Food Sci. Technol. Res., **14** (2), 205–210, 2008

Note

Antioxidant Activity of Traditional Chinese Medicinal Herbs

Ya-Chi LEE[1], Ai Mey CHUAH[1], Tomoko YAMAGUCHI[2], Hitoshi TAKAMURA[2, 3*] and Teruyoshi MATOBA[2]

[1] *Graduate School of Humanities and Sciences*

[2] *Department of Food Science and Nutrition*

[3] *KYOUSEI Science Center for Life and Nature, Nara Women's University, Kitauoya-nishimachi, Nara 630-8506, Japan*

Received August 28, 2007; Accepted December 23, 2007

Traditional Chinese medicinal herbs have been used for thousands of years to treat numerous ailments. In recent years, these herbs have been the focus of attention due to their medicinal and pharmacological benefits. In the present study, 25 types of herbs commonly used in Chinese medicinal culinary diets were quantitatively investigated for their various antioxidant activities. Using the 1,1-diphenyl-2-picrylhydrazyl high pressure liquid chromatography and 2´-deoxyguanosine oxidation methods, the highest antioxidant activity was found in chrysanthemum (Ju Hua), followed by hawthorn (Shan Zha), licorice root (Gan Cao), hibiscus (Luo Shen Hua), cassia seed (Jue Ming Zi) and Chinese wolfberry (Gou Gi), while poria (Fu Ling) and adlay (Yi Yi Ren) contained the lowest antioxidant activity. The same trend was also observed for total phenolic content using the Folin-Ciocalteu method. Ascorbic acid and tocopherol were only detected in trace amounts, suggesting that phenolic compounds may be the main contributors to herbal antioxidant activities.

Keywords: Chinese medicinal herbs, antioxidant, radical-scavenging activity, polyphenol

Introduction

The presence of excessive amount of free radicals in cells may trigger oxidative stress, which can lead to the manifestation of various diseases such as cancer, cardiovascular disease, immune-system decline, brain dysfunction and cataracts (Ames *et al.*, 1993). However, foods high in antioxidant activities, such as soy foods, green tea, fruits, vegetables, wines and herbs, are known for their ability to act against this oxidative damage by inhibiting the initiation or propagation of the oxidizing chain reactions (Velioglu *et al.*, 1998).

In Chinese society, medicinal herbs are extensively used not only for therapeutic but also culinary purposes. These tonic cuisines comprise dishes prepared primarily for their medicinal value, and draw on the vast array of herbs and botanicals found in the traditional Chinese apothecary. It is believed that a meal of tonic cuisine can restore the body's equilibrium and balance the flow of vital energy, or *qi*. Generally, the principle of "yin-yang" is consulted prior to the selection of Chinese medicinal herbs. The practice of traditional Chinese medicine always emphasizes the importance of keeping harmony between "yin" and "yang", *i.e.*, two opposing components essential in maintaining the balance of the homeostatic system to achieve physical health. It has been suggested that the concept of "yin" and "yang" is somewhat similar to the modern concept of antioxidant-oxidant balance (Ou *et al.*, 2003). "Yang-invigorating" action usually associates with immuno-enhancement and energy generation enhancement, i.e., through the enhancement of the mitochondrial oxidative process (Siu *et al.*, 2004), while "yin-nourishing" action suppresses the symptom of heat-fire or "yang", i.e., preventing the overoxidation process (Ou *et al.*, 2003).

Chinese medicinal herbs show a wide range of anti-cancer, anti-inflammatory, anti-microbial, anti-rheumatic, and immunomodulatory effects (Gu and Brandwein, 1998; Wong *et al.*, 2004). However, their complicated and multiple pharmacological effects were not understood until recently. Based on a recent large-scale research by Cai *et al.* (2004), it was found that traditional Chinese medicinal herbs associated with anti-cancer properties exhibited strong antioxidant activities and contained significantly high levels of phenolic compounds.

