Arch Pharm Res Vol 33, No 6, 867-873, 2010
DOI 10.1007/s12272-010-0609-3

Archives of Pharmacal Research
www.springer.com/12272

Peroxyl Radical Scavenging Capacity of Extracts and Isolated Components from Selected Medicinal Plants

Sun Ju Kim[1], Do Young Kwon[1], Yeong Shik Kim[1], and Young Chul Kim[1,2]

[1]College of Pharmacy, Seoul National University, Seoul 151-742, Korea and [2]Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, Korea

(Received December 22, 2009/Revised March 9, 2010/Accepted March 27, 2010)

We determined the ability of extracts and active components isolated from nine medicinal plants, *Poncirus trifoliata, Astragalus membranaceus, Magnolia obovata, Salvia miltiorrhiza, Angelica dahurica, Cornus officinalis, Cnidium officinale, Pueraria lobata* and *Ostericum koreanum*, to neutralize peroxyl radicals using the total oxyradical scavenging capacity (TOSC) assay. Peroxyl radicals were generated from thermal homolysis of 2,2'-azobis(2-methylpropionamidine) dihydrochloride, which oxidize α-keto-γ-methiolbutyric acid to yield ethylene, and the TOSC of the substances tested is quantified from their ability to inhibit ethylene formation. Extracts from *S. miltiorrhiza*, *M. obovata* and *P. lobata* were determined to be potent peroxyl radical scavenging agents with a specific TOSC (sTOSC) being at least three-fold greater than that of glutathione. Major constituents of the three plants, tanshinone, cryptotanshinone, 15,16-dihydrotanshinone, syringin, honokiol, magnolol, daidzein, puerarin and genistein, were examined for the antioxidant potential toward peroxyl radical. Puerarin and genistein were shown to have μM sTOSCs at least ten-fold greater than sTOSC of glutathione. Daidzein, syringin and honokiol demonstrated the peroxyl radical scavenging capacity comparable to that of glutathione. The implication of peroxyl radical in lipid peroxidation and other cellular damage suggests a possible protective role for the extracts and isolated components in oxidative stress caused by this reactive oxygen species.

Key words: Reactive oxygen species, Peroxyl Radical, Antioxidant, Total oxyradical scavenging capacity

Selected by Editors

INTRODUCTION

Reactive oxygen species (ROS) are generated constantly in aerobic organisms as an inevitable consequence of the coupling of oxidative phosphorylation of ADP with reduction of molecular oxygen to water. Other sources of ROS production include microsomal and mitochondrial electron transport, active phagocytosis, and the activity of several enzymes, e.g. xanthine oxidase, tryptophan dioxygenase, diamine oxidase, prostaglandin synthase, guanyl cyclase and glucose oxidase (Asada et al., 1974; Fridovich, 1978; Winston and Cederbaum, 1983; Cadenas et al., 1984). Xenobiotics and environmental pollutants may increase the intracellular formation of ROS through the Fenton reaction involving trace metals such as iron and copper (Halliwell and Gutteridge, 1984) or redox cycling of organic compounds (Kappus, 1986). Intracellular production of ROS does not necessarily result in cellular toxicity, but oxidative stress will occur when the balance between ROS formation and antioxidant defenses is disturbed. Oxidative stress has been implicated in several cellular toxicity processes, such as damage to proteins, enzyme inactivation, peroxidation of lipid membranes, DNA alteration and various pathologies including chemical carcinogenesis, heart disease, reperfusion injuries, rheumatoid arthritis, inflammation and aging (Cohen and d'Arcy Doherty,

Correspondence to: Young Chul Kim, College of Pharmacy, Seoul National University, Seoul 151-742, Korea
Tel: 82-2-880-7852, Fax: 82-2-871-7453
E-mail: youckim@snu.ac.kr

1987; Cutler, 1991).

The main ROS produced in these cellular processes comprise superoxide anion, hydrogen peroxide, hypochlorous acid, hydroxyl radical, peroxyl radical, alkoxyl radical and peroxynitrite. All of these species are strong oxidants, however, their reactivity toward biological macromolecules varies greatly; hydroxyl radical and superoxide anion are regarded as the most potent and weakest oxidant, respectively (Halliwell and Aruoma, 1991). Peroxyl radical formed via the reaction of carbon-centered radical with oxygen is a biologically relevant active species because of its likelihood to damage cellular constituents. Moreover, the pathological effects of peroxyl radicals have received much attention in connection with the chain-propagation mechanism of lipid peroxidation.

