Korean J. Plant Res. 28(3):305-311(2015)
http://dx.doi.org/10.7732/kjpr.2015.28.3.305

Print ISSN 1226-3591
Online ISSN 2287-8203

Original Research Article

Antioxidant, Anti-inflammatory and *Cytotoxicity* on Human Lung Epithelial A549 Cells of Jerusalem artichoke (*Helianthus tuberosus* L.) Tuber

Qin Zhang[1] and Hye-Young Kim[2]*

[1]Department of Medical Biotechnology, Kangwon National University, Chuncheon 200-701, Korea
[2]Department of Dental Hygiene, Kangwon National University, Samcheok 245-711, Korea

Abstract - This study investigated *in vitro* antioxidant, anti-inflammatory and cytotoxicity on human lung epithelial A549 cells of different solvent extracts from Jerusalem artichoke (*Helianthus tuberosus* L.) tuber. The EtOH extract contained amounts of phenolics (22.20 tannic acid equivalent ㎎/g) and exhibited the highest antioxidant activity and anti-inflammatory activity. Several methods were employed for measure the antioxidant activity: 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (IC_{50} = 206.79 ㎍/㎖), reducing power activity (21.26 ascorbic acid equivalent ㎎/g) and total antioxidant activity (19.05 ascorbic acid equivalent ㎎/g). Meantime, the EtOH extract inhibited the NO production completely with a concentration of 800 ㎍/㎖. Besides, the H_2O extract exhibited more potent effect on human lung epithelial A549 cells. This study suggested that Jerusalem artichoke tuber had antioxidant, anti-inflammatory and cytotoxicity on human lung epithelial A549 cells.

Key words - Jerusalem artichoke tuber, Antioxidant, Anti-inflammatory, Cytotoxicity

Introduction

In recent years, a large variety of phytonutrients are extracted from plants, of which many have protective biological properties. Phytonutrients used for food, cosmetic and pharmaceutical products not only have negligible amount of poison to our body, but also have strong biological activity (Ahn and Park, 2013). Most free radicals, being known as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are considered to be a fundamental cause of physiological diseases such as cancer, aging, diabetes mellitus, Alzheimer's disease and coronary heart ailment (Bulkley, 1983; Cheng *et al*., 2003; Finkel and Holbrook, 2000). Moreover, it was suggested that the antioxidant, anti-inflammatory and anti-cancer activities of the plant extracts could be helpful for the prevention and treatment of these diseases (Dufour *et al*., 2007).

Jerusalem artichoke (*Helianthus tuberosus* L.) belongs to the family Asteraceae and is a kind of herbaceous perennial plants that is cultivated in temperate regions for its tuber. Jerusalem artichoke tuber is spindly looking with 7.5-10 ㎝ long and 3-5 ㎝ thick, which equivocally resembles ginger root in appearance. The dried Jerusalem artichoke contains protein, crude fiber, mineral, starch, pectin substances and inulin; among them, the content of inulin is about up to 80% (Hu *et al*., 2006). The Jerusalem artichoke tuber is widely used as animal feed while it cannot be digested by human digestive system, as a result the tuber is suitable for patients in need of special diet such as diabetics (Aslan *et al*., 2010; Cheng *et al*., 2009). Besides, the antioxidant activity of the tuber has been reported to be changed through different extract temperatures, different extract methods and different production processes (Jeong *et al*., 2011; Lee *et al*., 2014). In our study, we were aimed to investigate the different antioxidant, anti-inflammatory and cytotoxicity on human lung epithelial A549 cells of Jerusalem artichoke tuber different solvent extracts.

Materials and Methods

Samples preparation

The dried tuber powder (20 g) was extracted by 600 ㎖ of five different solvents containing distilled water, methanol, 70% methanol, ethanol, 70% ethanol. After concentrating the

*Corresponding author. E-mail : khy0606@daum.net

filtrates with a rotary evaporator (CCA-110, Eyela, Tokyo, Japan), distilled water (H_2O) (18.03 g), methanol (MeOH) (2.81 g), 70% methanol (70% MeOH) (12.07 g), ethanol (EtOH) (1.16 g), 70% ethanol (70% EtOH) (10.70 g) extract was collected, respectively.

Chemicals and reagents

Ammonium molybdate, butylated hydroxyanisole (BHA), dimethyl sulfoxide (DMSO), 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferrous chloride, Folin-Ciocalteu reagent, potassium ferricyanide, sodium carbonate ($NaCO_3$), sodium phosphate, sulphuric acid, tannic acid, trichloroacetic acid (TCA) were purchased from Sigma (St, Louis, MO, USA). RPMI 1640 medium, DMEM medium and fetal bovine serum (FBS) were obtained from Gibco BRL (Grand Island, NY, USA). All culture supplies were obtained from BD-Falcon (BD, Franklin Lakes, NJ). All other chemicals were of analytical grade.

