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# Antioxidant Effect of Methanol Extracts from Lotus Plumule and Blossom (*Nelumbo nucifera* Gertn.)

**LISU WANG[1], JUI-HUNG YEN[1,2], HSIAO-LING LIANG[3] AND MING-JIUAN WU[1,3]***

[1.] *Institute of Biotechnology, Chia-Nan University of Pharmacy and Science, Tainan 717, Taiwan*

[2.] *ScinoPharm Biotech Ltd, Tainan Science-Based Industrial Park, Tainan 741, Taiwan*

[3.] *Department of Food Health, Chia-Nan University of Pharmacy and Science, Tainan 717, Taiwan*

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## ABSTRACT

Lotus (*Nelumbo nucifera* Gertn.) is a major economic aqueous plant in Tainan County. Lotus plumule contains various alkaloids and is used to remove "heat" or as tranquilizer and antihypertensive agent in folk medicine. Lotus blossom contains alkaloids, organic acids, amino acids, and $\beta$-carotenoid and is used to "warm" kidney and spleen or used as a cardiotonic in folk medicine. In an attempt to assess the possible antioxidant activities of methanol extracts of lotus plumule and blossom, several *in vitro* assays were conducted. We found that both lotus plumule and blossom possessed strong reducing powers and free radical scavenging abilities. However, only methanol extract of lotus plumule exhibited ferrous ion chelating capabilities, which might contribute to the difference in antioxidant activities between lotus plumule and lotus blossom when analyzing the preventive effects on fatty acid peroxidation and plasmid DNA damage. No mutagenicity in the methanol extract of lotus plumule or blossom was found for *Salmonella typhimurium* TA98 and TA100, either in the presence or absence of S9 mix.

Keywords: Lotus, Nelumbo nucifera Gertn., antioxidant activity

## INTRODUCTION

The formation of potentially toxic compounds caused by the oxidative deterioration of lipids in foods, is responsible for the decrease in food quality and safety[(1)]. It is necessary to suppress lipid peroxidation in food in order to preserve flavor, color and nutritional value. The addition of antioxidants to foods is the most effective way for delaying lipid peroxidation which is the reason for the unpleasant flavors. In the food industry, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ), are widely used because they are effective and less expensive than natural antioxidants. Their safety issues, however, are highly debated, thus generating the need to search for substitute materials from natural and safe sources as food antioxidants. Recently, much attention has been focused on vitamins C, E and carotenoids[(2-5)].

Antioxidants are also of interest to biologists and clinicians, because they may help to protect the human body against damages caused by reactive oxygen species (ROS)[(6)]. Various ROS such as singlet oxygen ($^1O_2$), superoxide radical ($O2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical ($^{\bullet}OH$) are generated as by-products during aerobic metabolism in cells[(7)]. These highly reactive species have a pontential for bringing about extensive damages, including lipid peroxidation, DNA lesions, and protein fragmentation within the cells of biological macromolecules. It's widely acknowledged that the accumulation of oxidative damages of intracellular macromolecules is an essential element in aging processes and in certain degenerated diseases[(8)].

A lot of studies have analyzed the antioxidant potential of a wide variety of vegetables, including cacao beans[(9)], potato, tomato, spinach, and legumes such as *Phaseolus vulgaris*[(10)]. Seeds, teas, and agricultural residues are also potential antioxidants[(11-13)]. Some of the natural products, especially polyphenolic compounds, have well-proven antioxidant abilities. The protective effects of these compounds have been attributed, in a large part, to their scavenging of free radicals, chelating of transition ions and/or modulating antioxidant proteins within cells[(14)].

Lotus (*Nelumbo nucifera* Gertn.) is a very important crop in Tainan County. Several new products have been developed recently in order to reduce the impact on local farmers after Taiwan joined WTO. In addition to the major edible parts, seeds and rhizomes, lotus blossom and plumule (lian zi xin) have been processed and sold as herbal beverages. Lotus plumule is used to remove "heat" in folk medicine. It is also used as tranquilizing and antihypertensive agents. It contains alkaloids including liensinine, isoliensinine, referine, lotusine, methylcorypalline, and demethylcoclaurine. Among them, referine has been shown to have a vasodilating effect and liensinine has antihypertensive and antiarrhymic abilities[(15)]. Lotus blossom contains alkaloids, organic acids, amino acids, and carotenoid. Lotus blossom is used to 'warm" kidney and spleen or as a cardiotonic agent in folk medicine.

Because there is limited information published to

* Author for correspondence. Tel:886-6-266-4911 ext. 220 ext. 193; Fax:886-6-266-6411; E-mail:imwu@mail.chna.edu.tw

prove the biological activities of lotus plumule and blossom, we attempt to assess their possible antioxidant activities in this research.

## MATERIALS AND METHODS

I. *Materials*

Lotus plumule and blossom (*Nelumbo nucifera* Gertn.) were purchased from local farms in Bei-Her, Tainan. They were sun dried, sealed in plastic bags and stored at 4˚C until use.

II. *Chemicals*

All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO), and solvents were from E. Merck (Darmstadt, Germany), unless otherwise indicated. All of the reagents were prepared in deioinized water to eliminate the contamination of metal ions.

