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Biological activity of functional foods and anti-microbial activity of phenolics from *Sambucus sieboldiana var. pendula* leaves

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Abstract This study aimed to investigate the biological and antibacterial activities of phenolics from *Sambucus sieboldiana var. pendula* leaf extract. The phenolic contents of the *S. sieboldiana var. pendula* extracted with water and ethanol was 11.60 and 12.39 mg/g, respectively. The inhibitory effects of the water and ethanol extracts on the angiotensin converting enzyme (ACE) were 71.94-92.08 and 48.42-78.33%, respectively, and on xanthine oxidase (XOase) were 42.11-100 and 35.61-100%, respectively, at a phenolic concentration of 50-200 μg/mL. All these effects were found to be concentration-dependent. Additionally, the leaf extracts exhibited excellent antibacterial activities against *Streptococcus mutans*, *Staphylococcus aureus*, and *Propionibacterium acnes*. Hence, *S. sieboldiana var. pendula* was confirmed to have excellent antihypertensive, antigout, and antimicrobial properties.

Keywords: antigout, antihypertensive, antimicrobial, *S. sieboldiana var. pendula*

Introduction

Environmental hormones, chemicals, and exhaust gas from internal combustion engines, have increased the atmospheric pollution. Owing to increasing pollution rates, individuals have started to focus on eco-friendly food products. In addition, the link between quality of life and diet has made it possible to discover the bioactive functions of plants widely distributed in nature and how to apply them to functional foods (Choi and Yang, 2008). The Nagoya Protocol of the 2010 Convention on Biological Diversity compiles information on each country's native plants how they are explored and developed. In Korea, attempts have been made to discover functional and medicinal products from plants (Han, 2010; Jeong et al., 2012; Kim and Kim, 1999; Lee et al., 2005; Seo et al., 2011). The importance of oriental medicines for the treatment or prevention of diseases has been highlighted in several studies conducted on various medicinal plants, wild plants, and wild vegetables (Chung et al., 2010; Im et al., 2011). Wild plants and vegetables contain various types of tannins, alkaloids, saponins, glycosides, and flavonoids and can be used as health foods owing to their positive pharmacological effects and low toxicity (Chung et al., 2011; Kim et al., 2012).

Sambucus sieboldiana var. pendula is a local plant native to the local forestry and openlands of Ulleungdo, Republic of Korea. It is a deciduous broad-leaved shrub that grows up to 4-5 m in height. Its yellowish-white flowers bloom in June, and its fruits ripen to vermilion in July. All plants of the genus *Sambucus* species, including *S. sieboldiana var. pendula*, are referred to as *S. racemosa* and are used as diuretics in kidney disease and hydrocephalus in Korea and Japan (Lee, 2014). Recent studies have reported the antioxidant effect (Chae and Cho, 2012), the effect on rheumatoid arthritis (Lee et al., 2012) and osteoporosis (Yoo and Jeong, 2000), the hepatocellular protective activity (Kim, 2013) and the anti-inflammatory activity of *S. racemosa* (Kim et al., 2013). However, the antibacterial activity of this plant remains unknown. Therefore, this study aimed to investigate the pharmacological activities of *S. sieboldiana var. pendula* leaves.

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Materials and Methods

Preparation of sample extracts

The *S. sieboldiana var. pendula* leaves were collected from Ulleungdo, dried at 45°C (Jeiotech, Daegu, Korea), and then powdered to 40-mesh size using a shredder machine (RT-08, Guohua ChME Inc., Tainan, Taiwan). To determine the optimal extraction conditions, 1 g of powdered *S. sieboldiana var. pendula* leaves was added to 100 mL of various solvents (distilled water, methanol, ethanol, acetone, and n-butanol) and shaken at room temperature for 24 h. The effect of the extraction solvent concentration was determined by changing its concentration from 10 to 100% using ethanol and distilled water.

