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Comparison of Hydroxyl Radical, Peroxyl Radical, and Peroxynitrite Scavenging Capacity of Extracts and Active Components from Selected Medicinal Plants

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The ability of 80% ethanol extracts from five medicinal plants, *Aralia continentalis*, *Paeonia suffruticosa*, *Magnolia denudata*, *Anemarrhena asphodeloides*, and *Schizonepeta tenuifolia*, to neutralize hydroxyl radical, peroxyl radical and peroxynitrite was examined using the total oxyradical scavenging capacity (TOSC) assay. Peroxyl radical was generated from thermal homolysis of 2,2′-azobis(2-methylpropionamidine) dihydrochloride (ABAP); hydroxyl radical by an iron-ascorbate Fenton reaction; peroxynitrite by spontaneous decomposition of 3-morpholinosydnonimine *N*-ethylcarbamide (SIN-1). The oxidants generated react with α-keto-γ-methiolbutyric acid (KMBA) to yield ethylene, and the TOSC of the substances tested is quantified from their ability to inhibit ethylene formation. Extracts from *P. suffruticosa, M. denudata,* and *S. tenuifolia* were determined to be potent peroxyl radical scavenging agents with a specific TOSC (sTOSC) being at least six-fold greater than that of glutathione (GSH). These three plants also showed sTOSCs toward peroxynitrite markedly greater than sTOSC of GSH, however, only *P. suffruticosa* revealed a significant hydroxyl radical scavenging capacity. Seven major active constituents isolated from *P. suffruticosa*, quercetin, (+)-catechin, methyl gallate, gallic acid, benzoic acid, benzoyl paeoniflorin and paeoniflorin, were determined for their antioxidant potential toward peroxynitrite, peroxyl and hydroxyl radicals. Quercetin, (+)-catechin, methyl gallate, and gallic acid exhibited sTOSCs 40~85 times greater than sTOSC of GSH. These four components also showed a peroxynitrite scavenging capacity higher than at least 10-fold of GSH. For antioxidant activity against hydroxyl radical, methyl gallate was greatest followed by gallic acid and quercetin. Further studies need to be conducted to substantiate the significance of scavenging a specific oxidant in the prevention of cellular injury and disease states caused by the reactive free radical species.

***Key words*:** Reactive oxygen species (ROS), Peroxyl radical, Hydroxyl radical, Peroxynitrite, Antioxidant, Total oxyradical scavenging capacity (TOSC)

INTRODUCTION

Aerobic organisms are constantly exposed to reactive oxygen species (ROS) that is generated as an inevitable consequence of the four-electron reduction of molecular oxygen to water coupled with oxidative phosphorylation of ADP. Other well known sources of ROS production include microsomal and mitochondrial electron transport, active phagocytosis, and the activity of several enzymes, such as xanthine oxidase, tryptophan dioxygenase, diamine oxidase, prostaglandin synthase, guanyl cyclase and glucose oxidase (Asada *et al*., 1974; Cadenas *et al*., 1984; Winston and Cederbaum, 1983). In addition, 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 imply tissue injury since damaging effects of ROS to biological tissues are normally counteracted by a complex array of specially adapted enzyme systems and small molecular

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List of Abbreviations: ABAP, 2,2'-azobis(2-methylpropionamidine) dihydrochloride; DTPA, diethylenetriaminepentaacetic acid; GSH, glutathione; KMBA, α-keto-γ-methiolbutyric acid; ROS, reactive oxygen species; SIN-1, 3-morpholinosydnonimine *N*-ethylcarbamide; TOSC, total oxyradical scavenging capacity

weight antioxidants. But oxidative stress will result when the ROS formation exceeds the antioxidant defense capability. Oxidative stress has been implicated in numerous cellular toxicity processes, such as damage to proteins, enzyme inactivation, lipid peroxidation of membranes, DNA alteration (Cohen and d'Arcy Doherty, 1987) and various pathologies including cellular reperfusion injury, chemical carcinogenesis, heart disease, atherosclerosis, rheumatoid arthritis, inflammation and aging (Gey *et al*., 1991; Cutler, 1991).

