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Antioxidant Activity of Main and Fine Roots of Ginseng (*Panax Ginseng* C.A. *Meyer*) Extracted with Various Solvents

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Abstract The objective of this study was to investigate antioxidant activities of freeze-dried, main root, and fine root of ginseng (*Panax ginseng* C.A. *Meyer*), which were extracted with various solvents including ethanol, methanol, and water. Ethanol extracts in both parts showed the most powerful scavenging activities against DPPH radicals. Especially, ethanol extract of fine root had higher reducing power and antioxidant capacity than that of main root. The highest antioxidant activity in linoleic acid emulsion system was also observed in fine root extracted with ethanol, followed by methanol and water. Both ferrous ion chelating activity and ferric reducing antioxidant power (FRAP) of extracts were increased with the increase of extracts concentration. These results suggest that ethanol extract of fine root of ginseng has the most effective antioxidant capacity compared to the methanol and water extracts tested in the present study. Thus it can be applied for the effective extraction of functional material from ginseng for the usage of pharmaceutical and/or food industries.

Keywords: ginseng, antioxidant activity, various solvent extract

Introduction

Ginseng (*Panax ginseng* C.A. *Meyer*) is a valuable herb that has been used extensively in eastern Asia, such as Korea, China, and Japan for more than 5,000 years. Ginseng is known as one of the most famous medicinal plants in the world and its efficacy as a medicine has been studied for a long time (1). Ginseng means literally 'the essence of the human' and is also known as the 'king of the herbs' (2). The ginseng genus (panax) consists of 17 species. Among them, 3 species including *P. ginseng* (ginseng), *P. quinquefolius* (American ginseng), and *P. notoginseng* (*sanchi*) are recognized as a medicine in China and thus they are well cultivated (3). *Panax ginseng* is categorized as either cultivated or wild, according to the different nurturing methods. Cultivated ginseng is systematically farmed on open land and harvested after 5-6 years when the growth rate and concentration of the active chemical constituents have peaked. On the other hand, wild ginseng is planted as seedlings in secluded mountain areas at an altitude between 800 to 1,500 m (4). Commercially available ginseng is classified into white and red ginseng. White ginseng is made by peeling the fresh ginseng roots and drying them without steaming. Red ginseng is made by drying the fresh ginseng with steaming, called transformation which used heat to preserve ginseng for an extended period of time (5).

Recently, there has been a renewed interest in investigating pharmacological activities of ginseng using biochemical and molecular biological techniques. The biochemical and pharmacological activities of the ginseng have been demonstrated in the central nervous system (CNS), cardiovascular, endocrine, and immune systems. Numerous studies show that ginseng with many active components does have beneficial effects, such as antiaging, antidiabetic, anticarcinogenic, analgesic, antipyretic, and antistress, antifatigue, tranquilizing activities, promotion of DNA, RNA, and protein synthesis activities, and antioxidant activity (6-8). All of the extracts of *Panax ginseng* roots possessed the antioxidant activity and inhibited autoxidation of methyl esters of unsaturated fatty acid (MEUFA) (9). Especially, the antioxidant and free radical scavenging effects of ginseng and some of its selected ingredients have been extensively investigated and well documented (10-12). In addition, ginseng extract was also reported to inhibit lipid peroxidation through transition metal chelation (11-13). However, few studies have been conducted to compare the antioxidant activities of fine and main roots extracts with different polarity. In this study, freeze-dried of main and fine roots of ginseng that extracted with ethanol, methanol, and water, were investigated for their antioxidant activities and compared to BHT, BHA, Trolox by measuring total phenolics, DPPH radical scavenging activity, ferrous ion chelating activity, reducing power, TBA-reactive species (TBARs) and ferric reducing antioxidant power (FRAP) assay.

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Materials and Methods

Plant material The 6-year-old ginseng plants were collected from Gaeseong Ginseng Cooperative Association (Gyeonggi, Korea). The average temperature and precipitation of the area of Gyeonggi-do throughout a year was 10.5°C and 1,300 mL, respectively. The ginseng was divided into main and fine roots, which were freeze-dried and stored in −70°C freezer.

