

# Antioxidant and ACE Inhibitory Activity of Cultivated and Wild *Angelica gigas* Nakai Extracts Prepared Using Different Extraction Conditions

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**ABSTRACT:** The purpose of this study was to investigate the biological activities of cultivated *Angelica gigas* Nakai (CAG) and wild *Angelica gigas* Nakai (WAG) extracts prepared by extraction with water, 30% ethanol, 60% ethanol, or 90% ethanol. The electron donating ability of the WAG extracts was higher than that of the CAG extracts and 0.1% and 1.0% solutions of the comparative substance, L-ascorbic acid. The superoxide dismutase-like activity of the CAG extracts was higher than that of WAG extracts. Superoxide dismutase-like activity was highest (33.95%) in the CAG water extract. The total polyphenol content was highest in the 60% ethanol extracts of WAG. The nitrite scavenging ability of the CAG and WAG extracts was highest at a pH of 1.2. The tyrosinase inhibitory effect was highest (43.72%) in the water extract of WAG. The angiotensin converting enzyme inhibitory activity was highest (83.84%) in the 60% ethanol extract of WAG. The results of the present study will be useful for understanding the antioxidant and angiotensin-converting enzyme inhibitory activities of *Angelica gigas* Nakai extracts.

**Keywords:** antioxidant, cultivated *Angelica gigas* Nakai, wild *Angelica gigas* Nakai, extraction condition

## INTRODUCTION

Dang-gwi (*Angelica gigas* Nakai; AG), a perennial plant in the Apiaceae family, can be found throughout Korea, China, and Japan. Based on its area of distribution, *Angelica gigas* is classified into different cultivars: AG, *Angelica sinensis* (Oliv.) Diels, and *Angelica acutiloba* Kitagawa. AG grows and is cultivated in the alpine region of Korea and is called Cham-dang-gwi in Korean (1). The main components of AG (i.e., decursin, decursinol, nodakenin,  $\alpha$ -pinene, limonene,  $\beta$ -eudesmol, and elemol) have various pharmacologic effects. Over the years, AG has been widely used for its various medicinal effects, including its anti-bacterial effect, sedative action, analgesic effect, and diuretic effect and its effects on uterine function, blood circulation, anemia, and hemostasis. Since the pharmacologic effects of AG became widely known, researchers have studied the extraction, chemical components (1), isolation and purification (2,3), anti-anemia effect (4), cytotoxic effect (5), and immune-enhancing effects (6) of AG. In addition, AG has been shown to have positive effects on leukemia, kidney toxicity, diabetic hypertension, and cancer (7). The biological activ-

ity and antioxidant effects of AG have also been studied (8-10). In addition, there have been several food patent development studies exploring the incorporation of AG into bread (11), fish cakes (12), kimchi (13), syrup, sikhye, and functional drinks. However, the study of the development of natural functional foods containing AG remains insufficient.

The present study will examine differences in the biological activities of cultivated *Angelica gigas* Nakai (CAG) and wild *Angelica gigas* Nakai (WAG). Of particular interest are the effects of the growing environment on the electron donating ability (EDA), superoxide dismutase (SOD)-like activity, total polyphenol content, tyrosinase inhibitory effect, nitrite-scavenging effect, and angiotensin-converting enzyme (ACE) inhibitory activity of AG. This information will provide fundamental data for the development of natural functional foods containing AG.

## MATERIALS AND METHODS

### Materials and extraction

Two kinds of AG were used in this study: cultivated and

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wild. CAG was purchased from the markets in Jeongseon, Gangwon and WAG was taken from Mt. Sobaek (Yeongju, Korea). After freeze-drying (FD 5512, Ilshin Lab Co., Ltd., Seoul, Korea), the AG were ground and stored frozen in a sealed 0.2 mm polyethylene film. Afterwards, they were extracted in 25 mL of water (i.e., 0% ethanol), 30% ethanol, 60% ethanol, or 90% ethanol with microwave assisted extraction (MAE; Soxwave-100, Prolabo, Paris, France) at 60 W for 5 min.

