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Changes in Antioxidant Activity with Temperature and Time in *Chrysanthemum indicum* L. (*Gamguk*) Teas During Elution Processes in Hot Water

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Abstract Determining the elution of water-soluble substances from herbal teas is an important factor in their efficient use in terms of taste, perfume, and content of health-related components. The antioxidant activity and content of catechins in commercial *Chrysanthemum indicum* (*gamguk*) teas were determined for optimum elution conditions. The water extract of *gamguk* teas did not differ significantly in yield compared to methanol extracts and showed stronger antioxidant activity. Catechin contents in *gamguk* teas were 8-18% of the extracts when individual peaks in high-performance liquid chromatography analysis were compared to standard catechin peaks. *Gamguk* teas exhibited faster release of antioxidants, and the antioxidant activity was positively correlated with the thermal treatments. *Gukhwacha* (GC) was the best tea for rapid release (30 sec) of antioxidants with the 50°C treatment, whereas antioxidants in other teas were relatively slower released.

Keywords: antioxidant, catechin, chrysanthemum, polyphenol, water-soluble component

Introduction

Chrysanthemum indicum L., or *gamguk*, is a perennial herb distributed widely both in low mountainous areas and along shorelines in Korea. The dried flowers have been popularly consumed as herbal teas and alcoholic beverages, and Oriental traditional medicine has applied *gamguk* to treat human ailments such as eye (1), inflammatory (2), and microbial diseases (3) as well as toothache (4).

Several studies have demonstrated that the inflorescence of chrysanthemum species contains antioxidant compounds expressing strong activity (5-8). Catechins, rich phenolic compounds in herbal teas, have been especially well investigated in green tea, and numerous studies have confirmed their health benefits (9,10). Catechins such as catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate have been routinely found in plants and represent a type of antioxidant, although Yilmaz and Toledo (11) reported that the antioxidant activities among certain catechins differed.

Most teas are packaged in teabags, and the easy release of tea components from tea substrates would be of greater benefit. Chemical components in plants are released differently from the plant substrate depending on which solvents are applied. In plant foliar tissues containing both hydrophobic and hydrophilic components, using alcoholic solvents tends to release more crude extracts compared to using water (12). Although a tea may contain a large quantity of health-promoting molecules, such as antioxidants, the conditions under which water-soluble components are released from the tea are important and may be influenced by eluting time and water temperature. Little research, however, on the eluting conditions of herbal teas has been conducted.

Thus, we sought to evaluate the antioxidant activity and catechin contents of *gamguk* teas eluted under different conditions using water.

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Materials and Methods

Materials Teas made from *C. indicum*, including 1.5 g *Gamro-soogukcha* (GSC), 0.4 g *Chamjoeun* (CJ), 0.5 g *Gukhwacha* (CG), 0.5 g *Hyanggiro-pin-gukhwacha* (HGC), and 0.5 g *Hyangee-joeun-gukhwacha* (HJGC), were purchased from a commercial market in Korea (Table 1). Dried *gamguk* (G) flowers (20 g) produced in Gangwon Province were obtained from a traditional medicinal market in Chuncheon (Gangwon) in Korea. Standard compounds of catechins were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Methanolic and aqueous extraction Two g of each *gamguk* sample were homogenized to a fine powder using a mortar and pestle. The homogenized samples were suspended into 200 mL of either methanol (80%, v/v) or distilled deionized water. The extraction process was performed using a vacuum reflux cooling system maintained at 80°C in a water bath. The extraction was conducted for 2 hr, followed by centrifugation at 13,000×g for 5 min. The supernatant was collected and the residues were resuspended in 200 mL of the same extraction solution. Extraction was repeated using the same procedures. The collected supernatant

Table 1. Chrysanthemum teas collected from commercial companies in Korea

Product name	Company	Characteristics
Gamguk (G)	An oriental medicinal market	Dried flowers
Gamro-soogukcha (GSC)	Gamrowon	Dried leaves + unpolished rice
Chamjoeun (CJ)	Kochshaem Food	Dried flowers
Gukhwacha (GC)	Chorokwon	Dried flowers
Hyanggiro-pin-gukhwacha (HGC)	Amore Pacific	Dried flowers
Hyangee-joeun-gukhwacha (HJGC)	TeaZen	Dried flowers

was filtered using No. 1 filter paper (Whatman, Florham Park, NJ, USA) and filtered again using No. 2 filter paper. The extraction solution was removed using a vacuum rotary evaporator (N-1000; Eyela, Tokyo, Japan) in a 40°C water bath. The pellets of each sample were collected into 10-mL glass vials using a minimal amount of 10% aqueous methanol and lyophilized. The lyophilized samples were kept in a freezer until analysis.