Chemicals 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 5,7, 8-tetramethychroman-2-carboxylic acid (Trolox, 97%), butylated hydroxytoluene (BHT), bulylated hydroxy anisole (BHA), Folin-Ciocalteu's phenol reagent, ferrozine, potassium ferricyanide, gallic acid, ferric chloride, trichloroacetic

acid, 2, 4, 6-tripyridyl-s-triazine (TPTZ), and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the chemicals were of the best available analytical grade.

Extraction Each solvent extracts of freeze-dried of main and fine roots were obtained as follows. Powdered ginseng (5.0 g) was suspended and extracted with each 100 mL of distilled water, ethanol, or methanol at 80°C for 2 hr. Each extracts was filtered through Whatman #2 filter paper (Whatman International Limited, Kent, England) and rinsed with 50 mL of each extraction solvent. Extraction of the residue was repeated using the same conditions as above. The 2 filtrates of methanol or ethanol were combined, respectively, and evaporated using a vacuum evaporator at 40°C. Water filtrate was frozen and lyophilized. The extracts were placed in a glass bottle and stored at −20°C until used.

Determination of percentage dry weight The main and fine roots were accurately weighed and dried to obtain constant weight in an oven at 105°C for 48 hr (14).

Determination of total phenolics Total phenolic substances in each solvent extracts was determined with Folin-Ciocalteu reagent according to the method of Singleton and Lamuela-Raventos (15). Dried solvent extracts (0.1 g) were extracted with 40 mL of 80% acetone at room temperature for 25 min in sonication bath followed by centrifugation at 3,800×g for 10 min. After the supernatant was collected, the precipitate was re-extracted as above. After the 1st and 2nd extraction, the supernatants were combined and the supernatants volume brought up to 100 mL with 80% acetone. One mL of extracts and 1.0 mL of diluted Folin-Ciocalteu reagent were mixed. After 3 min, 1.0 mL of 10% sodium carbonate was added to the mixture and was allowed to stand for 1 hr. Absorbance of the extract was measured at 760 nm and the read was compensated to standard gallic acid.

DPPH radical scavenging activity The free radical scavenging activity of each solvent extracts was measured by the DPPH method proposed by Brand-Williams *et al.* (16). Five-tenth mL of the sample at various concentrations was added to 1.0 mL of 0.1 mM solution of DPPH in ethanol and the mixture was shaken vigorously. The absorbance was measured at 525 nm after 20 min. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{DPPH radical scavenging activity (\%)}=[(A_0-A_1)/A_0]\times 100$$

where, A_0 was the absorbance of the control and A_1 was the absorbance with test compound.

Ferrous ion chelating activity Metal chelating activity was determined according to the method of Dinis *et al.* (17). The extract was added to 0.05 mL of 2 mM $FeCl_2$ solution. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated in the following equation:

$$\text{Ferrous ion chelating activity}=[(A_0-A_1)/A_0]\times 100$$

where, A_0 was the absorbance of the control and A_1 was the absorbance with the sample.

Reducing power The reducing power of each solvent extracts was determined according to the method of Oyaizu (18). One mL of extracts, 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1.0 mL potassium ferricyanide (10 mg/mL) were mixed and incubated at 50°C for 20 min. One mL of trichloroacetic acid (100 mg/mL) was added to the mixture and centrifuged at 12,000×g for 5 min. The supernatant was mixed with distilled water (1 : 1) and 0.1 mL of ferric chloride (1.0 mg/mL). The absorbance of the mixture was measured at 700 nm.

Antioxidant activity in linoleic acid emulsion system The antioxidant activity of each solvent extracts with different periods of incubation was measured in a linoleic acid model system according to the modified methods of Hayase and Kato (19) with some modifications. One g of linoleic acid was dissolved in 20 mL ethanol and added to a 25 mL of 0.2 M phosphate buffer (pH 7.0). Sample was added to a 1% of linoleic acid emulsion total weight. The mixture was incubated in a conical tube with a screw cap at 50±1°C for 12 days. The degree of oxidation was evaluated by measuring the peroxide values. The peroxide value was determined according to AOAC method (20) and expressed as meq O_2/kg lipid.

