capital letters (A,B) within the row indicate significant differences at $P<0.05$, as determined by Tukey's b test.
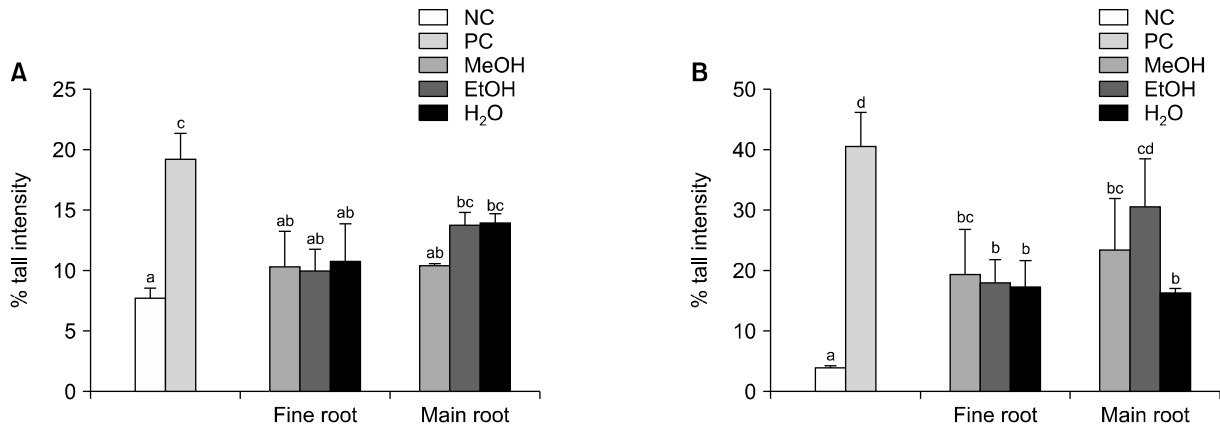
Fig. 3. Comet assay determination of antigenotoxic activity of different solvent extracts of ginseng fine and body main roots. Values represent means±standard deviation (n=3). Bars with different letters (a-d) are significantly different at $P<0.05$, as determined using Tukey's b test. NC, 1% dimethyl sulfoxide-treated negative control; PC, positive control. (A) 200 μM 4-hydroxynonenal-treated positive control; (B) 200 μM H_2O_2-treated positive control.

H_2O extracts were significantly higher than that of the EtOH extract. Sodrul et al. (2017) reported that the anti-cancer effects of ginseng could be attributed to saponin. In another study, Lee (2009) reported that proliferation of HT-29 cells is inhibited in a dose-dependent manner by 20(S)-Rg3 and 20(R)-Rg3, and is by approximately 78% following treatment with 600 μM 20(S)-Rg3. In contrast, 20(R)-Rg3, an isomer of 20(S)-Rg3, induces 10~20% lower activity.

Cancer cell death due to cell membrane damage was measured using an LDH release assay. All concentrations of all solvent extracts showed significantly higher cyto-toxicity than the negative control (NC). Nevertheless, at the highest extract concentration, LDH activity was found to be very low (approximately 10% cytotoxicity). Although this result differs slightly from those of two aforementioned studies, we determined that the anticancer activity of ginseng is, to a certain extent, influenced by the presence of ginsenosides.

Antigenotoxic capacity in human leukocytes
The protective effects of ginseng extracts against oxidative stress-induced DNA damage are shown in Fig. 3. HNE is formed by radical-initiated degradation of polyunsaturated fatty acids and is a sensitive marker of lipid peroxidation and oxidative stress (Deutsch, 1998). In contrast, H_2O_2 is believed to cause DNA strand breakage by generating hydroxyl radicals close to DNA molecules, via the Fenton reaction (Abrahamse, 1999). When the protective effects of 10 μg/mL ginseng root extracts on oxidative stress-induced DNA damage were examined in response to treatment with 200 μM HNE (Fig. 3A) and H_2O_2 (Fig. 3B), DNA damage was shown to be reduced in all groups. When leukocytes were subjected to HNE-induced oxidative stress in the presence of the solvent extracts, all fine root extracts exhibited strong inhibition of DNA damage from HNE-induced oxidative stress. Only