Recently there has been increasing interest in identifying natural antioxidants in plants to protect the human body from an assault of free radicals and to retard the progress of numerous chronic diseases associated with oxidative stress (Rice-Evans et al., 1996; Weisburger, 1999). In this study we determined the peroxyl radical scavenging capacity of extracts and active components isolated from nine herbal plants, *Poncirus trifoliata, Astragalus membranaceus, Magnolia obovata, Salvia miltiorrhiza, Angelica dahurica, Cornus officinalis, Cnidium officinale, Pueraria lobata,* and *Ostericum koreanum*. These plants have long been used for various therapeutical purposes in traditional medicine in East Asia. The claimed therapeutic efficacies of the selected plants are diverse, including anti-inflammation, anticancer, immunomodulation, hepatoprotection and anti-diabetic actions. The mechanisms underlying their beneficial effects are still unclear, but mostly suggested to be associated with antioxidant actions conferred by the ingredients in these medicinal plants. However, to characterize a substance as an antioxidant, its interaction with an oxygen species directly responsible for oxidative damage should be assessed. Some of these plants have been shown to scavenge superoxide and hydroxyl radical (Wenli et al., 2004; Youwei et al., 2008), and to interact with nitric oxide (Lee et al., 2005). However, extensive literature survey reveals that studies evaluating their antioxidant ability against peroxyl radical are scarce. Also there are several substances in these plants suggested to have significant antioxidant activities (Hu et al., 2007; Dikalov et al., 2008), but detailed information regarding the peroxyl radical scavenging capacity of the proposed major active components is still lacking.

Herein, we report on the antioxidant potential of extracts and isolated components from the selected medicinal plants determined by the total oxyradical scavenging capacity (TOSC) assay. The TOSC assay is based on the reaction between peroxyl radical and α-keto-γ-methiolbutyric acid (KMBA), which results in generation of ethylene. In the light of the above, it was of interest to determine the peroxyl radical scavenging capacity of antioxidants from the medicinal plants in this assay system and assign quantifiable values to their antioxidant capability.

MATERIALS AND METHODS

Materials

KMBA was purchased from Sigma Chemical Co.; 2,2'-azobis-aminopropane (ABAP) was obtained from Wako Pure Chemical Co. The 80% ethanol extracts from nine medicinal plants, *P. trifoliata, A. membranaceus, M. obovata, S. miltiorrhiza, A. dahurica, C. officinalis, C. officinale, P. lobata* and *O. koreanum*, were all provided by one of the authors (Y.S. Kim). The methods for isolation and purification of components in *M. obovata* (magnolol, honokiol, syringin), *P. lobata* (daidzein, puerarin, genistein), and *S. miltiorrhiza* (tanshinone I, crypto tanshinone, 15-16-dihydrotanshinone) were described elsewhere (Park et al., 2007; Cho et al., 2008; Jeon et al., 2008). All the other reagents and chemicals used in this study were of analytical reagent grade or better.

Total Oxyradical Scavenging Capacity (TOSC) assay

A slight modification of the method developed by Winston et al. (1998) was used to determine the TOSCs of the antioxidants. Peroxyl radicals were generated by thermal homolysis of ABAP. The incubation mixture consisted of 0.2 mM KMBA and 20 mM ABAP in 100 mM potassium phosphate buffer, pH 7.4, containing an aliquot of the test substance. Reactions were carried out at 37°C in sealed 10 mL vials in a final reaction volume of 1 mL. Ethylene production was measured by gas chromatographic analysis of a 400 μL aliquot taken directly from headspace of the reaction vial. Samples were collected at intervals of 20 min. Analyses were performed with a Varian 3300 Gas Chromatograph (Varian Instrument Division) equipped with a flame ionization detector and Porapak Q column (Supelco). The oven, injection and FID temperatures were set, respectively, at 60°, 180° and 180°C. Helium was used as the carrier gas at a flow rate of 30 mL/min.

The area under the kinetic curve was integrated from the curve that best fits the experimental points for the ethylene concentration *vs* time of incubation.