#### Electron donating ability

The reducing power of each extract was determined by measuring the EDA according to a modified method by Blois (14). Briefly, 0.2 mL of each extract was mixed with 0.8 mL of  $4 \times 10^{-4}$  M 1,1-diphenyl-2-picrylhydrazyl (DPPH) and dissolved in 2 mL of 99.9% ethanol to yield a total volume of 3 mL. After vortexing the mixtures for 10 s and incubating them at room temperature for 10 min, the absorbance of each mixture was measured at 525 nm using a SpectraMax M2 (Molecular Devices, LLC., Sunnyvale, CA, USA). Ascorbic acid was used as the positive control.

#### Superoxide dismutase (SOD)-like activity

To control the oxidation rate of the pyrogallol, which is oxidized by superoxide, the SOD-like activity of each extract was measured by the method of Marklund and Marklund (15). The pH of each sample was adjusted to 8.5 using a tris-HCl buffer [50 mM tris(hydroxymethyl)aminomethane, 10 mM EDTA]. Three milliliters of the tris-HCl buffer and 0.2 mL of 7.2 mM pyrogallol were added to 0.2 mL of each sample. The mixtures were incubated at 25°C for 10 min and then 0.2 mL of 1 N HCl was added to stop the reaction. The absorbance of the resulting mixtures was measured at 420 nm using a UV/VIS spectrometer (SpectraMax M2, Molecular Devices, LLC.). Ascorbic acid was used as the positive control.

#### Total polyphenol content

The total polyphenol content of each extract was measured by the method of Folin-Denis (16). Briefly, 0.5 mL of 1 N Folin-Ciocalteu reagent was added to 0.5 mL of each sample. The mixture was allowed to incubate for 3 min and then 10 mL of 2%  $\text{Na}_2\text{CO}_3$  solution was added. The resulting solution was mixed, incubated for 1 h, and then the absorbance was measured at 750 nm using a UV/VIS spectrometer (V-570, Jasco Inc., Hachioji, Japan). The total polyphenol content was determined from a tannic acid standard curve.

#### Tyrosinase inhibitory effect

The tyrosinase inhibitory effect was measured by the method of Wong et al. (17). A crude tyrosinase solution

was prepared by dissolving mushroom tyrosinase in 50 mM sodium phosphate buffer (pH 7.0). Subsequently, 0.2 mL of the crude tyrosinase solution and 0.1 mL of extract were added to 2.8 mL of 10 mM catechol solution. For the control sample, 50 mL of sodium phosphate buffer (pH 7.0) was added to the mixture instead the crude tyrosinase solution. The absorbance of each reaction mixture was measured at 420 nm by a UV/VIS spectrometer (V-570, Jasco Inc.). Ascorbic acid was used as the positive control.

#### Nitrites scavenging effect

The method described by Gray and Dugan (18) was used to measure the nitrite scavenging effect of each extract. Briefly, 0.1 mL of 1 mM  $\text{NaNO}_2$  solution was added to 0.2 mL of each extract, and the pH values of the resulting mixtures were adjusted to 1.2 (0.1 N HCl), 3.0, 4.2, or 6.0 using a 0.2 N of buffer solution. The final volume of each sample was adjusted to 1 mL. The samples were allowed to react at 37°C for 1 h, mixed thoroughly with 5 mL of 2% acetic acid and 0.4 mL of Griess reagent, and then incubated at room temperature for 15 min. The nitrite content of each mixture was determined by measuring the absorbance at 520 nm (SpectraMax M2, Molecular Devices, LLC.). The control was processed with the same method, but was prepared with 0.4 mL of distilled water instead of 0.4 mL Griess reagent. Ascorbic acid was used as the positive control.

#### ACE inhibitory activity

ACE inhibitory activity was measured by the modified method of Cushman (19). ACE crude enzyme extract was prepared by adding rabbit lung acetone powder (Sigma, St. Louis, MO, USA) to 300 mM NaCl-100 mM sodium borate buffer (pH 8.3) at a ratio of 0.2 g/10 mL (w/v). The mixture was incubated at 4°C for 24 h and then centrifuged (4°C, 8,000 rpm, 70 min) to obtain ACE crude enzyme extract.