III. *Extraction*

Lotus plumule and blossom were extracted with methanol at the ratio of 600 mL per 60 grams at room temperature overnight. The extracts were filtered and concentrated *in vacuo*. The crude extracts were weighed and dissolved in methanol, then packaged in nitrogen and stored at -20˚C until use.

IV. *Antioxidant Activity in a Hemoglobin-Induced Linoleic Acid System*

The antioxidant activities of lotus plumule and blossom were determined by a modified photometry assay[16]. Reaction mixtures (200 µL) containing various amount of extracts, 1 mM linoleic acid emulsion, 40 mM phosphate buffer, pH 6.5, and 0.0016% hemoglobin were incubated at 37˚C for 45 mins. At the end, 2.5 mL of 0.6% HCl in ethanol was added to stop lipid peroxidation. The peroxide value was measured in triplicate using the thiocyanate method by reading the absorbance at 480nm after coloring with 100 µL of 0.02 M $FeCl_2$ and 50 µL of 30% ammonium thiocyanate.

V. *Plasmid Relaxation Assay*

DNA strand damages were measured by converting circular double-stranded supercoiled DNA into nicked circular and linear forms[17]. Reactions were performed in 10 µL of solution containing supercoiled pUC18 plasmid (20 ng), 10 mM Tris-HCl buffer (pH 7.8), 1 mM hydrogen peroxide, 100 µM ferric chloride, 100 µM ascorbic acid and various amounts of the extracts. The mixtures were incubated at 37˚C for 30 mins. and the reactions were stopped by adding 1 µL of 0.5 M EDTA. The samples were separated on 0.7% agarose gel electrophoresis followed by ethidium bromide staining and captured by a CCD camera under UV (UVI, England).

VI. *Reducing Power*

Various concentrations of the extract (50 µL) were mixed with 200 µL of 0.2 M phosphate buffer, pH 6.5 and 200 µL of 1% potassium ferricyanide, then incubated at 50˚C for 20 mins. 10% trichloroacetic acid (250 µL) was added to the mixture and centrifuged at 3000 xg for 10 mins at room temperature. The resulting supernatant was taken and mixed with 500 µL of $ddH_2O$ and 100 µL of 0.1% ferric chloride then incubated at 37˚C for 10 mins. The absorbance at 700 nm was measured. This assay was done in triplicate. Increased absorbance indicated increased reducing power[18].

VII. *1, 1-Diphenyl-2-picrylhydrazyl (DPPH) Scavenging Effect*[19]

Reactions were performed in 1.25 mL of methanol containing 0.5 mM freshly made DPPH and various amounts of the extract. Reaction mixtures were incubated at 37˚C for 30 mins., and the absorbance at 517 nm was measured. This assay was done in triplicate.

IX. *Scavenging Effect on Hydroxyl Radicals*[20]

Reaction mixtures containing various concentrations of extract, 0.02 M phosphate buffer, pH 7.4, 2 mM $H_2O_2$, 0.05 mM ferric chloride, 0.05 mM ascorbate, 6 mM deoxyribose and 0.05 mM EDTA were incubated at 37˚C for 30 mins. The degree of deoxyribose oxidation was analyzed as thiobarbituric acid-reactive material.

X. *Ferrous Ion Chelating Effect*[21]

Reaction mixtures containing 100 µL of extract, 200 µL of 0.5 mM ferrous chloride and 200 µL of 5 mM ferrozine were incubated at 37˚C for 10 mins. After adding 1.5 mL of $ddH_2O$ to the mixture, the absorbance at 562 nm was measured. The lower absorbance at 562 nm indicated stronger chelating effect.

XI. *Mutagenicity Assay*

The mutagenecity assay was performed according to the Ames test[22]. The histidine-requiring strains of *Salmonella typhimurium* TA98 and TA100 were purchased from CCRC. The S9 mix was prepared from Sprague-Dawley male rats treated with Aroclor 1254, according to Ames *et al.*[23]. Various amounts of extract were added to overnight-cultured *Salmonella typhimurium* TA98 (0.1 mL) and S9 mix (0.5 mL) or phosphate buffer (0.5 mL) in place of S9 mix. The entire mixture was pre-incubated at 37˚C for 48 hours. Assay of each sample was determined in triplicate plates per run and data presented were means ± SD of

the three determinations. At least two runs of a single experiment were performed to validate reproducibility. The mutagenicity is expressed as the number of revertants per plate, at a given concentration of each sample. In this mutagenicity testing, the result was recognized as positive when the number exceeds twice the number of spontaneous revertants[23].

## RESULTS AND DISSCUSSION

The most commonly used method for determining antioxidant activity is to measure the inhibitory degree of autoxidation of linoleic acid, but the analysis takes 5-6 days[24]. Instead, we employed a modified rapid photometric assay as described by Kuo, Yeh, and Pan[16] to evaluate the antioxidant activity of methanol extracts of lotus plumule and blossom. As shown in Figure 1, the antioxidant activities are dose-dependent and reached a plateau (about 85-90% inhibition) when the concentration of lotus plumule exceeded 0.05 mg/mL (Figure 1A) or when the concentration of lotus blossom exceeded 0.6 mg/mL (Figure 1B). This result indicates that the antioxidant activity of lotus plumule is about ten folds higher than that of lotus blossom shown by the peroxide production in the presence of 1 mM linoleic acid.

