ANTIOXIDANT ACTIVITY OF *JACARANDA ACUTIFOLIA* FLOWER

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(Received 1st December 2006, accepted 25th April 2007)

Jacaranda acutifolia Humb. (Bignoniaceae) is a cultivated species in Taiwan area as an ornamental plant. The antioxidant activity of the acetone extract and its subfractions of *Jacaranda acutifolia* flower were evaluated using different antioxidant methodologies: DPPH free radical scavenging, chelating activity on ferrous ion and reducing power. The total phenolic content of these extracts was determined as the gallic acid equivalent. The antioxidant activities of all extracts were concentration-dependent and consistent with the total phenolic content. The ethyl acetate soluble fraction (EF) of *Jacaranda acutifolia* flower possessed the highest antioxidant activities, including effective DPPH radical scavenging (EC_{50} = 0.049 mg/mL) and ferric ion reducing activities (EC_{50} = 0.125 mg/mL). The total phenolic contents were estimated as 17.20 mg/g gallic acid equivalents. These results demonstrated the antioxidant potential of the extracts of *Jacaranda acutifolia* flower.

Key words: Antioxidant activity, *Jacaranda acutifolia*, Flower extracts.

INTRODUCTION

Free radicals and reactive oxygen species are byproducts in aerobic organism and have aroused significant interest among scientists in the past decade. It has been proposed that they could induce cellular damage and might be involved in several human diseases including cancer, arteriosclerosis, diabetic mellitus, hypertension, and AIDS and in aging processes[1-4]. Of various kinds of natural antioxidants, flavonoids and phenolic compounds have received much attention[5-8].

Jacaranda acutifolia Humb. (Bignoniaceae) is a cultured species in Taiwan area as a garden plant[9]. Various activities have been discovered in *Jacaranda* species such as anti-dyspeptic activity in *Jacaranda caroba*[10], the cytotoxic[11], leishmanicidal[12] and anti-malarial activity[13] in *Jacaranda caucana*, the lipoxygenase inhibitory activity

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in *Jacaranda filicifolia*[14], and the cardiovascular[15], cytotoxic[16] and antimicrobial activities[17-18] in *Jacaranda mimosaefolia*. However, little information is known about the antioxidant activity of *Jacaranda acutifolia*. This study intended to investigate the antioxidant activities of various extracts of *Jacaranda acutifolia* flower. The contents of the phenolic derivatives in the extracts were analyzed. The antioxidant activities, including the radical-scavenging effect, chelating activity on Fe^{2+} and reducing power were investigated, and the results were compared with those of the natural antioxidant, L-ascorbic acid (vitamin C).

MATERIALS AND METHODS

Plant Materials

The plant material was collected from the campus of Providence University (Taiwan) in July 2003. The voucher specimen was deposited at the herbarium of the Institute of Applied Chemistry, Providence University. The air-dried and powdered flowers of *Jacaranda acutifolia* (1.5 Kg) were extracted with acetone (10 liter × 3) at room temperature. After removal of the solvent, the syrup of the extracts was obtained (AE, 10.20%, w/w). Part of the extracts (80 g) was suspended in water and then partitioned with ethyl acetate and *n*-butanol sequentially to yield ethyl acetate fraction (EF, 6.03%, w/w), *n*-butanol fraction (BF, 1.82%, w/w) and aqueous fraction (WF, 2.35%, w/w).

Chemicals

α,α-Diphenyl-β-picrylhydrazyl (DPPH), L-ascorbic acid, gallic acid (GA), ferrous chloride, and potassium ferricyanide were purchased from Sigma chemicals Co. (USA). 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid monosodium salt (Ferrozine) and ferric chloride were obtained from Aldrich chemicals Co. (USA). All other chemicals were of analytical grade.

Total Phenolic Contents Analysis

Total phenolic content was analyzed using the Folin-Ciocalteu reagent method[19]. A mixture of an aliquot of the extracts (0.5 mL, 0.625 mg/mL), Folin-Ciocalteu reagent (0.5 mL) and 10% Na_2CO_3 (0.05 mL) was incubated at room temperature for 1 h, and then the absorbance was measured at 735 nm. Gallic acid was used as the standard for the calibration curve, and the total phenolic contents were expressed as mg gallic acid equivalents per gram of tested extracts.

