

**Antioxidant and anticancer activities of water and ethanol extracts obtained from *Sasa quelpaertensis* Nakai**

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**Abstract:** Antioxidant activities of *Sasa quelpaertensis* Nakai leaf extracts using two different solvents (water and ethanol) were determined by using *in vitro* antioxidant models including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, hydrogen peroxide and nitric oxide scavenging activities, and ferrous chelating and reducing power activities. Both extracts were shown to be antioxidants; however, ethanol extract exhibited higher antioxidant activity than that of water extract. The total phenolic and flavonoid contents of *Sasa quelpaertensis* Nakai leaf extract was positively correlated with the antioxidant activity. Significantly, the ethanol extract exerted the most potent cytotoxicity activity against human colon cancer HCT116 cells.

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## 1. Introduction

More than 1250 bamboo species, belonging to 75 genera, are distributed all over the world (Jiang, 2007; Wang et al., 2012). Most bamboo used as ornamental plant, but also used as a pharmaceutical intermediate and food additive. Bamboo leaves have been extensively used in folk medicine as an antifebrile and antihypertensive for centuries (Kweon et al., 2001; Wang et al., 2012). It was found that extract of bamboo leaves has multiple biological activities, such as cancer prevention (Seki et al., 2010; Seki and Maeda, 2010), antioxidant and antioxidation (Park et al., 2007; Mu et al., 2004). Bamboo leaves from *Sasa veitchii* and *Phyllostachys nigr* 'Henonis' are also used as an antioxidant food additive in Japan and China, respectively (Nakajima et al., 2003). Some functional components from bamboo leaves, such as flavonoids, lactones and phenolic acids, were reported (Zhang et al., 2008; Wang et al., 2012).

*Sasa quelpaertensis* Nakai is a type of bamboo grass widely distributed in Halla mountain, Jeju Island, Korea, which has been used as antidiabetic, diuretic and anti-inflammatory drugs (Ryou et al., 2012). *Sasa quelpaertensis* Nakai has recently gained considerable interest for its capacity to inhibit human leukemia HL-60 cell proliferation by inducing apoptosis (Jang et al., 2008). Other studies have demonstrated that *Sasa quelpaertensis*

Nakai leaf controls electrolyte and body fluid balance (Ryou et al., 2012), and reduces melanin production via inhibition of tyrosine hydroxylase (Yoon et al., 2007; Sultana et al., 2009).

In this study, we investigated the antioxidant and anticarcinogenic activities of *Sasa quelpaertensis* Nakai leaf extracts and demonstrated the potent bioactivities of the extracts suitable to be used as natural antioxidant compounds or pharmaceutical supplements.

## 2. Material and Methods

### 2.1. Plant material

The leaves of *Sasa quelpaertensis* Nakai used for the present study were collected from Halla mountain in February 2012. The leaves were dried in oven at 60 °C for 24 h and grounded into powder using a blender.

### 2.2. Extraction

Dried powder (25 g) of *Sasa quelpaertensis* Nakai leaf was extracted in a rotary shaker with 250 mL of water and 70% ethanol at 25 °C for 24 h. The extract was then purified by using a Sep-Pak C<sub>18</sub> cartridge and a 0.45 µm membrane filter (Waters, Milford, MA, US), concentrated using a rotary evaporator (Buchi Rotavapor R-200, New Castle, DE, US), freeze dried and finally stored at -20 °C until further use. The extraction yield of water

and ethanol extracts was determined to be  $13.6 \pm 0.34$  and  $11.8 \pm 0.13\%$ , respectively.

### 2.3. Determination of total phenolic and flavonoid contents

Total phenolic and flavonoid contents of *Sasa quelpaertensis* Nakai leaf extracts were determined by the method described previously (Kim et al., 2012a). Each experiment was performed at least in triplicate.

### 2.4. Analyses of antioxidant activities

#### 2.4.1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide and nitric oxide scavenging assays

DPPH radical, hydrogen peroxide and nitric oxide scavenging activities of *Sasa quelpaertensis* Nakai leaf extracts were determined as described in our recently published paper (Kim et al., 2012b). A dose response curve was plotted to determine the  $EC_{50}$  values.  $EC_{50}$  is defined as the effective concentration sufficient to obtain 50 % of a maximum scavenging capacity. All tests were performed in triplicate.

