

## Composition, antioxidant and antimicrobial activities of *Eleutherococcus senticosus* fruit extracts

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### ABSTRACT

This study was designed to examine the *in vitro* antioxidant and antimicrobial activities of essential oil and crude methanolic (MeOH) extract of *Eleutherococcus senticosus* (common name, Siberian ginseng) fruit and its partitioned fractions, including hexane, ethyl acetate (EtOAc), n-butanol (BuOH) and aqueous. 1,1-diphenyl-2-picryl-hydrazyl free radical scavenging and reducing power assay suggest that antioxidant activity of EtOAc and BuOH fractions is due to the reducing ability of the antioxidant against oxidative effects of reactive oxygen species. In addition, the essential oil of Siberian ginseng fruit showed remarkable antimicrobial activity against *Kocuria rhizophila* (MIC = 125 µg/ml), *Micrococcus luteus* (MIC = 500 µg/ml), and *Escherichia coli* (MIC = 63 µg/ml). The chemical compositions of the essential oil obtained by the simultaneous steam-distillation and extraction method were analyzed using GC-MS; trans-caryophyllene (21.7%), humulene (7.4%), bicylogermacrene (6.0%), (+) spathulenol (4.5%), germacrene-D (3.2%), tau-muurolol (2.5%) and delta-cadinene (2.3%) were found as main constituents. Furthermore, HPLC analysis identified ursolic acid as one of the principal components in Siberian ginseng fruit extract. Although this study has been carried out by *in vitro* assay, these results suggest that Siberian ginseng fruit may be a good candidate as a source of antioxidant and antimicrobial ingredients.

### INTRODUCTION

*Eleutherococcus senticosus* Maxim (synonym: *Acanthopanax senticosus*), common name Siberian ginseng, is a thorny shrub from the family Araliaceae distributed in Southeast Russia, Northeast China, Korea, and Japan (Hwang *et al.*, 2015). The root's and stem's cortex tissues of Siberian ginseng are widely used as a traditional Chinese medicinal ingredient used to invigorate *qi*, strengthen the spleen, and nourish kidney in the theory of Traditional Chinese Medicine, and to treat combined neurosis, coronary heart disease, inflammation, angina pectoris, stress-induced pathophysiological changes and menopausal syndrome (Huang *et al.*, 2011). In the extract of Siberian ginseng, triterpenoid saponins, lignans, coumarins, and flavones, among which, phenolic compounds such as syringin and eleutheroside E have been reported to have various medicinal anti-gout, anti-hepatitis, antihyperglycemic, anti-leishmania,

effects concerning antibacterial, anticancer, anti-inflammatory, antioxidant, haemostatic, immunostimulatory, and hypocholesterolemic effects (Huang *et al.*, 2011; Lee and Shin, 2002; Sun *et al.*, 2011). In addition, the seed's essential oil showed significant antioxidant activity and antimicrobial activity (Bajpai *et al.*, 2013a, 2013b), indicating that Siberian ginseng has potential as a crude drug and a dietary health supplement. The consumption of wild edible fruits, which gives an important contribution to the health of local communities in many developing countries, is gaining increasing interest. Fruits, together with wild edible fruits, are known to possess beneficial nutrients including minerals, polyphenols, and various vitamins that provide health benefits in addition to their nutritional value (Morales *et al.*, 2013). Although the root of ginseng, belonging to the family Araliaceae, is a commonly used herbal medicine, the berry of ginseng exhibits pharmacological activities that are significantly more potent than those of the root (Lee *et al.*, 2010). This is an example of the potential of wild edible fruits as beneficial materials for the food and pharmaceutical industry. Therefore, the wild fruits from many plant species have been investigated for pharmacological activities including antioxidant, antimicrobial and anticancer activities.

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However, there is still demand to find more information concerning the pharmacological potential of the wild fruits in terms of safety and bioactivity. Thus, in this study, we determined the antioxidant and antimicrobial activities of an essential oil, methanol (MeOH) extract and its fractions obtained from the dried fruit of Siberian ginseng.

