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Antioxidant Properties of the Extracts from Different Parts of Broccoli in Taiwan

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ABSTRACT

The flowers, stems, and leaves of broccoli (*Brassica oleracea* L var *italica Plenca*) cultivated in Taiwan were freeze-dried and extracted with methanol, water, or acetone. The antioxidant properties, including reducing power, ferrous ion chelating ability, and α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity, were tested in this study. The above antioxidant properties of broccoli extracts along with alpha-tocopherol and butylated hydroxyanisole (BHA) were compared. Results showed that the methanol and water extracts exhibited a higher reducing power in all three parts; while the acetone extract was the least. The stem extracts showed the highest reducing power, which was 1.3 times those alpha-tocopherol and BHA extracts, followed by the leaf extracts, which exhibited similar reducing power to alpha-tocopherol and BHA. The lowest reducing power was observed on flower extracts, which was only three fourth of the reducing power as compared to alpha-tocopherol and BHA. The methanol and water extracts of broccoli also exhibited high chelating ability; while the acetone extracts showed the lowest. The broccoli stem exhibited the highest chelating ability among three parts of broccoli. The acetone extracts from stems hardly showed any chelating ability as compared to alpha-tocopherol and BHA. The methanol extracts of broccoli showed the highest DPPH radical scavenging activity (>90%) among three different solvent extracts. Its DPPH radical scavenging activity was close to BHA and alpha-tocopherol. The water extracts showed only 43% DPPH radical scavenging activity; while the acetone extracts barely showed any DPPH radical scavenging activity.

Key words: Broccoli, antioxidant properties, reducing power, ferrous ion chelating ability, DPPH radical scavenging activity

INTRODUCTION

Vegetables are important to the human diet, and many studies have shown that a close relation exists between the intake of vegetables and cancer prevention[1-5]. Although the mechanism of cancer prevention by intake of vegetables is unclear, one important factor is the abundant natural antioxidants, such as vitamin A, C, E, and β-carotene, in vegetables. Vitamin C, a hydrophilic compound, is a major antioxidant to quench oxidation type of free radicals in blood. Both vitamin E and β-carotene exhibit antioxidant activity under lipophilic and hydrophilic conditions. Vitamin E is located in cell membranes and capable of reducing the free radicals in the cell membrane. β-Carotene is the major antioxidant in cell membranes and lipoproteins, although it shows a weaker antioxidant activity[6].

Vegetables contain not only the above nutritional antioxidants but also a great quantity of non-nutritional antioxidants, such as flavoniods, flavone, and polyphenol compounds[7-10]. Many studies have indicated that a frequent intake of cruciferous vegetables, such as broccoli, cauliflower, leaf mustard, cabbage, Chinese broccoli, and turnip, could protect against cancer[11,12]. Prochaska *et al.*[12] and Zhang *et al.*[13] have reported that a compound sulforaphane was isolated and identified from broccoli. Sulforaphane is able to induce some enzymes, such as quinone reductase and glutathione S-transferases, to metabolize xenobiotics. Thus, the intake of cruciferous vegetables could induce the detoxification enzymes, which accelerate the xenobiotics metabolism and therefore reduce the formation of tumor cells.

Research on cruciferous vegetables has focused on its edible parts; for example, the flower and tender stem parts of broccoli or cauliflower. The inedible parts are usually disposed of or used as organic fertilizers. In this study, the flowers, stems, and leaves of domestic broccoli were tested to understand their antioxidant properties.

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MATERIALS AND METHODS

I. *Materials*

(I) *Broccoli*

Broccoli (*Brassica oleracea* L var *italica Plenca*) purchased from a supermarket in Changhua, Taiwan, was cleaned and divided into three parts, flower, stem, and leaf. The materials were then freeze-dried, ground to powders, separately packed into plastic bags, and kept in a refrigerator at 4˚C until use.

