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Biological Activities of Water and Ethanolic Extracts from *Allium victorialis* L. Mature Leaves

Chunmei Li[1*], Young-Mee Lee[2*], Kyeong-Cheol Lee[3], Woong Han[1], Myeong-Hyeon Wang[1], and Sang-Sup Han[3†]

[1]College of Biomedical Science, and
[2]Division of Forest Resources, College of Forest and Environmental Sciences, Kangwon National University, Gangwon 200-701, Korea
[3]Department of Nursing, Kangwon National University, Gangwon 245-907, Korea

Abstract

***Allium victorialis* L. (*A. victorialis*) is a very popular vegetable in Korea. The most commonly used parts of this vegetable are the bulbs and young leaves. To determine if the mature leaves have any beneficial properties, we investigated antioxidant, anti-α-glucosidase, anti-inflammatory, and anticancer activities of water and ethanol extracts from *A. victorialis*. Antioxidant activity was evaluated by measuring total phenolic content, DPPH and superoxide radicals scavenging activities. The water extract from *A. victorialis* (W·*A. victorialis*) exhibited higher antioxidant ability than the ethanol extract (E·*A. victorialis*). Moreover, the water extract showed strong inhibitory effect on α-glucosidase. On the other hand, the ethanol extract had greater anti-inflammatory activity on murine macrophage cells (RAW 264.7) and greater anticancer activities against human colon cancer cells (HT-29). These results suggest that mature leaves from E·*A. victorialis* may have health-enhancing effects.**

Key words: *Allium victorialis* L., antioxidant, anti-α-glucosidase, anti-inflammatory, anticancer

INTRODUCTION

In the past few decades, much attention has been focused on the biological properties of natural foods and herbs (1-3). In pharmacology, biological activity describes the beneficial or adverse effects of a drug on living organisms. Many important bioactivities including antioxidant, anticancer, anticoagulant, antithrombotic, anti-diabetes, anti-inflammatory, antibacterial, and cytoprotective activities have been investigated in plant. Biological activities are correlated to the presence of certain compounds that may assist in predicting some traditional uses of medicinal plants (4). Based on the biological properties, we can assume that some plants can be less toxic to humans and can be used as medicine for the treatment of diseases.

Allium victorialis L. (*A. victorialis*) is a bulb geophyte belonging to the Amaryllidaceae family. *A. victorialis* has been widely used in the daily diet for a prophylactic anti-scorbutic remedy (5,6). In Korea, it is known as San-ma-neul or *Allium microdictyon* Prokh. Biological activities of *A. victorialis* have been investigated, including antiatherogenic (7), antimutagenic, anticancer (8), antihyperlipidemic (9), antioxidant, antimicrobial, and inhibitory activities against human thrombin, α-amylase and α-glucosidase (10). Furthermore, flavonoids such as anthocyanins and allivicin, and some furostanol glycosides have been isolated from *A. victorialis* extracts (11,12). As a vegetable, the bulbs and young leaves from *A. victorialis* are used. However, research on mature leaves was not conclusive. Thus, in this study, we evaluated the possible biologic activities such as antioxidant, anti-α-glucosidase, anticancer, and anti-inflammatory activities of mature leaves from *A. victorialis*.

MATERIALS AND METHODS

Sample preparation

Mature leaves, collected from wild type *A. victorialis* in Chuncheon, Korea, were shade-dried. Every 100 g of powder was extracted twice with 2 L ethanol or water. The dipped solutions were subjected to vacuum filtration (100-mm, Whatman, Maidstone, UK). The solvent was evaporated under reduced pressure using a vacuum rotary evaporator (CCA-1110, Eyela, Tokyo, Japan). Finally, the dried extracts were stored at -20°C for subsequent

*These two authors contributed equally to this work.
†Corresponding author. E-mail: sshan@kangwon.ac.kr
Phone: +82-33-250-8311, Fax: +82-33-252-8310

analysis.

Total phenolic content assay

The total phenolic contents of *A. victorialis* water extract (W·*A. victorialis*) and *A. victorialis* ethanol extract (E·*A. victorialis*) were determined with the Folin-Ciocalteu reagent using the method of Jung et al. (13) with some modifications. 0.1 mL of each extract (1 mg/mL), 0.5 mL of 0.1 N Folin-Ciocalteu reagent, and 0.4 mL of 7.5% sodium carbonate were added and incubated at room temperature for 30 min. The total phenolic content was quantified with respect to the standard, and was expressed as tannic acid equivalent. Results were expressed as milligrams of tannic acid equivalent per gram (mg/g).

