Antioxidant activity of extracts from *Acanthopanax senticosus*

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Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infectious and degenerative diseases. Literature shows that the antioxidant activity is high in medicinal plants. Realizing the fact that, this study was carried out to determine the antioxidant activity of water extract of *Acanthopanax senticosus*. Water extract (0.5 g/50 ml) of A. senticosus (ASE) were prepared and total phenol contents (TPC) and radical scavenging activity (RSA) of the extracts was determined for antioxidant activity. The TPC and RSA of ASE were 366.67 μM and 67.67%, respectively. In addition, the effect of ASE on DNA damage induced by H₂O₂ in human lymphocytes was also evaluated by Comet assay. The ASE showed strong inhibitory effect as its concentration increased from 0.125 to 1% by 65 to 81% against DNA damage induced by 200 μM of H₂O₂. These results suggest that water extract of commercial dried A. senticosus for tea showed significant antioxidant activity and protective effect against oxidative DNA damage.

Key words: *Acanthopanax senticosus*, antioxidant, total phenol contents, DPPH, DNA damage, comet assay.
INTRODUCTION

Many herbal plants contain antioxidants compounds and these compounds protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite (David et al., 2004; Dasgupta and De, 2004). An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Several authors demonstrated that antioxidant intake is inversely related to mortality from coronary heart disease and to the incidence of heart attacks (Hertog et al., 1993; Kris-Etherton and Krummel, 1993; Anderson et al., 1998). As well as antioxidant defense system of our body, antioxidants that are mainly supplied as dietary consumptions can also impede carcinogenesis by scavenging oxygen radicals or interfering with the binding of carcinogens to DNA which includes vitamin C, vitamin E (α-tocopherol, γ-tocopherol), carotenoids (β-carotene, α-carotene, β-cryptoxanthin, lutein, zeaxanthin, lycopene) and several polyphenolic compounds including flavonoids (catechins, flavonols, flavones, isoflavonoids) (Elsayed and Bendich, 2001; Stoner and Mukhtar, 1995). In particular, phenolic compounds have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals (Rice-Evans et al., 1995; Jorgensen et al., 1999).

The antioxidative potential of phenolic compounds can be attributed to their strong capability to transfer electrons to ROS/free radicals, chelating metal ions, activate antioxidant enzymes and inhibitory oxidases (Cos et al., 1998). In addition, free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to biomolecules, a process held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged lipids and proteins (Grune and Davies, 1997). DNA is probably the most biologically significant target of oxidative attack, and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age-related development of the major cancers (Totter, 1980; Ames et al., 1993).

Plant products are widely used in testing because of their low toxicity and great medicinal value. Much research has concentrated on different plant extracts’ abilities to induce antioxidant effects (Newman et al., 2000; Jeon et al., 2006). In the course of our screening program for antioxidant activity of various Korean traditional teas, we found that water extract of commercial dried Acanthopanax senticosus for tea has an antioxidative effect.

A. senticosus, also called the “Siberian Ginseng” or “Eleutherococcus senticosus”, has been used for rheumatism and prophylaxis to various diseases such as chronic bronchitis, hypertension and ischemia (Yi et al., 2001). A. senticosus is also known to be effective for reducing many kinds of stress (Gaffney et al., 2001) or fatigue (Dowling et al., 1996), and symptoms associated with allergic conditions (Yi et al., 2002), inflammation (Jung et al., 2003), and cancer (Hibasami et al., 2000). Today this oriental herb is called “adaptogen” in the US (Davydov and Krikorian, 2000). The major active components of A. senticosus are acanthoside, eleutheroside, chiisanoside, senticoside, triterpenic saponin, syringin, flavone, vitamin, minerals, β-sitosterol, sesamine and savinine (Davydov and Krikorian, 2000; Lee et al., 2004). Each chemical compound is known to produce diverse biological activiti-
es. In Korea, the extract of the A. senticosus plant is used as a component in traditional herbal Korean medicine, and is available as a functional beverage commercially marketed for reducing liver damage and accelerating alcohol detoxification. There are, however, no reports on the effect of the commercial dried A. senticosus for tea on antioxidative activity. Therefore, this work was aimed to evaluate the effect of water extract of dried A. senticosus for tea on antioxidant capacity in vitro and also to investigate the protective effect against oxidative DNA damage.