(II) *Reagents*

1. Potassium ferricyanide ($K_3Fe(CN)_6$, of purity 98.0%) was purchased from KATAYAMA Chemical Co., Ltd., Japan.
2. Ferric chloride ($FeCl_3 \cdot 6H_2O$, of purity 97.0%) was purchased from KATAYAMA Chemical Co., Ltd., Japan.
3. Butylated hydroxyanisole (BHA, of purity >90%) was obtained from SIGMA Chemical Co., USA.

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4. alpha-Tocopherol (of purity 95%) was obtained from SIGMA Chemical Co., USA.
5. Ferrous chloride ($FeCl_2 \cdot 4H_2O$, of purity >99%) was purchased from SIGMA Chemical Co., USA.
6. α,α-Diphenyl-β-picrylhydrazyl free radical (DPPH, TCI-GR) was obtained from TOKYO Chemical Inc., Japan.
7. 3-(2-Pyridyl)-5,6-di(p-sulfophenyl)-1,2,4-triazine, disodium salt (ferrozine) (TCI-GR) was obtained from TOKYO Chemical Inc., Japan.

II. *Methods*

(I) *Preparation of Sample Extracts*

Dry broccoli samples (100~1000 mg) were weighed and 50 mL of methanol, water, and acetone were then separately added into samples. The ratios of sample weight to solvent volume were 2, 4, 8, 12, 16, and 20 mg/mL. The extracts were then vacuum filtered, and tested for antioxidant activities.

(II) *Test for Reducing Power*

A method developed by Oyaizu[14] for reducing power test was used. The above broccoli extracts (10 mL) including methanol, water, and acetone solutions together with alpha-tocopherol and BHA methanolic solutions were spiked with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was then kept in a 50˚C water-bath for 20 min. The resulting solution was then cooled rapidly, spiked with 2.5 mL of 10% trichloroacetic acid, and centrifuged at 3000 rpm for 10 min. The supernatant (5 mL) was then mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride. The absorbance at 700 nm was then detected after reaction for 10 min. The higher the absorbance represents the stronger the reducing power.

(III) *Test for Ferrous Ion Chelating Ability*

A published method by Decker and Welch[15] was adopted. Five mL of the test solutions, including broccoli extract, alpha-tocopherol, and BHA solutions, were spiked with 0.1 mL of 2 mM $FeCl_2$ and 0.2 mL of 5 mM ferrozine solutions. After reaction for 10 min, the absorbance (at 562 nm) of resulting solutions was recorded. A complex of Fe^{+2} /ferrozine has a strong absorbance at 562 nm. The higher the ferrous ion chelating ability of the test sample gives the lower absorbance. The percentage of ferrous ion chelating ability is expressed by [1-(test sample absorbance / blank sample absorbance)] x 100.