Teabag extraction using water to evaluate temperature and time effects A teabag of each tea was added to 100 mL of distilled deionized water. Extraction temperatures were 50, 75, and 100°C, and extraction times were 30, 60, 120, and 180 sec. Each extracted sample was then filtered using a syringe filter (0.45 μm in diameter; Toyo Roshi Kaisha, Ltd., Tokyo, Japan).

Total phenolic and flavonoid contents The total phenolic contents of *gamguk* tea extracts were determined using tannic acid as the standard, following a method described by Kim *et al.* (13), with slight modifications. Briefly, 0.5 mL (2 mg/mL) of diluted sample or teabag extract was mixed with 1.8 mL of distilled deionized water, and 0.2 mL of 1 M phenol reagent was added. A reagent blank was prepared using distilled deionized water. After shaking the mixture and stabilizing it for 3 min, 0.4 mL of Na_2CO_3 (10% in water, v/v) was added to the sample and vortexed. Total volume (4 mL) was adjusted by adding 1.4 mL of distilled deionized water. The absorbance was measured at 725 nm after 1 hr incubation under ambient conditions.

Total flavonoid contents of the *gamguk* tea extracts were determined on the basis of quercetin as the standard, following a method described by Park *et al.* (14). One-half mL of sample was mixed with 0.1 mL aluminum nitrate in 1 M potassium acetate, and 4.3 mL of ethanol (80%, v/v) was then added. The absorbance was measured at 415 nm after 40 min incubation in a 37°C water bath.

Determination of antioxidant activity A test for 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of *gamguk* tea samples was carried out as described previously (15). A series of sample concentrations (1 mL), including 125, 250, 500, 1,000, and 2,000 μg/mL, was added to 4 mL of 1.5×10^{-4} M DPPH in methanol (80% in water, v/v). The mixed solution was shaken and incubated for 30 min at a room temperature. Absorbance was measured for DPPH remaining at 517 nm.

Content of catechins using HPLC *Gamguk* tea extracts were analyzed with a high-performance liquid chromatography (HPLC) system (CBM-20A; Shimadzu Co., Ltd., Kyoto, Japan) with 2 gradient pump systems (LC-20AT; Shimadzu), a UV-detector (SPD-10A; Shimadzu), and a column oven (CTO-20A; Shimadzu). A Gemini C18 column (3 μm, 100×4.6 mm; Phenomenex. Inc., Torrance, CA, USA) was used. The flow rate of the mobile phase solution was 1.0 mL/min. The mobile phase solution was run via a gradient system as follows: solution A (0.4%, v/v, formic acid in distilled deionized water) and solution B (0.4%, v/v, formic acid in acetonitrile), with a gradient elution programmed as follows: 0-10% of solution B for 0 to 10 min and 10-20% of solution B for 10 to 30 min. Sample injection volume was 10 μL, and peaks were monitored at 280 nm. Standard catechins, including catechin, epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate, were analyzed using HPLC as described above.

Statistical analysis Data are presented as means with standard errors for each treatment. Means of all data were subjected to standard ANOVA procedures using the SAS software (SAS version 8.02, SAS Institute Inc., Cary, NC, USA). Significant differences among treatment means were determined at the 5% level using Fisher's protected least significant difference (LSD) tests.

