Antioxidant and anticancer activity of extract from 
Betula platyphylla var. japonica

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Abstract

The antioxidant and anticancer properties of a medicinal plant, Betula platyphylla var. japonica were investigated. The total methanol extract of B. platyphylla var. japonica had protective effects against hydrogen peroxide (H₂O₂) in the Chinese hamster lung fibroblast (V79-4) cell line and induced apoptotic cell death in human promyelocytic leukemia (HL-60) cells, a cancer cell line. B. platyphylla var. japonica extract significantly increased cell viability against H₂O₂. The extract also showed high 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (IC₅₀ 2.4 μg/ml) and lipid peroxidation inhibitory activity (IC₅₀ below 4.0 μg/ml). Furthermore, B. platyphylla var. japonica extract reduced the number of V79-4 cells arrested in G₂/M in response to H₂O₂ treatment and increased the activities of several cellular antioxidant enzymes, including superoxide dismutase, catalase and glutathione peroxidase. Treatment with B. platyphylla var. japonica extract induced cytotoxicity and apoptosis in HL-60 cells, as shown by nucleosomal DNA fragmentation, increases in the subdiploid cell population, and fluorescence microscopy. B. platyphylla var. japonica extract gradually increased the expression of pro-apoptotic Bax and led to the activation of caspase-3 and cleavage of PARP. These findings suggest that B. platyphylla var. japonica exhibits potential antioxidant and anticancer properties.

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Introduction

The role of reactive oxygen species (ROS) has been implicated in many human degenerative diseases, including aging, cancer, and neurodegenerative disorders such as Alzheimer’s disease, Parkinson’s disease and Huntington’s disease (Harman, 1994; Simonian and Coyle, 1996). Hydrogen peroxide (H$_2$O$_2$), is a prominent ROS that causes lipid peroxidation and DNA damage in cells (Sies, 1985; Halliwell and Aruoma, 1991). In recent years, considerable effort has been directed towards identifying naturally occurring substances that can protect against oxidative stress.

Natural antioxidants have a wide range of biochemical activities, including inhibition of ROS generation, direct or indirect scavenging of free radicals, and alteration of intracellular redox potential (Finkel and Holbrook, 2000). Antioxidants have been used to inhibit apoptosis because apoptosis was initially thought to be mediated by oxidative stress (Hockenhery et al., 1993). Many antioxidant substances have anticancer or anticarcinogenic properties (Johnson et al., 1994; Dragsted et al., 1993). For example, resveratrol in grapes and other food products has been shown to protect cells from oxidative damage and cell death (Jang et al., 1997; Chanvitayapongs et al., 1997) and to prevent carcinogenesis in a murine model (Clement et al., 1998). Epigallocatechin-3-galate (EGCG) in green tea has been reported to scavenge free radicals (Hannasaki et al., 1994) and to inhibit carcinogen-induced tumors in the skin, lung, forestomach and colon of rodents (Stoner and Mukhtar, 1995). Kim et al. reported that ginsenosides Rb1 and Rg3 isolated from *Panax ginseng* protected cultured rat cortical cells from glutamate-induced neurodegeneration (Kim et al., 1998). The methanol extract of heat-processed neo-ginseng attenuated lipid peroxidation in rat brain homogenates induced by ferric ions or ferric ions plus ascorbic acid (Keum et al., 2000). Curcumin, a yellow coloring ingredient present in turmeric (*Curcuma longa* Linn, Zingiberaceae), has a diarylheptanoid moiety and has anticarcinogenic or antimutagenic effects in diverse animal models and in cultured cells (Rao et al., 1995).

Diarylheptanoids have been isolated from *Betula platyphylla* var. *japonica* together with other aromatic compounds (Matsuda et al., 1998). Earlier studies showed that *B. platyphylla* var. *japonica* could protect mice against CCL$_4$- or D-GalN/LPS (d-galactosamine/lipopolysaccharide)-induced liver damage (Soudamini and Kuttan, 1989). These findings suggest that *B. platyphylla* var. *japonica* most likely has both antioxidant and anticancer activity. Considerable attention has been focused on role of apoptosis or programmed cell death in the pathogenesis and treatment of human cancer (Thompson, 1995). Indeed, a variety of cytotoxic drugs have been reported to induce apoptosis of malignant cells in vitro (Planchon et al., 1995; Muller et al., 1997). The present study was therefore designed to investigate whether *B. platyphylla* var. *japonica* inhibits H$_2$O$_2$-induced oxidative stress in Chinese hamster lung fibroblast (V79-4) cells and to characterize the mechanism of its anticancer effects in human promyelocytic leukemia (HL-60) cells.

