Mol Cell Toxicol (2010) 6:279-285
DOI 10.1007/s13273-010-0038-x

ORIGINAL PAPER

A comparative study of antioxidant activity in some Korean medicinal plant used as food materials

Sun Im Kim[1], **Ki Hyeon Sim**[2] **& Hae-Yeon Choi**[3]

Received: 6 May 2009 / Accepted: 6 June 2010

Abstract The antioxidant properties of ten Korean medicinal plants were analyzed using various antioxidant assays, including their ability to scavenge the DPPH free radical, ABTS radical, superoxide anion, and nitrite, and their reducing powers. The contents of total phenolic compounds and flavonoids were also determined. Of the ten plants, *Ulmus pumila* L. (UP) and *Rubus coreanus* Miq. (RC) possessed strong antioxidant activity for all models tested. The half-maximal inhibitory concentration (IC_{50}) for DPPH free radicals of UP and RC was 4.16 and 4.87 μg/mL, respectively. The IC_{50} for superoxide anion of UP and RC was 145.7 and 136.3 μg/mL, respectively. In addition, the ABTS radical scavenging activity of these materials was higher than that of Trolox. The total phenol content of UP and RC was also significantly higher than in the other herbs. UP had the highest flavonoid content of the ten materials. A correlation was observed between the antioxidant activity and total phenol content.

Keywords *Ulmus pumila* Linne, *Rubus coreanus*, Antioxidant activity, Phenolic, Flavonoid

Recently, the beneficial effects of many foodstuffs and beverages on human health have been recognized to originate from the antioxidant activity of natural substances. Much attention has been focused on dietary natural antioxidants capable of inhibiting lipid peroxidation mediated by reactive oxygen radicals, which is involved in several pathological conditions such as atherosclerosis, cancer, and aging[1,2]. Reactive oxygen species (ROS) are forms of activated oxygen and include free radicals such as superoxide and hydroxyl. ROS cause severe oxidative damage that contributes to significant biological effects, such as carcinogenesis, mutagenesis, and cytotoxicity, as well as causing the deterioration of foods during storage[3]. Oxidative damage caused by free radicals may be related to aging and diseases such as atherosclerosis, cancer, and rheumatoid arthritis. In addition, ROS induce lipid peroxidation, causing the deterioration of foods, which induces the oxidation of lipids and DNA, resulting in membrane damage, increased membrane fluidity, and changes that lead to cancer via DNA mutation[4,5]. The consumption of fruits and vegetables containing antioxidants offers protection against these diseases. Dietary antioxidants can augment cellular defenses and help to prevent oxidative damage to cellular components. Besides playing an important role in physiological systems, antioxidants have been used in the food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats. The addition of synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone has been widely used industrially to control lipid oxidation in food. However, the use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity[6]. Consequently, an extensive search for novel natural antioxidants that scavenge radicals has been undertaken. The findings suggested that supplementation with antioxidants ameliorates the harmful effects of oxidative processes in living organisms.

Phenolic and flavonoid compounds, which are pre-

[1]Nano Bio-resources Center, Sookmyung Women's University, Hyochangwongil 52, Yongsan-gu, Seoul 140-742, Korea
[2]Traditional Dietary Life Food, Sookmyung Women's University, Hyochangwongil 52, Yongsan-gu, Seoul 140-742, Korea
[3]Department of Food and Nutrition, Sookmyung Women's University, Hyochangwongil 52, Yongsan-gu, Seoul 140-742, Korea
Correspondence and requests for materials should be addressed to H. Y. Choi (✉rndfood@paran.com)

sent naturally in vegetables, fruits, grains, and pulses, possess the ability to reduce oxidative damage associated with many diseases, including cancer, cardiovascular disease, immune deficiency disease, and aging[7,8]. The ability of phenolic acids and flavonoids to act as antioxidants has been studied extensively[9]. Flavonoids, tannins, and other phenolic constituents present in foods of plant origin have similar potential, and the phenolic constituents of various plants have potential medicinal properties, including antioxidant activities. Therefore, the study of the importance and role of non-nutrient compounds, particularly phenolic acid and flavonoids, as natural antioxidants and nutraceuticals has increased greatly.

