Bot. Bull. Acad. Sin. (2004) **45:** 285-290

Antioxidant activities of methanolic and hot-water extracts from leaves of three cultivars of Mai-Men-Dong (*Liriope spicata* L.)

Wen-Chi HOU[1,*], Wen-Chung WU[2], Chih-Yuan YANG[3], Hsien-Jung CHEN[4], Sin-Yie LIU[5], and Yaw-Huei LIN[6,*]

[1]*Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei, Taiwan 110*

[2]*School of Pharmacy, Taipei Medical University, Taipei, Taiwan 110*

[3]*Llife Science, Liberal Arts Center, HsiWu College, Taipei, Taiwan 224*

[4]*Department of Horticulture, Chinese Culture University, Taipei, Taiwan 111*

[5]*Taiwan Agricultural Research Institute, Council of Agriculture, Wu-Feng, Taichung, Taiwan 413*

[6]*Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan 115*

(Received June 30, 2004; Accepted August 2, 2004)

Abstract. 1,1-dipheny-2-picrylhydrazyl (DPPH) scavenging activities of the 80% methanolic leaf extracts of three cultivars (small leaf, SL; big leaf, BL; thin leaf, TL) of Mai-Men-Dong (*Liriope spicata* L.) are analyzed by spectrophotometry. The concentrations required for 50% inhibition (IC_{50}) of DPPH radicals were 81.08, 96.97, and 53.78 μg/mL, respectively. The methanolic extracts were further partitioned into three *n*-hexane-, ethylacetate-, and water-soluble fractions, among which the ethylacetate-soluble fraction exhibited the highest DPPH scavenging activity. The IC_{50} of ethylacetate-soluble fractions of SL, BL, and TL for DPPH radical scavenging activity were 41.55, 24.55, and 53.33 μg/mL, respectively. Each Mai-Men-Dong powder (1 g) was deposited in a tea bag and then dipped in hot water (100°C, 100 mL) for 3 min with triplicate samples. These hot-water extracts were then freeze-dried for an anti-DPPH radical capacity test, which found a positive correlation with the phenolic contents of each hot water extract. The IC_{50} of hot water extracts of SL, BL, and TL for DPPH radical scavenging activities were 378.97, 171.12, and 95.84 mg/mL, respectively. All three hot water extracts can effectively scavenge hydroxyl radical using electron spin resonance (ESR) spectrometry. The IC_{50} against hydroxyl radical were 80.8, 69.7, and 116 μg/mL, respectively, for the SL, BL, and TL cultivars.

Keywords: DPPH radical; Electron spin resonance (ESR); Hydroxyl radical; Hot water extracts; Mai-Men-Dong (*Liriope spicata* L.); Methanolic extracts.

Introduction

Active (or reactive) oxygen species and free radical-mediated reactions have been implicated in degenerative or pathological processes such as aging (Ames et al., 1993; Harman, 1995), cancer, coronary heart disease and Alzheimer's disease (Ames, 1983; Gey, 1990; Smith et al., 1996; Diaz et al., 1997). Meanwhile many epidemiological results point to an association between a diet rich in fresh fruit and vegetable and a decrease in the risk of cardiovascular diseases and certain forms of cancer (Salah et al., 1995) in humans. Several reports concern the antioxidant activities of natural compounds in fruits and vegetables. These include phenolic compounds (Rice-Evans et al., 1997), anthocyanin (Espin et al., 2000), echinacoside in *Echinaceae* root (Hu and Kitts, 2000), water extracts of roasted *Cassia tora* (Yen and Chuang, 2000), the storage proteins of sweet potato root (Hou et al., 2001a), yam tuber (Hou et al., 2001b), and potato tuber (Liu et al., 2003). In cells, metabolic pathways normally couple to degrade free radicals. If the generation rates of free radicals are faster than degradation rates under environmental stresses, cells suffer oxidative stresses. Two distinct pathways, nonenzymatic or enzymatic, were found in plant cells as routes of free radical scavengers. The former included ascorbate (Njus and Kelley, 1993) or chlorogenic acids (Kono et al., 1998) or vitamin E (Halliwell, 1999); the latter included different forms of SOD to metabolize superoxide free radical to hydrogen peroxide (Bowler et al., 1992; Lin et al., 1993). The hydrogen peroxide produced was further metabolized either by catalase or different forms of peroxidase such as glutathione peroxidase (EC 1.11.1.9).