Methods

Plant material

Dried bark from *Betula platyphylla* var. *japonica* (100 g) was extracted at 80°C in 70% methanol for 3 hr. The extract was then filtered and the filtrate was concentrated under low pressure using a vacuum rotary evaporator (Eyela, Japan). The remaining residue was lyophilized in a freezing-dryer (Ilsin,
Korea) and stored at –70 °C. Approximately 10 g of powdered extract was recovered. The powder was dissolved in dimethyl sulfoxide (DMSO) and diluted with phosphate buffered saline (PBS, pH 7.4) to give final concentrations of total extract ranging from 0.8 to 500 μg/ml.

Chemicals

The following chemicals were obtained from Sigma Chemical Co. (St Louis, MO, USA): dimethyl sulfoxide (DMSO), propidium iodide (PI), [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] (MTT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), 2,2′-di-p-nitrophenyl-5,5′-diphenyl-3,3′-(3,3′-dimethoxy-4,4′-diphenylene)-ditetrazolium chloride (NBT), nicotinamide adenine phosphor (NADPH), xanthine, xanthine oxidase, ethylenediaminetetraacetic acid sodium salt (Na-EDTA), pyridine, sodium azide, glutathione, glutathione reductase, ethidium bromide. Ethanol was obtained from Hayman Chemical Co. (Witham, Essex, UK). Hydrogen peroxide was purchased from Fluka Chemical Co. (Buchs, SG, Swiss). Cell culture materials were purchased from Gibco BRL (Gaithersburg, MD, USA). All other chemicals were of the highest analytical grade and were purchased from common sources.

DPPH free radical scavenging activity

In order to measure antioxidant activity, the DPPH free radical scavenging assays were carried out according to the previously described procedures (Blois, 1958; Lee et al., 2002).

Cell culture

The Chinese hamster lung cell line V79-4 (ATCC CCL-93) was grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), 2 mM glutamine, and antibiotics. The human promyelocytic leukemia cell line HL-60 (ATCC CCL-240) was grown in RPMI-1640 supplemented with 10% heat inactivated FBS, 2 mM glutamine, 100 unit/ml of penicillin and 100 μg/ml of streptomycin. Cultures were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO₂.

Cell viability and cytotoxicity analysis

Cell viability was estimated by the MTT assay (Hansen et al., 1989; Lee et al., 2002). V79-4 cells were treated with Betula platyphylla var. japonica extract for 1 hr prior to treatment with 100 μM of H₂O₂. The data are expressed as a mean percentage of viable cells as compared to the respective control cultures. Cytotoxicity of Betula platyphylla var. japonica on HL-60 cells was also evaluated by the MTT assay.

Lipid peroxidation inhibitory activity

Lipid peroxidation was determined by measuring malondialdehyde (MDA) (Ohkawa et al., 1979). V79-4 cells in culture were incubated with total extract of Betula platyphylla var. japonica at various concentrations (4, 20 and 100 μg/ml) for 60 min, followed by 100 μM of H₂O₂ for 60 min. Inhibitory activity towards lipid peroxidation was expressed as IC₅₀.
Analysis of nuclear morphology

V79-4 cells were seeded on sterilized cover glasses and treated with 100 μg/ml of Betula platyphylla var. japonica extract for 24 hr. Morphology of cellular nuclei were observed as described previously (Lee et al., 2003).

Flow cytometry

V79-4 cells were treated with 100 μg/ml of extract for 1 hr followed by the addition of 100 μM of H2O2 for 7 or 24 hr. HL-60 cells were treated with 500 μg/ml of extract for 0, 3, 7, 16, 24 or 48 hr. Cells were harvested and analyzed as described in Piao et al. (2001).

