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Changes in Antioxidant Activity and Total Phenolic Content of Water Spinach (*Ipomoea aquatic* Forsk.) under *In Vitro* Biomimicking System

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Abstract

The purpose of current study was to examine bioaccessibility of antioxidant activity and total phenolic content in each part of water spinach (*Ipomoea aquatic* Forsk.). *In vitro* biomimicking system simulated human digestive fluid was employed in order to measure bioavailable anti-oxidative effect and phenolic content. Antioxidant activity and total phenolic content was measured by using the DPPH method and the Folin-Ciocalteu assay, respectively. Stem of water spinach had a higher DPPH free radical scavenging effect (5.43 mg/mL for IC_{50}) than leaf (5.95 mg/mL for IC_{50}), while leaf had a greater level of total phenolic content (287.45 μg GAE/mL) than stem (216.45 μg GAE/mL). Bioaccessible antioxidant capacity and digestive stability of total phenolic content showed a similar pattern to what found in raw materials. Our result also indicated that total phenolic content was not found to be a major marker for prediction of antioxidant activity. It is plausible that other constituents such as vitamin E and C in water spinach could be contributors for antioxidant activities.

Key words: water spinach; antioxidant activity; total phenolic content; *in vitro* biomimicking

Introduction

Main role of antioxidants in human health is protecting from oxidative stress involved in the pathogenesis of diverse disorders and diseases such as coronary heart disease and cancer (Alia et al., 2003). Especially attention on natural antioxidants, which inhibit the adverse effects of the Reactive Oxygen Species(ROS) produced in plants, has been increasing since synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butyl hydroquinone (TBHQ), are suspected to be carcinogenic (Tyung et al., 2010; Hafidh et al., 2009). It may be beneficial to replace antioxidants from synthetic ones to natural ones because of functionality and implications related to emulsions in food systems (Moure et al., 2001).

Water spinach (*Ipomoea aquatic* Forsk.) is a fast-growing herbaceous aquatic or semi-aquatic green vegetable found in freshwater marshes and ponds throughout Southeast Asia and it belongs to the morning-glory family which is *Convolvulaceae* (Sivaraman et al., 2008; Prasad et al., 2006). It has a high level of nutrition value, including satisfactory quantities of essential amino acids, vitamin C, vitamin A, and iron and it is rich in carotenoids and chlorophylls (Marcussen et al., 2008; Prasad et al., 2006; Duc et al., 1999). In particular, all parts (leaf, stem) of water spinach can highly contribute to the total polyphenol intake involved in antioxidant activity around many countries (Thu et al., 2004).

Recently, interests on leafy vegetables containing water spinach have been rising and extensively investigated as a novel source of natural antioxidant in order to obtain health benefits for human (Prasad et al., 2006). However, there have been limited outcomes of water spinach carried out with all parts of its plant as well as using both raw and digestive material, so as to discover its bioavailability. Thus, the purpose of the present work was to determine the total phenolic content and antioxidant capacity in each part (leaf, stem) of water spinach by using both chemical and *in vitro* physiological approach.

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Materials and methods

Sample preparation

Water spinach (*Impoea aquatica* Forsk.) was harvested from

a local farm in Buchun-Si, Kyunggi-Do, South Korea in July, 2010. Water spinach was washed using distilled water and then divided into leaf and stem. Each part of water spinach was freeze-dried and grinded under the dark environment. It was stored at -20°C until analysis.

Chemicals and standards

All enzymes such as amylase, pepsin, lipase, pancreatin, and bile acid were purchased from Sigma (Sigma-Aldrich, Co., St. Louis, MO, USA). All of chemicals and standard were of analytical grade.