To measure ACE inhibitory activity, 100  $\mu\text{L}$  of 450 mM NaCl-100 mM sodium borate buffer (pH 8.3) and 50  $\mu\text{L}$  of 5 mM hippuryl-histidyl-leucine were added to 50  $\mu\text{L}$  of each AG extract, and the mixtures were pre-cultivated at 4°C for 10 min. Next, 50  $\mu\text{L}$  of the ACE crude enzyme extract was added to each mixture and the mixtures were incubated at 37°C for 30 min. Then 100  $\mu\text{L}$  of 1.75 N HCl and 1.5 mL of ethyl acetone were added to each mixture and 1 mL of supernatant was obtained. The supernatant was dried at 100°C for 1 h. Then 1 mL of distilled water was added to dissolve the residue, and the absorbance of the resulting solution was measured at 228 nm using a UV/VIS spectrometer (V-570, Jasco Inc.). The control was processed with the same method, but was prepared with 50  $\mu\text{L}$  of distilled water instead of the AG extract.

### Statistical analysis

Duncan's multiple range tests were used to examine the significance of the differences among the experimental groups (SAS version 8.0, SAS Institute Inc., Cary, NC, USA).

## RESULTS AND DISCUSSION

### Electron donating ability

Fig. 1 shows the DPPH radical scavenging activity of cultivated and wild AG extracts. For all extraction methods, the EDA of WAG was higher than the EDA of CAG (WAG: 88.05~96.03%, CAG: 68.02~93.94%). The EDA of the ethanol extracts was higher than the EDA of the water extracts; EDA increased as the ethanol content of the extracts increased. Among the extracts tested, the EDA of the 90% ethanol extract of WAG was the highest (96.03%). The EDA of the 90% ethanol extract of CAG and WAG was 93.94% and 96.03%, respectively, while the EDA of 0.1% L-ascorbic acid and 1% L-ascorbic acid, the comparative substance, was 86.97% and 90.83%, respectively.

According to the research of Chang et al. (20), the EDA of an AG extract by reflux condensing added to the water with 1:20 ratio (v/w) is 94.37%, which is higher than the EDA value measured in the AG water extracts in the present study. However, other authors have reported AG water extract EDA values that are lower than those measured in the present study. For example, Kang et al. (8) reported that the EDA of a water extract of AG was 66.8% and Ahn et al. (9) reported that the EDA of a water extract of AG was 27.89%. These differences in EDA may be due to differences in the extract to solvent ratio and differences in the extraction methods used.

The EDA values reported in the present study are

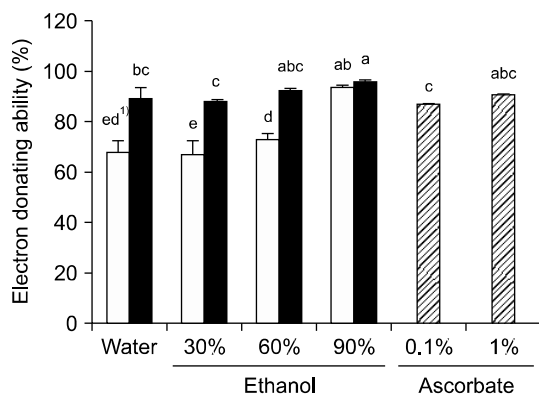


Fig. 1. Electron donating ability of microwaved *Angelica gigas* Nakai extracts.  $\square$ : cultivated *Angelica gigas* Nakai,  $\blacksquare$ : wild *Angelica gigas* Nakai. Microwave-assisted extraction (60 W, 5 min) was performed on a mixture composed of 2 g of sample and 50 mL of solvent. <sup>1</sup>Means with the same letter are not significantly different ( $P < 0.05$ ).

