

Antioxidant Activity of *Rubus coreanus* Fruit Extract: In Comparison to Green Tea Extract

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This study aimed to evaluate the antioxidant activity of Korean raspberry (*Rubus coreanus* Miq.) fruits, which are grown widely in the southwest area of Korea. Freeze-dried *Rubus coreanus* (RC) fruits and green tea (GT) were used to obtain water extracts (RCE and GTE, respectively). The total phenolic contents of the dried RC and GT were 126 $\mu\text{mol CE}$ (catechin equivalent)/g and 360 $\mu\text{mol CE/g}$, respectively. The free radical scavenging activity of RCE and GTE was expressed as SC_{50} (50% scavenging capacity) and compared. The scavenging activity of RC toward DPPH, superoxide, and hydroxyl radicals was lower than that of GT. The inhibitory activity of RCE and GTE on AAPH-induced lipid peroxidation was also analyzed. In contrast with the free radical scavenging activities, the lipid peroxidation-inhibiting activity of RC was superior to that of GT. These results suggest that RCE contains effective scavengers of peroxy radicals. In summary, RC contains less phenolics than does GT. Although RC has lower activity for scavenging DPPH, superoxide, and hydroxyl radicals than does GT, its activity to inhibit peroxy radical-lipid peroxidation was higher. These results suggest that RC could be used as a valuable natural source of antioxidants.

Key Words: *Rubus coreanus*; Antioxidant activity; Free radicals

Introduction

The flowers, leaves, fruits, and roots of plants contain numerous antioxidants, such as carotenoids, thiols, vitamins, flavonoids, and phenolic compounds. It has been reported that fruits and vegetables contain many of these antioxidants and that ingestion of them prevents or decreases the death rate from cancer and cardiac disorder by reducing oxidative stress.¹⁻⁵ Further

health effects provided by their ingestion include decreased blood pressure, higher immunity, detoxified pollutants or contaminants, and suppressed inflammation.^{6,7} The chemical composition of antioxidants from plants mainly includes phenol compounds, such as flavonoids like anthocyanin.⁸

Flavonoids have various biological functions other than antioxidant activity, such as anti-cancer, anti-bacterial, anti-viral, and anti-inflammatory actions; enzyme inhibition; and alteration of the immune system.⁹⁻¹¹

Based upon their antioxidant activity, flavonoids were recently shown to actively prevent and treat various diseases such as cancer, ischemic injury of the heart

Received: August 12, 2010, Accepted for Publication: October 25, 2010

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and brain, and AIDS.¹²⁻¹⁵ After mice were fed extracts of *Physalis angulata* flower and *Rosa hybrida* flower for 3 weeks, the activities of superoxide dismutase and catalase were increased and lipid peroxidation was inhibited in blood serum. Specifically, the concentration of HDL cholesterol was increased in the blood serum of mice fed extract of *Rosa hybrida* flower.¹⁶ Therefore, specific methods of how to analyze antioxidants and measure their activities in various plants have been studied.

Rubus coreanus Miq. (RC), a deciduous broadleaf shrub belonging to the family *Rosaceae*, is limited in growth to the far-east Asian countries (South Korea, China, and Japan).¹⁷ Until now, the biological function of constituents of *Rubus*, such as RC, were studied solely for their antioxidant and anti-inflammatory activities.¹⁸⁻²¹ In addition, anti-osteoporosis, anti-dementia, anti-stomach disorder, anti-rheumatism, and anti-naphylaxis activities of RC have been reported.^{20,22-24} However, the use of RC as a functional health food is usually based on older oriental medical literature and therefore is not proved scientifically.

Accordingly, this study aimed to investigate the antioxidant activity and reactive oxygen species (ROS)-scavenging activity of RC as compared with green tea (GT).

Materials and Methods

1. Chemicals

FCR (Folin-Ciocalteu Reagent), catechin, DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbate, EGCG ((-)-epigallocatechin gallate), PC (L- α -phosphatidylcholine), AAPH (3,2-azobis (2-amidinopropane) dihydrochloride), PMS (phenazine methosulfate), NBT (nitroblue tetrazolium), TBA (thiobarbituric acid), TCA (trichloroacetic acid), and MDA (malondialdehyde) were purchased from Sigma (St. Louis, MO, USA). CCA (coumarin-3-carboxylic acid) was purchased from Fluka (Buchs,

Switzerland). High-grade GT was purchased at a store.