MATERIALS AND METHODS

Materials

Commercial dried Acanthopanax senticosus for tea were supplied from Geolim Co. (Masan, Korea). Tannic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH), Histopaque 1077, fetal calf serum, low melting point agaroses, Triton X-100, disodium salt ethylenediaminetetraacetic acid, Tris-buffer, sodium chloride, sodium hydroxide, ethidium bromide, potassium chloride, potassium phosphate and sodium hydrogen carbonate were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Folin-Ciocalteu reagent from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Preparation of water extracts of A. senticosus

A. senticosus (0.5 g) was extracted for 3 h at 70°C with 50 ml of distilled water. Then the extracts were centrifuged at 1,000 × g for 15 min, and the supernatants were filtered through a Whatman No.1 filter paper. The water extract of A. senticosus was named as ASE

Total phenolic contents (TPC)

The TPC of the ASE were determined using the method of Gutfinger (Gutfinger, 1981). ASE (1 ml) was mixed with 1 ml of the 50% Folin-Ciocalteu reagent and 1 ml of 2% Na2CO3, centrifuged at 13, 400 × g for 5 min, and the absorbance was measured with a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) at 750 nm after 30 min incubation at room temperature. TPC were expressed as tannic acid equivalents.

DPPH radical scavenging activity

The DPPH radical scavenging activity of the ASE was estimated according to the method of Blois (Blois, 1958). After mixing 0.1 ml of ASE with 0.9 ml of 0.041 mM DPPH in ethanol for 10 min, the absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as percent inhibition and was calculated using the following formula:

% DPPH radical scavenging activity = \[(1 - \text{sample OD/control OD}) \times 100\]

Preparation of human leukocytes

Blood samples were obtained from three healthy male volunteers. 5 ml of fresh whole blood was added to 5 ml of phosphorous buffered saline (PBS) and layered onto 5 ml of Histopaque 1077. After centrifugation for 30 min at 400 × g at room temperature, the leukocytes were collected from the just above the boundary with the Histopaque 1077, washed with 5 ml PBS. Finally, they were
freshly used for comet assay or resuspended in freezing medium (90% fetal calf serum, 10% demethyl sulfoxide) at 6 × 10⁶ cells/ml. The cells were frozen to -80°C using a Nalgene Cryo 1°C freezing container (Nalgene, Rochester, NY) and stored in liquid nitrogen. The cell was thawed rapidly prior to each experiment in a water bath at 37°C.

Treatment of human leukocytes

Cells were incubated with ASE in three different treatments:

(1) Leukocytes were incubated with various concentrations of ASE (0, 0.125, 0.25, 0.5, 1%) for 30 min at 37°C in a dark incubator and then were resuspended in PBS with 200 μM H₂O₂ for 5 min on ice.
(2) Leukocytes were incubated simultaneously with 200 μM H₂O₂ and ASE. After each treatment, samples were centrifuged at 250 × g for 5 min and washed with PBS.
(3) Leukocytes were damaged oxidatively with 200 μM H₂O₂ for 5 min on ice and then incubated with ASE (0, 0.125, 0.25, 0.5, 1%) for 30 min at 37°C.

All the experiments were repeated twice with leukocytes from each of two donors on the separate day. PBS without oxidative stimulus was treated for negative control.

