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Nutritional Characteristics of *Calystegia japonica*

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메꽃(*Calystegia japonica*)의 영양학적 특성

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Abstract

In the present study, the proximate composition, sugar, minerals, total phenolic and flavonoid compounds, and amino acids in *Calystegia japonica* (*C. japonica*) were measured to determine if it can be used as a nutritional and functional material for the development of valuable foods. The mean crude protein, fat, and ash contents of the leaves were 5.75, 2.46, and 7.77%, respectively. The soluble-protein contents of the leaves and roots were 146.78 and 33.67 mg%, respectively. The reducing-sugar and free-sugar contents of the leaves were 682.70 and 166.00 mg%, respectively, and those of the roots were 2,934.89 and 37.70 mg%. The mineral content of the leaves was 3,122.13 mg%, and that of the roots was 1,540.85 mg%. The three elements Ca, K, and Mg were very rich in all their parts, with minerals accounting for 96-99% of their total mineral contents. The total phenolic compound of the leaves was 3,028.89 mg%, and the total flavonoid compound was 382.67 mg%. The phenolic and flavonoid compounds in the leaves were more than 7.6 times those in the roots. The free-amino acid levels in the leaves and roots were 2,467.15 and 1,334.81 mg%, respectively. The results of the comparison of the leaves and roots of *C. japonica* showed that the leaves had a rich proximate composition consisting of minerals, total phenolic and flavonoid compounds, and amino acid. This suggests that *C. japonica* leaves are potentially useful sources of functional and favorite foods and nutraceuticals.

Key words : *C. japonica*, proximate composition, sugar, mineral, amino acid

Introduction

Many current human health problems relate to diets. Micronutrients are involved in numerous biochemical processes and an adequate intake of certain micronutrients relates to the prevention of deficiency disease. Vegetables are valuable sources of useful material including minerals (1). Diets high in vegetables are also linked to decreased risk of disease (diabetes, cancer, etc) and their consumption should be encouraged (2).

The *Calystegia japonica*, also known as japanese bindweed of the Convolvulaceae family, is a perennial herb of climbing plant, it is similar to morning glory. *C. japonica* include traditional medicines that are derived from all parts of the plants, to function as diuretic, fatigue recovery, antipyretic, anitihypertension, blood sugar decline or laxative purpose in chinese and oriental herb medicine. It has been used in traditional medicine to cure diabetes and hypertension. Also *C. japonica* used for food, as side dish, green vegetable juice, rice cake or soup (3).

In preliminary studies, the water and ethanol extracts of *C. japonica* have a higher xanthine oxidase inhibition than ascorbic acid and butylated hydroxyanisole (4), beneficial effects on antioxidation (5). Also Kim *et al* (6) reported the genetical and morphological character of genus Calystegia, Lee *et al.* (7) was identified volatile flavor. Schimming *et al* (8) have been reported the chemotaxonomic significance of glycosidase inhibiting polyhydroxy-nortropanes for the 65

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convolvulaceous species. Also Tatsuzawa *et al* (9) reported the flower anthocyanins of genus Calystegia in Japan

The aim of the present study in this part was to determine the chemical composition and mineral contents of *C. japonica* leaves and roots in order to provide more comprehensive nutrient information about them. Furthermore, phenolic compound and flavonoid compound contents of these *C. japonica* were also investigated in order to evaluate their potential use nutraceuticals and medicines.

Materials and Methods

Sample preparation

Calystegia japonica was collected from a local collect in Gyeongsan Gyeongbuk Korea, in May 2007 and identified by NW Kim from the Daegu Haany University. The collecting sample, *C. japonica* was separated into leaves and roots. Leaves and roots were oven dried at 40℃ for 24 hours using heated-air dryer (DR-0160, Hankwang, Siheung, Korea). The dried leaves and roots were ground to a powder and stored at -70℃ until analyzed.

Proximate composition

The moisture, crude protein, crude fat and ash contents were determined following the standard Association of Official Analytical Chemists methods (10). Moisture content was determined after attaining constant weight at 105℃. Crude protein was determined by the micro-Kjeldahl procedure; the factor N×6.25 was used to convert nitrogen into crude protein. The crude fat content was obtained by the Soxhlet extraction method, sung diethyl ether. The ash content was determined by a muffle furnace at 550℃, and then the residue was quantitated gravimetrically. Total carbohydrate content was calculated as 100 - % moisture - % protein - % fat - % ash.