2. Preparation of water extracts from *Rubus coreanus* fruits and green tea

RC fruits were collected from Gochang, Korea. Aqueous extracts of RC and GT were prepared by the following method. RC fruits were frozen overnight at -80°C and then dried by using a lyophilizer (Hanil Science Industrial Co., Incheon, Korea). Dried fruits (5 g) and dried green tea (5 g, Hankook Tea Co., Gwangju, Korea) were homogenized in distilled water (200 mL) for 5 min by use of a Polytron homogenizer (model T-25B, IKA Korea Co, Anyang, Korea). The homogenates were shaken for 20 min at 80°C in a water bath and centrifuged at 20,000 rpm for 20 min at 4°C . Supernatants were individually collected and frozen overnight at -80°C , followed by drying with the lyophilizer. The weight of the lyophilized water extracts, RCE and GTE, were about 1.85 g and 2.00 g, respectively. RCE and GTE were dissolved in distilled water at a concentration of 10 mg/mL and stored at 4°C .

3. Quantitation of phenol groups

The phenol content of RCE and GTE was measured with FCR.²⁵ Aliquots of diluted RCE (200 μL) and GTE (200 μL) were mixed with 10% FCR (1 mL) in test tubes. The mixture was left to react for 3 min at room temperature, followed by the addition of 7.5% Na_2CO_3 (0.8 mL) and incubation for 2 h in a shaking water bath. After incubation, the absorbance of the mixture was measured at 760 nm by use of a UV-spectrophotometer (model UV-1650PC, Shimadzu, Japan). The phenolic content was quantified from the standard curve plotted with authentic catechin and expressed as μM CE (catechin equivalent) or mmol CE/g (dry weight).

4. Preparation of liposomes

Liposomes were prepared by following the method of Motoyama *et al.*²⁶ PC (20 mg) was dissolved in a mixture (2 mL) of chloroform and methanol (2 : 1).

The PC solution was dried in a rotary evaporator (Eyela N-N Series, Tokyo Rikakikai Co., Tokyo, Japan) under nitrogen purging. The lipid membrane was stripped by shaking with 20 mL of phosphate buffer solution (50 mM potassium phosphate/0.1 M KCl, pH 7.4). Liposomes were made by ultrasonication (20 kHz) for 15 s at 4°C under nitrogen purging. The amount of phospholipid in each liposome was evaluated by measuring the Pi (phosphorus) concentration according to the Ames method.²⁷

5. DPPH radical-scavenging activity

The antioxidant activity of RCE and GTE was analyzed by measuring the scavenging of DPPH radicals.²⁸ A mixture containing 250 μ L of 0.004% DPPH dissolved in ethanol and diluted with distilled water was mixed with 20 μ L of prediluted CFE in a 96-well plate and incubated at 37°C for 30 min. Scavenging of DPPH radicals by the antioxidant was analyzed by measuring the decrease in the absorbance at 517 nm with a microplate reader (model μ Quant, Bio-Tek Instruments, USA). The DPPH-scavenging capacity of each sample was compared to ascorbic acid and EGCG, as positive controls. Antioxidant activity was expressed as the concentration of each sample that decreased the radicals by half (SC₅₀, 50% scavenging capacity).

6. Superoxide radical-scavenging activity

Superoxide radicals (O_2^-) generated by autooxidation of PMS were quantified by the NBT reduction method.²⁹ The reaction mixture (total volume, 1.0 mL) containing 50 mM potassium phosphate (pH 7.4) buffer, 0.5 mM PMS, 0.15 mg Pi/ml PC liposomes, 0.1 mM NBT, and various concentrations of either EGCG, GTE, or CFE was incubated in a shaking water bath at 37°C for 1 h. The supernatant obtained after centrifugation was analyzed for a decrease in absorbance at 560 nm with a spectrophotometer (model UV-1650PC, Shimadzu, Japan).

7. Hydroxyl radical-scavenging activity

Hydroxyl radicals ($\cdot OH$) generated by Fenton reaction

of ascorbate and Cu (II) ions were quantified by 7-OHCCA (7-hydroxycoumarin-3-carboxylic acid) generation from CCA.³⁰ The reaction mixture (total volume, 250 μ L) containing 20 mM potassium phosphate (pH 7.4) buffer, 500 μ M ascorbate, 50 μ M CuCl₂, 0.1 mM CCA and various concentrations of either EGCG, GTE, or RCE was added to a microplate and incubated in a dark place at room temperature for 1 h. The reaction was stopped by the addition of 10 mM Tris base buffer (pH 9.0). The amount of generated 7-OHCCA was analyzed by a fluorescence microplate reader (Fluoroskan Ascent FL Type 374, Labsystems, Finland). The excitation wavelength was 380 nm and the emission wavelength was 460 nm.