The root of Mai-Men-Dong (*Liriope spicata* L.) is frequently used as a traditional Chinese herb, and the dried leaf powders are also used as tea drinks in Taiwan. In our previous report (Hou et al., 2003), the activities of super-

*Corresponding author. Prof. Wen-Chi Hou, Fax: 886-2-2378-0134; E-mail: wchou@tmu.edu.tw or Prof. Yaw-Huei Lin, Fax: 886-2-2782-7954; E-mail: boyhlin@gate.sinica.edu.tw

oxide dismutase and glutathione peroxidase were detected in leaves of three cultivars of Mai-Men-Dong. One of the three withstood an 80°C treatment for 30 min. In this research, we used 80% methanol to extract leaves of Mai-Men-Dong (small leaf, SL; big leaf, BL; thin leaf, TL). Meanwhile, the methanolic extracts were further partitioned into three fractions of hexane-soluble, ethylacetate-soluble, and water-soluble. These extracts were analyzed for their DPPH scavenging activities by spectrophotometry. Mai-Men-Dong powder was deposited in a tea bag and then dipped in hot water to get hot water-soluble extracts (Mai-Men-Dong tea), and we then evaluated the antioxidant effects by scavenging activities of DPPH by spectrophotometry and of hydroxyl radical by electron spin resonance (ESR) spectrometry.

Materials and Methods

Plant Materials

Fresh leaves of three cultivars (small leaf, SL; big leaf, BL; thin leaf, TL) of *Liriope spicata* L. were provided by Taiwan Agricultural Research Institute, Council of Agriculture, Wu-Feng, Taichung. After drying in 37°C oven, the dried leaves were cut into pieces for further investigations.

Methanolic Extracts of Mai-Men-Dong

The dried leaves of each cultivar of Mai-Men-Dong (250 g) were extracted twice with 500 mL of 80% methanol at 70°C for 4 h. The extracts were dried under reduced pressure. Each 80% methanolic extract was dissolved in small amounts of water and then was further partitioned in order into three fractions of hexane-soluble, ethylacetate-soluble, and water-soluble. These extracts were dried, and the DPPH scavenging activities were analyzed by spectrophotometry.

Hot Water Extract of Mai-Men-Dong

Triplicate samples of Mai-Men-Dong powder (1 g) were deposited in tea bags and then dipped in hot water (100 °C, 100 mL) for 3 min. The hot-water's extracts (Mai-Men-Dong tea drinks) were then freeze-dried and analyzed for DPPH scavenging activities by spectrophotometry and for hydroxyl radical scavenging activities by ESR spectrometry.

Scavenging Activitiy of 1,1-Dipheny-2-Picrylhydrazyl (DPPH) Radical by Spectrophotometry

The scavenging activity of each extract against DPPH radical was measured according to the method of Hou et al. (2001a, b). Each 0.3 mL of extract solution (dissolved in methanol or distilled water) was added to 0.1 mL of 1 M Tris-HCl buffer (pH 7.9) and then mixed with 0.6 mL of 100 mM DPPH in methanol for 20 min under light protection at room temperature. The absorbance at 517 nm was measured. Deionized water or methanol was used as a blank. The scavenging activity of DPPH radicals (%) was calculated following the equation: $(A517_{blank} - A517_{sample}) \div A517_{blank} \times 100\%$. The IC_{50} stands for the concentration required for 50% scavenging activity and was calculated from the above equation.