Soluble protein content

The dried *C. japonica* leaves and roots (10 g) were homogenized and extracted in 10 volumes of distilled water for 30 min, and the extracts were centrifuged at 1,500 rpm for 10 min and supernatant filtered through Whatman No 2 filter paper. Total soluble protein was quantified using the method of Lowry *et al* (11). Briefly, a 2 mL of extract were assayed with 1 mL mixed reagent(A : B = 50 : 1, A ; 2% Na_2CO_3 in 0.1 N NaOH, B ; 1% $C_4H_4KNaO_6$ in 0.5% $CuSO_4$ $5H_2O$) and allowed to react for 10 min in 30℃. And the mixture was mixed 0.1 mL of folin-ciocalteu's phenol reagent and kept at room temperature for 30 min. Then its absorption was read at measured at 750 nm (Shumadzu UV-1201, Kyoto, Japan). Bovine serum albumin (Sigma-Aldrich Co, St Louis, MO, USA) was used as standard to produce the calibration curve. The mean of three readings was used and the soluble protein content was expressed as bovine serum albumin equivalent, in mg% of dry weight. The experiment was repeated three times.

Reducing and free sugar contents

Reducing sugar was quantified according to the Somogyi method (12). The dried *C. japonica* (10 g) were extracted with 100 mL distilled water and filtered through Whatman No 2 filter paper. 1 mL of sample extract was added to 1 mL of the Somogyi reagent and boiled for 20 min, after cooling, 1 mL of Nelson reagent plus 5 mL of distilled water were added. The mixture was shaken and absorbance was measured at 520 nm. The reducing sugar content results were expressed as pure D-glucose (Sigma-Aldrich Co, St Louis, MO, USA) equivalent. The experiment was repeated three times.

For analysis of free sugar was determined using the method of Shim *et al* (13). The dried *C. japonica* (1 g) were extracted with distilled water (10 mL) at room temperature for 6 h and then centrifuged at 3,000 rpm for 15 min. The supernatant was filtered with a sep-pak cartridge C_{18} solid phase extraction cartridge (Waters, Milford, MA, USA) and this residue was then dissolved in water. The extract was determined by using system HPLC (High Performance Liquid Chromatography, Waters 600E controller, Milford, MA USA). The HPLC system was equipped with a Waters 2410 RI detector (Milford, MA, USA) and with a carbohydrate column (4.6×250 mm, 5 mm; Waters, Milford, MA, USA) at column temperature, 35℃. The mobile phase was acetonitrile/deionized water, 75:25 (v/v) at a flow rate of 1.0 mL/min. The results are expressed in mg% of dry weight, calculated by internal normalization of the HPLC peak area. The sugar standards used for identification were purchase from Sigma Chemical Co (St Louis, USA): D-fructose, D-glucose anhydrous, maltose, D-sucrose, D-trehalose and D-xylose.

Mineral analysis

The mineral content of *C. japonica* leaves and roots was analyzed using the method of Yun *et al* (14). Dry sample was digested with a microwave oven decomposition system (Milestone Ethos-1600, Monroe, CT, USA). Sample 0.5 g

was weighed and placed in a teflon digestion vessel with 6 mL of concentrated (65%) HNO_3 and 1 mL of 30% H_2O_2. Digests were left to cool and the sample solution was filtered through a membrane filter (pore size 0.45 μm), the volume made up to 50 mL with deionized water. For sample was analyzed using inductively coupled plasma optical emission spectrometers (ICP-OES) (IRIS Interpid II XSP, Thermo, MA, USA). The ICP-OES operating conditions: Nebulizer gas flow, 0.5 L/min at 20.1 psi pressure; Auxiliary gas flow, 0.5 L/min; Sample aspiration rate, 1.8 mL/min; Radio frequency (RF) power, 1.15 kW; Integration time, 30 sec; Relax pump time, 5 sec; gas, argon. All samples were measured in triplicate. Lines selected for the determination of the elements are listed in Table 3.