(IV) *Test for DPPH Radical Scavenging Activity*

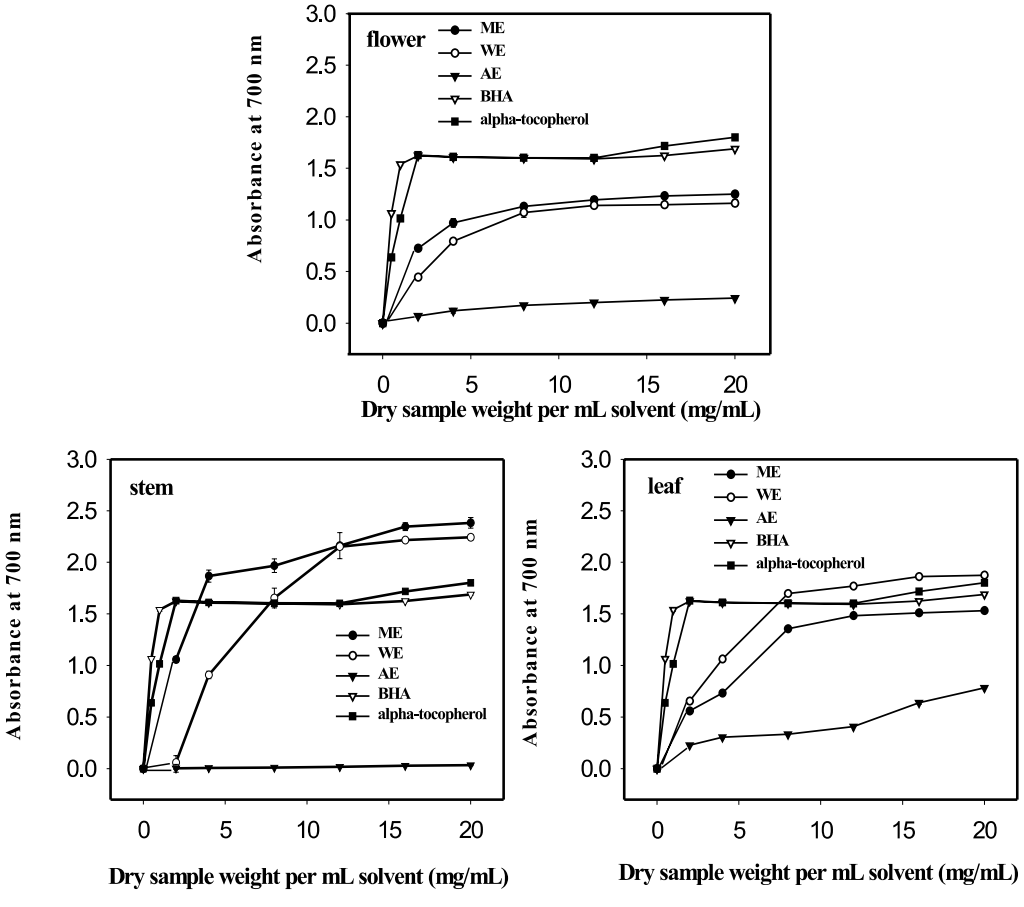

Figure 1. Reducing power of the extracts from different parts of broccoli using various solvents in comparison with BHA and alpha-tocopherol. ME: methanol extract; WE: water extract; AE: acetone extract.

