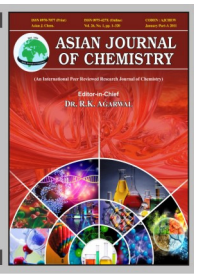
Asian Journal of Chemistry; Vol. 26, No. 3 (2014), 897-902

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16143A



Composition of Polyphenols and Antioxidant Activity of Garlic Bulbs Collected from Different Locations of Korea

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Received: 3 August 2013; *Accepted*: 9 October 2013; *Published online*: 30 January 2014; AJC-14663

The aqueous extracts of garlic bulbs collected from different locations of Korea *viz*., Namdo, Daeso, Godang, Hampyeong and Sangeol were analyzed for their phenolic profiles and screened for their antioxidant activities. Antioxidant activity was evaluated by four different methods namely DPPH radical scavenging activity, reducing power assay, metal chelating activity and phosphomolybdenum activity. Spectrophotometric analysis revealed that the highest levels of total phenolic content and total flavonoid content were found in the garlic bulbs collected from Hampyeong region. A total of 23 polyphenolic compounds were identified and quantified from garlic bulbs, including hydroxybenzoic acids, hydroxycinnamic acids, flavonols and other groups of phenolic compounds. The ultra performance liquid chromatography (UPLC) analysis of the phenolic compounds profile revealed that β-resorcylic acid was the dominant phenolic compound found in garlic bulb extracts collected from different locations; it constituted about 313.50 µg/g from Sangeol region to 452.10 µg/g from Godang region, followed by pyrogallol (139.35 to 418.80 µg/g), gallic acid (67.40 to 196.80 µg/g), rutin (51.35 to 90.45 µg/g), protocatechuic acid (31.75 to 76.25 µg/g) and quercetin (25.70 to 56.65 µg/g). The garlic bulbs collected from the Hampyeong region exhibited highest antioxidant activity assayed by four different methods except the phosphomolybdenum assay when compared with other garlic bulbs of different locations.

Keywords: Antioxidant activity, DPPH activity, Different locations, Garlic, Total flavonoid content, Total phenolic content, UPLC.

INTRODUCTION

Phytochemicals, particularly antioxidants from natural sources such as fruits, vegetables and herbs have gained popularity due to their protective properties against several chronic diseases such as cancer and cardiovascular diseases[1]. Among the natural compounds extracted from plants, polyphenols have received much attention due to their powerful antioxidant, antimicrobial and antiviral activities as well as their capacity to inhibit the proliferation of cancer cells, protect neuron against oxidative stress, stimulate vasodilation, reduce vascularization and improve insulin secretion[2]. Polyphenols are a diverse class of chemical compounds that share the ability to act as chain breaking antioxidants, which are proposed to protect against the damage caused by free radicals to DNA, cell membrane and cell components[3]. Moreover, they exhibit antibacterial, antiinflammatory, antiallergenic, antiarthrogenic and antithrombotic effects[4]. Recent research on the nutritional aspects have shown that polyphenols are able to modulate nutrient availability through the inhibition of digestive enzymes involved in lipid and starch breakdown, which could lead to beneficial effects on calorie intake, obesity[5], and blood glucose control[6]. Because of their many intriguing biological activities, polyphenols have been the subject of numerous investigations and substantial data on their extraction and analysis have been published[2,7].

Garlic (*Allium sativum* L., Alliaceae) has been playing one of the most important dietary and medicinal roles in human beings for centuries. It has been cultivated since ancient times, it is mainly used as spice and as flavouring agent and, due to its potential benefits in preventive and curative medicine, it has been used in ancient and modern cultures[8]. In the present day scenario also, the medicinal application of garlic is widespread and growing rapidly. Epidemiological, clinical and preclinical studies have demonstrated a close relation between dietary habits, including garlic intake and the occurrence of disease. Garlic is considered as one of the best disease-preventive foods, based on its potential and diversified effects[9]. A wide array of therapeutic effects, such as hypolipidaemic, antiatherosclerotic, hypoglycaemic, anticoagulant, antihypertensive, antimicrobial, antidote (for heavy metal poisoning) and hepatoprotective, has been reported[10,11]. Furthermore, garlic is used in the prevention of cold and flu symptoms through immune enhancement and exhibits anticancer and