greater than those reported for other medicinal plants. For example, Lee et al. (21) found that the EDA of *Angelica genuflexa* Nutt, another medicinal plant of the Apiaceae family, is between 0.51% and 49.79%, depending on the extract contents and solvents. According to Jeong et al. (22), the EDA of most of the medicinal plants that are available at the market is lower than the EDA of AG. For example, an 80% methanol (MeOH) extract of peony has an EDA of 57.1%, an 80% MeOH extract of ginger has an EDA of 48.3%, and an 80% MeOH extract of microphyll has an EDA 37.2%. In a study of *Angelica utilis* Makino, the EDA of red *Angelica utilis* Makino was higher than that of green *Angelica utilis* Makino, depending on kinds of *Angelica* sp. and extract solvents showed the highest (23) as AG's activity changes. Although the EDA of AG was different on kinds, extract methods, extract solvents, and extract ratio, the results of the current study indicate that the EDA of AG is greater than the EDA of other medicinal plants.

### SOD-like activity

The results of the SOD-like activity determinations are shown in Fig. 2. The SOD-like activity of CAG and WAG was under 34%, while the activity of 0.1% L-ascorbic acid and 1% of L-ascorbic acid was 60.65% and 75.93%, respectively. Overall, the SOD-like activity of CAG was higher than that of WAG, regardless of the solvent used. While SOD-like activity was higher in the water-extracted AG samples than in the ethanol-extracted AG samples, SOD-like activity was not affected by the concentration of ethanol in the extraction solvent.

Kim et al. (24) reported that the SOD-like activity of AG is 11.6%, which is lower than the SOD-like activity of AG measured in this study. This difference may be due to differences in the extraction method used and the contents of the extracts. The SOD-like activity measured

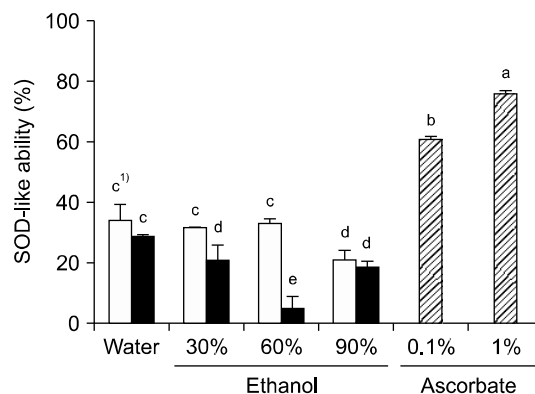


Fig. 2. SOD-like activity of microwaved *Angelica gigas* Nakai extracts.  $\square$ : cultivated *Angelica gigas* Nakai,  $\blacksquare$ : wild *Angelica gigas* Nakai. Microwave-assisted extraction (60 W, 5 min) was performed on a mixture composed of 2 g of sample and 50 mL of solvent. <sup>1</sup>Means with the same letter are not significantly different ( $P < 0.05$ ).

in the CAG and WAG extracts of the current study was lower than the SOD-like activity of extracts from other medicinal plants, such as *Epimedium koreanum* Nakai (42.4%), *Acanthopanax* (24.2%), and *Phlomis* root (21.3%), that were extracted under the same conditions as those used in the current study (7). The antioxidant activity of water extracts from elm tree and *Hemiptelea davidii* is 14.51~98.24% and the antioxidant activity of ethanol extracts from elm tree and *Hemiptelea davidii* is 0.34~80.00%. Antioxidant activity increased with the extracted content (25).

Previous work indicates that the SOD-like activity of water-extracted *Vitex* seed is higher than the SOD-like activity of ethanol-extracted *Vitex* seeds and upon the density of extracts (26). Similarly, it is expected that the SOD-like activity of AG will increase as the content of the extract increases. The SOD-like activity of an ethanol extract of *Angelica dahurica* root was higher than that of a water extract of *Angelica dahurica* root (23.24% vs. 15.86%, respectively). In addition, the SOD-like activity of the reflux extract of *Angelica dahurica* root was higher than that of the MAE extract of *Angelica dahurica* root (27). Together, these results indicate that the SOD-like activity of AG could be affected by the extract method and the extract contents.