Cell line and cell culture

Murine macrophage cell line RAW264.7 and human lung epithelial cell line A549 were purchased from the Korean Cell Line Bank (Seoul, Korea). The RAW 264.7 cells were cultured in RPMI 1640 medium. The A549 cells were cultured in DMEM medium. The cells were supplemented with 10% FBS and 100 U/㎖ of penicillin at 37°C in an incubator containing 5% CO_2 and 95% O_2.

Determination of total phenolic content

Total phenolic content was measured according to the Folin-Ciocalteu method. 100 ㎕ of sample solution (1 ㎎/㎖) was transferred into the tube containing 500 ㎕ of 10% (v/v) Folin-Ciocalteu reagent. After shaking adequately, 400 ㎕ of 7.5% (w/v) $NaCO_3$ solution was added. The mixture was allowed to keep for 30 min at room temperature. The absorbance was detected at 750 ㎚. Tannic acid was used as the standard to make a calibration curve.

Determination of DPPH radical scavenging activity

Five hundred㎕ of DPPH (0.2 M) methanol solution was added to the tube containing 500 ㎕ of sample solution at different concentrations (62.5-1000 ㎍/㎖). After shaking for 1 min, the mixed solution was kept for 30 min at room temperature away from light. The decrease of DPPH free radical was evaluated by measuring the absorbance at 515 ㎚. BHA and α-Tocopherol were used as the positive controls.

Determination of reducing power activity

Two hundred㎕ of sample solution (1 ㎎/㎖) was added into the mixture containing 500 ㎕ of 0.2 M phosphate buffer (pH 6.6) and 500 ㎕ of 1% (w/v) potassium ferricyanide, respectively. The resulting mixture was pre-incubated at 50°C for 30 min. Then, 500 ㎕ of 10% (w/v) TCA was added. After centrifuging at 3,000 rpm at room temperature for 10 min, 500 ㎕ of the upper layer of the mixture was removed, then, 500 ㎕ of distilled water and 100 ㎕ of 0.1% (w/v) ferrous chloride were added, successively. The absorbance was detected at 700 ㎚. Ascorbic acid was expressed as the standard to make a calibration curve.

Determination of total antioxidant activity

Two hundred㎕ of sample solution (1 ㎎/㎖) dissolved in ethanol was mixed with 200 ㎕ of 0.6 M sulphuric acid. Then, 200 ㎕ of 28 mM $NaCO_3$ was added into the mixture. Finally, 200 ㎕ of 4 mM ammonium molybdate solution was added. After sealing the test tube, the reaction mixture was incubated at 95°C for 90 min. The mixture was cooled to room temperature and measured at 695 ㎚. Ascorbic acid was expressed as the standard to make a calibration curve.

Determination of cytotoxicity on RAW264.7 cells

The cytotoxicity of sample on RAW264.7 cells was assessed by conventional MTT assay. The cells were placed in 96-well plates at a density of 5×10^4 cells/well with fresh medium. After pre-culture for 18 h, varying concentrations (100, 200, 400 and 800 ㎍/㎖) of sample were added into each well and cultured for another 24 h. Supernatants were then discarded. 10 ㎕ of MTT solution [5 ㎎/㎖ in phosphate-buffered saline (PBS)] and 90 ㎕ of non-FBS medium were added into each well to continuous culturing for 4 h until termination. The culture was terminated by addition of 200 ㎕ of DMSO to each well and the absorbance was measured at 550 ㎚.

Determination of NO production activity on RAW264.7 cells

The level of NO production was estimated by Griess reaction. The cells were seeded at 96-well plates at a density of 5×10^4 cells/well with fresh medium. After pre-culture for 18 h, different concentrations (100, 200, 400 and 800 ㎍/㎖) of sample were added into each well and the plates were incubated for 30 min. Subsequently, 1 ㎍/㎖ of LPS was added and the plates were further incubated for 24 h. After incubation, 100 ㎕ of supernatant and 100 ㎕ of Griess reagent (1% sulfanilamide in 5% phosphoric acid, 50 ㎕; 0.1% naphthyl-ethylenediamine dihydrochloride, 50 ㎕) was mixed into a new 96-well plate and kept for 10 min. The absorbance was measured at 550 ㎚.

Determination of cytotoxicity on A549 cells

MTT assay was performed on A549 cells to evaluate the cytotoxicity of sample. An amount of 1.5×10^5 cells were placed in each well of 96-well plates and allowed them to recover for 24 h. After pre-culture, sample with diverse concentrations (200, 400 and 800 ㎍/㎖) were added into each well and cultured again for 24 h. Supernatants were then removed. 10 ㎕ of MTT solution [5 ㎎/㎖ in phosphate-buffered saline (PBS)] and 90 ㎕ of non-FBS medium were added into each well. After a further culture for 4 h, 200 ㎕ of DMSO was added into each well to terminate the culture. The absorbance was measured at 550 ㎚.