S. sieboldiana var. pendula fleaves were prepared in two forms, phenolic extract and solid powder, for comparative analysis. The first type was a phenolic extract. To prepare samples for subsequent activity assays, 1 g of *S. sieboldiana var. pendula* leaf powder was placed in an erlenmeyer flask with 200 mL of distilled water and heated until the total volume reached 100 mL. Then, the mixture

was allowed to cool to room temperature and shaken for 24 h. Next, 100 mL of 10-100% ethanol added to 1 g of *S. sieboldiana var. pendula* leaf powder and the ethanol extract was shaken at room temperature for 24 h. The extract was filtered using a filter paper (Whatman No. 1, Whatman, Brentford, UK) and vacuum-dried in a rotary evaporator (Eyela NE, Tokyo, Japan) to the target concentration (50, 100, 150, and 200 μg gallic acid equivalent (GAE)/mL) for the experiment. The second type was a solid powder. This was prepared by drying extract of *S. sieboldiana var. pendula* fleaves at −80°C using a freeze dryer (FDS8518, Ilshin Bio Base Co. Ltd, Dongducheon, Korea).

Determination of total phenolic content

Phenolic content was measured using the Folin-Denis method (Folin and Denis, 1912). After mixing 1 mL of the extract with 1 mL of 95% ethanol and 5 mL of distilled water, 0.5 mL of 1 N Folin-Ciocalteu reagent was added to the mixture, until blue color was developed, and left for 5 min. A total of 1 mL of Na_2CO_3 was added and the absorbance at 725 nm was measured within 1 h. The total phenolic content was measured using a standard curve with gallic acid (mg GAE/g).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical inhibition was measured using the method by Blois (1958). The blank was mixed with 3 mL of 60 μM DPPH and 1 mL of distilled water by vortexing. Also 3 mL of 60 μM DPPH was added to 1 mL of samples and mixed. This mixture was stored at room temperature for 15 min then the absorbance was measured at 517 nm. The inhibition rate (%) was calculated as follows: 1−(absorbance of the sample/absorbance of the blank) ×100.

Angiotensin converting enzyme (ACE) inhibitory activity

The ACE inhibitory effect was measured as described by Cushman et al. (1977). Briefly, the blank was mixed with 0.15 mL of a 2.5 mM hippuryl-histidyl-leucine solution as a substrate in 0.1 M potassium phosphate buffer (pH 8.3, containing 0.3 M NaCl). As a control, 0.1 mL of the phosphate buffer was added instead of the sample, and reacted at 37°C for 30 min. To the test solutions, 0.35 mL of 1 N HCl was added to stop the reaction, and 3 mL of ethyl acetate was added to elute the resulting hippuric acid. The ethyl acetate layer was aliquoted, the solvent was distilled, and 2 mL of distilled water was further added to the remaining residue. The absorbance of hippuric acid obtained from the substrate was measured at 280 nm. Captopril was used as a positive control. The inhibition rate (%) was calculated as follows: 1−(hippuric acid content in sample/hippuric acid content in control) ×100.

Xanthin oxidase (XOase) inhibitory activity

The XOase inhibitory effect was measured as described by Stirpe (1969). Briefly, 0.3 mL of sample solution was mixed with 0.1 M potassium phosphate buffer (pH 7.5), 3 mL of substrate solution (2 mM xanthine solution), and 0.1 mL of enzyme solution. The control was prepared similaly but with 0.3 mL of distilled water used instead of the sample solution. Each sample was reacted at 37°C for 30 min. The reactions were stopped by adding 1 mL of 20% trichloroacetic acid (TCA) to the reaction mixture. Then the samples were centrifuged to remove proteins, following which the absorbance of uric acid was measured at 292 nm. Allupurinol was used as a positive control. The measured values were converted to the amount of uric acid using a standard curve of uric acid. The inhibition rate (%) was calculated as follows: 1−(uric acid production in the sample/uric acid production in the control)×100.