Scavenging Effect on DPPH Radical

The antioxidant activity of the plant extracts and the standard were assessed on the basis of the radical scavenging effect of the stable DPPH free radical[20]. A total of 750 μl aliquot of the extracts (4 mg/mL to 125

μg/mL in DMSO) or standard was added to 200 μl of DPPH in methanol solution. After incubation at 37 °C for 20 min, the absorbance of each solution was determined at 517 nm using a Shimadzu spectrometer. Percentage inhibition and the concentration of sample required for 50% scavenging of the DPPH free radical (EC_{50}) were determined in triplicate. L-Ascorbic acid was used as the standard compound.

The antioxidant activity constants (K) can be obtained from the slope of a ln ($1-A/A_0$) against antioxidant concentration plot[21] where A is the antioxidant activity at any given antioxidant concentration, and A_0 is the equilibrium antioxidant activity measured as the antioxidant activity remanding constant over a large antioxidant concentration. A higher K value represents a higher antioxidant activity.

Chelating Activity on Ferrous Ion

The Fe^{2+}-chelating ability of the plant extracts was determined according to the method reported by Decker and Welch[22]. An aliquot of the extracts (250 μ1, 0.094-3.0 mg/mL) in buffer solution (925 μl, pH 7.4) was treated with $FeCl_2$ (2 mM, 25 μl) and ferrozine (5 mM, 50 μl) for 10 min at room temperature. The absorbance of the resulting solution was measured at 562 nm. A lower absorbance of the reaction mixture indicated a higher Fe^{2+}-chelating ability. The capability to chelate the ferrous ion was calculated using the following equation:

Chelating activity% = 1 - (absorbance of sample at 562 nm - sample background at 562 nm/absorbance of control at 562 nm) × 100%

Reducing Power Test

The reducing power was evaluated by the Fe^{3+}-Fe^{2+} transformation in the presence of the samples using the method of Yen and Chen[20]. The mixture of the extracts (0.25-4.0 mg/mL) or L-ascorbic acid (0.002-0.062 mg/mL) in an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide was incubated at 50 °C for 20 min. The reaction was terminated by adding an equal volume of 1.0% trichloroacetic acid, and then the mixture was centrifuged at 2790 × *g* for 10 min. The supernatant was mixed with distilled water and 0.1% $FeCl_3$ at a ratio of 1:1:2 (v/v/v), and then absorbance was measured at 700 nm.

Statistical Analysis

Analysis of variance was performed by ANOVA procedures. The difference of means was determined by Duncan's new multiple-range test. Values of $p < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Total Phenolic Contents

The amounts of total phenolic contents of the extracts of *J. acutifolia* were depicted in Table 1. Phenolic

Table 1. The DPPH radical-scavenging activity, the antioxidant activity constant (K) of DPPH, ferric reducing activity and total phenolic contents of the *Jacaranda acutifolia* extracts

Extracts	DPPH radical-scavenging activity EC_{50} (mg/mL)	K (mL/mg)	Ferric reducing activity EC_{50} (mg/mL)	Total phenolic contents (mg GAE/g)
AE	0.111 ± 0.009^{a}	8.765 ± 0.108^{a}	N.D	25.743 ± 0.606^{a}
EF	0.049 ± 0.030^{b}	19.638 ± 0.378^{b}	0.125 ± 0.001^{b}	17.200 ± 0.295^{b}
BF	0.062 ± 0.008^{c}	23.225 ± 0.446^{c}	0.567 ± 0.012^{c}	33.576 ± 0.213^{c}
WF	0.538 ± 0.091^{d}	4.576 ± 0.946^{d}	1.492 ± 0.037^{d}	1.925 ± 0.018^{d}
Vit.C	0.006 ± 0.005^{e}	127.662 ± 1.863^{e}	0.172 ± 0.002^{e}	N.D

1. The DPPH radical-scavenging activity EC_{50}, antioxidant activity constant K and total phenolic contents are given as mean ± standard deviation, (n = 3). Ferric reducing activity EC_{50} is given as mean ± standard deviation, (n = 6). $^{a-e}$ Means in the column followed by different letters are significantly different ($p < 0.05$).
2. N.D expressed denotes not detectable.

compounds are considered to be the most important antioxidative plant components[5]. The highest amount was found in *n*-butanol soluble fraction (BF) (33.57 mg gallic acid equivalent/g extract).

