Journal of Culinary Research, Volume 20, Number 5, pp.29-33, 2014

http://www.culinary.re.kr

Antioxidant Activities of Naturaceuticals Extract *In Vitro*

Sung-Jin Park[1], Woo-Taeg Kwon[2] and Young-Ah Rha[3,¶]

[1]Dept. of Tourism & Food Service, Hallym Polytechnic University, Chuncheon 200-711, Korea
[2]Dept. of Environmental Health & Safety, Eulji University, Seongnam 461-713, Korea
[3]Dept. of Food Technology and Services , Eulji University, Seongnam 461-713, Korea

ABSTRACT: In this study, we examined the antioxidant activity of the naturaceutical extract *in vitro* using total phenolic contents, total flavonoids contents, DPPH radical scavenging activity, reducing power assay, and phenolic acid contents. The total phenolic and total flavonoids contents of naturaceutical extract were 5.46 mg/g, 2.21 mg/g, respectively. The DPPH radical scavenging activity of natraceutical extract varied from 18.77 (200 μg/mL) to 3.44 (1,000 μg/mL). The reducing power of the natraceutical extract absorbance varied from 1.07 (0.78 mg/mL) to 3.44 (12.5 mg/mL), and reducing power of extract presented a concentration-dependent activity increase. The highest amounts of *trans*-ferulic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, vanillin, vanillic acid, *p*-hydroxybenzaldehyde, and *trans*-cinnamic acid were observed in the naturaceutical extract at the levels of 750.79, 619.75, 531.34, 222.04, 219.28, 107.40, and 89.56 μg/g, respectively. The results imply that this antioxidant effect of the naturaceuticals extract could be harnessed in the management and prevention of degenerative diseases associated with oxidative stress.

Keywords: Naturaceuticals Extracts, Antioxidant, Total Phenolics, Total Flavonoids, High-Performance Liquid Chromatograpy

INTRODUCTION

It is commonly recognized that reactive oxygen species (ROS) are involved in a variety of physiological and pathological processed, including cellular signal transduction, cell proliferation, differentitation and apotosis, as well as ischemia - reperfusion, inflammation, and many neurodegenerative disorders (Ames BN *et al.,*1993; Bland JS, 1995). In healthy individuals, ROS production is continuously balanced by natural antioxidative defence systems. Oxidative stress is a proess where the physiological balance between pro-oxidants and antioxidants is disrupted in favour of the former, ensuing in potential damage for the organism (Halliwell B & Gutteridge JMC, 1990). ROS production can induce DNA damages, protein carbonylation, and lipid peroxidation, leading to a variety of chronic health problems, such as cancer, aging, Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis (Collin AR, 1999; Floyd RA, 1999). There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of the antioxidants activity of these compounds (Hertog ML *et al.,*1993; Surh YJ, 2002; Surh YJ *et al.,*1999). When added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life (Jaghav SJ *et al.,*1996). The antioxidant activity of extract of several plants, including their leaves, bark, roots (Mariod AA *et al.,* 2008), fruits, seeds (Liyana-Pathirana CM & Shahidi, F 2006; Malencic D *et al.,* 2008) and seedcake(Mariod AA *et al.,* 2006; Matthaűs B 2002) has been extensively studies. Tocopherol, tertiarybutylhydroquinone (TBHQ), butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA) are the most commonly used primary antioxidant in oils. However, many researchers reported the adverse effects of synthetic antioxidants such as toxicity and carcinogenicity (Williams GM *et al.,* 1999). Due to safety and limitation of synthetic antioxidant usage, natural antioxidants obtained from edible materials, edible by-products and residual sources have become alternately interesting (Moure A *et al.,* 2000). Dietary antioxidant intake may be an important strategy for inhibiting or delaying the oxidation of susceptible cellular substrates, and is thus relevant to disease prevention in many paradigms. Phenolic compounds such as flavonoids, phenolic acids, diterpenes and tannins have received attention for their high antioxidative activity (Rice-Evans CA *et al.,* 1996). Converging evidence from both experimental and epidemiological studies have demonstrated that cereals, vegetables, and fruits contain a myriad of phenolic compounds. Medicinal herbs continue to provide valuable therapeutic agents in both modern and traditional medicine. Traditional medicines have been used for improving the quality of human life(Park SJ & Rha YA, 2013) and its complications in many countries because plant drugs are often less toxic and have fewer side effects than synthetic drugs (Pari L & Umamaheswari J, 2000). In Asian countries, including Korea, China, and Japan, many herbal drugs are combined in the form of multiherbal formulas to enhance their functions (Son DW *et al.,* 2011; jeong SH *et al.,* 2012). The herbal constituents are selected to emphasize the therapeutic actions or to reduce the toxicity or side effects of compounds from other herbal species in the mixture (Bansky D & Barolet R, 1999). A wide variety of the traditional herbal remedies may present new avenues in the search for alternative drugs. Antioxidants posses the ability to protect the