Scavenging Activities Against Hydroxyl Radical by Electron Spin Resonance Spectrometry

The hydroxyl radical was generated by Fenton reaction according to the method of Kohno et al. (1991). The total 500-μL mixture contained hot-water extracts of each cultivar (0.042, 0.063, and 0.125 mg/mL), 5 mM 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), and 0.05 mM ferrous sulfate. After mixing, the solution was transferred to an ESR quartz cell and placed at the cavity of the ESR spectrometer. Hydrogen peroxide was then added to a final concentration of 0.25 mM. Deionized water was used instead of sample solution for blank experiments. After 40 s, the relative intensity of the signal of the DMPO-OH spin adducts was measured. All ESR spectra were recorded at the ambient temperature (298 K) on a Bruker EMX-6/1 EPR spectrometer equipped with WIN-EPR SimFonia software, Version 1.2. The conditions of ESR spectrometry were as follows: center field, 345.4 (5.0 mT; microwave power, 8 mW [9.416 Ghz]); modulation amplitude, 5 G; modulation frequency, 100 kHz; time constant, 0.6 s; scan time, 1.5 min.

Total Phenolic Contents

The total phenolic contents in each of the hot water extracts were calculated using the Folin-Ciocalteu reagent method (Lai et al., 2001). The gallic acid is used to plot the standard curve, and each extract is expressed as μg equivalent of gallic acid/100 μg sample.

Chemicals

All chemicals and reagents were from Sigma Chemical Co. (St. Louis, MO, USA) and were of the highest purity available.

Results and Discussion

DPPH radicals are widely used in the model system to investigate the scavenging activities of several natural compounds. When DPPH radical is scavenged, the color of the reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength 517 nm. Figure 1 shows the scavenging activity against DPPH radicals of 80% methanolic extracts of SL, BL, and TL cultivar of *Liriope spicata* L. All three methanolic extracts exhibit dose-dependent anti-DPPH radical activities. The IC_{50} values were 81.08, 96.76, and 53.78 μg/mL, respectively, for SL, BL, and TL cultivars.

Each 80% methanolic extract was further fractionated sequentially into hexane-, ethylacetate-, and water-soluble fractions. Using SL cultivar as an example, the recovery by dry weight of each fraction in order from 80% methanolic extracts was 2.85%, 11.31%, and 53%,

respectively. On the same weight basis (100 μg), the DPPH scavenging activity is 28.62%, 71.55%, and 60.56%, respectively, for the hexane-, ethylacetate-, and water-soluble fractions. We found that ethylacetate-soluble fraction exhibited the highest antiradical activity among them. Figure 2 shows the scavenging activity against DPPH radical from ethylacetate-soluble fraction of 80% methanolic extracts of the SL, BL, and TL cultivars of *Liriope spicata* L. The IC_{50} values were 41.55, 24.55, and 53.3 mg/mL, respectively, for the SL, BL, and TL cultivars. The IC_{50} of each ethylacetate-soluble fraction against DPPH radical is about one-half to one-fourth those of 80% methanolic extracts, except for TL cultivar.

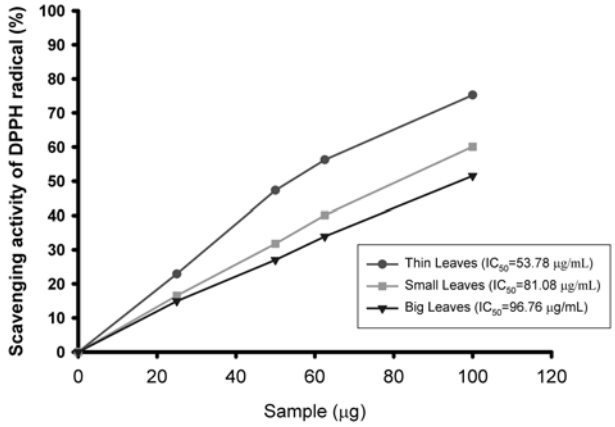

Figure 1. Scavenging activity against DPPH radical from 80% methanolic extracts of SL, BL, and TL cultivar of *Liriope spicata* L. The scavenging activity of DPPH radical (%) was calculated according to the following equation: $(A517_{blank} - A517_{sample}) \div A517_{blank} \times 100\%$.

