Natural Product Sciences
9(2) : 73-79 (2003)

Scavenging Effect of Korean Medicinal Plants on the Peroxynitrite and Total ROS

Hye Sook Kang, Hae Young Chung[1], Kun Ho Son[2], Sam Sik Kang[3], and Jae Sue Choi*

Faculty of Food Science and Biotechnology, Pukyong National University, Busan 608-737, Korea
[1]College of Pharmacy, Pusan National University, Busan 609-735, Korea
[2]Department of Food and Nutrition, Andong National University, Andong 760-749, Korea
[3]Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

Abstract – To discover the sources with antioxidative activity in traditional medicines, 100 extracts of Korean medicinal plants were screened for their scavenging effect on peroxynitrite ($ONOO^-$) and total reactive oxygen species (ROS). The potency of total ROS scavenging activity was shown in the extracts of 25 plants, and 4 of their species, *Macleaya cordata* R. Br., *Salvia plebeia* R. Br., *Cassia tora* L. and *Angelica gigas* Nakai, had a greater effect with IC_{50} values of 1.7±0.36, 4.3±1.08, 4.9±0.17 and 5.8±1.01 μg/ml, respectively, than that of trolox, positive control (7.61±0.12 μg/ml). Another 35 extracts exhibited inhibitory effect of below 50 percent at 100 μg/ml of sample concentrations on total ROS, while the rest observed total ROS generators rather than scavengers. The peroxynitrite scavenging activities were observed in the greater part of the plants tested. Five of them, *Schisandra chinensis* Baill, *Campsis grandiflora* (Thunb.) K. Schum., *Cedrela sinensis* A. Juss., *Pleuropterus multiflorus* Turcz. and *Veronica linariaefolia* Pall represented scavenging activities on peroxynitrite twice as strong with IC_{50} values of 0.48±0.10, 0.59±0.15, 0.60±0.10, 0.64±0.10 and 0.91±0.23 μg/ml, respectively, as that of penicillamine (1.72±0.05 μg/ml), positive control. Consequently, 25 species of the entire plants tested, exhibited scavenging activities on total ROS and $ONOO^-$, *Salvia plebeia* R. Br., *Macleaya cordata* R. Br., *Cassia tora* L. and *Angelica gigas* Nakai exerted potent scavenging activities on both radicals.
Key words – Korean medicinal plants, antioxidative activity, peroxynitrite, total ROS

Introduction

About one fourth of 1 kg of oxygen per day is used for respiration by a human. While the ultimate fate of this oxygen is conversion into water and carbon dioxide, in the process it undergoes many enzymatic reactions involving free radical formation. Two neutral free radicals, H·(hydrogen atom) and ·OH (hydroxyl radical), and charged free radicals can undergo further reactions to produce other radicals or reactive species as follows; singlet oxygen ($^1O_2$), superoxide anion ($\cdot O_2^-$), peroxy radicals (ROO·), ·OH, hydrogen peroxide (H_2O_2), and organic hydroperoxides (ROOH). Many essential biochemical reactions, for example, prostaglandin synthesis, peroxidase action, and phagocytosis proceed via free radicals. On the other hand, they are implicated in disease and toxic reactions, for example, in the toxicity of bipyridyl herbicides, radiobiological effects, effects of cigarette smoke, ischemic heart disease, and cancer (Miquel, 1989).

*Author for correspondence
Fax: 82-51-620-6330, E-mail: choijs@pknu.ac.kr

Oxidative stress can be defined as a disturbance in the prooxidant/antioxidant balance in favor of the former. Oxidative stress may lead to a variety of diseases and degenerative processes such as aging, immunodeficiencies, neurological disorders and carcinogenesis (Scandalios, 1997; Sies, 1991). The major cellular sources responsible for the generation of damaging-causing reactive oxygen species (ROS), such as $\cdot O_2^-$, H_2O_2, and ·OH, are mitochondria, peroxisomes and various other enzymes, cyclooxygenase (COX), NADPH dehydrogenase, and oxidases, including xanthine oxidase (XOD) which all contain ROS (Freeman, 1984).

Peroxynitrite ($ONOO^-$), a cytotoxic reactive nitrogen species (RNS) that can be formed by the combination of $\cdot O_2^-$ and nitiric oxide (·NO) radicals, is generated *in vivo* (Balavoine and Geletii, 1999) by endothelial cells, Kupffer cells, neutrophils and macrophages. $ONOO^-$ is a relatively stable species compared with free radicals, but it becomes highly reactive peroxynitrous acid (ONOOH) by protonation, readily decomposes with a very short half-life (1.9 s) at 37°C to form various cytotoxicants (Patel *et al.*, 1999).

