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Studies on the antioxidative activity of *Graptopetalum paraguayense* E. Walther

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Abstract

This study was aimed to evaluate the antioxidative activities of water (GWE), 50% ethanolic (GE50), and 95% ethanolic (GE95) extracts of *Graptopetalum paraguayense*. The antioxidant activities, including the radical-scavenging effect, reducing power, and antioxidative effect on Fe/ascorbate-induced lipid peroxidation in a liposome model system, were studied in vitro. The results showed that GWE, GE50 and GE95 possessed antioxidant characteristics including radical scavenging, reducing power, and lipid peroxidation inhibition. It was found that the antioxidative activities of all the extracts increased with increasing concentrations, and the activities correlated with both the total phenol and anthocyanin contents. A comparison of the 50% inhibition concentration (IC_{50}) values of different antioxidative reactions revealed that GE50 was more effective in scavenging α,α-diphenyl-β-picrylhydrazyl (DPPH) radical and showed a higher reducing power than GWE and GE95. However, there were no significant differences ($P > 0.05$) in the lipid peroxidation-prevention effects among the extracts.

Keywords: *Graptopetalum paraguayense*; Antioxidative activities; In vitro

1. Introduction

Reactive oxygen species (ROS), in the form of superoxide anion (O_2^-), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2), are generated in living organisms through many pathways. Accumulation of ROS in aerobic organisms is thought to cause oxidative damage in cells. Oxidative damage is believed to be strongly associated with certain human diseases such as carcinogenesis, mutagenesis, aging and atherosclerosis (Halliwell & Gutteridge, 1984). In addition, ROS are considered to induce lipid peroxidation causing the deterioration of foods (Duthie, 1993). Antioxidants that can neutralize free radicals may be used to protect the human body from diseases and retard lipid rancidity in foods (Kinsella, Frankel, German, & Kanner, 1993; Pryor, 1991). Many synthetic antioxidant components, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have shown toxic and/or mutagenic effects, which have shifted the attention to naturally occurring antioxidants (Branen, 1975; Ito, Fukushima, Hasegawa, Shibata, & Ogiso, 1983). Several research studies have demonstrated that herbal plants contain various potential antioxidants, and these antioxidative components may help the human body to reduce oxidative damages and prevent lipid peroxidation in foods (Cao, Sofic, & Prior, 1996; Liu & Ng, 2000; Zheng & Wang, 2001). Therefore, there is an increasing interest in finding natural herbal plants that exhibit antioxidative activity.

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Graptopetalum paraguayense E. Walther is a traditional Chinese herbal medicine belonging to the Crassvlaceae family. In folk medicine, it has several health benefits such as lowering of blood pressure, alleviating hepatic disorders and diuretic effects. However, little information is available about its antioxidative activity. Therefore, the objectives of this study were to investigate the antioxidative activites of the water (GWE), 50% ethanolic (GE50), and 95% ethanolic (GE95) extracts of *G. paraguayense*. The antioxidative activities, including the radical-scavenging effect, reducing power, and antioxidant effect on Fe/ascorbate-induced lipid peroxidation, were investigated in a liposome model system. In addition, results were compared with those of natural antioxidants, α-tocopherol (α-Toc) and gallic acid (GA). The levels of the total phenol and anthocyanin of the extracts were also determined.

2. Materials and methods

2.1. Chemicals

α,α-Diphenyl-β-picrylhydrazyl (DPPH), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), dihydronicotinamide adenine dinucleotide (NADH), α-tocopherol (α-Toc), L-ascorbic acid (L-AA), gallic acid (GA), thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), $FeCl_3$ and potassium ferricyanide were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Soybean lecithin was obtained from Wako Pure Chemicals Co. (Osaka, Japan). All other chemicals were reagent grade or purer.