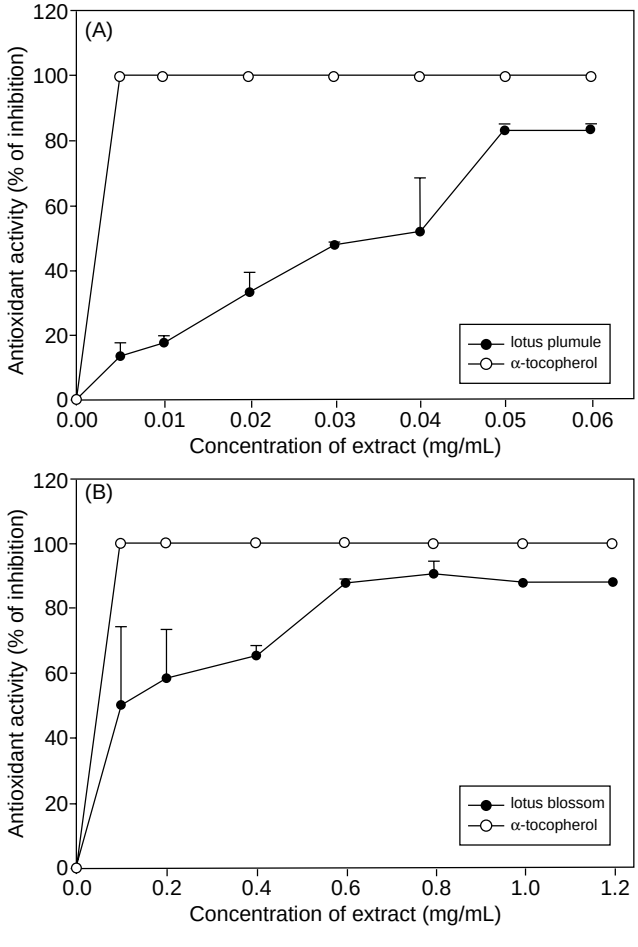


**Figure 1.** Antioxidant activity of the methanol extracts of lotus plumule and lotus blossom against linoleic acid peroxidaion induced by hemoglobin. The peroxide value was measured in triplicate by thiocyanate method as described in Materials and Methods. α-tocopherol was used as a positive control.

A substantial portion of ROS lethality involves DNA damage by oxidants generated from iron-mediated Fenton reaction[25]. We employed $H_2O_2$-$Fe^{+++}$-ascorbate system to induce oxidative damage of DNA. Plasmid relaxation assay was used to semi-quantitatively assess the DNA oxidative damage. In the process of DNA damage, DNA in supercoil was first nicked into open circular form, which was the product of single-stranded cleavage of supercoil DNA. The open circular DNA can be further cleaved by ROS into linear form, which was the result of double-stranded cleavage. Extensive oxidative damage would eventually cause DNA fragmentation and degradation subsequently. As shown in Figure 2, $H_2O_2$, $Fe^{+++}$, or ascorbate alone only caused a small portion of supercoil pUC18 DNA, which migrated the fastest, nicked to open circular form, which migrated the slowest (lanes 2, 3 and 4). However, in the presence of all three reagents together, DNA was hydrolyzed to linear DNA band, which migrated between supercoiled and open circular form (lane 5). In the presence of extract, the extent of DNA damage could be significantly reduced and the protective effects were dose-dependent (lanes 6-10). The strongest protective effect shown as open circular plasmid DNA was predominantly produced at 10 mg/ml extracts of lotus plumule and blossom (lane 6). When extract concentration decreased, the occurrence of open circular form of DNA decreased and the linear form of DNA progressively increased (lanes 6-10). We also observed that the linear form DNA was first present at 0.1 mg/mL of plumule extract (Figure 2A, lane 8) and at 1 mg/mL of blossom extract (Figure 2B, lane 7). This result suggested that the antioxidant effect of lotus plumule was stronger than lotus blossom.

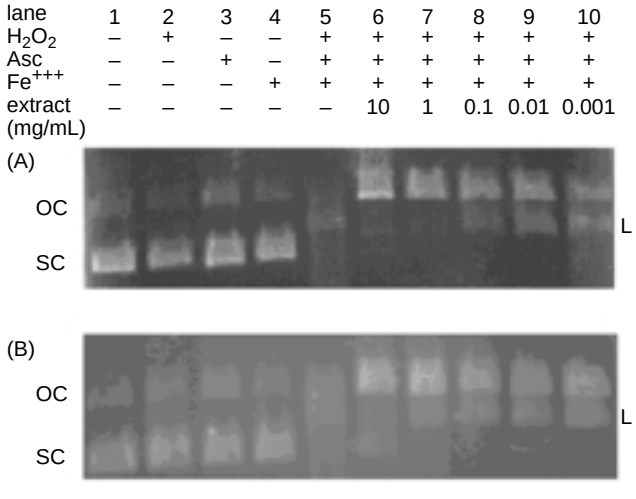


**Figure 2.** Agarose gel electrophoretic analysis of Fenton-mediated DNA oxidation. (A) inhibitory effect of methanol extracts of lotus plumule (B) inhibitory effect of methanol extract of lotus blossom. SC, supercoil form DNA; OC, open circular form DNA; L, linear form DNA.

