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Evaluation of In Vitro Antioxidant and Anti-Lipid Peroxidation Activities of Ching-Pien-Tsao (*Pteris multifida* Poiret) [1]

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Abstract

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The total antioxidant capacity (TAC), scavenging ability on 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) and superoxide anions, chelating effect on copper ions, and anti $FeCl_2/H_2O_2$-stimulated linoleic acid peroxidation of aqueous extract of *Pteris multifida* Poiret (AEPM), were determined in vitro. Results indicated that the AEPM showed a high TAC (86.7% at 20 mg/mL) with ferric thiocyanate method and exhibited the highest Trolox equivalent antioxidant capacity (1.42 mM at 10 mg/mL). The scavenging ability on superoxide anion and chelating effect on copper ions of AEPM were 95.4% and 44.7% at 20 mg/mL, respectively. The linoleic acid peroxidation inhibition percentage was increased with increasing concentrations and the IC_{50} was 1000 ± 42 μg/mL.

Key words: *Pteris multifida* Poiret, Antioxidant, Anti-lipid peroxidation activity.

Introduction

The herb *Petris multifida* Poiret, commonly called Ching-Pien-Tsao in China, is one of the folk medicines and also the most widespread used material in herb beverages in Taiwan (Cheng 2006). *P. multifida* Poiret has various flavonoids and possesses antipyretic, detoxified, antibiotic, anti-inflammation and antimutagenic activities (Lee & Lin 1988). *P. multifida* Poiret has been reported in traditional Chinese medicine books and in clinical application in Taiwan to be extensively used to treat various symptoms such as heat-shock. In the present study, in vitro antioxidant properties of aqueous extracts of *P. multifida* Poiret (AEPM) were assayed in terms of antioxidant activity by the ferric thiocyanate method, scavenging abilities on superoxide anion, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$), chelating ability on copper ions and anti $FeCl_2/H_2O_2$-stimulated linoleic acid peroxidation.

Materials and Methods

Chemicals

The chemicals which were used in this study including ascorbic acid, 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$), butylated hydroxyanisol (BHA), ethylenediaminetetraacetic acid (EDTA), linoleic acid, β-nicotinamide adenine dinucleotide, nitro blue tetrazolium, phenazine methosulfate, potassium persulfate, tetramethyl murexide, thiobarituric acid, α-tocopherol (Sigma Chemical Company, USA) and ammonium thiocyanate, chloroform, copper sulfate, ferrous chloride, potassium persulfate, trichloroacetic acid (E. Merck Inc., Germany).

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Preparation of aqueous extract of *Pteris multifida* Poiret (AEPM)

P. multifida Poiret was obtained from a local herb store and the voucher specimens were deposited in the herbarium of Faculty of Pharmacy, College of Pharmacy, Kaohsiung Medical University (Kaohsiung, Taiwan). For the aqueous extract preparation, whole plant was freeze-dried after washing, ground into powder and through a sieve (40-mesh) and then 100 g powder was boiled with 1 L of deionised water for 1 h, for a total of three times. The decoction was filtered, lyophilized, and kept at -20°C until use. The yield of AEPM was 5.34%.

Evaluation of total antioxidant activity of AEPM

The total antioxidant capacity (TAC) of AEPM was determined according to the method of Mitsuda *et al.* (1966). AEPM (0.5 mg) in 0.5 mL of deionised water were mixed with linoleic acid emulsion (2.5 mL, 0.02 M, pH 7.0) and phosphate buffer (2 mL, 0.2 M, pH 7.0). The mixed solution in a test tube was incubated at 37℃, and the peroxide value was determined by reading the absorbance at 500 nm after color development with ferrous chloride and ammonium thiocyanate at intervals during incubation. All test data are the average of triplicate analyses. The TAC was calculated as follows: TAC (%) = $[1 - (\Delta A_{500,\ sample})/(\Delta A_{500,\ control})] \times 100$. Butylated hydroxyanisol (BHA), ascorbic acid and α-tocopherol were used as controls.

Determination of Trolox equivalent antioxidant capacity of AEPM

The determination of $ABTS^{\cdot+}$ radical scavenging of AEPM was carried out as described by Re *et al.* (1998). The $ABTS^{\cdot+}$ radical was generated by reacting an $ABTS^{\cdot+}$ (7 mM) aqueous solution with potassium persulfate (2.45 mM, final concentration) in the dark for 12–16 h, at ambient temperature, and adjusting the Abs 734 nm to 0.700 (± 0.020) at 30℃. AEPM was diluted and a 10.0 mL sample when added to 1.0 mL $ABTS^{\cdot+}$ resulted in inhibition of the blank absorbance. After 1.0 mL $ABTS^{\cdot+}$ was added to 10.0 mL AEPM or Trolox standards, the absorbance at 734 nm was recorded 1 min after initial mixing and subsequently (for 10 min). All test data are the average of triplicate analyses. The Trolox equivalent antioxidant capacity (TEAC) was calculated against a Trolox calibration curve. Ascorbic acid and α-tocopherol were used as controls.