Ulmus pumila L. (UP), *Rubus coreanus* Miq. (RC), *Pinus densiflora* Sieb. et Zucc. (PD), *Carthamus tinctorius* L. (CT), *Artemisia* spp. (AS), *Zingiber officinale* Roscoe (ZO), *Curcuma longa* L. (CL), *Glycine soja* Sieb. et Zucc. (GS), *Glycine max* L. (Merr.) (GM), and *Lithospermum erythrorhizon* Sieb. et Zucc. (LE) are used as food in folk remedies either alone or in combination with other plants. UP occurs widely in Korea and China, and has antimutagenic effects. Consequently, it has long been used extensively in folk medicine in Korea. RC is a perennial shrub that grows in the southern part of the Korean Peninsula; the unripe fruit of RC is used in traditional herbal medicine for treating diabetes mellitus and sexual disinclination[10]. PD is one of the most important forest species in the Republic of Korea and has been used widely for promoting health as folk medicine or as food[11]. AS is effective in women's diseases, and is added to rice cakes and bean paste soup in Korea. CT contains a large amount of linoleic acid and is effective at preventing or treating atherosclerosis. Legumes are an important component of traditional diets in many regions worldwide. They are low in fat and are excellent sources of protein, dietary fiber, and a variety of micronutrients and phytochemicals[12]. The legume GS is called medicine legume in Korea; its seeds are used in foods and as medicinal materials and it contains isoflavone. GM inhibits mutagenicity and aging.

Therefore, this study determined the *in vitro* antioxidant activities of extracts of traditional food materials used in the Republic of Korea. The antioxidant activity was examined using antioxidant assays of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, 2,2-azinobis (3-ethyl benzothiazoline)-6-sulfonic acid (ABTS) radical scavenging, superoxide anion scavenging, nitrite scavenging, and reducing power. Furthermore, the total phenolic and flavonoid contents of the extracts were measured and the correlations with the antioxidant activities were determined. The results of this preliminary study will provide better understanding of the antioxidant properties of these materials and identify materials with high antioxidant activity for further investigation and development as value-added foods and nutraceuticals.

Table 1. Content of total polyphenol compound and flavonoid of Korean traditional food materials.

Materials	Total polyphenol contents (mg GAE/g)	Total flavonoid contents (mg RE/g)
UP	168.3 ± 5.75^{b}	77.1 ± 0.98^{a}
RC	221.0 ± 6.59^{a}	55.6 ± 1.70^{b}
PD	118.0 ± 1.19^{c}	28.2 ± 1.05^{d}
CT	67.19 ± 0.84^{d}	11.5 ± 0.48^{e}
AS	75.1 ± 2.76^{d}	48.2 ± 1.39^{c}
ZO	49.2 ± 2.46^{e}	26.7 ± 1.64^{d}
CL	40.3 ± 2.76^{f}	24.9 ± 0.60^{d}
GS	35.4 ± 3.29^{f}	6.2 ± 0.29^{f}
GM	22.0 ± 0.89^{g}	5.0 ± 0.68^{f}
LE	11.6 ± 0.24^{h}	10.1 ± 0.36^{e}

UP, *Ulmus pumila* Linne; RC, *Rubus coreanus*; PD, *Pinus densiflora* Sieb. and Zucc.; CT, *Carthamus tinctorius*; AS, *Artemisia* spp.; ZO, *Zingiber officinale* Roscoe; CL, *Curcuma Longa* L.; GS, *Glycine soja* Sieb. et Zucc; GM, *Glycine max* L. Mer.; LE, *Lithospermum erythrorhizon*. $^{a-h}$Differences between letters in the same column indicate significant differences ($P < 0.001$).