These ROS and RNS may act as potent oxidizing and nitrating agents to damage several cellular components, such as proteins, lipids and DNA. Its excessive formation may also be involved in several human diseases such as Alzheimers disease, rheumatoid arthritis, cancer and atherosclerosis (Squadrito and Pryor, 1998).

For several years, many researchers have investigated powerful and nontoxic antioxidants from natural sources, especially edible or medicinal plants to prevent the formation of the above reactive species related disorders in human as well as replace synthetic compounds, which may be carcinogenic and harmful to the lungs and liver (Branen, 1975). There are few reports on total ROS and $ONOO^-$ scavenging activities of Korean medicinal plants, except the leaves extracts of *Eriobotrya japonica* (Soung *et al.*, 1999), green tea (Van Dyke *et al.*, 2000; Chung *et al.*, 1998) and *Ginkgo biloba* (Cheung *et al.*, 1999).

In the present paper, we carried out the screening of Korean medicinal plant resources for its scavenging activities on total ROS and $ONOO^-$.

Materials and Methods

General experimental procedures – 3-Morpholinosydnonimine (SIN-1), DL-penicillamine (DL-2-amino-3-mercapto-3-methylbutanoic acid), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dihydrorhodamine 123 (DHR 123) and 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Molecular Probes (Eugene, Oregon, USA) and $ONOO^-$ was from Cayman Chemical Co. (Ann Arbor, MI, USA).

Plant material – All the plant materials used in this study were collected in May 2000 from Korea Forest Research Institute, Hongnung Arboretum in Seoul, Korea. They were extracted with MeOH at room temperature for 3 days. Then the solvent was evaporated, and the residue was concentrated and dried.

Measurement of the free radical generation (DCF Method) – The generation of ROS was assessed using the ROS-sensitive fluorescence indicator 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA). Test samples (final concentration, 0.5 mg/mL) were added to kidney postmitochondrial fraction in 50 mM potassium phosphate buffer and incubated at 37°C for 5 min. Then the mixture was loaded with DCFH-DA (5 μg/mL) in potassium phosphate buffer. DCFH-DA is a nonfluorescent probe that diffuses into cell. Mitochondrial esterase hydrolyzes DCFH-DA to 2', 7'-dichlorodihydrofluorescein (DCFH). ROS generated oxidize DCFH to the fluorescent dye 2', 7'-dichlorofluorescein (DCF). The fluorescence of DCF was measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm for 30 min on a microplate fluorescence spectrophotometer FL 500 (Bio-Tek instruments, USA). Trolox was used as a positive control.

Measurement of the $ONOO^-$ scavenging activity – The $ONOO^-$ scavenging activity was measured by monitoring the oxidation of dihydrorhodamine 123 by modifying the method of Kooy *et al.* (1994). A stock solution of DHR 123 (5 mM) in dimethylformamide was purged with nitrogen and stored at −80°C. A working solution of DHR 123 (final concentration, 5 μM) diluted from the stock solution was placed on ice and was not exposed to light prior to the study. The buffer of 90 mM sodium chloride, 50 mM sodium phosphate (pH 7.4), and 5 mM potassium chloride with 100 μM (f.c.) diethylenetriaminepentaacetic acid (DTPA) was purged with nitrogen and placed on ice before use. The $ONOO^-$ scavenging ability by the oxidation of DHR 123 was determined at room temperature on a microplate fluorescence spectrophotometer FL 500 (Bio-Tek instruments, USA) with excitation and emission wavelengths of 485 nm and 530 nm respectively. The background and final fluorescent intensities were measured 5 min after treatment with or without SIN-1 (f.c. 10 μM) or authentic $ONOO^-$ (f.c. 10 μM) in 0.3 M sodium hydroxide. Oxidation of DHR 123 by decomposition of SIN-1 gradually increased whereas authentic $ONOO^-$ rapidly oxidized DHR 123 with its final fluorescent intensity being stable over time. Penicillamine was used as a positive control.

Results and Discussion

Plant-food-derived antioxidants and active principles such as flavonoids, hydroxycinnamiates (ferulic acid, chlorogenic acids, vanillin, etc.), beta-carotene and other carotenoids, vitamin E, vitamin C, or rosemary, sage, tea and numerous extracts are increasingly being proposed as important dietary antioxidant substances (Aruoma, 1999).