Assays for antioxidant enzymes

V79-4 cells were treated with 4, 20 and 100 μg/ml of Betula platyphylla var. japonica extract for 60 min. The cells were then lysed in a lysis buffer appropriate for the requirements of each assay. Results are expressed as enzyme activity per mg protein, as compared to the corresponding control cultures. Superoxide dismutase activity was assayed by the nitroblue tetrazolium (NBT) method (Beauchamp and Fridovich, 1971). Catalase and glutathione peroxidase activity were assayed as previously described (Carrillo et al., 1991; Paglia and Valentine, 1967).

DNA fragmentation analysis

HL-60 cells were treated for different periods with various concentrations of Betula platyphylla var. japonica extract. Cells were then harvested and DNA fragmentation was analyzed as described (Hyun et al., 1997).

Western blot analysis

HL60 cells were treated with Betula platyphylla var. japonica extract and subjected to western blot analysis, as described previously (Piao et al., 2001). Blots were probed with mouse monoclonal anti-human anti-Bcl-2 (Oncogene Science, Cambridge, MA, USA), anti-caspase-3 (Transduction Laboratory, Lexington, KY, USA), and rabbit monoclonal anti-human anti-Bax and anti-PARP (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies. Immunoreactivity was detected using either an anti-mouse (Santa Cruz Biotechnology) or anti-rabbit (Amersham Biosciences, Buckinghanshire, UK) peroxidase-conjugated secondary IgG antibody and an enhanced chemiluminescence (ECL) detection kit (Amersham Biosciences).

Results

Antioxidant activity of Betula platyphylla var. japonica extract

The antioxidant activity of B. platyphylla var. japonica extract was evaluated on the basis of its DPPH free radical scavenging activity, by its protective effects on cell viability, and by the inhibition
of lipid peroxidation. The DPPH radical scavenging activity of the extract is shown in Fig. 1A. *Betula platyphylla* var. *japonica* extract showed relatively high DPPH radical scavenging activity, with an average IC$_{50}$ value of 2.4 µg/ml. As a control, resveratrol was used and IC$_{50}$ value obtained was 4.8 µg/ml (data not shown).

Fig. 1. Effects of *Betula platyphylla* var. *japonica* extract on (A) DPPH radical scavenging activity, (B) cell survival, (C) and lipid peroxidation inhibitory activity in V79-4 cells. Each experiment was performed at least 3 times and data are expressed as average percent change from control ± S.D.
We next measured the protective effect of *B. platyphylla* var. *japonica* extract on the survival in H$_2$O$_2$-treated cells. Cells were treated with extract for 1 hr prior to the addition of H$_2$O$_2$. Control cells were treated with H$_2$O$_2$ in the presence of vehicle (DMSO) only. There was no cytoprotective effect of DMSO at the concentrations used in this study (below 0.1%). The relative cell survival of V79-4 cells was decreased in response to treatment with various concentrations of H$_2$O$_2$ for 24 hr, with an average IC$_{50}$ value of about 100 μM (data not shown). Treatment with *B. platyphylla* var. *japonica* extract for 1 hr prior to the addition of 100 μM of H$_2$O$_2$ induced a dose-dependent increase in cell survival (Fig. 1B). At a dose of 100 μg/ml, the extract increased cell viability by 85%, as compared to control levels. The extract itself showed little cytotoxicity in V79-4 cells (IC$_{50}$>500 μg/ml). We also tested whether *B. platyphylla* var. *japonica* extract could inhibit lipid peroxidation in H$_2$O$_2$-treated V79-4 cells. As shown in Fig. 1C, cells pretreated with the extract exhibited a dose-dependent reduction in lipid peroxidation. Treatment of 4, 20 and 100 μg/ml of extract inhibited lipid peroxidation by 91%, 79% and 56%, respectively (IC$_{50}$ < 4.0 μg/ml). These values are similar to the values obtained from resveratrol. At concentrations of 4, 20 and 100 μg/ml of resveratrol, lipid peroxidation was inhibited by 74%, 72% and 65%, respectively (data not shown).