In addition, in order to better understand the biological activities of Siberian ginseng fruit, we analyzed the composition of its essential oil using GC-MS and determined the relationship between the amount of ursolic (3 $\beta$ -hydroxy-urs-12-en-28-oic acid) acid, which is a triterpene compound occurring in the plant kingdom as free acid or aglycone of triterpene saponins (Kowalski, 2007).

## MATERIALS AND METHODS

### Chemicals

Butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, potassium ferricyanide, ferric chloride, gallic acid, quercetin and ursolic acid were obtained from Sigma-Aldrich. MeOH, hexane, ethyl acetate (EtOAc) and n-butanol (water-saturated BuOH) were purchased from Merck. All reagents were of analytical grade or higher.

### Plant materials and extraction

Fresh fruits of Siberian ginseng (*E. senticosus*) were collected from Mt. Odae, Pyeongchang, Kangwon-do, Korea, during 2014. After harvesting, the fruits were washed with sterile water, and then air-dried for 3 days at room temperature. The dried fruits (100 g) of Siberian ginseng were soaked in methanol (1:10 dry weight material to MeOH (ml)) for 24 h and sonicated (1 h  $\times$  3 times) at 40 °C in an ultrasonic bath (Power sonic 420, Hwashin Co., Korea). The resulting extract was filtered and evaporated to dryness using a rotary vacuum evaporator at 40 °C to yield 16.65% (w/w).

The crude MeOH extract (16.65 g) was suspended in water and then fractionated by different solvents of increasing polarity using hexane, EtOAc and BuOH. The remaining aqueous extract was used as an aqueous fraction. Each fraction obtained was concentrated using a vacuum evaporator. The yields in different solvents were: 1.46 g; 8.77 % w/w for hexane, 0.83 g; 4.98 % w/w for EtOAc, 1.13 g; 7.81% w/w for BuOH and 68.95 g; 31% w/w for aqueous fractions. Then, 1 mg of MeOH extract and its fractions were re-dissolved in 1ml MeOH or water (only for aqueous fraction) for further analysis.

### Essential oil preparation

The essential oil was extracted using the simultaneous steam-distillation and extraction (SDE) method (Selli and Cayhan, 2009). The dried fruit (100 g) of Siberian ginseng was mixed with deionized water (1000 ml) in a round bottom flask, and the flask was attached to the arm of the SDE apparatus. A solvent mixture of *n*-pentane and diethyl ether (Merck, Darmstadt, Germany) was used as an extractant. The SDE process was allowed to proceed for

6 h, and the extract thereby obtained was dried over anhydrous sodium sulphate (Merck) and concentrated using N<sub>2</sub> gas. The resulting essential oil was weighed and stored at - 20 °C until chemical analyses and further analysis.

### Analysis of free radical scavenging activity

The free radical scavenging activity of each sample was evaluated with the DPPH free radical scavenging assay (Hyun *et al.*, 2013). Briefly, 0.8 mL of freshly prepared DPPH solution (0.4 mM in MeOH) was plated in 96-well plates, and 0.2 ml of the sample (or a control) was added followed by serial dilution to each well.

The mixture was incubated for 30 min in the dark at room temperature. Then the absorbance was measured at 520 nm using an iMark<sup>TM</sup> microplate reader (Bio-Rad). The RC<sub>50</sub> (50% reduction of DPPH radicals) was calculated from a graph of radical scavenging activity versus extract concentration. BHT was used as the standard.

### Determination of reducing power

The total reducing power of the essential oil, MeOH extract, and its fractions was determined using the method described previously (Lee *et al.*, 2013). Samples of various concentrations (100, 200, and 300  $\mu$ g/ml) were mixed with 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (1%, w/v), followed by incubation at 50°C for 20 min. The reaction was stopped by adding 0.5 ml of trichloroacetic acid solution (10%) and centrifuging the mixture at 2,500 rpm for 10 min.