*To whom correspondence should be addressed.
E-mail: takamura@cc.nara-wu.ac.jp

Since there are thousands of types of Chinese medicinal herbs available, optimal selection and intake may be important to achieve desirable effects on health. For this purpose, 25 types of commonly consumed traditional Chinese medicinal culinary herbs were screened for their individual radical-scavenging activity using the 1,1-diphenyl-2-picrylhydrazyl high pressure liquid chromatography (DPPH-HPLC) and 2´-deoxyguanosine (2´-dG) oxidation methods, as well as for their total phenolics, ascorbic acid and tocopherol contents.

Materials and Methods

Materials DPPH, L-ascorbic acid, pyrogallic acid, tris (hydroxymethyl)aminomethane (Tris), 1,4-dioxane, dimethyl sulfoxide (DMSO), Folin-Ciocalteu reagent and acetonitrile (HPLC grade) were obtained from Nacalai Tesque Inc. (Kyoto, Japan). 2,2´-Azobis(2-amidinopropane) dihydrochloride (AAPH), 2´-dG and 2-propanol (HPLC grade) were obtained from Wako Pure Chemical Industries (Osaka, Japan). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA). Standard tocopherols with purity of 98% (α-, β-, γ-, and δ-tocopherols) and 2,2,5,7,8-pentamethyl-6-chromanol (PMC) were from Eisai Ltd. (Tokyo, Japan). The water used in this experiment was purified with Milli-Q Labo equipment (Millipore Japan, Tokyo, Japan). All the other reagents were of chemical grade.

Preparation of sample and extracts Dried herbs were purchased from local oriental herbal stores in Kaoshiung, Taiwan, and were lyophilized in liquid nitrogen. The lyophilized samples were then ground into a fine powder with a food processor and kept at -80°C until analysis. Lyophilized sample powders (0.05-0.2 g) were extracted with 2 mL of methanol containing 5% acetic acid. The extracts were centrifuged at 1,500×g for 10 min. The extraction step was repeated three times and the resulting supernatants were combined, and subsequently dried under nitrogen atmosphere. The residue obtained was dissolved in 0.5-2 mL of 90% methanol and filtered with a 0.45-μm filter (Ekicrodisc 3 Syringe Filter, Nihon Pall Ltd., Tokyo, Japan). Analyses of the total phenol, ascorbic acid and DPPH were made after appropriate dilution with 90% methanol. As for the 2´-dG oxidation analysis, the residue was dissolved in DMSO.

Measurement of DPPH radical-scavenging activity Radical-scavenging activity was measured according to the DPPH-HPLC method (Yamaguchi *et al.*, 1998). The activity was expressed as μmol of Trolox equivalent per 100 g dry weight of sample.

Measurement of peroxyl radical-scavenging activity Peroxyl radical-scavenging activity was quantified according to the 2´-dG oxidation method of Sakakibara *et al.* (2002), whereby the suppression of 8-hydroperoxy-2´-deoxyguanosine (8-OOHdG) formation by the herbal extracts was measured. The radical-scavenging activity was evaluated by the difference in suppression of 8-OOHdG formations between a blank and a sample. The activity was expressed as μmol Trolox equivalent per 100 g dry weight of sample.

Measurement of ascorbic acid content Ascorbic acid content was measured according to the method of Kishida *et al.* (1992). The data were expressed as mg per 100 g dry weight of sample.

Measurement of tocopherol content Tocopherol content was measured according to the method of Ueda and Igarashi (1990) with slight modification. The result was expressed as mg tocopherol per 100 g dry weight of sample.

Measurement of total phenolic content Total phenolic content was measured according to the Folin-Ciocalteu method as modified by Singleton and Rossi (1965). The content was expressed as μmol of gallic acid equivalents per 100 g dry weight of sample.