Results and Discussion

Comparative yields of total phenolics (TPs), total flavonoids (TFs), and antioxidants between methanolic and aqueous extracts The water solubility of teas is one of their key characteristics. Both hydrophilic and hydrophobic phytochemicals dissolve in methanol, while hydrophobic components dissolve little if at all in water. Therefore, the chemical composition of extracts from plant tissues is highly dependent on the choice of extracting solvent. Eom *et al.* (12) reported that the total yield of crude methanol extract from foliar tissues of *Nepeta* × *faassenii* was 26-fold higher than the crude water extract, and that the methanol extract had lower biological activity in a plant-plant interaction. Unlike plant foliar tissues, the total yields in tea extracts of *gamguk* flowers were not significantly higher when methanol rather than water was used. Furthermore, certain *gamguk* tea extracts including G and HJGC gave significantly higher yields in water extracts (Table 2). For GSC manufactured by foliar tissues of *gamguk*, the yield of the methanol extract (222.2 mg/g d.w.) was much higher than that of the water extract (146.9 mg/g d.w.). Thus, we concluded that the content of water-soluble molecules in the flowers of *gamguk* was higher than that in foliar tissues.

TPs contents differed among *gamguk* teas. Duh and Yen (6) reported that the TPs content of water extract from the flower of *C. morifolium* Ramat was 17.2 mg/g (d.w.), with

Table 2. TP and TF contents and DPPH free radical scavenging activities (RSA) in commercial chrysanthemum teas extracted using 80% methanolic and aqueous solvents[1)]

Teas	TE (mg/g d.w.)		TPs (mg/g d.w.)		TFs (mg/g d.w.)		IC_{50} (mg/mL) of RSA	
	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O
G	418.6[b,2)]	476.9[a]	13.0±0.2	12.7±0.3	2.2±0.2	2.6±0.1	0.48±0.01	0.22±0.01
GSC	222.2[a]	146.9[b]	17.2±0.5	10.6±0.5	0.6±0.2	2.0±0.0	0.78±0.03	0.61±0.02
CJ	495.4[a]	501.9[a]	15.5±0.3	15.0±0.2	3.8±0.3	3.6±0.0	0.34±0.02	0.17±0.01
GC	337.4[a]	335.6[a]	15.6±0.3	11.3±0.4	3.4±0.3	2.9±0.0	0.36±0.01	0.24±0.02
HGC	233.2[a]	200.8[b]	15.9±0.6	12.2±0.4	3.1±0.3	2.8±0.0	0.38±0.03	0.24±0.02
HJGC	466.5[b]	495.8[a]	17.2±0.7	17.8±0.7	3.7±0.3	3.7±0.1	0.29±0.02	0.15±0.01

[1)]TE, total extract; TPs, total phenolics; TFs, total flavonoids; IC_{50} of RSA, inhibiting concentration of 50% DPPH free radical scavenging activity.
[2)]Different letters (a, b) in each row are not significantly different at the level of $p<0.05$; values are the average of triplicate experiments with standard error.

high antioxidant activity. Our results showed that the TPs content of *gamguk* teas ranged from 10 to 18 mg/g (d.w.). The TPs contents of *gamguk* teas were much higher in methanol extracts, except for HJGC. TFs contents also differed among *gamguk* teas: G and GSC showed higher yields in water extracts, GC and HGC had lower yields in water extracts, and CJ and HJGC had similar yields in both methanol and water extracts (Table 2).

Antioxidant activity measured by DPPH free radical scavenging activity was significantly higher in water extracts compared to methanol extracts. HJGC extracts had the highest DPPH free radical scavenging activity, with IC_{50} values of 0.29 mg/mL in methanol extract and 0.15 mg/mL in water extract, whereas GSC extracts had the lowest DPPH free radical scavenging activity, with IC_{50} values of 0.78 mg/mL in methanol extract and 0.61 mg/mL in water extract. As indicated above, although the amounts of total extracts among *gamguk* teas varied between methanolic and aqueous extracts, the components of water extract expressed more antioxidant activity.

It was founded that antioxidant activity in *gamguk* teas were highly correlated with the contents of TFs rather than TPs. TFs content and IC_{50} of DPPH free radical scavenging activity were negatively correlated, exhibiting $R^2 = 0.9665$ in methanol extracts and $R^2 = 0.7036$ in hot water extracts. Otherwise, TPs content in *gamguk* teas seemed not highly correlated with antioxidant activity.