chemopreventive activities[9,12,13]. It is generally considered that health-related functions are mostly attributed to the fresh garlic content, which is rich in γ-glutamylcysteine and many other sulfur-containing compounds in it, giving a characteristic flavour formed during storage and processing[12,14]. However, additional constituents of garlic include a wide range of primary and secondary non-sulfur biomolecules, such as steroidal glycosides[15], essential oil[16], flavonoids[17], anthocyanins[18], lectins[19], prostaglandins, fructan, pectin, adenosine, vitamins B1, B2, B6, C and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids and essential amino acids[20]. Many of these components work synergistically to provide different health benefits[9,12]. The antioxidant properties of garlic and different garlic preparations are well documented[12,14,21-25].

The aim of our study was to investigate the phenolic composition and antioxidant activity of aqueous garlic bulb extracts from different locations of Korea. According to the recommendations, the antioxidant effects in four different bioassays were studied, besides determination of total phenolics and flavonoids content. The study of polyphenolic composition is an important scientific agenda for food and nutritional sciences, which may contribute to the improvement of conventional foods with added health benefits being very useful to determine these chemicals in plants, in the field of nutrition, pharmacology and agronomy. To the best of our knowledge, this is the first report on the phenolic composition and antioxidant activity of aqueous garlic bulb extracts from different locations of Korea.

EXPERIMENTAL

Methanol, acetonitrile, glacial acetic acid and distilled water (HPLC grade) were purchased from J.T. Baker (USA) and hydrochloric acid was purchased from Daejung Co. (Daejung chemicals & Materials Co. Ltd, Siheung, Gyeonggi-Do, Korea). Dimethyl sulfoxide (DMSO), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, pyrogallol, protocatechuic acid, β-resorcylic acid, vanillic acid, caffeic acid, vanillin, *p*-coumaric acid, ferulic acid, *m*-coumaric acid, rutin, *o*-coumaric acid, chlorogenic acid, hesperedin, myricetin, resveratrol, quercetin, naringenin, kaempferol, formononetin, syringic acid, veratric acid and biochanin A were purchased from Sigma-Aldrich (USA).

Plant material and sample preparation: The garlic bulbs were collected from Namdo, Daeso, Godang, Hampyeong and Sangeol regions of South Korea. A voucher specimen is deposited in the Department of Applied Biosciences, Konkuk University, Seoul, South Korea. The garlic bulbs were washed thoroughly in tap water to remove adhering mud particles, rinsed in distilled water, drained and blotted to remove the water. The fresh bulbs were extracted using a method of maceration with distilled water for 76 h at room temperature. Solvent were removed in vacuo and extracts were obtained, respectively.

Extraction of phenolic compounds for the UPLC analysis: One g of the dried aqueous extract was extracted in 10 mL of acetonitrile and 2 mL of 0.1 N hydrochloric acid. The mixture was stirred for 2 h at room temperature. The extract was filtered through No. 42 Whatman filter paper and was concentrated using a vacuum evaporator. The residues were dissolved in 10 mL of 80 % aqueous methanol and filtered through a 0.45 μm membrane. The filtrate was used for the UPLC analysis.

