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Antioxidant activities of different wild bitter gourd (*Momordica charantia* L. var. *abbreviata* Seringe) cultivars

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ABSTRACT. Antioxidant activity assays were conducted using water (H) and methanolic (M) extracts of sixteen cultivars from indigenous wild bitter gourd (*Momordica charantia* L. var. *abbreviata* Seringe, MCA) in Taiwan. The scavenging activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals were different among MCA cultivars and the concentrations of 50% scavenging activity (IC_{50}) for the effective cultivar was 181 μg/mL (H) and 246 μg/mL (M) in the former extract and 148 μg/mL (H) and 37 μg/mL (M) in the latter. For inhibitory activities against Cu^{2+}-induced low-density-lipoprotein peroxidation, most MCA cultivars at 4000 μg/mL (especially for M extracts) showed protective activities and were equivalent to 0.8 mM Trolox by thiobarbituric acid reactive substance assays. These useful data may help promote the use and further research of MCA as an antioxidant in the health food industry

Keywords: *Momordica charantia* L. var. *abbreviata* Seringe (MCA); Antioxidant activity; 2,2-diphenyl-1-picrylhydrazyl (DPPH); Low-density-lipoprotein peroxidation.

Abbreviations: DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; **DPPH,** 2,2-diphenyl-1-picrylhydrazyl; **H extracts,** water extracts; **LDL,** low density lipoprotein; **M extracts,** methanolic extracts; **MC,** *Momordica charantia* L.; **MCA,** *Momordica charantia* L. var. *abbreviata*; **TBA,** thiobarbituric acid; **TBARS,** thiobarbituric acid reactive substance.

INTRODUCTION

Active oxygen species and free radical-mediated reactions are involved in degenerative and 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). Many epidemiological results indicate an association between people who have a diet rich in fresh fruits and vegetables and a decrease in the risk of cardiovascular diseases and certain forms of cancer (Salah et al., 1995). Several reports have focused on the antioxidant activities of natural compounds in fruits, vegetables, and herbal medicines, such as echinacoside in *Echinaceae* root (Hu and Kitts, 2000), anthocyanin (Espin et al., 2000), various phenolic compounds (Rice-Evans et al., 1997), and a hydrolysable tannin, geraniin, from *Phyllanthus urinaria* (Lin et al., 2008).

The wild bitter gourd (*Momordica charantia* L. var. *abbreviata* Seringe, MCA), normally smaller than domesticated bitter gourd (*Momordica charantia* L., MC), belongs to the family Cucurbitaceae. The fresh fruits of MC and MCA are frequently used as vegetables in Taiwan, MC also being used as a traditional medicine and listed in the Chinese pharmacopoeia *Ben Cao Kong Mu*. MC extract partitions reportedly show many pharmacological activities (Grover and Yadav, 2004), including hypoglycemic (Miura et al., 2001; Rathi et al., 2002; Kar et al., 2003), anti-bacterial (Omoregbe et al., 1996), anti-viral (Lee-Huang et al., 1990), cytotoxic (Lee-Huang et al., 1995),

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triglyceride-lowering (Senanayake et al., 2004), and anti-inflammatory activities (Kobori et al., 2008). The MCA extracts activated peroxisome proliferator-activated receptor α (Chao and Huang, 2003), and had anti-inflammatory (Lii et al., 2009), and antioxidant activities (Wu and Ng, 2008). Wu and Ng (2008) used one MCA cultivar to prepare separate hot-water and ethanolic extracts to investigate antioxidant and anti-radical activities. In this report, water (H) and methanolic (M) extracts of sixteen cultivars from indigenous wild bitter gourd (*Momordica charantia* L. var. *abbreviata* Seringe, MCA) in Taiwan were used for antioxidant activity assays. We found that scavenging activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals were different among MCA cultivars. These useful data might promote its use as an antioxidant worthy of further research in the health food industry.

MATERIALS AND METHODS

Materials

5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), DPPH, ferrous sulfate, and low density lipoprotein (LDL, 500 μg protein/mL) were purchased from Sigma Chemical Co. (St. Louis, MO). Hydrogen peroxide (33%) was from Wako Pure Chemical Industries (Osaka, Japan). Trolox and thiobarbituric acid (TBA) were purchased from E. Merck Inc. (Darmstadt, Germany). The other chemicals and reagents were from Sigma Chemical Co. (St. Louis, MO, USA).