Investigation of scavenging effect on superoxide anions of AEPM

The scavenging effect on superoxide anions of AEPM was determined according to the method of Robak & Gryglewski (1988). The reaction mixture, which contained 1.0 mL of AEPM (1–20 mg/mL) in deionised water, 1.0 mL of 120 μM phenazine methosulfate in phosphate buffer (0.1 M, pH 7.4), 1.0 mL of 936 mM β-nicotinamide adenine dinucleotide in phosphate buffer, and 1.0 mL of 300 μM nitro blue tetrazolium in phosphate buffer, was left standing at ambient temperature for 5 min, and the absorbance was measured at 560 nm against blank solution. All test data are the average of triplicate analyses. The percentage of scavenging effects (%) was calculated as follows: scavenging effect, $_{\text{superoxide anion}}$ (%) = $[1 - (\Delta A_{560,\ sample})/(\Delta A_{560,\ control})] \times 100$. BHA and α-tocopherol were used as controls.

Assay of chelating ability on copper ions of AEPM

Chelating ability on copper ions of AEPM was determined according to the method of Shimada *et al.* (1992). To 2.0 mL of the mixture consisting of 30 mmol/L hexamine, 30 mmol/L potassium chloride and 9 mmol/L copper sulfate was added to AEPM (0–20 mg/mL for copper ions) in 2 mL of deionised water and 0.2 mL of 1 mmol/L tetramethyl murexide. After 3 min at ambient temperature, the absorbance of the mixture was determined at 485 nm against a blank. All test data are the average of triplicate analyses. The percentage of chelating effects (%) was calculated as follows: chelating effect, $_{\text{copper ions}}$ (%) = $[1 - (\Delta A_{485,\ sample})/(\Delta A_{485,\ control})] \times 100$. Ethylene diamine tetraacetic acid (EDTA) and ascorbic acid were used as controls.

Evaluation of anti-lipid peroxidation activity of AEPM

The effect of anti-$FeCl_2/H_2O_2$-stimulated linoleic acid peroxidation of AEPM was determined by the method of Tamura & Shibamoto (1991). AEPM (0–1000 μg/mL) were added to a solution of 0.1 mol/L linoleic acid (0.2 mL), 2.0 mmol/L $FeCl_2(H_2O)_4$ (0.2 mL), 2.0 mmol/L H_2O_2 (0.2 mL) and 0.2 mol/L phosphate buffer (pH 7.4, 5 mL). The reaction mixture was incubated at 37℃ for 24 h. After incubation, 0.2 mL BHA (20 mg/mL), 1.0 mL thiobarituric acid (10 mg/mL) and 1.0 mL trichloroacetic acid (100 mg/mL) were added to the mixture, which was heated for 30 min in a boiling water bath. After cooling, 5.0 mL chloroform was added and the mixture was centrifuged at 1000× *g* to give a supernatant. The absorbance of supernatant was measured spectrophotometrically at 532 nm. All test data are the average of triplicate analyses. The inhibition of $FeCl_2/H_2O_2$-stimulated linoleic acid peroxidation (%) was calculated as follows: inhibition of peroxidation, $_{\text{linoleic acid}}$ (%) = $[1 - (\Delta A_{532,\ sample})/(\Delta A_{532,\ control})] \times 100$. The 50% of inhibitory concentration (IC_{50}) was also measured. BHA and α-tocopherol were used as controls.

Statistical analysis

Data represent the mean ± S. D. and differences from the treatment group mean were determined by ANOVA followed by Duncan's multiple range test. A value of $P < 0.05$ was considered statistically significant.

Results and Discussion

The in vivo toxicological study was done by 28-day oral administration of AEPM on rats in our previous report (Wang *et al.* 2007a). In that research, no oral toxicity or side effect of AEPM on rats was found. Base on the investigation, it seems that AEPM was safety when administrated by oral. On the other hand, it is well-known a steady health condition which balance between oxides and antioxidants in organism (McCord 1994). Unfortunately, the organism antioxidant system usually not prefect to inhibit oxidative damages and these damages are correlated to chronic disease such as cancer and cardiovascular disease (Ames 1989).

