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Chemical Composition and Electron Donating and Nitrite Scavenging Activities of *Glechoma hederacea var. longituba* NAKAI

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Abstract

This study was performed to investigate chemical and functional properties of *Glechoma hederacea* leaves in respect to its potential use as food material or as a medicinal herb. The chemical compositions on a dry basis were 20.38% in protein, 3.96% in fat, 59.58% in carbohydrate, 15.78% in ash, 5.36% in reducing sugar, 14.11% in total sugar and 0.26% in polyphenol, respectively. The free sugars were mainly composed of glucose, fructose and sucrose. In fatty acids compositions, linolenic acid showed the highest concentration at 45%, while the ratios of saturated to unsaturated fatty acids were 1 : 1.91. Seventeen kinds of total amino acids were determined, with the highest concentration (2,465.71 mg%) of glutamic acid. Among the free amino acids, proline showed the highest concentration (260.09 mg%), followed by glutamine, α-amino adipic acid, glutamic acid and valine. The contents of major minerals were 647.32 mg% in Na, 597.53 mg% in K and 239.75 mg% in Ca. The antioxidative activity of 10% water extract was similar to that of 50 ppm tocopherol. The nitrite scavenging ability reached the highest level at pH 1.2 and the lowest at pH 6.0.

Key words: *Glechoma hederacea* leaves, chemical constituents, nitrite scavenging activities

INTRODUCTION

Glechoma hederacea var. longituba NAKAI, belonging to a *Labiatae* family, is a perennial vine plant (1,2). *Glechoma hederacea*, called *kinbyeongkkochpul* in Korean, has a genus name like *hwalhyeoldan* and *yeonjeoncho* and is used as tubiflorous as well as bee plants. It is known that the leaves of *Glechoma hederacea* are used as a medicinal herb to treat diaphoresis, diuresis, defervescence, and dropsy and that they are also edible at an earlier stage (3).

Glechoma hederacea was scientifically proven to contain 3-O-α-L-rhamnopyranosyl (1-2)-β-D-galactopyranosyl, schaftoside, soya-saponin I , vicenin 1, vicenin 3, etc (1). Studies on the physiological activities of *Glechoma hederacea* include reports, on the Na^+-K^+-ATPase inhibitors of *Lysimachiae japonica* by Shoji et al. (4) and on effects of *Lysimachiae herba* extracts on the metabolic enzyme activities in galactosamine-intoxicated rats by Kim et al. (5). However, few attempts were made on the chemical and functional properties of *Glechoma hederacea* leaves.

This study was performed to investigate chemical and functional properties of *Glechoma hederacea* leaves in respect to its potential use as food material or as a medicinal herb.

MATERIALS AND METHODS

Material

The *Glechoma hederacea* leaves used were harvested in the fall of 1999 at Ganghwagun, Gyeonggido prior to being sun-dried. The leaves, passed through 20 mesh and stored at room temperature, were chemically analysed.

Determination of proximate composition and polyphenolic content

Proximate compositions of *Glechoma hederacea* leaves were determined according to the standard AOAC methods (6). The carbohydrate content was determined by subtracting the contents of moisture, crude protein, crude fat and crude ash from 100. The measurement of reducing sugar was based upon the modified Somogyi method (7) and the same method was used to determine total sugar following hydrolysis of the sample with 25% HCl. The content of total phenolics was determined by colorimetry using the Folin-Denis method (8). To prepare a sample solution, five grams of *Glechoma hederacea* leaves was extracted with 50 mL of distilled water at 25°C 12 hrs and then filtered. Absorbance was read at 700 nm and a standard curve was prepared with tannic acid. All measurements were repeated at least three times.

Determination of free sugar

Free sugars were extracted with 70% ethanol and analyzed using high-performance liquid chromatograph (HPLC) (9). HPLC was performed by Waters 400E system equipped with a refractive index detector and Sugar-PAK I (30 cm ×3.9 mm i.d.) by using a mobile phase made up of 50 mg Ca-EDTA/1 L H_2O.

Determination of fatty acid

Crude lipids were extracted with diethyl ether. Purified

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lipids were hydrolyzed with 1 N KOH/EtOH solution before being methyl esterificated using BF_3-methanol, as described by Metcalf et al. (10). Fatty acid esters were analyzed by gas chromatograph (Varian star 3400 CX) equipped with a flame ionization detector. A DB-FFAP column (30 m × 0.5 mm i.d.) was used for methyl ester separation. The column oven temperature was programed linearly from 150°C (5 min) to 240°C (20 min) at an increasing rate of 4°C/min.