TOSC is then quantified according to the Eq. (1), where ∫SA and ∫CA are the integrated areas for the sample and control reactions, respectively. Thus, a sample that displays no peroxyl radical scavenging capacity would give an area identical to the control reaction making the (∫SA/∫CA) equal to one and hence a corresponding TOSC value of zero. Conversely, as ∫SA approaches 0 the hypothetical TOSC approaches 100.

$$\text{TOSC} = 100 - \left(\frac{\int \text{SA}}{\int \text{CA}} \times 100\right) \quad (1)$$

Specific μg or μM TOSC (sTOSC) values were obtained from the linear regression lines for the experimental TOSC *vs* antioxidant concentration curves (see Fig. 1). Relative TOSC (rTOSC) values were calculated as shown in the Eq. (2) by dividing the sTOSC of the antioxidants tested by the sTOSC obtained for glutathione (GSH), thus establishing a scale based on GSH equivalents.

$$\text{rTOSC} = \frac{\text{sTOSC (antioxidant)}}{\text{sTOSC (GSH)}} \quad (2)$$

RESULTS

We determined the peroxyl radical scavenging capacity of extracts from nine herbal plants, *P. trifoliata, A. membranaceus, M. obovata, S. miltiorrhiza, A. dahurica, C. officinalis, C. officinale*, *P. lobata* and *O. koreanum* in comparison with that of GSH and ascorbic acid using the TOSC assay. A representative ethylene generation curve for the reaction with different amounts of the extract from *M. obovata* is shown in Fig. 1. A sTOSC value was derived from the slope of linear portion of TOSC *vs* the extract concentration plot (Fig. 1 insert). Here it can be seen that the extract from this plant inhibits ethylene production in a concentration-dependent manner.

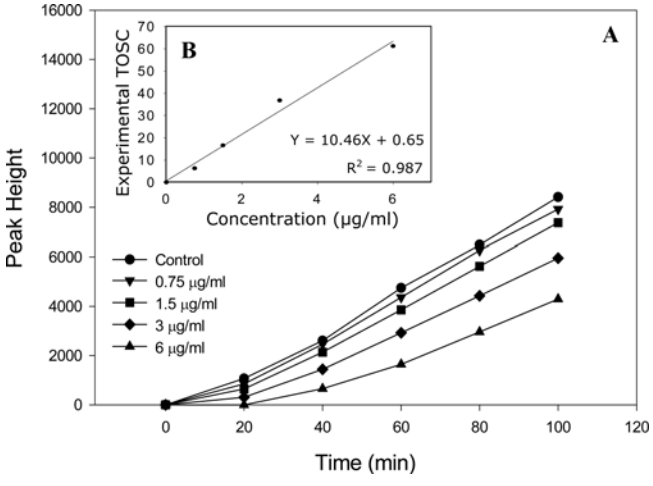

Fig. 1. (**A**) Time-course of ethylene generation from KMBA oxidation upon thermal homolysis of APAP in the presence of different concentrations of the extract from *M. obovata.* (**B**) Linear regression plot for the experimental TOSC values *vs* concentrations of *M. obovata.*

Extracts from all the plants tested in this study were shown to afford substantial antioxidant protection against KMBA oxidation by peroxyl radicals generated during thermal homolysis of ABAP. The slope and intercept of the regression lines were calculated from the linear portion of TOSC *vs* amount of the extracts (Table I). The sTOSC and rTOSC values are summarized in Table II. The rTOSC were expressed relative to GSH, an endogenous tripeptide that has the most important role in antioxidant defense in the body. The extracts of *M. obovata, S. miltiorrhiza* and *P. lobata* showed sTOSC values significantly greater than that of GSH. Especially, *M. obovata* demonstrated the peroxyl radical scavenging capacity as high as

Table I. Linear regression slopes and intercepts for the TOSC values *vs* concentrations of herbal extracts

	Y-int	Slope	R^2
Glutathione	5.83	1.71	0.947
Ascorbic acid	1.47	10.99	0.992
Poncirus trifoliata	3.84	0.58	0.984
Astragalus membranaceus	-0.09	0.70	0.984
Magnolia obovata	0.65	10.46	0.987
Salvia miltiorrhiza	0.32	6.31	0.992
Angelica dahurica	4.84	0.34	0.970
Pueraria lobata	2.31	5.24	0.983
Cnidium officinale	-3.11	1.58	0.978
Ostericum koreanum	3.70	1.35	0.976
Cornus officinalis	-0.01	0.74	0.985

The slope and intercept of the regression line were calculated from linear portion of the curve for the experimental TOSC values toward peroxyl radical *vs* the amounts of the extract used.