The most common method for detecting DNA damage in the cell-free system is enzymatic hydrolysis of DNA to nucleosides and chromatography of the formation of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-OHdG). However, some transition metals can induce DNA strand damage but do not generate 8-OHdG in DNA, thus plasmid relaxation technique has been suggested to be more sensitive[26]. The methanol extracts of lotus plumule and blossom apparently were strong inhibitors of oxidative damage of DNA because they can significantly protect DNA from Fenton-mediated DNA degradation even at concentration as low as 1µg/mL. Oxidative damage in DNA induced by Fenton reaction is thought to arise via a site-specific mechanism, i.e. involving the interaction of a transition metal ion with DNA prior to its reaction with hydrogen peroxide to produce the damaged DNA species[27]. It was suggested that single-strand damage in DNA was formed by hydroxyl radicals generated in free solution[28]. In contrast, the formation of double-strand breaks and 8-OHdG was strongly dependent on the binding of iron (II) to DNA between DNA bases; while the intra-strand cross-links might be formed following metal ion binding to phosphate groups[29]. Therefore, we speculated that part of the antioxidant activity of lotus plumule might arise from the blockage of iron from interacting with DNA so that the formation of more open-circular DNA and fewer linear DNA was observed when higher concentrations of extract was present (lanes 6 and 7 of Figure 2A).

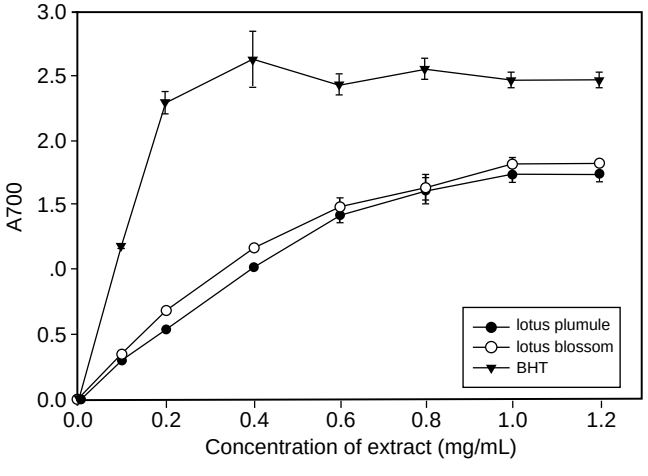


**Figure 3.** Reducing power of the methanol extracts of lotus plumule and blossom. The absorbance at 700 nm was measured in triplicate. BHT was used as a positive control.

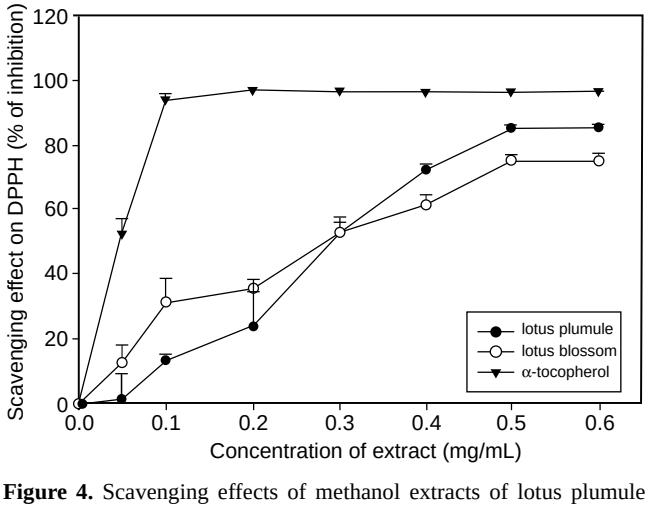


**Figure 4.** Scavenging effects of methanol extracts of lotus plumule and blossom on 1,1 diphenyl-2-picrylhydrazyl (DPPH) radicals. The absorbance at 517 nm was measured in triplicate.

The antioxidant activities of natural components may have a reciprocal correlation with their reducing powers[30-31]. The reducing powers of the methanol extracts of lotus plumule and blossom are shown in Figure 3. The reducing power increased as the extract concentration increased, indicating some compounds in lotus plumule and blossom were both electron donors and could react with free radicals to convert them into more stable products and to terminate radical chain reactions. The concentrations to attain one absorbance unit at 700 nm were 0.083 ± 0.012 mg/mL for BHT, 0.354 ± 0.054 mg/mL for lotus blossom, and 0.425 ± 0.064 mg/mL for lotus plumule. This result indicated that the reducing power of lotus blossom was slightly higher than lotus plumule but much less than BHT.

It is well known that antioxidants can seize the free-radical chain of oxidation and form stable free radicals, which would not initiate or propagate further oxidation. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) has been used extensively as a free radical to evaluate reducing substances [19,31]. In the current study, the scavenging activities of DPPH exerted by lotus plumule and blossom as well as α-tocopherol were summarized in Figure 4. At lower concentrations, lotus blossom showed slightly higher scavenging activity than lotus plumule, while the opposite effect was observed at higher concentrations. Linear response curves were also obtained and the $IC_{50}$ were estimated as 0.05 ± 0.01 mg/mL for α-tocopherol, 0.296 ± 0.047 mg/mL for lotus blossom and 0.298 ± 0.033 mg/mL for lotus plumule. These results demonstrated that methanol extracts of lotus blossom and plumule had similar free radical scavenging activities.