Determination of total amino acid

An analysis of the total amino acids was performed using an amino acid analyzer for the solution that was filtered through a 0.45 μm membrane after 6 N HCl hydrolysis (11). The instrument was Pharmacia model biochrom 20 and was equipped with a Sodium TEEK column (4.6 × 200 mm). The buffer was pH 3.20 sodium citrate, pH 4.25 sodium citrate, pH 6.45 sodium citrate and sodium hydroxide. The buffer flow rate was 25 and the ninhydrin was 20 mL/hr.

Determination of free amino acid

Free amino acids were extracted using 75% ethanol and then the protein sedimented with 25% trichloroacetic acid (TCA) solution was centrifuged. Diethyl ether was added and shaken to remove the residual lipids from the sample. The sample was dissolved in the loading buffer solution (0.2 N sodium citrate, pH 2.2) and then injected into the amino acid analyzer after filtration through a 0.45 μm membrane (12). The instrument and column were the same as above. The buffer was lithium buffer A, B, C Ⅱ, D Ⅱ, lithium buffer pH 3.55 and lithium hydroxide. The flow rate of buffer and the ninhydrin was 20 mL/hr.

Determination of mineral

Minerals were analyzed using an atomic absorption spectrophotometer (Varian Spectra AA 200 HT) according to the wet-digestion procedures (13). The analysis conditions for Fe, Cu, Mn, Zn, Mg, Ca, K, and Na were $A_{248.3}$, $A_{324.8}$, $A_{279.5}$, $A_{213.9}$, $A_{285.2}$, $A_{422.7}$, $A_{769.9}$, $A_{589.0}$, respectively.

Measurement of electron donating activity

The electron donating activity of *Glechoma hederacea* leaves' extract was measured according to α, α'-diphenyl-β-pycrylhydrazyl (DPPH) method (14). The water extract (1 mL) of the sample was added to the DPPH solution (5 mL) to measure the absorbance at 517 nm, thereby calculating the DPPH radical scavenging activity (%) with the following equation. The electron donating activities of α-tocopherol (50 ppm) and BHT (dibutyl hydroxytoluene, 50 ppm) were compared with those of different concentrations (0.5, 1.0 and 10%) of water extracts.

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{Abs}}{\text{Abc}}\right) \times 100$$

Where Abc is absorbance of DPPH solution without sample at 517 nm and Abs is absorbance of DPPH solution with sample at 517 nm.

Measurement of nitrite scavenging ability

According to the method described by Kato et al. (15) using Griess reagent, 10% of the water extract was used to determine the nitrite scavenging activity at different conditions (pH 1.2, 3.0, 4.2 and 6.0) by measuring the absorbance at 520 nm. Thus, the nitrite scavenging activity (%) was calculated with the following equation.

$$\text{Nitrite scavenging activity (\%)} = \left(1 - \frac{\text{Abs}}{\text{Abc}}\right) \times 100$$

Where Abc is absorbance of no treated at 520 nm and Abs is absorbance of treated sample at 520 nm.

RESULTS AND DISCUSSION

Proximate composition and polyphenolic content

The proximate chemical compositions in *Glechoma hederacea* leaves (moisture: 9.17%) are shown in Table 1. The proximate compositions were 20.38% in protein, 3.96% in fat, 59.58% in carbohydrate and 15.78% in ash on a dry basis, respectively. The polyphenolic compound of water extract from *Glechoma hederacea* leaves was 0.26%. Different contents of total polyphenolics were reported for the water extracts from various plant origins, such as 6.88% in green tea, 0.96% in cassiae semen, 0.95% in lycii fructus, 1.41% in solomon's seal, 0.56% in schizandrae fructus, and 0.03% in turnip (16,17). Lee et al. (18) reported similar contents of total phenolics in the unripe state of apples such as Fuji (0.11%) and Aori (0.12%). Many food phenolics have been reported to have both antimutagenic and anticarcinogenic activities in test animals (19). Furthermore, there has been a renewed interested in the natural sources of antioxidants and it has been found that they generally belong to the phenolic group of compounds (20). The antioxidant capacity was known to be strongly correlated with the content of total phenolics (21,22).

Free sugar

The affect of free sugars on food quality is concerned with the aroma production and the browning reaction by heating and is known to participate in flavor production (23). Table 2 shows the composition of the free sugars in *Glechoma hederacea*. Free sugars were primarily composed of glucose (4.61 mg/g), fructose (4.45 mg/g) and sucrose (3.34 mg/g).