Results and Discussion

Chinese medicinal herbs are used as ingredients in various kinds of teas (*Cha*) and soups (*Tang*). These teas and soups are usually consumed as a concoction of at least two different types of herbs and are named according to the herb that makes up the highest proportion. These tonic diets are commercially available as ready-to-consume teas or soups or are homemade. Among them, white dahlia root soup is a popular traditional remedy for anemia and fatigue, Chinese angelica root soup for hormone regulation, Chinese wild yam soup for indigestion and physical languor, chrysanthemum tea for summer weariness, hibiscus tea for appetite improvement and summer weariness, and cassia seed tea for constipation and improvement of visual acuity.

In order to understand the synergistic effect of the antioxidant activity of these concoctions, the contributions from an individual herb towards the total antioxidant activity warrant an investigation. Table 1 shows the 25 types of Chinese medicinal herbs used in this study. They are classified according to the parts of the plant from which the herbs are derived.

Measurement of radical-scavenging activity The radical-scavenging activity, as measured by DPPH and 2'-dG oxidation methods, of the 25 commercial Chinese medicinal herbs are listed in Table 2. As shown in Table 2, a wide range of antioxidant activity was detected. In general, DPPH method showed higher magnitude of radical-scavenging activity than 2´-dG oxidation method. However, both demonstrated almost similar trends in the magnitude of radical-scavenging activity. Dragland *et al.* (2003), in their research on the antioxidant activity of culinary and medicinal herbs, found a

Table 1. Classification of 25 Chinese medicinal herbs.

Common name	Chinese name	Scientific name	Family	Part used
Adlay	Yi Yi Ren	*Coix lachryma-jobi* L.var. ma-yuen Stapf.	Gramineae	Seed
American ginseng	Xi Yang Shen	*Panax quinquefolius* L.	Araliaceae	Root
Astragalus	Huang Qi	*Astragalus membranaceus* Bge.	Leguminosae	Root
Cassia seed	Jue Ming Zi	*Cassia obtusifolia* L.	Leguminosae	Seed
Chinese angelica root	Dang Gui	*Angelica sinensis* (Oliv.) Diels	Umbelliferae	Root
Chinese date	He Zao	*Ziziphus jujuba* Mill.	Rhamnaceae	Fruit
Chinese foxglove	Shou Di	*Rehmanniae glutinosa* Libosch.	Scrophulariaceae	Root
Chinese wild yam	Shan Yao	*Dioscorea opposita* Thunb.	Dioscoreaceae	Rootstock
Chinese wolfberry	Gou Gi	*Lycium barbarum* L.	Solanaceae	Fruit
Chrysanthemum	Ju Hua	*Chrysanthamum morifolium* Ramat	Compositae	Inflorescence
Cnidii rhizoma	Chuan Going	*Ligusticum chuaxiong* Hort.	Umbelliferae	Rootstock
Codonopsis root	Dang Shen	*Codonopsis pilosula* Nannf.	Campanulaceae	Root
Euryale seeds	Qian Shi	*Euryale ferox* Salib.	Nymphaeaceae	Seed
Ginseng root	Ren Shen	*Panax ginseng* C.A. Mey	Araliaceae	Root
Hawthorn	Shan Zha	*Crataegus pinnatifida* Bge.	Rosaceae	Fruit
Hibiscus	Luo Shen Hua	*Hibiscus sabdariffa* L.	Malvaceae	Calyx
Indian lotus	Lian Zi	*Nelumbo nucifera* Gaertn.	Nymphaeaceae	Seed
Jujube	Hong Zao	*Ziziphus jujuba* Mill.	Rhamnaceae	Fruit
Licorice root	Gan Cao	*Glycyrrhiza uralensis* Fisch.	Leguminosae	Root
Lily bulb	Bai He	*Lilium longiflorum* Thunb.	Liliaceae	Clove
Peong root	He Shou Wu	*Polygonum multiflorum* Thunb.	Polygalaceae	Root
Poria	Fu Ling	*Poria cocos* Wolf	Polyporaceae	Sclerotium
Tremellales	Mu Er	*Tremella fuciformis* Berk.	Tremellaceae	Fungus Body
Varnished conk	Lin Zhi	*Ganoderma lucidum* Karst.	Polyporaceae	Fungus Body
White dahlia root	Bai Shao	*Paeonia lactiflora* Pall.	Ranunculaceae	Root