§ Corresponding Author: Young-Ah Rha, Ph.D., Professor, Dept. of Food Technology and Services, Eulji University, Seongnam 461-713, Korea, Tel. +82-10-7758-7088, Fax. +82-31-740-7349, E-mail: yana@eulji.ac.kr
Received: 3 August, 2014, Revised: 14 October, 2014, Accepted: 20 October, 2014.

cellular organelles from damages caused by free radicals induced oxidative stress. Free radicals include hydroxyl radical, superoxide anion radical and hydrogen peroxide. Highly reactive free radicals which are formed by exogenous chemicals, stress or in the food system are capable of oxidizing biomolecules, resulting in cancer, coronary heart disease and hypertension (Tomson MJ, 1995). There, the objective of the present study was to evaluate the antioxidant activities of naturaceuticals extract. This study can help the food industry use it as a natural compound for antioxidant activities.

MATERIALS AND METHODS

Plant Materials

Sample is composed of seven medicinal herbs. This formula was organized to maximize the pharmacological effect in theoretical basis of traditional Oriental medicine. The ingredients of sample include sprout buckwheat, *Chrysanthemum indicum* L., *Akebia caulis*., *Ixeris dentate* Nakai, *Plantago asiatica* L., *Sasamorpha purpurascens*(Hackel) Nakai var. *borealis*(Hackel) Nakai., *Oenanthe stolonifera* DC. at the ratio of400:20:20:150:30:50:400.The mixing ration was based on a herbal medicine text (Shin MK, 1997). All herbs used in this study were purchased from Daekwangherb Co. (Chuncheon, Republic of Korea) and authenticated. These mixtures were boiled in water for 4 hours. The extracted solution was filtered, concentrated under reduced pressure (CCA-1100, Eyela, Tokyo, Japan), and finally freeze-dried (PVTFA 10AT, Ilsin, Korea). The yield of naturaceuticals extract was 40.5%. The powdered naturaceuticals extract was stored at –20℃ until used. The voucher specimen has been deposited in the same laboratory.

Chemicals and Reagents

Phenolic compounds(vanillic acid, vanillin, *p*-coumaric acid, *p*-hydroxybenzoic, *trans*-cinnamic acid, *p*-hydroxybenzaldehyde, *trans*-ferulic acid), Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethanol, methanol, and trifluoroacetic acid werepurchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonotrile was purchased from Merck KGaA (Darmstadt, Germany). HPLC grade water was purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). All chemicals and solvents were HPLC or analytical grade.

Total Phenolics and Total Flavonoids Contents.