Fatty acid

Seven fatty acids were determined in the leaves of *Gle-*

Table 1. Proximate composition and polyphenol content of *Glechoma hederacea* leaves

Composition	Content (%)
Moisture	9.17
Crude protein	18.51
Crude fat	3.60
Carbohydrate	54.39
Crude ash	14.33
Total sugar	12.82
Reducing sugar	4.87
Polyphenol	0.24

Table 2. Free sugar composition of *Glechoma hederacea* leaves

Content (mg/g, dry basis)			
Fructose	Glucose	Sucrose	Fructose/Glucose
4.45	4.61	3.33	0.97

choma hederacea (Table 3). Saturated fatty acids, including palmitic acid, arachidic acid and behenic acid, were 34% of the total fatty acids, whereas unsaturated fatty acids, such as oleic acid, linoleic acid and linolenic acid were 65%. Among the fatty acids identified from *Glechoma hederacea*, linolenic acid (n-3) showed the highest concentration of 45%. This tendency is similar to that of mugwort (24) and soybean leaf (25).

Total and free amino acid

The composition of total amino acids in *Glechoma hederacea* are presented in Table 4. The seventeen selected amino acids showed the ratio of essential to nonessential amino acids of 35.12 : 64.88. Glutamic acid showed the highest concentration of 2,465 mg%, followed by leucine, glysine, lysine, alanine and proline. Similar results on ginsengleaf tea were reported by Kwon et al. (26) finding that glutamic acid was of the highest concentration at 2,073 mg%, followed by aspartic acid and glycine.

Table 5 shows the composition of free amino acids in *Glechoma hederacea.* From the twenty four selected amino acids and their related compounds determined, the ratio of essential to nonessential amino acids were 16.67 : 83.33. Proline (260 mg%) was the highest concentration in all the free amino acids (971 mg%), followed by glutamine, α-aminoadipic acid, glutamic acid, valine and phenylalanine, which was different from the total amino acid in their profiles.

Mineral

Eight minerals were determined in the *Glechoma hederacea* leaves and their individual contents are provided in Table 6. The sample was mainly composed of Na (647.32 mg%), K (597.53 mg%) and Ca (239.75 mg%). This pattern was somewhat different from the reports by Hwang et al. (27) stating that K and Ca were predominant in the mineral compositions of most medicinal herbs marketed in Korea. It can be hypothesized that the composition and/or the content of minerals in the leaf may be affected by factors such as variety, nutrition status, fertilizer, season, environment and climate (28).

Electron donating activity

The electron donating ability is known to depress lipid

Table 3. Fatty acid composition of *Glechoma hederacea* leaves

Fatty acid	Content (rel.%)
Myristic acid (14 : 0)	0.91
Palmitic acid (16 : 0)	29.14
Oleic acid (18 : 1)	5.19
Linoleic acid (18 : 2)	14.59
Linolenic acid (18 : 3)	45.18
Arachidic acid (20 : 0)	1.75
Behenic acid (22 : 0)	2.41
Unknown	0.83
Total saturated fatty acid (TSFA)	34.21
Total unsaturated fatty acid (TUFA)	64.96

Table 4. Composition of total amino acid of *Glechoma hederacea* leaves

Total amino acid	Content (mg%, dry basis)
Threonine	375.16
Valine	442.17
Methionine	6.43
Isoleucine	242.80
Leucine	704.45
Phenylalanine	411.38
Lysine	645.77
Histidine	134.34
Serine	516.63
Glutamic acid	2,465.71
Proline	557.35
Glycine	695.54
Alanine	569.64
Cystine	37.42
Tyrosine	120.16
Ammonia	204.52
Arginine	324.93
Total	8,453.47

Table 5. Composition of free amino acid of *Glechoma hederacea* leaves

Free amino acid	Content (mg%, dry basis)
Threonine	14.56
Valine	52.08
Isoleucine	16.71
Leucine	20.17
Phenylalanine	45.95
Lysine	4.89
Histidine	8.89
Aspartic acid	15.70
Serine	28.62
Glutamic acid	59.12
Glutamine	195.93
α-Aminoadipic acid	130.40
Proline	260.09
Glycine	2.65
Alanine	37.39
Citruline	4.62
Cystine	2.25
Cystathionine	2.10
Tyrosine	4.35
β-Alanine	3.86
γ-Aminoisobutyric acid	36.70
Ammonia	15.29
Ornithine	0.51
Arginine	8.35
Total	971.18

