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Antioxidant Activity of Extracts from *Akebia quinata* Decne

– Research Note –

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Abstract

Antioxidant activity of *Akebia quinata* Decne was evaluated. Water extract (0.5 g/ 50 mL) of flowers and leaves of *A. quinata* were prepared and total phenol contents and radical scavenging activity of the extracts was determined for antioxidant activity. The total phenol contents of extracts from *A. quinata* flowers (FAQ) and leaves (LAQ) were 30.05 μM and 20.23 μM, while the radical scavenging activity of FAQ and LAQ were 60.51% and 52.97%, respectively. In addition, the effect of FAQ and LAQ extract on DNA damage induced by H_2O_2 in human lymphocytes was evaluated by comet assay. The FAQ and LAQ showed strong inhibitory effect against DNA damage induced by 200 μM of H_2O_2. These results suggest that water extracts of *A. quinata* Decne flowers and leaves showed significant ($p<0.05$) antioxidant activity and protective effect against oxidative DNA damage.

Key words: *Akebia quinata* Decne, antioxidant, total phenol contents, DPPH, DNA damage, comet assay

INTRODUCTION

Akebia quinata Decne (Korean name: *eu-rum*) is a twining vine, which widely inhabits in temperate eastern Asia. The genus *Akebia* was originated from the Japanese name from this plant, and *quinata* refers to the palmately compound leaf, composed of five obovate to broadly elliptic leaflets. *A. quinata*, especially stem (Korean name: *mok-tong*), has been used as one of important materials for oriental medicine (1,2). *Akebia* has separate male and female flowers on the same plant. An inflorescence has a few female flowers positioned beneath a raceme (unbranched stalk) of male flowers. While *A. quinata* has been traditionally used as an antiphlogistic, a diuretic, and an analgesic drug (3,4), the flowers and leaves of this plant were also used as materials for Korean traditional tea.

Antioxidants can protect peroxidaiton of biological active components. Although synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytolune have been widely used in foods, the use of these synthetic antioxidants is discouraged because of their toxicity (5,6) and carcinogenicity (7,8). A few natural antioxidants have attracted special interest because they can protect human body from free radicals, which may cause various diseases, carcinogenesis, and aging (9).

Natural antioxidants such as flavonoids, tannins, coumarins, curcuminoids, xanthons, phenolic and terpenoids are found in various plant products such as fruits, leaves, seeds, and oils (10), and some of these are as effective as synthetic antioxidants in model systems (11-13). *A. quinata* is a good source of saponins and oleanane disaccharides, which are involved in anti-nociceptove, anti-inflammatory (3), and anticancer activity (4). In this study, the antioxidative activity of flowers and leaves of *A. quinata* used as Korean traditional tea was evaluated.

MATERIALS AND METHODS

Materials

Commercial dried flowers and leaves of *A. quinata* Decne 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 phosphate 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. quinata*

Flowers or leaves of *A. quinata* (0.5 g) was extracted for 3 hours at 70°C with 50 mL of distilled water. Then, the extracts were centrifuged at 1,000×*g* for 15 min, and

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the supernatants were filtered through a Whatman No.1 filter paper. The water extract of flowers and leaves of *A. quinata* were named as FAQ and LAQ, respectively.

Total phenolic contents (TPC)

The TPC of the extracts were determined using the method of Gutfinger (14). FAQ or LAQ (1 mL) was mixed with 1 mL of the 50% Folin-Ciocalteu reagent and 1 mL of 2% Na_2CO_3, 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 extracts was estimated according to the method of Blois (15). After mixing 0.1 mL of FAQ or LAQ 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:

$$\% \text{ DPPH radical scavenging activity} = \left(1 - \frac{\text{Sample OD}}{\text{Control OD}}\right) \times 100$$

Preparation of human lymphocytes

Blood samples were obtained from two healthy male volunteers (non-smokers, 24 and 25 years old, respectively). Five mL of the 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 lymphocytes 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 6×10^6 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 cells were thawed rapidly prior to each experiment in a water bath at 37°C.

Treatment of A. *quinata* Decne extracts on human lymphocytes

Lymphocytes (2×10^4 cell/mL) were incubated with FAQ or LAQ dissolved in PBS and diluted into concentrations 0, 0.25, 0.5, 1 and 2% for 30 min at 37°C in a dark incubator. For oxidative stimulus they were then resuspended in PBS with 200 μM H_2O_2 for 5 min on ice. After each treatment, samples were centrifuged at 1,450 rpm for 5 min and washed with PBS. All the experiments were repeated twice with lymphocytes from each of two donors on the separate day.

Determination of DNA damage (comet assay)

The alkaline comet assay was conducted according to Singh et al. (16) with a 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, 100 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 Na_2EDTA (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 minutes before staining with 50 μL of ethidium bromide (20 μg/mL). Measurements were made by image analysis (Kinetic Imaging, Komet 5.0, UK) 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 (17). 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 was 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. The p-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

TPC and DPPH RSA of water extracts of A. *quinata* Decne

The fact that phytochemicals occurring in food and natural health products play a significant role in disease prevention and health promotion has been recognized. Bioactivities 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

Table 1. Total phenol contents (TPC) and DPPH radical scavenging activity (RSA) of water extract from flowers (FAQ) or leaves (LAQ) of *Akebia quinata*

	TPC (μM)	RSA (%)
FAQ	30.05[a]	60.51[a]
LAQ	20.23[b]	52.97[b]
SEM	0.298	0.113

SEM: Standard error of the means. Different letters (a,b) within a column indicate significantly different ($p<0.05$), n=3.

hydrogen or electrons but also they are stable radical intermediates which prevent various food ingredients from oxidation (18,19).