It is interesting that the commercial products of dried leaf powder of Mai-Men-Dong are used as tea drinks in Taiwan. For this reason, we freeze-dried hot-water extracts (simulating Mai-Men-Dong tea drinks), and the DPPH scavenging activity was analyzed by spectrophotometry. The recovery percentage of hot water extracts from each dried leaf powder (1 g) was 12.12%, 6.42%, and 7.04%, respectively, for the SL, BL, and TL cultivars. Figure 3 shows that all three hot water extracts exhibit dose-dependent anti-DPPH radical activities. The IC_{50} values are 378.97, 171.12, and 95.84 μg/mL, respectively, for the SL, BL, and TL cultivars. The simulated Mai-Men-Dong tea drinks clearly exhibited anti-DPPH radical activity though the scavenging efficiency was lower than that of ethylacetate-soluble fraction and 80% methanolic extracts (Table 1). The hot water extracts of TL cultivar exhibit the closest IC_{50} against DPPH radical compared with those of ethylacetate-soluble fraction and 80% methanolic extracts (Table 1). Table 2 shows the total phenolic contents in hot water extracts of the SL, BL, and TL cultivars of *Liriope spicata*. On the same weight basis (100 μg), the order of total phenoilc contents was TL > BL > SL, which is the

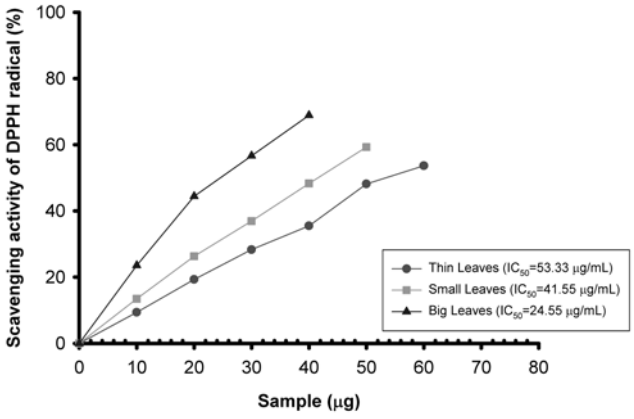

Figure 2. Scavenging activity against DPPH radical from ethylacetate-soluble fraction of 80% methanolic extracts of the SL, BL, and TL cultivarsof *Liriope spicata* L. The scavenging activity of DPPH radical (%) was calculated according to the following equation: $(A517_{blank} - A517_{sample}) \div A517_{blank} \times 100\%$.

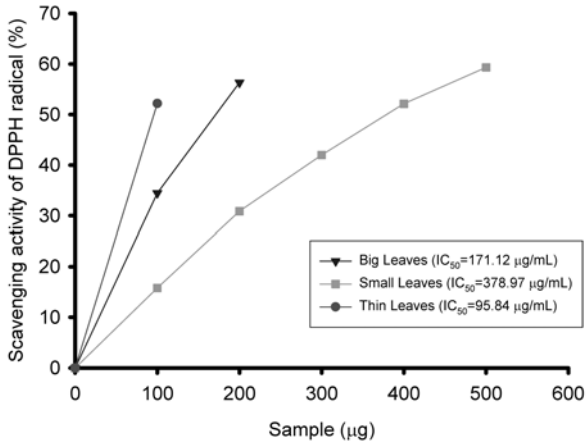

Figure 3. Scavenging activity against DPPH radical from hot water extracts of the SL, BL, and TL cultivars of *Liriope spicata* L. The scavenging activity of DPPH radical (%) was calculated according to the following equation: $(A517_{blank} - A517_{sample}) \div A517_{blank} \times 100\%$.

