

## Antioxidant and Antimicrobial Activities of Various Solvent Fractions of Fine Ginseng Root

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**Abstract** This study was carried out to investigate the changes of yield, total phenolics, saponin content and composition, antimicrobial, and antioxidant activities of various fractions of fine ginseng root (*Panax ginseng* C.A. Mayer) by maceration method in the order of increasing polarity (hexane, chloroform, ethyl acetate, butanol, and water). Butanol fraction showed the highest total saponin content compare to other fractions. Hexane fraction could harvest significantly high ginsenoside Rg2, Rg1, and Rf ( $p < 0.05$ ). And the contents of ginsenoside Rh1, Rg3, and Rg1 showed relatively higher in the fraction of ethyl acetate than other fractions. The system of hexane-chloroform-ethyl acetate-butanol showed relatively high content of ginsenoside Re, Rd, Rc, Rb3, and Rb1. However, the last fraction of water still remained lots of Rb2 content. The fraction of water was the highest phenolics. The 1,1-diphenyl-2-picrylhydrazil, superoxide, and hydroxyl radical scavenging activity of water fraction was higher than the other fractions. In antimicrobial activity, the fraction of hexane showed relatively high antimicrobial activity against *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli*. And the fractions of the chloroform and ethyl acetate showed higher antimicrobial activities than the other samples in against *P. aeruginosa* and *S. typhimurium*.

**Keywords:** fine ginseng root, solvent fraction, saponin content, antioxidant activity, antimicrobial activity

### Introduction

The roots of *Panax ginseng* have been commonly used in the formulation of tonics in Eastern Asia for over 2000 years. It is believed that the root of *P. ginseng* is a panacea, i.e., it is both a universal cure and it also promotes longevity. Various clinical and pharmacological effects of ginseng associated with its use have been reported, such as anti-cancer activity (1,2), anti-circulatory shock effects (3-5), promotion of hematopoiesis (6), and modulation of immune functions and cellular metabolic processes on carbohydrates, fats, and proteins (7). Polysaccharides, phenolics, flavonoids, fatty acid, and ginsenosides in ginseng also reported bioactive chemical compounds (8). Among these bioactive chemical compounds, more than 30 different ginsenosides have been isolated and characterized, and they have different pharmacological effects. However, their chemical components are not fully identified yet, and the compound of ginsenosides vary by species (9-11), the part of ginseng (8,12), and extraction method (13). Many investigators want to develop the method of increment of the active compound in ginseng and ginseng products, just like Rh1, Rg3, and Rg1. However, few studies have been conducted by using hydrolysis, alkaline degradation, enzymology, and fermentation method (14,15). In order to investigate proper fractionation process of ginseng extracts on the lines of physiological activity, we evaluated the yield, total phenolics, saponin content, ginsenoside compositions, antioxidant, and antimicrobial activities of extracted fine ginseng root in the various solvents.

### Materials and Methods

**Materials** Fine ginseng root were purchased from the local herbal shop (Geumsan, Chungnam, Korea). Ginsenosides Rh2, Rh1, Rg2, Rg3, Rg1, Rf, Re, Rd, Rb2, Rc, Rb3, and Rb1 were purchased from Chromadex, Inc. (Laguna Hill, CA, USA). High performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from J.T. Baker (Phillipsburg, NJ, USA). Mobile phases were degassed by sonication for 60 min before use. Solid phase extraction columns (Sep-Pak<sup>®</sup> Vac C18) were obtained from Waters (Milford, MA, USA). Phenolic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), ascorbic acid, butylated hydroxytoluene (BHT), Folin & Ciocalteu phenol reagent, 2-hydroxy-D-ribose, xanthine, xanthine oxidase, ferrozine, and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