Reduction of H$_2$O$_2$-induced nuclear fragmentation

In order to analyze the protective effect of *Betula platyphylla* var. *japonica* extract on H$_2$O$_2$-induced apoptosis, we used propidium iodide to stain nuclei of V79-4 cells treated with either H$_2$O$_2$ alone or with both *B. platyphylla* var. *japonica* extract and H$_2$O$_2$. As shown in Fig. 2A, control cells exhibited intact nuclei, but cells treated with 100 μM of H$_2$O$_2$ showed significant nuclear fragmentation (Fig. 2B). However, when cells were treated with *B. platyphylla* var. *japonica* extract for 1 hr prior to H$_2$O$_2$ treatment, a marked reduction in nuclear fragmentation was observed (Fig. 2C). In addition to these morphological observations, a protective effect of *B. platyphylla* var. *japonica* extract was confirmed using flow cytometry. DNA content was analyzed in V79-4 cells that were treated with H$_2$O$_2$ for 7 and 24 hr, with or without pretreatment with *B. platyphylla* var.

![Fig. 2. Betula platyphylla var. japonica extract reduces H$_2$O$_2$-induced apoptosis in V79-4 cells.](image-url)
japonica extract. As shown in Fig. 3, exposure to H₂O₂ for 7 and 24 hr induced cell cycle arrest in the G2/M phase in 42.7% and 33.4% of cells, respectively (Fig. 3A). Pretreatment of cells with B. platyphylla var. japonica extract (100 µg/ml) prior to H₂O₂ treatment reduced the number of cells in the G2/M phase to 35.6% and 25.9%, respectively (Fig. 3B). H₂O₂ treatment for 7 and 24 hr induced apoptosis in 6.1% and 4.2% of cells, and this was reduced to 0.4% and 0.8% in cells pretreated with extract.

Effect of Betula platyphylla var. japonica extract on antioxidant enzyme activity

In order to investigate whether these antioxidant properties of B. platyphylla var. japonica extract were mediated by an increase in antioxidant enzymes, we measured superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities in V79-4 cells treated with the extract (Fig. 4). Treatment with the extract at doses of 4, 20 and 100 µg/ml induced 21%, 31% and 36% increases, respectively, in SOD levels (Fig. 4A). Each sample dose-dependently increased SOD activity over a range of 4 to 100 µg/ml. The activity of SOD in control cells untreated with the extract was 24.9 ± 1.5 U/mg protein. B. platyphylla var. japonica extract also dose-dependently increased CAT activity, although this effect was not as robust as the effect on SOD activity (Fig. 4B). At doses of 4, 20 and 100 µg/ml, the extract increased CAT activity by 12%, 20% and 24%, respectively. CAT activity in untreated control cells was 14.9 ± 1.9 U/mg protein. GPX activity increased dramatically in response to treatment with extract (Fig. 4C). This occurred in a dose-dependent manner, in that 4, 20 and 100 µg/ml of extract increased GPX activity by 51%, 62% and 67%, respectively. The activity of GPX in control cells untreated with the extract was 11.7 ± 1.3 U/mg protein.

Fig. 3. Betula platyphylla var. japonica extract protects against H₂O₂-induced apoptosis in V79-4 cells. Flow cytometric DNA profiles of V79-4 cells treated with (A) 100 µM of H₂O₂ alone or with (B) 100 µg/ml of B. platyphylla var. japonica extract prior to the addition of H₂O₂ for 0, 7, or 24 hr, as indicated.
We evaluated the cytotoxicity of *Betula platyphylla* var. *japonica* extract in human promyelocytic leukemia (HL-60) cells with the MTT assay. When cells were treated for 2 days with 0, 20, 100, and 500 μg/ml of *B. platyphylla* var. *japonica* extract, the relative cell survival progressively decreased in a

**Cytotoxicity of Betula platyphylla var. japonica extract in cancer cell**

Fig. 4. Effect of *Betula platyphylla* var. *japonica* extract on various antioxidant enzymes. V79-4 cells were treated with 0, 4, 20 and 100 μg/ml of *B. platyphylla* var. *japonica* extract for 1 hr. (A) superoxide dismutase activity, (B) catalase activity, and (C) glutathione peroxidase activity were measured, as described in Methods. Each experiment was performed at least 3 times and the data are expressed as average enzyme units per mg protein from control ± S.D.
dose-dependent manner, as shown in Fig 5. The IC50 value for *B. platyphlla var. japonica* extract on HL-60 cells was 159.0 µg/ml.