2.2. Preparation of the water (GWE), 50% ethanolic (GE50) and 95% ethanolic (GE95) extracts of G. paraguayense

Graptopetalum paraguayense was planted in pots and kept at room temperature. The plant was harvested when the leaves had grown up to 5 cm long. Each 20 g of the plant was extracted with 700 ml of 95% ethanol at 75 °C, 50% ethanol at 85 °C, or with distilled water at 100 °C for 3 h. The decoctions were filtered, and then dried by a vacuum freeze-dryer. The extraction rates of the GWE, GE50 and GE95 were 3.84%, 1.95% and 2.50%, respectively. The extracts were sealed in plastic bottles and stored at −70 °C until used.

2.3. Total phenolic content assay

Total phenol content was analyzed using the Folin–Ciocalteu reagent method (Sato et al., 1996). An aliquot of the extracts (0.5 ml, 0.625 mg/ml) was mixed with 0.5 ml of Folin–Ciocalteu reagent and 0.05 ml of 10% Na_2CO_3, and then the absorbance was measured at 735 nm after 1 h of incubation at room temperature. Gallic acid was used as the standard for the calibration curve, and the total phenolic contents were expressed as mg gallic acid equivalents per gramme of tested extracts.

2.4. The anthocyanin content assay

The anthocyanin contents of the extracts from *G. paraguayense* were analyzed according to the method of Padmavati, Sakthivel, Thara, and Reddy (1997) with slight modification. The extracts were mixed with acidified methanol (1% HCl/methanol) for 2 h at room temperature in the dark, and then centrifuged at 1000 ×*g* for 15 min. The anthocyanin concentration in the supernatant was measured spectrophotometerically at 530 and 657 nm, respectively. The absorbance values for 530 and 657 nm were indicated as A_{530} and A_{657}, respectively. The extinction coefficient of 31.6 $M^{-1}cm^{-1}$ was used to convert absorbance values into concentrations of anthocyanin. The concentration was calculated using the following equation: anthocyanin contents (μmole/g) = $[(A_{530} - 0.33 \times A_{657})/31.6] \times$ [volume (ml)/weight (g)].

2.5. Measurement of superoxide radical-scavenging activity

Effects of the *G. paraguayense* extracts, α-Toc and GA on superoxide radical were determined by the PMS–NADH superoxide generating system (Nikishimi, Rao, & Yagi, 1972). The *G. paraguayense* extracts (0.08–5.0 mg/ml), α-Toc (0.125–1.0 mg/ml) or GA (0.008–0.25 mg/ml) was added to a solution mixture that contained 80 μM PMS, 624 μM NADH, and 200 μM NBT in 0.1 M phosphate buffer, pH 7.4. After 14 min of incubation at room temperature, the absorbance was measured at 560 nm. The abilities to scavenge the superoxide radical were calculated using the following equation: scavenging effect (%) = [1-(absorbance of sample at 560 nm/absorbance of control at 560 nm)] × 100%.

2.6. Measurement of the DPPH radical-scavenging activity

The DPPH radical-scavenging activity of the extracts from *G. paraguayens* was measured according to the method of Chung, Chang, Chao, Lin, and Chou (2002). An aliquot of the extracts (0.1 ml, 0.04–10.0 mg/ml), α-Toc (0.01–0.6 mg/ml), or gallic acid (0.008–0.25 mg/ml) was mixed with the 100 mM Tris–HCl buffer (0.4 ml, pH 7.4), and then added to 0.5 ml of 500 μM DPPH in ethanol (final concentration of 250 μM). The mixture was shaken vigorously and left in the dark at room temperature for 20 min. The absorbance of the mixture was measured spectrophometrically at 517 nm. The ability to scavenge DPPH radical was calculated by the following equation: scavenging effect (%) =

[1 – (absorbance of sample at 517 nm/absorbance of control at 517 nm)] × 100%.