8. Lipid peroxidation-inhibition capacity

Lipid peroxidation was measured by using the modified TBA assay method of Buege and Aust.³¹ Lipid peroxidation was induced by peroxyl radicals derived from AAPH, and inhibition mediated by EGCG, GTE, or RCE in PC liposomes was investigated. To induce lipid peroxidation, PC liposomes (0.3 mg/mL) and 500 μ M AAPH were added to 50 mM potassium phosphate buffer (pH 7.4). This reaction mixture (total volume: 1.0 mL) was treated with EGCG, GTE, or RCE and then incubated with shaking for 2 h at 37°C. To stop the reaction after incubation, each reaction mixture was maintained in ice. Two milliliters of TBA mixture (0.375% (w/v) TBA, 15% (w/v) TCA, and 0.25 N HCl) was mixed with the reaction by vortexing, followed by heating for 15 min at 100°C. The heated reaction mixture was allowed to cool in tap water and was then centrifuged at 2,500 rpm for 10 min. Absorbance of the supernatant was read at 535 nm with a spectrophotometer (model UV-1650PC, Shimadzu, Japan). The degree of LPO was expressed as the amount of MDA produced per unit amount of Pi in liposomes (nmol/ μ mol Pi) and was calculated by using the molecular extinction coefficient ($E_M = 1.56 \times 10^5$) of MDA.

9. Statistical analysis

Statistical differences between groups were calculated

by the one-way ANOVA test and Dunnett-t test. All values are presented as the mean \pm standard deviation.

Results

1. Quantification of phenol groups

About 1.85 g of RCE was prepared from 5 g of dried RC fruits and was dissolved in distilled water to a concentration of 10 mg/mL. The amount of phenol groups in the stock solution was quantified from the standard curve plotted previously with authentic catechin and was expressed as the concentration of CE phenol groups (Table 1). The phenol content of dried RC was 126 μ mol (36.5 mg) CE/g. The phenol content of RCE was 340 μ mol (98.6 mg) CE/g. Therefore, 1 mmol of CE phenol groups was contained in approximately 7.94 g of

dried RC or about 2.94 g of RCE, and 1 g of CE phenol groups was contained in approximately 27.4 g of dried RC or about 10.1 g of RCE. The phenol content of dried GT and GTE was measured by the same methods and were found to be 360 μ mol CE/g and 890 μ mol CE/g, respectively. An analysis of the phenol group content represented as GAE (gallic acid equivalents) from a gallic acid standard curve found the phenol content of RC to be 33.4 mg GAE/g and that of GT to be 95.4 mg GAE/g (data not shown). These results show that the content of phenol groups was less in RC than in GT, producing a ratio of about 1/2.9~1/2.6.

2. DPPH radical-scavenging activity

The scavenging of DPPH radicals by RCE was compared with that of GTE, as well as some well-known antioxidants such as ascorbic acid and EGCG (Fig. 1). Antioxidant activity was expressed as the concentration of antioxidant that scavenged free radicals by 50% (SC_{50}). The SC_{50} of ascorbic acid, EGCG, GTE, and RCE was 30.16 μ M (5.31 μ g/mL), 6.52 μ M (2.99 μ g/mL), 15.93 μ M CE (44.29 μ g dry GT/mL), and 17.68 μ M CE (140.4 μ g dry RC/mL), respectively. Therefore, the relative DPPH-scavenging activities were in the order of ascorbic acid < RCE < GTE < EGCG. The DPPH-scavenging activity of RCE was especially

Table 1. Total content of phenolics in water extract of RC and GT

RCE		GTE		Ratio (RCE/GTE)	
μ mol CE/ g dry RC	μ mol CE/ g RCE	μ mol CE/ g dry GT	μ mol CE/ g GTE	Dried Wt	Extract Wt
126	340	360	890	1/2.9	1/2.6

RCE, water extract of *Rubus coreanus* fruits; GTE, water extract of green tea; CE, catechin equivalent; RC, *Rubus coreanus* fruits; GT, green tea; Wt, weight.

