

## Antioxidant Activities of Various Solvent Extracts from Ginseng (*Panax ginseng* C.A. Meyer) Leaves

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### Abstract

Water, methanol and ethanol extracts of ginseng leaves were assayed for total phenolics and flavonoids, ascorbic acid, cupric and ferrous ion chelating activities, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP) assay and ABTS radical cation decolourization (TEAC) assay for their antioxidant properties. The ethanol extract of ginseng leaves contained significantly ( $p < 0.05$ ) higher amounts of total phenolics and flavonoids (600.57 and 1701 mg/100 g) than methanol (374.43 and 1512.64 mg/100 g) and water extracts (248.30 and 680.05 mg/100 g). Among solvent extracts of ginseng leaves, the ethanol extract showed the most powerful antioxidant activities. However, the ferrous ion chelating activity of ginseng leaf extracts were lower than the cupric ion chelating ability. These differences in concentrations of key antioxidants among various solvent extracts seemed to be responsible for their differences in antioxidant activities. These results suggest that ethanol extract of ginseng leaves 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 leaves for the usage of pharmaceutical and/or food industries.

**Key words:** ginseng leaves, antioxidant activities, various solvent extracts

### 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). 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 are well cultivated (2). 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. Traditionally, *P. ginseng* leaves have been consumed mostly in the form of tea (3). Most ginseng leaf tea produced in Korea is exported abroad and studies on its hygienic safety and quality has been reported (4,5).

Recently, there has been a renewed interest in investigating pharmacological activities of ginseng using biochemical and molecular biological techniques. Ginseng is composed of a mixture of glycosides, essential oils, and a variety of complex carbohydrates and phytosterols as well as amino acids and trace minerals (6). The principle active ingredients of ginseng are believed to be a

complex mixture of over 30 triterpenoid saponins commonly referred to as ginsenosides, which are present in the leaves, stems and berries in addition to the traditionally harvested roots (7). Many studies have been conducted to elucidate "magical" healing activities of ginsengs. Although most studies have been focused on ginseng roots, attention is now increasingly paid to ginseng leaves. Pharmaceutical activities of ginseng leaves mainly come from their abundant polysaccharides, phenolics, flavonoids and ginsenosides (8-10). However, few studies have been conducted to compare the antioxidant activities of ginseng leaf extracts with different polarities. In this study, water, methanol and ethanol extracts of ginseng leaves were investigated for their antioxidant activities, compared to BHA and Trolox, by measuring total phenolics and flavonoids, ascorbic acid, cupric and ferrous ion chelating activities, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP) assay and ABTS radical cation decolourization (TEAC) assay.

### MATERIALS AND METHODS

#### Chemicals

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>), kaempferol, Folin & Ciocalteu's phenol reagent, gallic acid,

(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) Trolox, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS<sup>•</sup>), pyridine, pyrocatechol violet and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were of analytical reagent grade.

#### Sample preparation

The ginseng leaves were collected from 6-year-old ginseng plants in the Gaeseong Ginseng Cooperative Association (Gyeonggi, Korea). The average temperature and precipitation of the area of Gyeonggi-do throughout the year was 10.5°C and 1,300 mL, respectively. Soon after collection, the ginseng leaves were freeze-dried by using a freeze dryer (FDU-1200, Eyela, Toyko, Japan), ground in a mill and passed through a 250 µm diameter mesh sieve. The powder was stored in -70°C freezer for further experiments.

#### Extraction of ginseng leaves

Each solvent extract of freeze-dried ginseng leaves were obtained as follows. Powdered ginseng leaves (5.0 g) were suspended and extracted with 20 volumes of distilled water, ethanol, or methanol at 80°C for 2 hr. The extracts were 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. The two filtrates each of methanol and ethanol were combined and evaporated using a vacuum evaporator at 40°C to dryness. Water filtrate was frozen and lyophilized. The extracts were placed in a glass bottle and stored at -20°C until used.

#### Determination of total phenolics

Total phenolic substances in ginseng leaf extracts were determined with Folin-Ciocalteu reagent according to the method of Singleton and Lamuela-Raventos (11). Dried solvent extracts (0.1 g) were extracted with 40 mL of 80% acetone at room temperature for 25 min in a sonication bath followed by centrifugation at 3800 rpm for 10 min. After the supernatant was collected, the precipitate was re-extracted as described above. The supernatants, which were obtained by 1st and 2nd extraction, were combined and made up to 100 mL with 80% acetone. One mL of extracts and 1.0 mL of diluted Folin-Ciocalteu's reagent were mixed. After 3 min, 1.0 mL of 10% sodium carbonate was added to the mixture and allowed to stand for 1 hr. The concentration of total phenolic contents was measured by reading absorbance at 760 nm and the read was compensated to standard gallic acid.