Table 6. Mineral composition of *Glechoma hederacea* leaves

Content (mg%, dry basis)							
Ca	Mg	Na	K	Fe	Cu	Mn	Zn
239.75	3.82	647.32	597.53	1.20	0.22	4.70	0.60

oxidation and inhibit aging of active radicals in body by donating electron to the active radicals (29). Hence, the DPPH radical scavenging method is used for measuring electron donating ability. Antioxidative activity is achieved by scavenging the radical and decolorizing when the DPPH with the relative stable radicals react upon the antioxidative substances (14). The electron donating ability of water extracts in *Glechoma hederacea* was measured by concentration and time. The activity of 0.5% or 1% water extract showed similar to that of 50 ppm BHT. Activity of 10% extract was similar to that of 50 ppm tocopherol after 10 min of reaction, as shown in Fig. 1. It seems that the antioxidative substances in *Glechoma hederacea* leaves react slowly with DPPH. This finding is supported by the reports by Cha et al. (30) who found that the absorbance of the DPPH solution decreased with time when mixed with the water extract of *Cudrania tricuspidata*.

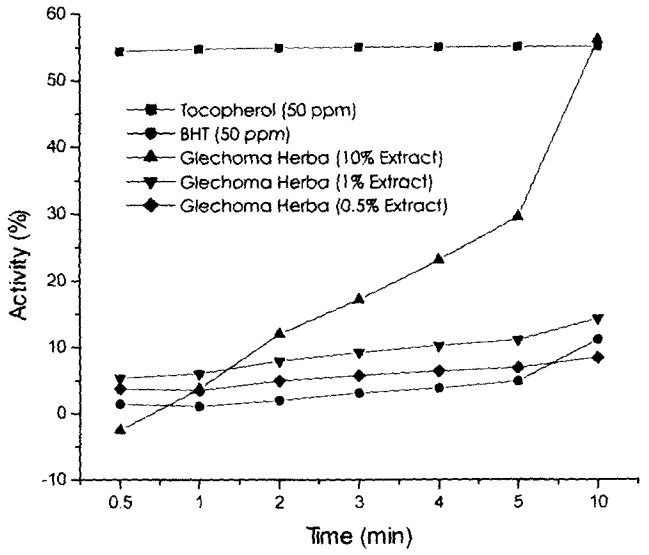

Fig. 1. DPPH radical scavenging activity of water extract from *Glechoma hederacea* leaves.

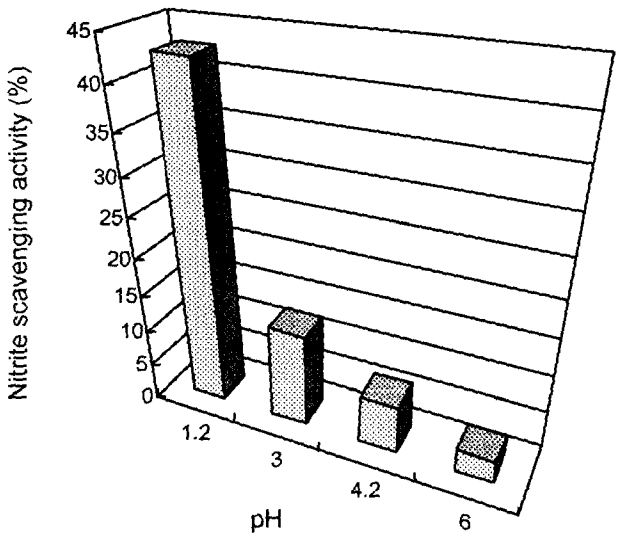

Fig. 2. Nitrite scavenging activity of water extract from *Glechoma hederacea* leaves under different pH conditions.

Nitrite scavenging activity

The nitrite scavenging activity of the sample increased as pH decreased, showing 42.80% at pH 1.2 (Fig. 2). This result was similar to those of *Ulmus devidiana* (31), pine leaf, mugwort and cassiae semen (32) in that the nitrite scavenging activity increased at a decreased pH. From both economical as well as practical points of view, nitrite is one of the most important food additives. However, the use of nitrite results in the occurrence of very low levels of nitrosamine, which at higher levels have been known to be carcinogenic in laboratory animals (33). Therefore, when functional materials that have potent nitrite scavenging activity are used in processed foods, it is expected that nitrosation inhibition might be feasible under stomach conditions (pH 1.2).

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