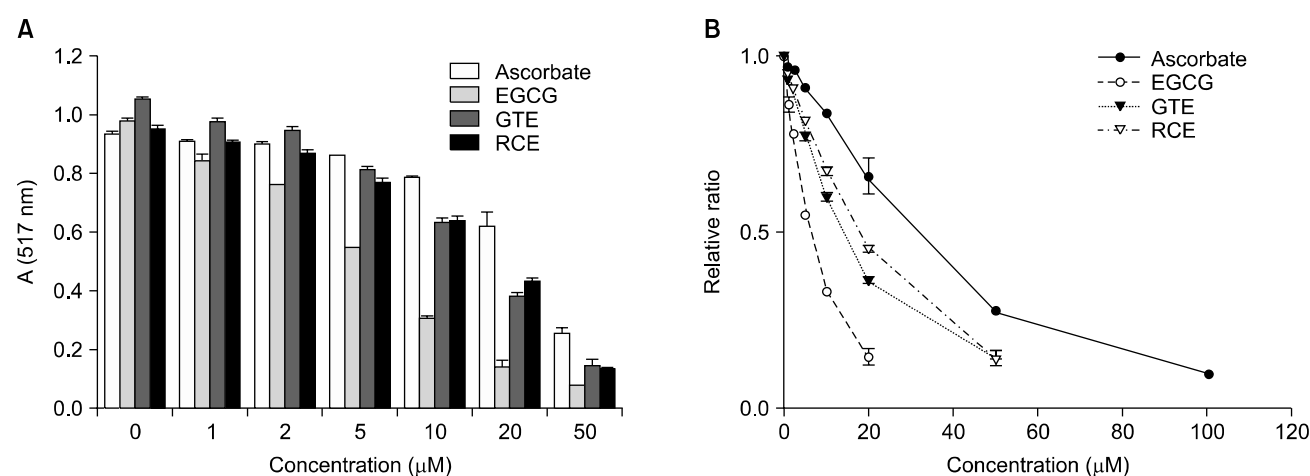


Fig. 1. DPPH radical-scavenging activity (A) and SC_{50} (B) of GTE and RCE. The concentrations of GTE and RCE were expressed as catechin equivalent (μ M CE). Each value is a mean \pm S.D. (n=3). SC_{50} of ascorbate, 30.16 mM CE; SC_{50} of each substance was as follows: EGCG, 6.52 mM CE; GTE, 15.93 mM CE; RCE, 17.68 mM CE. EGCG, (—)-Epigallocatechin gallate; GTE, water extract of green tea; RCE, te water extract of *Rubus coreanus* fruits; SC_{50} , 50% scavenging capacity of total radicals; CE, catechin equivalent.

lower than that of GTE by about 0.9-fold (Table 2). The SC₅₀ of GT and of RC was higher than that of ascorbic acid and of EGCG per dry weight/unit volume. This may be because ascorbic acid and EGCG are single compounds, whereas GT and RC are mixed compounds. Furthermore, the SC₅₀ of RC was 3-fold higher than that of GT (Table 2).

3. Superoxide radical-scavenging activity

Superoxide radicals (O₂⁻) produced by the autooxidation of PMS were scavenged by EGCG, GTE, and RCE. Their scavenging activities were analyzed and compared by measuring the formazan produced in the reduction of NBT (Fig. 2). The SC₅₀ was 29.17 μM (13.37 μg/mL) for EGCG, 59.33 μM CE (164.81 μg

dry GT/mL) for GTE, and 103.06 μM CE (818.30 μg dry RC/mL) for RCE. The superoxide radical-scavenging activity of RCE was lower than that of EGCG and GTE (Table 2).

4. Hydroxyl radical-scavenging activity

Hydroxyl radical-scavenging activities of EGCG, GTE, and RCE were compared with one another (Fig. 3). Radicals were produced by a metal-catalyzed oxidation system composed of ascorbic acid and Cu (II). The SC₅₀ of EGCG, GTE, and RCE was 29.92 μM (13.72 μg/mL), 39.34 μM CE (109.34 μg dry GT/mL), and 35.39 μM CE (280.87 μg dry RC/mL), respectively. As expected, the superoxide radical-scavenging activity of RCE was lower than that of EGCG and GTE (Table 2).