Preparations of MCA extracts

Sixteen fresh MCA cultivars were obtained fpr experiments from Jong-Ho Chyuan (Hualien District Agricultural Research and Extension Station, Hualien, Taiwan) and were assigned the letters A-P. Cultivars A and D contained the mature (AG and DG) and ripened (AY and DY) samples. After being washed, each de-seeded MCA (200 g) was cut into strips and blended with 200 mL deionized water. After 30 min centrifugation at 12500 ×*g*, the supernatants were saved as crude water extracts which were lyophilized as H extracts. Each de-seeded MCA (200 g) was cut into strips and blended with 200 mL methanol, and then left for 3-days. After filtering, the residue was extracted with methanol under the same procedure twice. The filtrates were collected, concentrated by rotary vacuum evaporator and then lyophilized as M extracts.

Scavenging activities of MCA extracts against DPPH radicals as detected by spectrophotometry

The 60 μl of MCA extracts (H extracts or M extracts, 25, 50, 100, and 200 μg in the final amounts were added to 20 μl of 1 M Tris-HCl buffer (pH 7.9), and then mixed with 120 μl of 100 μM DPPH in methanol to the final concentrations of 60 μM for 20 min under dark at room temperature (Hou et al., 2002; Lin et al., 2008). The decrease in absorbance at 517 nm was measured using ELISA reader (TECAN Sunrise microplate reader, Männedorf, Switzerland) and expressed as ΔA517. We measured means of triplicates and performed Trolox in parallel. We used deionized water or methanol as a blank experiment and calculated the scavenging activity of DPPH radicals (%) by the equation $(\Delta A517_{blank} - \Delta A517_{sample}) \div \Delta A517_{blank} \times 100\%$. IC_{50} stands for the concentration of 50% scavenging activity. We presented the quantitative data as the mean ± SD of three independent experiments.

Scavenging activities of MCA extracts against hydroxyl radicals as detected by electron spin resonance (ESR) spectrometry

The hydroxyl radical was generated by the Fenton reaction (Kohno et al., 1991; Liu et al., 2010). A total mixture of 500 μL included a fixed concentration of H extracts or M extracts at 500 μg/mL, 5 mM of DMPO, and 0.05 mM ferrous sulfate. The potent H extracts from cultivars D, J, and P or the M extract from cultivar F were then used to perform dose-dependent scavenging activity assays. After mixing, the solution was transferred to an ESR quartz cell, placed in the cavity of an ESR spectrometer, and hydrogen peroxide added to a final concentration of 0.25 mM. Deionized water or methanol was used instead of the sample solution for the control experiments. After 40 seconds, the relative intensity of the DMPO-OH spin-adduct signal was measured. All ESR spectra were recorded at ambient temperature (298 K) on a Bruker EMX-6/1 ESR spectrometer equipped with WIN-ESR 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 seconds and scan time, 1.5 min. The quantitative data were presented as the mean ± SD of three independent experiments

Protecting Cu^{2+} induced low density lipoprotein (LDL) peroxidation with MCA extracts

The capacity of H extracts or M extracts of MCA at a fixed concentration of 4000 μg/mL against Cu^{2+}-induced human LDL oxidation in a total 100 μL sample volume was measured by thiobarbituric acid reactive substances (TBARS) assay at wavelength 532 nm (Yan et al., 1995). The LDL (500 μg protein/mL) was incubated at 37°C under air in a 10 mM phosphate buffer (pH 7.4) containing 10 μM $CuSO_4$ for 24 h with or without MCA extracts. The reaction was stopped by adding EDTA with a final concentration of 100 μM. For TBARS determination, 100 μL of TBA solution (0.4 mg TBA in 80 mL of 10% trichloroacetic acid) was added to 20 μL of reaction mixture, mixed and heated at 90°C for 40 min. After centrifugation at 12500 ×*g* for 10 min, the 100 μL of supernatants were positioned at 96-wells and the absorbance at 532 nm was measured using ELISA reader. A student's *t*-test was used for comparison between two treatments. A difference between the blank and each treatment ($^{*}p < 0.05$; $^{**}p < 0.01$) or the blank and the control ($^{\#\#}p < 0.01$) was considered statistically significant.