Total phenol and flavonoid contents of the extracts

Generally, extracts that contain high amounts of phenols exhibit high antioxidant activity, and the total phenol content of the extracts was determined using the Folin-Ciocalteu phenol reagent. As shown in Table 1, RC possessed remarkably high amounts of phenols compared to the other samples. The total phenol content expressed as the mean ± SD in milligrams gallic acid equivalents per gram [(GAE)/g] was in the following order: RC (221 ± 6.6) > UP (168.3 ± 5.8) > AS (75.1 ± 2.8) > CT (67.1 ± 0.8) > GS (35.4 ± 3.3) > GM (22.0 ± 0.8) with all values significantly different at $P < 0.001$. The total flavonoid content was highest in UP at 77.1 ± 0.98 mg rutin equivalents (RE)/g extract. As one of the most diverse and widespread groups of natural compounds, flavonoids are likely the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities, including radical scavenging properties.

The content of phenolic compounds in the extracts was correlated with the DPPH radical scavenging activity and reducing power (r^2=0.9626, 0.9599) and was highest for RC (221 mg GAE/g) and lowest for LE (11.55 mg GAE/g).

DPPH radical scavenging activities

The free radical donor DPPH is used widely to evalu-

Table 2. Antioxidant activity of Korean traditional food materials by radical scavenging assay.

Materials	IC_{50}[1] (μg/mL)		
	DPPH radical	ABTS radical	Superoxide anion radical
UP	4.16±0.24	49.49±1.57	145.69±4.23
RC	4.87±0.18	79.54±2.94	136.34±3.29
PD	23.88±1.13	>1000	226.25±10.67
CT	32.55±2.89	>1000	>1000
AS	44.39±3.32	>1000	>1000
ZO	52.91±3.64	>1000	>1000
CL	83.53±4.47	>1000	>1000
GS	−	>1000	>1000
GM	−	>1000	>1000
LE	−	>1000	>1000
Positive control[2]	2.44±0.04	23.53±0.75	46.51±1.10

[1]Inhibitory activity was expressed as the mean of 50% inhibitory concentration of triplicate determines, obtained by interpolation of concentration-inhibition curve.
[2]Positive controls were used in DPPH assay, ABTS assay and Superoxide anion radical assay were ascorbic acid, trolox and tannic acid, respectively.
UP, *Ulmus pumila* Linne; RC, *Rubus coreanus*; PD, *Pinus densiflora* Sieb. and Zucc.; CT, *Carthamus tinctorius*; AS, *Artemisia* spp.; ZO, *Zingiber officinale* Roscoe; CL, *Curcuma Longa* L.; GS, *Glycine soja* Sieb. et Zucc; GM, *Glycine max* L. Mer.; LE, *Lithospermum erythrorhizon*.

ate the free radical scavenging effects of natural antioxidants. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of the DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. Ascorbic acid was used as a positive control. The extracts were able to reduce the stable radical DPPH to yellow diphenylpicrylhydrazine at various doses. Then, the DPPH radical scavenging activity was compared with the half-maximal inhibitory concentrations (IC_{50}). A smaller IC_{50} indicates greater antioxidant activity.

The scavenging activities of the extracts are shown in Table 2. Of the extracts, UP had the highest DPPH free radical scavenging activity and the lowest IC_{50} at 4.16 μg/mL, followed by RC (4.87 μg/mL) < PD (23 μg/mL), CT (32 μg/mL) < AS (44 μg/mL). The IC_{50} of ascorbic acid was 2.44 μg/mL. Based on this comparison, the extracts of UP and RC have strong antioxidant activity. Materials with a higher phenol content had greater antiradical activity (r^2=0.96262).

$ABTS^+$ scavenging activities

Table 2 shows the ABTS radical cation scavenging activity of the tested extracts. UP had the highest antioxidant activity. The IC_{50} of UP and RC was 49.49 and 79.54 μg/mL, respectively. The IC_{50} of the others exceeded 1 mg/mL. The antioxidant activity of UP (49.49 μg/mL) was similar to that of Trolox (0.094 mM). The Trolox equivalent antioxidant capacity (TEAC) value indicates more potent radical scavenging capacity. Therefore, the antioxidant activity of the extracts was calculated in terms of the TEAC. The TEAC of UP was 2.04 mM/mg, whereas that of GM was 0.021 mM/mg. The antioxidant activity of UP was 100 times greater than that of GM. In addition, the phenolic contents of the extracts were correlated with the $ABTS^+$ scavenging activity (r^2=0.7484), and was highest for UP (168 mg GAE/g) and lowest for GM (26.97 mg GAE/g).