Total phenolic compound contents

The leaves and roots of *C. japonica* (10 g) were extracted with 100 mL distilled water, and the supernatant filtered through Whatman No 2 filter paper. It used to determine the content of total phenolic compound using the Folin-Denis method (15). Briefly, the extracts (0.2 mL) were mixed with 1.8 mL of distilled water and 0.2 mL of Folin-ciocalteu's phenol reagent (Junsei Chemical Co, Tokyo, Japan) and allowed to react for 3 min, after which 0.4 mL of saturated Na2Co3 solution was added. The mixture was kept at room temperature for 1 hr and then its absorbance was measured at 725 nm (Shimadzu U-1201, Kyoto, Japan). Tannic acid (Sigma-Aldrich Co, St Louis, MO, USA) was used as a standard for preparing of the calibration curve ranging 1～1,000 μg/mL assay solution.

Total flavonoid compound content

The total flavonoid compound contents were measured by the method of Nieva Moreno *et al* (16). The dried *C. japonica* leaves and roots (5 g) was extracted with 100 mL of 80% ethanol and the supernatant filtered through Whatman No 2 filter paper. An aliquot of 0.5 mL was mixed with 10% aluminum nitrate (0.1 mL), 1 M potassium acetate 0.1 mL and added 80% ethanol (4.3 mL). The mixture was kept at 25oC for 40 min, and then its absorbance was measured at 415 nm. Total flavonoid concentration was calculated using quercetin as a standard (Sigma-Aldrich Co, St Louis, MO, USA) for preparing of the calibration curve ranging 1～1,000 μg/mL assay solution.

Free amino acid and amino acid derivatives analysis

The dried *C. japonica* leaves and roots (1 g) was extracted with 100 mL distilled water for 2 hours and filtered through 0.45 μm filter. The extract was analyzed by using automatic amino acid analyzer (Pharmacia LKB Biochrom Ltd, Cambridge, UK) with analyzer column (Lithium high resolution). The operating conditions were as follows: column temperature, 35℃～80℃: reaction temperature, 135℃; mobile phase, ninhydrin 25 mL/hr; flow rate 20 mL/hr. Identification and quantification of free amino acids and amino acid derivatives were achieved by comparing the retention times of the peaks with those standards.

Statistical analysis

C. japonica leaves and roots data are expressed as mean ± SD of at least three separate experiments. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests using version 15.0 for windows (Statistical Package for the Social Science, SPSS INC, Chicago, IL, USA). Probability values <0.01 were student's t-test, significantly different compared between two trial groups.

Result and Discussion

Proximate composition

C. japonica. were divided into two different parts: leaves and roots and the proximate compositions in these two parts were determined. As shown in Table 1, the carbohydrate ranged from 71.47% of dry weight in leaves and 75.35% in roots. The contents of the moisture contained 12.55% and 11.18 %, respectively. Crude ash content varied between 7.77% in leaves and 5.83% in roots. The content of protein from leaves was 5.75% and roots was 5.51%. Crude fat content were 2.46% in leaves and 2.13% in roots. The most abundant component is carbohydrate, crude ash is the second

Table 1. Proximate composition of leaves and root parts from *C. japonica*

(%-dry weight)

Composition	Leaves	Roots
Moisture	12.55 ± 1.18[1)]	11.18 ± 0.45
Crude protein	5.75 ± 0.15	5.51 ± 0.35
Crude fat	2.46 ± 0.31	2.13 ± 0.05
Crude ash	7.77 ± 0.00**	5.83 ± 0.00
Carbohydrate	71.47 ± 1.64	75.35 ± 0.85**

[1)]The results presents mean ± SD of triplicate determinations.
**p<0.01 (Student' t-test), significantly different compared between two trial groups.

abundant component. The carbohydrate contents of roots was higher than leaves, but other components in roots were contained lower than leaves.

Soluble protein, reducing sugar and free sugar contents

Table 2 shows the soluble protein, reducing sugar contents and free sugar composition in *C. japonica* leaves and roots. The soluble contents of the leaves were 146.78 mg%, which is significantly lower ($p<0.01$) than that observed in the roots (33.67 mg%). The content of reducing sugar in leaves was 682.70 mg%, the total amount of free sugar was 166.00 mg% including 165.45 mg% glucose and 0.55 mg% maltose. The reducing sugar of roots (2,934.89 mg%) were about 4.3 times greater that of leaves, free sugar of roots (37.70 mg%) including 36.75 mg% glucose and 0.95 maltose were lower than leaves. Total sugar in roots were 3.5 times higher than leaves ($p<0.01$). Free sugar of leaves and roots presented glucose as main sugars. Glucose was the over 97% as free sugar. This is in agreement with the results reported for the *Syneilesis palmata* (17). Also Kim *et al* (18) reported that the free sugar of *Acrorus calamus* leaf and root showed 990 mg% and 1,940 mg%, respectively. Among these, leaf and root glucose were 610 mg% and 1,080 mg%, fructose were 380 mg% and 860 mg%, respectively, in which glucose accounted for almost the whole of the free sugar determined. similar observations have been made in other plants (18).