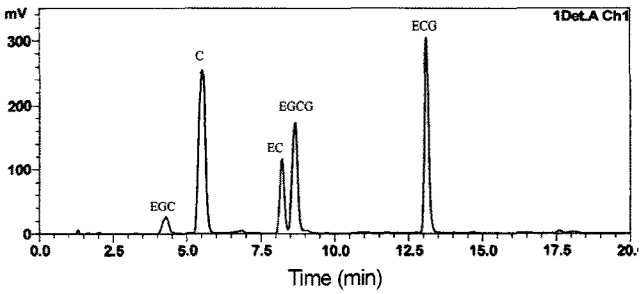

Fig 1. Chromatogram of standard catechins (0.2 mg/mL) at 280 nm produced using a UV detector. EGC, epigallocatechin; C, catechin; EC, epicatechin; EGCG, epigallochatechin gallate; and ECG, epicatechin gallate.

However, the correlation between TPs and DPPH free radical scavenging activity in tea extracts were shown to positive effect ($R^2 = 0.436$) in hot water extract. Otherwise, the correlation in methanol extract was shown to slight negative value ($R^2 = 0.0197$).

Content of catechins Extraction of phenolic compounds from plant materials is likely to be highly dependent on the choice of solvent. For example, Metivier *et al.* (16) reported that an adequate aqueous methanol extraction efficiently eluted flavonols, and several other studies demonstrated

Table 3. Content of catechins in extracts of *gamguk* teas

	Content of catechins (μg/mg extract)											
	Epigallocatechin		Catechin		Epicatechin		Epigallocatechin gallate		Epicatechin gallate		Total catechins (%) in extracts[1)]	
	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O
G	10.69[c,2)]	10.82[d]	8.00[b]	17.18[a]*	2.64[bc]	4.53[ab]*	2.53[bc]*	2.18[bc]	2.91[c]	2.77[c]	12.49	15.69
GSC	18.51[b]	39.57[a]*	4.98[cd]	4.38[e]	3.82[ab]*	3.14[c]	4.85[a]*	3.58[a]	1.16[d]*	0.65[d]	15.17	17.44
CJ	19.45[b]	20.24[c]	7.00[bc]	7.67[c]	3.17[b]	4.51[ab]*	1.83[c]	1.89[c]	4.78[a]	5.31[a]	9.07	8.36
GC	1.34[d]	26.49[b]*	3.92[d]	5.55[d]*	0.26[d]	5.10[a]*	0.22[d]	1.85[c]*	0.39[e]	3.96[b]*	8.45	8.30
HGC	ND[3)]	ND	4.12[cd]	5.65[d]*	0.23[d]	0.96[d]*	0.20[d]	0.35[d]	4.09[ab]*	0.40[e]	10.91	9.99
HJGC	23.75[a]	23.43[bc]	16.33[a]*	11.23[b]	4.21[a]*	3.01[c]	3.15[b]*	2.54[b]	2.70[c]	4.96[a]*	15.11	14.26

[1)]Calculated based on the percent peak area of 5 catechins vs. the percent peak area of whole peaks on HPLC chromatograms at 280 nm absorbance in tea extracts.
[2)]Different letters (a-e) in each column are not significantly different at the level of $p<0.05$; mark (*) in each of individual catechin comparison between extracts indicates significant difference at the level of $p<0.05$. Averages were calculated by triplicate experiments.
[3)]Not detected.

that an acetone and water mixture easily extracted tannins from plant tissues (17-19). However, water-soluble components in plants are valuable in teas (20). To determine the yield of water-soluble catechins that may affect antioxidant activity in teas as they are consumed, 5 samples of catechins in water extracts of *gamguk* teas were analyzed and compared with the catechin contents in methanol extracts. The contents of catechins in *gamguk* tea extracts were determined on the basis of standard catechin concentrations via HPLC (Fig. 1). Table 3 shows the catechin contents of *gamguk* teas extracted using methanol and water solvents. The total catechin contents in G, GSC, CJ, GC, and HGC were better released in hot water than in methanol. Otherwise, the contents in HGC and HJGC were higher in the methanol extracts. The major component of catechins in *gamguk* teas, except for HGC, in which it was not detected, was epigallocatechin. Interestingly, epigallocatechin content was very low in the methanol extract of GC (1.3 μg/mg), and yet the content was high in the water extract (26.5 μg/mg). GSC, which was manufactured using foliar tissues of *gamguk*, also showed a high content of epigallocatechin in its water extract. However, other catechin contents in GSC were lower in the water extract than in the methanol extract, while the contents in the other *gamguk* teas tended to be higher in the water extract, with certain exceptions for individual catechin contents.