The TPC in FAQ and LAQ were 30.05 μM and 20.23 μM, respectively (Table 1). Flowers of *A. quinata* used were held in groups of 2 to 5, and has purple-brown color. Generally, the chemicals in charge of purple-brown color are anthocyanins, one of phenolic compounds. These support that there is higher TPC in FAQ than in LAQ.

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 (20). 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 *A. quinata* extracts was investigated by a DPPH radical scavenging assay. The DPPH RSA of FAQ and LAQ were 60.51% and 52.97%, respectively (Table 1). The DPPH radical scavenging activity of FAQ was higher than that of LAQ, and it coincides with the TPC results. The studies conducted by Lu and Foo (21), Kim and Chung (22), and Siriwardhana et al. (23) reported higher correlations between DPPH radical scavenging activities and total polyphenolics.

Protective effect of water extracts of *A. quinata* Decne on oxidative DNA damage in human lymphocytes

The comet assay, which measures the breaking of the DNA strand at the level of single cells, is very easily applied to lymphocytes 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* (24). Hydrogen peroxide is believed to cause DNA strand breakage by generation of the hydroxyl radical (OH·) close to the DNA molecule, via the Fenton reaction (25).

The genotoxic effects of H_2O_2 and the protective ability of FAQ or LAQ were assessed in normal human lymphocytes by comet assay. The concentrations of FAQ or LAQ used (0~2%) were non-toxic, that is, had no effect on DNA strand breakage (Fig. 1A). Pretreatment of the cells for 30 min with *A. quinata* Decne significantly reduced the genotoxicity of hydrogen peroxide measured as DNA strand breaks (Fig. 1B). All the tested concentration of *A. quinata* Decne showed strong inhibitory activities more than 70% which was similar to PBS-treated negative control either in FAQ and LAQ. In lower concentration (0.25 and 0.5%), there were no statistical different between FAQ and LAQ, while LAQ showed significantly higher inhibitory activities than FAQ at higher concentration (1 and 2%). Although the inhibitory activities of FAQ decreased at the higher concentration compared to the lower concentration of FAQ or LAQ, they showed still higher inhibitory activities more than 70%.

The possible mechanism by which *A. quinata* Decne extract inhibited oxidative DNA damage in human lymphocytes can be ascribed to the chemical structure of the phenolic compound contained in *A. quinata* Decne. The phenolic compound in *A. quinata* Decne may work by providing hydrogen atoms from their phenolic hydroxyl groups to scavenge hydroxyl radical generated

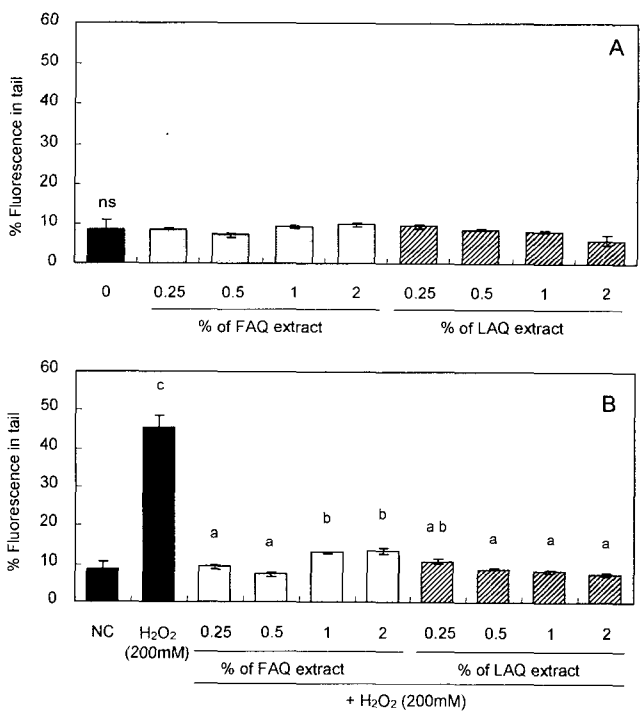

Fig. 1. The effect of supplementation *in vitro* with different concentration of *Akebia quinata* Decne extract on 200 μM H_2O_2-induced DNA damage in isolated human lymphocytes. Human lymphocytes were preincubated for 30 min with flowers (FAQ) or leaves (LAQ) of *A. quinata* Decne (A) and then exposed to H_2O_2 (B). NC: PBS treated normal control, H_2O_2 (200 μM): H_2O_2 treated positive control. Values are mean with standard error of triplicate experiments with lymphocytes from each of two different donors. ns: not significant. Bars not sharing the same letter are significantly different from one another ($p<0.05$), n=3.

from hydrogen peroxide. Although the main phenolic compound contained in *A. quinata* Decne need to be analyzed, many other phenolic phytochemicals, such as quercetin, myricetin and epigallocatechin gallate, work as antioxidants in this manner (26,27). Based on its higher total phenolic contents and better DPPH scavenging capability, FAQ would have been expected to be superior to LAQ in inhibiting oxidative DNA damage in human lymphocytes, but this was not the case. Despite being lower concentration of total phenol and weaker than FAQ in scavenging DPPH, LAQ was slightly higher inhibiting cellular DNA damage induced by H_2O_2 at the concentrations of 1 and 2%. This may explain that LAQ could contain higher concentration of another possible antioxidants, such as ascorbic acid, other than phenolic compounds compared to FAQ, which should be verified in the further study.

CONCLUSION

The water extract of flowers and leaves of *Akebia quinata*, names as FAQ and LAQ, respectively, showed strong DPPH radical scavenging activity, and inhibited hydrogen peroxide induced damage to cellular DNA in human lymphocytes, supporting protective effect against oxidative damage. These results indicated that Korean traditional teas made with *A. quinata* possessed antioxidant activity and can be a candidate for health foods.

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