Superoxide anion radical scavenging activity

A superoxide anion is a reduced form of molecular oxygen created by receiving one electron. The superoxide anion is the initial free radical formed in the mitochondrial electron transport system. Mitochondria generate energy using a four-electron chain reaction, reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria react directly with oxygen and form superoxide anion. Moreover, the conversion of superoxide and H_2O_2 into more reactive species (e.g., the hydroxyl radical) is considered to be one of the unfavorable effects of superoxide radicals.

The effects of the extracts on the superoxide radical were determined using the PMS-NADH superoxide generating system and the results are shown in Table 2. The IC_{50} of RC, UP, and PD was 136.34, 145.69, and 226.25 μg/mL, respectively. The IC_{50} of the other extracts exceeded 1,000 μg/mL. These results suggest that RC and UP had notably superior superoxide scavenging effects.

Nitrite scavenging activity

Nitrite or reactive nitrogen species are formed during reactions with oxygen or superoxides, such as NO_2, N_2O_4, N_3O_4, and NO_2^-, are very reactive. These compounds are responsible for altering the structural and functional behavior of many cellular components. From Table 3, the nitrite scavenging activities of UP and RC were 0.46 and 0.43 mM Trolox equivalents (TE)/mg, respectively. The IC_{50} of these extracts was 358 and 360 μg/mL, respectively. The IC_{50} of PD and CT was 417 and 404 μg/mL, respectively. The others had values exceeding 1,000 μg/mL. The phenolic contents of the extracts were correlated with the nitrite scavenging activity (r^2=0.8794) and was highest for RC (221 mg GAE/g) and UP, and lowest for LE (11.55 mg GAE/g).

Table 3. Nitrite scavenging activity and reducing power of Korean traditional food materials.

Materials	mM TE/mg[1]	
	Nitrite scavenging activity	Reducing power
UP	0.46±0.000[a]	0.58±0.003[a]
RC	0.43±0.002[b]	0.57±0.007[b]
PD	0.36±0.004[c]	0.35±0.003[c]
CT	0.34±0.033[c]	0.07±0.003[f]
AS	0.15±0.028[d]	0.22±0.002[d]
ZO	0.09±0.006[e]	0.12±0.005[e]
CL	0.13±0.012[d]	–
GS	0.01±0.005[f]	–
GM	–	–
LE	–	–

[1]TE: Trolox equivalent

[a-f]Differences between letters in the same column indicate significant differences ($P<0.001$).

UP, *Ulmus pumila* Linne; RC, *Rubus coreanus*; PD, *Pinus densiflora* Sieb. and Zucc.; CT, *Carthamus tinctorius*; AS, *Artemisia* spp.; ZO, *Zingiber officinale* Roscoe; CL, *Curcuma Longa* L.; GS, *Glycine soja* Sieb. et Zucc; GM, *Glycine max* L. Mer.; LE, *Lithospermum erythrorhizon*.

Reducing power

Other reports have indicated that the antioxidant activity was generally concomitant with the reducing power. The antioxidant potential of the extracts was investigated further by determining the reducing power. Table 3 shows the reducing power of the extracts using the potassium ferricyanide reducing method. The TEAC value of UP and RC was 0.58 and 0.57 mM TE/mg, respectively. The respective values for PD and AS were 0.35 and 0.22 mM TE/mg. The greatest reducing activity was observed for UP and RC, which had reducing power approximately 10 times greater than that of GM. In addition, the phenolic contents of the extracts were correlated with the reducing power (r^2=0.9599) and was highest for RC (221 mg GAE/g) and lowest for LE (11.55 mg GAE/g).

Discussion

This study evaluated the total phenolic contents and antioxidative properties of Korean medicinal plants using the Folin-Ciocalteu method, the scavenging activities of various radicals, such as DPPH, ABTS cation, and superoxide anion, the nitrite scavenging activity, and the reducing power.