2.7. Measurement of reducing power

The reducing powers of the extracts from *G. paraguayense*, α-Toc and GA were determined according to the method of Yen and Chen (1995). The extracts (0.04–2.5 mg/ml), α-Toc (0.005–0.6 mg/ml) or GA (0.002–0.062 mg/ml) were mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide, and then incubated at 50 °C for 20 min. An equal volume of 1% trichloroacetic acid was added to the mixture to stop the reaction, and then the mixture was centrifuged at 2790 ×*g* for 10 min. The supernatant was mixed with distilled water and 0.1% $FeCl_3$at a ratio of 1:1:2 (v/v/v), and then absorbance was measured at 700 nm. The reducing powers of the tested samples increased with the absorbance values.

2.8. Measurement of antioxidative effect on liposome peroxidation

The antioxidative effects of the extracts from *G. paraguayense* on liposome induced oxidation with $FeCl_3$–ascorbate and lipid peroxidation were quantified, based on MDA production, by the method described by Duh, Du, and Yen (1999). Liposomes were prepared from 300 mg soybean lecithin and 30 ml of 20 mM sodium phosphate buffer (pH 7.4), sonicated using an ultrasonic cleaner for 2 h. A mixture containing 1 ml of sonicated solution (10 mg lecithin/ml), 0.6 ml of sodium phosphate buffer, 0.05 ml of 25 mM $FeCl_3$, 0.05 ml of 25 mM ascorbic acid, and 0.25 ml of the extracts (5–0.31 mg/ml), α-Toc (0.005–0.6 mg/ml) or GA (0.002–0.062 mg/ml) was incubated for 1 h at 37 °C. After incubation, the solution was mixed with TBA (0.4% in 0.2 M HCl) and BHT (0.2% in 95% ethanol) at a ratio of 1:2:0.3 (v/v/v), and then heated at 100 °C for 20 min. After cooling of the mixture, an equal volume of *n*-butanol was added to extract the chromogen in the mixture. The absorbance of the *n*-butanol layer was measured spectrophometrically at 532 nm. The ability to inhibit MDA formation was calculated by the following equation: inhibition effect (%) = [1-(absorbance of sample at 532 nm/absorbance of control at 532 nm)] × 100%.

2.9. Statistical analysis

All data were expressed as means ± SD. Analysis of variance was performed by ANOVA procedures. Duncan's new multiple-range test was used to determine the differences of means, and $P < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Phenolic contents

The total phenolic contents of GWE, GE50, and GE95 were 22.7 ± 1.10, 34.0 ± 1.3 and 11.0 ± 0.4 mg gallic acid equivalent/g, respectively, while the anthocyanin levels of GWE, GE50, and GE95 were 0.54, 1.29, and 0.03 μmol/g, respectively. It was noted that GE50 had the highest total phenol and anthocyanin contents among the three extracts of *G. paraguayense*.

3.2. Scavenging effect on superoxide radical

Superoxide radical is known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to tissue damage and various diseases (Halliwell & Gutteridge, 1999). In a biological system, its toxic role can be eliminated by superoxide dismutase (SOD). There is an increasing interest in finding natural compounds from plants and seeds that can exhibit activity similar to SOD. Fig. 1 shows the dose-response curves of superoxide-scavenging activities of the extracts from *G. paraguayense* by the PMS–NADH superoxide-generating system. It was found that the superoxide-scavenging activities of all the extracts increased with the increase of their concentrations. The results showed that GE95 had the highest superoxide radical-scavenging activity among the extracts, followed by GWE, and GE50 had the lowest. At a concentration of 0.625 mg/ml, the scavenging activity of GE95 reached a plateau of 90.6% while, at the same concentration, the scavenging effects of GWE and GE50 were only 79.1% and 40.4%, respectively. However, the scavenging effect of GA at 0.25 mg/ml was 90.2% and α-Toc showed no detectable superoxide radical-scavenging effect. As compared by the