One hundred Korean medicinal plants were screened to evaluate their total ROS and peroxynitrite scavenging activities as seen in Table 1. The generation of ROS was assessed using the ROS-sensitive fluorescence indicator 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA). The DCFH-DA is a nonfluorescent probe that diffuses into the cell. Mitochondrial esterase hydrolyzes DCFH-DA to 2', 7'-dichlorodihydrofluorescein (DCFH). The ROS generated oxidize DCFH to the fluorescent dye 2', 7'-dichlorofluorescein (DCF). In the present study, 25 of 100 extracts showed total ROS scavenging activity and 4 species of them, *Macleaya cordata* R. Br., *Salvia plebeia* R. Br., *Cassia*

Table 1. Scavenging effects of Korean medicinal plants on total ROS and $ONOO^-$

Family	Scientific name	Part used	ROS IC_{50} (μg/ml)	$ONOO^-$ IC_{50} (μg/ml)
Actinidiaceae	*Actinidia arguta* Planch.	LF	–*	7.28±0.88
Apocynaceae	*Trachelospermum asiaticum* var. *intermedium* Nakai	AP	38.0±6.05	2.67±0.39
Araceae	*Acorus calamus* L. var. *angustatus* Bess.	LF	–*	8.22±0.96
Bignoniaceae	*Campsis grandiflora* (Thunb.) K. Schum.	LF	>>100	0.59±0.15
Boraginaceae	*Symphytum officinale* L.	AP	>>100	2.36±0.12
Campanulaceae	*Platycodon grandiflorum* (Jacq.) A. DC.	AP	–*	4.24±0.54
Caryophyllaceae	*Dianthus sinensis* L.	AP	–*	29.15±5.15
Compositae	*Achillea sibirica* Ledeb.	AP	–*	1.83±0.12
	Artemisia capillaris Thunb.	AP	52.5±1.40	5.86±0.73
	Aster koraiensis Nakai	AP	–*	2.78±0.32
	Aster tataricus L.	AP	94.1±25.49	2.47±0.25
	Atractylodes japonica Koidz.	AP	>>100	2.89±0.55
	Chrysanthemum zawadskii var. *latilobum* (Max.) Kitagawa	AP	–*	6.29±0.85
	Echinops setifer Iljin	AP	>>100	5.30±0.99
	Inula helenium L.	AP	35.2±5.38	4.53±0.18
	Ixeris dentata (Thunb.) Nakai	AP	96.9±15.34	3.54±0.27
	Ixeris dentata (Thunb.) Nakai	WP	>>100	1.35±0.08
Crassulaceae	*Sedum kamtschaticum* Fisch.	AP	>>100	6.43±0.27
Ericaceae	*Rhododendron mucronulatum* Turcz.	AP	–*	1.72±0.08
Geraniaceae	*Geranium sibiricum* L.	AP	41.1±13.95	2.39±0.28
Haemodoraceae	*Anemarrhena asphodeloides* Bunge	AP	–*	9.20±0.46
Iridaceae	*Belamcanda chinensis* (L.) DC.	FR	>>100	7.39±0.37
	Iris koreana Nakai	AP	>>100	14.18±2.78
	Iris nertschinskia Lodd.	AP	95.6±18.18	7.82±0.34
	Iris pallasii var. *chinensis* Fisch.	LF	>>100	10.61±0.57
Lamiaceae	*Leonurus sibiricus* L.	AP	–*	6.45±0.49
	Mentha arvensis var. *piperascens* Malinv.	AP	18.7±4.65	2.91±0.68
	Perilla frutescens Brit. var. *crispa* Decne	AP	>>100	1.47±0.16
	Salvia plebeia R. Br.	AP	4.3±1.08	1.20±0.04
Lardizabalaceae	*Akebia quinata* (Thunb.) Decaisne.	LF	>>100	1.65±0.20
Leguminosae	*Astragalus membranaceus* Bunge	AP	–*	11.12±0.35
	Caragana sinica (Buchoz) Rehder	AP	>>100	19.46±8.83
	Cassia occidentalis L.	AP	59.8±2.70	5.97±1.09
	Cassia tora L.	AP	4.9±0.17	7.76±0.27
	Glycyrrhiza uralensis Fisch.	AP	>>100	12.87±0.51
	Glycyrrhiza uralensis Fisch.	SD	–*	22.97±1.62
	Lotus corniculatus var. *japonicus* Regel.	AP	>>100	41.36±2.45
Liliaceae	*Allium thunbergii* G. Don.	AP	>>100	12.75±0.86
	Allium tuberosum Roth.	AP	>>100	5.41±0.51
	Asparagus cochinchinensis Merr.	AP	–*	8.67±3.84
	Asparagus oligoclonos Max.	AP	–*	4.21±0.36
	Asparagus oligoclonos Max.	ST	–*	12.75±0.93
	Disporum sessile D. Don ssp. flavens Kitagawa	AP	–*	14.76±1.06
	Hemerocallis fulva L.	AP	>>100	8.96±0.21
	Hemerocallis fulva var. *kwanso* Regel	AP	>>100	10.33±0.79
	Hemerocallis lilioasphodelus L.	AP	>>100	5.93±0.80
	Hosta lancifolia Engl.	AP	–*	38.95±2.13
	Liriope platyphylla Wang *et* Tang	LF	–*	62.83±1.96
	Liriope platyphylla Wang *et* Tang	WP	–*	15.08±0.33
	Polygonatum odoratum (Miller) Druce var. *pluriflorum* (Miq.) Ohwi.	AP	–*	8.68±0.70
	Rohdea japonica Roth.	LF	>>100	32.23±0.66
	Scilla scilloides (Lindl.) Druce.	AP	–*	14.54±0.76
Lythraceae	*Lythrum anceps* (Koehne) Makino	AP	21.5±0.89	1.63±0.04
Magnoliaceae	*Schisandra chinensis* Baill.	LF	–*	0.48±0.10
Malvaceae	*Althaea rosea* Cav.	AP	>>100	12.15±0.24
	Gossypium indicum Lam.	LF	>>100	61.21±0.10
	Hibiscus manihot L.	AP	–*	4.16±0.60
Meliaceae	*Cedrela sinensis* A. Juss.	LF	–*	0.60±0.10
Moraceae	*Ficus carica* L.	AP	–*	11.15±0.94
	Ficus carica L.	LF	86.7±12.6	5.72±0.42