Table 1. Comparison of the scavenging efficiency against DPPH radical between hot water extracts and 80% methanolic extracts or ethylacetate-soluble fraction of the SL, BL, and TL cultivars of *Liriope spicata.*

	SL cultivar	BL cultivar	TL cultivar
Hot water extracts/ethylacetate-soluble	9.12[a]	6.97	1.80
Hot water extracts/80% methanolic extracts	4.67	1.77	1.78

[a]Ratio of IC_{50} against DPPH radical.

Table 2. The total phenolic contents in hot water extracts of the SL, BL, and TL cultivars of *Liriope spicata.*

	SL cultivar	BL cultivar	TL cultivar
Total phenolic contents (μg equivalent of gallic acid/100 μg sample)	1.01	2.01	3.01

same as that of IC_{50} against DPPH radical. The methanol extracts of *Echinacea* root from three species exhibited different DPPH scavenging activities, and the IC_{50} values all exceeded 400 μg/mL (Hu and Kitts, 2000). The IC_{50} of the DPPH scavenging activity of bicarbonate extracts from hsian-tsan was 510 μg/mL (Lai et al., 2001).

The hydroxyl radical was generated by Fenton reaction and was trapped by DMPO to form DMPO-OH adduct. The intensities of DMPO-OH spin signal in ESR spectrometry were used to evaluate the scavenging activity of hot water extracts against hydroxyl radical. Figure 4 shows scavenging activities against hydroxyl radicals of hot water extracts of SL (A), BL (B), and TL (C) cultivars of *Liriope spicata* L. at different concentrations (0.042, 0.063, and 0.125 mg/mL). The scavenging activities against hydroxyl radical are 4.80, 37.63, and 80.62% (for SL cultivar); 10.59, 47.46, and 70.91% (for BL cultivar); and 5.54, 31.63, and 53.12% (for TL cultivar), respectively, at 0.042, 0.063, and 0.125 mg/mL. All three hot water extracts can effectively scavenge hydroxyl radical. IC_{50} values against hydroxyl radical are 0.0808, 0.0697, and 0.116 mg/mL, respectively, for SL, BL, and TL cultivars.

In conclusion, the ethylacetate-soluble fraction from 80% methanolic extracts exhibits the highest DPPH scavenging activity. The hot water extracts (simulated Mai-Men-Dong tea drinks) exhibit dose-dependent scavenging activities against both DPPH and hydroxyl radicals. Together with the previous report of superoxide dismutase and glutathione peroxidase (heat stable) activities in leaf extracts of different cultivars of *Liriope spicata*, the potential use of *Liriope spicata* for health food deserves further investigation.

Acknowledgments. The authors want to thank the National Science Council, Republic of China for its financial support (NSC93-2313-B038-001).

Literature Cited

Ames, B.N. 1983. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. Science **221:** 1256-1264.

Ames, B.N., M.K. Shigena, and T.M. Hegen. 1993. Oxidants, antioxidants and the degenerative diseases of aging. Proc. Nat. Acad. Sci. USA **90:** 7915-7922.

Bowler, C., M.V. Montagu, and D. Inze. 1992. Superoxide dismutase and stress tolerance. Ann. Rev. Plant Physiol. Plant Mol. Biol. **43:** 83-116.