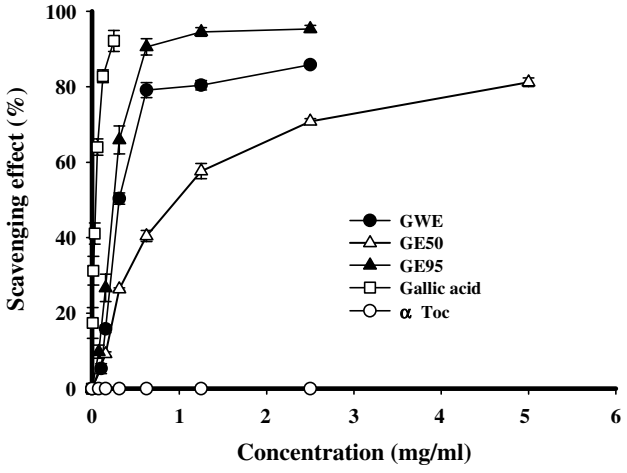

Fig. 1. Scavenging effects of the *G. paraguayense* extracts on superoxide radical. Each value represents means ± SD ($n = 6$).

half- inhibition concentration (IC_{50}) values, the *G. paraguayense* extracts showed less superoxide radical-scavenging effect than GA but much better than α-Toc (Table 1). Although the superoxide radical-scavenging abilities of the extracts were significantly less than that of GA, it was evident that the extracts did show the SOD-like ability.

3.3. Scavenging effect on DPPH radical

The proton-radical scavenging action has been known as an important mechanism of antioxidation. Fig. 2 shows the dose-response curves of α,α-diphenyl-β-picrylhydrazyl (DPPH) radical-scavenging activities of the extracts from *G. paraguayense*. It was found that the radical-scavenging activities of all the extracts increased with the concentrations. The results showed that GE50 had the highest DPPH-scavenging activity among the extracts, followed by GE95, and GWE had the lowest. At a concentration of 1.25 mg/ml, the scavenging activity of GE50 reached a plateau of 85.0% while, at the same concentration, the scavenging effects of GE95 and GWE were only 43.8% and 39.3%, respectively. From the half-inhibition concentrations (IC_{50}) of the extracts, it was also seen that GE50 had the highest DPPH-scavenging activity as shown by the lowest value of IC_{50}, while GWE had the least activity (Table 1). To obtain 80% DPPH-scavenging activity, the concentrations needed for GE50, GE95, GWE, α-tocopherol (α-Toc), and gallic acid (GA) were 1.02, 3.29, 5.00, 0.44, and 0.08 mg/ml, respectively. In other words, to reach a similar extent of DPPH-scavenging effect, the concentration required for *G. paraguayense* extracts was significantly higher than that required for α-Toc or GA. Although the DPPH radical-scavenging abilities of the extracts were significantly below those of α-Toc or GA, it was evident that the extracts did show the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

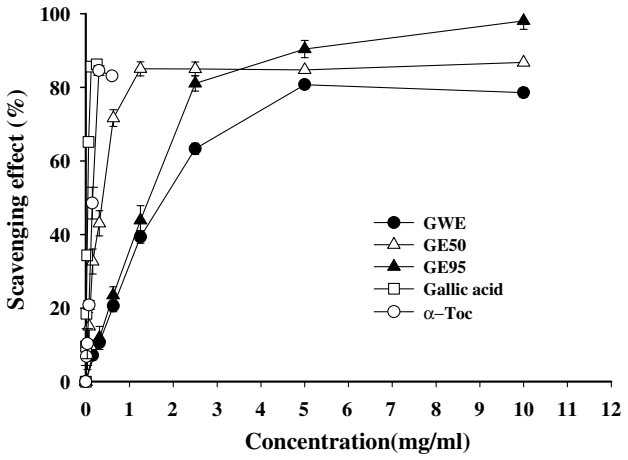

Fig. 2. Scavenging effects of the *G. paraguayense* extracts on DPPH-radical. Each value represents means ± SD ($n = 6$).