There has been increasing interest in identifying antioxidant substances originated from plants to protect the human body from an assault of free radicals and to retard the progression of various chronic human diseases associated with oxidative stress (Rice-Evans *et al*., 1996; Weisburger, 1999). The medicinal plants selected in this study, *Aralia continentalis*, *Paeonia suffruticosa*, *Magnolia denudata*, *Anemarrhena asphodeloides* and *Schizonepeta tenuifolia*, have long been used for various therapeutical purposes in traditional medicine in East Asia. The claimed therapeutic efficacies are diverse, including anti-inflammation, anticancer, immunomodulation, hepatoprotection and anti-diabetic actions. With respect to the molecular mechanism underlying the beneficial actions of these medicinal plants, an antioxidant effect has been frequently suggested to have a significant role. However, to characterize a substance as an antioxidant, its interaction with an oxygen species directly responsible for the oxidative damage should be assessed. Some of these plants have been shown to scavenge superoxide and hydroxyl radical (Liu and Ng, 2000; Cai *et al*., 2004; Youwei *et al*., 2008), and to interact with nitric oxide (Liao *et al*., 2007). However, extensive literature survey reveals that studies evaluating their antioxidant ability against different oxygen species are limited.

In this study we evaluated the antioxidant potential of extracts and isolated components from the selected medicinal plants using the total oxyradical scavenging capacity (TOSC) assay. The TOSC assay is based on the reaction between an oxidant and α-keto-γ-methiolbutyric acid (KMBA) that is oxidized to ethylene upon exposure to ROS. We employed three different oxidant generating systems in order to produce peroxynitrite, hydroxyl radical, and peroxyl radical. Thus, the relative efficiency of various antioxidants from these plants could be compared with respect to their ability to eliminate the different oxidants.

MATERIALS AND METHODS

Chemicals and reagents. Ascorbic acid, KMBA, diethylenetriaminepentaacetic acid (DTPA), and reduced glutathione (GSH) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); 2-2′-azo-bis-(2 methylpropionamidine)-dihydrochloride (ABAP) was obtained from Wako Pure Chemical Co. (Osaka, Japan); 3-morpholinosydnonimine *N*-ethylcarbamide (SIN-1) from Alexis Corporation (San Diego, CA, U.S.A.). The 80% ethanol extracts from the five medicinal plants, *A. continentalis*, *P. suffruticosa*, *M. denudata*, *A. asphodeloides* and *S. tenuifolia*, and purified components from *P. suffruticosa* (benzoic acid, benzoyl paeoniflorin, (+)-catechin, gallic acid, methyl gallate, paeniflorin, and quercetin) were provided by Dr. Y.S. Kim at Seoul National University. All the other reagents and chemicals used in this study were of analytical reagent grade or better.

Total oxyradical scavenging capacity (TOSC) assay. A slightly modified method originally developed by Regoli and Winston (1999) was used to determine the TOSCs of the antioxidants. Three oxidant generating systems designed to generate independently peroxyl radical, hydroxyl radical and peroxynitrite were used to compare the antioxidant scavenging capacity of the extracts and active components of the plants. Holding the substrate KMBA at a constant concentration appropriate assay conditions were developed in which each oxidant produced an equivalent yield of ethylene production throughout the reaction time-course. Peroxyl radicals were produced by the thermal homolysis of ABAP, hydroxyl radicals were generated by an iron-ascorbate Fenton reaction, and peroxynitrite was generated from the spontaneous decomposition of SIN-1, a molecule that releases concomitantly nitric oxide and superoxide anion, which rapidly combine to form HOONO. Final assay conditions were: (a) 0.2 mM KMBA, 20 mM ABAP in 100 mM potassium phosphate buffer, pH 7.4 for peroxyl radicals; (b) 1.8 μM Fe^{3+}, 3.6 μM EDTA, 0.2 mM KMBA, 180 μM ascorbic acid in 100 mM potassium phosphate buffer, pH 7.4 for hydroxyl radicals; (c) 0.2 mM KMBA and 80 μM SIN-1 in 100 mM potassium phosphate buffer, pH 7.4 with 0.1 mM DTPA for peroxynitrite.