Table 1. Continued

Family	Scientific name	Part used	ROS IC_{50} (μg/ml)	$ONOO^-$ IC_{50} (μg/ml)
Oleaceae	*Forsythia viridissima* Lindl.	AP	>>100	5.07±0.92
Onagraceae	*Oenothera odorata* Jacq.	AP	>>100	2.24±0.05
Orchidaceae	*Dendrobium moniliforme* (L.) Sw.	AP	>>100	9.02±1.58
Papaveraceae	*Macleaya cordata* R. Br.	AP	1.7±0.36	4.06±0.18
Plantaginaceae	*Plantago asiatica* L.	AP	60.8±8.12	1.54±0.11
	Plantago asiatica L.	SD	>>100	3.51±0.20
Polygonaceae	*Rumex acetocella* L.	AP	25.0±5.62	6.36±0.94
	Pleuropterus multiflorus Turcz.	AP	12.8±0.67	0.64±0.10
	Reynoutria elliptica (Koidz.) Migo	LF	10.7±1.00	1.47±0.19
	Rumex crispus L.	AP	>>100	10.25±0.62
Primulaceae	*Lysimachia vulgaris* var. *davurica* (Led.) R.Knuth	AP	>>100	19.67±1.41
	Lysimachia Christina Hance	WP	–*	31.72±0.12
Pteridaceae	*Pteridium aquilinum* var. *latiusculum* (Desv.) Underw	AP	>>100	11.80±0.46
Ranunculaceae	*Aconitum carmichaeli* Debx.	AP	–*	10.81±0.08
	Paeonia suffruticosa Andr.	AP	11.3±0.98	1.95±0.12
	Paeonia suffruticosa Andr.	FL	9.4±0.53	1.56±0.17
	Pulsatilla koreana Nakai	AP	>>100	4.14±0.42
Rosaceae	*Agrimonia pilosa* Ledeb.	AP	–*	7.79±1.33
	Potentilla discolor Bunge	AP	>>100	5.68±0.22
Rubiaceae	*Galium verum* L. var. *asiaticum* Nakai	AP	60.9±5.56	4.18±0.08
Rutaceae	*Dictamnus dasycarpus* Turcz.	AP	>>100	2.28±0.17
	Ruta graveolens L.	AP	>>100	10.80±0.29
	Zanthoxylum schinifolium S. *et* Z.	LF	–*	3.02±0.83
	Zanthoxylum schinifolium S. *et* Z.	AP	–*	5.68±0.34
Saururaceae	*Houttuynia cordata* Thunb.	AP	42.2±1.23	1.27±0.01
	Saururus chinensis Baill.	AP	–*	2.92±0.16
Scrophulariceae	*Veronica linariaefolia* Pall	AP	>>100	0.91±0.23
Solanaceae	*Physalis alkekengi* var. *francheti* (Masters) Hort.	AP	–*	1.10±0.10
Umbelliferaeceae	*Angelica dahurica* (Fisch.) Benth. *et* Hooker *f.*	AP	>>100	4.35±0.62
	Angelica gigas Nakai	AP	5.8±1.01	2.61±0.13
	Angelica gigas Nakai	FR	–*	8.25±0.51
	Angelica gigas Nakai	LF	–*	3.21±0.20
	Foeniculum vulgare Gaertner	AP	–*	5.85±1.26
	Ligusticum tenuissimum Nakai (Kitagawa)	AP	–*	5.80±0.51
	Ostericum koreanum (Max.) Kitagawa	AP	22.2±2.45	2.36±0.39
	Peucedanum japonicum Thunb.	AP	–*	18.10±1.23
Valerianaceae	*Patrinia scabiosaefolia* Fischer.	AP	–*	16.19±1.27
	Patrinia villosa (Thunb.) Juss.	AP	52.3±6.02	1.49±0.13
Violaceae	*Viola mandshurica* W. Becker.	AP	–*	13.34±0.31
Zingiberaceae	*Zingiber mioga* (Thunb.) Rosc.	AP	–*	5.62±1.46
Penicillamine				1.72±0.05
Trolox			7.61±0.12	