The deoxyribose method is a simple assay to determine the rate constants for reactions of hydroxyl radicals[20]. When the mixture of $FeCl_3$-EDTA, $H_2O_2$ and ascorbate were incubated with deoxyribose in phosphate buffer (pH 7.4), the hydroxyl radicals generated attack the deoxyribose and result in a series of reactions that cause the formation of MDA. Any hydroxyl radical scavenger added to the reaction would compete with deoxyribose for the availability of hydroxyl radicals, thus reducing the amount of MDA formation. We herein tested the scavenging activities of lotus blossom and plumule along with positive control, DMSO. In Figure 5, we found that the maximum scavenging capacity on hydroxyl radicals (60%) could be achieved when methanol extract concentrations of lotus plumule were more than 0.02 mg/mL and when methanol extract concentrations of lotus blossom were more than 0.06 mg/mL. Nevertheless, DMSO, a well-known hydroxyl radical scavenger, had 70% scavenging activity at the low-

est concentration tested at 0.01 mg/mL. This result suggested that the scavenging potential on hydroxyl radicals decreased in the order of DMSO > lotus plumule > lotus blossom.

We further tested the ferrous ion chelating activity of methanol extracts of lotus plumule and lotus blossom. We found that only lotus plumule possessed noticeable chelating activity of ferrous ion (Figure 6), while no detectable chelating ability for lotus blossom could be found (data not shown). Linear response curve was also obtained and the $IC_{50}$ was estimated as 4.82 ± 0.39 mg/mL. Although this concentration is much higher than what can be achieved under physiological condition, it may be significant because it minimizes the concentration of metal in lipid peroxidation and DNA oxidation. This may explain why the antioxidant activity of lotus plumule is higher than that of lotus blossom as shown in Figures 1 and 2.

To test the mutagenicity of methanol extracts of lotus plumule and blossom, Ames tests were performed as described in Materials and Methods. For testing doses at 0 to 2.5 mg per plate, no mutagenicity in *Salmonella typhimurium* TA98 or TA100, either with or without S9 mix in the presence of extracts of lotus plumule and lotus blossom, was observed as shown in Table 1. This result indicated that extracts of neither lotus parts were potential mutagens toward *Salmonella typhimurium* TA98 and TA100.

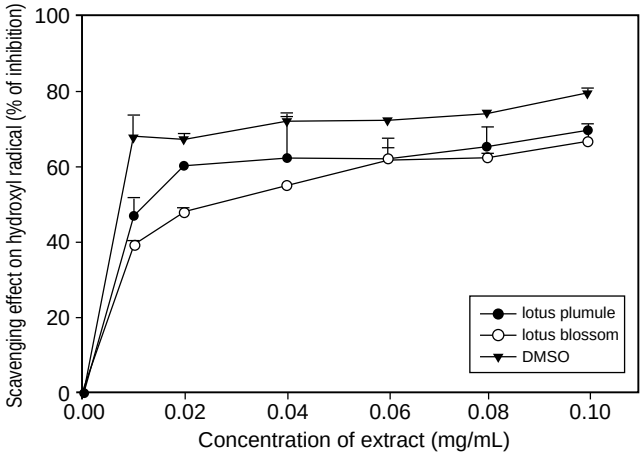


**Figure 5.** Scavenging effects of methanol extracts of lotus plumule and blossom on hydroxyl radicals. The degree of deoxyribose oxidation was analyzed as thiobarbituric acid-reactivity. DMSO was used as positive control.

Herein, we demonstrated that both lotus plumule and lotus blossom contain antioxidant components while the exact phytochemical characteristics related to their activities are still under investigation.

## CONCLUSIONS

The objective of this study was to obtain information on the antioxidant potentials of methanol extracts of lotus plumule and blossom (*Nelumbo nucifera* Gertn.). We employed traditional cell-free assays to investigate the antioxidant activities preventing lipid peroxidation and non-lipid oxidative damages. A modified linoleic acid peroxidation induced by hemoglobin was first performed to evaluate the potential of antioxidant activity of methanol extract of lotus leaves. It has been shown that the products derived from hemoglobin-catalyzed peroxidation of linoleic acid were 9-hydroperoxy-10, 12-octadecadienoic acid (9-HPODE) and 13-hydroperoxy-9,11-octadecadienoic acid (13-HPODE), which were similar to those obtained from autoxidation[16]. Therefore, this procedure can be used as a rapid screening substitute for traditional autoxidation analysis. The degree of peroxidation was then measured by ferric thiocyanate assay, which is based on the complex of ferric ion with thiocyanate and xylenol orange. It has been shown to be an easy, rapid and sensitive method of measuring LOOHs in lipids[32].