Total phenolic compounds of extracts were determined using Folin-Ciocalteu reagent described by Singleton (Singleton VL & Rossi JA, 1965) with slight modifications. Each extract (0.1 g) was diluted to 1 mL with distilled water. The diluted samples were mixed with 1 mL of diluted (1:10) Folin-Ciocalteu reagent and incubated at 22℃ for 5 min. The mixtures were reacted with 1 mL of 10% sodium carbonate solution and allowed to stand at 22 ℃ for 1h. The absorbance was measured using a microplate reader at 760 nm. A standard curve was prepared at 0.5, 1.0, 1.5, 2.5, 5.0, 7.5, and 10.0 mg/mL of gallic acid in the same manner as described for the extracted samples. Total phenolic concentrations were expressed as mg of gallic acid equivalent (GAE) per g of extract. Total flavonoid content was determined using a spectrometric method (Moreno MIN *et al.,* 2000). Each extract (0.5 mL) was serially mixed with 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M aqueous potassium acetate, and 4.3 mL of ethanol. The mixture was allowed to stand at room temperature for 40 min, and then the absorbance was measured spectrophotometrically at 415 nm. A standard curve was prepared at 0, 2, 4, 6, 8, and 10 mg/mL of quercetin as described above. Flavonoid contents were expressed as mg of quercetin equivalent (QE) per g of extract.

DPPH Radical Scavenging Activity

The antioxidant activity of the fermented samples and control was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Shimada K *et al.,*1992). Two mL of the extracted sample (1%, w/v) was mixed with 1 mL of 0.2 mM DPPH radical solution in 95% ethanol. The mixture was incubated at 25℃ for 30 min and measured the absorbance at 517 nm. The scavenging activity of DPPH radical was calculated by the following equation:

$$\text{DPPH scavenging activity (\%)} = [1-(A_t/A_c)]\times 100$$

Where A_c is the absorbance of the control reaction and A_t is the absorbance of the extract.

Reducing Power

The reducing power was determined by the method of Oyaizu (Oyaizu M, 1986). About 1 mL of extract of varying concentrations was mixed with 0.2 M sodium phosphate buffer (1 mL, pH 6.6) and 1% aqueous potassium ferricyanide (1 mL, $K_3Fe(CN)_6$). The mixture was incubated at 50℃ for 20 min, immediately cooled to room temperature, and mixed with 1 mL of 15% trichloroacetic acid, which was centrifuged at 3,000 ×*g* for 10 min. The upper layer was mixed with deionized water and $FeCl_3$, then, the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

HPLC Analysis of Phenolic Acids

Phenolic monomers and dimers were analyzed using the high-performance liquid chromatography (HPLC, Waters M600E; Milfold, MA, USA) system equipped with a UV absorbance detector (280 nm, 0.05 AUFS). The separations of free phenolic acids were achieved in the reverse phase mode using Waters Spherisorb ODS2 column (250 mm × 4.6 mm I.D., 5 μm). The mobile phases were solvent A, 1 mM trifluoroacetic acid (TFA) in 10% (v/v) acetonitrile; solvent B, 1 mM TFA in 40% (v/v) methanol and 40% (v/v) acetonitrile. The gradient profile was used as follows (Waldron KW *et al.,*1996) : A 90%, B 10% (initial); A 90%, B 10% (0~10 min); A 60%, B 40% (10~15 min); A 60%, B 40% (15~24 min); A 0%, B 5%, C 100%,(24~40 min); A 90%, B 10%, C 100%,(40~45 min); A 90%, B 10%, C 100%,(45~50 min) at a flow rate of 1mL/min. Peaks were identified by retention times established from standard solutions. Pure phenolic acid standards (*p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, vanillic acid, vanillin, coumaric acid, *trans*-ferulic acid, and *trans*-cinnamic acid) were dissolved in methanol and serially diluted to 10, 50, 100 mg/ml. Dilutions and peak areas were used to establish standard curves.

Statistical Analysis

The experimental results are expressed as means±standard deviation (SD) of three measurements at least. Statistical Package for Social Science (SPSS 13) was used to analyses the variance

Table 1. Total phenols (mg GAE/g) and flavonoids (mg QE/g) in the natraceuticals extracts

	Total phenols	Flavonoids
Extract	5.46±0.56[a]	2.21±0.23[a]

[a] Each value is the mean±SD of triplicate measurements.