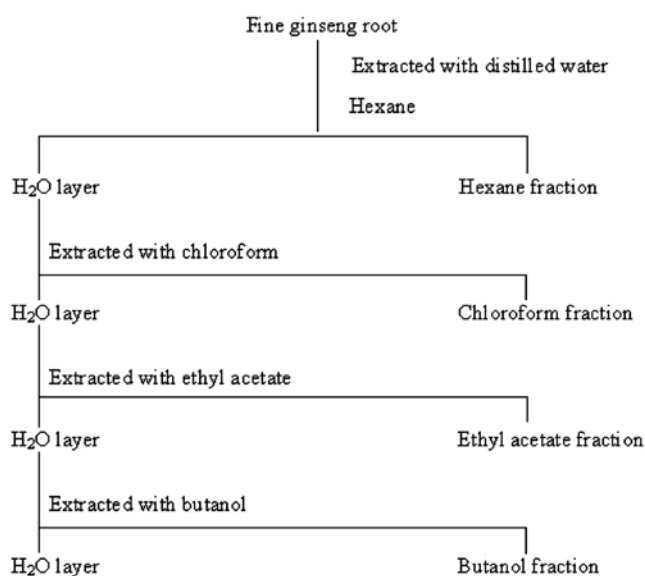
**Preparation of fine ginseng root fractions** The fine root of ginseng was washed with tap water, dried in oven at 50°C for 48 hr to about 4% moisture (dry basis) and ground to a fine powder (60 mesh). The dried sample (50 g) was extracted 3 times with 500 mL distilled water at 100°C in a Soxhlet apparatus for 3 hr, and the extract were filtered with Whatman filter paper No. 2 (Whatman International Ltd., Springfield Mill, Kent, England). To 500 mL of water extract of ginseng, 1,000 mL of each solvent viz. hexane, chloroform, ethyl acetate, butanol, and water was added serially (Fig. 1). After filtering through filter paper, the supernatant in different solvents was recovered. This process was repeated and the respective solvent from the supernatant was evaporated through vacuum rotary evaporator to have the crude extract. For checking the phenolics, saponin content, antioxidant activity, and antimicrobial activity, the each fraction was dried.

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**Fig. 1.** The flow chart of fine ginseng root extracts with various solvents.

**Determination of saponin content** To test the saponin content, the each dried sample was dissolved in 10 mL 40% acetonitrile in water. One mL of the aqueous sample solution was applied to a Sep-Pak® Vac C18 column (Maxi-Clean Cartridge Columns 600 mg C18; Alltech, Deerfield, IL, USA) to remove polar compounds. The sample was eluted sequentially with water (3 mL) and 100% methanol (3 mL). The 100% methanol elution was filtered through a 0.45- $\mu$ m syringe filter (Acrodisc 13-mm HPLC Syringe Filter, CR PTFE, Alltech, Whatman, Brentford, Middlesex, UK), and 25  $\mu$ L filtrate was injected into the HPLC system. The content of each ginsenoside was determined from the corresponding calibration curves.

**Determination of total phenolics** The Folin-Ciocalteu method was performed as described by Singleton and Ross (16). An aliquot of 50  $\mu$ L of the diluted sample solution (50 mg/mL) was mixed with 50  $\mu$ L of commercial Folin-Ciocalteu reagent and 50  $\mu$ L of 20% sodium carbonate aqueous solution. The final volume was adjusted to 300  $\mu$ L with deionized water. The color generated was read after about 2 hr at room temperature at 725 nm. A calibration plot of absorbance versus phenolic concentration was made using gallic acid as a standard. The phenolic content in the samples was evaluated from the generated absorbance value and the results were expressed as gallic acid equivalents (GAE mg of extract powders). The equation of the standard curve was followed: Phenolic content = 0.069  $\times$  OD + 0.123 ( $R^2 = 0.994$ )