DPPH free radical scavenging activity assay

The 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of W·*A. victorialis* and E·*A. victorialis* were evaluated according to the method Zhang et al. (14) with modifications. Well vortex-mixed solutions of 0.1 mM DPPH and varying concentrations of extracts were incubated at room temperature for 30 min. The absorbance was measured at 515 nm. Inhibition (IC_{50}) was calculated from the graph of DPPH scavenging activity.

Superoxide anion radical ($O_2\cdot^-$) scavenging activity assay

The $O_2\cdot^-$ scavenging activity was determined by the method described in Ewing and Janero (15). 50 μL aliquot of each sample solution, 1 mL of 0.1 M phosphate buffer solvent (pH 7.4, 0.1 mM ethylenediamine-tetraacetic acid (EDTA), 50 μM nitro blue tetrazolium (NBT), 78 μM β-nicotinamide adenine dinucleotide (NADH), and 3.3 μM phenazine methosulfate (PMS)) were mixed and incubated at 25°C for 8 min. The absorbance was measured at 560 nm.

α-Glucosidase inhibitory activity assay

The α-glucosidase inhibitory activity was determined as described by Nishioka et al. (16) with slight modification. Extracts with concentration of 10, 50, 100, 500, and 1000 μg/mL were mixed with 0.075 units of α-glucosidase. The reaction of the mixture was started with the substrate 3 mM p-nitrophenyl glucopyranoside (pNPG) in phosphate buffer. After incubation at 37°C for 30 min, the reaction was stopped by adding 2 mL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the p-nitrophenol release from pNPG at 400 nm.

Cell lines and cell culture

Murine macrophage RAW 264.7 cell and human colon cancer cell (HT-29) lines were purchased from the Korean Cell Line Bank (Seoul, Korea) and grown in Roswell Park Memorial Institute medium 1640 (RPMI 1640), supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were cultured in a humidified atmosphere and incubated at 37°C in 5% CO_2.

MTT assay for cell viability

RAW 264.7 and HT-29 cells were seeded in 96-well plates (1.5×10^5 cells/well) and incubated in RPMI 1640 for 24 hr. Then, RAW 264.7 cells were treated with LPS (2 μg/mL) in the presence of 25, 50, 100, 200, and 400 μg/mL of *A. victorialis* extracts for 24 hr. HT-29 were pretreated with 25, 50, 100, 200, and 400 μg/mL of *A. victorialis* extracts for 24 hr. After incubation, the cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MTT is a pale yellow substrate that was reduced by living cells to yield a dark blue formazan product. After reacting for 4 hr, the supernatant was removed and the formazan crystals were dissolved in dimethyl sulfoxide (DMSO). Absorbance was measured at 550 nm.

Nitric oxide assay

After pre-incubation of RAW 264.7 cells with LPS (2 μg/mL) for 24 hr, the quantity of nitrite in the culture medium was measured as an indicator of NO production. Aliquots of 100 μL cell culture medium were mixed with 50 μL of 1% sulfanilamide (in 5% phosphoric acid) and 50 μL of 0.1% naphthyl-ethylenediamine dihydrochloride. Subsequently, the mixture was incubated at room temperature for 10 min and the absorbance was measured at 550 nm.

Statistical analysis

All experiments were carried out independently in triplicate (n=3). The data are expressed as the mean ± standard derivation (SD). Statistical significance was evaluated with analysis of variance (ANOVA) using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Results were considered to be significant when $p<0.05$.

