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Chemical Composition and Antioxidant Activity of Ramie Leaf (*Boehmeria nivea* L.)

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Abstract This study investigated change in the chemical components and antioxidant activity of ramie (*Boehmeria nivea*) leaves (RL) for the development of functional foods. Proximate compositions of protein, crude ash, and crude fat were 24.49, 11.41, and 4.89%, respectively. Contents of minerals of calcium (Ca), potassium (K), magnesium (Mg), and iron (Fe) were 1,874, 1,433, 362, and 16 mg/100 g, respectively. α, β, and γ-Tocopherol contents were 9.79, 0.18, and 1.44 mg/100 g, respectively. Linoleic and linolenic acid contents were higher than those of palmatic and stearic acid. Total phenolic and flavonoids contents showed the high level of 149 and 49 mg/g. The IC_{50} values of 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, and superoxide radical scavenging of RL extracts were 688, 424, and 596 μg/mL, respectively, while the radical scavenging values by butylated hydroxylanisole (BHA) were 92, 58, and 98 μg/mL, respectively. Thus, RL has the potential to be used as a healthy and functional food ingredient.

Keywords: ramie leaf (*Boehmeria nivea* L.), proximate composition, mineral, tocopherol, radical scavenging activity

Introduction

Phytochemicals are bioactive substances of plants that have been associated with the protection of human health against chronic degenerative diseases (1). The major groups of phytochemicals that contribute to the total antioxidant capacity (TAC) of plant foods include polyphenols, carotenoids, and traditional antioxidant vitamins like vitamin C and E (2). Thus, it is important to increase the antioxidant intake in the diet and search for natural antioxidant sources among plants used as food additives.

Ramie, also called China grass [*Boehmeria nivea* (L.) Gaud.], is a hardy perennial herbaceous plant of the Urticaceae family (3). Today, it is mainly planted in China and other Asian countries including Philippines, India, South Korea, and Thailand (3). Besides some impurities called gum such as hemicellulose (13.1-16.7%), pectin (1.9%), and wax (0.3%), ramie is mainly composed of cellulose (68.6-76.2%) (4).

As an environmental friendly natural material ramie fabric possesses various functions. Currently, it has been widely and mostly used as a refreshing material for summer because it is excellent in absorbing and evaporation sweat has a coarse tactile sensation (5). In addition, 25% of protein is contained in the stems and leaves of a 'ramie' so that they have been widely used as feedstuff in the Central and South America and Taiwan. In the thin-skinned shell waste of ramie, there are various ingredients such as 3% of nitrogen, 1% of lactic acid, l7% of lime; hence, it has been used as an organic fertilizer (6). In Korea, there have been some researches on the use of its smooth leaves in foods such as various *tteok* (traditional Korean rice cakes) (7). In particular, since it has been found that the green leaves of plants are rich in nutritional ingredients such as vitamins, mineral matters, and proteins and in various bioactive materials (8), there have been continuous researches on their developments as the types of teas and health foods using mugwort (9), persimmon leaves (10), and jujube leaves (11). Nevertheless, there has been a lack of researches on the nutritional components and antioxidant effects concerning the leaves of a 'ramie'.

In this regard, this study measured the chemical composition and antioxidant activity for the feasibility of food source in the leaves of a 'ramie'. For chemical composition, it measured proximate composition, mineral, fatty acid, vitamin E content, and the content of polyphenol, flavonoids electron donating abilities to the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, and their capacities to scavenge hydroxyl and superoxide radicals of 70% ethanol extracts. The data obtained may be used to enhance the nutritional value of the leaves of a 'ramie'.

Materials and Methods

Materials Ramie (*Boehmeria nivea* L.) leaves (RL) were purchased from the Ramie Association, Hansan-myeon, Seocheon-gun, Chungnam, South Korea in June 2007. The samples were stored at –20°C until analysis. L-Ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxylanisole (BHA), nitro blue tetrazolium (NBT), ethylenediamine tetraacetic (EDTA), bovine serum albumin, 2-deoxyribose, and ferrous sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the reagents were of analytical grade.