Table II. sTOSC (per μg) and rTOSC of nine herbal extracts toward peroxyl radical

	sTOSC	rTOSC
Glutathione	1.71	1
Ascorbic acid	10.99	6.43
Poncirus trifoliata	0.58	0.34
Astragalus membranaceus	0.70	0.41
Magnolia obovata	10.46	6.12
Salvia miltiorrhiza	6.31	3.69
Angelica dahurica	0.34	0.20
Pueraria lobata	5.24	3.06
Cnidium officinale	1.58	0.92
Ostericum koreanum	1.35	0.79
Cornus officinalis	0.74	0.43

Table III. Linear regression slopes and intercepts for the TOSC values *vs* concentrations of selected components

	Y-int	Slope	R^2
Glutathione	5.83	1.71	0.947
Ascorbic acid	1.47	10.99	0.992
Magnolia obovata			
Syringin	1.82	2.04	0.980
Honokiol	3.19	1.75	0.977
Magnolol	0.23	1.10	0.997
Salvia miltiorrhiza			
Tanshinone I	0.64	0.20	0.875
Cryptotanshinone	3.10	0.46	0.840
15,16-Dihydrotanshinone	1.88	0.63	0.933
Pueraria lobata			
Daidzein	1.09	2.55	0.992
Puerarin	9.49	10.88	0.901
Genistein	4.52	27.15	0.923

The slope and intercept of the regression line were calculated from linear portion of the curve for the experimental TOSC values toward peroxyl radical *vs* the amounts of the isolated component used.

six-fold of the value provided by GSH, which was followed by *S. miltiorrhiza* and *P. lobata*. The μg sTOSC of ascorbic acid was equal to that of *M. obovata*, and markedly greater than the sTOSCs of the other extracts. Since the extracts from *M. obovata, S. miltiorrhiza* and *P. lobata* demonstrated the peroxyl radical scavenging capacity significantly greater than the others, we proceeded to determine the antioxidant potential of the components that are suggested to be associated with biological activities of the three medicinal plants.

Syringin, honokiol and magnolol were probed as the active components in *M. obovata*; tanshinone, cryptotanshinone and 15,16-dihydrotanshinone in *S. miltiorrhiza*; daidzein, puerarin and genistein in *P. lobata*. The slopes and intercepts of the regression lines from TOSC *vs* amount of each extract curves are listed in Table III. Table IV shows sTOSC and rTOSC, per μg and per μM, of the components isolated from the three plants. Among the substances examined, genistein revealed the greatest sTOSC and rTOSC per μg followed by puerarin originated from the same plant, *P. lobata*. In contrast, the μM sTOSC toward peroxyl radical was found to be highest for puerarin. This value is 16-fold greater than sTOSC of GSH, and 4.5-fold greater than that of ascorbic acid. Genistein displays the rTOSC value of 13.85 per μM relative to GSH. Syringin and honokiol, major active components derived from *M. obovata*, revealed the peroxyl radical scavenging capacity comparable to that of GSH. Other components did not exhibit significant antioxidant ability to neutralize peroxyl radicals.

DISCUSSION

Generation of peroxyl radicals is common to all organisms and may be especially high under oxidative stress. The scavenging of peroxyl radicals is a key step in the prevention of lipid peroxidation by breaking the chain of propagation of free radical reactions; thus, prompting the study of many compounds with respect to their ability to scavenge these radicals (Wayner et al., 1985; DeLange and Glazer, 1989; Cao et al., 1993). The TOSC assay has been proven effective in the determination of peroxyl radical scavenging capacity

Table IV. sTOSC and rTOSC of the isolated components toward peroxyl radical

	sTOSC	rTOSC	sTOSC	rTOSC
	(per μg)		(per μM)	
Glutathione	1.71	1	0.53	1
Ascorbic acid	10.99	6.43	1.93	3.64
Magnolia obovata				
Syringin	2.04	1.19	0.73	1.38
Honokiol	1.75	1.02	0.47	0.89
Magnolol	1.10	0.64	0.29	0.55
Salvia miltiorrhiza				
Tanshinone I	0.20	0.12	0.08	0.15
Cryptotanshinone	0.46	0.27	0.14	0.26
15,16-Dihydrotanshinone	0.63	0.37	0.17	0.32
Pueraria lobata				
Daidzein	2.55	1.49	0.65	1.23
Puerarin	10.88	6.36	8.57	16.16
Genistein	27.15	15.88	7.34	13.85