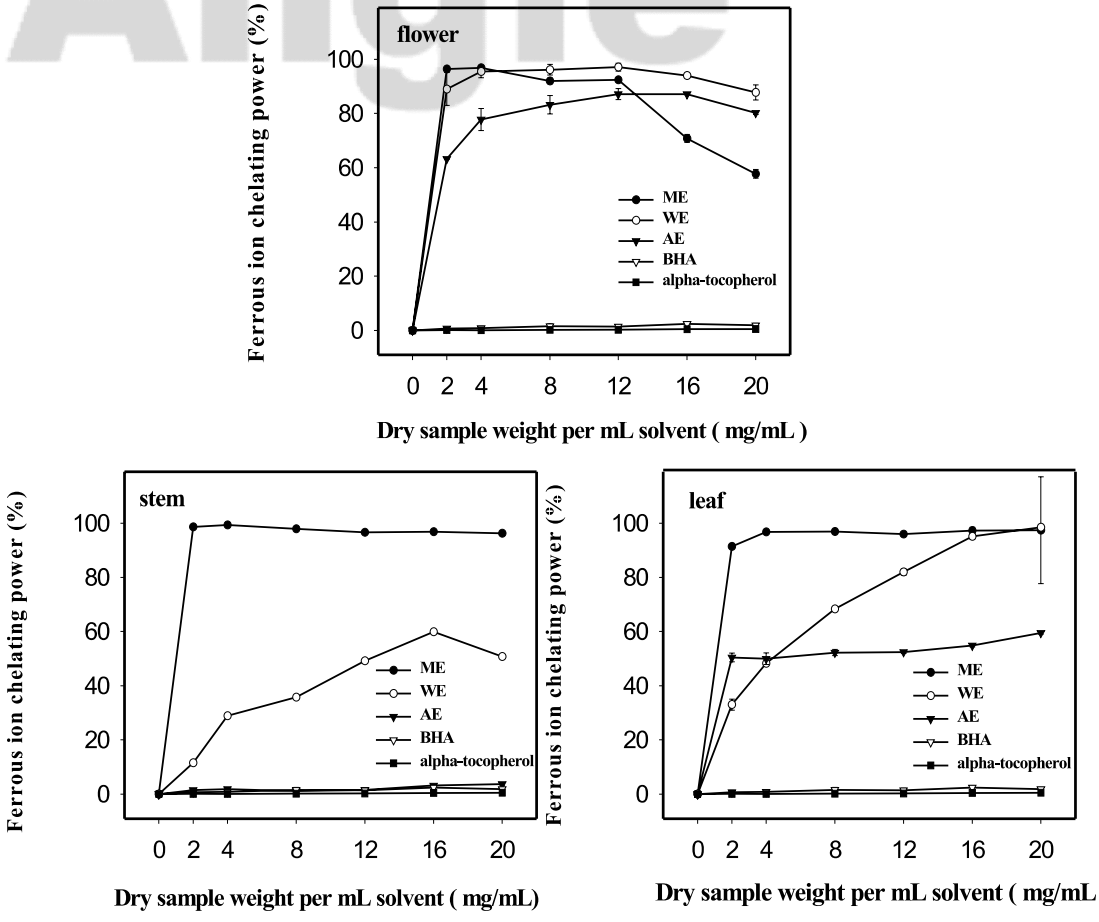

Figure 2. Ferrous ion chelating power of the extracts from different parts of broccoli using various solvents in comparison with BHA and alpha-tocopherol. ME: methanol extract; WE: water extract; AE: acetone extract.

A method according to Shimada *et al.*[16] was used to test for DPPH radical scavenging activity. Five mL of the test solutions, including broccoli methanolic, water, and acetone extract solutions accompanied with 5 mL of alpha-tocopherol, and BHA methanolic solutions were mixed with 1 mL of freshly prepared 1 mM DPPH methanolic solution. The resulting solutions were then left to stand for 30 min prior to being spectrophotometrically detected at 517 nm. The lower the absorbance at 517 nm represents the higher DPPH scavenging activity. The percentage of DPPH scavenging activity is expressed by [1-(test sample absorbance / blank sample absorbance)] x 100.

RESULTS AND DISCUSSION

I. *Reducing Power of Broccoli Extracts*

The reducing powers of broccoli extract solutions, alpha-tocopherol and BHA are shown in Figure 1. At a concentration of 2 mg/mL, relatively high reducing powers of both alpha-tocopherol and BHA were observed. Methanol and water extracts of all 3 parts of broccoli exhibited higher reducing powers than the acetone extract. Their reducing power was increased by increasing the sample concentration. The methanolic stem extract with concentrations higher than 4 mg/mL showed a higher reducing power than alpha-tocopherol and BHA. The maximum absorbance for methanolic stem extract was up to 2.38, compared to 1.80 and 1.69 for alpha-tocopherol and BHA, respectively. The reducing power of the water extract of stems was next to that of methanolic stem extract; while the flower extract showed the lowest reducing power.

It has been reported that the methanolic extracts of several Chinese traditional edible plants, such as Jew's ear, lotus seed, and Job's tears exhibit less reducing power than alpha-tocopherol and BHA[17]. Our study showed that the methanol and water extracts of both the stem and leaf of domestic broccoli had higher reducing power than alpha-tocopherol and BHA at concentrations ranging at 4~8 mg/mL. These results suggest that the stems and leaves of broccoli cultivated in Taiwan possess higher reducing power than several edible plants traditionally consumed by Chinese people.

II. *Ferrous Ion Chelating Ability of Broccoli Extracts*

Figure 2 demonstrates the ferrous ion chelating ability of the broccoli extracts, BHA and alpha-tocopherol. As can be seen, both BHA and alpha-tocopherol hardly carried the ferrous ion chelating ability due to their chemical structure properties. Among 3 different solvent extracts, methanolic