**Ginsenosides analysis by HPLC-eveporative light scattering detector (ELSD)** HPLC analysis was carried out by using a PU-2089 PLUS pump (PU-2089 PLUS; Jasco, Tokyo, Japan) coupled with a SoftA 200S ELSD (SoftA Corp., Westminster, CO, USA) and a AS-2057 PLUS auto injector (Jasco). A Prevail Carbohydrate ES column (250  $\times$  4.6 mm, 5  $\mu$ m particle size) from Alltech was used for all separations. HPLC conditions were as

follows: solvent A (acetonitrile:water:IPA = 80:5:15), solvent B (acetonitrile:water:IPA = 67:21:12; gradient, 0-28 (0-10% B), 28-35 (10-85% B), 35-45 (85-80% B), 45-50 (80-75% B), 50-55 (75-100% B), 55-57 (100-100% B), 57-58 (100-10% B), and 58-70 min (10-10% B). The column was then washed with 100% B for 10 min at a flow rate of 0.8 mL/min. ELSD was set to a probe temperature of 70°C, and the nebulizer for nitrogen gas was adjusted to 2.5 L/min. Each concentration of standard of ginsenoside was injected for the HPLC analysis, and peaks were assigned by comparing their retention times with that of each reference compound. The standard solutions containing 10-200 ppm of ginsenosides were injected into the HPLC. Calibration curves were plotted as the peak area ratio versus the amount of each analytic.

**DPPH radical scavenging activity** Free radical scavenging activity of the extracts/fractions was evaluated with the modified DPPH assay (17). Scavenging of DPPH represents the free radical reducing activity of extract/fractions based on a one-electron reduction. Briefly, the reaction mixture contained 300  $\mu$ L of extract/fraction concentrations (1-100 g/mL) and 2 mL of DPPH (0.1 mM in ethanol solutions). The reaction mixture was then placed in the cuvette holder of spectrophotometer (V-530; Jasco) against the blank, which did not contain the extract/fraction. The L-ascorbic acid was used as the positive control. The percentage DPPH decolorization of the sample was calculated by the equation:

$$\% \text{ Inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{sample}}} \times 100$$

**Superoxide anion radical scavenging activity** Superoxide radicals were generated by a modified method of Gotoh and Niki (18) with a slight modification. Different concentrations of the samples were added to the reaction solution containing 100  $\mu$ L of 30 mM ethylenediamine tetraacetic acid (EDTA, pH 7.4), 10  $\mu$ L of 30 mM hypoxanthine in 50 mM NaOH, and 200  $\mu$ L of 1.42 mM nitro blue tetrazolium (NBT). After the solution was pre-incubated at room temperature for 3 min, 100  $\mu$ L of 0.5 U/mL xanthine oxidase was added to the mixture and the volume was brought up to 3 mL with 50 mM phosphate buffer (pH 7.4). After the solution was incubated at room temperature for 20 min, absorbance was measured at 560 nm. The reaction mixture without xanthine oxidase was used as a blank. The samples were added to the reaction mixture, in which  $\text{O}_2^{\cdot -}$  was scavenged, thereby inhibiting the NBT reduction. Absorbance was measured and the decrease in  $\text{O}_2^{\cdot -}$  was represented. The scavenging activity on superoxide anion radical was calculated by the following equation:

$$\% \text{ Scavenging activity} = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}}{\text{Abs}_{\text{blank}}} \times 100$$

**Hydroxyl radical scavenging activity** The scavenging activity of samples on the hydroxyl radical (OH) was measured by the deoxyribose method (19) and ammonium thiocyanate method (20) with a slight modification. The deoxyribose assay was performed in 10 mM phosphate buffer (pH 7.4) containing 2.5 mM deoxyribose, 1.5 mM

H<sub>2</sub>O<sub>2</sub>, 100 μM FeCl<sub>3</sub>, 104 μM EDTA, and the test sample (250 μg/mL). The reaction was started by adding ascorbic acid to a final concentration of 100 μM. The reaction mixture was incubated for 1 hr at 37°C in a water-bath. After incubation, the color was developed by addition of 0.5% thiobarbituric acid followed by ice-cold 2.8% trichloroacetic acid in 25 mM NaOH and heating for 30 min at 80°C. A control was performed without samples. The sample was cooled on ice and the absorbance was measured at 532 nm. The scavenging activity on hydroxyl radicals was calculated by the following equation:

$$\% \text{ Scavenging activity} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