RESULTS AND DISCUSSION

Antioxidant compounds play an important role as a health-protecting factor in food and medicine. Antioxidants can reduce the risk of many diseases, such as inflammation, diabetes, cancer, and cardiovascular disease, as well as certain degenerative diseases and aging (17-20). Phenolic acids, which are abundant in plants, are well known for their antioxidant characteristics (21). Such phenolics may function as free-radical scavengers, complexers of prooxidant metals, reducing agents, and

quenchers of singlet-oxygen formation (22). Thus, the amounts of phenolics in plants are often considered to be a significant indicator of antioxidant activity. In this study, W·*A. victorialis* and E·*A. victorialis* were extracted from the dried mature leaves (yields were 38.0 and 45.2%, respectively). The phenolic contents of these two extracts were expressed as tannic acid equivalents (Table 1). W·*A. victorialis* and E·*A. victorialis* exhibited phenolic content of 75.82 and 42.49 mg tannic acid/g. Kwon et al. (10) also reported about 23.21 and 10.59 mg/g total polyphenolic content in *A. victorialis* fresh juice and 70°C-treated juice, respectively. Our result indicated that W·*A. victorialis* had higher phenolic content than E·*A. victorialis*. Since phenolics indicate the presence of antioxidants and are free-radical scavengers, we hypothesize that the W·*A. victorialis* may have a greater effect in free radical scavenging activity.

In the free radical scavenging activity assay, the DPPH free radical and superoxide anion radical were used as targets. As a well-characterized radical, DPPH has been used extensively in determining the free radical scavenging ability of various samples (23). Superoxide anion radical is a major member of reactive oxygen species (ROS). ROS such as superoxide, hydroxyl, and peroxyl radicals are generated under oxidative stress conditions. The DPPH free radical scavenging activity of *A. victorialis* is shown in Table 1. As an important chain-breaking antioxidant, α-tocopherol was used as a positive control. Compared to α-tocopherol, the DPPH free radical scavenging activity of *A. victorialis* extracts was not significant. At a concentration of 100 μg/mL, the scavenging activity of W·*A. victorialis* and E·*A. victorialis* was 25.7 and 10.1%, respectively (data not shown). This activity is weaker than that of *A. victorialis* free juice (10). Superoxide anion radical scavenging activity of *A. victorialis* extract is shown in Fig. 1. Gallic acid was used as the positive control, and scavenged about 91.3% of all radicals at 100 μg/mL (data was not shown in Fig. 1). E·*A. victorialis* showed very weak superoxide anion radical scavenging activity. In contrast, W·*A. victorialis* exhibited considerable effect on E·*A. victorialis*. Most phenolic compounds have significant antioxidant activity. The higher free radical scavenging activity of W·*A. victorialis* may be attributed to its higher phenolic content. Since free radicals are related to the etiology of cardiovascular disease, diabetes mellitus, gastric ulcers, cancer, arthritis, Alzheimer's disease, Parkinson's disease, and inflammation (24), the W·*A. victorialis* may have the potential to prevent free radical-related diseases.

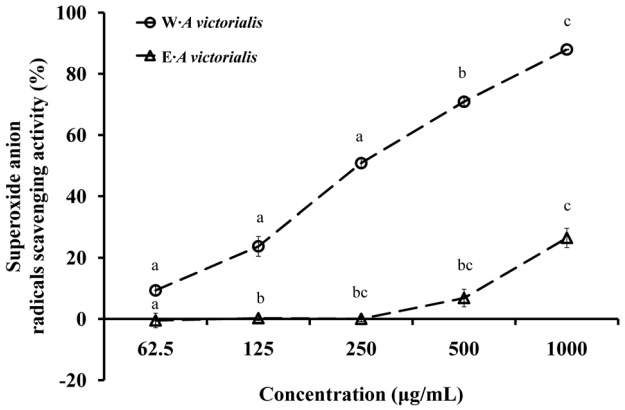

Fig. 1. Superoxide anion radical scavenging activity of different concentrations of W·*A. victorialis* and E·*A. victorialis*. Data represent the mean±SD of three independent experiments.

Diabetes has become a common disease all over the world (25). Strict control of blood glucose levels can effectively decrease the incidence of diabetes-related complications (26). Thus, inhibiting α-glucosidase are usually been projected in preventing type 2 diabetes (Noninsulin dependent mellitus, NIDDM) (27,28). Antidiabetic agents can control the α-glucosidase to delay carbohydrate absorption. Different concentrations of each type of *A. victorialis* extract were used to evaluate the α-glucosidase inhibitory effect. Kwon et al. (10) demonstrated that *A. victorialis* fresh juice had negligible inhibitory activity against α-glucosidase; however, in our study, the W·*A. victorialis* inhibited α-glucosidase activist in a dose-dependent manner (Fig. 2) while there was no effect with E·*A. victorialis*. These results suggest that W·*A. victorialis* is a better choice for consideration as an anti-diabetic agent in food.