**Effect of Betula platyphlla var. japonica extract on DNA and nuclear fragmentation in HL-60 cells**

One of the mechanisms by which cell growth is suppressed is apoptotic cell death. Therefore, the effect of *B. platyphlla var. japonica* extract on DNA fragmentation was examined in HL-60 cells (Fig. 6). As shown in Fig. 6A, nucleosomal DNA fragmentation was observed when cells were treated with 100 and 500 µg/ml of *B. platyphlla var. japonica* extract for 24 hr. Exposure to the higher dose of *B. platyphlla var. japonica* extract (500 µg/ml) induced DNA fragmentation in as little as 3 hr (Fig. 6B). The profile for *B. platyphlla var. japonica* extract-induced apoptosis closely correlated with its growth suppressive effects. Thus, the growth suppression induced by *B. platyphlla var. japonica* extract in HL-60 cells may be related to the induction of apoptosis.

The apoptotic effects of *B. platyphlla var. japonica* extract were confirmed by flow cytometric analysis. The extract clearly induced apoptosis in a time- and dose-dependent manner, as shown in Fig. 7. HL-60 cells were incubated with increasing concentrations of *B. platyphlla var. japonica* extract for 24 hr. The percentages of apoptotic cells observed at 4, 20, 100 and 500 µg/ml of extract were 0, 5.7%, 12.2% and 68.5%, respectively (Fig. 7A). When HL-60 cells were incubated with 500 µg/ml of *B. platyphlla var. japonica* extract for 3, 7, 16, and 24 hr, the relative percentage of apoptotic cells observed were 25.5%, 46.3% 58.2% and 68.5%, respectively (Fig. 7B).

**Effects Betula platyphlla var. japonica extract on apoptosis-related protein levels HL-60 cells**

To understand the molecular mechanisms by which the *B. platyphlla var. japonica* extract induced apoptosis, we examined various apoptosis-related proteins. HL-60 cells were cultured in media...
containing 500 µg/ml of *B. platyphlla var. japonica* extract for 0, 1, 3, 7, 16, and 24 hr. At each time point, total protein was isolated and Bcl-2, Bax, caspase-3, and PARP (poly(ADP-ribosyl)polymerase) immunoreactivity levels were measured by Western blotting (Fig. 8). The pro-apoptotic protein, Bax was
increased in a time-dependent manner in response to extract, whereas the levels of the anti-apoptotic molecule Bcl-2 were unchanged; thus, the ratio of Bcl-2/Bax progressively decreased. Pro-caspase-3 levels were also decreased, implying that the levels of active caspase-3 were increased. To investigate the enzymatic activation of caspase-3, we measured the cleavage of PARP, which is a caspase-3 substrate. When cells were treated with 500 μg/ml of *Betula platyphylla var. japonica* extract, a time-dependent increase in the formation of the 85 kDa fragment and a decrease in the formation of the 116 kDa PARP were observed.

**Discussion**

Reactive oxygen species generated by mitochondria or from other intracellular or extracellular sites can cause cell damage and initiate various degradation processes (Davies and Hochstein, 1982). Mild amounts of oxidative damage may actually stimulate physiological mitochondrial biogenesis, via the production of superoxide from ubisemiquinone. However, more severe or more prolonged oxidative damage clearly induces toxic reactions that can contribute significantly to the aging process (Cadenas and Davies, 2000). Oxidative damage induced by the intracellular production of H$_2$O$_2$ occurs during many physiological and pathological processes. The cytotoxic effects of H$_2$O$_2$ on Hep G2 cells included a potent inhibition of cell growth, elevated lactate dehydrogenase leakage, and MDA formation (Yang et al., 1999). H$_2$O$_2$ itself is not highly reactive; the primary mechanism by which H$_2$O$_2$ induces cytotoxicity during oxidative stress is via the formation of a highly reactive species in the presence of transition metal ions, or through other mechanisms (Halliwell et al., 1992).