(AVONA).

RESULTS AND DISCUSSION

The total phenolic content and total flavonoids content in natraceutical extracts is presented Table 1. The total phenolic contentsin extract was 5.46 mg GAE/g, and total flavonoids content was 2.21 mg QE/g. It is reported that intense heat from hot water was able to release cell wall phenolics or bound phenolic due to the breakdown of celluar consistuents, thus casuing polyphenols to be extracted (Lim YY & Murtijaya J, 2007). Free radicals are involved in a number of diseases involving the failure of cellular function due to oxidative damage to DNA, lipids, and proteins (Chen FA *et al.,* 2006).

The effect of antioxidants on DPPH radical scavenging was thoughtto be due to their hydrogen-donating ability. DPPH has been used extensively as a free radical to evaluate reducing substances (Leong LP & Shui G, 2002). As evidenced in Table 2, the extract presented a concentration-dependent activity increase. The DPPH radical scavenging activity of natraceutical extract varied from 18.77 (200 μg/mL) to 3.44 (1,000 μg/mL). This variation of free radical scavenging activity in the extracts may be due to the differences in the total phenolic contents. Such observation agreed with several previous findings (Zheng W & Wang SY, 2001; Malenciæ D *et al.,* 2008).

The reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Meir S *et al.,*1995). Fe^{3+} reduction is often used as indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action that is strongly correlated with other antioxidant properties (Koear M *et al.,* 2005). In this assay system, the presence of antioxidants causes the reduction of the Fe^{3+}/ferricyanate complex to the ferrous form (Fe^{2+}) monitored at 700 nm. As evidenced in Fig. 1, the reducing power of extract presented a concentration-dependent activity increase. The natraceutical extract absorbance varied from 1.07 (0.78 mg/mL) to 3.44 (12.5 mg/mL). Indeed, some phenolic compounds such as flavonoids and phenolic acids exhibited antioxidant activity through their reductive capacity in a Fe^{3+}-Fe^{2+} system (Zhao HF *et al.,* 2006). Antioxidant activity is primarily associated with the content of polyphenolic components, glycosides, and flavonoids(Merken HM *et al.,* 2001).

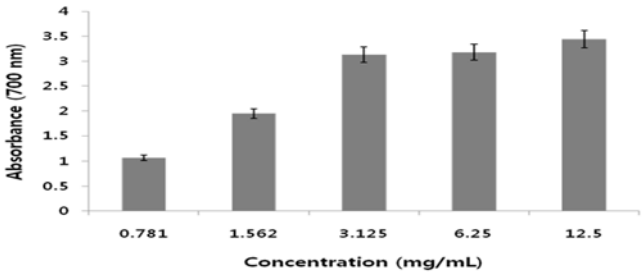

Fig. 1. Reducing power of natraceutical extract.

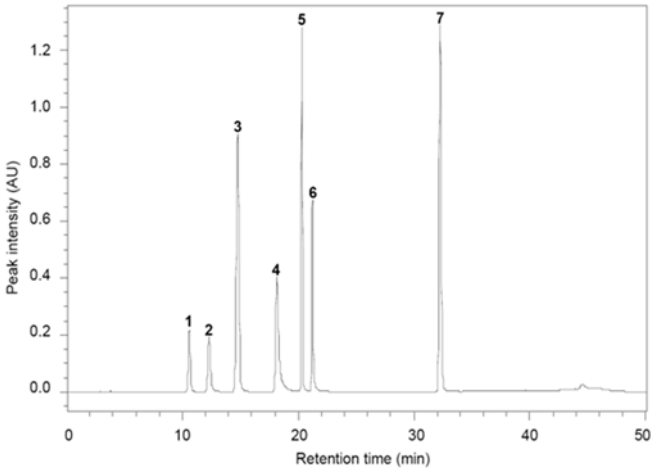

Fig. 2. Typical HPLC chromatogram of phenolic acid standards. Peak identification: (1) *p*-hydroxybenzoic acid; (2) vanillic acid; (3) *p*-hydroxybenzaldehyde; (4) vanillin; (5) *trans*-*p*-coumaric acid; (6) *trans*-ferulic acid; (7) *trans*-cinnamic acid.