Results are the mean±S.E. (n=3).
Penicillamine and Trolox were used as positive controls on peroxynitrite and total ROS.
–*showed the total ROS generation under the experimental conditions.
Abbreviations used: AP, aerial part; FL, flower; FR, fruit; LF, leaves; SD, seed; ST, stem; WP, whole plant.

tora L. and *Angelica gigas* Nakai, with IC_{50} values of 1.7±0.36, 4.3±1.08, 4.9±0.17 and 5.8±1.01 μg/ml, respectively, had a greater effect than that of trolox, positive control (7.61±0.12 μg/ml). Another 35 extracts exhibited an inhibitory effect of below 50 percent even at the maximum concentration tested (100 μg/ml) on the total ROS. On the other hand, the rest of the 40 plants were observed as total ROS generators rather than scavengers.

The formation of the $ONOO^-$ was detectable by the oxidation of DHR 123 to rhodamine 123 from decomposition of SIN-1 or authentic $ONOO^-$ was scavenged by the plant extracts to a variable degree. Among them, five of *Schisandra chinensis* Baill, *Campsis grandiflora* (Thunb.) K. Schum., *Cedrela sinensis* A. Juss., *Pleuropterus multiflorus* Turcz. and *Veronica linariaefolia* Pall represented scavenging activities on peroxynitrite twice as strong with IC_{50} values of 0.48±0.10, 0.59±0.15, 0.60±0.10, 0.64±0.10 and 0.91 ±0.23 μg/ml, respectively, the same as that of penicillamine

(1.72±0.05 μg/ml), positive control. In addition, 14 species of them with IC_{50} values ranging from 1.10±0.10 to 1.95 ±0.12 μg/ml were comparable to that of the positive control. IC_{50} values of the rest were divided into 4 groups as followed; 24 species with 2.24±0.05~4.53±0.18 μg/ml, 28 with 5.07±0.92~9.20±0.46 μg/ml, 21 with 10.25±0.62 ~19.67±1.41 μg/ml, and 8 with 22.97± 1.62~62.83±1.96 μg/ml. As a result, 4 species of the entire Korean medicinal plants used, *Salvia plebeia* R. Br., *Macleaya cordata* R. Br., *Cassia tora* L., and *Angelica gigas* Nakai exhibited potent scavenging activities both on the total ROS and $ONOO^-$.

