

The antioxidant and cytotoxic activities of *Sonchus oleraceus* L. extracts*

Jie Yin, Gu-Joong Kwon and Myeong-Hyeon Wang[§]

School of Biotechnology, Kangwon National University, Chuncheon, Kangwon-Do 200-701, Korea

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Abstract

This study investigated *in vitro* antioxidant activity of *Sonchus oleraceus* L. by extraction solvent, which were examined by reducing power, hydroxyl radical-scavenging activity(HRSA) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays. 70% MeOH extract had the greatest reducing power while EtOH extract had the greatest HRSA. The antioxidant activity of *S. oleraceus* extracts was concentration dependent and its IC₅₀ values ranged from 47.1 to 210.5 µg/ml and IC₅₀ of 70% MeOH, boiling water and 70% EtOH extracts were 47.1, 52.7 and 56.5 µg/ml, respectively. 70% MeOH extract of *S. oleraceus* contained the greatest amount of both phenolic and flavonoid contents. The extracts tested had greater nitrite scavenging effects at lower pH conditions. The cytotoxic activity showed that EtOH extract had the best activity against the growth of stomach cancer cell. These results suggest that *S. oleraceus* extract could be used as a potential source of natural antioxidants.

Key Words: *Sonchus oleraceus* L, Antioxidant, Cytotoxic, Phenolic content, Nitrite scavenging activity

Introduction

Free radicals lead to a variety of biochemical and physiological lesions (Ames, 1998) and are implicated in the etiology of degenerative diseases, including coronary artery disease, stroke, diabetes and cancer (Halliwell *et al.*, 1992). Antioxidants delay or prevent oxidation of the substrate (Halliwell, 1995). Interest in finding naturally occurring antioxidants in foods or medicines to replace synthetic antioxidants has increased considerably, given that synthetic antioxidants are being restricted due to their side effects (Ito *et al.*, 1983; Zeng & Wang, 2001). Therefore, attention has been directed toward the development and isolation of natural antioxidants from plant sources. The antioxidants in some plants including ascorbic acid, carotenoids, flavonoids and hydrolysable tannins play important roles in preventing diseases induced by free radicals (Huxley & Neil, 2003). The antioxidant activity of plant materials is correlated with their phenolic compound content (Velioğlu *et al.*, 1998). Polyphenols belong to a heterogeneous class of compounds with a variety of antioxidant actions. The antioxidant and radical scavenging activities have been studied using medicinal plants and fruits (Singh *et al.*, 2002). The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals (Decker, 1997). In addition, flavonoids can act as free radical scavengers and terminate the radical chain reactions that occur during the oxidation of triglycerides (Das & Pereira, 1990). It has been reported that phenolic compounds have a nitrite scavenging effect at low pH (Kang *et al.*, 1996). The antioxidant

and anticancer activities of extracts from medicinal plants and herbs are associated with phenolic compounds (Cai *et al.*, 2004).

S. oleraceus has rough, thorny petioles that embrace the main stem. It blossoms yellow flowers from May to September, and the seeds ripen in July. The seeds are used for medicine and young leaves are edible. It was suggested that the amount of polyphenol and the antioxidant activity in plants depend on environment factors such as growing season (Howard *et al.*, 2002) and location (Ma *et al.*, 2003). The nutritional composition of *S. oleraceus* was reported previously (Guil-Guerrero *et al.*, 1998). However, detailed information on antioxidative activities of the *S. oleraceus* was not sufficiently available.

Therefore, this study was initiated to investigate the antioxidant activities of *S. oleraceus* by extraction solvent, including the free radical scavenging activity, reducing power, nitrite scavenging activity, total phenolic content, and flavonoid content using a number of classical assays. We evaluated whether this species could be used as a source of natural antioxidants.

