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RESEARCH NOTE

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# *Sasa quelpaertensis* Leaf Extracts Induce Apoptosis in Human Leukemia HL-60 Cells

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**Abstract** In east Asia, the leaves of various *Sasa* species have been used in folk medicine for centuries. The effects of the methanolic extract and its subsequent fractions derived from the leaves of *Sasa quelpaertensis* Nakai on cell proliferation in human leukemia HL-60 cells were evaluated. The ethyl acetate fraction of this extract (ESQL) significantly reduced cell viability in a dose-dependent manner (0-250 μg/mL). ESQL ($IC_{50}$=24.8 μg/mL) exhibited growth inhibition comparable to the main constituent of green tea, epigallocatechin ($IC_{50}$=26.2 μg/mL), which was used as a positive control. ESQL treatment induced apoptosis, which was confirmed by the presence of nuclear condensation and annexin V-staining. These results demonstrate that ESQL contains chemopreventive phytochemicals that may be useful in neutraceutical applications.

**Keywords:** apoptosis, bamboo grass, cytotoxicity, HL-60 cell, *Sasa quelpaertensis*

## Introduction

A large number of chemopreventive phytochemicals show preventive effects in oxidative damage-related diseases, including cancer. For example, curcumin, tea polyphenols, and genistein possess anticancer properties partially attributable to their apoptosis-inducing activities (1,2). The use of natural medicines and functional foods as chemopreventive agents is beneficial in that they are already applied in human dietary requirements.

The genus *Sasa* (family Gramineae) comprises plants commonly known as bamboos. These well-known perennials are distributed throughout Asia, and are used to treat burns, hemoptysis, and uremia in traditional medicine (3,4). *Sasa albomarginata* extract has shown anticancer activity (5), and the lignin and polysaccharide preparations from *Sasa kurilensis* inhibited the growth of sarcoma-180 cells implanted in mice (6). *Sasa senanensis* extracts, which contain polysaccharides, chlorophylline, lignin, and flavonoids, possess antioxidant, anti-inflammatory, and antitumor properties (7-11). Recently, the phytochemical components and pharmacological effects of *Sasa borealis* leaves were investigated, which revealed the presence of 4 antioxidant flavone glycosides and 2 phenolic compounds (12,13).

*Sasa quelpaertensis* Nakai is a bamboo grass endemic to the area surrounding Hallasan(Mt.) on Jeju Island, Korea (14). While the leaves of this plant have been increasingly harvested for bamboo tea, their functional properties have not been investigated in detail, with the exception of our previous studies revealing anti-inflammatory effects (15). We evaluated the effects of the methanolic extract and its subsequent fractions derived from *S. quelpaertensis* leaves on cell growth in human leukemia HL-60 cells.

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## Materials and Methods

**Plant materials, methanol extraction, and fractionation** The leaves of *S. quelpaertensis* were collected during May 2004 on Jeju Island, Korea. The leaves were cleaned, dried at room temperature for 2 weeks, and ground into a fine powder. The dried leaves (1.5 kg) were extracted with 80% methanol (MeOH) and then concentrated under a vacuum. The resulting MeOH extract (64.07 g) was suspended in water (1 L), and successively partitioned with hexane (1 L ×3), ethyl acetate (EtOAc; 1 L×3), and *n*-butanol (BuOH; 1 L×3), yielding the following fractions: hexane (4.36 g), EtOAc (3.6 g), *n*-BuOH (23.67 g), and $H_2O$ (19.07 g). Each fraction was evaporated and freeze-dried.

**Cell culture** Acute leukemia cells were obtained from the Korean Cell Line Bank. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics (penicillin, 100 U/mL; streptomycin 100 μg/mL; Gibco, Grand Island, NY, USA). The cultures were maintained at 37°C in a humidified atmosphere of 5% $CO_2$. Cells in the exponential growth phase were used throughout the experiments.

**Cytotoxicity tests** Cytotoxicity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to measure metabolic activity (16). HL-60 cells ($2.0\times10^5$/mL) cultured in 96-microwell plates were exposed to the test materials for 4 days. After incubation, 0.1 mg of MTT was added to each well and the cells were incubated at 37°C for 4 hr. The plates were centrifuged at 10,000×g for 5 min at room temperature, and the medium was carefully aspirated. Then 150 μL of dimethyl sulfoxide was added to each well to solubilize the formazan crystals. The plates were read immediately at 540 nm on a microplate reader (Amersham Pharmacia Biotech, Uppsala, Sweden). All experiments were repeated 3 times and the mean absorbance

values were calculated. The results are expressed as the percent inhibition, which was calculated from the decrease in absorbance in treated cells compared to the control.