In this work, the antioxidant activities of *Betula platyphylla var. japonica* extract were determined by measuring its protective effects on cell viability, DPPH radical scavenging activity, inhibition of lipid peroxidation activity, the fluorescent staining of nuclei, and by flow cytometry. The total extract from *B. platyphylla var. japonica* exhibited significant DPPH free radical scavenging activity and enhanced cell
viability in V79-4 cells exposed to H2O2. The protective effects of B. platyphylla var. japonica extract on H2O2-induced apoptosis were observed with the aid of a fluorescence microscope and a flow cytometer. Cells exposed to H2O2 exhibit distinct morphological features of programmed cell death, such as nuclear fragmentation and an increase in the percentage of cells with a sub-G1 DNA content. However, cells pretreated with B. platyphylla var. japonica extract exhibited significantly lower levels of these characteristics of apoptotic cells. The morphological features and DNA profiles of extract-treated cells were very similar to those of control cells. Treatment with B. platyphylla var. japonica extract also decreased the DNA content of G2/M phase cells. These data suggested that B. platyphylla var. japonica extract inhibited H2O2-induced apoptosis.

We also found that the B. platyphylla var. japonica extract increased the activity of three antioxidant enzymes, SOD, CAT and GPX, that are altered in various diseases involving free radical attack (Halliwell and Gutteridge, 1998). Thus, maintaining the balance between the rates of radical generation and radical scavenging is an essential part of biological homeostasis. It is of particular interest to note that SOD catalyzes the breakdown of O2- to O2 and H2O2 and prevents the formation of OH-. Thus, SOD has been implicated as playing an essential defensive role against potential oxygen toxicity. The ROS scavenging activity of SOD is effective only when it is followed by the actions of CAT and GPX, because the dismutase activity of SOD generates H2O2, which needs to be further scavenged by CAT and GPX. B. platyphylla var. japonica extract modestly activated SOD and strongly activated GPX. It appears that B. platyphylla var. japonica extract can effectively scavenge H2O2 generated by SOD.

A vast variety of naturally occurring substances have been shown to protect against experimental carcinogenesis. Thus, it is becoming increasingly evident that certain phytochemicals, particularly those included in our daily diet, many have important cancer chemopreventive properties (Sanaha et al., 1997). Some anti-inflammatory chemopreventive agents have been found to suppress growth and proliferation of transformed or malignant cells through induction of programmed cell death or apoptosis (Bellosillo et al., 1998). Our present results demonstrated that extract from B. platyphylla var. japonica induced apoptosis in HL-60 cells at relatively higher concentrations than those that mediate its growth inhibitory and antiproliferative activities. The induction of apoptotic cell death was accompanied by characteristic morphological and structural changes. Internucleosomal DNA fragmentation, as determined by agarose gel electrophoretic analysis, were consistent with apoptosis in cells treated with the B. platyphylla var. japonica extract. The flow cytometry data more clearly confirmed apoptosis in B. platyphylla var. japonica extract-treated HL-60 cells.

Apoptosis is a tightly regulated process, which involves changes in the expression of distinct genes. One of the major genes that regulates apoptosis is the protooncogene bcl-2, which encodes a 26 kDa mitochondria-associated protein. The bcl-2 gene product prolongs cell survival by blocking apoptosis induced by a wide variety of stimuli (Okura et al., 1998; Kuo et al., 1996; Finucane et al., 1999). In our study, there was no change in Bcl-2 protein levels in HL-60 cells treated with B. platyphylla var. japonica extract, but the levels of Bax, a pro-apoptotic protein, were increased in a time-dependent manner, resulting in a decrease in the ratio of Bcl-2/Bax. Cytoplasmic aspartate-specific cysteine proteases of the ICE/CED-3 family, known as caspases, play an important role in apoptosis (Klaus et al., 1998). Caspase-3, one of the caspase family enzymes, can inactivate PARP by proteolytically cleaving this 116 kDa enzyme into an 85 kDa fragment (Nigata, 2000; Duriez and Shah, 1997; Li and Darzynkiewicz, 2000). When HL-60 cells were treated with B. platyphylla var. japonica extract, procaspase-3 was activated and the caspase substrate PARP was proteolytically cleaved.
Taken together, these findings suggest that *B. platyphylla* var. *japonica* extract exhibits antioxidant activity at lower concentrations and apoptotic effects at higher concentrations, through the down-regulation of Bcl-2/Bax and activation of caspase-3. Further studies will be needed to identify the active compounds that confer the antioxidant and/or anticancer activities of the *B. platyphylla* var. *japonica* extract. Once such compounds are identified, the mechanisms by which they exert their effects can begin to be characterized.

References