The correlations between DPPH free radical scavenging activity and catechin contents in tea extracts were not shown to distinguishable effects. However, higher amounts of catechin, epicatechin, and epicatechin gallate in hot water extracts increased the DPPH free radical scavenging activity, with negative correlations (R^2 = 0.2399, 0.0215, and 0.4483).

Effects of temperature and time on antioxidant activity of tea in a teabag Teas are generally manufactured in several forms, including ground powder, chipped tissue, or packed teabags. In the case of *gamguk* teas, the most prevalent form is a teabag in which ground tissues are wrapped in a water-permeable paper. The individual teabag weights were 1.5 g for GSC, 0.4 g for CJ, 0.5 g for CG, 0.5 g for HGC, and 0.5 g for HJGC. Figure 2 shows DPPH free radical scavenging activity in the water-soluble extracts of each teabag at different temperatures and times. CG and HJGC were efficiently extracted, reaching more than 80% of DPPH radical scavenging activity within 30 min regardless of the temperature. At 50°C, each tea showed different degrees of antioxidant release between 30 and 180 sec, with the lowest scavenging activity in GSC. At 75°C, CJ, GC, and HJGC quickly released antioxidants, exhibiting over than 90% of their scavenging activity within 30 sec. At 100°C treatments, most teas, except for GSC, showed faster release of antioxidants, expressing more than 90% of their scavenging activity within 30 sec. GSC showed more than 80% of the scavenging activity at either 180 sec at 75°C or 120 sec at 100°C. Thus, we found that antioxidants were quickly released from most *gamguk* teas manufactured using the flowers and easily released at relatively low temperatures. However, the release of antioxidants from GSC, which is manufactured by using the foliar tissues with unpolished rice grains added, requires a higher