Salvia plebeia belonging to the Lamiaceae, is a small herb which grows indigenously in wet places throughout almost all of China and India. Weng *et al.* (2000) reported that 6-methoxy-luteolin-7-glucoside, β-sitosterol and 2'-hydroxy-5'-methoxybiochanin A, isolated from this plant, had strong antioxidant activities contributing to an electron donating group in lard with an oxidative stability instrument (OSI) at 100°C. In addition, aqueous extract of *S. plebeia* had strong activity on the mast cell mediated-type allergic reactions in rats (Shi and Kim, 2002). In the previous study, 3-(3',4'-dihydroxyphenyl)-(2*R*)-lactamide, dimethyl lithospermate from *Salvia miltiorrhiza*, the same genus along with the above plant, inhibited significantly the generation of free radicals of hepatocyte by the DCF method (Kang, *et al.*, 1997). In addition, salviamiltamide, a new cyclic phenyllactamide, was found to be a DPPH radical scavenger (Choi, *et al.*, 2001). A furanofuranoid lignan glycoside, with radical scavenging on $ONOO^-$, total ROS and DPPH radical was also isolated from the same source, and characterized as (+)-1-hydroxypinoresinol-1-*O*-β-D-glucoside based on spectroscopic evidence. Since the compounds with more than one catechol moiety increase the resonance stability, this stability may have influenced the inhibitory effect of the compounds (Kang *et al.*, 1997; Kang *et al.*, 2003).

Macleaya cordata R. Br. (Papaveraceae) has been used for improvement of antitumor, antiphlogistic, detoxification and sterilization, and for healing ulcers, tympanitis and burns in Korea (Kim, 1997). This plant is a producer of antimicrobial benzo[*c*]phenanthridine alkaloids, such as sanguinarin, chelerithrin, dihydrosanguinarin and dihydrochelirubin, used for aesthetic, antiseptic, antitumor and fungicide (Fonin *et al.*, 1995).

The seeds of *Cassia tora* L. (Leguminosae) are used to improve vision in Chinese herbal medicine and are reputed for their medicinal value as an asperient, antiasthenic and diuretic agent (Namba, 1980). Crysophanol, chryso-obtusin, alaternin and aurantio-obtusin from the CH_2Cl_2 fraction and cassiaside, isorubrofuasrin gentiobioside and rubro-fusarine gentiobioside from the *n*-BuOH fraction of *C. tora* were isolated, and identified. Each of these compounds demonstrated significant antimutagenic activity (Choi *et al.*, 1997; Choi *et al.*, 1998). Lee *et al.* (1998) reported nor-rubrofusarin as having a strong radical scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. In addition, Hatano *et al.* (1999) reported that torachrysone, toralactone, aloe-emodin, rhein and emodin showed noticeable antibacterial effects on four strains of the methicillin-resistant *Staphylococcus aureus* with a minimum inhibitory concentration of 2-64 μg/ml. And, both alaternin and emodin were found to inhibit the peroxidation of linoleic acid by the thiocyanate method in a dose-dependent manner. Whereas the former showed inhibitory activities in total ROS and $ONOO^-$, the latter did not (Choi, *et al* 2000a).

Angelical gigas Nakai (Umbelliferae) has been used traditionally in Korean herbal medicine under the Korean names "Zam Dang Gui" not only for the treatment of anemia but also as a sedative, an anodyne, or a tonic agent (Han, 1992). The following coumarins have been isolated from the aerial parts of *A. gigas*: decursin, a 2:1 mixture of decursinol and its enantiomer aegelinol, a racemic mixture of (+)- and (−)-agasyllin, prenyletin, nodakenetin and gigasol (Porzel A, and Huneck, 1991). As well, Kang *et al.* (2001) reported that the MeOH extract of the underground part of this plant inhibited significant acetylcholinesterase (AchE) activity, and isolated 12 coumarins such as decursinol, marmesin, xanthotoxin, 7-demethylsuberosine, umbelliferone, isoimperatorin, xanthyletin, 7-methoxy-5-prenyloxycoumarin, decursin, 7-hydroxy-6-(2-(*R*)-hydroxy-3-methylbut-3-enyl)coumarin, nodakenin and peucedanone. They suggested that the coumarin skelecton containing a pyrone moiety plays an important role in the inhibitory activity against AChE. Also, two polyacetylenes isolated from this plant and their peracetate, inhibited the production of nitric oxide (NO) in LPS-activated RAW 264.7 cells by suppressing the inducible nitric oxide synthase (i-NOS) enzyme expression (Choi *et al.*, 2000b).