UPLC analysis of the phenolic compounds: UPLC was performed using the Thermo Accela UPLC (Thermo, New York, USA) system. Separation was primarily achieved using a HALO C18 (2.7 μm, 2.1 × 100 mm) column and the absorbance was measured at 280 nm. The mobile phases were 0.1 % glacial acetic acid in distilled water (solvent A) and 0.1 % glacial acetic acid in acetonitrile (solvent B). The injection volume was 4 μL and the linear gradient of UPLC solvents was as follows: 0 min, 92 % A : 8 % B; 0-2.2 min, 90 % A : 10 % B; 2.2-5 min, 85 % A : 15 % B; 5-7.5 min, 84.5 % A : 15.5 % B; 7.5-8.5 min, 82.2 % A : 17.8 % B; 8.5-13 min, 55 % A : 45 % B; 13-14 min, 0 % A : 100 % B; and 14-15 min, 92 % A : 8 % B. The run time was 15 min and the flow rate was 500 μL/min.

Solutions of available pure known compounds, gallic acid, pyrogallol, protocatechuic acid, β-resorcylic acid, vanillic acid, caffeic acid, vanillin, *p*-coumaric acid, ferulic acid, *m*-coumaric acid, rutin, *o*-coumaric acid, chlorogenic acid, hesperedin, myricetin, resveratrol, quercetin, naringenin, kaempferol, formononetin, syringic acid, veratric acid and biochanin A were chromatographed as external standards. All standards were dissolved in methanol before injections in the analytical UPLC system. Their ranges of concentration used were 25, 50, 100, 150 μg/mL. Phenolic compounds of garlic bulb extracts was identified by comparing their retention times with those of pure compounds. The results were expressed as μg/g of each compound from the total phenolic compounds.

Determination of total phenolic content: The amount of total phenolic compounds in the extracts was determined spectrophotometrically with the Folin-Ciocalteu (FC) reagent, using slightly modified method of Fukumoto and Mazza[26]. The reaction mixture contained 500 μL of extract, 2.5 mL of freshly prepared 0.2 M FC reagent and 2 mL of sodium carbonate solution and was kept in the dark under ambient conditions for 0.5 h to complete the reaction. The absorbance of the resulting solution was measured at 760 nm in a UV-visble spectrophotometer (Optizen 2120, Mecasys, Korea). The concentration of total phenolic compounds was expressed as mg of gallic acid equivalents (GAE) per g of dried extract, using a standard curve of gallic acid. All measurements were carried out in three replicates.

Determination of total flavonoid content: Measurement of total flavonoid content in the investigated extracts was determined spectrophotometrically according to Jia *et al.*[27], using a method based on the formation of complex flavonoid aluminium with the absorbtivity maximum at 430 nm. The diluted sample solutions, in the amount of 1 mL, were separately mixed with 1 mL of 2 % $AlCl_3 \times 6H_2O$. After incubation at room temperature for 15 min, the absorbance of the reaction mixtures was measured at 430 nm. The flavonoids content was expressed as mg of quercetin equivalents (QE) per g of dried extract, by using a standard graph. All measurements were carried out in three replicates.

DPPH radical scavenging activity: The DPPH assay was performed as previously described[8]. The samples (ranging from 50 to 250 μg/mL) were mixed with 1 mL of 90 μM DPPH

solution and made up with 95 % MeOH to a final volume of 4 mL. The absorbance of the resulting solutions and the blank (with same chemicals, except sample) was recorded after 1 h at room temperature (37 °C). For each sample, three replicates were recorded. The disappearance of DPPH was measured spectrophotometrically at 515 nm. Radical scavenging capacity (RSC), expressed as a percentage, was calculated by the following equation:

$$RSC\ (\%) = [(A_0 - A_s)/ A_0] \times 100$$

where A_0 is the absorbance of the control and A_s is the absorbance of the sample.

Reducing power assay: The reducing power of the extracts was determined according to the method of Oyaizu[28]. Different extracts of concentration (100 to 500 µg/mL) in 1 mL of distilled water was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide $[K_3Fe(CN)_6]$ and the mixture was incubated at 50 °C for 20 min. Then, 2.5 mL of 10 % trichloroacetic acid was added to the mixture and centrifuged at 650 g for 10 min. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % ferric chloride and the absorbance was measured at 700 nm against a blank. Increased absorbance of the reaction mixture indicated increased reducing power. All analysis were run in triplicate and averaged.