difference of more than 1000-fold among the various herbs investigated. From our results, chrysanthemum and hawthorn consistently showed the highest activity among the tested samples for both the DPPH and 2´-dG oxidation methods. This is in agreement with the results of Kim and Lee (2005) who identified two new dicaffeoylquinic acids from chrysanthemum that showed strong DPPH radical-scavenging activity. In contrast, adlay and poria showed the weakest antioxidant activity. Hibiscus, Chinese wolfberry, peong root, licorice root, Chinese foxglove and Cnidii rhizoma can be grouped into the category with the next-highest level of antioxidants, although not in the same order for both methods. Anthocyanins found in hibiscus were able to quench 50% of the DPPH free radical at 0.20 mg/mL, indicating that it may prevent living systems from oxidative damage (Wang *et al.*, 2000), thus confirming our result.

The variations in the assays were not surprising, as different methods of evaluation can give varying results depending on the specificity of the free radical being used as a reactant (Wu *et al.*, 2004). DPPH is a stable organic nitrogen radical capable of being reduced to DPPH-H when the odd electron of the DPPH radical is paired with a hydrogen from a radical-scavenging antioxidant. This method of utilizing DPPH as the oxidizing radical is based on the amount of antioxidant required for a 50% decrease in the initial concentration of DPPH radical and the time needed for the DPPH radical concentration to reach a steady state (Sanchez-Moreno *et al.*, 1998). However, in the 2´-dG oxidation method, AAPH produced a molecular oxygen radical via an intermediary AAPH-peroxyl radical with 2´-dG as the target. The forma-

Table 2. DPPH and peroxyl radical scavenging activity, total phenolics, tocopherol and ascorbic cid contents of Chinese medicinal herbs.

Name of herb	DPPH radical scavenging activity (μmol Trolox eq. /100 g DW)[1,2]	Peroxyl radical scavenging activity (μmol Trolox eq. /100 g DW)[1,2]	Total phenolics (μmol gallic acid eq. /100 g DW)[1,2]	Tocopherol (mg/100 g DW)[1,2]	Ascorbic acid (mg/100 g DW)[1,2]
Chrysanthemum	38463 ± 1261	17760 ± 772	13505 ± 1248	15.6 ± 2.2	4.1 ± 0.2
Hawthorn	23624 ± 363	13599 ± 693	11429 ± 766	15.8 ± 1.4	5.7 ± 0.3
Hibiscus	17656 ± 849	11559 ± 897	9336 ± 510	3.6 ± 0.0	3.2 ± 0.4
Cassia seed	13201 ± 1148	8437 ± 789	7135 ± 112	33.5 ± 3.1	23.2 ± 2.3
White dahlia root	9851 ± 268	3493 ± 376	4602 ± 274	1.7 ± 0.0	trace
Peong root	9521 ± 190	6603 ± 150	4580 ± 230	trace	trace
Licorice root	9195 ± 138	13322 ± 705	10064 ± 102	trace	6.1 ± 0.2
Chinese foxglove	6078 ± 398	7207 ± 430	5588 ± 236	6.9 ± 0.3	6.1 ± 0.2
Chinese wolfberry	5614 ± 408	7420 ± 767	6172 ± 793	7.3 ± 1.5	11.5 ± 2.9
Cnidii rhizoma	5152 ± 386	6110 ± 484	4920 ± 282	1.0 ± 0.1	4.0 ± 1.1
Chinese date	3311 ± 81	4031 ± 142	4458 ± 248	trace	7.7 ± 0.6
Jujube	2826 ± 244	3642 ± 285	3125 ± 17	trace	8.6 ± 0.1
Chinese angelica root	1616 ± 55	3498 ± 76	3013 ± 197	trace	3.9 ± 0.1
Varnished conk	1266 ± 17	1206 ± 117	1156 ± 6	trace	trace
Ginseng root	1110 ± 62	2257 ± 48	1935 ± 23	2.7 ± 0.1	5.3 ± 0.5
Euryale seeds	856 ± 24	443 ± 30	410 ± 24	708.3 ± 48.0	trace
Astragalus	807 ± 41	3349 ± 235	1276 ± 77	ND[3]	4.1 ± 0.8
Indian lotus	587 ± 38	746 ± 33	1034 ± 6	2.0 ± 0.1	trace
American ginseng	569 ± 30	2040 ± 27	1020 ± 17	2.2 ± 0.1	2.8 ± 0.2
Lily bulb	352 ± 26	471 ± 49	408 ± 22	trace	trace
Tremellales	247 ± 11	392 ± 17	329 ± 32	ND	ND
Chinese wild yam	217 ± 18	567 ± 57	225 ± 12	trace	ND
Codonopsis root	203 ± 2	1712 ± 132	721 ± 35	8.3 ± 0.5	3.6 ± 0.3
Adlay	173 ± 8	167 ± 24	95 ± 0	1.8 ± 0.0	trace
Poria	37 ± 4	206 ± 33	98 ± 13	ND	ND