Materials and Methods

Extraction of S. oleraceus

S. oleraceus were collected at Chuncheon in South Korea in August, 2006. They were dried in the shade at room temperature and cut into small pieces, followed by being grinded into powder. Dried *S. oleraceus* powder (100 g) was soaked in 2 L of six

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[§] Corresponding Author: Myeong-Hyeon Wang, Tel. 82-33-250-6486, Fax. 82-33-241-6480, Email. mhwang@kangwon.ac.kr

different solvents including water, ethanol, 70% ethanol, methanol and 70% methanol, and kept in a shaking incubator at 25°C for 2 days. For the extraction with boiling water, the powder was heated at 100°C for 3 h. After filtering the extracts in vacuum with Whatman No. 1 filter paper, the residue was re-extracted, filtered, and evaporated using a rotary evaporator at 45°C. The yields of these dried extracts were as follows: water extract 8.1%, boiling water extract 12.2%, ethanol extract 3.8%, 70% ethanol extract 8.7%, methanol extract 8.5%, 70% methanol extract 11.5% and chloroform extract 3.0%. All of extracts were kept in the refrigerator prior to further experiments.

Reducing power assay

The reducing power assay was determined according to the method of Oyaizu (1986). Different concentrations of *S. oleraceus* extract in 1 ml of each solvent including water, ethanol and methanol were mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide, respectively. The mixture was incubated at 50°C for 30 min. The mixture was centrifuged at $395 \times g$ for 10 min at room temperature after 2.5 ml of 10% trichloroacetic acid (w/v) was added. The upper layer (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride, and the absorbance was measured at 700 nm. Vitamin E was used as the standard.

Hydroxyl radical ($\cdot OH$) scavenging activity

The scavenging activity of the *S. oleraceus* extracts on the hydroxyl radical ($\cdot OH$) was based on the deoxyribose method (Aruoma, 1994). Hydroxyl radicals were generated by direct addition of iron (II) salts to the reaction mixture. The reaction mixture contained 200 μl of 10 mM $FeSO_4 \cdot 7 H_2O$, 200 μl of 10 mM EDTA, 200 μl of 10 mM 2-deoxyribose, 200 μl of 10 mM H_2O_2 , mixed with 1.2 ml of 100 mM phosphate buffer (pH 7.4) containing 200 μl of *S. oleraceus* extract. The reaction mixture was incubated for 4 h at 37°C in a water bath. After incubation, 1 ml of 1% thiobarbituric acid and 1 ml of ice-cold 2.8% trichloroacetic acid were added to the resultant reaction mixture followed by being heated in a boiling water bath (95°C -100°C) for 10 min. After cooling down to room temperature and being centrifuged at $395 \times g$ for 5 min, the absorbance at 532 nm was measured. Butylated hydroxytoluene (BHT) and Vitamin E were used as the positive control.

Radical scavenging activity (RSA)

The free radical scavenging activity was determined according to the method of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical (Kilani *et al.*, 2005). Different concentrations (0.025-0.4 mg/ml) of *S. oleraceus* extract (0.5 ml) were mixed with 0.5 ml freshly prepared DPPH in MeOH (final concentration 65 μM). The absorbance of the mixture was measured at 517 nm after

incubation for 30 min in the dark condition at room temperature. The IC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated by using the percent scavenging activities of serially diluted extract concentration.

Determination of total phenolic and flavonoid contents

The total phenolic content was estimated by Folin-Denis reagent. One ml of each extract at different concentrations was mixed with 2 ml of Folin-Denis reagent and 2 ml of 35% sodium carbonate. The mixtures were shaken thoroughly and made up to 10 ml with distilled water. The absorbance at 760 nm was determined after incubation at room temperature for 30 min. Phenolic content was estimated from a standard curve determined from different concentrations of tannic acid.