#### 2.4.2. Ferrous ion chelating assay

This methodology was performed using a microplate reader (Spectra MR, Dynex, VA, US). An aliquot (250  $\mu$ L) of each extract (concentration range 12.5 to 200  $\mu$ g/mL) in the 96-well plate was mixed with 5  $\mu$ L of 2 mM ferrous chloride solution. The reaction was initiated by the addition of 10  $\mu$ L of 5 mM ferrozine solution. After incubation at room temperature for 10 min, the absorbance was measured at 562 nm.

#### 2.4.3. Reducing power assay

This methodology was performed using a microplate reader described above. The water and ethanol extract solutions (concentration range 12.5 to 200  $\mu$ g/mL) were mixed with 200 mM sodium phosphate buffer (pH 6.6) containing 1% (w/v) potassium ferricyanide. The mixture was incubated at 50 °C for 20 min, and 10% (w/v) trichloroacetic acid was added. The mixture was poured into the each well of 96-well plate, and the absorbance was measured at 700 nm.  $EC_{50}$  value ( $\mu$ g/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis (Huang et al., 2006).

### 2.5. Analysis of anticancer activity

#### 2.5.1. Cell line and culture

Human colon cancer HCT116 cells were a

kind gift from Dr. G.N. Wogan (Massachusetts Institute of Technology, MA, US), and were maintained in McCoy's 5A medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100  $\mu$ g/mL streptomycin and 2 mM L-glutamine at 37 °C with 5% CO<sub>2</sub> in a humidified atmosphere.

#### 2.5.2. Determination of the cell viability

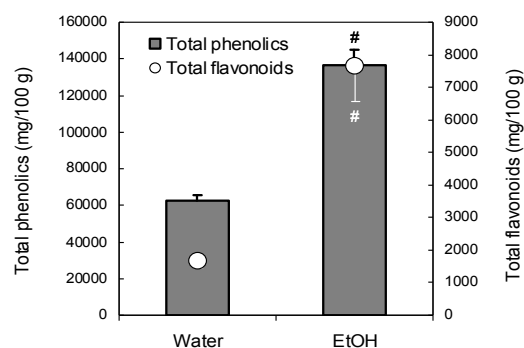
The inhibitory effect of the *Sasa quelpaertensis* Nakai leaf extracts on the proliferation of human colon cancer cells was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, HCT116 cells ( $2 \times 10^3$ /well) were loaded into 96-well culture plates and treated with fresh medium containing various concentrations (0–200  $\mu$ M) of each extract for 24 h. Cells were washed once with phosphate-buffered saline and reacted with the MTT solution (Boehringer Mannheim, Indianapolis, IN) at 37 °C for 4 h to produce the formazan salt. Finally, the formazan salt formed in each cultured cells was dissolved in DMSO, and the optical density (OD) value of each solution was measured at 540 nm using a microplate reader. The OD value detected for the control (cells without treatment with any samples) from the treated cells was plotted on the x-axis, designated as proliferation (% of control), to demonstrate the effect of each extract on the viability of the cells.

### 2.6. Statistical analysis

Student's *t*-test or one-way analysis of variance (ANOVA) with the Bonferroni post test was used to determine the statistical significance of data ( $p < 0.05$ ).

## 3. Results and Discussion

Figure 1 shows the total phenolic and flavonoid contents of water and ethanol extracts of *Sasa quelpaertensis* Nakai. Phenolic compounds, aromatic secondary plant metabolites, which mainly include flavonoids, phenolic acids, stilbenes, coumarins and tannins (Robbins, 2003). From the general concept, the more phenolic and flavonoid substances the sample contains, the higher antioxidant activity it shows. In this study, the contents of total phenols and flavonoids were significantly higher for ethanol extract (136267.1 and 7650 mg/g, respectively) than that for water extract (62267.3 and 1670.2 mg/g, respectively) ( $p < 0.05$ ) (Figure 1). This indicates that ethanol is relatively more efficient extraction solvent of polyphenolic compounds from *Sasa quelpaertensis* Nakai.