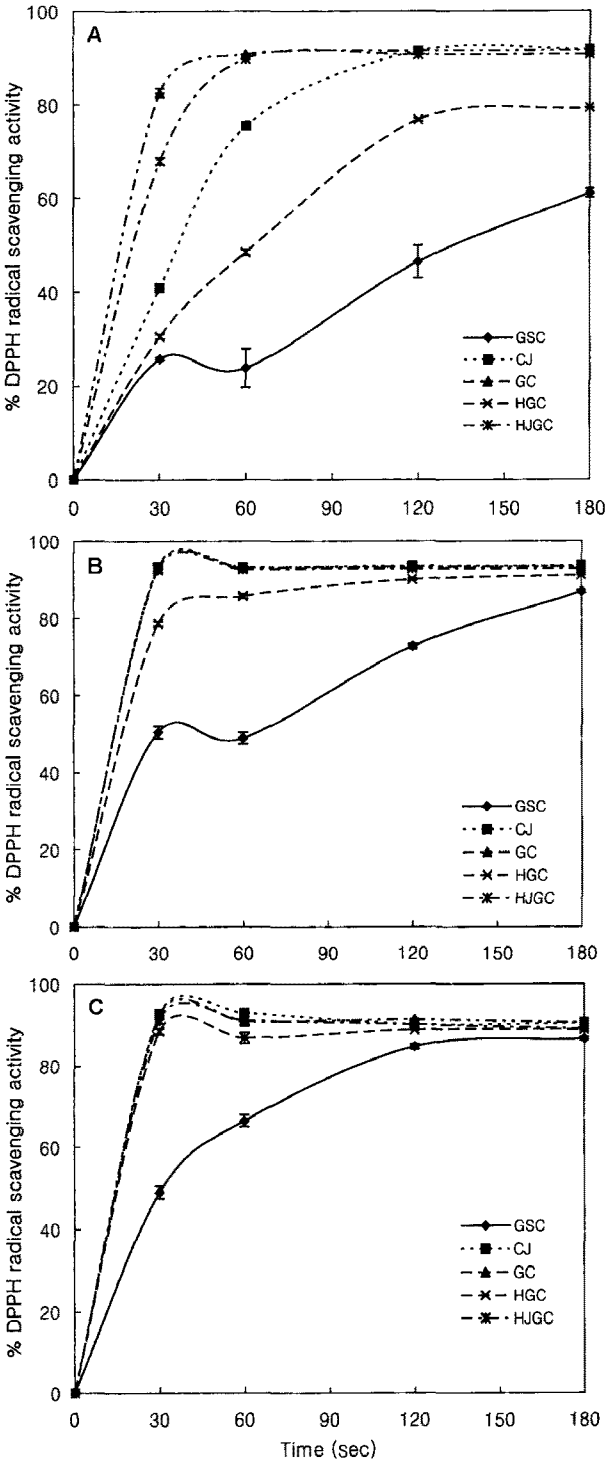

Fig. 2. Effects of time and thermal treatments on DPPH free radical scavenging activity of each chrysanthemum teabag. Each teabag was dissolved in 100 mL water at 50 (A), 75 (B), and 100°C (C). One mL of each extract was analyzed; error bars indicate standard errors of triplicate-average.

temperature and longer extraction time.

Table 3 shows the required amount of water-soluble *gamguk* tea extracts to express 50% DPPH radical scavenging activity when the extraction was conducted at different temperatures for 3 min in 100°C. The results indicated that the teas manufactured using flowers required relatively small amounts of extracts (2.82 mg) to express

Table 4. Temperature effect on antioxidant activities in water-soluble *gamguk* tea extracts[1)]

Tea	Required sample (mg/mL) for IC_{50} of RSA		
	50°C	75°C	100°C
G	0.84[a]	0.82[a]	0.79[b]
GSC	11.06[a]	8.23[b]	6.25[c]
CJ	0.84[a]	0.75[b]	0.79[b]
GC	1.63[a]	1.39[b]	1.33[b]
HGC	2.82[a]	2.60[b]	2.66[b]
HJGC	1.23[a]	1.05[b]	1.09[b]

[1)]Extraction time was 3 min in water; values with different letters (a-c) in each row are significantly differed at the level of $p<0.05$. Averages were calculated by triplicate experiments.

the 50% inhibition concentration of DPPH (IC_{50}), while GSC extract required a much larger amount (6.25 mg). In Table 2, which indicates the IC_{50} values of fully water-extracted samples, we see that antioxidants are eluted more slowly from GSC than from the other teas.

In conclusion, better condition of tea elution seems not easily defined because the preference of consumers is so variable. However, consumers may have interest to the optimum elution condition of teas for the purpose of health effect (21). Thus, the indication of better eluting condition should be beneficial to consumers. In the consideration described above, it is concluded the following results: 1. Hot water extracts of *gamguk* teas were similar in quantity to methanol extracts and exhibited better DPPH free radical scavenging activity. 2. Catechin contents of *gamguk* teas ranged from 8 to 18% of the extracts, when peak areas at 280 nm were analyzed via HPLC. 3. *Gamguk* teas that originated from the flowers showed faster release of antioxidants, and the antioxidant activity was positively correlated with thermal treatments. 4. GC had the fastest release (30 sec) of antioxidants during the 50°C thermal treatment. At 75°C, CJ, GC, and HJGC developed more than 90% DPPH free radical scavenging activity within 30 sec. At 100°C, all teas except for GSC developed this activity within 30 sec. In the case of GSC, the optimum time for the best release of antioxidants was more than 180 sec at 50°C, about 180 sec at 75°C, and approximately 120 sec at 100°C.