Antimicrobial activity

Staphylococcus aureus, *Escherichia coli*, *Propionebacterium acnes*, *Streptococcus mutans*, and *Helicobacter pylori* were used to determine the antibacterial activity of *S. sieboldiana var. pendula*. Nutrient agar medium (Difco., Plymouth, MI, USA) was used to incubate *S. aureus*, *E. coli*, and *P. acnes*. Bacteria were inoculated on agar plates and incubated at 37°C for 24-48 h. Brain heart infusion medium was used for *S. mutans* and optimal medium was used for *H. pylori* (0.5 g special peptone, 0.75 g agar, 0.25 g NaCl, 0.25 g yeast extract, 0.2 g beef extract, and 0.025 g pyruvic acid per in 50 mL medium). To maintain microaerobic conditions, the plates were incubated at 10% CO_2 incubator at 37°C for 48-72 h with a humidity of >95% (Ju and Cho, 2009). The antibacterial activity was measured using the paper disc method to measure the inhibitory zone (clear zone). The diameter of inhibitory zone surrounding paper discs and contact area of paper discs with agar surface were measured. Inhibitory zone was calculated by subtracting overall clear zone by diameter of the paper disc.

Statistical analysis

The experiment were performed in triplicates and the results are expressed as means ± standard deviation. Analysis of variance and duncan's multiple range test were used, and a 95% confidence level was assumed to indicate statistical significance. All statistical analysis were performed using SPSS 7.5 (Statistical Package for Social Science, Chicago, IL, USA).

Results and Discussion

Change of phenolic content in extracts by solvent type and concentration

The phenolic compounds, secondary metabolites produced in defense responses by plants, bind to proteins via their hydroxyl group. Owing to their properties, phenolic compounds have various biological functions (Choi et al., 1998; Choi et al., 2003). The potential pharmacological activity of *S. sieboldiana var. pendula* was evaluated in the phenolic components isolated from the leaves of this plant. Then, the amount of phenolics extracted was determined using various solvents (distilled water, methanol, ethanol, acetone, and n-butanol). As shown in Fig. 1A, when distilled water and ethanol were used as solvents, the phenolic content was the highest at 11.60 and 12.39 mg GAE/g, respectively. The content of phenolics extracted using ethanol was higher than that extracted using other solvents. In addition, a