Reactions were carried out at 37°C in sealed 10 m*l* vials in a final reaction volume of 1 m*l*. Ethylene production was measured by gas chromatographic analysis of a 400 μ*l* aliquot taken directly from headspace of the reaction vial. Samples were collected at intervals of 16 min. Analyses were performed with a Varian 3300 gas chromatograph (Varian Instrument Division, Palo Alto, CA, U.S.A.) equipped with a flame ionization detector and Porapak Q column (Supelco, Bellefonte, PA, U.S.A.). 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 m*l*/min.

The area under the kinetic curve was integrated from the curve that best fits the experimental points for the ethylene concentration vs incubation time. The TOSC is then quantified according to the equation (1), where $\int$SA and $\int$CA are the integrated areas for the sample and control reactions, respectively. Thus, a TOSC value of 0 ($\int$SA / $\int$CA equals to one) indicates a sample with no scavenging capacity (i.e. no inhibition of ethylene formation), while a maximum theo-

retical TOSC value of 100 would correspond to a total inhibition of ethylene formation throughout the assay ($\int$SA equals to zero).

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

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

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

For comparative purposes, the effects of GSH and ascorbic acid, both potent scavengers of ROS in the body, were also determined in these systems.

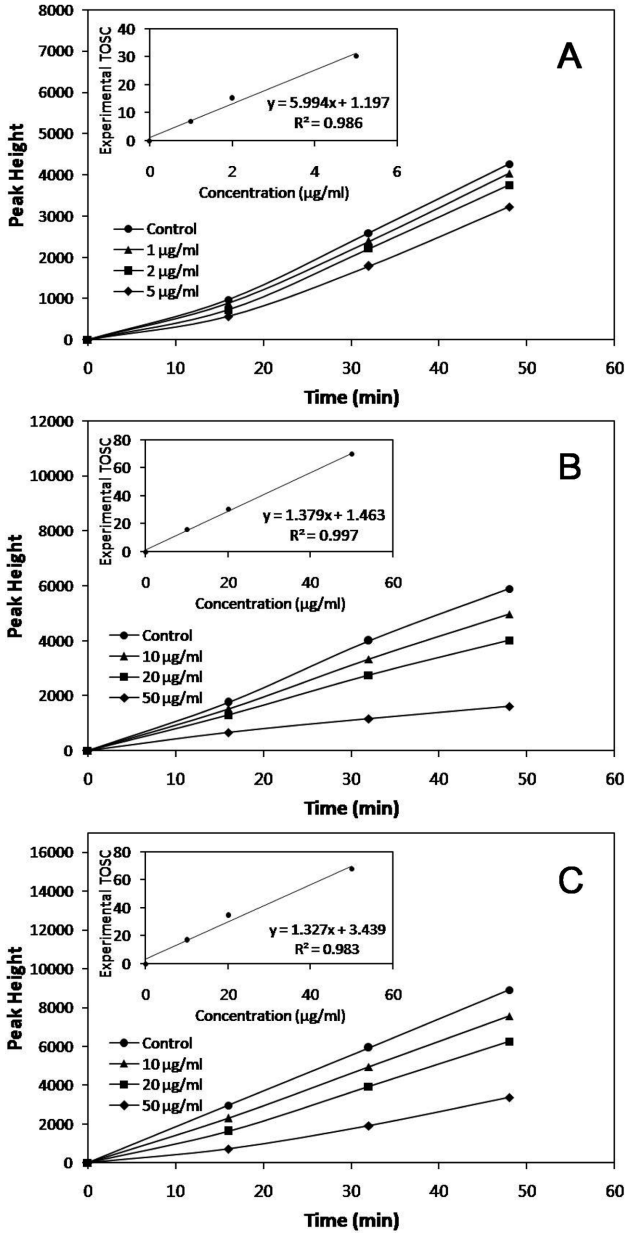

Fig. 1. Time-course of ethylene generation from KMBA oxidation by peroxyl radical (A), hydroxyl radical (B), and peroxynitrite (C), in the presence of different concentrations of *P. suffruticosa*. Each insert shows linear regression plot for the experimental TOSC values vs concentrations of *P. suffruticosa*.