Antioxidant activity by FRAP assay The FRAP assay was done according to the modified Benzie and Strain method (21) with some modifications. The stock solutions included 300 mM acetate buffer, pH 3.6 (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL $C_2H_4O_2$), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solution and then warmed at 37°C before using. One-hundred-fifty mL of ginseng fine root extracts were allowed to react with 2,850 mL of the FRAP solution for 30 min in the dark. Readings of the colored products (ferrous tripyridyltriazine complex) were then taken at 593 nm. The standard curve was linear between 25 and 800 mM Trolox. Results are expressed in mM/mg fresh mass.

Statistical analysis All experimental data were analyzed using analysis of variance (ANOVA) and significant differences ($p<0.05$) among means from triplicate analysis were determined by Duncan's multiple range test using the statistical analysis system (SPSS version 12.0).

Results and Discussion

Moisture content, yield, and contents of total phenolic compounds Phenolic compounds are commonly found in both edible and inedible plants and they have been reported to have multiple biological effects, including antioxidant activity (22). Table 1 shows the effect of various solvents on yield and total phenolic content in fine

Table 1. Effect of extract solvents on yield and total phenolic contents in fine and main roots[1)]

Solvent	Fine root		Main root	
Extracts	Yield[2)]	Total phenolics[3)]	Yield	Total phenolics
EtOH	15.43±2.19[c]	499.06±67.87[a]	10.68±1.24[c]	447.06±43.37[a]
MeOH	36.39±1.64[b]	413.17±17.35[b]	24.55±1.35[b]	362.89±16.15[b]
H_2O	53.09±1.33[a]	330.11±25.46[c]	44.61±2.46[a]	325.39±21.32[c]

[1)]Values are mean±SD (n=3); [a-c]means in a column followed by different superscripts are significantly different at the $p<0.05$ level.
[2)]g/100 g of ginseng, dry matter basis.
[3)]mg/100 g of extract, dry matter basis.

and main roots. Moisture content of fine and main roots were 79.44 and 75.38%, respectively (data not shown). Water extracts produced the highest yields of 53.09 g for fine root and 44.61 g for main root. However, ethanol extracts produced the highest amounts of phenolics from both main root (447.06 mg) and fine root (499.06 mg), which are significantly ($p<0.05$) different from those of methanol and water extract. The enrichment of phenolic compounds within plant extracts is correlated with their enhanced antioxidant activity (23,24). This is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (25). Wee *et al.* (26,27) isolated and identified 4 phenolic acids (salicylic acids, *p*-coumaric acid, gentisic acid, and caffeic acid) from antioxidant fractions in Korean ginseng. Wee *et al.* (28) identified 2 more phenolic acids (*p*-hydroxybenzoic acid and 1H-indole-2-carboxylic acid) in Korean ginseng. Kim *et al.* (29) isolated and identified 2 free phenolic acids (ferulic and vanillic acid) from Korean ginseng. Phenolic acids are reportedly present as 3 different forms of free, esterified, and insoluble-bound phenolic acids in plants (30-32). For the effective utilization of ginseng as a medicinal herb or functional food component, qualitative and quantitative information on the phenolic acids in ginsengs is essential.