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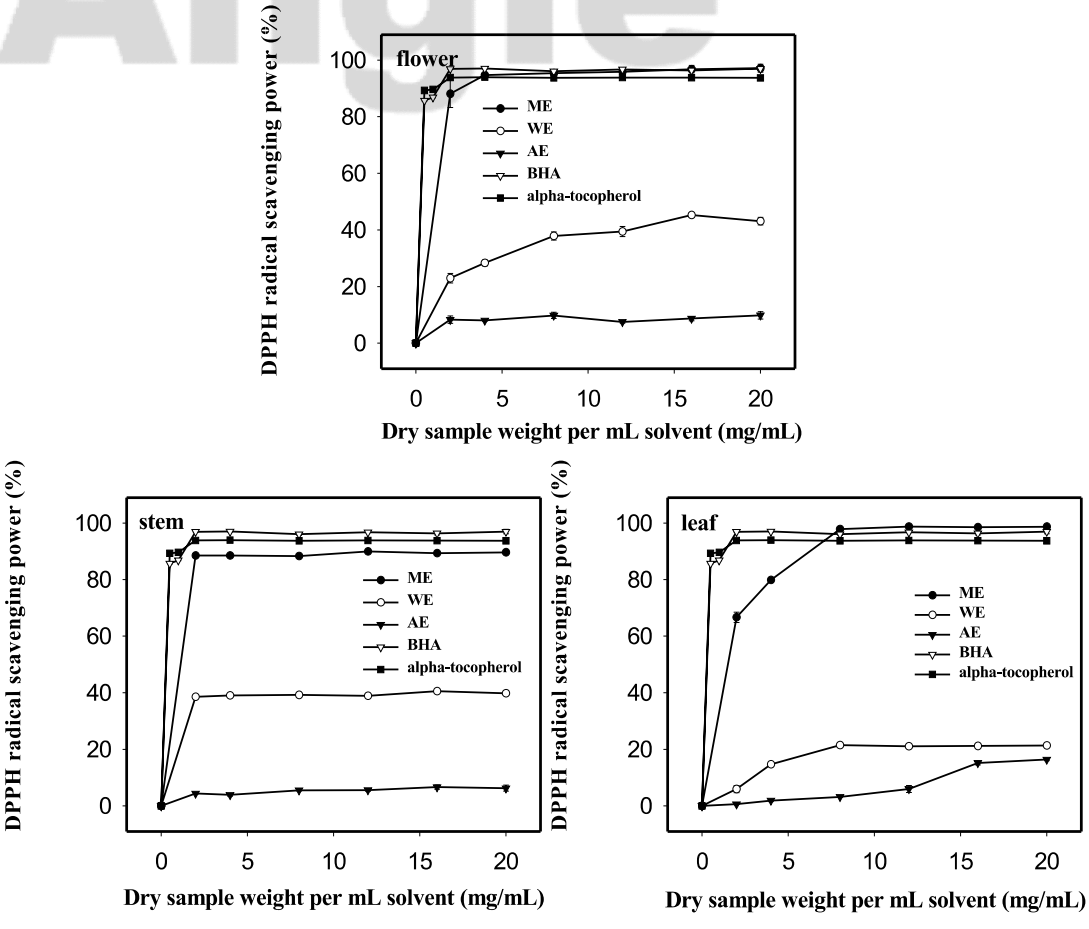

Figure 3. DPPH radical scavenging effect of the extracts from different parts of broccoli using various solvents in comparison with BHA and tocopherol. ME: methanol extract; WE: water extract; AE: acetone extract.

extract showed the highest ferrous ion chelating ability followed by water and acetone extracts. However, a decrease in ferrous ion chelating ability was observed when the methanolic flower extract at higher concentrations was tested. The methanolic stem extract performed the best ferrous ion chelating ability (up to 99%) among the other parts of broccoli extracts.

The methanolic extract of broccoli at 2 mg/mL could reach more than 90% ferrous ion chelating ability. Using a soybean sprout extract, the concentration at 3 mg/mL was required to obtain the same level of ferrous ion chelating ability[18]. The methanolic extracts of mungbean sprouts and radish sprouts only exhibited a chelating ability of 60% and 40%, respectively, at a concentration of 3 mg/mL. The concentration unit used in this study is expressed by the ratio of crude sample weight per solvent volume, instead of extract weight per solvent volume. Based on the differences between the two concentration definitions, domestic broccoli is estimated to possess a higher ferrous ion chelating ability than soybean, mungbean, and radish sprouts.