#### Determination of ascorbic acid content

Ascorbic acid was quantitatively determined according to the 2,6-dichloroindophenol-Na dye method described by Kirk and Sawyer (12) with slight modifications. Dried solvent extracts (1.0 g) were sonicated for 5 min by 6% metaphosphoric acid (v/v). The extract was made up accurately to a suitable volume (10 mL), mixed and centrifuged at 3000 rpm for 15 min at room temperature. Five milliliters of the supernatant was titrated against a standard 2,6-dichloroindophenol dye. To standardize, 0.053 g of ascorbic acid was dissolved in 90 mL of 20% metaphosphoric acid and diluted with water to 100 mL. 10 mL of this solution was pipetted into a small conical flask and titrated with indophenol's solution until a faint pink colour persisted for 15 sec. A portion of the 2 mL of ginseng leaf extracts was pipetted into a conical flask with 5 mL of 20% metaphosphoric acid, and made up to 10 mL with water. The solution was titrated with the indophenols solution until a faint pink colour persisted for 15 sec. Results are presented on a dry matter basis.

#### Determination of total flavonoid content

Total flavonoid content was determined by using a colorimetric method described previously (13). Dried solvent extracts (0.2 g) were dissolved in 20 mL of 80% methanol, extracted for 2 hr at room temperature and centrifuged at 3000 rpm for 15 min. The volume of the extract was made up to 100 mL with 80% methanol. A portion of 0.5 mL was taken and 0.5 mL of 2% AlCl<sub>3</sub> ethanol solution was added to it. After 1 hr at room temperature, the absorbance was measured at 420 nm. Total flavonoid contents were calculated as kaempferol from a calibration curve.

#### Metal ions chelating activity

The ability of ginseng leaf extracts to chelate the pro-oxidative transitional metal ions Cu<sup>2+</sup> and Fe<sup>2+</sup> was investigated according to Wang and Xiong (14) and Dinis et al. (15), respectively, with slight modifications. For the Cu<sup>2+</sup> chelation experiment, 1 mL of 2 mM CuSO<sub>4</sub> was mixed with 1 mL of pyridine (pH 7.0) and 20 µL of 0.1% pyrocatechol violet. After the addition of 1 mL of samples in different concentrations, the disappearance of the blue color, due to dissociation of Cu<sup>2+</sup>, was recorded by measuring the absorbance at 632 nm with a spectrophotometer (Epoch, Biotech Instruments, Vermont IL, USA) after the reaction proceeded for 5 min. For comparison, the reaction mixture, containing 1 mL of distilled water instead of sample, served as control. For the Fe<sup>2+</sup> chelation experiment, 100 µL of samples in different concentrations were added with 600 µL of distilled water and 100 µL of 0.2 mM FeCl<sub>2</sub> · 4H<sub>2</sub>O. The mixture was

allowed to rest at room temperature for 30 sec. The reaction mixture, containing 100  $\mu\text{L}$  of distilled water instead of sample, served as control. The reaction mixture obtained was later added with 200  $\mu\text{L}$  of 1 mM ferrozine and changes in color were monitored at 562 nm with a spectrophotometer (Epoch, Biotech Instruments), after a 10 min resting time at room temperature. The  $\text{Cu}^{2+}$  and  $\text{Fe}^{2+}$  chelating activities were calculated using the following equation:

$$\text{Chelation activity (\%)} = [(A_0 - A_s) / A_0] \times 100$$

where  $A_0$  is absorbance of the control and  $A_s$  is absorbance of the sample.

#### DPPH radical scavenging activity

The free radical scavenging activity of ginseng leaf extracts was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH $\cdot$ ). This activity was measured by the procedure described by Yen and Hsieh (16) wherein the bleaching rate of a stable free radical, DPPH $\cdot$ , is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH $\cdot$  absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.12 mM solution of DPPH $\cdot$  in methanol was prepared daily and protected from light. An aliquot of 2 mL of this solution was added to 80  $\mu\text{L}$  of samples, in different concentrations, and 320  $\mu\text{L}$  of distilled water. The solution was then mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of mixtures was measured at 517 nm using a spectrophotometer (Epoch, Biotech Instruments). Aqueous solutions of trolox at various concentrations were used to perform the calibration curves (0.15~1.15 mM). The results were expressed both as  $\mu\text{M}$  equivalents of trolox per mL of sample.