DPPH radical scavenging activity The DPPH radical scavenging activities of various solvent extracts from fine and main roots are shown in Fig. 1. The stable radical DPPH activity has been widely used for the determination of primary antioxidant activity for pure antioxidant compounds, plant and fruit extracts and food materials (33). The antiradical activity of phenolic compounds depends on their molecular structure, such as availability of phenolic hydrogens and the possibility of stable phenoxyl radicals formation (34,35). A close to linear correlation between DPPH radical scavenging activity and concentrations of polyphenolic compounds in various vegetable and fruits have been reported (36,37). In the present study, DPPH radical scavenging activity of fine root extract was significantly ($p<0.05$) higher than that of main root extract, regardless of solvents. This indicates that fine root of ginseng can be a better source for the antioxidant compounds. Among the solvents, ethanol extract showed the most powerful scavenging activities against DPPH radicals. However, its effect was not greater than that of BHT or BHA. These results suggest that the amount of phenolics extracted the most from fine root with ethanol is related to DPPH radical scavenging ability of ginseng.

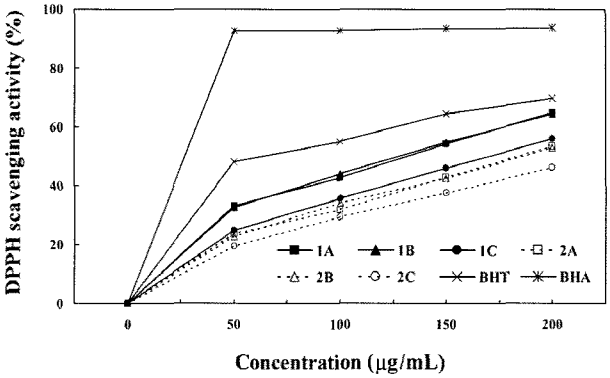

Fig. 1. DPPH radical scavenging activity of various solvent extracts from ginseng fine and main roots. 1A, Ethanol extract of fine root; 1B, methanol extract of fine root; 1C, water extract of fine root. 2A, Ethanol extract of main root; 2B, methanol extract of main root; 2C, water extract of main root.

Ethanol extract of ginseng is also known as primary antioxidants, which is the major initiator of the autoxidation chain of fat, thereby terminating the chain reaction (38,39).

Ferrous ion chelating activity Transition metals such as iron can stimulate lipid peroxidation by generating hydroxyl radicals through Fenton reaction. In addition, they can accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals, thus force the reaction of lipid peroxidation, which is implicated in many human diseases (40). The method of chelating activity is based on chelating of Fe^{2+} by the reagent ferrozine (17). In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased (41). We investigated ferrous ion chelating activity of ginseng extract. Result is shown that chelating activity of the ethanol extract is slightly higher than that of water or methanol extracts. As observed in DPPH radical scavenging activity, fine root has significantly ($p<0.05$) higher chelating activity than main root, regardless of solvent. Overall, chelating activity of the ethanol extract of fine root with ferrous ion was improved with the increasing concentration of extract; 22.64% at 2 mg/mL was increased up to 59.56% at 8.0 mg/mL (Fig. 2). Strong ferrous ion chelating activity of ethanol extract has been also observed in *P. notoginseng* (42). Ferrous ions are the most effective pro-oxidants in food system (43), thus effective Fe^{3+} ions chelating ability of ethanol extracts from ginseng can explain the superior antioxidant activity.

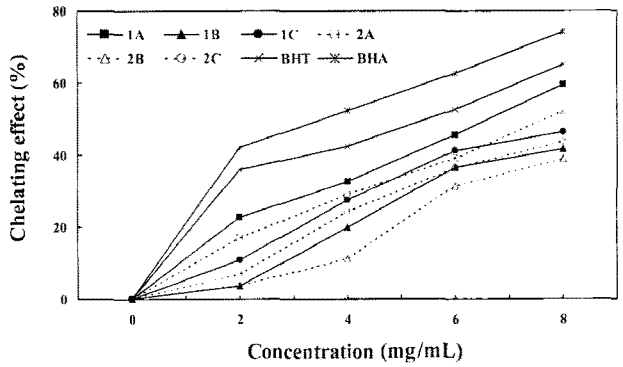

Fig. 2. Ferrous ion chelating activity of various solvent extracts from ginseng fine and main roots. 1A, Ethanol extract of fine root; 1B, methanol extract of fine root; 1C, water extract of fine root. 2A, Ethanol extract of main root; 2B, methanol extract of main root; 2C, water extract of main root.