### Total polyphenol content

The total polyphenol content of each extract (in mg%) is shown in Fig. 3. The total polyphenol content for WAG was 114.97~146.41 mg% and 133.05~178.02 mg%. The total polyphenol content of all of the extracts was higher than 100 mg%, but at least two times lower than the activity of the comparative substance, L-ascorbic acid. The 60% ethanol extracts of CAG and WAG had the highest total polyphenol content (144.88 mg% and 178.02 mg%, respectively), while the 90% ethanol ex-

tracts of CAG and WAG had the lowest total polyphenol content (114.97 mg% and 133.05 mg%, respectively). The rank order (highest to lowest) of the total polyphenol content of the extracts tested in this study is: 60% ethanol extracts > 30% ethanol extracts > water extracts > 90% ethanol extracts.

Chang et al. (20) reported that the total polyphenol content of a hot water extract of AG is 5.77 mg/g and the total polyphenol content of a hot water extract treated with cold ethanol and partly refined is 6.16 mg/g. Similar to the results of the present study, the total polyphenol content of a 60% ethanol extract of rosemary is greater than the total polyphenol content of a water extract of rosemary (25.7 mg/g vs. 24.3 mg/g, respectively) (28). Likewise, the total polyphenol content of an 80% ethanol extract of *Morus bombycis* Koidzumi is greater than that of a water extract of *Morus bombycis* Koidzumi (29). The rank order of the total polyphenol content of fresh *Pleurotus eryngii* is as follows: hot water extract > 50% ethanol extract > 100% ethanol extract (30). We hypothesize that these differences in polyphenol content occur because polyphenol components exist in various forms and kinds in plants and therefore elute differently under different extraction conditions (31). Lee et al. (25) and Kroyer et al. (32) report that DPPH radical scavenging activity increases with total polyphenol content. However, this study was to estimate the correlation between the electron donating ability and polyphenols.

### Tyrosinase inhibitory effect

The tyrosinase inhibitory effect of AG extracts is shown in Fig. 4. The tyrosinase inhibitory effect of WAG extracts increased from 26.58% to 37.56% as the ethanol concentration of the extraction solvent increased. In contrast, the tyrosinase inhibitory effect of CAG extracts

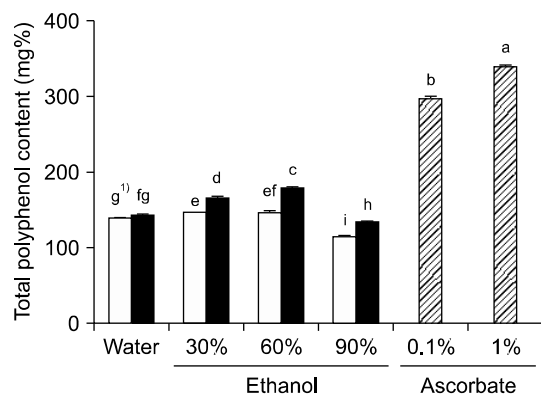


Fig. 3. Total polyphenol contents (mg%) of microwaved *Angelica gigas* Nakai extracts. □: cultivated *Angelica gigas* Nakai, ■: wild *Angelica gigas* Nakai. Microwave-assisted extraction (60 W, 5 min) was performed on a mixture composed of 2 g of sample and 50 mL of solvent. <sup>1</sup>Means with the same letter are not significantly different ( $P < 0.05$ ).

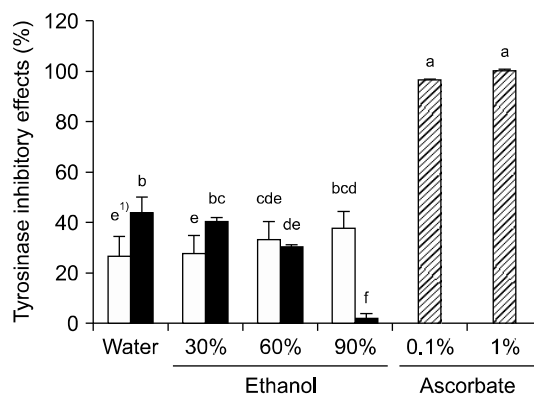


Fig. 4. Tyrosinase inhibitory effects of microwaved *Angelica gigas* Nakai extracts. □: cultivated *Angelica gigas* Nakai, ■: wild *Angelica gigas* Nakai. Microwave-assisted extraction (60 W, 5 min) was performed on a mixture composed of 2 g of sample and 50 mL of solvent. <sup>1</sup>Means with the same letter are not significantly different ( $P < 0.05$ ).

decreased from 43.72% to 1.84% as the ethanol concentration of the extraction solvent increased. The highest tyrosinase inhibitory effect (43.72%) was two times lower than the tyrosinase inhibitory effect of the comparative solutions, 0.1% L-ascorbic acid (96.71%) and 1% L-ascorbic acid (100.47%).