**Assays for apoptosis** To examine the effect of the EtOAc fraction on morphological changes, HL-60 cells ($2.5\times10^5$ cells/mL) were treated with various concentrations of the test materials for 48 hr. The treated cells were stained with a DNA-specific fluorescent dye (Hoechst 33342; Sigma, St. Louis, MO, USA), and then observed under a fluorescence microscope (IX-71; Olympus, Tokyo, Japan) equipped with a JENOPTIK color digital camera (Carl Zeiss, Jena, Germany). We tested for apoptotic cell death using the Annexin V-Apoptosis Detection kit (Becton Dickinson, Franklin Lakes, NJ, USA). Briefly, the cells were washed twice with phosphate-buffered saline and resuspended in binding buffer. The cells were then stained with annexin V-FITC and propidium iodide (PI, 2 mg/mL) for 15 min in the dark at room temperature, followed by flow cytometric analysis of 10,000 cells from each treatment. Annexin-V binds to cells expressing phosphatidylserine on the cell membrane surface. Data analysis was performed using flow cytometry (BD FACSCalibur; Becton Dickinson).

**Western blot analysis** HL-60 cells ($2.5\times10^5$ cells/mL) were treated with different concentrations of the EtOAc fraction for 24 hr and then harvested. The total cell lysate (40 μg) was electrophoresed on an 8-15% SDS-polyacrylamide gel and transferred to immobilon polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA). The membrane was blocked with 5% nonfat dried milk for 1 hr and then washed. The membrane was incubated overnight at 4°C with the following primary antibodies: anti-procaspase-3, anti-poly ADP-ribose polymerase (PARP) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-caspase-3 (Cell Signaling Technology, Danvers, MA, USA), and anti-β-actin (Sigma). The blots were washed 4 times and then treated with peroxidase-conjugated anti-mouse IgG. The proteins were visualized using an enhanced chemiluminescence western blot detection kit (Amersham Pharmacia Biotech).

**Statistical analysis** Statistical analysis was performed using Student's *t*-test for comparisons between two or more groups. The results are expressed as the mean± standard deviation (SD).

## Results and Discussion

**Anti-proliferative effect of *S. quelpaertensis* leaf extract** The methanolic extract of *S. quelpaertensis* leaves and its fractions significantly reduced the viability of HL-60 cells at a concentration of 100 μg/mL after incubation for 96 hr (Fig. 1A). The ethyl acetate fraction of the leaves (ESQL) potently inhibited the proliferation of HL-60 cells in a dose-dependent manner (Fig. 2B). The inhibitory effect of ESQL ($IC_{50}$ value=24.8 μg/mL) was similar to that of epigallocatechin gallate (EGCG, $IC_{50}$ value=26.2 μg/mL;

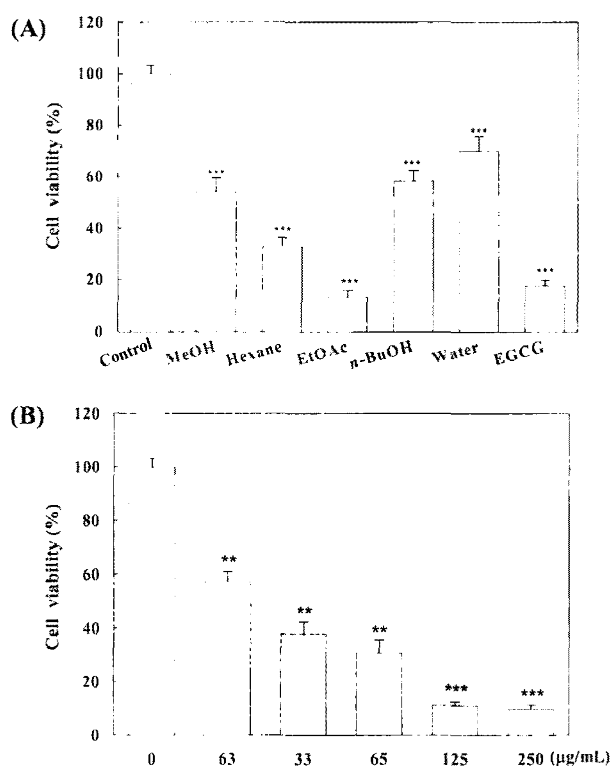


**Fig. 1. (A) Anti-proliferative effect of the methanolic extract and its fractions derived from the leaves of *Sasa quelpaertensis*. (B) Dose-dependent anti-proliferative effect of the ethyl acetate fraction from the leaves of *S. quelpaertensis* (ESQL) in HL-60 cells.** Data are represented as the mean±SE. *$p$<0.05; **$p$<0.01; ***$p$<0.001.