Analysis of proximate composition and minerals The proximate composition of RL was determined by AOAC (12). Moisture and ash content were determined gravimetrically by desiccation at 105°C and incineration at

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550°C in an electric furance (F-2F; Kong Soung Co., Seoul, Korea), respectively. Crude protein content was obtained by micro-Kjeldahl method. Lipids were extracted using a Soxhelt-extraction apparatus (Dong Ha-Tech., Seoul, Korea). The minerals of RL were measured by wet digestion method. Ashes of RL were added to $HCl:H_2O$ (1:3) and dried in water bath. Sample solutions were subsequently analyzed by inductively coupled plasma-atomic emission spectroscope (ICP-AES, Thermo Jarrell Ash, Franklin, MA, USA).

Analysis of fatty acids Fatty acid compositions of RL were determined by (13). For lipid extraction, 50 g of RL were homogenized in 150 mL chloroform:methanol (1:2, v/v) mixture and reacted at 100°C for 30 min filling N_2, and then filtrated through Whatman No. 1 filter paper. Extracted lipid from each sample was saponified in 0.5 N NaOH methanolic solution and esterified in BF_3-methanol. Fatty acids were determined using a gas chromatography (Varian star 3600; Varian Inc., Walnutcreek, CA, USA) equipped with flame ionization detector and Omagawax 205 fused-silica bond capillary column (30 m×0.32 mm, i.d.×0.25 μm film thickness). Temperature of oven, injection port, and detector were 140, 250, and 260°C, respectively, and nitrogen flow rate was 50 mL/min.

Analysis of vitamin E The vitamin E content of methanolic extracts of RL was determined according to the procedure described by Lee *et al.* (14), with some modifications. In brief, an aliquot of each methanolic extract was evaporated under nitrogen gas. The residues were redissolved in *n*-hexane, filtered, and analyzed by normal phase high performance liquid chromatography (HPLC, Younglin Inc., Anyang, Korea). The analysis of tocopherols and tocotrienols was performed on an LiChrosphere-Diol 100 column (4.0×250 mm, i.d., 5 μm) using a mobile phase of hexane: isopropanol of 98.7:1.3 (v/v) at a flow rate of 1 mL/min. Peaks were detected by fluorescence using an excitation wavelength of 290 nm and an emission wavelength of 330 nm.

Total polyphenol and flavonoids contents Total polyphenol content of RL was determined according to the modified Folin-Ciocalteu method (15). The RL was extracted with 70% ethanol and then measured using a UV-visible spectrophotometer (DU-650; Beckman Coulter, Fullerton, CA, USA) at a wavelength of 700 nm. The tannic acid level was used as a reference, and the total polyphenol content was calculated based on a standard curve. Total flavonoid content was evaluated using the method of Jia *et al.* (16) method. The RL was extracted with 70% ethanol and then measured using a UV-visible spectrophotometer at a wavelength of 510 nm, the catechin level was used as a reference, and the total flavonoid content was calculated based on a standard curve.

DPPH radical scavenging activity The scavenging activity for the DPPH radical was evaluated using the method of Tepe *et al.* (17), at a wavelength of 517 nm with a UV-visible spectrophotometer. The radical scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [1-(Abs_{sample})/Abs_{blank}]\times 100$$

Sample concentration providing 50% inhibition concentration (IC_{50}) was calculated from the graph plotting inhibition percentage against sample concentration. All samples were analyzed in triplicate.

Hydroxyl radical scavenging activity The scavenging activity for the hydroxyl radical was evaluated using the method of Halliwell *et al.* (18) at a wavelength of 520 nm with a UV-visible spectrophotometer. The radical scavenging activity was calculated using the following equation:

$$\text{Hydroxyl radical scavenging activity (\%)} = [1-(Abs-Abo)/(Abc-Abo)]\times 100$$

Where Abo is the absorbance at 520 nm with no treatment, Abc is the absorbance of the treated control at 520 nm, and Abs is the absorbance of the treated sample at 520 nm.

Superoxide radical scavenging activity The scavenging activity for the superoxide radical was evaluated using the following equation at a wavelength of 560 nm according to the xanthine-xanthine oxidase method (19):

$$\text{Superoxide radical scavenging activity (\%)} = [1-(Abs_{sample})/Abs_{blank}]\times 100$$

Calculation of IC_{50} The concentration of the extract (in μg/mL) that is required to scavenge IC_{50} of reactive oxygen species (ROS) was calculated by using the percent scavenging activities of 5 different extract concentrations.