of a homologous series of bioflavonoids (Dugas et al., 2000), apple tissue extracts (Eberhardt et al., 2000), various biogenic and synthetic antioxidants (Winston et al., 1998), cytosolic and microsomal fractions of rat and marine organisms (Regoli et al., 2000; Kwon et al., 2009a; Kim et al., 2009), and a variety of commercial beverages (Kwon et al., 2009b). The present report shows the adequacy of this assay in the assignment of a quantifiable parameter (the TOSC value) for the relative peroxyl radical scavenging capacity of the extracts and active components isolated from nine medicinal plants widely used in East Asia.

The role of antioxidants in disease prevention is a topic of increasing research interest. In particular, the flavonoids and polyphenolic compounds in various plants have received significant attention for their potential protective role in oxidative stress-mediated diseases. We have been studying the antioxidative effects of various medicinal plants and their therapeutic implications in chronic diseases, and herein, report on the peroxyl radical scavenging capacity of the extracts and active components derived from nine medicinal plants. All of the extracts prepared from these plants showed considerable peroxyl radical scavenging ability to neutralize peroxyl radical in the TOSC assay. Especially, the extracts of *M. obovata, S. miltiorrhiza* and *P. lobata* showed significantly greater sTOSC values in comparison with GSH, the major endogenous antioxidant in animal life. The extract from *M. obovata* demonstrated the peroxyl radical scavenging capacity almost as high as that of ascorbic acid. In the meantime, the extracts from other plants tested showed much smaller sTOSC values. Therefore, we selected the three medicinal plants for examination of antioxidant components in the extracts.

The stem bark of *M. obovata* (Magnoliaceae) has long been used in traditional medicine for treatment of gastrointestinal disorders, anxiety and allergic diseases (Fujita et al., 1972). Previous chemical studies have revealed neolignans, sesquiterpenes, sesquiterpene-neolignans, phenylpropanoids and alkaloids as the major constituents, and among them, syringin, honokiol and magnolol are suggested to be responsible for its biological activity. The root of *S. miltiorrhiza* Bunge (Labiatae), according to the therapeutic theory of oriental herb medicine, is effective in alleviating blood circulation and anti-inflammation (Chan et al., 2004). Heterocyclic quinones such as tanshinone I, cryptotanshinone and 15,16-dihydrotanshinone are known to be the active components. Isoflavonoids such as puerarin, daidzein and genistein are frequently suggested to account for the chemoprevention, antioxidation and cardioprotection activities associated with the root extract of *P. lobata* Ohwi (Leguminosae) (Chiang et al., 2005). Thus, we proceeded to determine the peroxyl radical scavenging capacity of these substances as the active components in the three plants as alluded to above.

The components isolated from the three plants showed a large difference in the antioxidant potential as determined by the TOSC assay. Generally the substances from *S. miltiorrhiza* demonstrated weaker radical scavenging capacity than the others. The μg sTOSCs for tanshinone I, cryptotanshinone and 15,16-dihydrotanshinone were approximately one half or less of the μg sTOSC for GSH, which suggests that some other components in the same plant, but not tested in this study, would better account for the high peroxyl radical scavenging capacity of the extract prepared from *S. miltiorrhiza*. It is also implied that the peroxyl radical scavenging capacity may not play a critical role in the biological effects of these substances which are frequently suggested to be the active components in this plant. Among the three components derived from *M. obovata*, syringin showed the greatest peroxyl radical scavenging capacity, followed by honokiol and magnolol. The μg sTOSC for honokiol was essentially that for GSH (1.75 *vs* 1.71), however, the antioxidant potential of magnolol was significantly less than that of honokiol, a polyphenol with similar chemical structure. The greatest antioxidant capacity was demonstrated by the constituents of *P. lobata*. The sTOSC for genistein was 27.15 per μg, a value almost 16-fold greater than the sTOSC for GSH or 2.5-fold greater than that for ascorbic acid. Puerarin, a major isoflavonoid in *P. lobata*, also showed a μg sTOSC value 6-fold greater than that of GSH, but its aglycon, daidzein, was only slightly more potent than GSH per μg basis. However, a μM sTOSC value was greatest for puerarin, revealing the peroxyl radical scavenging capacity 16 times more potent than that of GSH. The presence of these isoflavonoids most probably plays a significant role in the expressed antioxidant ability of the extract from *P. lobata*. Lupeol, a pentacyclic triterpene, also isolated from *P. lobata* and suggested to be an active component of this herbal plant, has only negligible antioxidant ability toward peroxyl radical (data not shown).