Thus, the reports described above supported well our results that *Salvia plebeia* R. Br., *Macleaya cordata* R. Br., *Cassia tora* L., and *Angelica gigas* Nakai exhibited potent scavenging activities both on the total ROS and $ONOO^-$.

Free or non-free radicals including ROS and RNS cause a variety of diseases such as inflammation, cardiovascular diseases, cancer, Alzheimers disease, rheumatoid arthritis, and atherosclerosis (Beckman *et al.*, 1996; Podrez *et al.*, 1999). These diseases have been reported to be ameliorated by radical scavengers (Aruoma, 1999; Hermann *et al.*,

1999). Therefore, the Korean medicinal plants tested with radical scavenging activities can be useful in the prevention and treatment of free radical related disease. With further study, we may be able to find potential beneficial effects, active components and action mechanism of these Korean medicinal plants to prevent and treat the free radical related diseases.

Acknowledgements

This research was supported by two grants (PF002201-07 and -08) from the Plant Diversity Research Center of the 21st Century Frontier Research Program funded by the Korean Ministry of Science and Technology.

References

Aruoma, O. I., Antioxidant actions of plant foods: use of oxidative DNA damage as a tool for studying antioxidant efficacy. *Free Radical Res.* **30**, 419-427 (1999).

Balavoine, G. G., and Geletii, Y. V., Peroxynitrite scavenging by different antioxidants. Part I: convenient assay. *Nitric Oxide* **3**, 40-54 (1999).

Beckman, J. S. and Koppenol, W. H., Nitric oxide, superoxide and peroxynitrite: the good, the bad and the ugly. *Am. J. Physiol.* **271**, C1424-C1437 (1996).

Branen, A.L., Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *J. Am. Oil Chem. Soc.* **52**, 59-63 (1975).

Cheung, F., Siow, Y. L., Chen, W. Z., and O, K., Inhibitory effect of *Ginkgo biloba* extract on the expression of inducible nitric oxide synthase in endothelial cells. *Biochem. Pharmacol.* **58**, 1665-1673 (1999).

Choi, J. S., Chung, H. Y., Jung, H. A., Park, H. J., and Yokozawa, T., Comparative evaluation of antioxidant potential of alaternin (2-hydroxyemodin) and emodin. *J. Agric. Food Chem.* **48**, 6347-6351 (2000a).

Choi, J. S., Kang, H. S., Jung, H. A., Jung, J. H., and Kang, S. S., A new cyclic phenyllactamide from *Salvia miltiorrhiza. Fitoterapia* **72**, 30-34 (2001).

Choi, J. S., Lee, H. J., Park, K. Y., Ha, J. O., and Kang, S. S., *In vitro* antimutagenic effects of anthraquinone aglycones and naphthopyrone glycosides from *Cassia tora. Planta Med.* **63**(1), 11-14 (1997).

Choi, J. S., Lee, H. J., Park, K. Y., and Jung, G. O., *In vitro* antimutagenic effects of alaternin and isorubrofusarin gentiobioside from roasted *Cassia tora. Nat. Prod. Sci.* **4**(2), 100-104 (1998).

Choi, Y. E., Ahn, H., Ryu, J. H., Polyacetylenes from *Angelica gigas* and their inhibitory activity on nitric oxide synthesis in activated macrophages. *Biol. Pharm. Bull.* **23**(7), 884-886 (2000b).

Chung, H. Y., Yokozawa, T., Soung, D. Y., Kye, I. S., and Baek, B. S., Peroxynitrite-scavenging activity of green tea tannin. *J. Agric. Food Chem.* **46**, 4484-4486 (1998).

Fonin, V. S., Sheichenko, V. I., Savina, A. A., Litovskaya, V. I. and Tolkachev, O. N., Cell cultured of *Macleaya cordata*-a producer of antimicrobial benzo[*c*]phenanthridine alkaloids. *Antibiot Khimioter* **40**(8), 17-22 (1995).

Freeman, B. A., Biological sites and mechanism of free radical production, in Armstrong, D., Sohal, R., Culter, R. G., Slater, T. (eds.), *Free radicals in molecular biology, aging, and disease*, Raven Press, New York, 1984, pp. 43-52.

Han, K. S., Pharmacognosy, 4th ed. Dongmyungsa Press, Seoul, 1992, pp. 201-202.