the MeOH extract of the main root extracts exhibited a significant difference compared to the PC. Fine and main root extracts showed maximum and minimum suppressions of 48% (EtOH extract of fine root) and 27% (H_2O extract of main root), against DNA damage caused by HNE-induced oxidative stress. All extracts significantly inhibited DNA damage induced by oxidative stress with H_2O_2; the lowest effects were observed for EtOH (17.9%) and H_2O (17.2%) extracts. Main root extracts showed the lowest antigenotoxic capacities; the activity of H_2O and MeOH extracts were 16.3%, and 23.3%, respectively, whereas, the activity of the EtOH extract (30.4%) did not significantly differ significantly from that of the PC (39.9 %). These results reveal that ginseng extracts have protective effects against DNA damage mediated by oxidative stress induced by HNE and H_2O_2; however, the differences between the sources is not clear. This is consistent with the trend reported by Yoo and Lee (2009) who found that ginseng extracts significantly reduced DNA damage in lymphocytes treated with 30 μM phenanthrene. Thus, it appears that ginseng extracts suppress
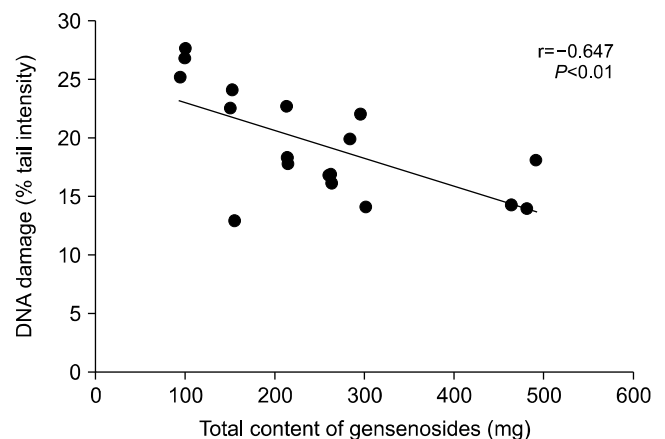

Fig. 4. Correlations between total ginsenoside and antigenotoxic activity against hydrogen peroxide-induced oxidative stress.

DNA damage caused by oxidative stress induced by a variety of substances. The correlation between ginsenoside contents and inhibition of DNA damage from H_2O_2-induced oxidative stress is shown in Fig. 4. We observed a negative correlation between total ginsenosides and antigenotoxic activity (r=-0.647, $P<0.01$), therefore concluding that inhibition of DNA damage in lymphocytes is directly affected by levels of ginsenoside.

In Asian countries, ginseng preparations are renowned tonics that are considered to be effective for promoting a better quality of health and for even prolonging life (Yun, 2001). In recent years, ginseng has become the preferred functional food since it has been evaluated as the most efficacious among natural health foods (Baeg and So, 2013). Previous studies of ginseng have certain limitations, which we sought to overcome by extracting the active ingredient ginsenoside from two different parts ginseng plants using three different solvents. Furthermore, various indexes were used to examine the antioxidant, antiproliferative, and antigenotoxic activities.

This study shows that ginseng has antioxidant, antiproliferative, and antigenotoxic effects, and that the activity differs according to the part of the plant and extraction solvent. We hypothesized that high ginsenoside levels could also contribute to high antioxidant activity. However, the results of this study were not entirely consistent.

Nevertheless, there was a positive correlation between ORAC activity and Rg3, and a negative correlation between total ginsenoside content and inhibition of DNA damage caused by H_2O_2-induced oxidative stress. Therefore, it is highly likely that ginsenosides have some effects on antioxidative activity. A limitation of this study is that other various active constituents of ginseng were not considered. Ginseng also contains numerous diverse and potentially active constituents, including phenolic compounds, polyacetylene, alkaloids, essential oil components, proteins and peptides, free sugars, and fatty acids (Kim, 2011). Therefore, future studies should provide reliable basic data on the efficacy of ginseng by examining the contents and activities of the various components, and by performing a more comprehensive correlation analysis.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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