Total flavonoid contents were determined according to the aluminum chloride colorimetric method (Chang *et al.*, 2002). Quercetin was used as a standard to make the calibration curve. The sample solution (0.5 ml) was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride hexahydrate ($AlCl_3$), 0.1 ml of 1 M potassium acetate (CH_3COOK) and 2.8 ml of distilled water. After incubation at room temperature for 40 min, the absorbance of reaction mixture was measured at 415 nm. The same amount of distilled water substituted for the amount of 10% aluminum chloride as the blank. Using a seven point standard curve (0-500 mg/l), the total flavonoid content of extracts was determined in independent triplicate (n=3).

Measurement of nitrite scavenging ability (NSA)

One ml of each extract sample was mixed with 1 ml of 1 mM nitrite sodium and followed by addition of 8 ml solution at pH 1.2 adjusted with 0.1 N HCl or 0.2 M citrate buffer at pH 4.2 and 6.0. Then 1 ml from this mixture was withdrawn and added to 2 ml of 2% acetic acid and 0.4 ml of Griess reagent (1% sulfanilic acid and 1% naphthylamine in a methanol solution containing 30% acetic acid) after incubation in a water bath at 37°C for 1 h. After vigorously mixed with a vortex, the reactant was placed at room temperature for 15 min, and the absorbance was measured at 520 nm. A blank was prepared by adding 0.4 ml distilled water instead of the Griess reagent.

In vitro cytotoxic activity (MTT assay)

The cytotoxicity of *S. oleraceus* extracts to the stomach cancer cell line (NCI-N87) was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT, Sigma) assay (Sun *et al.*, 2005). The stomach cancer cell NCI-N87 was seeded into a 96-well plate at 1×10^6 cells/ml in 200 μl RPMI complete medium containing each extract from *S. oleraceus*. After 48 h of incubation at 37°C in humidified chamber adjusted with 5% CO_2 , 50 μl of MTT solution (2 mg/ml) was added to each well

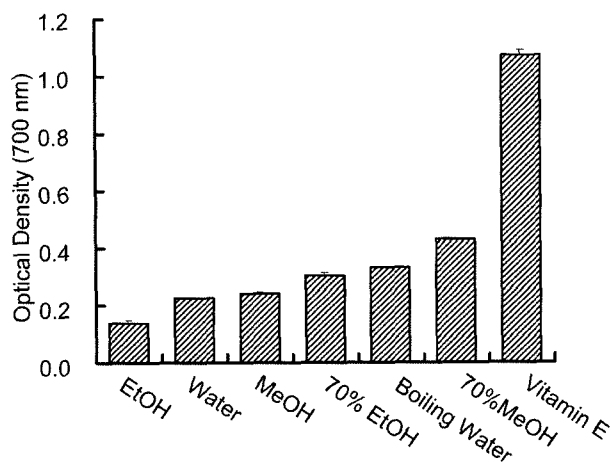


Fig. 1. Effects of different solvents used to produce extracts from *S. oleraceus* on reducing power. The concentrations of these extracts were the same as 500 $\mu\text{g/ml}$. Vitamin E at the same concentration of 500 $\mu\text{g/ml}$ was used as the positive control.

and incubated 4 h further. The supernatant was carefully removed using a needle or syringe and 100 μl of DMSO was added to each well followed by pipetting up and down to dissolve crystals. The plate was placed in a 37°C incubator for 5 min to dissolve air bubbles. The absorbance at 570 nm was measured with a microplate reader. All experiments were performed in triplicate.

Statistical analysis

All experiments were conducted in independent triplicate ($n=3$) and data were expressed as mean \pm standard deviation for a number of experiments. Statistical significance was evaluated by one-way analysis of variance using SPSS 7.5 (SPSS Institute, Cary, NC, USA) and individual comparisons were obtained by Duncan's multiple-range test which was used to determine the difference of means, and $p < 0.05$ was considered to be statistically significant.

Results

Reducing power assay

Although all of the extracts had less reducing power activity than Vitamin E at the same concentration, extracts from the sample had considerable reducing activity. As shown in the results, the reducing activity of six extracts, in the descending order, was 70% MeOH > boiling water > 70% EtOH > MeOH > water > EtOH (Fig. 1). This is in accord with the order of the free radical scavenging activity and may be associated with the relationship between the antioxidant activity and reducing power of plant extracts.