#### Antioxidant capacity by ferric reducing/antioxidant power (FRAP) assay

The FRAP assay was done according to the modified Benzie and Strain method (17) with some modifications. Briefly, 900  $\mu\text{L}$  of FRAP reagent, freshly prepared and warmed at 37°C, were mixed with 90  $\mu\text{L}$  distilled water and either 30  $\mu\text{L}$  of samples in different concentrations or standard or appropriate blank reagent. The FRAP reagent contained 2.5 mL of a 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, plus 2.5 mL of 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  and 25 mL of 0.3 mM acetate buffer pH 3.6. Readings at the absorption maximum (593 nm) were taken every 15 sec, using a spectrophotometer (Epoch, Biotech Instruments). Temperature was maintained at 37°C. The readings at 30 min were selected for calculation of FRAP values.

#### ABTS radical cation decolorization assay

The spectrophotometric analysis of ABTS $^{+\cdot}$  radical scavenging activity of ginseng leaf extracts was determined according to the method described by Re et al. (18) with slight modifications. This method is based on the reaction between ABTS and potassium persulfate giving blue/green ABTS radicals (ABTS $^{+\cdot}$ ). With the addition of the antioxidants, decolorization is attained and measured spectrophotometrically at 734 nm. Briefly, ABTS $^{+\cdot}$  was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12~16 hr before use. The ABTS $^{+\cdot}$  solution (stable for two days) was diluted with 5 mM phosphate buffered saline (pH 7.4) to an absorbance of  $0.70 \pm 0.02$  at 734 nm and equilibrated at 30°C. For the photometric assay, 3 mL of the ABTS $^{+\cdot}$  solution and 30  $\mu\text{L}$  of samples in different concentrations were mixed for 45 sec and measured immediately after 5 min at 734 nm (absorbance did not change significantly up to 10 min).

#### Statistical analysis

All experimental data were analyzed by analysis of variance (ANOVA) and significant differences among means from triplicate analysis at ( $p < 0.05$ ) were determined by Duncan's multiple range tests using the statistical analysis system (SPSS 17.0, IBM Inc, Armonk, NY, USA).

## RESULTS AND DISCUSSION

#### Contents of ascorbic acid, total phenolics and total flavonoids in ginseng leaf extracts

Phenolic compounds are known powerful chain breaking antioxidants (19) and because of their scavenging ability, due to their hydroxyl groups, and contribute directly to antioxidative action (20). Phenolic compounds are also effective hydrogen donors, which makes them good antioxidants (21). In addition, flavonoids are one of the most powerful antioxidants found in plants. Typically, they possess one or more of the following structural elements that are considered important to their antioxidant activities: an *o*-diphenol group in ring B; a 2-3-double bond conjugated with the 4-oxo function, and hydroxyl groups at positions 3 and 5 (22). In the present study, total phenolics, flavonoids and ascorbic acid contents in various solvent extracts of ginseng leaves with various concentrations are shown in Table 1. The ethanol extract of ginseng leaves contained significantly ( $p < 0.05$ ) higher amounts of total phenolics and flavonoids (600.57 and 1701.71 mg/100 g) than methanol (374.43 and 1512.64 mg/100 g) and water extracts (248.30 and

**Table 1.** Contents of ascorbic acid, total phenolics and total flavonoids in water, ethanol and methanol extracts of ginseng leaves (mg/100 g of extract, dry matter basis)

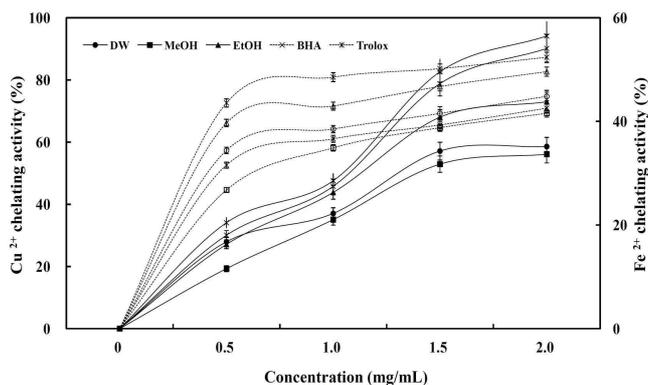
Components	Water	Methanol	Ethanol
Ascorbic acid	24.17 ± 1.76 <sup>a1)</sup>	13.71 ± 1.45 <sup>b</sup>	10.69 ± 1.07 <sup>c</sup>
Total phenolics	248.30 ± 2.18 <sup>c</sup>	374.43 ± 6.26 <sup>b</sup>	600.57 ± 11.50 <sup>a</sup>
Total flavonoids	680.05 ± 0.90 <sup>c</sup>	1512.64 ± 4.66 <sup>b</sup>	1701.71 ± 6.32 <sup>a</sup>