Phenolic compounds are common in both edible and inedible plants, and have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolic compounds is due mainly to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and decomposing peroxides[13]. Crude extracts of fruits, herbs, vegetables, cereals, nuts, and other plant materials rich in phenolics are of increasing interest to the food industry. The importance of the antioxidant constituents of plant materials in maintaining health and protecting from coronary heart disease and cancer is also gaining interest among scientists, food manufacturers, and consumers[14]. A study has suggested that polyphenolic compounds inhibit mutagenesis and carcinogenesis in humans, when up to 1.0 g is ingested daily from a diet rich in fruits and vegetables[15]. These compounds possess a broad spectrum of chemical and biological activities, including radical scavenging properties. Among the fractions of *Acacia confusa* Merr. bark extracts, the fraction with the highest phenol content had the highest scavenging activity[16]. Recent studies have shown that flavonoids and related phenols contribute significantly to the total antioxidant activity of many fruits and vegetables[17]. These data concur with reports showing that a high total phenol content increases the antioxidant activity and that a linear correlation exists between the phenolic content and antioxidant activity[18-20]. Siddhuraju *et al*. reported that the high concentration of tannins extracted from the stem bark of *Cassia fistula* L. possessed elevated DPPH free radical quenching capacity[21]. Similarly, Amarowicz *et al*. reported that the tannins extracted from canola and rapeseed hulls exhibited high scavenging efficiency toward DPPH free radicals[22]. In this study, we observed that the DPPH free radical scavenging activity was correlated with the phenol content. Kumaran and Karunakaran reported that the DPPH free radical scavenging effect of an extract of *Phyllanthus debilis* Klein ex Willd from India was 87.24% at a dose of 25 μg/mL[23]. Zhao *et al*. reported the antioxidant activity of an extract of *Salvia miltiorrhiza* Bunge, a traditional Chinese medicinal herb[24]. Extensive investigations of the antiradical and antioxidant activities of phenolics, including flavonoids and phenolic acids, have been reported[9]. Nabasree and Bratati studied the superoxide radical scavenging activity of 11 leafy vegetables from India[25]. The IC_{50} of these extracts ranged from 204 to 1,388 μg/mL. Zhao *et al*. reported that the IC_{50} of an extract of *S. miltiorrhiza* was 1.76 mg/mL[24]. Nitric oxide (NO) is implicated in inflammation, cancer, and other pathological conditions. Plants and plant products may counteract the effects of NO formation and may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Furthermore, the scavenging activity may also help to arrest the chain of reactions initiated by the excess generation of NO that is detrimental to human health.

The reducing properties are generally associated with the presence of reductones. The antioxidant action of reductones is based on breaking the free radical chain by donating a hydrogen atom[26,27]. Reductones also react with certain precursors of peroxide, preventing peroxide formation. Our data suggest that the reducing power of UP and RC likely contributes significantly toward the observed antioxidant effect. The results for various free radical scavenging systems revealed that Korean medicinal plants have antioxidant activities. Considering all our results, we concluded that UP and RC have good antioxidant activity. UP was the greatest scavenger of ABTS radicals, DPPH radicals, superoxide anion, and nitrite. The reducing power of UP, which had the highest total flavonoid content, was similar to that of RC, which had the highest total phenol content. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the antioxidant activity of many fruits and vegetables. We found a correlation between antioxidant activity and total phenol content, as determined by the squared regression coefficient. The antioxidant activity of Korean medicinal plants was in the order UP > RC > PD > CT.

From these results, we concluded that UP contained large amounts of phenols and flavonoids, and had high antioxidative activity. Further studies to isolate and identify individual phenolic and flavonoid compounds are warranted and *in vivo* studies are needed to better understand their mechanisms of action as antioxidants.