Metal chelating activity: The chelating effect was determined according to the method of Dinis *et al*.,[29]. Briefly, 2 mL of various concentrations (50 to 250 µg/mL) of the extracts in methanol was added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was measured spectrophotometrically at 562 nm. The inhibition percentage of ferrozine-Fe^{2+} complex formation was calculated by using the formula given below:

$$\text{Metal chelating effect}\ (\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where $A_{control}$ is the absorbance of control (the control contains $FeCl_2$ and ferrozine complex formation molecules) and A_{sample} is the absorbance of the test compound. EDTA was used as a standard.

Evaluation of antioxidant capacity by phosphomolybdenum method: The total antioxidant capacity of garlic bulb extracts was evaluated by the method of Prieto *et al*.,[30]. An aliquot of 0.1 mL of sample solution (1 mg/mL) was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as the rest of the sample. For samples of unknown composition, water soluble antioxidant capacity was expressed as equivalents of α-tocopherol (mg/g of extract).

Experimental results are expressed as means ± SD. Each sample was analyzed three times as well as experiments were replicated three times (n = 3).

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents: Although most antioxidant activities from plant sources are derived from phenolic-type compounds[31], these effects do not always correlate with the presence of large quantities of phenolics. Therefore, both sets of data need to be examined together. With respect to this, the investigated garlic extracts were analyzed for total phenolic and flavonoid contents. The amount of total phenolics varied widely from different locations and ranged from 33.50 to 49.89 mg GAE/g, the highest phenolic content was observed in the garlic bulbs collected from Hampyeong (49.89 mg GAE/g) and the lowest was observed from the Namdo region (33.50 mg GAE/g) (Table-1). Furthermore, the results obtained from evaluation of total flavonoid content also indicate some variations (Table-1). In the garlic bulbs collected from Hampyeong, the content of quercetin equivalents was notably higher (1.56 mg QE/g), followed by Daeso (1.43 mg QE/g) and the lowest was from the Namdo region (1.02 mg QE/g). The decreases of the total phenolic and flavonoid contents are most probably caused by the increase of sulphur compounds and terpenoids substances present in the essential oil of mature garlic bulbs. The levels of total phenolics determined in this way are not absolute measurements of the amounts of phenolic compounds, but are in fact based on their chemical reducing capacity relative to gallic acid[32].

TABLE -1
TOTAL PHENOLIC (EXPRESSED AS GALLIC ACID EQUIVALENTS) AND FLAVONOID CONTENT (EXPRESSED AS MG QUERCETIN/G) OF GARLIC BULB EXTRACTS COLLECTED FROM DIFFERENT LOCATIONS OF KOREA

Extract	Total phenolic content (mg/g)	Total flavonoid content (mg/g)
Namdo	33.50 ± 0.54	1.02 ± 0.023
Daeso	39.27 ± 0.87	1.43 ± 0.260
Godang	43.53 ± 1.38	1.05 ± 0.030
Hampyeong	49.89 ± 1.66	1.56 ± 0.037
Sangeol	41.86 ± 0.94	1.14 ± 0.042

Values are means of triplicates ± SD

UPLC separation and determination of phenolic compounds in garlic bulb extract: The phenolic compounds like flavonols, hydroxybenzoic acids and hydroxycinnamic acids which are present in most of the vegetables, herbs and spices are considered as an important therapeutic agent because of their beneficial effects on human health, such as protection of certain types of cancers, aging and cardiovascular diseases[33]. Therefore qualitative and quantitative analysis of the garlic bulb extracts was made using ultra performance liquid chromatography (UPLC) as described in the experimental part and the results are presented in Table-2. The phenolic compounds in the garlic bulb extracts were identified by comparisons to the retention time and UV spectra of authentic standards while the quantitative data were calculated from the calibration curves. Garlic bulbs collected from Namdo region had 58.3 µg/g of dry matter of flavonols, 90.05 µg/g of dry matter of hydroxycinnamic acid, 455.05 µg/g of dry matter of hydroxybenzoic acid and 282.45 µg/g of dry matter of other phenolic compounds. The other garlic bulbs collected from other regions