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References

1. Matsuda H, Morikawa T, Toguchida I, Harima S, Yoshikawa M. Medicinal flowers. VI. Absolute stereostructures of two new flavanone glycosides and a phenylbutanoid glycoside from the flowers of *Chrysanthemum indicum* L.: Their inhibitory activities for rat lens aldose reductase. Chem. Pharm. Bull. 50: 972-975 (2002)
2. Cheng W, Li J, You T, Hu C. Anti-inflammatory and immunomodulatory activities of the extracts from the inflorescence of *Chrysanthemum indicum* Linné. J. Ethnopharmacol. 101: 334-337 (2005)
3. Zhu S, Yang Y, Yu H, Ying Y, Zou G. Chemical composition and antimicrobial activity of the essential oils of *Chrysanthemum indicum*. J. Ethnopharmacol. 96: 151-158 (2005)
4. Kim SJ, Park YM, Jung ST. Anticariogenic effects and inhibition of glucosyltransferase activity of *Chrysanthemum indicum* L. extracts. Korean J. Food Culture 20: 341-345 (2005)
5. Bartoli CG, Simontacchi M, Montaldi ER, Puntarulo S. Oxidants and antioxidants during aging of chrysanthemum petals. Plant Sci. 129: 157-165 (1997)
6. Duh PD, Yen GC. Antioxidant activity of three herbal water extracts. Food Chem. 60: 639-645 (1997)
7. Duh PD. Antioxidant activity of water extract of four *Harng Jyur* (*Chrysanthemum morifolium* Ramat) varieties in soybean oil emulsion. Food Chem. 66: 471-476 (1999)
8. Chung HS. Phenolic compounds with antioxidant activity on DPPH free radical scavenging and inhibition of xanthine/xanthine oxidase from the flowers of *Chrysanthemum morifolium*. J. Food Sci. Nutr. 11: 198-203 (2006)
9. Bell JRC, Donovan JL, Wong R, Waterhouse AL, German JB, Walzem RL, Kasim-Karakas SE. (+)-Catechin in human plasma after ingestion of a single serving of reconstituted red wine. Am. J. Clin. Nutr. 71: 103-108 (2000)
10. Graf BA, Milbury PE, Blumberg JB. Flavonols, flavones, flavanones, and human health: Epidemiological evidence. J. Med. Food 8: 281-290 (2005)
11. Yilmaz Y, Toledo RT. Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. J. Agr. Food Chem. 52: 255-260 (2004)
12. Eom SH, Yang HS, Weston LA. An evaluation of the allelopathic potential of selected perennial groundcovers: Foliar volatiles of catmint (*Nepeta* × *faassenii*) inhibit seedling growth. J. Chem. Ecol. 32: 1835-1848 (2006)
13. Kim KT, Yoo KM, Lee JW, Eom SH, Hwang IK, Lee CY. Protective effect of steamed American ginseng (*Panax quinquefolius* L.) on V79-4 cells induced by oxidative stress. J. Ethnopharmacol. 111: 443-450 (2007)
14. Park YK, Koo MH, Ikegaki M, Contado JL. Comparison of the flavonoid aglycone contents of *Apis mellifera propolis* from various regions of Brazil. Arq. Biol. Tecnol. 40: 97-106 (1997)
15. Brand-Willams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28: 25-30 (1995)
16. Metivier RP, Francis FJ, Clydesdale FM. Solvent extraction of anthocyanins from wine pomace. J. Food Sci. 45: 1099-1100 (1980)
17. Lim HA, Jang CH, Kim JH, Kim JR, Ha YR, Song YS, Kim YK, Kim JS. Antiproliferative and anticarcinogenic enzyme-inducing activities of green tea seed extract in hepotoma cells. Food Sci. Biotechnol. 15: 914-919 (2006)
18. Foo L, Porter L. The structure of tannins of some edible fruits. J. Sci. Food Agr. 32: 711-716 (1981)
19. Yilmaz Y, Toledo RT. Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. J. Food Compos. Anal. 19: 41-48 (2006)
20. Jeon JR, Choi JH. Protective effect of water extract of *Fraxinus rhynchophylla* leaves on acetaminophen-induced nephrotoxicity in mice and its phenolic compounds. Food Sci. Biotechnol. 16: 988-993 (2007)
21. Hwang IG, Woo KS, Kim DJ, Hong JT, Hwang BY, Lee YR, Jeong HS. Isolation and identification of an antioxidant substance from heated garlic (*Allium sativum* L.). Food Sci. Biotechnol. 16: 963-966 (2007)