Materials & Methods

Samples

The several Korean food materials were purchased from Kyoung Dong Markets located in Seoul, Korea. The materials that were investigated include *Ulmus pumila* Linne (UP), *Rubus coreanus* (RC), *Pinus densiflora* Sieb. and Zucc. (PD), *Carthamus tinctorius* (CT), *Artemisia* spp. (AS), *Zingiber officinale* Roscoe (ZO), *Curcuma Longa* L. (CL), *Glycine soja* Sieb. et Zucc (GS), *Glycine max* L. Mer. (GM), and *Lithospermum erythrorhizon* (LE). The samples were kept at −20°C until ready for extraction.

Chemicals

Folin-Ciocalteu phenol reagent, 1,1-diphenyl-2picrylhydrazyl free radical (DPPH), L-ascorbic acid, Gallic acid, Rutin, Nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), β-dihydronicotinamide adenine dinucleotide (β-NADH), 2,2′-azino-bis (3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, 1,10-phenanthroline, potassium hexacyanoferrate, 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), 2-deoxyribose, hydroperoxide, Trizma base, ferrous chloride and ferric chloride were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Trolox was purchased from Sigma-Aldrich Chemical Co. (GmbH, Germany). All other chemicals used were of at least analytical grade.

Preparation of extracts from Korean food material

Dried samples were ground using a domestic blender and 100 g of this material was refluxed extracted for 3 hr at 80°C using 1 L of 70% ethanol. After this procedure, the samples were cooled and filtered under vacuum using Whatman No. 1. The residues were re-extracted with an additional 70% ethanol, as described above. The solvent of the combined extract was evaporated under rotary vacuum-evaporator at 60°C and the remaining water was removed by freeze drying. The freeze-dried extracts were ground to a fine powder (particle size of about 0.15 mm) and stores in screw cap bottles at −20°C before analysis.

The amounts of total phenolic compounds and flavonoids

The total phenolic content of the extracts were determined using Folin-Ciocalteu method with some modfications and changes[28]. The extracts (200 μL) were mixed with 400 μL of 2 N Folin-Ciocalteu reagent and 0.8 mL of 10% sodium carbonate. The mixtures were shaken thoroughly and allowed to stand for 1 hr. Next, the absorbance at 750 nm was determined. The phenolic contents were determined using a standard curve obtained from various concentrations of gallic acid. Total phenolics were expressed as mg gallic acid equivalent (GAE)/g extract.

The total flavonoid content of extracts were determined by Davis deformed method with some modification. The extracts (1.0 mL) were mixed with 10 mL of diethylene glycol and 1 mL of 1 N NaOH. The mixtures were shaken thoroughly and allowed to stand for 1 hr at 37°C. Then the absorbance at 420 nm was determined. The flavonoid contents were acquired using a standard curve obtained from various concentrations of rutin. The flavonoids were expressed as mg rutin equivalent (RE)/g extract.

DPPH free radicals scavenging activity

The scavenging activity for DPPH free radicals was measured according to the method of Blois[29]. Briefly, 4 mL of extract solution and 1 mL of 1.5×10^{-4} M DPPH solution were mixed. The mixture was shaken vigorously and allowed to stand at room temperature

for 30 min. The control was prepared as above without any extract, and ethanol was used for the baseline correction. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. IC_{50} values were determined by regression analysis of the results obtained at three different concentrations of the sample. IC_{50} value is inversely related to the activity.

Antioxidant activity by the $ABTS^{\cdot+}$ assay

The total antioxidant activity of extracts was measured by the $ABTS^{\cdot+}$ radical cation decolorization assay involving preformed $ABTS^{\cdot+}$ radical cation[30]. ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 hr before use. Prior to assay, the solution was diluted with ethanol (99.5%) so that its absorbance was 0.70 $\pm$0.02 at 734 nm. To determine the scavenging activity, 0.9 mL ABTS reagent was mixed with 0.1 mL of each sample and the absorbance was measured at 734 nm after 6 min of reaction at room temperature, using 75% ethanol as a control. Trolox was used as positive control.