**Antimicrobial activity** The Gram-positive bacteria *Salmonella aureus* KFRI 240, *Bacillus cereus* KFRI 181, and *Listeria monocytogenes* KFRI 799, and the Gram-negative bacteria *Escherichia coli* KFRI 836, *Staphylococcus typhimurium* KFRI 191, and *Pseudomonas aeruginosa* KFRI 252 were obtained from Korea Food Research Institute (KFRI, Seongnam, Korea). The extracts/fractions were dissolved in 0.1% dimethyl sulfoxide (DMSO) solvent to a final concentration of 100 mg/mL and sterilized through filtration by 0.45-μm Millipore filters. Antimicrobial test was then carried out by disc diffusion method (21) using 100 mL of suspension containing 10<sup>8</sup> CFU/mL of bacteria spread on nutrient agar (Difco, Lab., Detroit, MI, USA) medium. The 8-mm diameter disc (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) were impregnated with 1, 2, 5, 10, 15, 20, and 50 μL of 100 mg/mL each solvent fraction (dry base) and placed on the inoculated agar. The inoculated plates were incubated at 37°C for 24 hr for clinical bacterial strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated twice.

## Results and Discussion

**Yield of extracts** The water fraction of ginseng extracts produced most high yield as 86.67%. As shown in Table 1, the yield of dried water fractions of ginseng extracts is summarized. The yield of each solvent viz. hexane, chloroform, ethyl acetate, butanol, and water was 0.82, 3.51, 5.19, 3.81, and 86.67% of total extract, respectively. From the results, the fraction of water of ginseng extract showed the highest yield comparing other harvested samples used for this study ( $p < 0.05$ ). The yields of fractions of ginseng were in the order: water fraction > ethyl acetate fraction > butanol, and chloroform fraction > hexane fraction, respectively.

**Total phenolics** The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites that possess an aromatic ring bearing one or more hydroxyl constituents. Current interest in them stems from their antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic activity (29-33). Total phenolics were measured for all the fractions from the water extracts of dried fine root of ginseng (Table 2). The total phenolic content of the extract/fractions (50 mg/mL) was done by Folin-Ciocalteu method. The phenolics followed the order:

**Table 1. The extract amount and yield of various solvents from fine ginseng root**

Fractions <sup>1)</sup>	Yield <sup>2)</sup> (%)
Hexane	0.82±0.08 <sup>c</sup>
Chloroform	3.51±0.01 <sup>d</sup>
Ethyl acetate	5.19±0.01 <sup>b</sup>
Butanol	3.81±0.01 <sup>c</sup>
Water	86.67±0.13 <sup>a</sup>

<sup>1)</sup>Fractions are described in Fig. 1.

<sup>2)</sup><sup>a-c</sup>Mean±SD is significantly different in the same column ( $p < 0.05$ ).

**Table 2. The total polyphenolic content of various solvents from fine ginseng root**

Fractions <sup>1)</sup>	Total phenolic compound <sup>2)</sup> (mg gallic acid/g sample dry base)
Hexane	12.97±0.98 <sup>c</sup>
Chloroform	13.17±0.16 <sup>b</sup>
Ethyl acetate	12.85±0.28 <sup>c</sup>
Butanol	12.89±0.37 <sup>c</sup>
Water	13.51±0.02 <sup>a</sup>

<sup>1)</sup>Fractions are described in Fig. 1.

<sup>2)</sup><sup>a-c</sup>Mean±SD is significantly different in the same column ( $p < 0.05$ ).

water fraction (13.51 mg), chloroform fraction (13.17 mg), butanol fraction (12.89 mg), ethyl acetate fraction (12.85 mg), and hexane fraction (12.97 mg), respectively, expressed as gallic acid equivalents (GAE)/g of fractions. This results shows that chloroform, ethyl acetate, and water extract/fraction contained relatively higher phenolics than hexane, and butanol extract/fractions than the other fractions. The difference in the antioxidant activity of the different extract/fractions may be ascribed to the difference in the phenolics as well as the phenolic compositions. Further studies are needed on the isolation and characterization of individual phenolic compounds to elucidate their different antioxidant mechanisms and the existence of possible synergism, if any, among the compounds.