Scavenging Effect on DPPH Radical

The proton-radical scavenging action has been known as an important mechanism of antioxidation. As shown in Fig. 1, the DPPH radical-scavenging activities of the extracts from *J. acutifolia* were dose-dependent; a higher concentration of the extracts resulted in the higher radical-scavenging activity. The ethyl acetate soluble fraction (EF) exhibited the highest DPPH-scavenging activity (EC_{50} = 0.049 mg/mL) while the aqueous fraction had the lowest activity (EC_{50} = 0.538 mg/mL). Although the extracts showed lower DPPH radical-scavenging abilities compared to L-ascorbic acid (EC_{50} = 0.006 mg/mL), the extracts did show the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants[20].

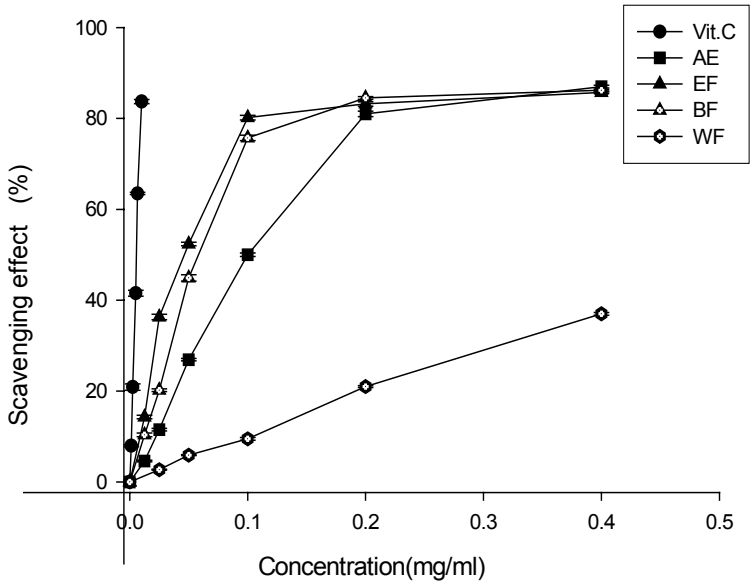

Fig. 1. The scavenging effect of *Jacaranda acutifolia* extracts on DPPH radical. Each value is expressed as mean ± standard deviation (n = 3).

Chelating Activity on Ferrous Ion

Many plant phenolic compounds have been described as antioxidants due to their chelating ability to iron ion. As shown in Fig. 2, the plant extracts displayed the Fe^{2+}-chelating effect in a concentration-dependent manner. The EF extracts had the highest Fe^{2+}-chelating effect (EC_{50} = 0.125 mg/mL) while the WF extract had the lowest effect (EC_{50} = 1.492 mg/mL). *Jacaranda acutifolia* extracts may afford protection against oxidative damage even though their Fe^{2+}-chelating activities were higher than that of L-ascorbic acid (EC_{50} = 0.172 mg/mL).

Reducing Power

The presence of reductants (antioxidants) would result in the reduction of Fe^{3+}/ferricyanide complex to its ferrous form which can be monitored by the formation of Perl's prussian blue at 700 nm. Dose-response curves (Fig. 3) were obtained from the extracts of *J. acutifolia* and the ethyl acetate fraction (EF) had the highest reducing power among all extracts.

At a dosage of 4.0 mg/mL, high reducing power values of 2.85 and 1.88 were found in EF and BF, respectively, while a low value of 0.69 was obtained in WF. This indicated that the *J. acutifolia* extracts could serve as electron donors and react with free radicals, converting them to more stable products and terminating the radical chain reaction[20]. The reducing power of *J. acutifolia* extracts was less pronounced than that of Vit. C. At a concentration of 2.0 mg/mL, the reducing power values were 3.59 and 1.68 in vitamin C and EF, respectively.