Statistical analyses

All tests were carried out in triplicate (n = 3). Results are expressed as mean ± standard deviation (SD). One-way analysis of variance with Duncan's multiple-range test was used to determine the significant differences between the groups. Value of $p < 0.05$ was considered significant. All analyses were performed using SPSS 21.0 (SPSS Institute, Cary, NC, USA).

Results

Yield

The effect of Jerusalem artichoke tuber was measured after extraction with five different solvents. As shown in Table 1, the extraction yields of the five extracts were as follows: H_2O extract (90.15%), MeOH extract (14.05%), 70% MeOH extract (60.35%), EtOH extract (5.80%) and 70% EtOH extract (53.50%).

Total phenolic content

Total phenolic content of the five extracts from Jerusalem artichoke tuber was determined as tannic acid equivalent in milligram per gram (mg TAE/g) in Table 2. The phenolic content was ranked as: EtOH extract > MeOH extract > 70% MeOH extract > H_2O extract > 70% EtOH extract. The EtOH extract was richest in phenolic content.

Table 1. Yield and DPPH radical scavenging activity of different solvent extracts from Jerusalem artichoke tuber

Extracts	Yield (%)[z]	DPPH scavenging ability (IC_{50}: ㎍/㎖)[y]
H_2O	90.15%	1874.37 ± 146.25[a]
MeOH	14.05%	261.64 ± 5.99[c]
70%MeOH	60.35%	296.59 ± 0.87[c]
EtOH	5.80%	206.79 ± 5.99[c]
70%EtOH	53.50%	806.76 ± 15.78[b]
Positive control		
α-Tocopherol	-	9.25 ± 0.20[d]
BHA	-	4.59 ± 0.13[d]

[z]Extraction yield (%) is expressed as: (sample fraction weight / sample extract weight) × 100.
[y]IC_{50}: The effective concentration at which DPPH radicals were scavenging by 50%.
Each value is expressed as the mean ± SD (n = 3). Means not sharing the same letter are significantly different at $P < 0.05$ probability level at same concentration by Duncan's multiple-range test.

Table 2. Total phenolic content (TPC), reducing power activity (RPA) and total antioxidant activity (TAA) of different solvent extracts from Jerusalem artichoke tuber

Extracts	TPC (TAE ㎎/g)[z]	RPA (AAE ㎎/g)[y]	TAA (AAE ㎎/g)[y]
H_2O	6.60 ± 0.40[d]	4.44 ± 0.51[d]	18.48 ± 0.22[a]
MeOH	18.20 ± 0.00[b]	15.93 ± 0.36[b]	18.60 ± 0.58[a]
70%MeOH	9.67 ± 0.23[c]	9.00 ± 0.38[c]	17.43 ± 0.62[b]
EtOH	22.20 ± 0.4[a]	21.26 ± 0.17[a]	19.05 ± 0.36[a]
70%EtOH	5.67 ± 0.23[e]	3.85 ± 0.13[d]	15.48 ± 0.33[c]

[z]TAE: tannic acid was expressed as the standard to make a calibration curve.
[y]AAE: asocebic acid was expressed as the standard to make a calibration curve.
Each value is expressed as the mean ± SD (n = 3). Means not sharing the same letter are significantly different at P < 0.05 probability level at same concentration by Duncan's multiple-range test.