Excessive free radicals are produced during oxidative stress and to cause damage to biomolecules. Antioxidant studies indicated that AEPM should possess the ability of inhibiting free radical formation or scavenging free radical. AEPM showed a rapid, dose-dependant and increase tendency of TAC (86.7% at 20 mg/mL) and as equal as ascorbic acid at 10 mg/mL (Fig. 1); however, BHA significantly exhibited the highest ($P < 0.05$) inhibition ability at very low concentrations (0.1–0.5 mg/mL). Superoxide anion is one of the most important reactive oxygen in living cells and possesses very strong oxidative ability and can cause huge damages to cells (Halliwell & Gutteridge 1990). AEPM also exhibited high scavenging superoxide anion ability (95.3% at 20 mg/mL) though lower than that of α-tocopherol and BHA (Fig. 2). $ABTS^{\cdot+}$ is one of positive free radicals and could be quenched by α-tocopherol, and the TEAC assay is a simple and quick detection method for $ABTS^{\cdot+}$ (Arts *et al.* 2004). AEPM could scavenge $ABTS^{\cdot+}$ radical cation, the TEAC value of AEPM was equal to 1.42 mM Trolox at 10 mg/mL and was significant higher ($P < 0.05$) than that of Trolox, α-tocopherol and ascorbic acid, respectively (Fig. 3). Transition metal ions, such as copper ions, are known to catalyze the formation of free radicals and a minority of metal ions could accelerate the lipid peroxidation was reported (Gordon 1996). The chelating effect on copper ions of AEPM were increased with increasing concentrations and exhibited 44.7% chelating ability at 20 mg/mL and much higher ($P < 0.05$) than that of ascorbic acid though poor than EDTA at all tested concentrations (Fig. 4). In $FeCl_2/H_2O_2$-stimulated linoleic acid peroxidation system, the IC_{50} value of AEPM was 1000 ± 42 μg/mL and significant lower ($P < 0.05$) than that of BHA (Fig. 5); however, the α-tocopherol exhibited a pro-oxidant activity at lower concentrations (between 100–600 μg/mL) and its inhibitory effect were lower than that of AEPM at higher concentrations (above 800 μg/mL). The pro-oxidant phenomenon might come from α-tocopherol trapping

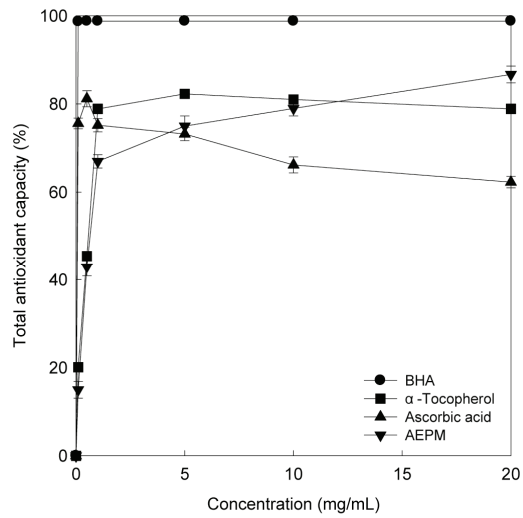

Fig. 1. The total antioxidant capacity of aqueous extracts of *P. multifida* Poiret (AEPM). Each value is expressed as mean ± standard deviation (n = 3).

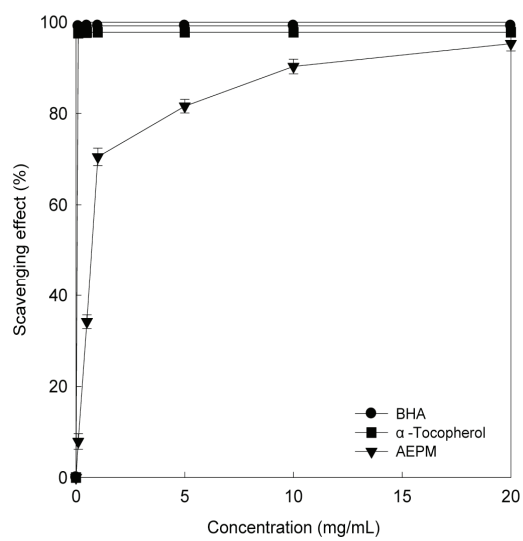

Fig. 2. Scavenging effects of aqueous extracts of *P. multifida* Poiret (AEPM) on superoxide anions. Each value is expressed as mean ± standard deviation (n = 3).

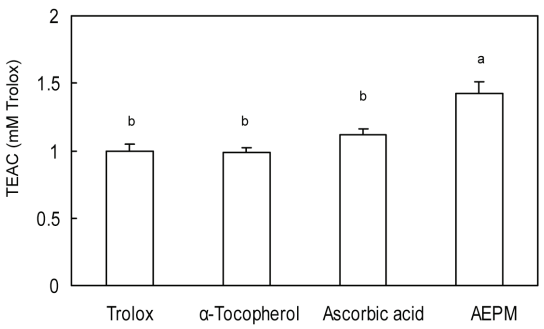

Fig. 3. The TEAC value of aqueous extracts of *P. multifida* Poiret (AEPM). The concentrations of Trolox, α-tocopherol, ascorbic acid and AEPM were 1 mM, 1mM, 1mM and 10 mg/mL, respectively. Each value is expressed as mean ± standard deviation (n = 3).