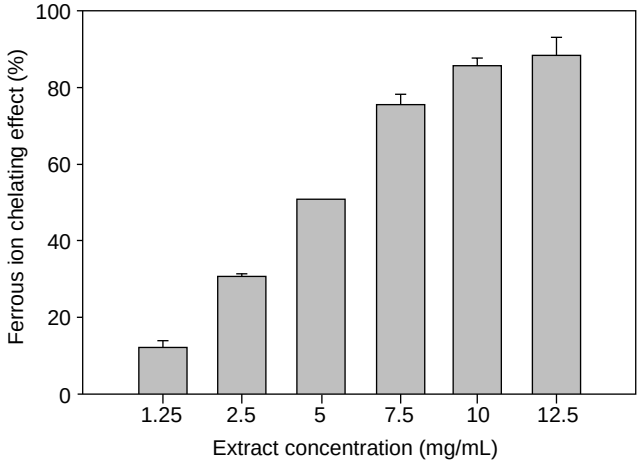


**Figure 6.** Ferrous ion chelating effect of the methanol extracts of lotus plumule. The absorbance at 562 nm was measured in triplicate.

**Table 1.** Mutagenicity of methanol extracts of lotus plumule and lotus blossom toward *Salmonella typhimurium* TA98 and TA100 with or without S9 mix

| | His$^+$ revertants/plate | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | lotus plumule | | | | lotus blossom | | | |
| Concentration | TA98 | | TA100 | | TA98 | | TA100 | |
| (mg/plate) | + S9 | – S9 | + S9 | – S9 | + S9 | – S9 | + S9 | – S9 |
| Control | 43 ± 4 | 15 ± 2 | 56 ± 11 | 25 ± 3 | 28 ± 2 | 11 ± 3 | 106 ± 3 | 76 ± 4 |
| 0.5 | 48 ± 1 | 18 ± 1 | 45 ± 15 | 30 ± 8 | 40 ± 2 | 14 ± 3 | 79 ± 3 | 76 ± 6 |
| 1.0 | 45 ± 3 | 17 ± 3 | 43 ± 7 | 22 ± 6 | 37 ± 3 | 14 ± 7 | 112 ± 11 | 60 ± 6 |
| 2.5 | 46 ± 5 | 17 ± 3 | 45 ± 7 | 27 ± 3 | 34 ± 4 | 16 ± 2 | 107 ± 5 | 53 ± 2 |

The reducing power increased when the concentrations of methanol extracts of lotus plumule and lotus blossom increased, indicating they were electron donors and could react with free radicals to convert them to more stable products and terminate radical chain reactions. It has been shown that the antioxidant effect exponentially increased as a function of the development of the reducing power, suggesting that the antioxidant properties be associated with the development of the reducing power[33]. Therefore, the antioxidant activities in methanol extracts of lotus plumule and blossom may be related to their reducing powers. In conclusion, reducing power, hydroxyl radical and free radical scavenging effects and/or ferrous ion chelating ability may account for the antioxidant abilities of methanol extracts of lotus plumule and blossom in cell-free systems.

Herbs have played a significant role in maintaining human health and improving the quality of life for thousands of years. They have served as valuable ingredients for seasonings, beverages, cosmetics, dyes, and medicines. Many active phytochemicals, including flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides have been identified[34]. Numerous *in vitro* studies have shown that some of the phytochemicals are potent antioxidants, metal chelators or free radical scavengers, which may account for their health-promoting properties[35].

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## REFERENCES

1. Moure, A., Cruz, J. M., Franco, D., Dominguex, J. et. al. 2001. Nature antioxidants from residual sources. Food Chem. 72: 145-171.
2. Frankel, E. N. 1996. Antioxdants in lipid foods and their impact on food quality. Food Chem. 57:51-55.
3. Mallet, J. F. 1994. Antioxidant activity of plant leaves in relation to their alpha-tocopherol content. Food Chem. 49: 61-65.
4. Palozza, P. and Krinsky, N. I. 1992. β-Carotene and alpha-tocopherol are synergistic antioxidants. Arch. Biochem. Biophys. 297: 184-187.
5. Tsuchihashi, H. Kigoshi, M. and Iwatsuki, E. 1995. Action of beta-carotene as an antioxidant against lipid peroxidation. Arch. Biochem. Biophys. 323: 137-147.
6. Halliwell, B. 1995. Oxygen radicals, nitric oxide and human inflammatory joint disease. Ann. Rheum. Dis. 54: 505-510.
7. Gutteridge, J. M. 1994. Biological origin of free radicals, and mechanisms of antioxidant protection. Chem. Biol. Interact. 91: 133-140.
8. Vaughan, M. 1997. Oxidative modification of macromolecules minireview series. J. Biol. Chem. 272:18513-18513.
9. Sanbongi, C. 1998. Antioxidative polyphenols isolated from *Theobroma cacao*. J. Agric. Food Chem. 46: 454-457.
10. Ganthavorn, C. and Hughes, J. S. 1997. Inhibition of soybean oil oxidation by extracts of dry beans (*Phaseolus vulgaris*). J. Am. Oil Chem. Soc. 74: 1035-1030.
11. Tsuda, T., Watanabe, M., Ohshima, K., Yamamoto, A., Kawakishi, S., and Osawa, T. 1994. Antioxidative components isolated from the seed of tamarind (*Tamarindus indica* L.) J. Agric. Food Chem. 42: 2671-2674.
12. Benzie, I. F. and Szeto, Y. T. 1999. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. J. Agric. Food Chem. 47:633-636.
13. Gabrielska, J. 1997. Protective effect of plant flavonoids on the oxidation of lecithin liposomes. Pharemazie 52:2-3.
14. Kameoka, S. Leavitt, P. Chang, C and Kuo, S. M. 1999. Expression of antioxidant proteins in human intestinal Caco-2 cells treated with dietary flavonoids. Cancer Lett. 146: 161-167.
15. Huang, K. C. 1999. Lian zi xin. In “the pharmacology of Chinese Herbs”. 2nd ed. pp 192-193. Huang, K. C. ed. CRC Press, New York, U. S. A..
16. Kuo, J. M., Yeh, D. B. and Pan, B. S. 1999. Rapid photometric assay evaluating antioxidative activity in edible plant material. J. Agric. Food Chem. 47: 3206-3209.
17. Kobayashi, S., Ueda, K. and Komano, T. 1990. The effects of metal ions on the DNA damage induced dy hydrogen peroxide. Agric. Biol. Chem. 54: 69-76.
18. Oyaizu, M. 1986. Antioxidative activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. Nippon ShoKuhin Kogyo Gakkaishi 35: 771-775.
19. Schimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. 40: 945-948.
20. Halliwell, B., Gutteridge, J. M. C. and Arurma, O. I. 1987. The deoxyribose method: a simple “test-tube” ssay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem. 165: 215-219.
21. Dinis, T. C. P., Madeira, V. M. C. and Almeida, L. M. 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315:161-169.
22. Maron, D. M. and Ames, B. N. 1983. Revised methods for the Salmonella mutagenicity test. Mutant. Res.113: 173-215.
23. Ames, B. N., McCann, J. and Yamasaki, E. 1975. Methods for detecting carcinogens and mutagens with