[1] DW: Dry weight
[2] Data are mean values ± SD of 3 determinations
[3] ND: Not detected.

tion of 8-OOHdG starts only after the antioxidant source has been fully consumed. Since the kinetics of reactions in these two methods are different, they are also expected to give different results. Moreover, some recent studies demonstrated that the number and position of hydroxyl groups, related glycosylation and other substitutions largely determine the radical-scavenging activities of phenolic compounds (Son and Lewis 2002; Cai *et al.*, 2006). Yokozawa *et al.* (1998) reported that the affinity of DPPH towards flavonoids-like apigenin was low despite its high affinity to tannins. In the 2´-dG oxidation method, this assay was found to be more sensitive than DPPH to flavonoids as well as biological components such as glutathione (Sakakibara *et al.*, 2002). Therefore, the variation and complexity, as well as the possession of hydrophilic and lipophilic properties of polyphenols, might cause the differences in magnitude between the tested samples.

Measurement of total phenolic, tocopherol and ascorbic acid content The total phenolic content of Chinese medicinal herbs were measured along with tocopherol and ascorbic acid content, as shown in Table 2. Chrysanthemum showed the highest phenolic content, followed by hawthorn, licorice root, hibiscus, cassia seed and Chinese wolfberry, whereas poria and adlay showed the lowest phenolic content. This result is consistent with the results obtained for the radical-scavenging activity shown earlier, which indicate that the higher the total phenolic content, the stronger the antioxidant activity. Cai *et al.* (2004) reported that the total phenolic content in aqueous extracts of chrysanthemum, hawthorn and licorice root were 3.16, 1.01 and 0.89 g/100 g dry weight, respectively. The differences in the phenolic content in plant might be affected by harvest season and cultivars, as suggested by Łata *et al.* (2005).

In contrast, only trace amounts of tocopherol and ascorbic acid were observed in the tested samples. With the exception of euryale seed, cassia seed, hawthorn and chrysanthemum, tocopherol contents were below 10 mg/100 g or was not detected at all. As for ascorbic acid content, cassia seed showed the highest ascorbic acid content (~23 mg/100 g), followed by Chinese wolfberry (~12 mg/100 g). In the other

materials, ascorbic acid content was below 10 mg/100 g or was not detected at all. When compared to the total phenolic content, it appeared that tocopherol content and ascorbic acid did not contribute significantly to antioxidant activity. Chinese medicinal herbs are usually sun-dried for long-term preservation. In the original fresh plant, ascorbic acid might be present in higher levels, but the sun-drying treatment might have been overly detrimental to its stability. Oboh and Akindahunsi (2004) revealed that sun drying of green leafy vegetables caused a significant decrease in the vitamin C content. In addition, Goffman and Möllers (2000) observed no tocopherol degradation in intact rapeseed oil, except for a slight decrease in seeds after incubation at 40 °C and after exposure to air. These studies indicated that ascorbic acid is unstable and might be reduced markedly as compared to tocopherol and phenolic compounds after sun-drying treatment. Therefore, only a trace amount of ascorbic acid was detected in the tested samples. However, the tocopherol content of euryale seed remained at a high level.