Jung et al. (33) measured the tyrosinase inhibitory effect of medicinal plant extracts (2 g dry weight extracted with 100 mL water). They found that the tyrosinase inhibitory effect of AG is 39%, which is slightly higher than the maximum tyrosinase inhibitory effect measured in the 1:25 ratio (v/w) WAG extracts of the present study. The tyrosinase inhibitory effect of a snake beard water extract is 22.80%, which is lower than that of the AG measured in the present study (34). Previous reports indicate that the tyrosinase inhibitory effect of plum is 88.0%~100%, which is similar to that of the comparative substance, L-ascorbic acid, used in the present study (35).

Song et al. (23) reports that the whitening effect of tyrosinase observed in *Angelica utilis* Makino is affected by the total polyphenol content. Likewise, previous work indicates that the highest tyrosinase inhibitory effect is observed in the white sandalwood extracts with the highest total polyphenol content (36). However, the results of the present study did not reveal a correlative relationship between the tyrosinase inhibitory effect and the total polyphenol content of AG.

### Nitrite scavenging effect

As shown in Table 1, the nitrite scavenging effect of the AG extracts was affected by pH. The nitrite-scavenging effect of CAG and WAG was highest at a pH of 1.2 and decreased as pH increased, indicating that the nitrite scavenging effect was higher in more acidic conditions. In addition, at a pH of 1.2, the scavenging effect of all of the extracts tested was higher than that of the comparative solutions, 0.1% L-ascorbic acid and 1% of L-ascorbic acid (84.34% and 84.93%, respectively). There was no distinct difference in the nitrite scavenging effects of CAG and WAG in the present study. The highest

scavenging ability was observed in the 30% ethanol extracts of CAG and WAG. It demonstrated a higher nitrite scavenging effect at water extract of CAG and ethanol extract of WAG.

The present study provides support for previous reports that AG has a superior nitrite scavenging effect. Earlier studies report that AG water extracts and methanol extracts have a very high nitrite scavenging effect at a pH of 1.2 (92.87~96.51% and 93.00~98.86%, respectively) (10). The results of the current study also support previous reports that the nitrite scavenging effect of medicinal herbs varies with pH, and that there is no difference in the nitrite scavenging effect of water and ethanol AG extracts (37). However in the previous study, the nitrite scavenging effect of water and ethanol AG extracts with a pH of 1.2 was 37% and 35%, respectively. Previous reports indicate that the nitrite scavenging effect of Korean mountain ginseng, *Rubus coreanus*, and *Angelica dahurica* leaves decreases with increasing pH and that the nitrite scavenging effect of these leaves is at least two times greater than that of AG (38-40).

### ACE inhibitory activity

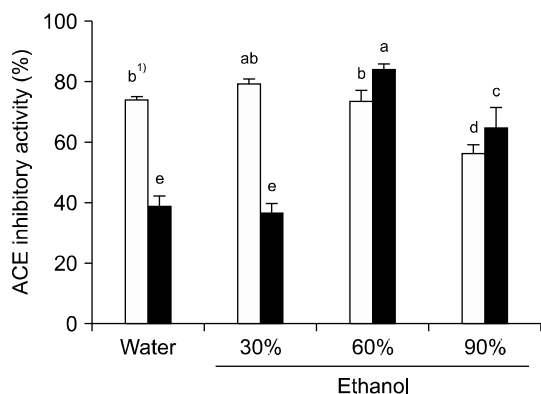
Angiotensin II increases blood pressure by inducing blood vessel contraction and increases the secretion of aldosterone into body fluids. Angiotensin II is produced by ACE when rennin, an enzyme, acts on angiotensinogen to produce angiotensin I (41). To prevent blood pressure, it is required to inhibition of ACE. The ACE inhibitory activity of CAG extracts was measured in this study. As shown in Fig. 5, the rank order of the ACE inhibitory activity of CAG extracts was: 30% ethanol extract (79.13%) > water extract (73.58%) > 60% ethanol extract (73.28%) > 90% ethanol extract (56.17%). In contrast, the rank order of the ACE inhibitory activity of WAG extracts was: 60% ethanol extract (83.84%) > 90% ethanol extract (64.67%) > water extract (38.76%) > 30% ethanol extract (36.29%).