Phenolic compounds are classified as primary or chain-breaking antioxidants based on their mechanisms of antioxidant activities, including electron- or hydrogen-donating, metal-chelating, free radical scavenging, lipoxygenase-inhibiting abilities (Decker EA, 1997).

The radical scavenging capacity and reducing power potential of extracts were positively correlated with the content of total phenolic compounds. Vanillic acid, *p*-hydroxybenzoic, *p*-hydroxybenzaldehyde, vanillin, *trans*-*p*-coumaric acid, *trans*-ferulic acid, and *trans*-cinnamic acid were used for phenolic acid quantification. The representative HPLC profile of selected phenolic standards is shown in Fig. 2.

The contents of phenolic acids in natraceutical extracts are shown in Fig. 3. The highest amounts of *trans*-ferulic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, vanillin, vanillic acid, *p*-hydroxybenzaldehyde, and *trans*-cinnamic acid were observed in the naturaceutical extract at the levels of 750.79, 619.75, 531.34, 222.04, 219.28, 107.40, and 89.56 μg/g, respectively. Phenolic acids are classified as hydroxybenzoic acids (salicylic, gallic, and vanillic

Table 2. DPPH radical scavenging activity of the natraceuticals extracts

	Concentration(μg/mL)				
	200	400	600	800	1,000
DPPH radical scavenging activity(%)[a]	18.77±0.68[a]	30.58±0.47[a]	57.32±0.17[a]	76.27±0.31[a]	82.35±0.5

[a] Each value is the mean±SD of triplicate measurements.

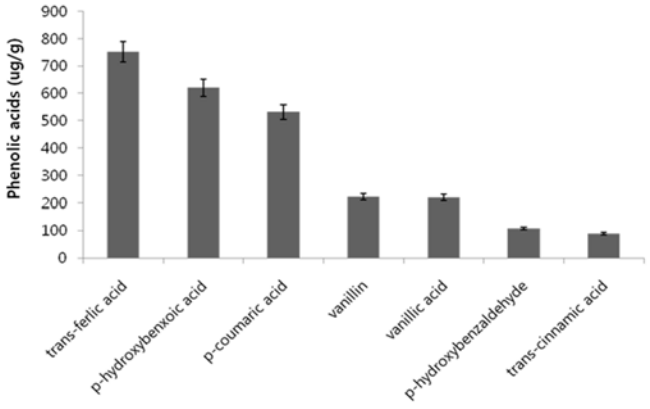

Fig. 3. Phenolic acids (g/g) in the extracts of natraceutical.

acids) and hydroxycinnamic acids (caffeic, chlorogenic, sinapic, fertaric, coumaric, and ferulic acids), which are responsible for sensory quality, antioxidant activity, and other physiological benefits (Edlin *et al.,*1998; Shahidi F & Naczk M, 2003); Cabrita MJ *et al.,* 2008). The hydroxycinnamic acids are known to be more effective antioxidants than the hydroxybenzoic acids(Larson RA, 1998).

The results in the present work indicate the presence of phenolic compound and antioxidant potential in natraceutical extract. Total phenolic contents were determined using a spectrophotometic technique and calculated as gallic acid equivalents/g. The total phenolic and total flavonoids contents of naturaceutical extract were 5.46 mg/g, 2.21 mg/g, respectively. The DPPH radical scavenging activity of natraceutical extract varied from 18.77 (200 µg/mL) to 3.44 (1,000 µg/mL). The reducing power of the natraceutical extract absorbance varied from 1.07 (0.78 mg/mL) to 3.44 (12.5 mg/mL), and reducing power of extract presented a concentration-dependent activity increase. The highest amounts of *trans*-ferulic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, vanillin, vanillic acid, *p*-hydroxybenzaldehyde, and *trans*-cinnamic acid were observed in the naturaceutical extract at the levels of 750.79, 619.75, 531.34, 222.04, 219.28, 107.40, and 89.56 µg/g, respectively. Based on the results of this *in vitro* study, natraceutical extract is a significant source of potent dietary antioxidants.