the Salmonella mammalian microsome mutagenicity test. Mutat. Res. 31: 347-364.
24. Mitsuda, H., Yuasumoto, K. and Iwami, K. 1996. Antioxidative action of indole compounds during the autoxidation of linoleic acid. Eiyoto Shokuryo 19: 210-214.
25. Mello-Filho, A. C. and Meneghini, R. 1991. Iron is the intracellular metal involved in the production of DNA damage by oxygen radicals. Mutat. Res. 251: 109-113.
26. Yang, J. L., Wang, L. C., Chang, C. Y. and Liu, T. Y. 1999. Singlet oxygen is the major species participating in the induction of DNA strand breakage and 8-hydroxydeoxyguanosine adduct by lead acetate. Environ. Mol. Mutagen. 33: 194-201.
27. Chevion, M. 1988. A site-specific mechanism for free radical induced biological damage: the essential role of redox-active transition metals. Free Radic. Biol. Med. 5:27-37.
28. Lloyd, D. R., Phillips, D. H. and Carmichael, P. L. 1997. Generation of putative intrastrand cross-links and strand breaks in DNA by transition metal ion-mediated oxygen radical attack. Chem. Res. Toxicol. 10: 393-400.
29. Lloyd, D. R. and Phillips, D. H. 1999. Oxidative DNA damage mediated by copper (II), iron(II) and nickel(II) Fenton reactions: evidence for site-specific mechanisms in the formation of double-strand breaks, 8-hydroxydeoxyguanosine and putative intrastrand cross-links. Mutat. Res. 424: 23-36.
30. Yen, G. C. and Duh, P. D. 1995. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. J. Agric. Food Chem. 42: 629-632.
31. Duh, P.-D and Yen, G C. 1997. Antioxidative activity of three herbal water extracts. Food Chem. 60: 639-645.
32. Shantha, N. C. and Decker, E. A. 1994. Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. J. AOAC Int. 77: 421-424.
33. Tanaka, M., Kuei, C. W., Nagashima, Y. and Taguchi, T. 1988. Application of antioxidative maillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishi* 54: 1509-1414.
34. Craig, W. J. 1999. Health-promoting properties of common herbs. Am. J. Clin. Nutr. 70(suppl):491S-499S.
35. Cotell, N., Bernier, J. L., Catteau, J. P, Pommery, J., Wallet, J. C. and Gaydou, E. M. 1996. Antioxidant properties of hydroxy-flavones. Free Radic. Biol. Med. 20:35-43.

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## 以高效液相層析法分析包裝水中低分子量醛類含量

蔡佳芬　蕭惠文　李樹其　周薰修*

行政院衛生署藥物食品檢驗局
台北市115南港區昆陽街161-2號

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### 摘　要

利用高效液相層析法（high performance liquid chromatography, HPLC）分析包裝水中甲醛、乙醛及丙醛含量之檢驗方法業已建立。水樣檢體以DNPH (2,4-dinitrophenylhydrazine) 試劑0.5 mL及2 M過氯酸0.1 mL於55°C反應60分鐘，再以C18之玻璃淨化管柱吸附，利用乙腈5 mL將其沖提，進行HPLC分析。甲醛、乙醛及丙醛之DNPH衍生物以Cosmosil 5C18-MS為分離管，乙腈／去離子水（55/45, v/v）為移動相，可獲得較佳之分離效果，檢測波長為紫外光360 nm。去離子水中同時添加甲醛、乙醛及丙醛各2-200 ppb，其平均回收率為84.4 – 103.2%、90.2 – 122.1%及60.8 – 100.4%，最低檢出限量均為1 ppb。以此方法檢驗市售包裝水63件，包括國產品43件及進口品20件，檢測結果均遠低於世界衛生組織對飲用水品質所訂之900 μg/L限量。