Table 2. Comparison of the antioxidant activities (SC₅₀) of RC and GT

Scavenging parameter	SC ₅₀				SC ₅₀ ratio (RC/GT)	
	RC		GT		Molar ratio	Dry wt ratio
	μ M CE	μ g/mL	μ M CE	μ g/mL		
DPPH radicals	17.68	140.32	15.93	44.29	1.11	3.17
Superoxide radicals	103.06	818.30	59.33	164.81	1.74	4.97
Hydroxyl radicals	35.39	280.87	39.34	109.34	0.90	2.57
Lipid peroxidation	0.52	1.70	0.70	1.94	0.74	0.88

RC, *Rubus coreanus* fruits; GT, green tea; SC₅₀, 50% scavenging capacity of total radicals; DPPH, 2,2-diphenyl-1-picrylhydrazyl; CE, catechin equivalent; wt, weight.

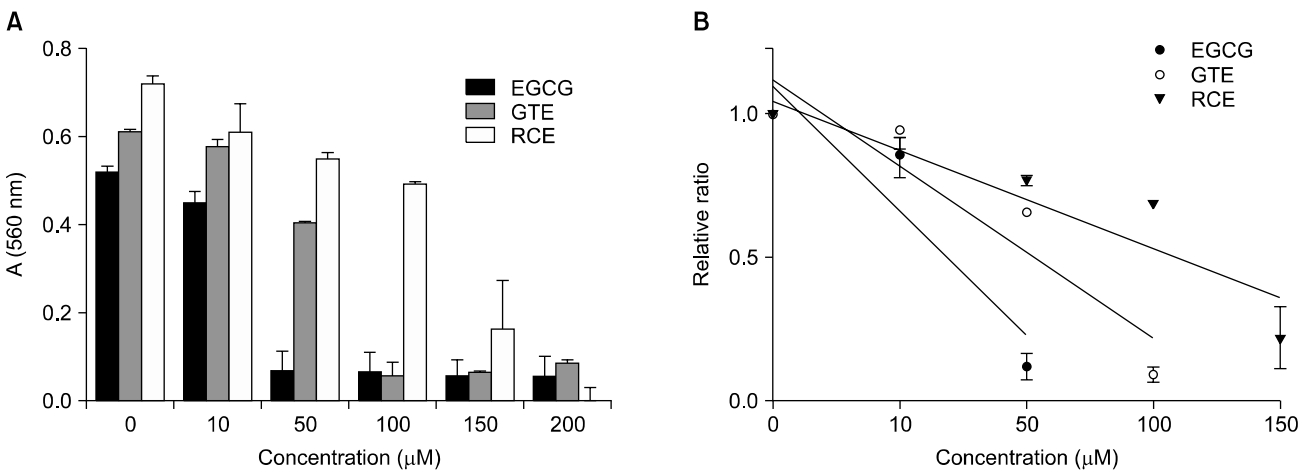


Fig. 2. Superoxide radical-scavenging activity (A) and SC₅₀ (B) of GTE and RCE. Superoxide radicals were generated from the reaction of 0.5 mM PMS and 0.15 mg Pi/mL PC liposomes. The concentrations of GTE and RCE were expressed as catechin equivalent (μM CE). Each value is a mean±S.D. (n=3). SC₅₀ of each substance was as follows: EGCG, 29.17 μM CE; GTE, 59.33 μM CE; RCE, 103.06 μM CE. Abbreviations: EGCG, (–)-Epigallocatechin gallate; GTE, water extract of green tea; RCE, water extract of *Rubus coreanus* fruits; SC₅₀, 50% scavenging capacity of total radicals; CE, catechin equivalent.