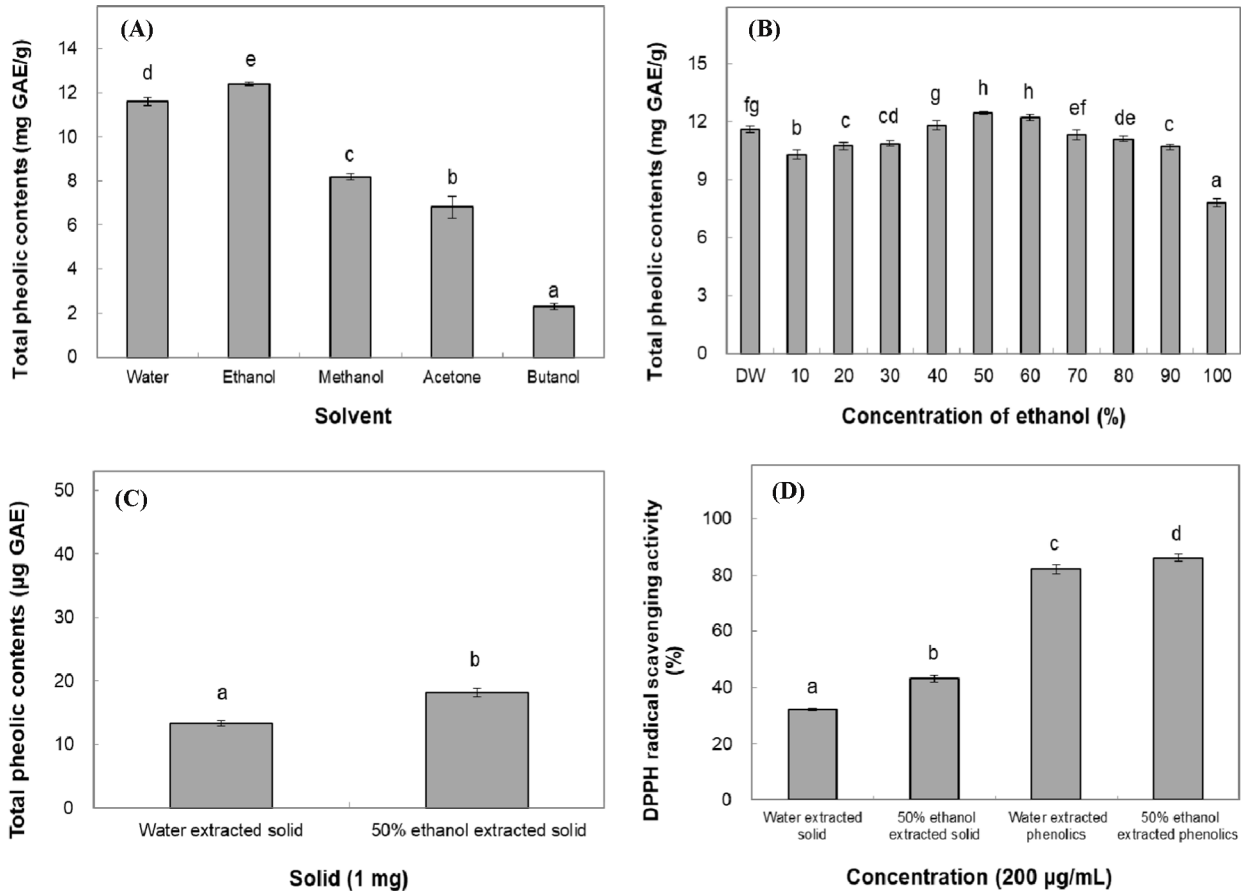

Fig. 1. The extracted phenolic content from *S. sieboldiana var. pendula* leaf by various solvents (A) and ethanol concentration (B). Total phenolic contents in extracted solid (C), DPPH radical scavenging activity by extracted solid and phenolics (D). Data are expressed as the mean±standard deviation (n=6). *p<0.05. GAE: gallic acid equivalents.

mixture of 50% ethanol in water resulted in the highest extraction of phenolics (12.43 mg GAE/g) as shown in Fig. 1B. According to these results, the extract of *S. sieboldiana var. pendula* using 50% ethanol and water, which is harmless to the human body, was used for the following studies.

In this experiment, extracts of two types were divided into solid and phenolics. The total phenolic contents contained in the water and 50% ethanol extracts of *S. sieboldiana var. pendula* were 13.3 and 18.1 μg per 1 mg of the solid, respectively (Fig. 1C). The antioxidant activity of solid and phenolic extracts at the same concentration of 200 μg/mL was tested by DPPH assay. Compared to the 40% of antioxidant activity caused by the extracted solid, the level of antioxidant activity in extracted phenolics was higher than 80% (Fig. 1D). Therefore, we judged that the phenolics of extract in *S. sieboldiana var. pendula* were related to the physiological activity. Based on these results, the extracted phenolics of *S. sieboldiana var. pendula* was selected to confirm physiological activity.

ACE inhibitory activity

ACE plays a role in the production of angiotensin II, which can induce hypertension. Therefore, ACE inhibitors inhibit the production of angiotensin II, decrease the production of aldosterone, and increase the production of bradykinin. Consequently, blood pressure reduces through the increase in sodium excretion caused by the expansion of renal blood vessels (Oh et al., 1997). In this study, a high ACE inhibitory activity of 71.94 to 92.08% was observed for 50-200 μg/mL phenolics extracted in water (Table 1). An ACE inhibitory activity of 48.42 to 78.33% was measured for 50-200 μg/mL phenolics in extracted ethanol, and all these effects were concentration-dependent. Oh et al. (2010) who reported ACE inhibition rates of 11.8, 52.7, 2.3, 14.3, and 4.3% in extracts of four plants, including camellia, the antihypertensive activity of *S. sieboldiana var. pendula* leaf extract in our study was superior.