TABLE -2
MAJOR PHENOLIC COMPOUNDS IDENTIFIED IN THE GARLIC BULB EXTRACT COLLECTED FROM DIFFERENT LOCATIONS OF KOREA BY UPLC ANALYSIS

Compounds	Concentration (μg/g)				
	Namdo	Daeso	Godang	Hampyeong	Sangeol
Flavonols					
Myricetin	22.40 ± 0.424	20.20 ± 0.212	21.10 ± 0.141	21.55 ± 2.192	19.20 ± 0.141
Quercetin	25.70 ± 0.848	36.45 ± 0.636	46.35 ± 0.919	56.65 ± 3.181	43.5 ± 4.525
Kaempferol	10.20 ± 0.141	9.10 ± 0.141	9.45 ± 0.070	9.10 ± 0.282	8.95 ± 0.070
Hydroxycinnamic acid					
Caffeic acid	37.05 ± 0.212	39.30 ± 0.141	39.55 ± 0.212	31.05 ± 0.070	28.30 ± 0.070
p-Coumaric acid	10.95 ± 0.070	11.05 ± 0.212	9.05 ± 0.070	8.90 ± 0.070	12.45 ± 0.212
Ferulic acid	28.05 ± 0.070	28.30 ± 0.070	26.75 ± 0.070	34.20 ± 0.353	28.05 ± 0.212
m-Coumaric acid	0.60 ± 0.007	2.6 ± 0.141	4.55 ± 0.070	0.95 ± 0.070	0.15 ± 0.070
o-Coumaric acid	10.70 ± 0.070	10.30 ± 0.070	11.50 ± 0.141	11.75 ± 0.070	12.40 ± 1.131
Chlorogenic acid	2.70 ± 0.707	nd	nd	8.25 ± 0.212	1.50 ± 0.282
Hydroxybenzoic acid					
Gallic acid	67.40 ± 0.282	87.40 ± 0.282	130.25 ± 1.131	196.80 ± 0.424	98.70 ± 0.565
Protocatechuic acid	31.75 ± 0.070	34.2 ± 5.656	53.00 ± 1.555	76.25 ± 1.484	38.3 ± 0.707
β-Resorcylic acid	317.25 ± 0.212	355.15 ± 2.757	452.10 ± 3.535	413.5 ± 1.272	313.5 ± 0.141
Vanillic acid	25.80 ± 0.070	27.35 ± 0.212	29.55 ± 0.353	27.10 ± 0.141	26.75 ± 0.070
Syringic acid	12.85 ± 0.070	14.55 ± 0.070	16.05 ± 0.070	21.1 ± 0.141	13.00 ± 0.141
Other Phenolic compounds					
Pyrogallol	139.35 ± 1.767	144.35 ± 1.060	361.75 ± 5.727	418.80 ± 9.353	207.15 ± 3.181
Rutin	52.80 ± 0.424	90.45 ± 0.919	51.35 ± 0.353	80.15 ± 2.899	58.45 ± 0.070
Vanillin	17.10 ± 0.070	36.6 ± 3.111	13.00 ± 0.707	46.45 ± 0.353	15.65 ± 0.070
Veratric acid	14.85 ± 0.212	13.85 ± 0.070	14.65 ± 0.353	15.35 ± 0.353	15.95 ± 0.353
Hesperidin	14.25 ± 0.070	13.45 ± 0.212	13.35 ± 0.070	14.65 ± 0.212	12.75 ± 0.070
Resveratrol	17.10 ± 0.141	18.00 ± 0.424	18.20 ± 1.131	17.50 ± 0.070	17.75 ± 0.212
Naringenin	10.60 ± 0.565	26.10 ± 0.565	10.90 ± 1.838	16.45 ± 0.212	11.35 ± 0.212
Formononetin	11.30 ± 0.565	10.70 ± 0.141	10.75 ± 0.212	11.20 ± 0.141	10.80 ± 0.070
Biochanin A	5.10 ± 0.565	4.30 ± 0.141	4.25 ± 0.212	4.95 ± 0.353	4.25 ± 0.070