Taken as a whole, among the extracts prepared from the nine selected medicinal plants, *S. miltiorrhiza*, *M. obovata* and *P. lobata* were shown to be potent peroxyl radical scavenging agents in the TOSC assay. Substances frequently suggested to be the active components in these plants were examined for their antioxidant potential toward peroxyl radical. Of the nine substances isolated from the plants, puerarin and

genistein revealed the greatest sTOSC values per μM, more than 10 times larger than the sTOSC of GSH. In the light of the potential role of peroxyl radical as a mediator of tumor initiation and promotion (Marnett, 1987), the radical scavenging capacity of these natural substances suggests their possible therapeutic role in cancer prevention as well as in other chronic diseases that may be linked to oxidative stress.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Korea Food and Drug Administration for "Studies on the Identification of the Efficacy of Biologically Active Components and Biomarker Genes from Oriental Herbal Medicines" (2008).

REFERENCES

Asada, K., Kiso, K., and Yoshikawa, K., Univalent reduction of molecular oxygen by spinach chloroplasts on illumination. *J. Biol. Chem.*, 249, 2175-2181 (1974).

Cadenas, E., Boveries, A., and Chance, B., Low-level chemiluminescence of biological systems, In Pryor, W.A. (Ed.). Free Radicals in Biology. Academic Press, San Diego, pp. 211-242, (1984).

Cao, G., Alessio, H. M., and Cutler, R. G., Oxygen-radical absorbance capacity assay for antioxidants. *Free Radic. Biol. Med.*, 14, 303-311 (1993).

Chan, K., Chui, S. H., Wong, D. Y. L., Ha, W. Y., Chan, C. L., and Wong, R. N. S., Protective effects of Danshensu from the aqueous extract of Salvia miltiorrhiza (Danshen) against homocysteine-induced endothelial dysfunction. *Life Sci.*, 75, 3157-3171 (2004).

Chiang, H. M., Fang, S. H., Wen, K. C., Hsiu, S. L., Tsai, S. Y., Hou, Y. C., Chi, Y. C., and Chao, P. D., Life-threatening interaction between the root extract of Pueraria lobata and methotrexate in rats. *Toxicol. Appl. Pharmacol.*, 209, 263-268 (2005).

Cho, S. Y., Lee, J. H., Bae, K. H., Kim, Y. S., and Jeong, C. S., Anti-gastric effects of magnolol and honokiol from the stem bark of *Magnolia obovata. Biomol. Ther.*, 16, 270-276 (2008).

Cohen, G. M. and d'Arcy Doherty, M., Free radical mediated cell toxicity by redox cycling chemicals. *Br. J. Cancer*, Suppl., 8, 46-52 (1987).

Cutler, R. G., Recent progress in testing the longevity determinant and dysdifferentiation hypotheses of aging. *Arch. Gerontol. Geriatr.*, 12, 75-98 (1991).

DeLange, R. J. and Glazer, A. N., Phycoerythrin fluorescence-based assay for peroxy radicals: a screen for biologically relevant protective agents. *Anal. Biochem.*, 177, 300-306 (1989).

Dikalov, S., Losik, T., and Arbiser, J. L., Honokiol is a potent scavenger of superoxide and peroxyl radicals. *Biochem. Pharmacol.*, 76, 589-596 (2008).

Dugas, A. J. Jr., Castaneda-Acosta, J., Bonin, G. C., Price, K. L., Fischer, N. H., and Winston, G. W., Evaluation of the Total Peroxyl Radical-Scavenging Capacity of flavonoids: structure-activity relatioships. *J. Nat. Prod.*, 63, 327-331 (2000).

Eberhardt, M. V., Lee, C. Y., and Rui, H. L., Antioxidant activity of fresh apples. *Nature*, 405, 903-904 (2000).

Fridovich, I., The biology of oxygen radicals. *Science*, 201, 875-880 (1978).

Fujita, M., Itokawa, H., and Sashida, Y., Honokiol, a new phenolic compound isolated from the bark of *Magnolia obovata* Thunb. *Chem. Pharm. Bull.*, 20, 212-213 (1972).