III. *DPPH Radical Scavenging Activity of Broccoli Extracts*

The DPPH radical scavenging activities of broccoli extracts, alpha-tocopherol, and BHA are presented in Figure 3. The results showed that alpha-tocopherol and BHA possessed up to 93.9% and 96.9%, respectively, of DPPH radical scavenging activities at a concentration of 2 mg/mL. The methanolic extract of broccoli gave the highest DPPH radical scavenging activity followed by water and acetone extracts. The acetone extracts showed the least scavenging activity (<20%). This activity was increased by increasing the concentrations of broccoli samples. The flower and stem parts of broccoli had higher DPPH radical scavenging activity than leaf parts. Their methanolic extract solutions at 4 mg/mL could reach up to 94% scavenging activity, which was comparable to alpha-tocopherol and BHA.

In comparison with the test results of Wong and Yen[18], the methanolic extracts of broccoli at 2 mg/mL possessed higher DPPH radical scavenging activity than the methanolic extract (2 mg/mL) of mungbean and soybean sprouts, and equal to that of radish sprouts. Based on the different concentration definitions described above, domestic broccoli is estimated to have higher DPPH radical scavenging activity than soybean, mungbean, and radish sprouts.

The results presented in Figures 1, 2, and 3 showed that both methanol and water could give the broccoli extracts a higher reducing power. In ferrous ion chelating ability and DPPH radical scavenging activity, using methanol for broccoli extraction could also produce a satisfactory result.

Therefore, it is concluded that methanol is the best solvent to extract antioxidants from broccoli. This result is in accordance with the finding of other reports[17-22] in solvent selection for antioxidant extraction from botanicals.

CONCLUSIONS

The results of this study indicate that different parts of broccoli carry certain levels of antioxidant activities. As compared to BHA and alpha-tocopherol, the stem part has stronger reducing power. All three parts of broccoli possess higher ferrous ion chelating ability than BHA and alpha-tocopherol, which hardly show any ferrous ion chelating ability due to the properties of chemical structures. The decline in ferrous ion chelating ability at higher concentrations was observed, but the reason is unclear and needs to be further studied. In DPPH radical scavenging activity, both the flower and stem parts of broccoli show comparable scavenging activity to BHA and alpha-tocopherol.

This study has shown that leaf and stem parts of broccoli exhibit certain levels of antioxidant properties although they are inedible. Therefore, a proper stem or leaf processing or treatment to develop a new type of product could enhance the utilization of broccoli.

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省產青花菜不同部位之抗氧化性

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摘　要

本研究將省產青花菜（*Brassica oleracea* L var *italica Plenca,* broccoli）分為花、莖、葉三部分，經由冷凍乾燥處理之後，利用甲醇、水、丙酮三種溶劑萃取，並測其萃取液之抗氧化性。抗氧化性測定項目包括還原力、亞鐵離子螯合能力及α,α-diphenyl-β-picrylhydrazyl（DPPH）自由基之清除能力，並與BHA及alpha-tocopherol作比較。在還原力方面，花、莖、葉之甲醇及水萃取液均有良好之還原能力，其中以莖萃取液之還原能力最強，約為alpha-tocopherol及BHA之1.3倍；其次為葉萃取液，其還原力與alpha-tocopherol及BHA相當；花萃取液之還原力較差，約為alpha-tocopherol及BHA之四分之三。丙酮之萃取效果不佳。在亞鐵離子螯合能力方面，仍以甲醇及水之萃取效果較佳，丙酮之萃取效果不佳，而花、莖、葉三部分中以莖萃取液之螯合力最強，而莖之丙酮萃取液與alpha-tocopherol及BHA一樣不具螯合力。在DPPH自由基的清除能力方面，花、莖、葉三部位均以甲醇之萃取效果最佳，其DPPH自由基的清除能力與alpha-tocopherol及BHA相當，達90%以上，水萃取液之清除能力低於43%，丙酮萃取效果則不顯著。

關鍵詞：青花菜，抗氧化性，還原力，鐵離子螯合力， DPPH自由基清除能力