**Saponin content** The differences in peak area due to the analyzed described in methods and materials. The area of each peak corresponds to the calibration curve of each sample is shown in Table 3. The content of total and individual ginsenosides of tested fractions harvested from water extract in dried fine root of ginseng is shown in Table 4. The total saponin contents/g of hexane, chloroform, ethyl acetate, butanol, and water extract/fractions were 50.92, 58.03, 71.18, 98.20, and 55.43 mg/g, respectively. This result indicated that the butanol extract/fraction showed the highest total saponin content comparing other fractions and the fraction of hexane could improve ginsenoside Rg2, Rg1, and Rf. The order of Rb2 content in the tested different fraction was; water fraction > ethyl acetate fraction > hexane fraction > chloroform fraction > butanol fraction. The content of Rh2, Rg2, Rg1, and Rf in hexane fraction were significantly higher than the other fractions (0.37, 5.70, 11.62, and 1.21 mg/g, respectively). The ethyl acetate fraction could possess relatively high content of ginsenoside Rh1, Rg3, and Rg1 (2.38, 3.92, and 11.10 mg/g, respectively) comparing the other fractions.

**Table 3. The quadratic regression of each saponin standard**

Index	Parameter			R <sup>2</sup>	Range
	a <sup>1)</sup>	b	c		
Rh2	0.26	148.70	37.86	0.917	25-100
Rh1	3.185	124.9	-3×10 <sup>11</sup>	0.997	25-50
Rg2	7.255	11.61	-98.81	0.988	8-50
Rg3	4.452	118.8	7.548	0.999	8-50
Rg1	0.824	219	-440.2	0.995	25-100
Rf	-	325.4	11.15	0.993	9-50
Re	4.67	33.5	144.1	0.936	9-50
Rd	2.668	153.2	-76.65	0.999	25-100
Rb2	0.046	309.4	-1084.0	0.996	25-100
Rc	0.794	287.3	-780.7	0.990	25-100
Rb3	0.34	262.2	-630.2	0.990	25-100
Rb1	0.377	304.3	-431	0.996	25-50

<sup>1)</sup>Parameter estimates of table can be obtained from  $y=ax^2+bx+c$ .

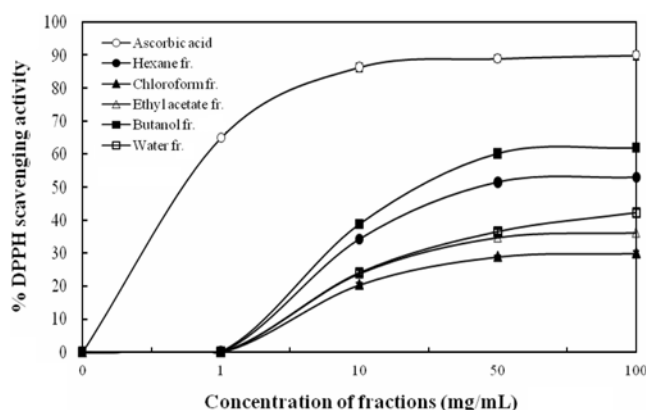
**Table 4. The saponin contents of various solvents of fine ginseng root extract (mg/g sample)**