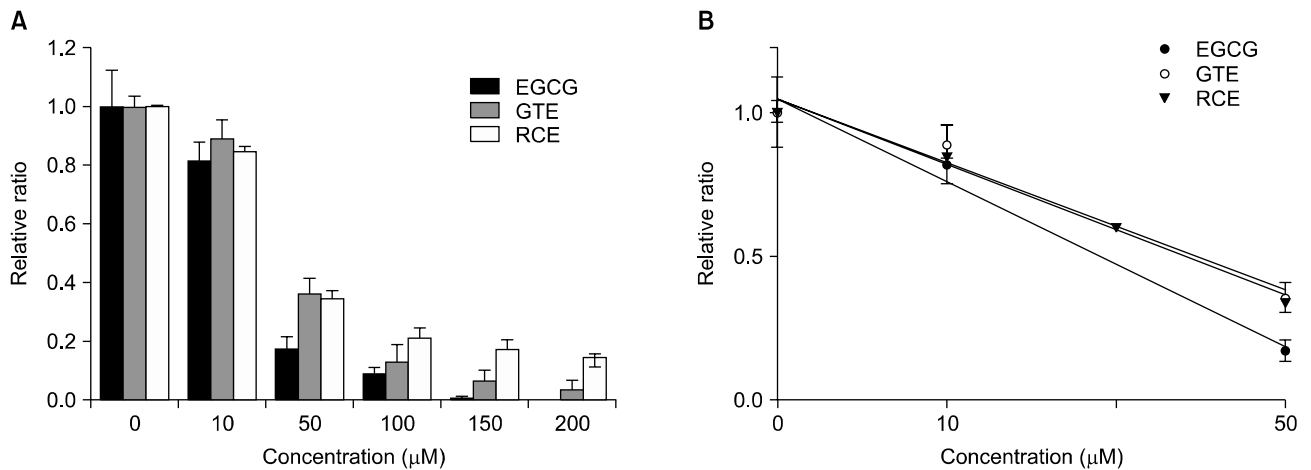


Fig. 3. Hydroxyl radical-scavenging activity (A) and SC₅₀ (B) of RCE and GTE. Hydroxyl radicals were generated by a mixed-function oxidation system containing 0.5 mM ascorbate and 50 μM Cu (II). Concentrations of GTE and RCE were expressed as catechin equivalent (μM CE). Each value is a mean ± S.D. (n=3). SC₅₀ of each substance was as follows: EGCG, 29.92 μM CE; GTE, 39.34 μM CE; RCE, 35.39 μM CE. EGCG, (–)-Epigallocatechin gallate; GTE, water extract of green tea; RCE, water extract of *Rubus coreanus* fruits; SC₅₀, 50% scavenging capacity of total radicals; CE, catechin equivalent.

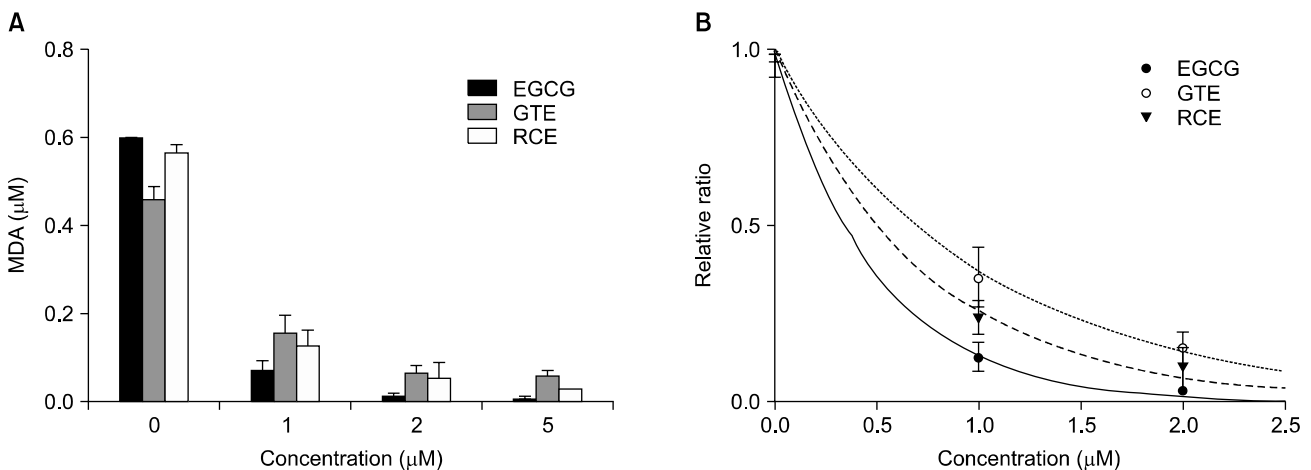


Fig. 4. Lipid peroxidation-inhibiting activity (A) and SC₅₀ (B) of RCE and GTE. Lipid peroxidation of PC liposomes was induced with 500 μM AAPH at 37°C for 1 h. Concentrations of GTE and RCE were expressed as catechin equivalent (μM CE). Each value is a mean ± S.D. (n=3). SC₅₀ of each substance was as follows: EGCG, 0.34 μM CE; GTE, 0.70 μM CE; RCE, 0.52 μM CE. EGCG, (–)-Epigallocatechin gallate; GTE, water extract of green tea; RCE, water extract of *Rubus coreanus* fruits; SC₅₀, 50% scavenging capacity of total radicals; CE, catechin equivalent; MDA, malondialdehyde.