REFERENCES

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences*, *90*, 7915-7937.

Bland, J. S. (1995). Oxidants and antioxidants in clinical medicine: Past, present and future potential. *Journal of Nutritional & Environmental Medicine,5*, 255-280.

Bansky, D., & Barolet, R. (1999). *Chiness Herbal Medeicine Formulas and Strategies*. Seattle, Eastland Press, pp.7-8.

Cabrita, M. J., Torres, M., Palma, V., Alves, E., Pat, R., & Costa, F. A. M. (2008). Impact of malolactic fermentation on low molecular weight phenolic compounds. *Talanta,74*, 1281-1286.

Chen, F. A., Wu, A. B., Shieh, P., Kuo, D. H., & Hsieh, C. Y. (2006). Evaluation of the antioxidant activity of *Ruellia tuberose*. *Food Chemistry, 94*, 14-18.

Collin, A. R. (1999). Oxidative DAN damage, antioxidants, and cancer. *Bio-Essays, 21*, 238-246.

Decker, E. A. (1997). Phenolics: Prooxidants or antioxidants?. *Nutrition Reviews, 55*, 396-407.

Edlin, D. A. N., Narbad, A., Gasson, M. J., Dickinson, J. R., & Lloyd, D. (1998). Purification and characterization of hydroxycinnamate decarboxylase from *Brettanomyces anomalus*. *Enzyme and Microbial Technology, 22*, 232-239.

Floyd, R. A. (1999). Antioxidants, oxidative stress, and degenerative neurological disorders. *Experimental Biology and Medicine, 222*, 236-245.

Halliwell, B., & Gutteridge, J. M. C. (1990). Role of free radicals and catalytic metal ions in human disease: An overview. *Methods in Enzymology,186*, 1-85.

Hertog, M. L., Feskens, E. M., Hollman, P. H., Katanm, M. B., & Kromhout, D. (1993). Dietary antioxidant flavonoids and risk of coronary heart-diseases the Zutphen elderly study. *Lancet*., *342*, 1007-1011.

Jaghav, S. J., Nimbalkar, S. S., Kulkarni, A. D., & Madhavi, D. L. (1996). Lipid oxidation in biological and food systems. In D. L. Madhavi,; S. S.; Deshpande.; D. K. Salunkhe (Eds.), *Food Antioxidants*. Dekker Press, New York, pp. 5-63.

Jeong, S. H., Kim, S. I., Sim, K. H., Jin, S. Y., & Kim, M. H. (2012). Antioxidant and antidiabet activity of *Jehotang* according to different cooking methods. *The Korean Journal of Culinary Research,18*, 233-242.

Koear, M., Dorman, H. J. D., & Hiltunen, R. (2005). Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. *Food Chemistry, 91*, 525-533.

Larson, R. A. (1988). The antioxidants of higher plants. *Phytochemistry*, *27*, 969-978.

Liyana-Pathirana, C. M., & Shahidi, F. (2006). Importance of insoluble bound phenolics to antioxidant properties of wheat. *Journal of Agricultural and Food Chemistry, 54*, 1256-1264.

Lim, Y. Y., & Murtijaya, J. (2007). Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. *LWT-Food Science Technology*, *40*, 1664-1669.

Leong, L. P., & Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry,76*, 69-75.

Malencic, D., Maksimovix, Z., Popovic, M., & Miladinovic, J. (2008). Polyphenol contents and antioxidant activity of soybean seed extracts. *Bioresource Technology, 99*, 6688-6691.

Mariod, A. A., Matthaűs, B., & Hussein, I. H. (2008). Antioxidant properties of methanolic extracts from different parts of *Sclerocarya birrea*. *International Journal of Food Science & Technology, 43*, 921-926.