Hatano, T., Uebayashi, H., Ito, H., Shiota, S., Tsuchiya, T., and Yoshida, T., Phenolic constituents of Cassia seeds and antibacterial effect of some naphthalenes and anthraquinones on methicillin-resistant *Staphylococcus aureus*. *Chem. Pharm. Bull.* **47**(8), 1121-1127 (1999).

Hermann, M., Kapiotis, S., Hofbauer, R., Exner, M., Seelos, C., Held, I. and Gmeiner, B., Salicylate inhibits LDL oxidation initiated by superoxide/nitric oxide radicals. *FEBS Lett.*, **445**, 212-214 (1999).

Kang, H. S., Chung, H. Y., Jung, J. H., Kang, S. S., and Choi, J. S., Antioxidant effect of *Salvia miltiorrhiza. Arch. Pharm. Res.* **20**(5), 496-500 (1997).

Kang, H. S., Chung, H. Y., Byun, D. S., and Choi, J. S., Further isolation of antioxidative (+)-1-hydroxypinoresinol-1-*O*-β-D-glucoside from the rhizome of *Salvia miltiorrhiza* that acts on peroxynitrite, total ROS and 1,1-diphenyl-2-picrylhydrazyl radical. *Arch. Pharm. Res.* **26**(1), 24-27 (2003).

Kang, S. Y., Lee, K. Y., Sung, S. H., Park, M. J., and Kim, Y. C., Coumarins isolated from *Angelica gigas* inhibit acetylcholinesterase: Structure-activity relationships. *J. Nat. Prod.* **64**, 683-685 (2001).

Kim, J. K., Illustrated natural drugs encyclopedia (color ed.) vol. 2, Namsandang, Seoul, 1997, pp. 38.

Kooy, N. W., Royall, J. A., Ischiropoulos, H., and Beckman, J. S., Peroxynitrite mediated oxidation of dihydrorhodamine 123. *Free Radic. Biol. Med.* **16**, 149-156 (1994).

Lee, H. J., Park, J. C., and Choi, J. S., The ^{13}C-NMR assignment of nor-rubrofusarin having strong radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radical. *Nat. Prod. Sci.* **4**(2), 95-99 (1998).

Miquel, J., CRC handbook of free radicals and antioxidants in biomedicine Vol. I, CRC press, Inc., Boca Raton, Florida, 1989, pp. 17-25.

Namba, T., Colored Illustrations of Wakan-Yaku, Vol. 1, Hoikusha Publishing Co. Ltd., 1980, pp 226.

Patel, R. P., McAndrew, J., Sellak, H., White, C. R., Jo, H., Freeman, B. A., and Darley-Usmar, V. M., Biological aspects of reactive nitrogen species. *Biochem. Biophys. Acta.* **1411**, 385-400 (1999).

Podrez, E. A., Schmitt, D., Hoff, H. F. and Hazen, S. L., Myeloperoxidase-generated reactive nitrogen species convert LDL into an atherogenic form in vitro. *J. Clin. Invest.*, **103**, 1547-1560 (1999).

Porzel A, D. J. J., and Huneck, S., Gigasol and other coumarins from *Angelica gigas*. *Phytochemistry* **30**(2), 710-712 (1991).

Scandalios, J. G., Oxidative stress and the molecular biology of antioxidant defenses. Cold Spring Harbor Laboratory Press. New York, 1997, pp. 890.

Shi, T. Y., and Kim H.M., Inhibition of immediate-type allergic reactions by the aqueous extract of *Salvia plebeia*. *Immunopharmacol. Immunotoxicol.* **24**(2), 303-314 (2002).

Sies, H., Oxidative stress. Academic Press. Inc., San Diego, 1991, pp. 650.

Soung, D. Y., Kim, J. S., Chung, H. Y., Jung, H. A., Park, J. C., Choi, J. S., Flavonoids and chlorogenic acid from *Eriobotrya joponica* scavenge peroxynitrite. *Nat. Prod. Sci.* **5**, 80-84 (1999).

Squadrito, G. L., and Pryon, W. A., Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radic. Biol. Med.* **25**, 392-403 (1998).

Van Dyke, K., McConnell, P., and Marquardt, L., Green tea extract and its polyphenols markedly inhibit luminol-dependent chemiluminescence activate, by peroxynitrite or SIN-1. *Luminescence* **15**, 37-43 (2000).

Weng, X. C., and Wang, W., Antioxidant activity of compounds isolated from *Salvia plebeia*. *Food Chemistry* **71**, 489-493 (2000).

(Accepted May 28, 2003)