XOase inhibitory activity

XOase, which is involved in the purine metabolism, forms uric acid from xanthine or hypoxanthine. When the generated uric acid increases in the plasma, it accumulates in the joints causing gout. This disease causes severe pain and joint swelling. Therefore, the inhibition of XOase enzyme activity inhibits the generation of free radicals that may occur during lipid oxidation (Hatano et al., 1991). The XOase inhibitory effect of *S. sieboldiana var. pendula* leaf extract at a phenolic concentration of 50-200 μg/mL of water and ethanol extract, were 42.11-100 and 35.61-100%, respectively (Table 2). In addition, compared to allopurinol, a gout suppression

Table 1. Inhibitory activity of *S. sieboldiana var. pendula* leaf phenolics against angiotensin converting enzyme

Total phenolic content (μg GAE/mL)	Sample					
	Water extract		50% ethanol extract		Positive control (Captopril)	
	Hippuric acid (μg/mL)	Inhibitory activity (%)	Hippuric acid (μg/mL)	Inhibitory activity (%)	Hippuric acid (μg/mL)	Inhibitory activity (%)
Control	8.11±0.06	-	8.11±0.06	-	8.11±0.06	-
50	2.96±0.12	71.94±1.51[a1)]	4.87±0.08	48.42±1.04[a]	6.35±0.05	30.09±0.63[a]
100	1.94±0.03	84.61±0.42[b]	3.63±0.08	63.74±0.96[b]	5.70±0.07	38.17±0.84[b]
150	1.36±0.06	91.72±0.75[c]	2.79±0.03	74.11±0.42[c]	4.86±0.03	48.54±0.36[c]
200	1.33±0.09	92.08±1.11[c]	2.44±0.04	78.33±0.55[d]	3.60±0.03	64.10±0.36[d]

[1)]The data were expressed as the mean±standard deviation (n=6). *p<0.05.

Table 2. Inhibitory activity of *S. sieboldiana var. pendula* leaf extract against xanthine oxidase

Total phenolic content (μg GAE/mL)	Sample					
	Water extract		50% ethanol extract		Positive control (Allopurinol)	
	Uric acid (ug/ml)	Inhibitory activity (%)	Uric acid (ug/ml)	Inhibitory activity (%)	Uric acid (ug/ml)	Inhibitory activity (%)
Control	17.89±0.14	0	17.89±0.14	0	17.89±0.14	0
50	10.36±0.21	42.11±1.16[a1)]	11.52±0.61	35.61±3.40[a]	7.17±1.30	59.90±7.25[a]
100	3.16±0.18	82.36±0.96[b]	6.04±0.34	66.26±1.89[b]	6.42±1.10	64.12±6.15[a b]
150	-	100.0±2.43[c]	2.02±0.28	88.83±1.52[c]	5.85±0.06	67.28±0.32[b]
200	-	100.0±0.96[c]	-	100.0±1.06[d]	5.30±0.19	70.37±1.04[c]

[1)]The data were expressed as the mean±standard deviation (n=6). *p<0.05.