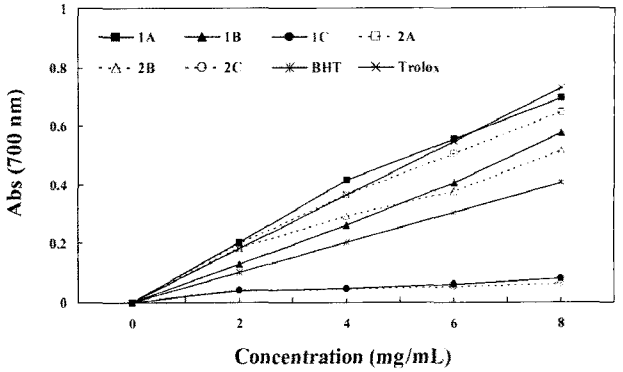

Fig. 3. Reducing power of various solvent extracts from ginseng fine and main roots. 1A, Ethanol extract of fine root; 1B, methanol extract of fine root; 1C, water extract of fine root. 2A, Ethanol extract of main root; 2B, methanol extract of main root; 2C, water extract of main root. BHT and Trolox was dissolved to a concentration of 0-0.8 mg/mL.

Reducing power In the reducing power assay, the presence of reductants (antioxidants) in the test samples reduce Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}), which can be monitored by measuring the formation of Perl's Prussian blue color at 700 nm (44). Numerous studies observed a direct correlation between antioxidant activity and reducing power of certain plant extracts (45,46). The reducing power is generally associated with the presence of reductones (45), which break the free radical chain by donating a hydrogen atom (47) or react with certain precursors of peroxide, thus preventing peroxide formation (48). In the present study, the Fe^{3+} to Fe^{2+} reduction in the presence of the extracts was investigated to examine the reducing power of various solvent extracts from main and fine roots, which were compared to those of BHT and Trolox. The highest reducing power was also found in ethanol extract of fine root. The reducing power of ethanol and methanol extracts increased with the increase of extract concentration, while the reducing power of BHT and Trolox did not change above the concentration of 2 mg/mL. The reducing power of ginseng was significantly lower than those of BHT and Trolox at even at high concentration of 8 mg/mL. As expected, water extract from both parts had the weakest reducing power, which did not change with the concentration of extract (Fig. 3). Water extract of *P. notoginseng* exhibited no reducing power in a Fe^{3+}-Fe^{2+} system (42). The ginseng extract examined in this study demonstrated good reducing capacity thereby can be potentially used as efficient reductones.

Antioxidant activity in linoleic acid emulsion system Lipid peroxidation leads to rapid development of rancid and stale flavors and is considered as a primary mechanism of quality deterioration in lipid foods and oils (49). Antioxidant activity in linoleic acid emulsion system involves lipid peroxidation of biological tissue (oxidative degradation of polyunsaturated fatty acid in the cell membranes), which cause cell membrane destruction and damage and generates a number of degradation products, such as malondialdehyde (MDA) (50). The peroxide values of all ginseng extracts in linoleic acid emulsion system showed similar pattern as a function of time. Compared to methanol and water extracts, ethanol extracts exhibited lower peroxide values. Overall, peroxide value in ethanol extract of root is significantly ($p<0.05$) lower than that of main root (Table 2). In addition, peroxide values

Table 2. Peroxide values of various solvent extracts from fine and main roots as a function of time[1)]