Table 3. Mineral composition and contents of leaves and roots of *C. japonica*

(mg%-dry weight)

Minerals(nm)	Leaves	Roots
Al(398.152)	nd[1)]	15.67 ± 0.12[2)]
Co(228.616)	nd	nd
Cu(324.754)	0.59 ± 0.00	0.55 ± 0.00
Li(670.784)	0.06 ± 0.01	0.04 ± 0.00
Mn(257.610)	2.32 ± 0.01	1.03 ± 0.01
Ni(231.604)	0.05 ± 0.00	0.07 ± 0.00
Zn(213.856)	1.96 ± 0.00	1.33 ± 0.00
Ca(184.006)	1,925.20 ± 3.52**	112.83 ± 0.15
Cr(283.563)	nd	nd
Fe(238.204)	8.38 ± 0.04	12.53 ± 0.11
K(766.491)	732.87 ± 4.70	1,282.13 ± 4.12**
Mg(285.213)	440.67 ± 0.42**	91.30 ± 0.22
Na(330.237)	9.99 ± 0.02	23.37 ± 0.03
Se(196.090)	0.04 ± 0.00	nd
Total	3,122.13	1,540.85

[1)]nd is not detected.
[2)]The results presents mean ± SD of triplicate determinations.
**$p<0.05$ (Student' t-test), significantly different compared between two trial groups.

Table 2. Contents of soluble protein, reducing sugar and free sugar of leaves and root parts from *C. japonica*

(mg%-dry weight)

Parts	Soluble protein	Reducing sugar	Free sugar			
			Glucose	Sucrose	Maltose	Total
Leaves	146.78 ± 3.77[1)**]	682.70 ± 6.29	165.45	tr[2)]	0.55	166.00**
Roots	33.67 ± 2.67	2,934.89 ± 15.73**	36.75	0.95	tr	37.70

[1)]The results presents mean ± SD of triplicate determinations.
**$p<0.05$ (Student' t-test), significantly different compared between two trial groups.
[2)]tr: trace.

Mineral content

The mineral contents of herbal plant and food are gaining importance because of toxicological as well as their nutritional viewpoints. Dietary intake is considered to be the major supplier of these elements of the body (19). In this study, the mineral composition of *C. japonica* leaves and roots, performed by ICP-OES, are displayed in Table 3. The mineral composition and contents of *C. japonica* leaves and root were difference, with the detected total 12 minerals. The total mineral contents of leaves were 3,122.13 mg% and roots were 1,540 mg%, the leaves were twice more than roots. The three elements Ca, K, and Mg which account for 96% to 99% of total mineral contents, were very rich in all parts. Ca content in leaves were the highest (1,925.20 mg%), comprising more than 61% of the total mineral content, followed by 732.87 mg% for K. And the K in roots (1,282.13 mg%) were more than 83% of the roots total mineral content, and followed by Ca (122.83 mg%). The Co, Ge and Cr were not detected in the *C. japonica*. Ca and Mg are the major component of bone and assists in teeth development (20), helping immune system. Especially Mg is a necessary nutrient for the

normalization of the function of various endogenous enzymes, for helping the energy generation and for the normalization of the blood circulation. K is effective mineral for diuretic effect. In sum, *C. japonica* can be effective and useful herbal materials as metabolic and physiological activity

Total phenolic and flavonoid compound contents

As phenolic compounds have been shown to possess strong antioxidant activity, and flavonoids are one of the most diverse and widespread group of natural phenolic compounds, flavonoids are likely to be the most important natural polyphenols (21). Especially phenolic compounds have strong antioxidative activity (22). Thus, the phenolic compound content including flavonoid compound content could be used as an important indicator of antioxidant capacity (23). Antioxidant play an important role in decreasing DNA damage, diminishing lipid peroxidation, maintaining immune function, and inhibiting malignant transformation or proliferation in vitro, which are thought to prevent some diseases, cancer, diabetes, hypertension, heart disease and age related degenerative conditions. The total phenolic and flavonoid compound contents of the leaves and roots from *C. japonica* are shown in Table 4. The leaves had a higher phenolic and flavonoid compound contents than roots. The phenolic compound contents of leaves and roots were 3,028.89 mg% and 381.11 mg%, and the flavonoid compound contents were 382.67 mg% and 50.49 mg%, respectively. Kim *et al*, (2004) reported that the phenolic compound contents of Korean ginseng and *Polydonati* rhizoma showed 397 mg% and 262 mg%, the flavonoid compound contents were 591 mg% and 51 mg%, respectively. This *C. japonica* results were higher phenolic compound than the Kim *et al*, (24) reported.