Superoxide anion scavenging activity

The superoxide anion scavenging activity of samples was measured by the method of Siddhuraju and Becker with some modification[31]. Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH, and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments the superoxide anion was generated in 3 mL of Tris-HCl buffer (100 mM, pH 7.4) containing 1 mL of NBT (150 μM) solution, 1 mL of NADH (468 μM) solution and 1 mL of the extract (1 mg/mL). The reaction was started by adding 1 mL of PMS solution (60 μM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured. The tannin acid was used as positive control.

Nitrite scavenging activity

Nitrite was generated from sodium nitroprusside and measured by the Greiss reaction according to the method described by Kato, Lee, Chyen, Kim, & Hayase[32]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by using Greiss reagent. Scavengers of NO compete with oxygen, leading to reduced production of NO[33]. Using Griess reagent, extracts (1.0 mg/mL) were used to determine nitrite scavenging activity at different conditions (pH 1.2) by measuring the absorbance at 520 nm. The sodium nitrite (1 mM) was mixed with different concentrations of various extracts dissolved in the suitable solvent systems, incubated at 37°C for 1 hr, and then reacted with Greiss reagent (1% sulphanilamidein in 30% acetic acid, and 0.1% naphthylethylenediamine dihydrochloride in 30% acetic acid). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and the subsequent coupling with naphthylethylenediamine was read at 520 nm, and referred to the absorbance of potassium nitrite standard solutions that were similarly treated with Griess reagent. Trolox was used as a standard and results were also expressed as trolox equivalent antioxidant capacity in mM trolox/mg of extract.

Reducing power

The reducing power of the extracts was determined by the method of Oyaizu with slightly modified[34]. The extract 0.75 mL was mixed with 0.75 mL of potassium hexacyanoferrate, followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 mL of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 3,000 rpm/min for 10 min. 1.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power. Trolox was used as positive control. The value was derived as TEAC (trolox equivalent antioxidant capacity).

Statistical analysis

Tests were carried out in triplicate for 3-5 separate experiments. Values are presented as means $\pm$ SD. Statistical analysis was carried out with three groups using one-way analysis of variance (ANOVA) and Duncan's multiple range test. The values of $P < 0.001$ were considered statistically significant.

Acknowledgements This Research was financed by Seoul Research and Business Development Program (No. 10582), Republic of Korea.

References

1. Frei, B. Nonenzymatic antioxidant defense systems. In *Natural antioxidants in human health and disease*. Briviba K., Sies H, eds. Academic Press, London, UK,