Inflammation is a complex biological response to pathogens and damaged cells. However, chronic and uncontrolled inflammation may serve as an important and common pattern in various diseases (29). Therapy of inflammatory diseases is usually directed at the inflamma-

Table 1. Yield, total phenolic and DPPH free radical scavenging activity of water and enthanol extracts from *Allium victorialis* L.

Extracts	Yield (%)	Total phenolic content (tannic mg/g)[1)]	DPPH radical scavenging activity (IC_{50}: μg/mL)[2)]
W·*A. victorialis*	38.0	75.82±1.35	287.44±22.36
E·*A. victorialis*	45.2	42.49±0.80	483.57±3.19
α-Tocopherol (positive control)	−	−	11.21±0.75

[1)]Total phenolic content is expressed as tannic equivalent mg/g ($p<0.05$).
[2)]IC_{50}: the effective concentration at which DPPH radicals were scavenged by 50% ($p<0.05$).

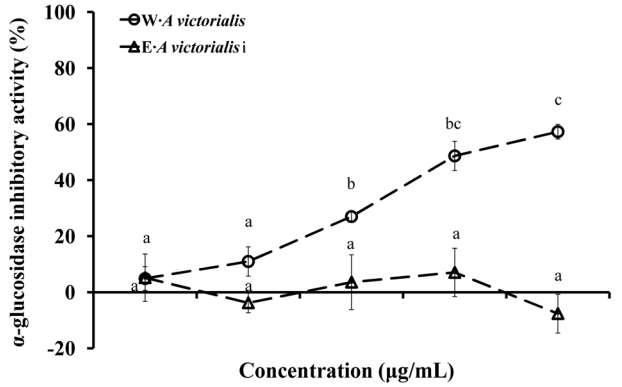

Fig. 2. Anti-α-glucosidase activity of different concentrations of W·*A. victorialis* and E·*A. victorialis*. Data represent the mean±SD of three independent experiments.

tory processes. Many anti-inflammatory drugs have been prepared and marketed (30); however, these complex drugs are known to provoke gastrointestinal irritation. Thus, more gentle anti-inflammatory natural herbs are being investigated. In our study, the anti-inflammatory activity of *A. victorialis* extracts was measured on lipopolysaccharide (LPS)-induced RAW 264.7 cells. LPS can activate immune cells to upregulate inflammatory states. The level of nitric oxide (NO) produced is an important indicator of the inflammatory process. The overproduction of NO can create cytotoxicity and tissue damage in an organism (31,32). Compared to the group with LPS treatment, the E·*A. victorialis* reduced NO product at higher dosages (Fig. 3A). LPS can induce apoptosis in the cells, with a viability of 61.42% (Fig. 3B). E·*A. victorialis* protected the cells against LPS-induced apoptosis. After treatment with E·*A. victorialis*, the viability of LPS-stimulated cells increased to 100%. However, W·*A. victorialis* did not protect the cells against LPS-induced cell death. The groups treated W·*A. victorialis* and LPS showed lower viability than the LPS-stimulated group. While the anti-inflammatory effect of E·*A. victorialis* is considerable, the results suggest that W·*A. victorialis* is not suitable to be used as an anti-inflammatory agent. The molecular mechanism of the anti-inflammatory effect elicited by E·*A. victorialis* will be investigated in further study.

Cancers cause about 13% of all human deaths worldwide. Many of the cancers are induced by factors such as smoking, diet and obesity, infections, radiation, stress, lack of physical activity, and pollutants (33), suggesting that cancer is largely a preventable disease (34). In addition to controlling the above factors to protect from diseases like cancer, having some fruits and vegetables on daily basis is also very important. In this study, the cytotoxicity of *A. victorialis* on HT-29 cells was assessed. The viability of HT-29 cells after incubation with 25,