3.4. Reducing power of G. paraguayense extracts

In the reducing power assay, the presence of reductants (antioxidants) in the samples would result in the reduction of the Fe^{3+}/ferricyanide complex to its ferrous form. Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's prussian blue at 700 nm. Fig. 3 shows the dose-response curves for the reducing powers of the extracts from *G. paraguayense*. It was found that the reducing powers of all the extracts also increased with the increase of their concentrations. At a dosage of 1.25 mg/ml, GE50 and GWE showed high reducing power values of 1.53 and 1.21, respectively, however, GE95 only exhibited a low reducing power value of 0.40. These results revealed that the extracts of *G. paraguayense* were electron donors and also could react with free radicals, converting them to more stable products and terminating the radical chain reaction (Yen & Chen, 1995). From a comparison of the absorbance at 700 nm, the reducing powers of GA and α-Toc were found to be significantly more pronounced than those of *G. paraguayense* extracts. At a concentration of 0.03 mg/ml, the reducing powers of GA and

Table 1
The 50% inhibition concentration (IC_{50}) for superoxide, DPPH radical-scavenging activity and lipid peroxidation inhibition of the *G. paraguayense* extracts

Extract/antioxidant	Superoxide radical-scavenging activity IC_{50}(mg/ml)	DPPH radical-scavenging activity	Lipid peroxidation inhibition
GWE	0.27 ± 0.01^{b}	1.65 ± 0.08^{a}	1.29 ± 0.20^{a}
GE50	1.07 ± 0.24^{a}	0.29 ± 0.05^{c}	1.49 ± 0.17^{a}
GE95	0.19 ± 0.01^{b}	1.44 ± 0.16^{b}	1.34 ± 0.20^{a}
α-Toc	ND	0.14 ± 0.02^{d}	0.10 ± 0.02^{b}
Gallic acid	0.04 ± 0.01^{c}	0.03 ± 0.00^{e}	0.27 ± 0.02^{b}

Values are given as means ± SD ($n = 6$).
ND, not detectable.
[a–e] Means in the same column followed by different letters are significantly different ($p < 0.01$).

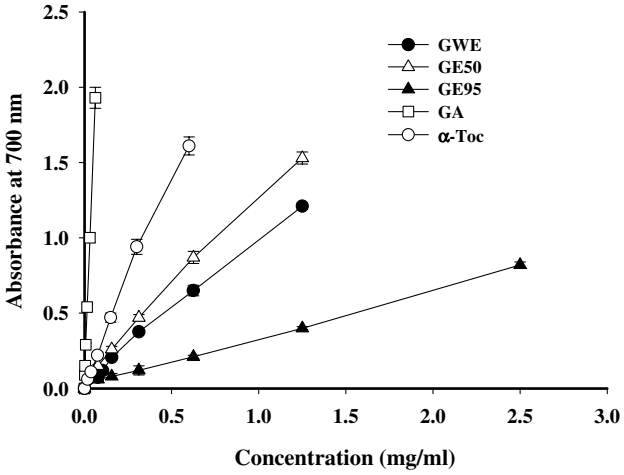

Fig. 3. Reducing power of the *G. paraguayense* extracts with different concentrations. Each value represents means ± SD ($n = 6$). Increase in the absorbance at 700 nm indicates the reducing power.

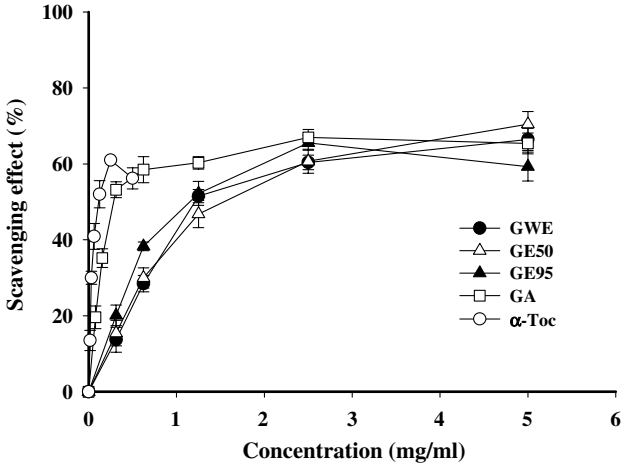

Fig. 4. Inhibition of the *G. paraguayense* extracts on $FeCl_3$/ascorbic acid-induced lipid peroxidation in a liposome model system. Each value represents means ± SD ($n = 6$).