Hydroxyl($\cdot\text{OH}$) radical scavenging activity

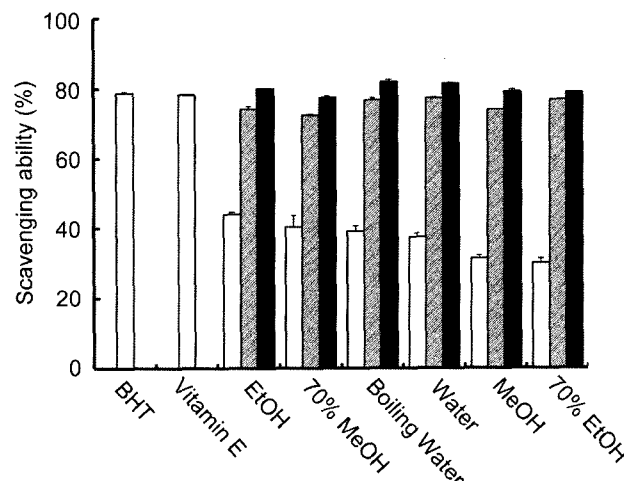


Fig. 2. Effects of different solvents used to produce extracts from *S. oleraceus* on Hydroxyl radical ($\cdot\text{OH}$) scavenging assay. The concentrations of different solvent extracts were 100 (\square), 500 (▨) and 1000 (\blacksquare) $\mu\text{g/ml}$, respectively. Vitamin E and BHT at the concentration of 100 $\mu\text{g/ml}$ were used as the positive control.

Table 1. DPPH free radical scavenging activity of various extracts from *S. oleraceus* expressed as IC_{50} . Each value is expressed as a mean of three determinations

Samples	DPPH radical activity (IC_{50} : $\mu\text{g/ml}$)
70% MeOH ext.	47.1
Boiling water ext.	52.7
70% EtOH ext.	56.5
MeOH ext.	106.8
Water ext.	116.9
EtOH ext.	210.5
Positive control antioxidants	
L-Ascorbic acid	4.5
BHA	3.6

IC_{50} : the effective concentration at which DPPH radicals were scavenged by 50%.

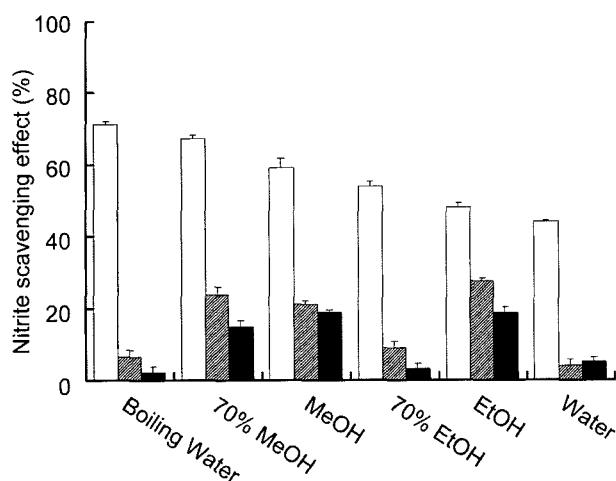
The hydroxyl radical scavenging activity of *S. oleraceus* extracts was increased with the sample concentrations (Fig. 2). Nearly all of the extracts showed a considerable hydroxyl radical scavenging activity, although the activity was less than those of the positive controls at the same concentration (Vitamin E and BHT scavenged 78.2 and 78.7% of the available free radicals, respectively, at the concentration of 100 $\mu\text{g/ml}$). At concentrations from 500 to 1,000 $\mu\text{g/ml}$, all of the extracts scavenged more than 70% of the available free radicals.