<sup>1)</sup>Different superscripts (a-c) within a row mean significant differences at the  $p < 0.05$  level.

680.05 mg/100 g). Moreover, ascorbic acid was more effectively extracted by water (24.17 mg/100 g) than methanol (13.71 mg/100 g) and ethanol extracts (10.69 mg/100 g). This result was in agreement with that of Jung et al. (23), who reported that ethanol extracts of wild ginseng leaves contained more phenolics and flavonoids than other extracts. The positive correlation between phenolic content and antioxidant potential of various plant extracts have been well demonstrated in prior reports (24,25). The polarity of the solvent had significant impact on the extraction of phytochemicals such as antioxidants, phenolics and flavonoids (26). Therefore, the high content of total phenolics and flavonoids in ginseng leaf extracts indicated the strong antioxidant properties. The results confirmed the possibility of recovering high amounts of phenolics with antioxidant properties from ginseng leaves.

#### Metal ions chelating activity

Actually, hydroxyl radicals can be generated by the Fenton reaction ( $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^-$ ), in which iron participates as a catalyst in body. The superoxide radical participates in the Haber-Weiss reaction ( $\cdot\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \cdot\text{OH} + \text{OH}^-$ ) which combines a Fenton reaction and the reduction of  $\text{Fe}^{3+}$  by superoxide, yielding  $\text{Fe}^{2+}$  and oxygen ( $\text{Fe}^{3+} + \cdot\text{O}_2^- \rightarrow \text{Fe}^{2+} + \text{O}_2$ ). As ferrous ions and other transition metal ions (e.g.  $\text{Cu}^{2+}$ ) can catalyze the oxidation in body, it is significant to investigate the metal chelating activity of an antioxidant (27). The cupric and ferrous ion chelating activity in various solvent extracts of ginseng leaves with various concentrations are shown in Fig. 1. The cupric and ferrous ion chelating activity of ginseng leaf extracts increased according to the increasing concentration of extract. The ethanol extracts showed the highest chelating activity, followed by those of water and methanol extracts. The cupric ion chelating activity of the ethanol extracts was 27.09% at 0.5 mg/mL and increased up to be 73% at 2.0 mg/mL. Furthermore, chelating activity of the ethanol extracts with ferrous ions was improved with increasing concentration of the extract; 36.69% at 0.5 mg/mL was increased up to 49.61% at 2.0 mg/mL. However, the ferrous ion chelating activity of ginseng leaf extracts was lower than the cupric ion chelating ability. This re-



**Fig. 1.** The cupric and ferrous ion chelating activity in various solvent extracts of ginseng leaves with various concentrations. Bars indicate the standard deviation from triplicate determinations (solid line:  $\text{Cu}^{2+}$  chelating ability, dotted line:  $\text{Fe}^{2+}$  chelating ability).

sult was in agreement with that of Kitts et al. (28), who reported that ginseng extracts were effective at chelating cupric ion, while having a relatively low affinity for ferrous and ferric ions. In addition, flavonoids were known to retain free radical scavenging capacity by forming complexes with metal ions (29). Potent chelating activities with metal ions might come from abundant contents of kaempferol in the extracts. Cheng and Breen (30) compared four flavonoids for their ability to suppress Fenton reaction of the iron-ATP complex and proposed that the greater iron chelating activity was due to the 3',4'-dihydroxy (catechol) grouping on the B ring. Similarly, Grinberg et al. (31) suggested that the protective activity of tea polyphenols against OH dependent salicylate hydroxylation was as a result of iron chelation. The literature on the adverse effects of polyphenols on iron bioavailability have emphasized that the binding of iron to the flavonoid antioxidants can suppress the accessibility of the iron to oxygen molecules and non-flavonoid polyphenolics can reduce iron and then form  $\text{Fe}^{2+}$  polyphenol complexes that are inert (32). Therefore, the result revealed that all ginseng leaf extracts demonstrate a marked capacity for transition metal binding, suggesting that their action as peroxidation protectors may be related to their metal binding capacity.