α-Toc reached 1.00 and 0.94, respectively. In other words, GA showed the highest reducing power, followed by α-Toc, GE50, GWE, and GE95, in decreasing order.

In addition, the total phenolic compounds and anthocyanin contents correlated significantly with the DPPH radical-scavenging activity ($r = 0.64$, $P < 0.01$ and $r = 0.72$, $P < 0.01$, respectively) and the reducing power ($r = 0.92$, $P < 0.01$ and $r = 0.96$, $P < 0.01$, respectively) of the *G. paraguayense* extracts. Plant phenols and polyphenolic compounds, such as flavonoids and anthocyanins, are widely distributed in the human diet through vegetables, fruits, beans, cereals, tea, coffee, natural herb and spice extracts, and they have been found to possess significant antioxidant activities (van Acker et al., 1996; Zheng & Wang, 2001) that are associated with lower incidence of and lower mortality rates from certain human diseases (Hertog, Hollman, & van de Putte, 1993; Huang & Ferraro, 1992; Igarashi, Yuriko, & Asako, 2000). In this study, the total phenolic compounds and anthocyanins of the *G. paraguayense* extracts were found to be in the ranges of 11.0–34.0 mg/g (as gallic acid) and 0.03–1.29 μmol/g, respectively; and they may be attributed, in a significant part, at least, to the radical-scavenging activities and reducing powers of the *G. paraguayense* extracts.

3.5. Antioxidant effect on liposome peroxidation

Cellular membranes, which contain abundant phospholipids, such as phosphatidylcholine (lecithin), are major targets subjected to the damage caused by free radicals. Cellular damage due to lipid peroxidation is strongly associated with aging, carcinogenesis and other diseases (Yagi, 1987). In addition, lipid peroxidation is an important deteriorative reaction in the processing and storage of lipid-containing foods (Duthie, 1993). Liposomes have been used extensively as biological models for in vitro lipid peroxidation studies (Duh et al., 1999; Westerlund, Östlund-Lindqvist, Sainsbury, Shertzer, & Sjöquist, 1996). Therefore, we used the liposomal system to evaluate the inhibitory activities of the *G. paraguayense* extracts toward lipid peroxidation.

As shown in Fig. 4, the *G. paraguayense* extracts exhibited dose-dependent inhibitory activities on $FeCl_3$/ascorbate-induced lipid peroxidation in a liposome model. The extracts, at dosages of 0.31–5.0 mg/ml, showed 13.7–70.4% inhibition toward the lipid peroxidation. Among the extracts, there were no significantly differences in their activities in inhibiting the lipid peroxidation, as shown by the small differences of the IC_{50} values (Table 1). As compared with the IC_{50} value, GA was the most effective lipid peroxidation inhibitor, followed by α-Toc, and then the extracts. Although the extracts showed less inhibitory effects against lipid peroxidation than α-Toc or GA, it was evident that the extracts did have anti-lipid peroxidation activities. The mechanism of the inhibitory effects, by which the extracts protect against lipid peroxidation, may involve radical-scavenging and reducing ability. Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acid in the cell membranes that leads to cellular damage. There is now increasing interest in finding natural herbal plants that can prevent, not only lipid peroxidation in foods, but also oxidative damage to living cells. The results presented in this study merit further investigation of the inhibitory effects of the extracts against oxidative damage in vivo.