In vitro biomimicking system

An *in vitro* biomimicking system with three steps was followed from Shim et al. (2010) with slight modifications. Aliquots (0.2 g) of freeze-dried samples were dissolved with 20 mM phosphate buffer (5 mL). The salivary phase was initiated by adding amylase and an initial pH was adjusted to pH 6.9 with 20 mM phosphate buffer. Samples were required to incubate at 37°C and 250 rpm in a shaking bath for 5 min, after treating with nitrogen gas. The gastric phase was initiated by adding pepsin solution (2 mL) and its pH was recorded to pH 2.0 with 1 M HCl. Samples were allowed to incubate under the following condition in a shaking bath (250 rpm) at 37°C for 1 hr. The small intestinal phase was initiated with sodium bicarbonate (1 M) used to adjust pH to 5.3. Bile acid (0.5 mL) and small intestinal enzyme solution including 0.75 mL of lipase and pancreatin respectively were added and then its pH was increased to 7.0 with 1 M NaOH. After three stage of *in vitro* digestive model system, all of sample volumes were required to be equal with 20 mM phosphate buffer. In addition, samples were treated with nitrogen gas and then incubated under the condition in a shaking bath (250 rpm) at 37°C for 2 hr. Each supernatant of water spinach was separated using a centrifuge (3000 rpm) at 4°C for 30 min and stored at -80°C until further analysis.

Scavenging effects on DPPH free radicals

To detect anti-oxidative capacity of raw material and digesta, 2, 2-diphenylpicrylhydrazyl (DPPH) method was assayed from Bor et al. (2006) with some modifications. Aliquots (0.3 mL), which were each part of raw material and digesta, negative control (MeOH), and positive control (Vitamin C) respectively, were added to 0.1 mM DPPH (0.7 mL). Samples were mixed by vortex and then placed at room temperature in the dark for 20 min. The absorbance of each sample was measured by UV-Vis spectrophotometer (Genesys 10 UV, Madison, W.I., U.S.A.) at 517 nm. Antioxidant activity to scavenge DPPH free radical was calculated as IC_{50} (Concentration required to acquire a 50% radical scavenging ability). Inhibition percentage (%) was also calculated using the following equation from Ozsoy et al., (2008): DPPH free radical scavenging activity (%) = [(Absorbance of control - Absorbance of sample)/ Absorbance of control] × 100

UV-Vis spectral method for quantification analysis of total phenolic content

Folin-Ciocalteu method obtained by Bor et al. (2006) was adjusted with some modifications in order to determine the total phenolic content. Total phenolic content was calculated and expressed using gallic acid as a standard. In terms of raw material, aliquots (0.1 g) of freeze-dried samples were added to 10 mL of 0.3 HCl in MeOH/H_2O (60:40, v/v) and thoroughly stirred up in a dark place for 1 hour. A 1.0 mL of 2.0% Na_2CO_3 solutions was added to 50 μL of the resulting solution (10 mg/mL). After 2min, 50 μL of prepared 50% Folin-Ciocalteu reagent was added to the mixture and immediately shaken by vortex. The samples were covered and incubated at room temperature for 30 min. The absorbance of all samples was measured with the UV-Vis spectrometer set to 750 nm. In terms of digesta, there was only one difference compared with the method of raw material in the first step; A 0.2 mL of all digesta (10 mg/mL in phosphate buffer) was added to 0.2 mL of 0.3% HCl in MeOH/H_2O (60:40, v/v), respectively. Without this step, all steps were same as the procedure of raw material.

Data analysis

Results are presented as representative data carried out in triplicate. Data are expressed as mean±standard error of mean (SEM). To assess statistical significance for each individual factor in each part of samples, analysis of variance was performed by Tukey's post hoc test using SAS (version 9.1.3, SAS Institute, Cary, NC, USA). Differences in means between raw materials and digesta were considered statistically significant at $p<0.05$.