Radial scavenging activity (RSA)

The free radical scavenging capacities of the extracts, measured by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method, are shown in Table 1. The boiling water, 70% MeOH, and 70% EtOH extracts had stronger antioxidant activities than any other extract from *S. oleraceus*.

Table 2. Total phenolic and flavonoid contents of different extracts from *S. oleraceus*

Solvent extract	Phenolic content (mg/g)	Flavonoid content (mg/g)
Water ext.	40.5 ± 0.0	14.1 ± 2.3
Boiling water ext.	71.2 ± 0.5	125.2 ± 11.2
EtOH ext.	39.5 ± 0.5	82.2 ± 1.3
70% EtOH ext.	73.6 ± 0.7	158.9 ± 7.9
MeOH ext.	54.1 ± 0.3	118.1 ± 27.0
70% MeOH ext.	74.8 ± 0.4	170.0 ± 11.1

**Fig. 3.** Nitrite scavenging activity of various extracts from *S. oleraceus*. The pH of different solvent extracts was 1.2 (□), 4.2 (▨) and 6.0 (■), respectively.

Determination of the Total Phenolic and Flavonoid Contents

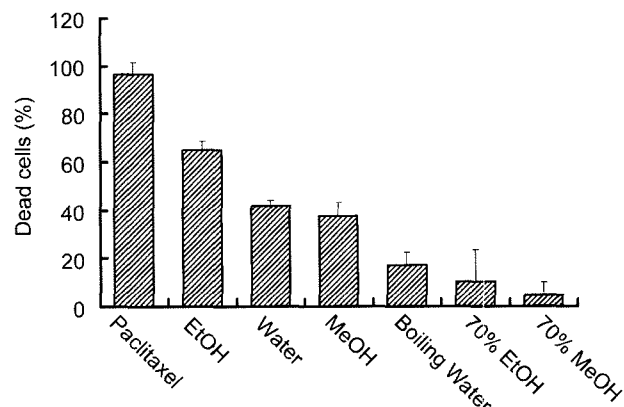
The contents of the 70% MeOH, 70% EtOH, boiling water, MeOH, water and EtOH extracts were 74.8, 73.6, 71.2, 54.1, 40.5 and 39.5 mg/g, respectively (Table 2). The flavonoid contents of the extracts are shown in Table 2, and they were in accord with the total phenolic content.

Measurement of the nitrite scavenging ability (NSA)

The nitrite scavenging effect of *S. oleraceus* extracts was examined over a range of acidic conditions (pH 1.2, 4.2 and 6.0). The scavenging effect was the highest at pH 1.2 (Fig. 3). However, the nitrite scavenging activity in the tested extracts was not in the same order at different pH levels. The scavenging rates of these six extracts at pH 1.2 were shown to be in order as boiling water (71%), 70% EtOH (67%), MeOH (59%), 70% MeOH (54%), EtOH (48%) and water (44%). Moreover, the nitrite scavenging effect was the greatest at lower pH.

In vitro cytotoxic activity (MTT assay)

The cytotoxic activity of the extracts from *S. oleraceus* against stomach cancer cell (NCI-N87) is shown in Fig. 4. The percentage of growth inhibition at various concentrations was

**Fig. 4.** Inhibitory effect of various extracts from *S. oleraceus* on the growth of NCI-N87 in MTT assay. The concentration of all of the tested extracts was the same as 100 µg/ml. Paclitaxel at the concentration of 100 µg/ml was used as the positive control.

determined as a percentage of viable treated cells in comparison with viable cells of untreated controls. The EtOH extract had the best activity against the proliferation of stomach cancer cells. The maximal inhibition of cell growth was 65.0% in the EtOH extract compared to 96.5% for paclitaxel at the same concentration of 100 µg/ml.