RESULTS AND DISCUSSION

Generation of ROS is common to all living organisms and may be especially high under oxidative stress. The major ROS produced in the cellular processes include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\bullet$), peroxyl radical ($ROO^\bullet$), alkoxyl radical ($RO^\bullet$), and peroxynitrite (HOONO). All of these species are strong oxidants, however, their reactivity toward biological macromolecules varies greatly; hydroxyl radical and superoxide anion, respectively, are generally regarded as the most potent and weakest oxidants (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. However, evaluating the true oxidant scavenging capacity of antioxidants is complicated by the different efficiencies that antioxidants exhibit toward different oxidants. Thus, extending the measure of antioxidant behavior to a wider range of oxidants would be useful to better understand the resistance or susceptibility of a tissue to oxidative stress under different conditions. The TOSC assay was originally designed to evaluate the scavenging capacity of tissues from different marine invertebrates toward peroxyl radical (Regoli *et al.*, 1998; Regoli and Winston, 1998), and later, extended to include the SIN-1 and iron-ascorbate systems to evaluate oxidant scavenging capacity toward peroxynitrite and hydroxyl radical as well (Regoli and Winston, 1999).

The TOSC assay employed in this study has been proven effective in determination of the oxygen radical scavenging capacity of a homologous series of bioflavonoids (Dugas *et al*., 2000), apple tissue extracts (Eberhardt *et al*., 2000), extracts from selected medicinal plants (Kim *et al*., 2010), 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 results show the adequacy of this assay in assignment of a quantifiable parameter (the TOSC

Table 1. Linear regression slopes and intercepts for the TOSC values vs concentrations of plant extracts

	Peroxyl radical			Hydroxyl radical			Peroxynitrite		
	Y-int	Slope	R^2	Y-int	Slope	R^2	Y-int	Slope	R^2
GSH	4.43	2.00	0.970	3.90	0.50	0.986	1.34	0.45	0.974
Ascorbic acid	3.73	13.94	0.963		N.D.		4.04	15.97	0.988
A. continentalis	0.83	2.41	0.950		N.E.		0.29	1.02	0.990
P. suffruticosa	1.20	5.99	0.986	1.46	1.38	0.997	3.44	1.33	0.983
M. denudata	0.33	7.07	0.999		N.E.		0.32	4.83	0.987
A. asphodeloides	–0.21	3.17	0.999		N.E.		0.91	0.56	0.987
S. tenuifolia	3.81	11.81	0.978		N.E.		0.33	2.00	0.999

The slope and intercept of the regression line were calculated from linear portion of the curve for the experimental TOSC values toward peroxyl radical, hydroxyl radical, and peroxynitrite vs the amounts of the plant extract used. N.D.: Not Determined. N.E.: Not Effective.

Table 2. sTOSC (per μg) and rTOSC of five plant extracts toward peroxyl radical, hydroxyl radical, and peroxynitrite

	Peroxyl radical		Hydroxyl radical		Peroxynitrite	
	sTOSC	rTOSC	sTOSC	rTOSC	sTOSC	rTOSC
GSH	2.00	1	0.50	1	0.45	1
Ascorbic acid	13.94	6.97	N.D.		15.97	35.49
A. continentalis	2.41	1.21	N.E.		1.02	2.27
P. suffruticosa	5.99	3.00	1.38	2.76	1.33	2.96
M. denudata	7.07	3.54	N.E.		4.83	10.73
A. asphodeloides	3.17	1.59	N.E.		0.56	1.24
S. tenuifolia	11.81	5.91	N.E.		2.00	4.44

N.D.: Not Determined. N.E.: Not Effective

value) for the relative oxygen radical scavenging capacity of extracts and components isolated from the selected plants toward peroxynitrite, hydroxyl and peroxyl radicals.