Statistical analysis Mean and standard deviation (SD) were calculated using SPSS PC+ software (version 11.5; SPSS Inc., Chicago, IL, USA). All data were presented as the mean±SD.

Results and Discussion

Proximate composition Proximate compositions of RL are shown in Table 1. The crude protein was very high content 24.49% compared with fat (4.89%) and ash (11.41%). Ahn and Yang (20) reported that the highest protein content found in bangah leaves 14.9% and lipid and ash contents were 7.77 and 8.43%, respectively. Choi *et al.* (21) reported that the levels of protein, lipid, and ash of dried ginseng leaf were 17.12, 1.90, and 6.73%, respectively. Lee *et al.* (22) reported that the levels of protein, lipid, and ash of mulberry leaves were 12, 4.9, and 8.74%, respectively. The RL had a higher amount of protein than bangah, ginseng, and mulberry leaves. Meanwhile, if the defoliation time for green tea is late, it has been reported that protein content is likely to be low; therefore, it has been believed that there should be studies on the changing aspects of the leaves of ramie depending on the defoliation time.

Mineral The mineral contents of RL are shown in Table 2. It has been found that RL were rich in Ca, K, and Mg. The values for the Ca, K, and Mg increased significantly from 1,874, 1,433, and 362 mg/100 g, respectively. Jang *et al.* (23) reported that barley leaf tea and green tea had the

Table 1. Proximate compositions of the ramie leaves

Overall composition (%)			
Moisture	Crude lipid	Crude protein	Crude ash
7.94±0.03[1)]	4.89±0.04	24.29±0.37	11.47±0.39

[1)]Results were mean±SD (n=3).

Table 2. Mineral contents of the ramie leaves

Mineral	Ramie leaves (mg/100 g)	Mineral	Ramie leaves (mg/100 g)
Ca	1,874.83±98.87[1)]	Na	15.64±7.11
K	1,433.86±37.81	Zn	4.15±1.25
Mg	362.52±1.91	Cu	0.79±0.04
Fe	16.81±0.55	-	-

[1)]Results were mean±SD (n=3).

highest Ca content necessary to control the muscles and neurological functions. Choi *et al.* (21) report that content of Ca of dried ginseng leaves higher than K. The contents of minerals were in order of Mg, P, Na, Fe, Mn, Zn, and Cu, abundantly. Ryo and Cha (24) reported that chicory had a high amount of calcium content and parsley was rich in potassium content so that they were useful in low sodium diet. It has been shown that dried ramie leaves also had some minerals such as Ca, K, and Mg, which were beneficial to human body so that they were thought to be used as food materials useful in health.

Fatty acids Fatty acids compositions of RL are shown in Table 3. A total of 11 fatty acids ranging from C_8 to C_{22} were identified. The RL was an increase in the content of unsaturated fatty acids such as linoleic acid (30%) and linolenic acid (33%) rather than in that of saturated fatty acids such as palmitic acid (11%) and steric acid (4%). Ahn and Yang (20) reported that 15 fatty acids in bangah leaves were identified and the major fatty acids were linolenic acid, palmatic acid, lauric acid, and linoleic acid. Oh and Wang (25) reported that fatty acid of herb leaves, linolenic and linoleic acid was higher than palmitic acid and steric acid. Linoleic acid and linolenic acid which have been known to be effective in lowering the concentration of cholesterol causing arteriosclerosis led to their possible use as the source for health foods.

Vitamin E Natural vitamin E consists of 4 tocopherols and 4 tocotrienol homologs, i.e., α, β, γ, and δ, that all have antioxidant, anticancer, and cholesterol-lowering activity (26). Tocotrienols reportedly inhibit cholesterol synthesis, lower serum cholesterol levels in animal models, and suppress tumor cell proliferation; the γ- and δ-horologes have greater potency than the α-homolog (27). The vitamin E contents of RL are shown in Table 4. The RL has α-, β-,

Table 3. Fatty acid compositions of the ramie leaves

Fatty acid	Composition (%)	Fatty acid	Composition (%)
Caprylic acid	0.07±0.01[1)]	Oleic acid	8.46±0.34
Capric acid	0.90±0.01	Linoleic acid	30.39±0.93
Myristic acid	0.76±0.01	Linolenic acid	33.14±1.04
Palmitic acid	11.78±0.07	Arachidic acid	0.21±0.01
Palmitoleic acid	2.07±0.29	Behenic acid	1.46±0.16
Stearic acid	4.34±0.01	Others	6.41±0.09