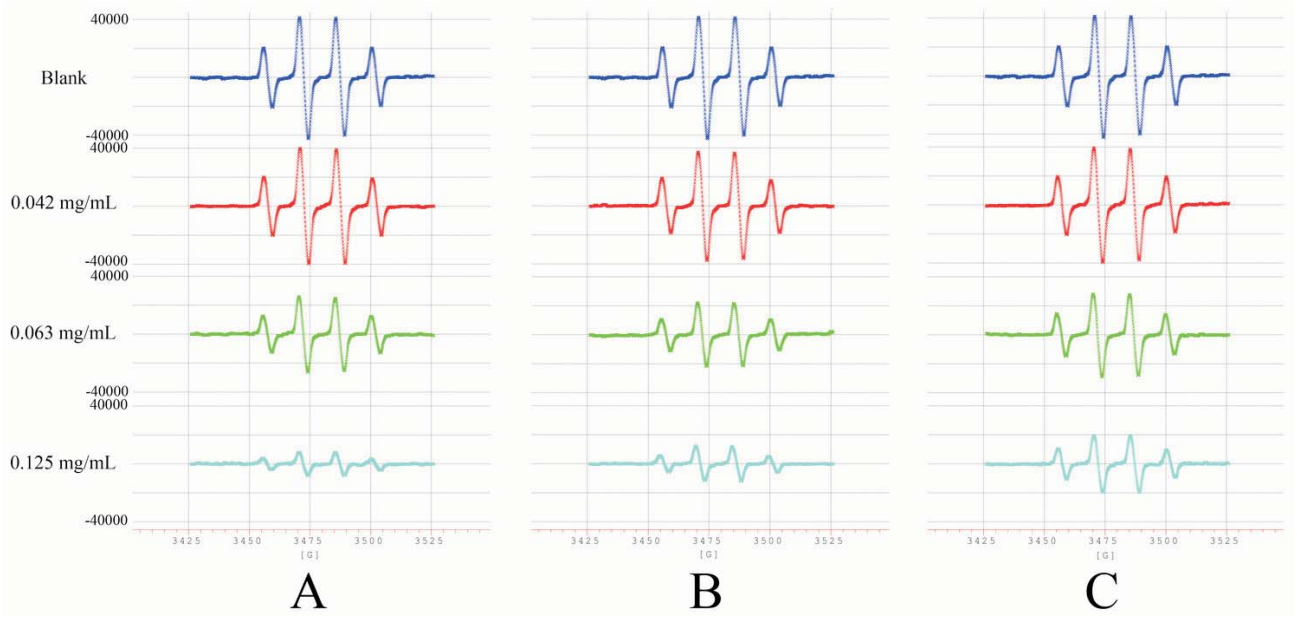

Figure 4. Scavenging activities against hydroxyl radical from hot water extracts of the SL (A), BL (B), and TL (C) cultivars of *Liriope spicata* L. at different concentrations (0.042, 0.063, and 0.125 mg/mL). The signal intensities of DMPO-OH adduct were determined by electron spin resonance spectrometry.

Diaz, M.N., B. Frei, J.A. Vita, and J.F. Keaney. 1997. Antioxidants and atherosclerotic heart disease. N. Engl. J. Med. **337:** 408-416.

Espin, J.C., C. Soler-Rivas, H.J. Wichers, and C. Viguera-Garcia. 2000. Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuff. J. Agric. Food Chem. **48:** 1588-1592.

Halliwell, B. 1999. Food-derived antioxidants. Evaluating their importance in food and in vivo. Food Sci. Agric. Chem. **1:** 67-109.

Harman, D. 1995. Role of antioxidant nutrients in aging: overview. Age **18:** 51-62.

Hou, W.C., Y.C. Chen, H.J. Chen, Y.H. Lin, L.L. Yang, and M. H. Lee. 2001a. Antioxidant activities of trypsin inhibitor, a 33 kDa root storage protein of sweet potato (*Ipomoea batatas* (L.) Lam cv. Tainong 57). J. Agric. Food Chem. **49:** 2978-2981.

Hou, W.C., M.H. Lee, H.J. Chen, W.L. Liang, C.H. Han, Y.W. Liu, and Y.H. Lin. 2001b. Antioxidant activities of dioscorin, the storage protein of yam (*Dioscorea batatas* Decne) tuber. J. Agric. Food Chem. **49:** 4956-4960.