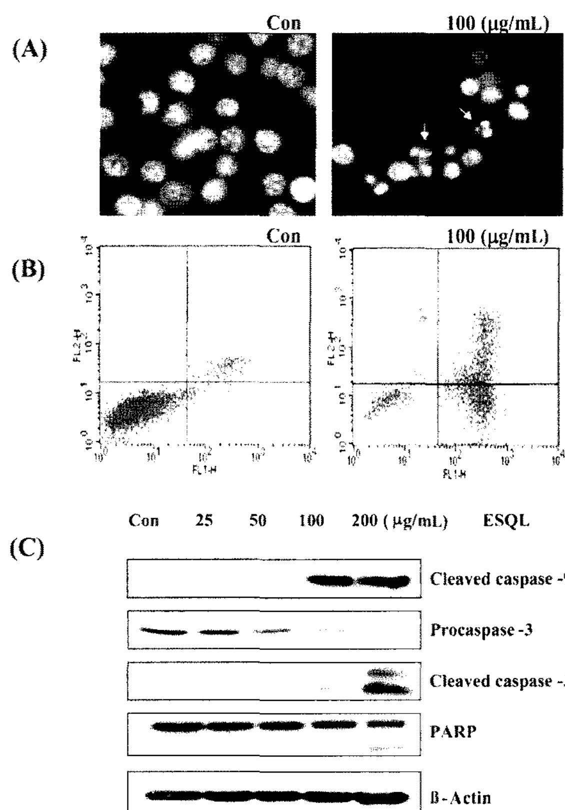


**Fig. 2. (A) Identification of nuclear condensation in apoptotic cells. (B) Assessment of apoptosis by annexin-V and PI double-staining. (C) Western blot analysis of apoptosis-related proteins in HL-60 cells.** The arrow indicates the apoptotic bodies in HL-60 cells undergoing cell death.

Fig. 1A), which was used as a positive control. Numerous reports have demonstrated that the anticancer and anti-proliferative effects of EGCG are related to the induction of apoptosis and cell cycle arrest, the regulation of transcription factors, enzyme modulation, antioxidant activity, and the inhibition of carcinogenesis, gene expression, angiogenesis, and metastasis (17). Recently, Nakazato *et al.* (18) suggested that EGCG may inhibit cell proliferation via the modulation of reactive oxygen species. We demonstrated that ESQL has potent antioxidant effects via the scavenging of DPPH radicals, nitric oxide, and superoxide radicals (data not shown), suggesting that the inhibitory effect of ESQL on HL-60 cell growth is at least partly related to its potency as an antioxidant.

**The induction of apoptosis by the EtOAc fraction from *S. quelpaertensis* leaves** Cell cycle-mediated apoptosis has recently received considerable attention because of its regulatory role in the growth of various cancers. To characterize ESQL-induced cell death, we examined several characteristics of apoptosis. After treatment with 100 μg/mL of ESQL for 48 hr, apoptotic cells were identified by the presence of condensed nuclei (Fig. 2A). Consistent with these results, ESQL-treated cells showed a significant increase in annexin V-positive/PI-negative cells (42%), indicating an increase in apoptosis compared to the untreated control (2%). Western blots revealed that ESQL treatment caused increased expression of activated caspase-3 and -9, decreased expression of procaspase-3, and the degradation of the 116 kDa PARP molecule into 85-kDa fragments. These responses occurred in a dose-dependent manner (Fig. 2C).

Many natural compounds have the ability to initiate apoptosis in tumor cells without the toxic effects associated with synthetic drugs, suggesting that they may be valuable as chemopreventive and chemotherapeutic agents (19). ESQL contains several phytochemicals, such as polyphenols, and possesses many of the desirable qualities of an anticancer treatment. However, other *Sasa* species have also shown therapeutic potential. Sasa Health, an alkaline extract derived from the leaves of *S. senanensis*, contains polysaccharides, chlorophyllin, lignin, and flavonoids, and has shown a protective effect on Her2/NeuN cells in mammary tumorigenesis (11). In addition, 4 antioxidant flavone glycosides (tricin-7-*O*-β-D-glucopyranoside, isoorientin, apigenin 6-*C*-β-D-xylopyranosyl-8-*C*-β-D-glucopyranoside, isoorientin 2-*O*-α-L-rhamnoside) have been isolated from the leaves of *S. borealis* (12). Two phenolic compounds, (-)-syringaresinol and tricin, isolated from the leaves of *S. borealis*, exhibited P-glycoprotein inhibition in the adrimiamycin-resistant human breast cancer cell line, MCF-7/ARD (13). We recently reported that the hot water extract of *S. quelpaertensis* leaves has an anti-inflammatory effect on LPS-stimulated RAW 264.7 cells (15). Here, we demonstrated that ESQL has an anti-proliferative effect in human leukemia HL-60 cells via the induction of apoptosis, which suggests that ESQL may be useful as a chemopreventive therapy.

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