Then, 0.5 ml of the obtained supernatant was mixed with 0.5 ml of distilled water and 0.1 ml of ferric chloride solution (0.1%, w/v). The absorbance was measured at 750 nm using a microplate reader.

### Determination of antimicrobial activity

The bacterial test strains used in this study were *Kocuria rhizophila* (KACC 14744) and *Micrococcus luteus* (KACC 14819), *Klebsiella pneumonia* (KACC 14816), *Escherichia coli* (KACC14818), *Enterobacter cloacae* (KACC11958), *Salmonella enterica* subsp. *enterica* (KACC 10769), and *Pseudomonas aeruginosa* (KACC 10186). All the strains were obtained from the Korean Agricultural Culture Collection (KACC) in South Korea. The degree of antimicrobial activity was assayed by the serial twofold dilution method to determine the minimum inhibitory concentration (MIC) of essential oil and MeOH extract and its fractions. The growth of the bacteria and yeast was evaluated based on the degree of turbidity of the culture using the naked eye.

### Estimation of total phenolic and flavonoid contents

The content of total phenolic compounds in MeOH extract and its fractions were determined using Folin-Ciocalteu reagent, with gallic acid as a standard phenolic compound (Hyun *et al.*, 2013). Each extract was incubated with 50  $\mu$ l of 2 N Folin-

Ciocalteu reagent for 5 min at room temperature, followed by the addition of 0.3 ml of 20% sodium carbonate and incubation for 15 min at room temperature.

After mixing with 1 ml of distilled water, absorbance was measured at 750 nm using a microplate reader. The total phenolic content (TPC) in the MeOH extract and its fractions were calculated in milligrams of gallic acid equivalents (GAE) per gram of extract using the equation obtained from the calibration curve of gallic acid.

To determine the total flavonoid content (TFC) in each extract, 0.5 ml of each extract was mixed with 0.1 ml of aluminum nitrate (10%, w/v), 0.1 ml of 1 M potassium acetate, and 4.3 ml of 80% ethanol as described by Hyun *et al.* (2013). After incubation at room temperature for 40 min, absorbance was determined at 415 nm. TFC was determined as milligrams of quercetin equivalents (QE) per gram of extract.

### Essential oil analysis

The essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS). The analytical GC was carried out on an Agilent 6890N GC (Agilent Technologies, Santa Clara, CA, USA) equipped with the Agilent 5975 inert mass selective detector. A DB-5MS fused silica capillary column (30 m × 250 μm, 0.25 μm film thickness) was used. The initial oven temperature of 50 °C was maintained for 2 min, and then increased to 250 °C at a rate of 10 °C/min followed by holding at 250 °C for 10 min.

The carrier gas was helium with a flow rate of 1.0 ml/min and samples (in methanol) of 1.0 ml were injected. MS were taken at 70 eV and electron scanning ranges was 15500 amu. Identification of essential oil components was based on GC retention time on a DB-5 capillary column relative to computer matching of mass spectra using the Wiley/7n mass spectral database (Hewlett-Packard Co., Palo Alto, CA, USA).

### High-performance liquid chromatography (HPLC) analysis

The HPLC analysis was carried out with an Agilent 1200 chromatograph, equipped with a diode array detector (Agilent Technologies, Waldbronn, Germany). The column temperature was 40°C.

To determine the concentration of ursolic acid in the extracts, an Eclipse XDB-C18 column (4.6 × 150 mm, particle size 5.0 μm) was applied, with MeOH/H<sub>2</sub>O (95:5) serving as the mobile phase at a flow rate of 0.4 ml/min, and the reading was taken at 215 nm (Xu *et al.*, 2012). A standard calibration curve of ursolic acid was constructed in the concentration range of 10 ng to 1000 ng ( $y = 0.5511x - 1.8572$ ,  $R^2 = 0.9995$ ). The quantification was performed by peak integration using the external standard method.