Halliwell, B. and Aruoma, O. I., DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Lett.*, 281, 9-19 (1991).

Halliwell, B. and Gutteridge, J. M. C., Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.*, 219, 1-14 (1984).

Hu, C., Yuan, Y. V., and Kitts, D. D., Antioxidant activities of the flaxseed lignan secoisolariciresinol diglucoside, its aglycone secoisolariciresinol and the mammalian lignans enterodiol and enterolactone *in vitro. Food Chem. Toxicol.*, 45, 2219-2227 (2007).

Jeon, S. J., Son, K. H., Kim, Y. S., Choi, Y. H., and Kim, H. P., Inhibition of prostaglandin and nitric oxide production in lipopolysaccharide-treated RAW 264.7 cells by tanshinones from the roots of *Salvia miltiorrhiza* bunge. *Arch. Pharm. Res.*, 31, 758-763 (2008).

Kappus, H., Overview of enzyme systems involved in bioreduction of drugs and in redox cycling. *Biochem. Pharmacol.*, 35, 1-6 (1986).

Kim, S. K., Seo, J. M., Chae, Y. R., Jung, Y. S., Park, J. H., and Kim, Y. C., Alleviation of dimethylnitrosamine-induced liver injury and fibrosis by betaine supplementation in rats. *Chem. Biol. Interact.*, 177, 204-211 (2009).

Kwon, D. Y., Jung, Y. S., Kim, S. J., Park, H. K., Park, J. H., and Kim, Y. C., Impaired sulfur-amino acid metabolism and oxidative stress in nonalcoholic fatty liver are alleviated by betaine supplementation in rats. *J. Nutr.*, 139, 63-68 (2009a).

Kwon, D. Y., Choi, K. H., Kim, S. J., Choi, D. W., Kim, Y. S., and Kim, Y. C., Comparison of peroxyl radical scavenging capacity of commonly consumed beverages. *Arch. Pharm. Res.*, 32, 283-287 (2009b).

Lee, Y. S., Han, O. K., Park, C. W., Yang, C. H., Jeon, T. W., Yoo, W. K., Kim, S. H., and Kim, H. J., Pro-inflammatory cytokine gene expression and nitric oxide regulation of aqueous extracted Astragali radix in RAW 264.7 macrophage cells. *J. Ethnopharmacol.*, 100, 289-294 (2005).

Marnett, L. J., Peroxyl free radicals: potential mediators of tumor initiation and promotion. *Carcinogenesis*, 8, 1365-1373 (1987).

Park, J. G., Cheon, H. J., Kim, Y. S., Kang, S. S., Choi, J. S., and Lee, S. M., Hepatoprotective activities of daidzein, geenistein and puerarin in primary cultures rat hepato-

cytes. *Yakhakhoe Chi*, 51, 115-125 (2007).
Regoli, F., Total oxyradical scavenging capacity (TOSC) in polluted and translocated mussels: a predictive biomarker of oxidative stress. *Aquat. Toxicol.*, 50, 351-361 (2000).
Rice-Evans, C. A., Miller, N. J., and Paganda, G., Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.*, 20, 933-956 (1996).
Wayner, D. D., Burton, G. W., Ingold, K. U., and Locke, S., Quantitative measurement of the total peroxyl radical-trapping antioxidant capacity of human plasma by controlled peroxidation. *FEBS Lett.*, 187, 33-37 (1985).
Weisburger, J. H., Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea. *Food Chem. Toxicol.*, 37, 943-948 (1999).
Wenli, Y., Yaping, Z., and Bo, S., The radical scavenging activities of radix puerariae isoflavonoids: a chemiluminescence study. *Food Chem.*, 86, 525-529 (2004).
Winston, G. W. and Cederbaum, A. I., NADPH-dependent production of oxy radicals by purified components of the rat liver mixed function oxidase system. II. Role in microsomal oxidation of ethanol. *J. Biol. Chem.*, 258, 1514-1519 (1983).
Winston, G. W., Regoli, F., Dugas, A. J. Jr., Fong, J. H., and Blanchard, K. A., A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Radic. Biol. Med.*, 24, 480-493 (1998).
Youwei, Z., Jinlian, Z., and Yonghong, P., A comparative study on the free radical scavenging activities of some fresh flowers in southern China. *LWT-Food Sci. Technol.*, 41, 1586-1591 (2008).