RESULTS AND DISCUSSION

Cultivars A and D contained the mature (green flesh, AG and DG) and ripe (yellow flesh, AY and DY) samples. Table 1 shows the recovery rate of H and M extracts from de-seeded MCA. The recovery rate was around 2%, and the M extracts had higher recovery rate than did H extracts, except for cultivars F, H, I, J, and N.

Table 1. The recovery rate of water (H) and methanolic (M) extracts of sixteen indigenous wild bitter gourd (*Momordica charantia* L. var. *abbreviata* Seringe, MCA) cultivars from Taiwan.

No.	Extracts	Flesh weight (g)	Extract weight (g)	Recovery (%)
AG (matured)	M	203.6	4.0340	1.98
	H	208.0	4.1111	1.98
AY (ripen)	M	219.3	4.2451	1.94
	H	197.8	3.2808	1.66
B	M	196.4	3.3131	1.69
	H	207.4	2.9549	1.42
C	M	200.2	3.3338	1.67
	H	236.9	2.5125	1.06
DG (matured)	M	208.5	5.3540	2.57
	H	205.5	3.4029	1.66
DY (ripen)	M	208.2	4.4179	2.12
	H	216.7	2.5205	1.16
E	M	108.6	2.4727	2.28
	H	207.1	4.1341	2.00
F	M	53.7	1.1295	2.10
	H	52.5	1.5951	3.04
G	M	165.5	3.4774	2.10
	H	214.0	4.2893	2.00
H	M	210.3	4.1944	1.99
	H	250.1	5.4477	2.18
I	M	200.0	3.6930	1.85
	H	200.0	4.1726	2.09
J	M	145.2	2.7742	1.91
	H	202.5	5.4629	2.70
K	M	236.5	6.8875	2.91
	H	131.2	3.5497	2.71
L	M	201.4	4.6538	2.31
	H	162.8	1.2791	0.79
M	M	204.2	4.9257	2.41
	H	221.0	1.2620	0.57
N	M	223.9	4.8464	2.16
	H	212.8	4.8912	2.30
O	M	178.8	3.1145	1.74
	H	183.2	3.0729	1.68
P	M	180.5	3.9271	2.18
	H	193.7	3.4371	1.77

Active oxygen species and free radical-mediated reactions are involved in degenerative and 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). Several *in vitro* assay systems are used to evaluate the antioxidant potential in foods. DPPH radicals are widely used in model systems, to investigate the scavenging activities of several natural compounds. The color of DPPH radicals changed from purple to yellow, and the absorbance at a wavelength of 517 nm was decreased. Table 2 shows the results of IC_{50} of H and M extracts against DPPH radicals. We found that the M extracts generally showed better DPPH scavenging activities than H extracts did, and that extracts from mature flesh had better DPPH scavenging activities than extracts from ripe flesh. Among 16 MCA cultivars, the H extracts (IC_{50}, 181 μg/mL) or M extracts (IC_{50}, 246 μg/mL) of cultivar N exhibited the best DPPH scavenging activities, equivalent to that of 25.5 μg Trolox/g of H extracts and 16.88 μg Trolox/g of M extracts. Wu and Ng (2008) reported that hot water extracts and ethanolic extracts from one MCA showed DPPH scavenging activities (IC_{50}) of 129.94 and 156.89 μg/mL, respectively. These differences

Table 2. The concentration for 50% inhibition (IC_{50}) against DPPH radicals from water (H) and methanolic (M) extracts of sixteen wild bitter gourd cultivars.

No.	H extracts	M extracts
	IC_{50} (μg/mL)	IC_{50} (μg/mL)
AG	521 ±6	562 ±19
AY	726 ±26	> 1000
B	570 ±34	> 1000
C	519 ±23	519 ±5
DG	> 1000	405 ±14
DY	> 1000	672 ±20
E	> 1000	384 ±7
F	528 ±34	398 ±6
G	> 1000	> 1000
H	446 ±14	364 ±4
I	> 1000	902 ±28
J	677 ±30	734 ±7
K	> 1000	259 ±8
L	> 1000	> 1000
M	> 1000	> 1000
N	181 ±6	246 ±8
O	> 1000	766 ±24
P	720 ±19	460 ±15

might be due to a different cultivar or the 25 μM DPPH concentrations Wu and Ng (2008) used compared to our presently reported 60 μM.