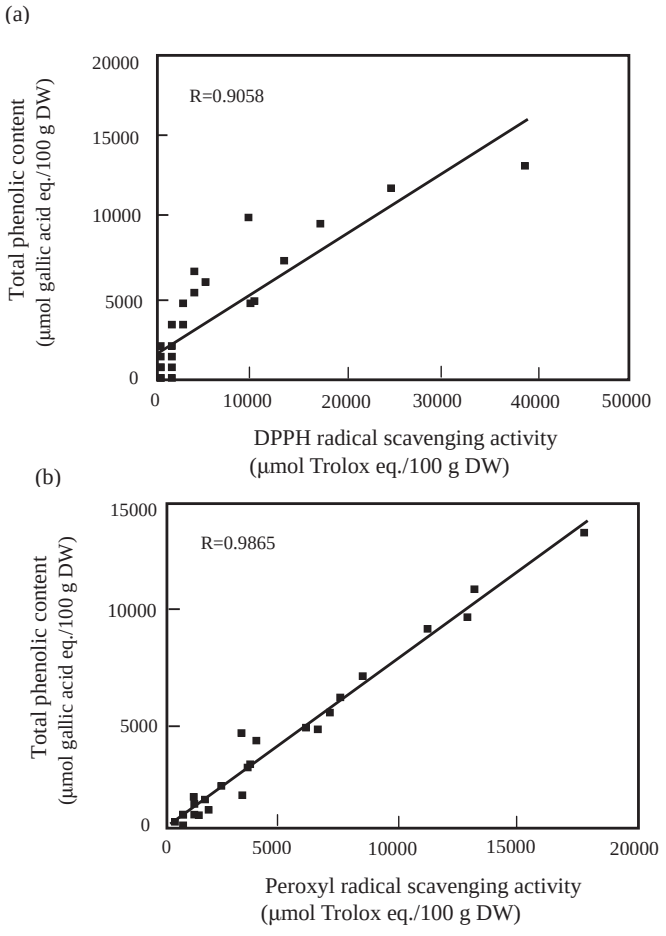

Fig. 1. Relationship between total phenolic content and radical-scavenging activities of 25 Chinese medicinal herbs. (a) Total phenolic content and DPPH radical-scavenging activity, (b) total phenolic content and peroxyl radical-scavenging activity.

The contribution of phenolic compounds to the radical-scavenging activity Figure 1 shows the relationship between total phenolic content and DPPH radical-scavenging activity, as well as peroxyl radical-scavenging activity, of the 25 types of Chinese medicinal herbs. Both DPPH and peroxyl radical-scavenging activity showed high correlation coefficients of 0.9058 and 0.9865, respectively, confirming that the phenolic compounds are likely to contribute to the radical-scavenging activity of the herbs. Cai *et al.* (2004) and Miliauskas *et al.* (2004) also observed a positive correlation between the ABTS assay antioxidant activity and the total phenolic content of the herbal and aromatic plant extracts in their studies, in agreement with our results.

Conclusion

The 25 types of Chinese medicinal herbs tested showed a wide range of antioxidant activity. A linear relationship existed between antioxidant activity and phenolic content, suggesting that the higher the phenolic content, the stronger the antioxidant activity. In contrast, only a trace amount of tocopherol and ascorbic acid was detected, confirming that the main contributors to the radical-scavenging activity in these herbs are likely to be that of phenolic compounds.

References

Ames, B. N., Shigenaga, M. K. and Hagen, T. M. (1993). Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA*, **90**, 7915-7922.

Cai, Y. Z., 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.

Cai, Y. Z., Sun, M., Xing, J., Luo, Q. and Corke, H. (2006). Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Sci.*, **78**, 2872-2788.