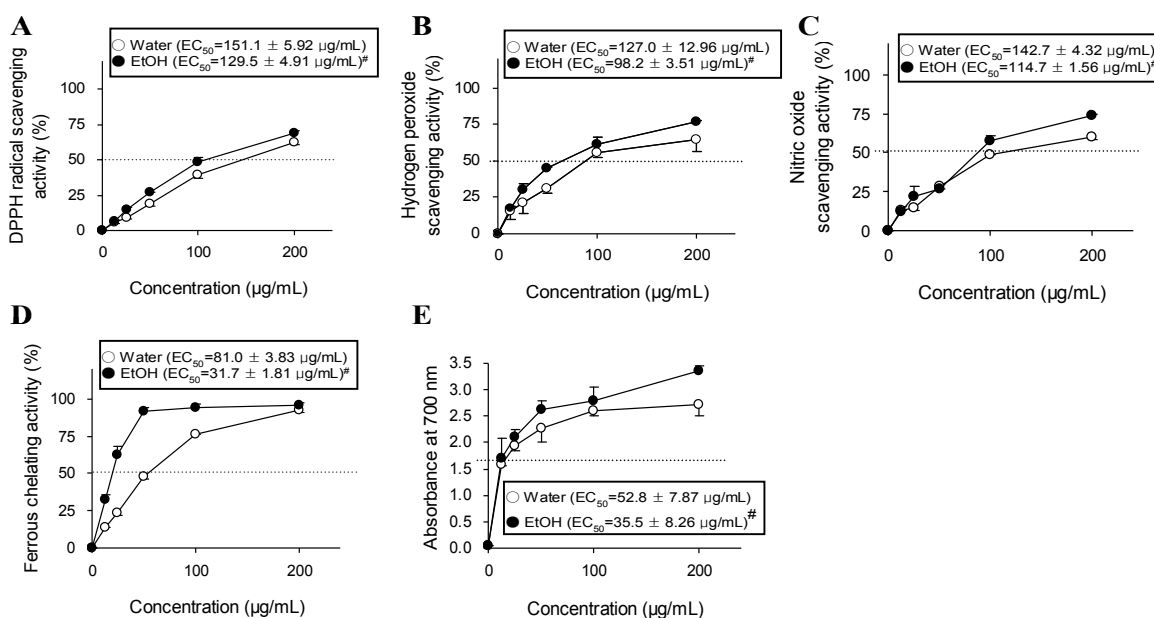
**Fig. 1.** Total phenolic and flavonoid compositions of the water and ethanol extracts obtained from *Sasa quelpaertensis* Nakai. Each value is expressed as mean  $\pm$  standard deviation ( $n = 3$ ). <sup>#</sup> $p < 0.05$  compared to water extract by Student's  $t$ -test.

The DPPH radical is usually used as a substrate to evaluate the antioxidative action of antioxidants by determining the free radical-scavenging ability of various samples (Amarowicz et al., 2004). The DPPH radical scavenging activities of water and ethanol extracts obtained from *Sasa*

*quelpaertensis* Nakai showed increasing trends with increase of extract concentration (Figure 1A). The half effective concentration ( $EC_{50}$ ) value for DPPH radical scavenging activity of ethanol extract (129.5  $\mu\text{g/mL}$ ) was significantly lower than that of water extract (151.1  $\mu\text{g/mL}$ ) (Figure 1A).

The results of hydrogen peroxide inhibition by the water and ethanol extracts of *Sasa quelpaertensis* Nakai are presented in Figure 1B. Obviously, the hydrogen peroxide scavenging activities were concentration-dependent for both extracts. The  $EC_{50}$  values of Ethanol extract was 98.2  $\mu\text{g/mL}$ , which were lower than that of water extract (127  $\mu\text{g/mL}$ ) (Figure 1B).

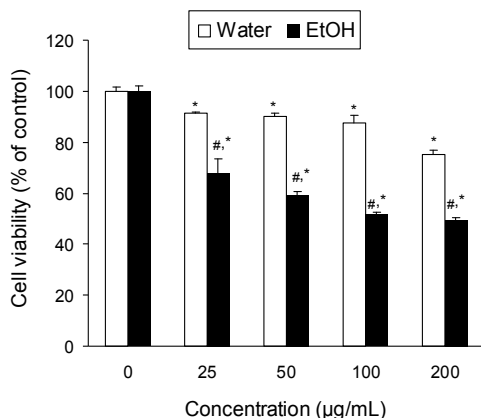
Nitric oxide is an essential bioregulatory molecule required for several physiological processes. The elevation of the nitric oxide results in inflammation, cancer and other pathological conditions (Kim et al., 2012b). The same trend was observed for the level of nitric oxide scavenging effects in tested *Sasa quelpaertensis* Nakai leaf extracts; both extracts scavenged nitric oxide in a dose-dependent manner, and ethanolic extract (114.7  $\mu\text{g/mL}$   $EC_{50}$ ) had higher nitric oxide scavenging ability than water extract (142.7  $\mu\text{g/mL}$   $EC_{50}$ ) (Figure 1C).