Hou, W.C., Y.L. Lu, S.Y. Liu, and Y.H. Lin. 2003. Activities of superoxide dismutase and glutathione peroxidase in leaves of different cultivars of *Liriope spicata* L. on 10% SDS-PAGE gels. Bot. Bull. Acad. Sin. **44:** 37-41.

Hu, C. and D.D. Kitts. 2000. Studies on the antioxidant activity of Echinaceae root extract. J. Agric. Food Chem. **48:** 1466-1472.

Gey, K.F. 1990. The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms. Biochem. Soc. Trans. **18:** 1041-1045.

Kohno, M., M. Yamada, K. Mitsuta, Y. Mizuta, and T. Yoshikawa. 1991. Spin-trapping studies on the reaction of iron complexes with peroxides and the effects of water-soluble antioxidants. Bull. Chem. Soc. Jpn. **64:** 1447-1453.

Kono, Y., S. Kashine, T. Yoneyama, Y. Sakamoto, Y. Matsui, and H. Shibata. 1998. Iron chelation by chlorogenic acid as a natural antioxidant. Biosci. Biotechnol. Biochem. **62:** 22-27.

Lai, L. S., S.T. Chou, and W.W. Chao. 2001. Studies on the antioxidant activities of Hsian-Tsao (*Mesona procumbens* Hemsl). J. Agric. Food Chem. **49:** 963-968.

Lin, C.T., K.W. Yeh, M.C. Kao, and J.F. Shaw. 1993. Cloning and characterization of a cDNA encoding the copper/zinc-superoxide dismutase from sweet potato tuberous root. Plant Mol. Biol. **3:** 911-913.

Liu, Y.W., C.H. Han, M.H. Lee, F.L. Hsu, and W.C. Hou. 2003. Patatin, the tuber storage protein of potato (*Solanum tuberosum* L.), exhibits antioxidant activity in vitro. J. Agric. Food Chem. **51:** 4389-4393.

Njus, D. and P.M. Kelley. 1993. The secretary-vesicle ascorbate-regenerating system: a chain of concerted H^+/e^--transfer reaction. Arch. Biophys. Acta. **1144:** 235-248.

Rice-Evans, C.A., N.J. Miller, and G. Paganga. 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. **2:** 152-159.

Salah, N., N.J. Miller, G. Paganga, L. Tijburg, G.P. Biolwell, and C. Rice-Evans. 1995. Polyphenolic flavonols as scavenger of aqueous phase radicals and as chain breaking antioxidants. Arch. Biochem. Biophys. **322:** 339-346.

Smith, M.A., G. Perry, P.L. Richey, L.M. Sayre, V. Anderson, M.F. Beal, and N. Kowal. 1996. Oxidative damage in Alzheimer's. Nature **382:** 120-121.

Yen, G.C. and D.Y. Chuang. 2000. Antioxidant properties of water extracts from *Cassia tora* L. in relation to the degree of roasting. J. Agric. Food Chem. **48:** 2760-2765.

麥門冬葉子的甲醇與熱水抽取物抗氧化活性之研究

侯文琪[1] 吳紋君[2] 楊智元[3] 陳顯榮[4] 劉新裕[5] 林耀輝[6]

[1] 台北醫學大學生藥學研究所
[2] 台北醫學大學藥學系
[3] 醒吾技術學院通識中心
[4] 文化大學園藝系
[5] 行政院農委會農業試驗所
[6] 中央研究院植物研究所

三種麥門冬的抽取物，無論是 80% 甲醇抽取物（進一步以正己烷、乙酸乙酯、及水依次分配抽取）或是模仿茶粉泡茶方式(即以熱水浸泡麥門冬粉)，均具有 DPPH 自由基（以分光光度方法測定）和氫氧自由基（以 ESR，即電子自旋共振光譜方法測定）的清除能力，且與濃度具有相關性。熱水抽取物的 DPPH 自由基的清除能力與其內含的總酚量有密切關連性。

關鍵詞：麥門冬；抗氧化活性；DPPH 自由基；氫氧自由基。