The hydroxyl radical was generated by the Fenton reaction, and was trapped by DMPO to form DMPO-OH adducts. We used the intensities of the DMPO-OH spin signals on ESR spectrometry to evaluate the scavenging activities of water (Figure 1A) and methanolic (Figure 1B) extracts from 16 MCA cultivars at a fixed concentration of 500 μg/mL. Based on the results of DMPO-OH intensities

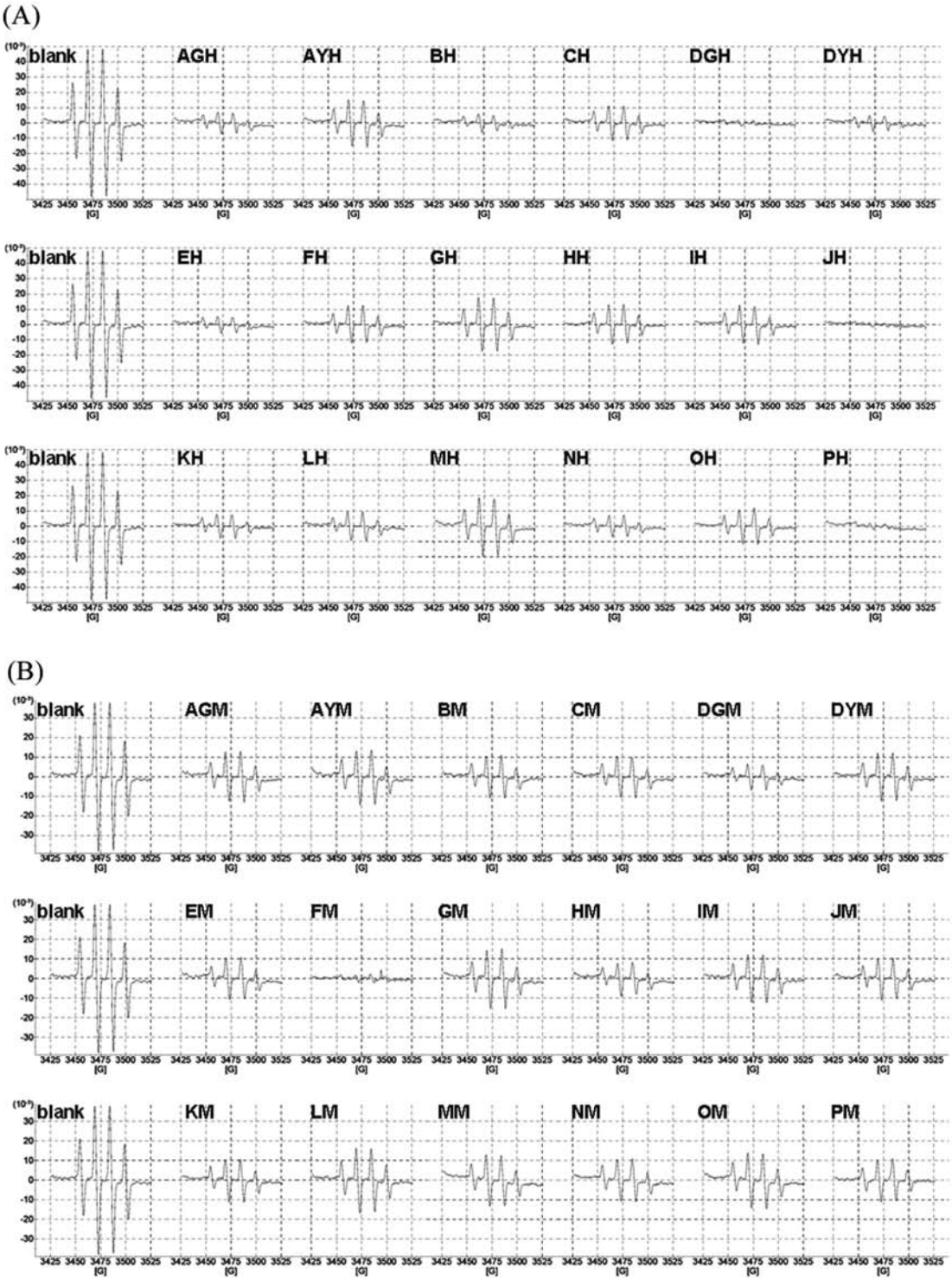

Figure 1. The intensity changes of (A) water and (B) methanolic extracts (500 μg/mL) of sixteen wild bitter gourd cultivars against DMPO-OH adducts by electron spin resonance. Sixteen MCA cultivars (A to P) were used in experiments with cultivars A and D containing the mature (AG and DG) and ripe (AY and DY) samples. Deionized water was used instead of sample (water extracts, H; methanolic extracts, M) for the control experiments.