**Fig. 2.** Scavenging effects of the water and ethanol extracts obtained from *Sasa quelpaertensis* Nakai on DPPH (A), hydrogen peroxide (B) and nitric oxide (C), and activities of ferrous chelating (D) and reducing power (E).  $EC_{50}$  means the effective concentration at which the antioxidant activity was 50%, the absorbance was 1.75 for reducing power, the DPPH, hydrogen and nitric oxide radicals were scavenged by 50%, and ferrous ions were chelated by 50%, respectively.  $EC_{50}$  was obtained by interpolation from linear regression analysis. Each value is expressed as mean  $\pm$  standard deviation ( $n = 3$ ). <sup>#</sup> $p < 0.05$  compared to water extract by Student's  $t$ -test.

Chelating effect on ferrous ions of *Sasa quelpaertensis* Nakai extracts were 13–33% and 24–96% at 12.5 and 25–200 µg/mL, respectively (Figure 2D). The EC<sub>50</sub> values for the water and ethanol extracts were 81 and 31.7 mg µg/mL, respectively (Figure 2D), indicating that extraction solvent affected the chelating effect of *quelpaertensis* Nakai.

The antioxidant activity has been reported to be concomitant with reducing power (Lee et al., 2003). The reducing power is generally associated with the presence of reductones, which exert antioxidant action by breaking the free radical chains by donating a hydrogen atom. In this assay, the presence of reductants in the antioxidant sample causes the reduction of the Fe<sup>3+</sup>/ferricyanide complex to the Fe<sup>2+</sup>/ferrous form, so the reducing power of the sample can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Singh and Rajini, 2004). As shown in Figure 2E, reducing power of *Sasa quelpaertensis* Nakai extracts increased with concentration. With regard to reducing power, ethanol extract (35.5 µg/mL EC<sub>50</sub>) was more effective than water extract (52.8 µg/mL EC<sub>50</sub>) (Figure 2E). The reducing power of *Sasa quelpaertensis* Nakai leaf extracts is probably due to the presence of phenolic compounds which might act as electron donors.



**Fig. 3.** The percentage of viable HCT116 cells after *Sasa quelpaertensis* Nakai bamboo leaves treatment. Cell viability was determined by MTT assay after treatment with 0, 25, 50, 100 and 200 µg/mL of water and ethanol extracts for 24 h. Each point is the mean ± SD of three experiments. <sup>#</sup>*p* < 0.05 compared to water extract and <sup>\*</sup>*p* < 0.05 compared to vehicle (DMSO) control by Student's *t*-test.

In the present study, the anticancer activity of *Sasa quelpaertensis* Nakai extracts was investigated using an 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide assay on human colon cancer cell line, HCT116. Cells were treated with various concentrations (0–200 µg/mL) of water and ethanol extracts for 24 h, and then analyzed by MTT cell viability assay for the cell viability, proliferation (% control), as shown in Figure 3. The formazan product of MTT assay was analyzed for quantification of the viability of cells. Both water and ethanol extracts were found to exhibit significantly growth inhibitory effect in HCT116 cells, and ethanol extract exhibited the most significant antiproliferative effect: it led to a maximum decrease of 49% at 200 µg/mL (Figure 3). Polyphenolic compounds might inhibit cancer cells by xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens, while some flavonoids could also alter hormone production and inhibit aromatase to prevent the development of cancer cells (Zhao et al., 2007). The mechanism of action of anticancer activity of phenolics could be by disturbing the cellular division during mitosis at the telophase stage. It was also reported that phenolics reduced the amount of cellular protein and mitotic index, and the colony formation during cell proliferation of cancer cells (Gawron and Kruk, 1992). Therefore, it is of interest to characterize the cytotoxic and apoptotic mechanisms in HCT116 cells induced by *Sasa quelpaertensis* Nakai extracts. This work is in progress.

In this study, application of different solvents to extract antioxidant and anticancer compounds from *Sasa quelpaertensis* Nakai was investigated. The study indicated that the ethanol extract possessed the highest phenolic and flavonoid contents and exhibited strong antioxidant and anticancer activities. This seems that *Sasa quelpaertensis* Nakai extract can be used as natural antioxidant and anticancer agent.

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