Saponine	Fractions				
	Hexane	Chloroform	Ethyl acetate	Butanol	Water
Rh2	0.37 <sup>sv</sup>	0.22 <sup>lw</sup>	<sup>1)</sup> ND <sup>kx2)</sup>	ND <sup>ix</sup>	ND <sup>hx</sup>
Rh1	0.09 <sup>hx</sup>	0.29 <sup>kw</sup>	2.38 <sup>hv</sup>	0.09 <sup>ijx</sup>	0.09 <sup>gx</sup>
Rg2	5.70 <sup>ev</sup>	3.62 <sup>fx</sup>	4.32 <sup>dy</sup>	4.60 <sup>ex</sup>	5.30 <sup>bw</sup>
Rg3	1.35 <sup>dw</sup>	0.34 <sup>ix</sup>	3.92 <sup>ev</sup>	0.29 <sup>hixy</sup>	ND <sup>hy</sup>
Rg1	11.62 <sup>bv</sup>	7.20 <sup>bx</sup>	11.10 <sup>bw</sup>	2.31 <sup>gy</sup>	0.35 <sup>fx</sup>
Rf	1.21 <sup>fv</sup>	0.47 <sup>iw</sup>	0.39 <sup>ix</sup>	0.23 <sup>hy</sup>	0.39 <sup>ex</sup>
Re	1.27 <sup>fy</sup>	3.29 <sup>gw</sup>	2.42 <sup>hx</sup>	8.48 <sup>dv</sup>	0.83 <sup>cz</sup>
Rd	0.09 <sup>hy</sup>	3.76 <sup>ew</sup>	2.53 <sup>gx</sup>	4.53 <sup>ev</sup>	0.09 <sup>gy</sup>
Rb2	27.56 <sup>ax</sup>	26.70 <sup>ay</sup>	33.46 <sup>aw</sup>	18.95 <sup>cz</sup>	47.04 <sup>av</sup>
Rc	0.59 <sup>fx</sup>	5.24 <sup>dw</sup>	3.60 <sup>fx</sup>	24.83 <sup>bv</sup>	0.66 <sup>dy</sup>
Rb3	0.54 <sup>fx</sup>	1.46 <sup>hx</sup>	2.04 <sup>iw</sup>	3.24 <sup>fv</sup>	0.68 <sup>dy</sup>
Rb1	0.53 <sup>fy</sup>	5.42 <sup>cw</sup>	5.03 <sup>cx</sup>	30.65 <sup>av</sup>	ND <sup>hz</sup>
Total	50.92 <sup>z</sup>	58.03 <sup>x</sup>	71.18 <sup>w</sup>	98.20 <sup>v</sup>	55.43 <sup>y</sup>

<sup>1)</sup>Not detected.

<sup>2)</sup>a-k, v-z Different letter within the same column and row, respectively, differ significantly ( $p<0.05$ ).

And ginsenoside Rh1, Rg3, and Rg1 showed relatively high content in the fraction of ethyl acetate. The system of hexane-chloroform-ethyl acetate-butanol could harvest relatively high content of ginsenoside Re, Rd, Rc, Rb3, and Rb1, showed 8.48, 4.53, 24.83, 3.24, and 30.65 mg/g, respectively. The system of hexane-chloroform-ethyl acetate-butanol could relatively high content of ginsenoside Re, Rd, Rc, Rb3, and Rb1. Although the water fraction still contained high amount of Rb2, the content of other kinds of ginsenosides, except ginsenoside Rb2, showed unsatisfactory in the water fractions. In this results, the butanol fraction could harvest the higher content of Rb1 comparing other fractions, therefore, when the system of hexane-chloroform-ethyl acetate-butanol-water was used for further separation, the pure content of Rb1 could be attained. In addition, the higher content of Re could be harvested in the hexane-chloroform-ethyl acetate-butanol system.