(Figure 1), the scavenging activities against hydroxyl radicals among MCA cultivars were very different, as shown in Table 3. H extracts generally showed better hydroxyl radical scavenging activities than M extracts did, and extracts from mature flesh had better results than those from ripe flesh. The H extracts from mature D, J, and P cultivars had hydroxyl radical scavenging activities over 90% at 500 μg/mL, and M extracts from cultivar F had the highest, at 500 μg/mL (Table 3). Therefore, the different concentrations of the above-mentioned extracts were used to calculate the concentration of 50% hydroxyl radical scavenging activity. The IC_{50} was 151, 190, and 148 μg/mL, respectively, for H extracts of mature D, J, and P cultivars and 37 μg/mL for M extracts of cultivar F.

Free radicals can damage macromolecules in cells, such as DNA, proteins and lipids in membranes (Halliwell, 1999). Lipid peroxidation products (such as malondialdehyde) can damage proteins and DNA (Esterbauer et al., 1991). LDL peroxidation contributes to the development of atherosclerosis (Steinbrecher, 1987) and its delay or prevention is an important antioxidant function. The TBARS assay reveals the degrees of Cu^{2+}-induced human LDL peroxidation. Figure 2 shows the protective effect of water (A) and methanolic (B) extracts from 16 MCA cultivars at concentrations of 4 mg/mL against Cu^{2+}-induced human LDL peroxidation, using 0.8 mM Trolox (T) as comparison. Either H or M extracts exhibited protective effects against LDL peroxidation with significant differences to the blank (*p < 0.05; **p < 0.01, Figure 2). M extracts (Figure 2B) generally showed better protective effects against LDL peroxidation than H extracts did (Figure 2A). Most M extracts (4000 μg/mL) from the 16 MCA cultivars showed equally protective effects as 0.8 mM Trolox and almost retarded LDL peroxidations compared to the control. Wu and Ng (2008) used a single MCA cultivar to prepare hot-water and ethanolic extracts to investigate antioxidant and anti-radical activities. They found that hot-water extracts contained higher total flavonoids (62 mg/g) than that of ethanolic extracts (44.0 mg/g), however, the former contained lower phenolic

Table 3. The inhibition (%) against hydroxyl radicals from water (H) and methanolic (M) extracts (500 μg/mL) of sixteen wild bitter gourd cultivars.

No.	H extracts (500 μg/mL)	M extracts (500 μg/mL)
	Inhibition (%)	Inhibition (%)
AG	86.75 ± 1.90	64.38 ± 1.72
AY	66.07 ± 3.37	63.34 ± 0.44
B	86.37 ± 2.04	69.97 ± 0.95
C	74.21 ± 3.90	70.77 ± 1.45
DG	93.63 ± 2.39	82.56 ± 0.30
DY	87.47 ± 3.75	66.24 ± 1.81
E	87.60 ± 2.38	71.99 ± 0.86
F	70.39 ± 5.22	89.55 ± 1.32
G	62.03 ± 1.56	60.24 ± 0.84
H	68.51 ± 5.49	79.34 ± 1.41
I	72.15 ± 1.99	67.43 ± 0.61
J	94.83 ± 1.01	78.08 ± 8.12
K	83.00 ± 1.51	68.46 ± 4.65
L	83.01 ± 3.78	62.93 ± 8.72
M	60.51 ± 1.36	70.50 ± 6.88
N	82.44 ± 3.49	68.48 ± 3.83
O	76.12 ± 1.26	67.79 ± 6.97
P	92.17 ± 0.77	66.86 ± 6.00