Table 4. Contents of the total phenolic and flavonoid compound from leaves and root parts from *C. japonica*

(mg%-dry weight)

Composition	Leaves	Roots
Total phenolic compound	3,028.89 ± 20.37[1)]	391.11 ± 21.43
Total flavonoid compound	382.67 ± 3.04	50.49 ± 2.68

[1)]The results presents mean ± SD of triplicate determinations.

Free amino acid and amino acid derivatives analysis

The amino acid and derivatives composition of leaves and roots of *C. japonica* is shown in Table 5. The results showed that leaves and roots contained 17 known amino acids, including all of the essential amino acids and 15 known amino acid derivatives. The leaves and roots amino acid contents were 2,467.15 mg% and 1,334.81 mg%, respectively. The amino acid derivatives were 1,515.10 mg% (leaves) and 1,140.92 mg% (roots). The leaves amino acid and derivatives

Table 5. Contents of the free amino acid and amino acid derivatives of aerial and root parts from *C. japonica*

(mg%-dry weight)

Free amino acids		Leaves	Roots
Essential amino acid	Threonine	198.09	51.28
	Methionine	6.61	2.92
	Isoleucine	140.39	93.61
	Leucine	179.31	61.98
	Phenylalanine	144.84	47.29
	Valine	394.05	168.44
	Lysine	49.67	13.92
Non-essential amino acid	Aspartic acid	125.42	375.27
	Serine	26.09	49.63
	Glutamic acid	432.16	185.09
	Glycine	102.07	22.96
	Alanine	503.30	142.46
	Cystine	tr[1)]	tr
	Tyrosine	165.15	42.72
	Histidine	tr	4.02
	Arginine	tr	41.63
	Proline	tr	31.59
Total amino acids		2,467.15	1,303.22
Amino acid derivatives	Phosphoserine	351.12	182.13
	Taurine	tr	161.53
	Phosphoethanolamine	tr	134.03
	Sarcosine	tr	11.38
	α-aminoadipic acid	41.90	382.01
	Citruline	nd[2)]	4.57
	β-alanine	30.67	19.85
	α-Aminoisobutyric acid	25.57	nd
	ɣ-Aminoisobutyric acid	746.20	134.30
	DL-5-Hydroxylysine	36.85	15.88
	Cystathionine	16.48	35.86
	1-Methylhistidine	nd	6.42
	Ornithine	24.95	4.21
	Anserine	nd	30.03
	Carnosine	241.36	18.72
Total		3,742.890	2,425.42

[1)]tr is trace.
[2)]nd is not detected.

contents were higher than roots. The leaves had higher contents of glutamic acid, glycine, alanine, tyrosine and essential amino acids than that of roots. The roots was higher contents of aspartic acid, serine, histidine, arginine and proline. The percentage of essential amino acids in total amino acids was 45.11% (1,112.96 mg%) in leaves and 32.92% (439.44 mg%) in roots. The percentage of savoury amino acids, aspartic acid and glutamic acid, to total amino acid in roots were as high as 28.11% and 13.87%, the sweet (alanine) and savoury (glutamic acid) amino acid to total amino acid in leaves were as high as 20.40% and 17.52%, respectively. The percentage of ɣ-aminoisobutyric acid in total amino acid derivatives was 49.25% in leaves. the roots were including α-aminoadipic (33.48%) acid in total amino acid derivatives.

Conclusion

According to the results of the present study, we found that *C. japonica* leaves are higher than roots, as protein, minerals, total phenolic compounds, flavonoid compound contents, free amino acid and amino acid derivatives. This suggests that *C. japonica* leaves are a good source of nutrient and micronutrients for health, *C. japonica* is a potentially useful of resource for the functional and favorite food and nutraceutical.

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