Dragland, S., Senoo, H., Wake, K., Holte, K. and Blomhoff, R. (2003). Several culinary and medicinal herbs are important sources of dietary antioxidants. *J. Nutr.*, **133**, 1286-1290.

Goffman, F. D. and Möllers, C. (2000). Changes in tocopherol and plastochromanol-8 contents in seeds and oilseed rape (*Brassica napus* L.) during storage as influenced by temperature and air oxygen. *J. Agric. Food Chem.*, **48**, 1605-1609.

Gu, W.-Z. and Brandwein, S.R. (1998). Inhibition of type II collagen-induced arthritis in rats by triptolide. *Int. J. Immunopharmacol.*, **20**, 389-400.

Kim, H. J. and Lee, Y. S. (2005). Identification of new dicaffeoylquinic acids from *Chrysanthemum morifolium* and their antioxidant activities. *Planta Med.*, **71**, 871-876.

Kishida, E., Nishimoto, Y. and Kojo, S. (1992). Specific determination of ascorbic acid with chemical derivatization and high per-

formance liquid chromatography. *Anal. Chem.*, **64**, 1505-1507.

Łata, B., Przeradzka, M. and Bińkowska, M. (2005). Great differences in antioxidant properties exist between 56 apple cultivars and vegetation seasons. *J. Agric. Food Chem.*, **53**, 8970-8978.

Miliauskas, G., Venskutonis, P. R. and van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.*, **85**, 231-237.

Oboh, G. and Akindahunsi, A. A. (2004). Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. *Nutr. Health*, **18**, 29-36.

Ou, B., Huang, D., Hampsch-Woodwill, M., & Flanagan, J. A. (2003). When east meets west: the relationship between yin-yang and antioxidation-oxidation. *FASEB J.*, **17**, 127-129.

Sakakibara, H., Ashida, H. and Kanazawa, K. (2002). A novel method using 8-hydroperoxy-2´-deoxyguanosine formation for evaluating antioxidative potency. *Free Radic. Res.*, **36**, 307-316.

Sanchez-Moreno, C., Larrauri, J. A. and Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. *J. Sci. Food Agric.*, **76**, 270-276.

Siu, K. M., Mak, D. H. F., Chiu, P. Y., Poon, M. K. T., Du, Y. and Ko, K. M. (2004). Pharmacological basis of 'Yin-nourishing' and 'Yang-invigorating' actions of Cordyceps, a Chinese tonifying herb. *Life Sci.*, **76**, 385-395.

Singleton, V. L. and Rossi, J. A. Jr. (1965). Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, **16**, 144-158.

Son, S. and Lewis, B. A. (2002). Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues: structure-activity relationship. *J. Agric. Food Chem.*, **50**, 468-472.

Ueda, T. and Igarashi, O. (1990). Determination of vitamin E in biological specimens and foods by HPLC-pretreatment of sample and extraction of tocopherols. *J. Micronutr. Anal.*, **7**, 79-96.

Velioglu, Y. S., Mazza, G., Gao, L. and Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.*, **46**, 4113-4117.

Wang, C.-J., Wang, J.-M., Lin, W.-L., Chu, C.-Y., Chou, F.-P. and Tseng, T.-H. (2000). Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. *Food Chem. Toxicol.*, **38**, 411-416.

Wong, C. K., Tse, P. S., Wong, E. L., Leung, P. C., Fung, K. P. and Lam, C. W. (2004). Immunomodulatory effects of yun zhi and danshen capsules in health subjects - a randomized, double-blind, placebo-controlled, crossover study. *Int. Immunopharmacol.*, **4**, 201-211.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E. and Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.*, **52**, 4026-4037.

Yamaguchi, T., Takamura, H., Matoba, T. and Terao, J. (1998). HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci., Biotechnol. Biochem.*, **62**, 1201-1204.

Yokozawa, T., Chen, C. P., Dong, E., Tanaka, T., Nonaka, G. and Nishioka, I. (1998). Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2-picrylhydrazyl radical. *Biochem. Pharmacol.*, **56**, 213-222.