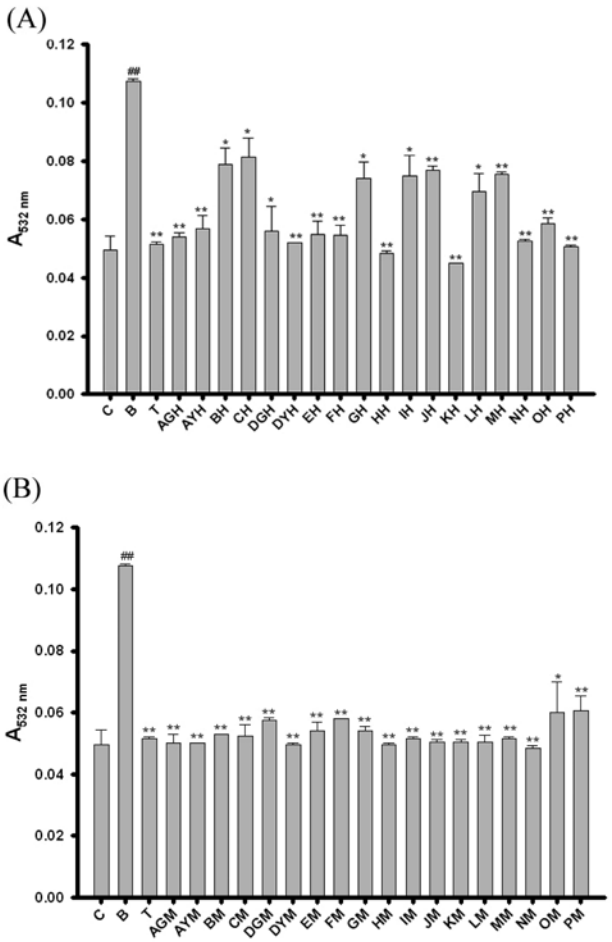

Figure 2. Effects of (A) water and (B) methanolic extracts (4000 μg/mL) of sixteen wild bitter gourd cultivars against Cu^{2+}-induced LDL peroxidation determined by thiobarbituric acid methods. Sixteen MCA cultivars (A to P) were used in experiments with cultivars A and D containing the mature (AG and DG) and ripe (AY and DY) samples. Lipid peroxidation was determined as TBARS ($A_{532\ nm}$). The C (control), B (blank) and T (0.8 mM Trolox) were added for comparison. The quantitative data were presented as mean ± SD of two independent experiments. A student's *t*-test was used for comparison between two treatments. A difference between the blank and each treatment (*p < 0.05; **p < 0.01) or the blank and the control ($^{\#\#}p < 0.01$) was considered statistically significant.

contents (51.6 mg/g) than that of the latter (68.8 mg/g). Our report made clear that the anti-radical and antioxidant activities are very different among cultivars. Kubola and Siriamornpun (2008) reported that gallic acid, tannic acid, (+)-catechin, caffeic acid, *p*-coumaric acid, ferulic acid, benzoic acid, and other unidentified compounds may contribute to the total antioxidant activities in different parts of MC fractions.

In conclusion, active oxygen species and free radical-mediated reactions are involved in degenerative and pathological processes. There has been a recent emphasis on the problems associated with aging-related diseases, including neurodegenerative diseases (e.g., Alzheimer's, Parkinsons, and Huntington's disease). The intricate causes of the aging process are still a matter of extensive speculation, and give rise to many theories. In particular, the role of reactive oxygen species is a prerequisite nowadays for understanding this process (Abrass, 1990; Bickford et al., 2000; Schulz et al., 2000). Our study shows that sixteen different indigenous wild bitter gourd cultivars in Taiwan exhibited different antioxidant and anti-radical activities. The potential antioxidant activities of these compounds should be further investigated for possible use in producing health foods.

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不同品種山苦瓜抗氧化活性研究

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本實驗以花蓮區農業改良場提供之臺灣原生 16 種不同品種山苦瓜 (*Momordica charantia* L. var. *abbreviata* Seringe, MCA)，分別以冷水（水抽物，H）與甲醇（甲醇抽取物，M）抽取，進行抗氧化活性分析。結果發現品種之間清除 DPPH 自由基與氫氧自由基的活性都不相同，最有效品種之 50%有效濃度清除 DPPH 自由基為 181 μg/mL (H) 及 246 μg/mL (M)；最有效品種之 50%有效濃度清除氫氧自由基為 148 μg/mL (H) 及 37 μg/mL (M)。在抑制銅離子誘導低密度脂蛋白過氧化活性表現，特別是甲醇抽取物（M）在 4000 μg/mL 濃度下，表現出保護效果（以 TBARS 表示）幾乎與 0.8 mM Trolox 效果相當。以上的結果顯示，山苦瓜抽取物未來也許可以開發為天然抗氧化保健食品。

關鍵詞：山苦瓜；抗氧化活性；DPPH 自由基；低密度脂蛋白過氧化。