This study brings attention to the antioxidant potential of *G. paraguayense*; however, the components responsible for the antioxidative activities of the extracts are unknown. Therefore, further research is needed for further isolation and identification of the antioxidative components in the extracts.

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References

van Acker, S. A. B. E., van Den Berg, D. J., Tromp, M. N. J. L., Griffioen, D. H., van Bennekom, W. P., van der Vijgh, W. J. F., et al. (1996). Structural aspects of antioxidant activity of flavanoids. *Free Radical Biology and Medicine, 20*(3), 331–342.

Branen, A. L. (1975). Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *Journal of the American Oil Chemists' Society, 52*(2), 59–63.

Cao, G., Sofic, E., & Prior, R. L. (1996). Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry, 44*(11), 3426–3431.

Chung, Y. C., Chang, C. T., Chao, W. W., Lin, C. F., & Chou, S. T. (2002). Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. *Journal of Agricultural and Food Chemistry, 50*(8), 2454–2458.

Duh, P. D., Du, P. C., & Yen, G. G. (1999). Action of methanolic extract of mung bean hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage. *Food and Chemical Toxicology, 37*(11), 1055–1061.

Duthie, G. G. (1993). Lipid peroxidation. *European Journal of Clinical Nutrition, 47*(11), 759–764.

Halliwell, B., & Gutteridge, J. M. C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *The Biochemical Journal, 219*, 1–4.

Halliwell, B., & Gutteridge, J. M. C. (1999). *Free radicals in biology and medicine*. Oxford: Oxford University Press.

Hertog, M. G. L., Hollman, P. C. H., & van de Putte, B. (1993). Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *Journal of Agricultural and Food Chemistry, 41*(8), 1242–1246.

Huang, M. T., & Ferraro, T. (1992). Phenolic compounds in food and cancer prevention. In M. T. Huang, C. T. Ho, & C. Y. Lee (Eds.), *Phenolic compounds in food and their effects on health II* (pp. 8–34). Washington: American Chemical Society.

Igarashi, K., Yuriko, K., & Asako, T. (2000). Preventive effects of dietary cabbage acylated anthocyanins on paraquat-induced oxidative stress in rats. *Bioscience, Biotechnology, and Biochemistry, 64*(8), 1600–1607.

Ito, N., Fukushima, S., Hasegawa, A., Shibata, M., & Ogiso, T. (1983). Carcinogenicity of butylated hydroxyanisole in F344 rats. *Journal of the National Cancer Institute, 70*, 343–347.

Kinsella, J. E., Frankel, E., German, B., & Kanner, J. (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technology, 47*(4), 85–89.

Liu, F., & Ng, T. B. (2000). Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sciences, 66*(8), 725–735.

Nikishimi, M., Rao, N., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochemical and Biophysical Research Communication, 46*, 849–854.

Padmavati, M., Sakthivel, N., Thara, K. V., & Reddy, A. R. (1997). Differential sensitivity of rice pathogens to growth inhibition by flavonoids. *Phytochemistry, 46*(3), 499–502.

Pryor, W. A. (1991). The antioxidant nutrients and disease prevention-what do we know and what do we need to find out?. *The American Journal of Clinical Nutrition, 53*, S391–S393.

Sato, M., Ramarathnam, N., Suzuki, Y., Ohkubo, T., Takeuchi, M., & Ochi, H. (1996). Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. *Journal of Agricultural and Food Chemistry, 44*(1), 37–41.

Westerlund, C., Östlund-Lindqvist, A. M., Sainsbury, M., Shertzer, H. G., & Sjöquist, P. O. (1996). Characterization of novel indenoindoles. Part I. Structure-activity relationships in different model systems of lipid peroxidation. *Biochemical Pharmacology, 51*(10), 1397–1402.

Yagi, K. (1987). Lipid peroxides and human disease. *Chemistry and Physics of Lipids, 45*, 337–341.

Yen, G. C., & Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry, 43*(1), 27–32.

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry, 49*(11), 5165–5170.