**Fig. 2. DPPH radical scavenging activities of fractions in fine ginseng root extracts.**

**DPPH radical scavenging activity** DPPH is a stable free radical and has been widely used to test the ability of compounds or plant extracts to act as free radical scavengers or hydrogen donors (23,24). The DPPH radical scavenging activities of *P. ginseng* root extracted with different solvents by maceration method in the order of increasing polarity. Various dried solvent fractions from ginseng roots had in a dose-dependant manner from 1 to 100 mg/mL (Fig. 2). Butanol fraction from ginseng extracts on DPPH radical scavenging activity was found to be significantly higher than other fractions ( $p<0.05$ ). The DPPH radical scavenging activity of each fraction was in the order: butanol fraction>hexane fraction>water and ethyl acetate fraction>chloroform fraction, respectively. In general, the polyphenol concentrations are positively correlated with antioxidant activity due to their hydrogen donating abilities. A close to linear correlation between DPPH radical scavenging activity and concentrations of polyphenolic compounds in various vegetable and fruits have been reported (24-26). These results might be mostly affected by the presence and position of phenolic hydroxyl group. However, in this study, the correlation between DPPH radical scavenging activity and phenolics in ginseng fractions were not similar to ones obtained by previous reports. The phenolics and DPPH radical scavenging activity of each fraction of ginseng showed insignificant linear correlation ( $r=-0.493$ ,  $p>0.05$ ).

**Superoxide radical scavenging activity** Among the oxygen radicals, hydroxyl radicals are the most reactive and induce severe damage to the adjacent biomolecules (27). The hydroxyl radical scavenging activity was investigated by using the Fenton reaction. As shown in Fig. 3, all of the fractions of ginseng extracts had scavenging activity toward superoxide radicals in a dose-dependent manner (1-100 mg/mL). It is interesting to note that, unlike results of the previous experiments, water extract showed the best superoxide radical scavenging activities at <0.1 mg/mL dosage. Although lower than scavenging activities of ascorbic acid in entire dosage ranges, the inhibition rates of superoxide formation reached to 64.23%, when up to 50 mg/mL of water fraction was added. It is interesting to note that, unlike results of the previous experiments, water extract showed the best superoxide radical scavenging

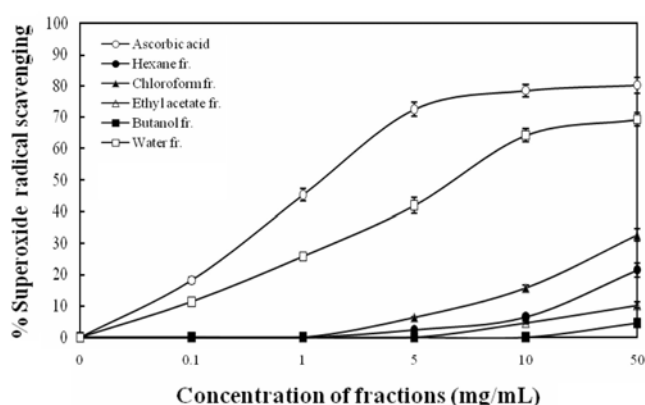


Fig. 3. Superoxide anion radical scavenging activities of fractions in fine ginseng root extracts.

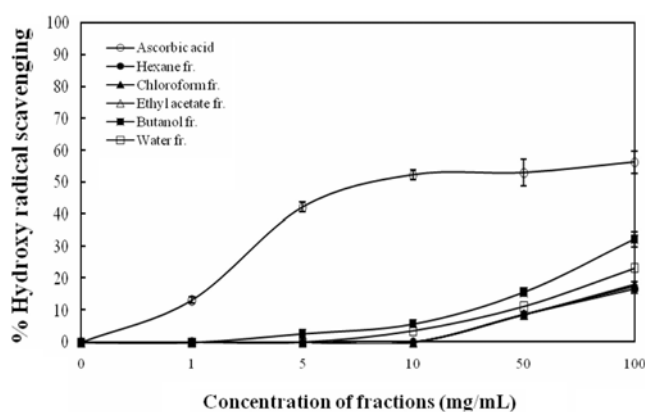


Fig. 4. Hydroxyl radical scavenging activities of fractions in fine ginseng root extracts.

activities at <0.1 mg/mL dosage.