### Statistical analysis

Statistical analyses were performed using ANOVA testing. Duncan's test was used to determine the significance of differences between the groups. Differences at  $p < 0.05$  were considered significant.

## RESULTS AND DISCUSSION

### Antioxidant properties of Siberian ginseng fruit extract

The investigation of antioxidant properties of plants is considered an important issue in developing novel antioxidant agents with low toxicity and side effects. Therefore, to investigate the antioxidant properties in the Siberian ginseng fruit, its essential oil and crude MeOH extract and its fractions were analyzed for antioxidant activities based on DPPH free radical scavenging activity and reducing power.

As shown in Table 1, the highest DPPH free radical scavenging activity was in the EtOAc fraction ( $RC_{50} = 403.29 \pm 15.17$  μg/ml extract), followed by the BuOH fraction ( $RC_{50} = 459.27 \pm 15.61$  μg/ml extract) and the crude MeOH extract ( $RC_{50} = 713.42 \pm 11.55$  μg/ml) (Table 1).

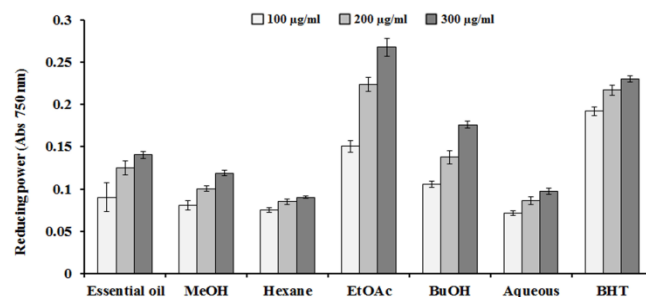
**Table 1:** DPPH free radical scavenging activity of essential oil, crude MeOH extract, and its fractions.

Sample	$RC_{50}^{1)}$
Essential oil	1699.34±218.16d <sup>2)</sup>
MeOH extract	713.42±11.55c
Hexane fraction	1718.50±142.48d
EtOAc fraction	403.29±15.17b
BuOH fraction	459.27±15.61b
Aqueous fraction	756.11±56.57c
BHT	186.97±12.62a

<sup>1)</sup>  $RC_{50}$ : Amount required for a 50% reduction of DPPH after 30 min; each value is mean ± standard derivation of triplicate experiments.

<sup>2)</sup> Each value represents the mean ± SD, and the means were significantly different as calculated from a paired Duncan's test at  $p < 0.05$ .

In addition, we determined the antioxidant property using reducing power assay, which measures the ability of the substance to reduce  $Fe^{3+}$  to  $Fe^{2+}$  and is known as a robust method for measuring total antioxidant capacities (Benzie and Strain, 1996). The results showed that the reducing power of the EtOAc and BuOH fractions was higher than those of other samples (Fig. 1).



**Fig. 1:** Reducing power of *Eleutherococcus senticosus* fruit extracts. Values are the average of triplicate experiments and represented as mean ± standard deviation.

The 300 μg/ml EtOAc fraction exhibited an  $OD_{750}$  value of  $0.268 \pm 0.01$ , whereas BHT displayed an  $OD_{750}$  value of  $0.23 \pm 0.003$ . The reducing power assay is based on the reduction of  $Fe^{3+}$  to  $Fe^{2+}$  by the action of electron donating antioxidants (Szydłowska-Czerniak *et al.*, 2008), whereas the DPPH-free radical scavenging assay is believed to involved hydrogen atom transfer reaction (Huang *et al.*, 2005).

As shown in Fig 1, the EtOAc fraction possessed a strong reducing activity compared with synthetic antioxidant BHT, whereas DPPH free radical scavenging activity of EtOAc fraction lower than that of BHT was determined (Table 1). This indicates that antioxidant activity of Siberian ginseng fruit extracts is mainly due to the inactivation of oxidants by reducing ability rather than hydrogen atom transfer reaction.