5. Lipid peroxidation-inhibition capacity

Inhibition of lipid peroxidation by EGCG, GTE, or RCE was compared (Fig. 4). Lipid peroxidation was induced with peroxy radicals derived from AAPH and was measured by the TBA assay. The SC₅₀ of EGCG, GTE, and RCE was 0.34 μM (0.16 μg/mL), 0.70 μM CE (1.94 μg dry GT/mL), and 0.52 μM CE (1.70 μg dry RC/mL), respectively. Furthermore, the SC₅₀ values

of the lipid peroxidation-inhibition activity in dry weight per unit volume were compared (Table 2); that of RCE was lower than that of EGCG, but higher than that of GTE.

Discussion

Polyphenol compounds have various physiological

functions such as antioxidant activity. Thousands of these compounds have been identified in plants, and many studies aim to find new polyphenol compounds from plants and elucidate their functions.

This study investigated the antioxidant activity of RCE compared with GTE, which is a well-known antioxidant source. The phenol content of RC (126 $\mu\text{mol CE/g}$) was only about one third that of GT (360 $\mu\text{mol CE/g}$). Other reports have detailed the phenol content of some *Rubus* species fruits from the USA. Wang and Lin¹⁸ reported the phenol contents of water extracts of some berries (that is, blackberry, black raspberry, red raspberry, and strawberry) to be 4.00~21.66 mg GAE/g. When the phenol content is expressed as GAE, that of RC was 33.4 mg GAE/g and that of GT was 95.4 mg GAE/g. These results suggest that the phenol content of RC was higher than that of a similar species from the USA. Wada and Ou¹⁹ reported the phenol content of methanol extracts of various berries (evergreen blackberries, red raspberries, boysenberries, marion berries, and black raspberries) to be 30.94~57.65 mg GAE/g dry weight. These results show that methanol extract contains provably more phenol compounds than water extract. However, we used water extract in the present study because of the obvious relevance of evaluating fruits as a functional food. Wang and Lin¹⁸ also reported that phenol compounds in *Rubus* species are higher in the leaves (47.2~129.2 mg GAE/g) than in fruits. Therefore, further insights could be gained by extracting polyphenol compounds from leaves of RC.

The free radical-scavenging activities of RC, expressed as SC_{50} , were compared with those of GT (Table 2). When antioxidant activities are expressed as dry weight per unit volume, a 2.5~5-fold higher amount of RC than of GT is required to attain a similar SC_{50} when scavenging DPPH, superoxide, or hydroxyl radicals. However, when antioxidant activities are expressed as the molarity of CE, nearly similar amounts of dried extracts of RC and GT were needed to scavenge DPPH (1.11-fold) and hydroxyl radical (0.9-fold), but much

more dried extract of RC than of GT was needed to scavenge superoxide radical (1.74-fold). That is, RC and GT showed nearly similar activities in scavenging DPPH and hydroxyl radicals, but RC showed much less activity than GT in scavenging superoxide radical.

By the way, the lipid peroxidation inhibition by RC was slightly stronger than that by GT when expressed as both the molarity of CE and the dry weight per unit volume. This result means that RC might have better peroxyl radical-scavenging activity than GT because lipid peroxidation was induced by peroxyl radicals. It is quite possible that each antioxidant shows different scavenging activities to various radicals.

These results show that the antioxidant activity of the polyphenols of RC is almost equipotential to that of GT. However, RC itself has a relatively lower content of antioxidant compounds than does GT. Therefore, the amount of RC required may be 2.5~5 fold higher than that of GT in order to produce a similar level of scavenging activity. Last, although the antioxidant activity of *Rubus* species fruits is lower than that of GT, they have an advantage upon ingestion. A daily intake of GT should be limited because of high caffeine content. However, daily intake of *Rubus* species of fruits would have a much lower limit.

Acknowledgments

This work was supported by a research grant from The Research Institute of Medical Sciences, Chonnam National University (2008-CURIMS-DR006).

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