Previously, Do et al. (42) reported that the ACE inhibitory activity of 10 mg/mL *Psoralea corylifolia* L. is

**Table 1.** Nitrite scavenging effect of microwaved *Angelica gigas* Nakai extracts

	Solvent	Nitrite scavenging ability (%)			
		pH 1.2	pH 3.0	pH 4.2	pH 6.0
Cultivated <i>Angelica gigas</i> Nakai	Water	97.50±0.35 <sup>a1)</sup>	66.71±0.52 <sup>b</sup>	25.59±1.56 <sup>c</sup>	13.44±2.67 <sup>d</sup>
	30% ethanol	98.63±0.18 <sup>a</sup>	82.66±0.41 <sup>b</sup>	28.80±2.01 <sup>c</sup>	11.33±2.12 <sup>d</sup>
	60% ethanol	97.60±0.09 <sup>a</sup>	70.22±0.26 <sup>b</sup>	25.91±1.57 <sup>c</sup>	13.51±0.67 <sup>d</sup>
	90% ethanol	92.04±0.22 <sup>a</sup>	62.33±0.15 <sup>b</sup>	21.37±1.47 <sup>c</sup>	7.45±3.26 <sup>d</sup>
Wild <i>Angelica gigas</i> Nakai	Water	86.35±0.35 <sup>a</sup>	50.64±0.53 <sup>b</sup>	20.04±0.76 <sup>c</sup>	12.42±0.42 <sup>d</sup>
	30% ethanol	101.20±0.83 <sup>a</sup>	88.10±0.30 <sup>b</sup>	32.34±9.33 <sup>c</sup>	6.73±1.21 <sup>d</sup>
	60% ethanol	101.07±1.64 <sup>a</sup>	88.08±5.06 <sup>b</sup>	33.53±1.88 <sup>c</sup>	15.60±2.41 <sup>d</sup>
	90% ethanol	93.55±1.08 <sup>a</sup>	71.82±1.56 <sup>b</sup>	25.32±1.49 <sup>c</sup>	15.69±1.63 <sup>d</sup>
0.1% L-ascorbic acid		84.38±0.14 <sup>a</sup>	50.48±1.87 <sup>b</sup>	30.68±1.37 <sup>c</sup>	14.78±1.36 <sup>d</sup>
1% L-ascorbic acid		84.93±0.24 <sup>a</sup>	85.01±0.05 <sup>a</sup>	84.34±0.23 <sup>b</sup>	76.49±0.46 <sup>c</sup>

<sup>1)</sup>Within each row, means with the same letters are not significantly different ( $P < 0.05$ ).



**Fig. 5.** ACE inhibitory ability of microwaved *Angelica gigas* Nakai extracts. □: cultivated *Angelica gigas* Nakai, ■: wild *Angelica gigas* Nakai. Microwave-assisted extraction (60 W, 5 min) was performed on a mixture composed of 2 g of sample and 50 mL of solvent. <sup>1)</sup>Means with the same letter are not significantly different ( $P < 0.05$ ).

65.2%, and Kang et al. (43) noted that the ACE inhibitory activity of pine needles and a hot water extract of mugwort are 61.0% and 75.1%, respectively. In addition, the ACE inhibitory activity of *Phellinus ribis* is relatively low (12.0%) (44). Therefore, the results of the present study indicate that AG has notable ACE inhibitory activity.

## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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