### Antimicrobial activities of Siberian ginseng fruit extract

Although pharmaceutical companies have produced a number of antimicrobial drugs in recent years, the development of bacterial super resistant strains to antimicrobial drugs in use has attracted the attention of the scientific community regarding the search for new cost-effective agents from natural sources (Höfling *et al.*, 2010; Pai *et al.*, 2004). Therefore, there is renewed interest in natural sources with antimicrobial activities to develop antibacterial agents. To investigate the antimicrobial activity of Siberian ginseng fruit, we determined the MIC of its essential oil and MeOH extract and its fractions using the serial twofold dilution method, and the results are expressed as MIC values (Table 2). In general, essential oil exhibited good antimicrobial activity against *K. rhizophila* (MIC = 125 µg/ml), *M. luteus* (MIC = 500 µg/ml), and *E. coli* (MIC = 63 µg/ml). The hexane and EtOAc fractions showed significant antimicrobial activity against *E. cloacae* (MIC = 125 µg/ml), whereas crude MeOH extract and its BuOH and aqueous fractions exerted no effect on all the tested bacteria (Table 2). In addition, crude MeOH extract and its fraction of Siberian ginseng fruit showed greater activity against Gram-negative bacteria than against Gram-positive bacteria (Table 2), whereas Gram-positive bacteria are highly sensitive to essential oil of Siberian ginseng fruit.

The difference in sensitivity between the Gram-negative and Gram-positive bacteria against essential oil might be due to the structural differences in the bacterial cell walls of Gram-positive and Gram-negative bacteria. Therefore, we hypothesize that Gram-negative bacteria are resistant to essential oil of Siberian ginseng fruit because the outer layer of Gram-negative bacteria outer membrane is composed of lipopolysaccharide, which forms a hydrophilic permeability barrier, as discussed by Mann *et al.* (2000) and Smith-Plamer *et al.* (1998).

The chemical composition of essential oil extracted from Siberian ginseng fruit was analyzed by GC-MS. A total of 33 different compounds were identified, which constituted 67.7% of total oils. Trans-caryophyllene (21.7%), humulene (7.4%), bicyclogermacrene (6.0%), (+) spathulenol (4.5%), germacrene-D (3.2%), tau-murolol (2.5%), and delta-cadinene (2.3%) were found as main constituents (Table 3).

In microwave-assisted seed essential oil of Siberian ginseng, the main components were ethyl phthalate (31.73%) and d-mannitol (20.06%), but trans-caryophyllene was not identified (Bajpai *et al.*, 2013a). Similarly, it has been shown that the main identified constituents of essential oil from ginseng roots were athulenol and 2-*epi*-(E)-β-caryophyllene (Smigielskia *et al.*, 2006), whereas palmitic acid and beta-farnesene were found as the main compounds in essential oil from ginseng leaves (Jiang *et al.*, 2014). This indicates differences in the chemical composition of the essential oil in the different plant organs. Trans-caryophyllene has been reported to possess many pharmacological effects including anti-inflammatory activity, antimicrobial activity, and analgesic activity (Astani *et al.*, 2011; Chavan *et al.*, 2010; Fernandes *et al.*, 2007).

In addition, sesquiterpene hydrocarbons such as humulene, germacrene-D, dcadinene, α-cubebene, α-copaene, γ-cadinene, and β-elemene have shown antimicrobial activity (Haznedaroglu *et al.*, 2001; Rahman *et al.*, 2008; Solis *et al.*, 2004). This indicates that antimicrobial activity of essential oil extracted from Siberian ginseng fruit might be due to the presence of sesquiterpene hydrocarbons as main constituents.