#### DPPH radical scavenging activity

Stable radical DPPH has been widely used for the de-

**Table 2.** Antioxidant activity determined by the DPPH methods<sup>1)</sup>

Concentration (mg/mL)	Water	Methanol	Ethanol
0.5	38.79 ± 6.95 <sup>cB2)</sup>	45.36 ± 8.04 <sup>dB</sup>	84.38 ± 3.65 <sup>cA</sup>
1.0	48.74 ± 7.65 <sup>bcB</sup>	56.97 ± 6.04 <sup>cB</sup>	107.44 ± 5.27 <sup>bA</sup>
1.5	58.21 ± 6.90 <sup>bB</sup>	68.97 ± 6.14 <sup>bB</sup>	116.06 ± 9.69 <sup>abA</sup>
2.0	70.73 ± 5.05 <sup>aC</sup>	78.76 ± 3.82 <sup>aB</sup>	121.98 ± 1.17 <sup>aA</sup>

<sup>1)</sup>Data expressed as  $\mu\text{M}$  equivalents of trolox per 1 mL of ginseng extracts.

<sup>2)</sup>Different superscripts within a column (a-d) and row (A-C), respectively, indicate significant difference at  $p < 0.05$  level.

termination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts, and food materials (33). The DPPH radical scavenging activity in various solvent extracts of ginseng leaves with various concentrations are shown in Table 2. The DPPH radical scavenging activity is expressed as  $\mu\text{M}$  equivalents of trolox per one mL of ginseng extracts. The DPPH radical scavenging activity of ginseng leaf extracts correlated with increasing concentrations. The DPPH radical scavenging activity of the ethanol extracts with various concentrations ranged from 84.38 to 121.98  $\mu\text{M}$  trolox equivalents, while water and methanol extracts ranged from 38.79 to 70.73 and 45.36 to 78.76  $\mu\text{M}$  trolox equivalents, respectively. Ethanol extracts from ginseng leaves on DPPH radical scavenging activity were found to be significantly ( $p < 0.05$ ) higher than water and methanol extracts. This result was in agreement with that of Jung et al. (34), who reported that ethanol extracts of wild ginseng leaves exhibited better radical-scavenging activities than those of other extracts. Moreover, a linear correlation between DPPH radical scavenging activity and concentrations of polyphenolic compounds in various vegetables and fruits have been reported (35,36). According to Marinova and Yanishlieva (37), antioxidant activity of extracts is strongly dependent on the types of solvents used due to compounds with different polarities exhibiting differing rates of antioxidant potential. Thus, this result suggested that the DPPH radical scavenging ability of extracts from ginseng leaves was related to various amount of antioxidants.

#### Ferric reducing/antioxidant power assay

The FRAP assay measures the reduction of ferric iron ( $\text{Fe}^{3+}$ ) to ferrous iron ( $\text{Fe}^{2+}$ ) in the presence of anti-

oxidants, which are reductants with half-reaction reduction potentials above  $\text{Fe}^{3+}/\text{Fe}^{2+}$ . This assay is also commonly used for the routine analysis of single antioxidants and total antioxidative activity. The FRAP values in various solvent extracts of ginseng leaves with various concentrations are shown in Table 3. The FRAP values for the ethanol extract of ginseng leaves were high, ranging from 50.76 to 230.41  $\mu\text{M}$  trolox equivalents, as compared with the water and methanol extracts ranged from 32.11 to 156.04 and 27.38 to 149.43  $\mu\text{M}$  trolox equivalents, respectively. The FRAP values of different extracts were significantly ( $p < 0.05$ ) different from one another. These results are in agreement with those obtained for the antioxidant activity determined by the DPPH radical scavenging assay. However, the ginseng leaf extracts exhibited a higher capacity in reducing ferric ion ( $\text{Fe}^{3+}$ ) to ferrous ion ( $\text{Fe}^{2+}$ ) than scavenging free radicals. The reducing activities are generally associated with the presence of reductones (38), which have been shown to use antioxidant activity to break the free radical chain by donating a hydrogen atom (39). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation (40).

Moreover, according to Oktay et al. (41), a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species. It is known that the antioxidant potential of phenolics depends not only on the quantity but also on the nature of phenolics, such as the molecular weight, the number of aromatic rings, and the nature of hydroxyl group substitution (42). Therefore, the ginseng leaf extracts examined in this study demonstrated good reducing capacity thereby acting as efficient reductones.