In this study we first determined the oxidant scavenging capacity of extracts from the five medicinal plants, *A. continentalis*, *P. suffruticosa*, *M. denudata*, *A. asphodeloides* and *S. tenuifolia*, in comparison with that of GSH and ascorbic acid using the TOSC assay. Fig. 1 illustrates a representative ethylene generation curve from oxidation of KMBA in the oxidant generating systems with different amounts of the extract from *P. suffruticosa*. Comparative time-courses of ethylene production over a 48-min incubation of 0.2 mM KMBA in the peroxyl radical, hrdroxyl radical, and peroxynitrite generating systems were shown (Fig. 1A, B, C). Different amounts of the *P. suffruticosa* extract reduced the ethylene formation proportionally, indicating a linear relationship between TOSC values and the concentration of the plant extract (Fig. 1, insert). It is evident that the *P. suffruticosa* extract inhibits ethylene production from a reaction between KMBA and peroxynitrite, hydroxyl, or peroxyl radicals in a concentration-dependent manner.

All the extracts from the five medicinal plants tested in this study were shown to afford substantial inhibition of KMBA oxidation either by peroxyl radical generated during thermal homolysis of ABAP or by peroxynitrite generated from spontaneous decomposition of SIN-1. The slope and intercept of regression lines were calculated from the linear portion of experimental TOSC vs the extract concentration plot (Table 1). A sTOSC value was then derived from the slope of the linear regression curve. The sTOSC and rTOSC values are summarized in Table 2. The rTOSC values were expressed relative to GSH, an endogenous tripeptide that has the most important role in antioxidant defense in the body. The *S. tenuifolia* extract showed the greatest sTOSC value, almost six times higher than that of GSH, against peroxyl radical, followed by *M. denudata* and *P. suffruticosa*. Likewise, the extracts of these three plants, *M. denudate*, *S. tenuifolia*, and *P. suffruticosa*, showed significantly greater oxidant scavenging capacities toward peroxynitrite than the others. However, for the hydroxyl radical scavenging activity, only the *P. suffruticosa* extract appeared to be effective (Table 1, 2). None of the others showed any appreciable oxidant scavenging capacity toward hydroxyl radical. In fact the *P. suffruticosa* extract demonstrated an antioxidant activity against all the reactive species tested in this study almost three times greater than that of GSH. Therefore, we proceeded to determine the antioxidant potential of the isolated components that are suggested to be associated with biological efficacy of this medicinal plant.

The seven components isolated from *P. suffruticosa* showed a large difference in the antioxidant potential against the three reactive species as determined by the TOSC assay (Table 3). Quercetin exhibited the greatest peroxyl radical scavenging capacity, almost as high as 85 times of GSH,

Table 3. Linear regression slopes and intercepts for the TOSC values vs concentrations of the isolated components from *P. suffruticosa*

	Peroxyl radical			Hydroxyl radical			Peroxynitrite		
	Y-int	Slope	R^2	Y-int	Slope	R^2	Y-int	Slope	R^2
GSH	4.43	2.00	0.970	3.90	0.50	0.986	1.34	0.45	0.974
Ascorbic acid	3.73	13.94	0.963		N.D.		4.04	15.97	0.988
Benzoic acid		N.E.		5.33	1.00	0.913	2.82	0.43	0.874
Benzoyl paeoniflorin		N.E.			N.E.		0.10	0.38	0.999
(+)-Catechin	0.47	118.70	0.994	1.98	0.81	0.980	4.23	13.25	0.953
Gallic acid	0.48	79.05	0.986	0.49	1.30	0.999	–0.83	28.85	0.997
Methyl gallate	0.82	128.90	0.998	3.09	7.11	0.977	7.26	16.17	0.911
Paeoniflorin	1.55	1.25	0.994		N.E.		–0.47	1.95	0.993
Quercetin	–0.41	169.90	0.997	1.04	1.09	0.973	3.26	5.99	0.965