### Determination of total phenolic content, total flavonoid content and glycones of triterpene saponins

Plant-derived polyphenolic compounds exhibit a wide range of pharmaceutical properties, such as antioxidant, antimicrobial, anti-inflammatory, and anti-cancer properties (Balasundram *et al.*, 2006). Accumulated evidences by *in vitro* test demonstrated that the antioxidant and antimicrobial activities of plant extracts is positively correlated with the TPC and the TFC (Canadanovic-Brunet *et al.*, 2005; Hyun *et al.*, 2013; Oboh *et al.*, 2008), indicating that the high level of antioxidant activity in the EtOAc and BuOH fractions of Siberian ginseng fruit might be due to the presence of high amounts of phenolic acids and flavonoids.

**Table 2:** Antibacterial activity of essential oil, crude MeOH extract, and its fractions.

Extract and fractions	MIC (µg/ml) <sup>1)</sup>						
	K.r. <sup>2)</sup>	M.l. <sup>2)</sup>	Es.c. <sup>2)</sup>	K.p. <sup>2)</sup>	E.c. <sup>2)</sup>	S.e. <sup>2)</sup>	P.a. <sup>2)</sup>
Essential oil	125	500	63	>1000	>1000	1000	1000
MeOH extract	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Hexane fraction	1000	1000	>1000	>1000	125	500	500
EtOAc fraction	1000	1000	>1000	>1000	125	500	500
BuOH fraction	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Aqueous fraction	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Tetracycline	8	8	8	8	8	8	8

<sup>1)</sup> MIC values against bacteria and yeast were determined by the serial twofold dilution method.

<sup>2)</sup> K.r.: *Kocuria rhizophila* KACC 14744, M.l.: *Micrococcus luteus* KACC 14819, Es.c.: *Escherichia coli* KACC14818, K.p. *Klebsiella pneumonia* KACC 14816, E.c.: *Enterobacter cloacae* KACC11958, S.e.: *Salmonella enterica* subsp. enterica KACC 10769, P.a.: *Pseudomonas aeruginosa* KACC 10186.

**Table 3:** Composition of *Eleutherococcus senticosus* fruit essential oils (%) obtained by gas chromatography/mass spectrometry.

No.	RT	SI <sup>a</sup>	Major compounds	Percentage concentration
1	3.3723	17889	2,2-dimethoxybutane	0.6776
2	4.4993	6150	Furfural	0.3851
3	6.2694	32176	alpha-Pinene	0.6368
4	7.0639	32202	beta-Pinene	0.3429
5	7.2006	31858	beta-Myrcene	0.3822
6	7.4482	24725	Octanal	0.3128
7	7.8473	29891	p-Cimene	0.1563
8	7.9249	31986	dl-Limonene	0.6127
9	7.9692	32057	beta-Phellandrene	1.5271
10	8.1724	18805	Benzeneacetaldehyde	0.7135
11	10.2049	73571	Epoxylinolol	0.2688
12	10.3971	52813	4-Terpineol	0.2107
13	10.5079	34041	Cryptone	0.4291
14	10.6077	53283	alpha-Terpineol	0.6795
15	11.4465	14302	Caprolactam	0.1686
16	11.8641	49547	Phellandral	1.8908
17	13.2277	121683	Copaene	0.3131
18	13.3828	121794	beta-Elementene	2.2658
19	13.8522	121314	trans-Caryophyllene	21.6658
20	13.9667	121697	beta-Cubebene	0.4366
21	14.3178	121252	Humulene	7.4055
22	14.6282	121792	Germacrene-d	3.1866
23	14.7427	121611	Aromadendrene	2.1779
24	14.8093	121761	Bicyclogermacrene	6.0434
25	15.0383	121465	delta-Cadinene	2.3401
26	15.3599	116031	alpha-calacorene	0.405
27	15.818	145074	(+) Spathulenol	4.5157
28	16.0509	121608	(+)-Aromadendrene	1.8174
29	16.3945	121346	(+)-Cyclosativene	1.4603
30	16.5756	148446	tau-Muurolol	2.4604
31	16.7862	121746	beta-Selinene	1.1041
32	19.4616	213890	Methyl palmitate	0.2538
33	19.772	195431	n-Hexadecanoic acid	0.4228

<sup>a</sup>SI: Library search purity value.