Determination of DNA damage (Comet assay)

The alkaline comet assay was conducted according to Singh et al. (1988) with little modification. The cell suspension was mixed with 75 μl of 0.5% low melting agarose (LMA), and added to the slides precoated with 1.0% normal melting agarose. After solidification of the agarose, slides were covered with another 75 μl of 0.5% LMA, and then immersed in lysis solution (2.5 M NaCl, 10 mM EDTA, 10 mM Tris, and 1% sodium laurylasarcosine; 1% Triton X-100 and 10% DMSO) for 1 h at 4°C. The slides were next placed into an electrophoresis tank containing 300 mM NaOH and 10 mM Na₂EDTA (pH 13.0) for 40 min for DNA unwinding. For electrophoresis of the DNA, an electric current of 25 V/300 mA was applied for 20 min at 4°C. The slides were washed three times with a neutralizing buffer (0.4 M Tris, pH 7.5) for 5 min at 4°C, and then treated with ethanol for another 5 min before staining with 50 μl of ethidium bromide (20 μg/ml). Measurements were made by image analysis (Kinetic Imaging, Komet 5.0, U.K) and fluorescence microscope (LEICA DMLB, Germany), determining the percentage of fluorescence in the tail (tail intensity, TI; 50 cells from each of two replicate slides). Cell viability measured by trypan blue exclusion test was above 95% for all treatments.

Statistical analysis

Experiments for TPC and DPPH RSA measurements were done in triplicate, and analysis of variance was conducted by the procedure of General Linear Model using SAS software. Student-Newman-Keul’s multiple range tests were used to compare the significant differences of the mean values among treatments (P < 0.05). The data for Comet assay are the means of three determinations and were analyzed using the SPSS package for Windows (Version 11.5). The mean values of the DNA damage (tail intensity) from each treatment were compared using one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test. P-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

TPC and DPPH RSA of water extracts of A. senticosus

The fact that phytochemicals occurring in food and natu-
Table 1. Total phenol contents (TPC) and DPPH radical scavenging activity (RSA) of water extract from Acanthopanax senticosus.

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<th>TPC (µM)</th>
<th>RSA (%)</th>
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<tr>
<td>Acanthopanax senticosus</td>
<td>366.67</td>
<td>67.67</td>
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Phytochemicals and medicinal plants play a significant role in disease prevention and health promotion has been recognized. Biological activities in herbal and nutraceutical products constitute a myriad of chemical compounds, among which phenolic substances often play a primary or a synergistic function. Phenolic compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also they are stable radical intermediates, which prevent various food ingredients from oxidation (Cuvelier et al., 1992; Maillard et al., 1996).

The TPC in ASE was 366.67 µM (Table 1). A. senticosus used held in groups of 2 to 5, and has purple-brown color. Generally, the chemicals in charge of purple-brown color are Eleutheroside as a lignan component and flavones, a phenolic compounds (Lee et al., 2004).

Radical scavengers were evaluated by their reactivity toward a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH•). Free radicals are produced continuously in cells, either as by-products of metabolism or deliberately as in phagocytosis (Cheeseman and Slater, 1993). The organic compound DPPH is a radical, in which there is an unpaired/odd electron located on one of the nitrogen atoms. The free radical scavenging activity of ASE was investigated by a DPPH radical scavenging assay. The DPPH RSA of ASE was 67.67% (Table 1).

Lee et al. (2004) reported that eleutheroside B, a lignan component, isolated from A. senticosus was found to cause a moderate free radical scavenging effect on DPPH. Furthermore, the studies conducted by Lu and Foo (2000), Kim and Chung (2002), and Siriwardhana et al. (2003) reported higher correlations between DPPH radical scavenging activities and total polyphenolics. Therefore, this result suggested that the commercial dried A. senticosus for tea possess the antioxidative activity.

Protective effect of ASE on oxidative DNA damage in human leukocytes

The comet assay, which measures the breaking of the DNA strand at the level of single cells, is very easily applied to leukocytes and therefore lends itself to human bio-monitoring studies. It has become a standard technology for the measurement of oxidative DNA damage both in vitro and in vivo (Kassie et al., 2000). Hydrogen peroxide is believed to cause DNA strand breakage by generating the hydroxyl radical (OH•) close to the DNA molecule, via the Fenton reaction (Diplock, 1991).