Mariod, A. A., Matthaűs, B., Eichner, K., & Hussein, I. H. (2006). Antioxidant activity of extracts from *Sclerocarya birrea* kernel oil cake. *Grasas y. Aceites*., *57,* 361-366.

Matthaűs, B. (2002). Antioxidant activity of extracts obtained from residues of different oilseeds. *Journal of Agricultural and Food Chemistry, 50*, 3444-3452.

Merken, H. M., Merken, C. D., & Beecher, G. R. (2001). Kinetics method for the quantitation of anthocyanidins, flavonols, and flavones in foods. *Journal of Agricultural and Food Chemistry, 49*, 2727-2732.

Meir, S., Kanner, J., Akiri, B., & Philosoph-Hadas, S. (1995). Deter-

mination and involvement of aqueous reducing compounds in oxidative. *Lebensm.-Wiss Technology, 29*, 238-244.

Moure, A., Franco, D., Sineiro, J., Dominguez, H., Nunez, M. J., & Lema, J. M. (2000). Evaluation of extracts from Gevuina hulls as antioxidants. *Journal of Agricultural and Food Chemistry, 48*, 3890-3897.

Moreno, M. I. N., Isla, M. I., Sampietro, A. R., & Vattuone, M. A. (2000). Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. *Journal of Ethnopharmacology, 71*, 109-114.

Oyaizu, M. (1986). Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. *Japan Journal of Nutrition, 44,* 307-315.

Park, S. J., & Rha, Y. A. (2013). Component analysis and digestive enzyme activities of fermented *Crataegi fructus e*xtracts. *The Korean Journal of Culinary Research,19,* 136-145.

Pari, L., & Umamaheswari, J. (2000). Antihyperglycemic activity of *Musa sapientum* flowers: Effect on lipid peroxidation in alloxan diabetic rats. *Phytotherapy Research, 14,* 1-3.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine, 20*, 933-956.

Shahidi, F., & Naczk, M. (2003). *Phenolics in Food and Nutraceuticals*. London, CRC Press.

Shin, M. K. (1997). *Clinical Traditional Herbalogy*, 5thed. Younglimsa, Seoul.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry, 40*, 945-948.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Wnology and Viticulture, 16*, 144-158.

Son, D. W., Kim, D. G., & Lee, S. J. (2011). Effect of natural functional mixture on the descent of blood glucose level in streptozotocin-induced diabetic(type I) rats(II). *The Korean Journal of Culinary Research, 17*, 238-247.

Surh, Y. J. (2002). Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: A short review. *Food and Chemical Toxicology, 40*, 1091-1097.

Surh, Y. J., Hurh, Y. J., Jang, Y. Y., Lee, E., Kong, G., & Lee, S. J. (1999). Resveratrol, an antioxidant present in red wine, induces apotosis in human promyelocytic leukemia (HL-60) cells. *Cancer Letters, 140,* 1-20.

Thomson, M. J. (1995). The role of free radicals and antioxidants: How do we know that they are working. *Critival Revies in Food Science and Nutrition, 35*, 21-39.

Waldron, K. W., Parr, A. J., Ng, A., & Ralph, J. (1996). Cell wall esterified phenolic dimers: Identification and quantification by reverse phase high performance liquid chromatography and diode array detection. *Phytochemical Analysis, 7*, 305-312.

Williams, G. M., Iatropoulos, M. J., & Whysner, J. (1999). Safety assessment to butylated hydroxyanisol and butylated hydroxytoluene as antioxidant food additives. *Food and Chemical Toxicology, 37,* 1027-1038.

Zhao, H. F., Dong, J. J., Lu, J., Chen, J., Li, Y. Y., & Shan, I. J. (2006). Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley(*Hordeum vulgare* L.). *Journal of Agricultural and Food Chemistry, 54,* 7277-7286.

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry, 49*, 5156-5170.