Results and Discussion

Determination of anti-oxidative effect and total phenolic content

Antioxidant activity detected by DPPH free-radical scavenging effect and total phenolic content determined by the Folin-Ciocalteu method were shown in Table 1. Considering chemical approach of water spinach, the order of antioxidant

Table 1. Antioxidant activity and total phenolic content before and after *in vitro* digestion of water spinach

	DPPH free radical scavenging (IC_{50}, mg/ mL) [1]		Total phenolic content (ìg GAE/mL) [2]	
	Raw material [3]	Digesta [4]	Raw material	Digesta
Leaf	5.95±0.02	48.07±1.07 *	287.45±10.46	137.66±4.24 *
Stem	5.43±0.01	14.02±0.05 *	216.45±4.41	85.71±2.12 *
Vit C [6]	0.0018			

[1] Results of DPPH free radical scavenging effect were expressed as IC_{50}.
[2] Results of total phenolic content were expressed as gallic acid equivalents determined by Folin-Ciocalteu method.
[3] Before digestion samples of water spinach
[4] After digestion samples of water spinach
[5] Mean±SEM (n=3)
[6] Ascorbic acid as a positive control; * means significant decrease in digesta relative to raw material ($p<0.05$).

activity in raw material was stem>leaf, while that of total phenolic content in raw water spinach was surprisingly adverse against the result of antioxidant activity. According to the previous study from Huange et al. (2005), there were comparable results showing that stem had higher radical scavenging activity than leaf, and that outcomes of total phenolic content might be different depending on which extract fraction was used such as water and ethanol. For example, water extract of leaf had the higher level of total phenolic content than that of stem, although ethanol extract of leaf was lower than that of stem when it comes to total phenolic content.

Regarding *in vitro* physiological approach, changes in both antioxidant activity and total phenolic content in leaf and stem from water spinach were also investigated. Antioxidant activity and total phenolic content in all samples showed overall decreasing pattern after simulated *in vitro* digestion model system. In particular, stem was much higher than leaf in the bioavailability of antioxidant effect on the basis of Table 1. For instance, stem was only decreasing around 2.6 times while leaf was significantly decreasing around 8 times in terms of scavenging effect on DPPH free-radical after digestion. Bioaccessiblility of total phenolic contents from leaf and stem after three steps of *in vitro* digestion model system were around 47.89 and 39.59%, respectively. It meant that phenolic compounds in leaf were more stable and then become bioavailable than those in stem.

Relationship between anti-oxidative effect and total phenolic content

Fig. 1 shows seemingly a positive correlation (r^2=0.6163, $p<0.05$) between total phenolic content(gallic acid equivalent, GAE) and antioxidant activity (% of DPPH free radical scavenging effect) with all samples of water spinach. On the other hand, we had to take into account carefully with the

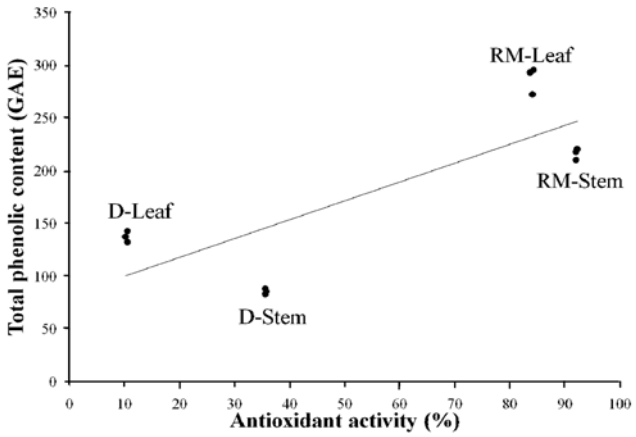

Fig. 1. Correlation between antioxidant ability detected by the DPPH method and total phenolic content determined by the Folin-Ciocalteu method.

interesting fact that stem of raw material had higher antioxidant ability than leaf of that, even though total phenolic content of raw water spinach stem was lower than that of leaf. It indicated that every relationship between total phenolic content and antioxidant activity was not shown to be positive since some constituents such as vitamin E and C might be efficient antioxidants and make strongly a difference on antioxidant activity investigated in the finding by Park et al. (1994). Thus, this study implies that total phenolic content might not be a main indicator to predict how high level of antioxidant activity is in water spinach. Our study suggests that other components in water spinach mainly responsible for antioxidant activity have to be profiled for improving their bioavailability.

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