Values are means of triplicates ± SD

having the same number of total phenolic compounds, contained between 65.75 to 87.3 μg/g of dry matter of flavonols, between 82.85 to 95.10 μg/g of dry matter of hydroxycinnamic acids, between 490.25 to 734.75 μg/g dry matter of hydroxybenzoic acids and 354.10 to 625.50 μg/g of other phenolic compounds.

β-Resorcylic acid was the dominant phenolic compound found in garlic bulb extracts collected from different locations; it constituted about 313.50 μg/g from Sangeol region to 452.10 μg/g from Godang region, followed by pyrogallol (139.35/g to 418.80 μg/g), gallic acid (67.40 to 196.80 μg/g), rutin (51.35 to 90.45 μg/g), protocatechuic acid (31.75 to 76.25 μg/g) and quercetin (25.70 to 56.65 μg/g). The three flavonols identified in the analysis were myricetin, quercetin and kaempferol. Quercetin was the most dominant flavonols which was present in all the garlic bulb extracts collected from different geographical regions and it accounted for the largest proportion of the total flavonols content, followed by myricetin and kaempferol was detected in lower amounts in all the garlic bulb extracts (Table-2). In the hydroxycinnamic acid group, caffeic acid was the most dominant hydroxycinnamic acid present in all the garlic bulb extracts which ranged from 28.30 to 39.55 μg/g dry matter, followed by ferulic acid, which ranged from 26.75 to 34.20 μg/g. *p*- and *o*-Coumaric acid were present in moderate quantities and *m*-coumaric acid was present in low quantities in all the garlic bulb extracts. Chlorogenic acid was not detected in Daeso and Godang garlic bulbs (Table-2). β-resorcylic acid was the dominant compound in the hydroxybenzoic acid group followed by gallic acid, protocatechuic acid and vanillic acid. Previous work has established that the antioxidant properties of some plants are partly due to low molecular mass phenolic compounds, particularly flavonoids, which are known to be potent antioxidants[34]. The results suggest that flavonols like quercetin, myricetin, together with hydroxybenzoic acid, hydroxycinnamic acid and other group of phenolic acids play a predominant role in the garlic bulbs collected from different locations. In humans, the presence of flavonoids may contribute to the neutralization of cell-damaging free radicals and the maintenance of heart health[33]. The presence of hydroxycinnamic and hydroxybenzoic acids in our diets may also contribute to bolster cellular antioxidant defenses and to maintain a healthy vision. Although flavonoids are increasingly recognized as playing important roles as antioxidant, further work is necessary to uncover the full potential of these compounds in the improvement of human health.

Antioxidant activity: The antioxidant potential of different plant extracts and pure compounds can be measured using numerous *in vitro* assays. Each of these tests is based on one feature of the antioxidant, such as the ability to scavenge free radicals, or the inhibition of lipid peroxidation. However, a single method is not recommended for the evaluation of the antioxidant activities of different plant products, due to their complex composition[8]. Therefore, the antioxidant effects of plant products must be evaluated by two or more different *in vitro* assays to get relevant data. With respect to this, the antioxidant properties of the examined garlic extracts were

evaluated, as free radical-scavenging capacity (RSC), assay of reductive potential, chelation of metal ions and reduction of molybdenum(VI) to molybdenum(V).