The slope and intercept of the regression line were calculated from linear portion of the curve for the experimental TOSC values toward peroxyl radical, hydroxyl radical, and peroxynitrite vs the concentrations of the isolated component used. N.D.: Not Determined. N.E.: Not Effective.

Table 4. sTOSC and rTOSC of the isolated components from *P. suffruticosa* toward peroxyl radical, hydroxyl radical and peroxynitrite

	Peroxyl radical		Hydroxyl radical		Peroxynitrite	
	sTOSC	rTOSC	sTOSC	rTOSC	sTOSC	rTOSC
	(per μg)		(per μg)		(per μg)	
GSH	2.00	1	0.50	1	0.45	1
Ascorbic acid	13.94	6.97	N.D.		15.97	35.49
Benzoic acid	N.E.		1.00	2.00	0.43	0.96
Benzoyl paeoniflorin	N.E.		N.E.		0.38	0.84
(+)-Catechin	118.70	59.35	0.81	1.62	13.25	29.44
Gallic acid	79.05	39.53	1.30	2.60	28.85	64.11
Methyl gallate	128.90	64.45	7.11	14.22	16.17	35.93
Paeoniflorin	1.25	0.63	N.E.		1.95	4.33
Quercetin	169.90	84.95	1.09	2.18	5.99	13.31

N.D.: Not Determined. N.E.: Not Effective.

followed by methyl gallate, (+)-catechin, and gallic acid (Table 4). The other components, benzoic acid, benzoyl paeoniflorin and paeoniflorin, did not show appreciable peroxyl radical scavenging capacities. Similar, but not identical, trends were noted for the peroxynitrite scavenging capacity. Gallic acid showed sTOSC toward peroxynitrite 60 times higher than that of GSH, followed by methyl gallate, (+)-catechin, and quercetin. In the meantime, methyl gallate showed the greatest sTOSC toward hydroxyl radical that is 14 times of sTOSC provided by GSH. The four active components isolated from *P. suffruticosa*, quercetin (+)-catechin, methyl gallate and gallic acid, revealed significantly greater sTOSCs toward hydroxyl radical as well as peroxynitrite and peroxyl radical compared with the other components in the plant extract. Therefore, it appears that the antioxidant potential of *P. suffruticosa* is provided mostly by these constituents. Also the results clearly indicate that antioxidants exhibit different efficiencies in scavenging different reactive oxygen species.

The antioxidant capacity of specific chemical scavengers and their activities with different oxidants are fundamental in understanding the susceptibility to oxidative stress of biological tissues. However, the complexity of cellular balance between oxidant challenge and antioxidant response often precludes generalization regarding the potential of ROS-mediated impact based on the response to a single oxidant. This is typically true for the assays that measure the ability of antioxidants to scavenge peroxyl radical generated by thermal homolysis of azo compounds (Wayner *et al*., 1985; De Lange and Glazer, 1989; Cao *et al*., 1993; Chevion *et al*., 1997). Because the oxidant scavenging capacity of antioxidants varies with the kinds of oxidants that interact with them, the ability of antioxidants to scavenge alkyl peroxyl radicals, such as those generated from ABAP, should be considerably different toward other oxidants, for example, hydroxyl radical and peroxynitrite and/or its secondary decomposition products (Hogg *et al*., 1992; Squadrito *et al*., 1996). This view is in good agreement with the present results. In conclusion the findings in this study emphasize the importance of better characterization of different antioxidants, either in the pure form or as constituents of more crude fractions, which differ greatly with respect to their

abilities to confer protection against different reactive free radical species. Further studies to elucidate the significance of scavenging a specific oxidant in the prevention of cellular injury and disease states caused by the reactive free radical species are warranted.