[1)]Results were mean±SD (n=3).

and γ-tocopherol and those of content were 9.79, 0.18, and 1.44 mg/100 g, respectively. The α-tocopherol out of tocopherol isomers has the strongest vitamin E activity and the greatest activity against singlet oxygen species (28). Bae *et al.* (29) reported that α-tocopherol content of persimmon leaves, green tea, cassia semen, mulberry leaves, pine needle, and lycii fructus were 58.02, 21.04, 6.89, 5.48, 7.94, and 58.02 mg/100 g, respectively. The RL had less α-tocopherol content than persimmon leaves or green tea, but they had more α-tocopherol content than cassia semen, mulberry leaves, pine needle, and lycii fructus. The RL presented a content of 0.11 mg/100 g of α-tocotrienols, 0.04 mg/100 g of γ-tocotrienols, and 0.20 mg/100 g of δ-tocotrienols.

Antioxidant compounds The polyphenol, flavonoids contents of the 70% ethanol extracts from the RL are shown in Table 5. Phenolic compounds are secondary metabolic products that occur throughout the plant kingdom. They contain the phenolic hydroxyl group, which has an antioxidative effect via interactions with the phenol ring and its resonance stabilization effect (30). The total polyphenol and flavonoid contents in RL extracts were 149 and 49 mg/g, respectively. The RL had a higher amount of polyphenol than persimmon leaves (5.80 mg/g), *sanghwang* mushrooms (17.93 mg/g), green tea (10.98 mg/g), and *ddangdurup* (*Aralia cordata* Thunb, 29.93 mg/g) and had the similar amount of polyphenol to the leaves of Island Common Thistle (120 μg/mL), one of wild edible plants (31).

ROS scavenging activity The decrease in the absorbance of the DPPH radical caused by antioxidants is due to the scavenging of the radicals by hydrogen donation; this is a visible change from purple to colorless. The IC_{50} values of DPPH radical, hydroxyl radical, and superoxide radical scavenging of RL extracts were 688, 424, and 596 μg/mL, respectively, while the radical scavenging values by BHA was 92, 58, and 98 μg/mL, respectively (Table 6). When comparing DPPH scavenging activities of RL to the plants for medicinal use indigenous to Korea, they were higher

Table 4. Vitamin E content of the ramie leaves

Tocopherol (mg/100 g)			Tocotrienol (mg/100 g)		
α	β	γ	α	γ	δ
9.79±0.11[1)]	0.18±0.03	1.44±0.02	0.11±0.07	0.04±0.01	0.20±0.03

[1)]Results were mean±SD (n=3).

Table 5. The total polyphenol and flavonoid contents of the 70% EtOH extracts from ramie leaves

Antioxidant activity	
Polyphenol (mg/g)	Flavonoids (mg/g)
149.58±1.62[1)]	49.24±0.38

[1)]Results were mean±SD (n=3).

Table 6. Antioxidative activities of the 70% EtOH extracts from ramie leaves

	IC_{50} (μg/mL)		
	DPPH	OH	O_2
BHA	92.18±0.31[1)]	58.52±0.77	98.38±1.44
Ramie leaves	688.24±41.27	424.56±5.19	596.84±47.55

[1)]Results were mean±SD (n=3).

than the leaves of Chinese quince (*Chaenomeles sinensis*, 46 μg/mL), ginko (*Ginko biloba*, 342 μg/mL), mulberry tree (*Morus alba*, 294 μg/mL) but lower than those of *deoduk* (*Codonopsis lanceolata* T., 1,000 μg/mL), fernbrake (*Coniogramme intermedia* H., 893 μg/mL), and yam (*Dioscorea batatas* D., 1,000 μg/mL) (31). *Soimurup* (*Achyanthes japonica*), thistle (*Cirsium nipponicum*), *seodulchui* (*Saussurea grandifolia*) at 1 mg/mL scavenged 84.85, 94.31, and 75.49% of the hydroxyl radicals, respectively (32). Eventually, it has been confirmed that RL had antioxidant effects equivalent to the plants for medicinal use. The present study suggests that RL are useful nutritional antioxidants for the nutraceutical industry. But, further studies are needed to isolate and identify the antioxidant components within RL.

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