DPPH radical-scavenging capacity: The radical scavenging capacity was evaluated by measuring the scavenging activity of examined garlic extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radicals and the results were compared with BHT (Fig. 1). DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The method is based on the reduction of methanolic $DPPH^-$ solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The aqueous garlic bulb extracts were able to reduce the stable radical DPPH• to the yellow colored diphenyl picrylhydrazine. The IC_{50} values of the garlic bulb extracts were Hampyeong (95.04 µg/mL), Daeso (99.44 µg/mL), Sangeol (122.72 µg/mL), Godang (141.93 µg/mL) and Namdo (166.92 µg/mL) respectively. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (*e.g.*, hydroquinone, pyrogallol, gallic acid) and aromatic amines (*e.g.*, *p*-phenylene diamine, *p*-aminophenol), reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability[35]. The positive correlation between polyphenolic content of the extracts and its antioxidant activity is well documented[36]. Therefore, the content of total phenolic compounds in the extracts might explain their high antioxidant activities. In this study, the extracts exhibited a concentration dependent anti-radical activity by inhibiting DPPH• radical (Fig. 1). Of the different extracts, Hampyeong garlic bulb extract exhibited the highest antioxidant activity of 85.77 % at 250 µg/mL concentration, followed by Daeso (80.5 %), Sangeol (75.92 %), Godang (73.98) and Namdo (61.94 %), respectively at the same concentration (Fig. 1). One of the possible mechanisms is polyphenolic associated compounds. Those kinds of phenolic compounds show antioxidant activity due to their redox properties, which play an important role in absorbing and neutralizing free radical, quenching singlet and triple oxygen or decomposing peroxide. Butylated hydroxytoluene (BHT) showed similar degree of free radical scavenging activity with that of the extracts at low concentration points. The DPPH activity of BHT exhibited 92.04 % at 50 µg/mL concentration.

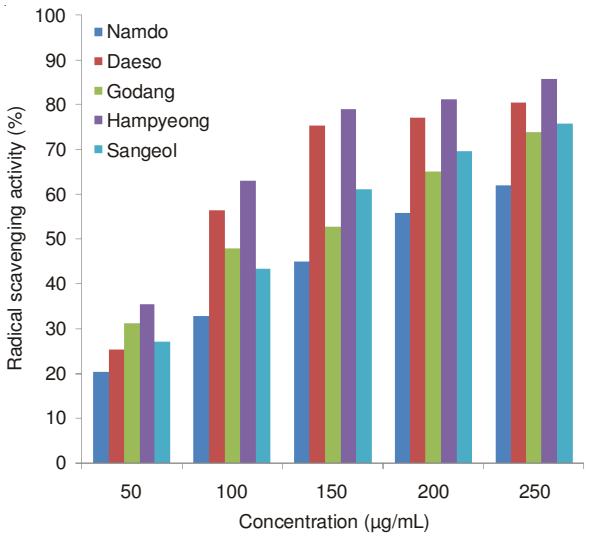

Fig. 1. Free radical-scavenging activity of the aqueous garlic bulb extract at different concentrations by DPPH method collected from different locations of Korea. Each sample was assayed in triplicate for each concentration. Experimental results were means ± SD of three parallel measurements

Reducing power assay: Various mechanisms, including reducing capacity, prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging have been claimed to explain the antioxidant activities[37]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In the present study, the extracts exhibited effective reducing capacity at all concentration points. The reducing capacity of the extracts increased with increase in the concentration (Fig. 2). The reducing power of the different location garlic bulb extracts followed the order of Hampyeong > Daeso > Sangeol > Godang > Namdo. The reducing properties are generally associated with the presence of reductones[38], which have been shown to exert antioxidant action by breaking the free radical chain, by donating a hydrogen atom[39]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation.