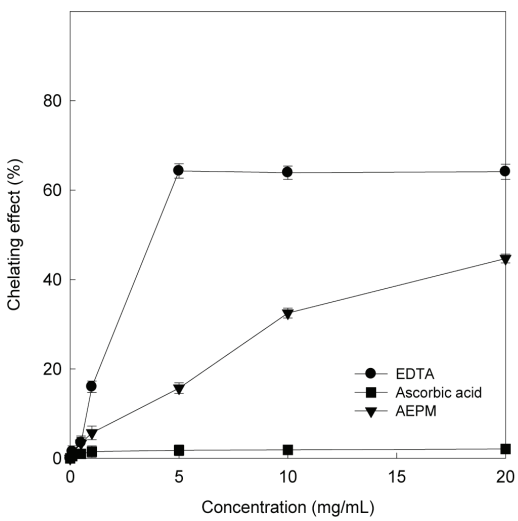

Fig. 4. Chealting effects of aqueous extracts of *P. multifida* Poiret (AEPM) on copper ions. Each value is expressed as mean ± standard deviation (n = 3).

hydrogen to form a new radical (Schuler 1990) and cause free radical chain reaction (Cillard & Cillard 1980).

In conclusion, many antioxidant capacities of aqueous extracts from various herbs were observed (Dorman *et al.* 2003; Kumaran & Karunakaran 2006; Chyau *et al* 2006). On the other hand, extract with water is a cheap, easy and safe method (Møller *et al* 1999) and more accordant with the actual habit, especially, it is the prior choice when developing health herb drink (Duh 1999). According to our previous report (Wang *et al* 2007b) and based on above results in this research, it could be concluded that AEPM

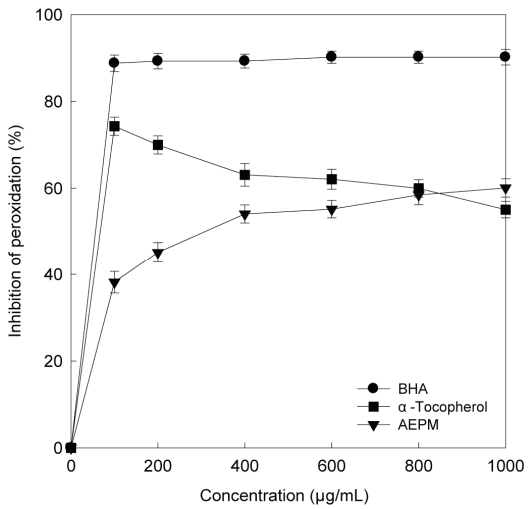

Fig. 5. Inhibition of peroxidation effect of aqueous extracts of *P. multifida* Poiret (AEPM) in $FeCl_2/H_2O_2$-stimulated linoleic acid peroxidation system. Each value is expressed as mean ± standard deviation (n = 3).

is a potent antioxidant. The antioxidant mechanism of AEPM could be thorough scavenging free radicals, chelating transition metal ions and break chain reaction of lipid peroxidation. Meanwhile, its antioxidant activity may result from its various antioxidants, e.g. flavonoids (Lu *et al.* 1999) and phenols (Hu & Zheng 2004). Further and detailed researches, such as specific phenolic compositions and physiological functions of *P. multifida*, are going on in our laboratory.

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井邊草體外抗氧化與抗脂質過氧化活性之評估[1]

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摘 要

王子慶、李後易、楊季清。2009。井邊草體外抗氧化與抗脂質過氧化活性之評估。台灣農業研究 58:55–60。

本研究藉由硫氰酸鐵法、清除 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$)、超氧陰離子、螯合銅離子與抑制 $FeCl_2/H_2O_2$ 所誘導之亞麻油酸過氧化等方法，評估井邊草水萃取物 (aqueous extract of *P. multifida* Poiret, AEPM) 體外總抗氧化能力 (total antioxidant capacity, TAC) 與抗脂質過氧化活性。結果顯示，AEPM 具有高 TAC (在 20 mg/mL 濃度下之 TAC 爲 86.7%) 與最高之水溶性維他命 E (Trolox) 等價抗氧化能力 (在 10 mg/mL 濃度下爲 1.42 mM)。AEPM 濃度爲 20 mg/mL 時，清除超氧陰離子與螯合銅離子之能力分別爲 95.4%及 44.7%，亞麻油酸脂質過氧化抑制率則會隨著濃度之增加而增加，其 IC_{50} 是 1000 ± 42 μg/mL。

關鍵詞：井邊草、抗氧化、抗脂質過氧化活性。

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