107 (1994).
2. Halliwell, B. & Gutteridge, J. M. C. Free radicals, ageing and disease. In *Free radicals in biology and medicine*. Clarendon Press, London, UK, 416 (1996).
3. Southorn, P. A. Free radicals in medicine. II. Involvement in human disease. *Mayo Clin Proc* **63**:390-408 (1988).
4. Duthie, D. D. Lipid peroxidation. *Eur J Clin Invest* **45**:759-764 (1993).
5. Pietta, P. G. Flavonoids as antioxidants. *J Nat Prod* **63**:1035-1042 (2000).
6. Sherwin, E. R., Branen, A. L., Davidson, P. M. & Salminen, S. Food additives. New York, Marcel Dekker, USA, p. 139-193 (1990).
7. Middeleton, E., Kandaswamy, C. & Theoharides, T. S. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacol Rev* **52**:673-751 (2000).
8. Ishikawa, Y., Morimoto, K. & Hamasaki, T. Falvoglaucin, a metabolite of *Eurotium chevalieri*, its antioxidation and synergism with tocopherol. *J Am Oil Chem Soc* **61**:1864-1868 (1984).
9. Rice-Evans, C., Miller, N. J. & Paganda, G. Structure antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* **20**:933-956 (1996).
10. Moon, G. S. Constituents and uses of medicinal herbs. Ilweolseogak, Seoul, Korea, p. 310-311 (1991).
11. Song, H. J. Introductory oriental medicine at home. Kuk IL Press, Seoul, South Korea, p. 173-201 (1993).
12. Anderson, J. W., Smith, B. M. & Washnock, C. S. Cardiovascular and renal benefits of dry bean and soybean intake. *Am J Clin Nutr* **70(Suppl)**:464S-474S (1999).
13. Osawa, T. Novel natural antioxidants for utilization in food and biological systems. In Uritani, I., Garcia, V. V. & Mendoza, E. M. (Eds.), *Postharvest biochemistry of plant food-materals in the tropics*. Tokyo, Japan: Japan Scientific Societies Press, 241-251 (1994).
14. Loliger, J. The use of antioxidants in food. In Aruoma, O. I. & Halliwell, B. (eds.), *Free radicals and food additives*. Taylor and Francis, London, UK, 129-150 (1991).
15. Tanaka, M., Kuie, C. W., Nagashima, Y. & Taguchi, T. Applications of antioxidative Maillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishi* **54**:1409-1414 (1998).
16. Tung, Y. T., Wu, J. H. & Chang, S. T. Antioxidant activities of natural phenolic compounds from *Acacia confusa* bark. *Biores Technol* **98**:1120-1123 (2007).
17. Luo, X. D., Basile, M. J. & Kennelly, E. J. Polyphenolic antioxidants from the fruits of *Chrysophyllum cainito* L. (star apple). *J Agric Food Chem* **50**:1379-1382 (2002).
18. Holasova, M. *et al*. Buckwheat-the source of antioxidant activity in functional foods. *Food Res Int* **35**:207-211 (2002).
19. Velioglu, Y. S., Mazza, G., Gao, L. & Oomah, B. D. Antioxidants activity and total phenolics in selected fruits, vegetables, and grain products. *J Agr Food Chem* **46**:4113-4117 (1998).
20. Gheldof, N. & Engeseth, N. J. Antioxidants capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of vitro lipoprotein oxidation in human serum samples. *J Agr Food Chem* **50**:3050-3055 (2002).
21. Siddhuraju, P., Mohan, P. S. & Becker, K. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chem* **79**:61-67 (2002).
22. Amarowicz, R., Naczk, M. & Shahidi, F. Antioxidant activity of crude tannins of Canola and Rapeseed hulls. *J Am Oil Chem Soc* **77**:957-961 (2002).
23. Kumaran, A. & Karunakaran, R. J. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. *LWT-Food Sci Tech* **40**:344-352 (2007).
24. Zhao, G. R. *et al*. Antioxidant activities of Salvia miltiorrhiza and Panax notoginseng. *Food Chem* **99**:767-774 (2007).
25. Nabasree, D. & Bratati, D. Antioxidant activity of some leafy vegetables of India: A comparative study. *Food Chem* **101**:471-474 (2007).
26. Moncada, A., Palmet, R. M. J. & Higgs, E. A. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharm Rev* **43**:109-142 (1991).
27. Gordon, M. F. The mechanism of antioxidant action in vitro. In Hudson, B. J. F. (Ed.), *Food Antioxidants*, Elsevier Applied Science, London, UK, 1-18 (1990).
28. Takashi, K., Makiko, T., Tomoko, H. & Yoko, A. Antioxidant properties of dried 'kayamo-nori', a brown alga Scytosiphon lomentaria (*Scytosiphonales, Phaeophyceae*). *Food Chem* **89**:617-622 (2005).
29. Blois, M. S. Antioxidants determination by the use of a stable free radical. *Nat* **4617**:1199-1200 (1958).
30. Re, R. *et al*. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* **26**:1231-1237 (1999).
31. Siddhuraju, P. & Becker, K. The antioxidant and free radical scavenging activityes of processed cowpea (*Vigna unguiculata* Walp.) seed extracts. *Food Chem* **101**:10-19 (2007).
32. Kato, H. *et al*. Uninhibitory of nitrosamine formation by nondialyzable melanoidins. *Agr Bio Chem* **51**:1333-1338 (1987).
33. Manjeshwar, S. B., Ganesh, C. J., Shaival, K. R. & Kiran, B. S. Evaluation of nitric oxide scavenging activity of certain spices in vitro: A preliminary study. *Nahrung/Food* **47**:261-264 (2003).
34. Oyaizu, M. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. *Jap J Nutr* **44**:307-315 (1986).