The genotoxic effects of H₂O₂ and the protective ability of ASE were assessed in normal human leukocytes by comet assay. Pretreatment of the cells for 30 min with ASE significantly reduced the genotoxicity of hydrogen peroxide measured as DNA strand breaks (Figure 1A). The protective effect of ASE increased as its concentration increased from 0.125 to 1% by 65 to 81% in ASE of H₂O₂ treated positive control. Especially, the highest concentration (1%) was shown that there was no statistical difference compared to PBS-treated negative control. The antigenotoxic effect of ASE was the case when the
Figure 1. Effects of supplementation in vitro with different concentration of ASE on 200 μM H2O2-induced DNA damage in isolated human leukocytes. Leukocytes were incubated with various concentrations of ASE (0, 0.125, 0.25, 0.5, and 1%) for 30 min at 37°C in a dark incubator and then were resuspended in PBS with 200 μM H2O2 for 5 min on ice (A). Leukocytes were incubated simultaneously with 200 μM H2O2 and ASE (B). Leukocytes were damaged oxidatively with 200 μM H2O2 for 5 min on ice and then incubated with ASE for 30 min at 37°C (C). NC, PBS-treated negative control; Values are expressed as mean ± standard error of duplicate experiments with leukocytes from each of two different donors. Values not sharing the same letter are significantly different from one another ($P < 0.05$).
ASE and H$_2$O$_2$ were treated simultaneously on ice for 5 min (Figure 1B).

The possible mechanism by which ASE inhibited oxidative DNA damage in human leukocytes can be ascribed to the chemical structure of the phenolic compound contained in *A. senticosus*, such as isofraxidin, eleutherosides B and E from the stem barks (Nishibe et al., 1990), eleutheroside E2 and isomaltol 3-O-α-D-glucopyranoside from the roots (Li et al., 2001), and chiisanoside, chiisanogenin and hyperin form the leaves (Lee et al., 2003) and protocatechuic acid, syringin, chlorogenic acid, caffeic acid, liriodendrin, and isofraxidin in ethanol extract of whole *A. senticosus* (Li et al., 2006). These phenolic compounds in *A. senticosus* may work by providing hydrogen atoms from their phenolic hydroxyl groups to scavenge hydroxyl radical generated from hydrogen peroxide (Croft, 1998) and thus protect leukocytic DNA from damage induced by H$_2$O$_2$. The protective effect of procatechuic acid, chlorogenic acid, caffeic acid on oxidative DNA damage measured by comet assay has been demonstrated in several *in vitro* and *in vivo* studies (Szeto and Benzie, 2002; Guglielmi et al., 2003; Schaefer et al., 2006).

When human leukocytes were post-incubated with ASE for 30 min after exposure to hydrogen peroxide, the protective ability of the ASE was not changed (Figure 1C). The damaged DNA by ROS can be repaired by DNA repair pathway and it is clear that individual variations in repair capability would have a bearing on cancer risk (Collins and Horvathova, 2000; Torbergsen and Collins, 2000). Collins et al. (1995) reported that when fresh isolated human leukocytes were incubated after hydrogen peroxide treatment, repair of strand breaks appears to be unusually slow. In the present study, however, hydrogen peroxide induced DNA damage in human leukocytes was effectively repaired to almost similar level as PBS treated negative control by post-treatment of ASE for 30 min. Although the exact mechanism for DNA repair activity needs to be elucidated, the ASE may contribute to stimulation of DNA repair.

These results indicate that *A. senticosus* supplementation by tea to human leukocytes could inhibit H$_2$O$_2$ induced damage to cellular DNA, supporting a protective effect of *A. senticosus* against oxidative damage.

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**REFERENCES**