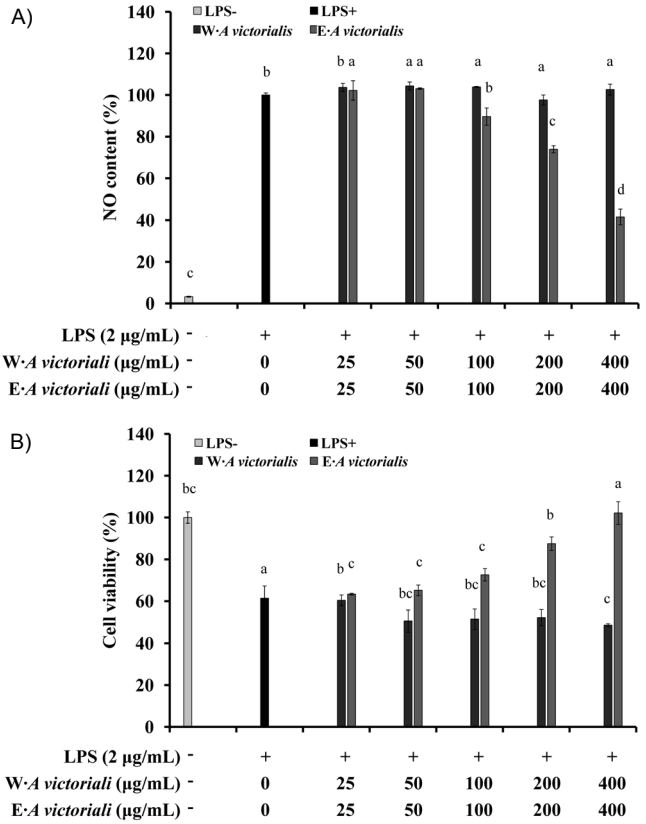

Fig. 3. NO scavenging activity and cytotoxicity of W·*A. victorialis* and E·*A. victorialis* on LPS-stimulated RAW 264.7 cells. A). RAW 264.7 cells were treated with LPS (2 μg/mL) in the presence of 25, 50, 100, 200, and 400 μg/mL W·*A. victorialis* and E·*A. victorialis* for 24 hr. B) Cytotoxicity of W·*A. victorialis* and E·*A. victorialis* on LPS-stimulated RAW 264.7 cells. Data represent the mean±SD of three independent experiments.

50, 100, 200, and 400 μg/mL W·*A. victorialis* was not changed remarkably (Fig. 4); however, 400 μg/mL of E·*A. victorialis* killed 37.12 % of all cancer cells. These results are in contrast with the result of Ham et al. (8),

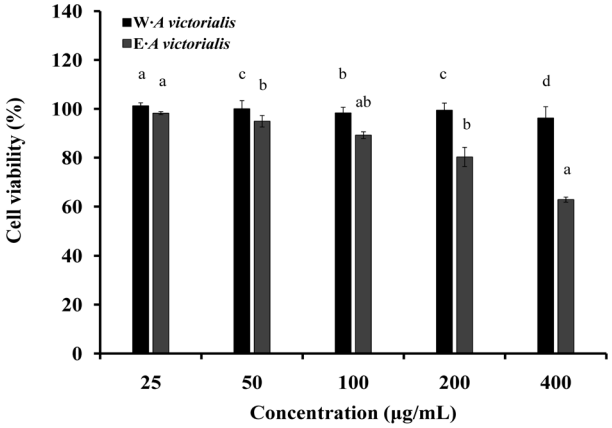

Fig. 4. Cell viabilities of human colon cancer cells (HT-29) after incubated with 25, 50, 100, 200, and 400 μg/mL W·*A. victorialis* and E·*A. victorialis* for 24 hr. Data represent the mean±SD of three independent experiments.

where very low dosage of E·*A. victorialis* showed strong cytotoxicity against human lung carcinoma (A549), human breast adenocarcinoma (MCF-7), and human gastric carcinoma (KATOⅢ). The difference maybe related to different compounds present in each tissue.

CONCLUSION

Two extracts from *A. victorialis* mature leaves showed different biological activities in this study. The W·*A. victorialis* showed higher phenolic, free radical scavenging, and anti-α-glucosidase activities. E·*A. victorialis* showed lower antioxidant activity, no anti-α-glucosidase activity, but evidenced higher anti-inflammatory and anticancer activities. These results suggest that W·*A. victorialis* should be investigated for further research of its antioxidant and anti-diabetes activity. The mechanisms of anti-inflammatory and anticancer effects elicited by E·*A. victorialis* will be explored in further studies.

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