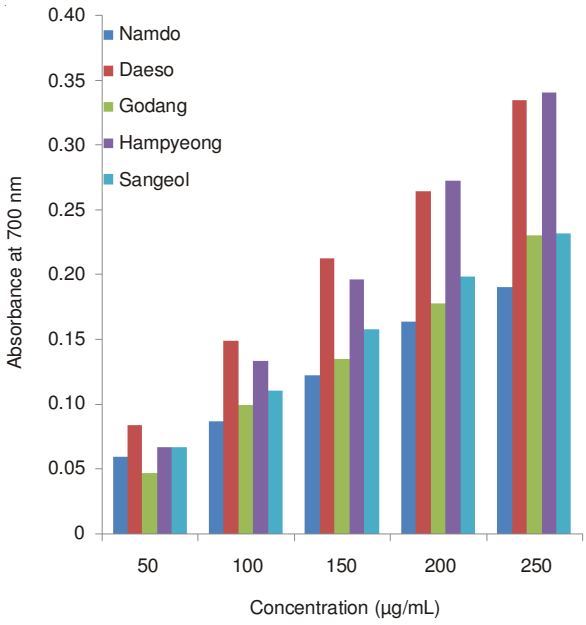

Fig. 2. Reducing power of the aqueous garlic bulb extract at different concentrations collected from different locations of Korea. Each sample was assayed in triplicate for each concentration. Experimental results were means ± SD of three parallel measurements

Metal chelating activity: Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease in the red color of the complex. Measurement of color reduction therefore allows estimating the metal chelating activity of the coexisting chelator[40]. It is reported that chelating agents which form s-bonds with metal, are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion[41]. In this assay, both the extracts and standard compounds interfered with the formation of ferrous complex with the reagent ferrozine, suggesting that

it has potential chelating activity and captures the ferrous ion before ferrozine. The absorbance of Fe^{2+}-ferrozine complex is linearly decreased with dose taken (50 to 250 µg/mL). The percentages of metal chelating capacity at 250 µg/mL doses of the garlic bulb extracts collected from different locations of Korea were found to be Hampyeong (90.45 %), Daeso (84.67 %), Godang (81.0 %), Sangeol (75.08 %) and Namdo (45.57 %), respectively. The standard EDTA exhibited 98.59 % activity at 100 µg/mL concentration. The data obtained reveals that the extract from Hampyeong demonstrated an effective capacity for iron binding, suggesting that its action as antioxidant may be related to its iron-binding capacity and the extract from Namdo demonstrated weak iron-binding capacity.

Evaluation of antioxidant capacity by phosphomolybdenum method: The antioxidant capacity of the garlic bulb extracts collected from different locations was measured spectrophotometrically through phosphomolybdenum method, which is based on the reduction of Mo(VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate/Mo(V) compounds with a maximum absorption at 695 nm. The antioxidant capacity of the extracts was found to decrease in the order: Namdo > Daeso > Hampyeong > Sangeol > Godang (Table-3).

TABLE -3
ANTIOXIDANT CAPACITY OF GARLIC BULB EXTRACTS COLLECTED FROM DIFFERENT LOCATIONS OF KOREA BY PHOSPHOMOLYBDENUM METHOD

Extract	Antioxidant capacity [as equivalent to α-tocopherol (mg/g)]
Namdo	37.22 ± 1.514
Daeso	38.55 ± 2.343
Godang	79.00 ± 3.533
Hampyeong	46.25 ± 2.413
Sangeol	77.88 ± 7.591

Values are means of triplicates ± SD

Conclusion

In the present study, the total phenolic and flavonoid contents of the garlic extracts differed with different geographical regions. The highest phenolic and flavonoid content was observed in the garlic bulbs collected from Hampyeong region (49.89 mg GAE/g and 1.56 mg QE/g) and the lowest was recorded from the Namdo region (33.50 mg GAE/g and 1.02 mg QE/g). The UPLC analysis of the phenolic compounds profile revealed that flavonols, hydroxycinnamic acid, hydroxybenzoic acid and other phenolic acid groups varied in their compositions. The garlic bulbs collected from the Hampyeong region exhibited highest antioxidant activity assayed by four different methods except the phosphomolybdenum assay when compared with other garlic bulbs of different geographical regions.

ACKNOWLEDGEMENTS

The author Praveen Nagella is recipient of the Konkuk University SMART Research Professor Program, Konkuk University, Seoul, South Korea.

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