Table 3. Antimicrobial activity *S. sieboldiana var. pendula* leaf phenolics

Name of micro organism	Clear zone (cm)									
	Total phenolic content (μg GAE/100 μL)									
	Water extract					50% ethanol extract				
	0[1)]	50[2)]	100[3)]	150[4)]	200[5)]	0[1)]	50[2)]	100[3)]	150[4)]	200[5)]
H. pylori	ND[6)]	ND	ND	ND	ND	ND	ND	ND	ND	ND
P. acnes	ND	ND	ND	ND	1.4	ND	ND	ND	ND	ND
S. aureus	ND	0.7	0.9	1.0	1.2	ND	ND	ND	ND	ND
E. coli	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S. mutans	ND	0.9	1.0	1.1	1.3	ND	0.75	0.9	1.0	1.1

[1)]0 μg/100 μL phenolics, [2)]50 μg/100 μL phenolics, [3)]100 μg/100 μL phenolics, [4)]150 μg/100 μL phenolics, [5)]200 μg/100 μL phenolics, [6)]ND is not detected

prescription medicine that was used as a positive control, showed 59.90-70.37% XOase inhibitory activity at a treatment concentration of 50-200 μg/mL. Compared to the results of other studies that reported 20 and 10% XOase inhibitory activity in white lotus extract (Lee et al., 2010) and cratagi extract (An et al., 2002), respectively, the gout inhibitory effect of *S. sieboldiana var. pendula* leaf extract in our syudy was superior.

In this study, the inhibitory effect of ACE and XOase were higher in the water extract with a slightly lower total phenolic contents than the 50% ethanol extract. Also, Song et al. (2013) reported that study of antioxidant activity using ethanol and hot-water extracts of citrus pomace, the activity was higher in the ethanol extract with a lower total phenolic contents than in the hot-water extract. These results were also consistent with this study, it was judged that because the ethanol extract contained relatively more polyphenol components contributing to the antioxidant activity (Song et al., 2013).

Antimicrobial activity

The antibacterial activity of extracts of *S. sieboldiana var. pendula* leaf extract against *H. pyroli*, *S. aureus*, *E. coli*, *P. acnes*, and *S. mutans* was evaluated (Table 3). No significant antibacterial activity was found against *H. pyroli* and *E. coli*. *P. acnes* is found on human skin and causes acne. The antibacterial effect on *P. acnes* was found only in the water extract at a concentration of 200 μg/100 μL, with a wide clear zone of 1.4 mm. In addition, 50-200 μg/100 μL of the water extract showed antibacterial effects, demonstrationg a clear zone of 0.7-1.2 mm. The antibacterial

effects of the water and ethanol extracts on *S. mutans*, the causative agent of caries, was found at a concentration range of 50-200 μg/100 μL, exhibiting clear zones of 0.9-1.3 and 0.75-1.1 mm, respectively. Thus, *S. sieboldiana var. pendula* leaf extract showed a significant antibacterial activity in a concentration-dependent manner and can therefore be used for the treatment of diseases, such as caries, food poisoning, and acne. Based on the above mentioned results, *S. sieboldiana var. pendula* could be used as a functional food owing to its excellent biological and antimicrobial activities.

Conclusion

The phenolics of *Sambucus sieboldiana var. pendula* leaf extract for functional resources were examined on inhibitory activity against biological enzyme and antimicrobial activity. The amount of phenolic compounds were 11.60 and 12.39 mg/g by water and 50% ethanol extraction, respectively. The inhibition activities on ACE were 92.08 and 78.33% at 200 μg/mL phenolic concentration in water and 50% ethanol extracts, respectively. The XOase inhibitory activity were 100.00% at 200 μg/mL phenolic both water and ethanol extracts. These results were higher than 70.37% of allopurinol as positive control at 200 μg/mL. The water extract from *S. sieboldiana var. pendula* leaves showed antibacterial activity on the *Streptococcus mutans*, *Staphylococcus aureus*, and *Propionibacterium acnes* in water extract and *S. mutans* in ethanol extract. Therefore, we judged that the phenolic compounds of extract in *S. sieboldiana var. pendula* leaf were related to the physiological activity. This result suggests that phenolic from *S. sieboldiana var. pendula* leaves are suitable as functional foods with antihypertension, anti-gout and anti-microorganism activities.

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