**Table 3.** Antioxidant activity determined by the FRAP methods<sup>1)</sup>

Concentration (mg/mL)	Water	Methanol	Ethanol
0.5	32.11 ± 2.99 <sup>dB2)</sup>	27.38 ± 3.18 <sup>dB</sup>	50.76 ± 4.03 <sup>dA</sup>
1.0	76.96 ± 3.66 <sup>cB</sup>	76.02 ± 4.91 <sup>cB</sup>	118.51 ± 5.64 <sup>cA</sup>
1.5	112.37 ± 5.72 <sup>bB</sup>	112.13 ± 5.68 <sup>bB</sup>	181.54 ± 6.04 <sup>bA</sup>
2.0	156.04 ± 6.04 <sup>aB</sup>	149.43 ± 7.45 <sup>aB</sup>	230.41 ± 7.19 <sup>aA</sup>

<sup>1)</sup>Data expressed as  $\mu\text{M}$  equivalents of trolox per 1 mL of ginseng extracts.

<sup>2)</sup>Different superscripts within a column (a-d) and row (A,B), respectively, indicate significant difference at  $p < 0.05$  level.

**Table 4.** Antioxidant activity determined by the ABTS methods<sup>1)</sup>

Concentration (mg/mL)	Water	Methanol	Ethanol
0.5	934.15 ± 51.09 <sup>dB2)</sup>	1007.29 ± 60.48 <sup>dB</sup>	1183.93 ± 51.42 <sup>dA</sup>
1.0	1199.56 ± 65.01 <sup>cC</sup>	1448.22 ± 64.36 <sup>cB</sup>	1718.83 ± 82.79 <sup>eA</sup>
1.5	1399.26 ± 76.00 <sup>bC</sup>	1774.86 ± 77.29 <sup>bB</sup>	2264.15 ± 74.63 <sup>bA</sup>
2.0	1663.18 ± 88.61 <sup>aC</sup>	2098.53 ± 87.64 <sup>aB</sup>	2527.91 ± 88.11 <sup>aA</sup>

<sup>1)</sup>Data expressed as  $\mu\text{M}$  equivalents of trolox per 1 mL of ginseng extracts.

<sup>2)</sup>Different superscripts within a column (a-d) and row (A-C), respectively, indicate significant difference at  $p < 0.05$  level.

### ABTS<sup>•+</sup> radical scavenging activity

The ABTS systems have been commonly used to measure the total antioxidative status of various biological specimens with measuring radical scavenging by electron donation (43). The ABTS radical scavenging activity in various solvent extracts of ginseng leaves with various concentrations are shown in Table 4. Ethanol extract from ginseng leaves on ABTS radical scavenging activity were found to be significantly ( $p < 0.05$ ) higher than water and methanol extracts. The ABTS radical scavenging activity for the ethanol extracts with various concentrations ranged from 1183.93 to 2527.91  $\mu\text{M}$  trolox equivalents, while water and methanol extracts ranged from 934.15 to 1663.18 and 1007.29 to 2098.53  $\mu\text{M}$  trolox equivalents, respectively. Hagerman et al. (44) reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals (ABTS<sup>•+</sup>). Antioxidant activity measured by the FRAP assay showed the same relationships as did the ABTS<sup>•+</sup> method, but TEAC values were higher. This difference in the antioxidant activity could be due to the different reaction mechanisms involved. Moreover, it is assumed that the difference in the radical scavenging activity is due to the different reaction media; aqueous and methanolic for ABTS and DPPH, respectively (43).

In conclusion, in this study, all solvent extracts of ginseng leaves exhibited scavenging activities toward DPPH and ABTS radicals. They also showed cupric and ferrous chelating activity. In particular, among the solvents tested in this study, ethanol extracts of ginseng leaves had the highest potential antioxidant and free radical scavenging activity, although its effectiveness as an antioxidant was not superior to chemical antioxidants, such as BHA and Trolox. In light of these valuable bioactivities, ginseng leaves, considered as waste materials, have good commercial potential to be utilized as promising natural antioxidants in the food, pharmaceutical or cosmetic industries for the large amounts available. Hence, detailed studies on the role of individual phytochemicals involved in the antioxidant activity of ginseng leaves are required for their use as functional foods and in the pharmaceutical industry.

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