Discussion

Antioxidant activity is reported to be concomitant with the reducing power, or the capability of reducing oxidized intermediates of the lipid peroxidation processes (Ordonez *et al.*, 2006). The reducing power indicates compounds that are electron donors, which can act as primary and secondary antioxidants (Yen & Chen, 1995). Studies have shown that reducing activity is associated with the presence of reductones (Duh, 1998), which exert their antioxidant effect by donating a hydrogen atom and breaking the free radical chain (Gordon, 1990). Our study showed that all of extracts had less reducing power activity than vitamin E at the same concentration and there were considerable reducing activities in the extracts from our sample. The reducing activity of six extracts was as follows in the descending order: 70% MeOH > Boiling water > 70% EtOH > MeOH > Water > EtOH. Since the reducing activity contributes significantly to the antioxidant activity, we plan to investigate whether the sample contains antioxidants like reductones. As antioxidants donate protons to these radicals, the absorbance is decreased. The decrease in absorbance is used to measure the extent of radical scavenging (Aziz *et al.*, 2007). DPPH is a stable free radical that accepts an electron or hydrogen radical and becomes a stable diamagnetic molecule (Soares *et al.*, 1997). In addition, antioxidant activity has been reported to have a linear relationship with the total phenolic or anthocyanin content in some plants (Kalt *et al.*, 1999). As shown in the results, boiling water extract, 70% MeOH extract and 70% EtOH extract had stronger

antioxidant activities (IC_{50} ranged from 47.1 to 56.5 $\mu\text{g/ml}$, Table 1) than any other extract from *S. Oleraceus* (vit. C and BHA with an IC_{50} of 4.5 and 3.6 $\mu\text{g/ml}$, respectively), which were in the descending order as: 70% MeOH > Boiling water > 70% EtOH > MeOH > Water > EtOH extract.

Phenolic compounds are found in both edible and inedible plants, which have multiple biological effects including antioxidant activity. The antioxidant activity of phenolic compounds is due mainly to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching single and triple oxygen, and decomposing peroxides (Osawa, 1994; Siriwardhana *et al.*, 2003). Therefore, we hypothesized that the significant hydroxyl radical scavenging activity of the *S. oleraceus* extracts is likely to be due to phenolic compounds present in the extracts. In our hydroxyl radical scavenging activity assay, nearly all of the extracts showed considerable hydroxyl radical scavenging activity, though less than that of the positive control at the same concentration (100 $\mu\text{g/ml}$), and the activity order was shown here as: BHT > Ve > EtOH > 70% MeOH > Boiling water > Water > MeOH > 70% EtOH. In addition, the key role of phenolic compounds as free-radical scavengers has been emphasized in several reports (Komali *et al.*, 1999; Moller *et al.*, 1999). The phenolic compounds may contribute to the antioxidative action directly (Duh *et al.*, 1999). A study of *Hypericum hyssopifolium* found that the antioxidant activity was derived from flavonoid-type compounds (Cakir *et al.*, 2003). It is thought that the high free radical-scavenging activity and total antioxidant activity result from the existence of both phenolic and flavonoid-type compounds. The higher content of total phenolic and flavonoid compounds in *S. oleraceus* may account for the observed reducing power and free radical scavenging effect.

Nitrite ions in the acidic environment of the stomach induce mutagenic and cell-damaging reactions (Kato & Puck, 1971). Exposure to excess dietary nitrites has been implicated as a potential etiological factor in the development of stomach and colorectal cancers. Nitrite is toxic and the consumption of excess nitrites over time results in the oxidization of hemoglobin, which can lead to methemoglobinemia (Jeon *et al.*, 2002). It has been reported that phenolic compounds have a greater nitrite scavenging effect in environments with low pH (Noh *et al.*, 2002).

In conclusion, based on the results of reducing power, hydroxyl radical scavenging, and DPPH free radical scavenging assays, we showed that extracts from *S. oleraceus* had effective antioxidant activity. In addition, the extracts may have considerable activity against the growth of the stomach cancer cells. Therefore, *S. oleraceus* might be a source of food and natural antioxidants.

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