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REFERENCES

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

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

Cai, Y., Luo, Q., Sun, M. and Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.*, **74**, 2157-2184.

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

Chevion, S., Berry, E.M., Kitrossky, N. and Kohen, R. (1997). Evaluation of plasma low molecular weight antioxidant capacity by cyclic voltammetry. *Free Radic. Biol. Med.*, **22**, 411-421.

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

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

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

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

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

Gey, K.F., Puska, P., Jordan, P. and Moser, U.K. (1991). Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *Am. J. Clin. Nutr.*, **53**, 326S-334S.

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

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

Hogg, N., Darley-Usmar, V.M., Wilson, M.T. and Moncada, S. (1992). Production of hydroxyl radicals from the simultaneous generation of superoxide and nitric oxide. *Biochem J.*, **281**, 419-424.

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

Kim, S.J., Kwon, D.Y., Kim, Y.S. and Kim, Y.C. (2010). Peroxyl radical scavenging capacity of extracts and isolated components from selected medicinal plants. *Arch. Pharm. Res.*, **33**, 867-873.

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

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

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

Liao, H., Banbury, L.K. and Leach, D.N. (2007). Elucidation of danzhixiaoyao wan and its constituent herbs on antioxidant activity and inhibition of nitric oxide production. *Evid. Based Complement. Alternat. Med.*, **4**, 425-430.

Liu, F. and Ng, T.B. (2000). Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sci.*, **66**, 725-735.

Regoli, F. and Winston, G.W. (1998). Applications of a new method for measuring the total oxyradical scavenging capacity in marine invertebrates. *Mar. Environ. Res.*, **46**, 439-442.

Regoli, F. and Winston, G.W. (1999). Quantification of total oxidant scavenging capacity of antioxidants for peroxynitrite, peroxyl radicals, and hydroxyl radicals. *Toxicol. Appl. Pharmacol.*, **156**, 96-105.

Regoli, F., Nigro, M., Bompadre, S. and Winston, G.W. (2000). Total oxidant scavenging capacity (TOSC) of microsomal and cytosolic fractions from Antarctic, Arctic and Mediterranean scallops: differentiation between three potent oxidants. *Aquat. Toxicol.*, **49**, 13-25.

Regoli, F., Winston, G.W., Mastrangelo, V. and Bompadre, S. (1998). Total oxyradical scavenging capacity in mussel *Mytilus* sp. as a new index of biological resistance to oxidative stress. *Chemosphere*, **37**, 2773-2783.

Rice-Evans, C.A., Miller, N.J. and Paganda, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.*, **20**, 933-956.

Squadrito, G.L., Jin, X., Uppu, R.M. and Pryor, W.A. (1996). Distinguishing reactivities of peroxynitrite and hydroxyl radical. *Methods. Enzymol.*, **269**, 366-374.

Wayner, D.D.M., Burton, G.W., Ingold, K.U. and Locke, S. (1985). Quantitative measurement of the total peroxyl radical-trapping antioxidant capacity of human plasma by controlled peroxidation. *FEBS Lett.*, **187**, 33-37.

Weisburger, J.H. (1999). Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea. *Food. Chem. Toxicol.*, **37**, 943-948.

Winston, G.W. and Cederbaum, A.I. (1983). Oxyradical production by purified components of the liver microsomal mixed

function oxidase system II. Role in ethanol oxidation. *J. Biol. Chem.*, **258**, 1514-1519.

Winston, G.W., Regoli, F., Dugas, A.J., Jr., Fong, J.H. and Blanchard, K.A. (1998). A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Radic. Biol. Med.*, **24**, 480-493.

Youwei, Z., Jinlian, Z. and Yonghong, P. (2008). A comparative study on the free radical scavenging activities of some fresh flowers in southern China. *LWT-Food Sci. Technol.*, **41**, 1586-1591.