Thus, to determine relation between antioxidant activity and TFC and TPC of crude MeOH extract and its fractions, we determined the TPC and TFC of each test sample. The highest amount of phenolic compounds ( $334.36 \pm 12.80$  mg GAE/g) was found in the EtOAc fraction containing higher antioxidant activity compared with other samples, whereas the aqueous fraction contained the lowest level of TPC ( $197.98 \pm 4.26$  mg GAE/g) (Table 4).

**Table 4:** Total phenolic content and total flavonoid content of crude MeOH extract and its fractions.

Extract and fractions	Total phenol (mg GAE/g) <sup>1)</sup>	Total flavonoid (mg QE/g) <sup>2)</sup>
MeOH extract	$229.83 \pm 9.34b^{3)}$	$62.25 \pm 7.12a$
Hexane fraction	$314.98 \pm 33.24d$	$203.75 \pm 20.15c$
EtOAc fraction	$334.36 \pm 12.80d$	$198.25 \pm 23.50b$
BuOH fraction	$287.98 \pm 9.64c$	$45.75 \pm 9.88a$
Aqueous fraction	$197.98 \pm 4.26a$	$41.25 \pm 8.53a$

<sup>1)</sup> Total phenolic content analyzed as gallic acid equivalent (GAE) mg/g of extract; values are the average of triplicates.

<sup>2)</sup> Total flavonoid content analyzed as quercetin equivalent (QE) mg/g of extract; values are the average of triplicates.

<sup>3)</sup> Each value represents the mean  $\pm$  SD, and the means were significantly different as calculated from a paired Duncan's test at  $p < 0.05$ .

The highest TFC was found in the hexane fraction ( $203.75 \pm 20.15$  mg QE/g), followed by the EtOAc ( $198.25 \pm 23.50$  mg QE/g) fraction. Although BuOH fraction contained

lower level of TPC and TFC compared with hexane fraction (Table 4), BuOH fraction exhibited strong antioxidant activity (Table 1 and Fig. 1). Such variation in antioxidant activity between different fractions might be due to the different composition of plant-derived compounds including the phenolic and flavonoid compounds rather than TFC and TPC in each fraction.

**Table 5:** Ursolic acid contents of *Eleutherococcus senticosus* fruit extracts.

	Ursolic acid <sup>1)</sup>
MeOH extract	$0.21 \pm 0.027$
Hexane fraction	$3.91 \pm 0.109$
EtOAc fraction	$5.33 \pm 0.328$
BuOH fraction	<sup>2)</sup>
Aqueous fraction	<sup>2)</sup>

<sup>1)</sup> mg/g of extract values are the average of triplicate experiments.

<sup>2)</sup> not detectable.

The triterpenoid saponin is known as an active compound in Siberian ginseng, and ursolic acid is aglycone of triterpene saponins (Hwang *et al.*, 2015). Ursolic acid is known to possess a wide range of biological activities such as anti-inflammatory, antimicrobial and anticancer (do Nascimento *et al.*, 2014). As shown in Table 5, the EtOAc fraction contained the highest amount of ursolic acid ( $5.33 \pm 0.328$  mg/g of extract), followed by the hexane ( $3.91 \pm 0.109$  mg/g of extract) fraction. A correlation between antimicrobial activity and amount of ursolic acid indicates

that ursolic acid could be one of potential active compounds in Siberian ginseng fruit extract.

## CONCLUSION

Overall, we analyzed the antioxidant, antimicrobial, chemical composition of the essential oil and the level of ursolic acid, and suggested that Siberian ginseng fruit is rich source of naturally antioxidant and antimicrobial activities. In addition, the presence of sesquiterpene hydrocarbons and ursolic acid in the extract of Siberian ginseng fruit indicates the potential of Siberian ginseng fruit as a crude drug and dietary health supplement. Further studies on the isolation and characterization of the plant extract as well as the toxicity should be tested to confirm the safety use.

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