Part	Solvent Extracts	Incubation time (day) meq O_2/kg 0	3	6	9	12
	Control[2)]	0	418.75±27.26^{a}	468.92±48.25^{a}	406.72±34.06^{a}	501.42±49.02^{a}
	BHT	0	206.42±30.70^{de}	256.42±17.48^{ef}	231.42±17.48^{e}	336.67±12.51^{bc}
	Trolox	0	168.25±18.15^{e}	206.42±25.28^{f}	208.42±24.67^{e}	323.92±28.59^{c}
	EtOH	0	281.42±35.25^{bc}	306.08±41.03^{de}	293.72±37.60^{d}	368.25±26.61^{bc}
Fine root	MeOH	0	312.67±20.53^{b}	369.58±27.75^{bc}	346.42±27.12^{bcd}	451.75±35.86^{a}
	H_2O	0	318.42±26.27^{b}	375.18±35.59^{bc}	356.25±44.44^{abc}	461.25±34.04^{a}
	EtOH	0	218.25±28.16^{de}	326.25±45.60^{cd}	313.25±37.75^{cd}	383.92±34.48^{b}
Main root	MeOH	0	243.58±24.85^{cd}	388.58±26.39^{b}	369.42±26.36^{abc}	473.58±26.39^{a}
	H_2O	0	256.08±35.11^{cd}	401.75±17.30^{b}	386.75±20.57^{ab}	481.42±25.85^{a}

[1)]Values are mean±SD (n=3); $^{a-f}$means in a column followed by different superscripts are significantly different at the $p<0.05$ level.
[2)]No sample was added to the linoleic acid emulsion.

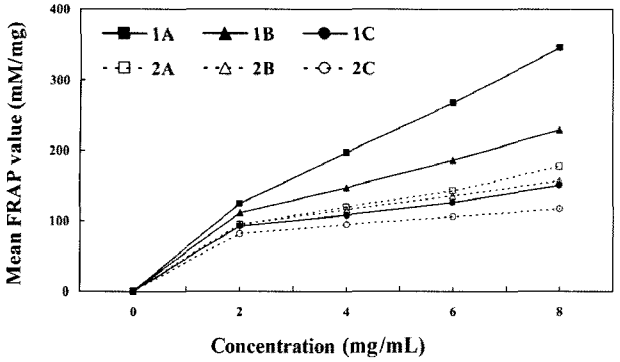

Fig. 4. FRAP values of various solvent extracts from fine and main roots. 1A, Ethanol extract of fine root; 1B, methanol extract of fine root; 1C, water extract of fine root. 2A, Ethanol extract of main root; 2B, methanol extract of main root; 2C, water extract of main root.

were increased as the increase of incubation time but decrease of peroxide values was observed on the 9th day and increased again thereafter. This result might be due to the conversion of peroxides into secondary oxidation products. In this assay, we have noticed that the inhibition degree of lipid peroxidation was varied according to the kinds of the extract solvent used.

Antioxidant capacity by FRAP assay Several methods have been developed to evaluate the total antioxidant activity of fruits or other plants and animal tissues. Among them, Trolox equivalent antioxidant capacity (51), total radical absorption potentials (52), oxygen radical absorption capacity assays (53,54), the ferric reducing ability of FRAP assay (21) are commonly used in various investigations. In the present study, we selected the FRAP assay to evaluate the reductants (antioxidant) activities of fine root and main root extracts with different polarity. The FRAP assay measures the antioxidant effect of any substance in the reaction medium as reducing ability as well as measures the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants (55). This assay is also commonly used for the routine analysis of single antioxidants and total antioxidant activity of plant extracts (55-57). As shown in Fig. 4, the FRAP value of ginseng extracts increased as the concentration of extracts increased. As we noticed in other tests, the ethanol extract showed higher FRAP value than methanol and water extract, indicating strong reducing activity of ethanol extracts. Superior reductants activity was also observed in fine root.

In conclusion, we investigated various solvents for their effectiveness to extract antioxidant compounds from freeze-dried, main and fine roots of ginseng and compared to BHT, BHA, and Trolox. Overall, all extracts from fine root demonstrated better antioxidant activities compared to those of main root. Among the solvents tested in this study, ethanol extract of fine root of ginseng has the highest antioxidant activities although its effectiveness as an antioxidant was not superior to chemical antioxidants, such as BHT, BHA, and Trolox. However, the ginseng extracts, especially from fine root demonstrated good antioxidant capacities, thereby can be potentially used as production of the value-added functional food products for food industries.

Acknowledgments

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