**Hydroxyl radical scavenging activity** Hydroxyl radical scavenging activity of various solvent fractions from ginseng roots was determined by the xanthine-xanthine oxidase system. Figure 4 shows the % inhibition of hydroxyl radical generated by addition of 1-100 mg/mL of solvent extracts of ginseng. The hydroxyl radical scavenging activity of each fraction was in the order: butanol fraction > water fraction > chloroform fraction > hexane, and ethyl acetate fraction, respectively. Although lower than scavenging activities of ascorbic acid in entire dosage ranges, the inhibition rates of hydroxyl formation reached to 15.68 and 32.25%, respectively, when up to 50 and 100 mg/mL of butanol fractions were added.

**Antimicrobial activity** The antimicrobial activities of the fractions against *E. coli*, *L. monocytogenes*, *S. typhimurium*, *B. cereus*, *S. aureus*, and *P. aeruginosa* at concentration of 1, 5, 10, 20, and 50  $\mu$ L of 10 mg/mL are shown in Table 5. There was no antimicrobial activity against tested all microorganisms at low concentration (<1  $\mu$ L of 10 mg/mL) of 5 kinds of fractions, and the fractions of butanol and water showed no antimicrobial activity against all kinds of microorganisms. From the concentration of 5  $\mu$ L of 10 mg/mL of chloroform fraction was shown

Table 5. The antimicrobial activities of fine ginseng root in the various solvents

Fractions	Loading amount ( $\mu$ L)	Microorganisms <sup>1)</sup>					
		STA	ECO	PSE	LIS	BAC	SAL
Hexane fraction	1	- <sup>2)</sup>	-	-	-	-	-
	5	-	-	-	-	-	-
	10	-	-	~	-	-	-
	20	~	-	++	-	~	+
	50	+	~	+++	-	+	++
Chloroform fraction	1	-	-	-	-	-	-
	5	-	-	~	-	-	-
	10	-	-	~	-	-	-
	20	-	-	+	-	-	~
	50	-	-	+++	-	-	+
Ethyl acetate fraction	1	-	-	-	-	-	-
	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	20	-	-	+	-	-	~
	50	-	-	++	-	-	+
Butanol & water fraction	1	-	-	-	-	-	-
	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	20	-	-	-	-	-	-
	50	-	-	-	-	-	-

<sup>1)</sup>STA, *Staphylococcus aureus* KFRI 240; ECO, *Escherichia coli* KFRI 836; PSE, *Pseudomonas aeruginosa* KFRI 252; LIS, *Listeria monocytogenes* KFRI 799; BAC, *Bacillus cereus* KFRI 181; SAL, *Salmonella typhimurium* KFRI 191.

<sup>2)</sup>-, No antimicrobial activity; ~, Slight antimicrobial activity inhibition zone (I.Z) of sample 8-9 mm; +, Moderate antimicrobial activity I.Z of sample 9.1-12 mm; ++, Clear antimicrobial activity I.Z of sample 12.1-15 mm; +++, Strong antimicrobial activity I.Z of sample >15 mm.

antimicrobial activity against *P. aeruginosa*. However, same concentrations of other fractions showed no effect on the 6 kinds of microorganism. In these results, the strain of *P. aeruginosa* was the highest antimicrobial activity on the hexane, chloroform, and ethyl acetate fractions in the tested fractions. This result indicated that the chloroform fraction among the fractions showed the highest antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *B. cereus*, and *S. typhimurium* among the fractions. And the chloroform and ethyl acetate fractions were showed only against *P. aeruginosa* and *S. typhimurium* on the antimicrobial activity, and the hexane fraction showed higher antimicrobial activity against *S. typhimurium* than other fractions.

In conclusion, the present study provided the potential antioxidant and antimicrobial properties of various solvent fractions from water fraction of fine ginseng root. The water fraction of fine ginseng root has shown better effect on antioxidant activity, while the hexane fraction of fine ginseng root possessed was more effective than other fractions on antimicrobial activity. These result suggested that fine ginseng root fractions could have antioxidant and antimicrobial effects, which are expected to be applied to the food and pharmaceutical industry related to ginseng.

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