In summary, the ethyl acetate soluble fraction (EF) of *J. acutifolia* flower demonstrated the highest anti-oxidant activities, include scavenging effect on DPPH radical (EC_{50} = 0.049 mg/mL), ferric reducing activity (EC_{50} = 0.125 mg/mL), and total phenolic contents (17.20 mg/g as gallic acid). All antioxidant activities of the extracts performed in a concentration-dependent manner. The antioxidant potential of *Jacaranda acutifolia*

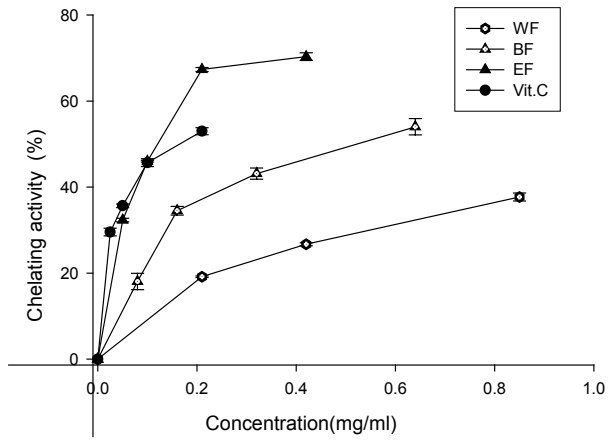

Fig. 2. The ferric chelating activity of *Jacaranda acutifolia* extracts. Each value is expressed as mean ± standard deviation (n = 6).

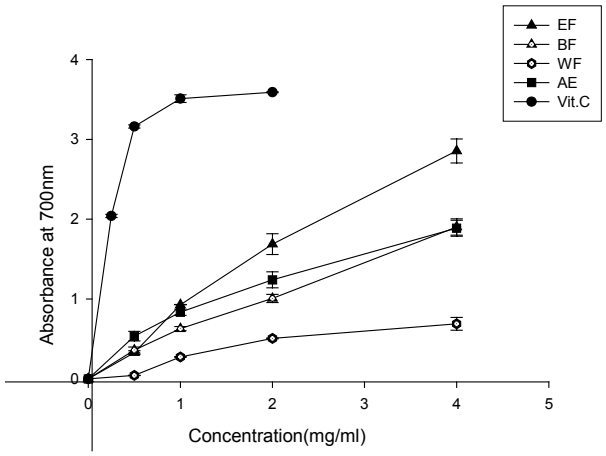

Fig. 3. Reducing power of *Jacaranda acutifolia* extracts. Each value is expressed as mean ± standard deviation (n=6).

flower was illustrated in this study. However, the components responsible for the antioxidant activities remain unknown. Further isolation and identification of the antioxidant components are necessary.

ACKNOWLEDGEMENTS

The authors are grateful to Providence University for financial support (PU-93-11100-5162-006-010). The authors are also indebted to Prof. Kuoh-Cheng Yang (Department of Ecology, Providence University) for the identification of the plant and Dr. Grace Shiahuy Chen (Department of Applied Chemistry, Providence University) for her kindly suggestions of this research.

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J Chin Med 17(4): 143-150, 2006

藍花楹花部抗氧化活性之研究

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(95 年 12 月 1 日受理，96 年 4 月 25 日接受刊載)

藍花楹（*Jacaranda acutifolia* Humb.）為紫葳科落葉喬木，原產於美洲熱帶地區，為世界著名觀賞樹木，台灣引進栽培為庭院觀賞植物，亦可供行道、街道植栽之用。本研究以藍花楹花部丙酮萃取物進行抗氧化活性測試。測定項目包含自由基（2,2-二苯基苦味肼基團，DPPH）清除能力，螯合亞鐵離子之能力（chelating ability on ferrous ions）、還原力（reducing power）及總酚類含量（total phenol content）評估。結果顯示乙酸乙酯可溶層擁有較高的抗氧化活性，其清除 DPPH 自由基能力為（EC_{50} = 0.049 mg/mL）及螯合亞鐵離子能力為（EC_{50} = 0.125 mg/mL），總酚類含量為（17.20 mg/g as gallic acid）。此研究證實藍花楹花部具有抗氧化活性研究開發的潛力。

關鍵詞：藍花楹，花部，抗氧化活性。

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