**關鍵詞**：包裝水，甲醛，乙醛，丙醛，DNPH衍生物

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## 台灣中部地區十年來（1991-2000）細菌性食品中毒趨勢分析

張洳楣　陳泰華*

行政院衛生署藥物食品檢驗局中部檢驗站
台中市文心南三路20號

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### 摘　要

食品中毒是全球飲食衛生之重要課題，尤以細菌性食品中毒為首。本文回顧台灣中部地區1991-2000年細菌性食品中毒狀況並分析其地區性特色及飲食型態。十年間中部地區發生食品中毒案共274件（不含無檢體送驗案件），中毒人數共12845人，死亡人數三人。食品中毒案中有171件（62.4%）是屬於細菌性食品中毒，其檢出順序以仙人掌桿菌居首（41.2%）、其次是金黃色葡萄球菌（17.9%）及腸炎弧菌（15.7%）。死亡人數三人，都發生在彰化縣，死亡原因均為誤食有毒河豚致死。引起食品中毒主要涉嫌食品有海鮮類（32.5%）、肉類製品（23.5%）及穀類製品（15.6%）。食品中毒案最常發生的場所是自宅（41.2%），其次是學校（34.3%）及餐廳（12.0%）。自20件食品中毒案分離所得病原性大腸桿菌O型血清共有15種型別，以O18型最多（15%）。自中毒食品分離所得腸炎弧菌（*Vibrio parahaemolyticus*）具K型血清55株（51.9%），另51株未檢出K型（48.1%），血清型K6腸炎弧菌於1997年分離14株居首，以件數而言，49案食品中毒中K28型檢出4件（8.2%）最高。155株金黃色葡萄球菌株中有121株（78.0%）具產毒能力，34株（22.0%）不具產毒能力。分離所得之金黃色葡萄球菌腸毒素型歸納分類成七型：A、B、C、D、A&B、A&D、A&B&C，以A型腸毒素檢出92株（76.0%）佔多數。

**關鍵詞**：食品中毒，仙人掌桿菌，金黃色葡萄球菌，腸炎弧菌

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## 蓮子心及蓮花甲醇抽出物的抗氧化性探討

王麗淑[1]　顏瑞鴻[1,2]　梁小玲[3]　吳明娟[1,3*]

[1] 嘉南藥理科技大學　生物科技研究所
台南縣仁德鄉保安村二仁路一段60號
[2] 神隆生物科技公司　台南科學工業園區
台南縣南科八路一號三樓
[3] 嘉南藥理科技大學　食品衛生系
台南縣仁德鄉保安村二仁路一段60號

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### 摘　要

蓮是台南縣極具經濟價值之作物。蓮子心含各種生物鹼，在中醫上主要功能為去熱、鎮靜、降血壓等。蓮花含各種生物鹼、有機酸、氨基酸、及胡蘿蔔素，在中醫上主要功能為溫補腎、脾、及當作強心劑。本實驗主旨為利用試管內的分析，來探討蓮子心及蓮花的甲醇抽出物的抗氧化性。我們發現，蓮子心甲醇抽出物在抑制亞麻油酸的過氧化和質體DNA的氧化傷害方面，皆比蓮花的甲醇抽出物效果好。當分析其還原力與氫氧基、自由基的清除能力時，蓮子心與蓮花差異不大。然而只有蓮子心的甲醇抽出物具有亞鐵離子的螯合能力，因此金屬離子螯合力的強弱可能正是影響蓮子心及蓮花抗氧化性強弱的原因。此外，以Ames test作安全性探討時發現，蓮子心及蓮花的甲醇抽出物，不論有無添加S9 mix，在所測試之濃度（0～2.5mg/plate）內對*Salmonella typhimurium* TA 98和TA100皆沒有致突變性。

**關鍵詞**：蓮、抗氧化、致突變性

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## 蒼耳子成分之高效能液相層析與毛細管電泳分析

許順吉[1]　徐鳳麟[2]　戴火木[3]　許明志[3]　黃明星[3*]

[1] 國立台灣師範大學 化學系
[2] 台北醫學大學 生藥技術系
[3] 嘉南藥理科技大學 生活保健系

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### 摘　要

本研究開發CE及HPLC方法，分析定量蒼耳子的二個指標成分。以毛細管區帶電泳層析（CZE）分析技術，使用硼酸鹽與醋酸鈉緩衝液系統，可成功的於50分鐘內完成蒼耳子藥材之分析；在HPLC分析部分，使用磷酸鹽緩衝溶液與氯甲烷水溶液為沖提液，可於60分鐘內，成功地分離蒼耳子藥材的成分。除探討分析條件之確效，並比較CE及HPLC方法之優劣。

**關鍵詞**：蒼耳子，蒼耳，高效液相層析，毛細管電泳