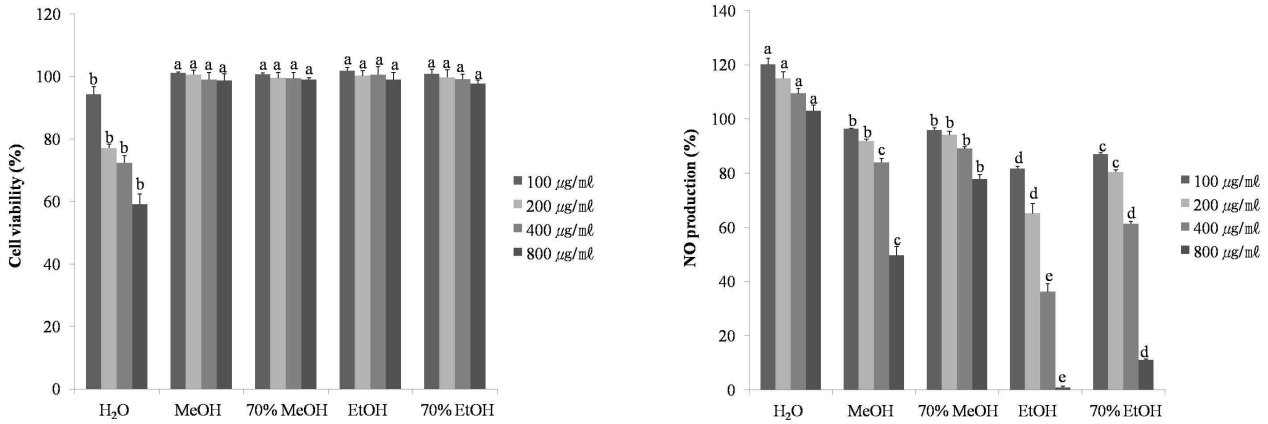

Fig. 1. Cytotoxicity (A) and NO production activity (B) on LPS-stimulated RAW264.7 cells of different solvent extracts from Jerusalem artichoke tuber. Each value is expressed as the mean ± SD (n = 3). Means not sharing the same letter are significantly different at P < 0.05 probability level at same concentration by Duncan's multiple-range test.

DPPH free radical scavenging activity

The DPPH radical scavenging ability of the five extracts from Jerusalem artichoke tuber increased in a dose-dependent manner. As shown in Table 1, the IC_{50} order of the five extracts was as follows: EtOH extract < MeOH extract < 70%MeOH extract < 70% EtOH extract < H_2O extract; lower IC_{50} reflected stronger scavenging activity. The EtOH extract proved the highest DPPH radical scavenging activity.

Reducing power activity

The reducing power activity of the five extracts from Jerusalem artichoke tuber was presented as the weight of ascorbic acid equivalent (mg AAE/g) in Table 2. The ability was presented in a decending order as: EtOH extract > MeOH extract > 70% MeOH extract > H_2O extract > 70% EtOH extract. The EtOH extract had more noticeable reducing power activity.

Total antioxidant activity

The total antioxidant activity of the five extracts from Jerusalem artichoke tuber was described as the weight of ascorbic acid equivalent (mg AAE/g) in Table 2. The total antioxidant abilities of EtOH extract, MeOH extract and H_2O extract were significantly higher than that of 70% EtOH extract and 70%MeOH extract. The EtOH extract had slightly strong total antioxidant activity.

Cytotoxicity and NO production activity on RAW264.7 cells

The cytotoxicity of the five extracts from Jerusalem artichoke tuber on RAW264.7 cells was shown in Fig. 1A. In addition to the H_2O extract, the other four extracts at the test

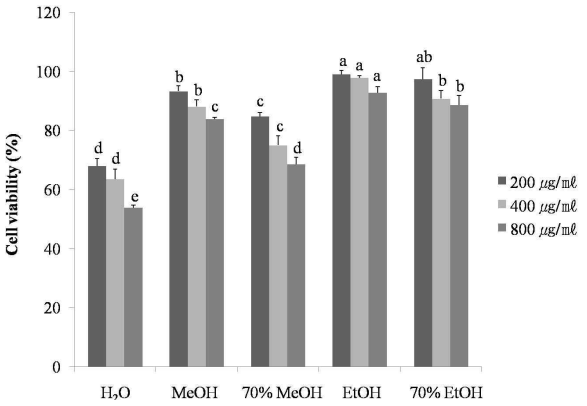

Fig. 2. Cytotoxicity on A549 cells of different solvent extracts from Jerusalem artichoke tuber for 24 h. Each value is expressed as the mean ± SD (n = 3). Means not sharing the same letter are significantly different at $P < 0.05$ probability level at same concentration by Duncan's multiple-range test.

concentrations exhibited scarcely any toxicity to cell viability. The NO production ability was measured by using RAW264.7 cells was shown in Fig. 1B. With the extract test concentration increasing, the suppression of NO production was strengthened, which showed a dose-effect relationship. Especially, the EtOH extract exhibited the strongest inhibition ability of NO production.

Cytotoxicity on A549 cells

The cytotoxicity of the five extracts from Jerusalem artichoke tuber on A549 cells was shown in Fig. 2. The ability of cell growth was inhibited in an increasing trend with the increasing concentration of the extract. All of five extracts inhibited the cell growth at test concentrations, and in particular the H_2O extract revealed the highest cytotoxicity.

Discussion

Inulin is an energy reserving material in more than 36,000 species of plants such as wheat, bananas, garlic, asparagus, onion and chicory. The high content and excellent solubility of inulin in Jerusalem artichoke tuber may contribute to the highest yield of water extract. The phenolic content exhibits various medicinal properties, such as antioxidant, anticancer, antiallergenic, anti-inflammatory and antiviral (Luthria, 2008; Pietta, 2000). Phenolic content consists of a hydroxyl group and plays an important role in the antioxidant capacity by donating hydrogen and forming stable radical intermediates (Kim and Roh, 2013). Furthermore, the phenolic content has been found to have certain linear relationship with antioxidant activity and may contribute to the oxidation-resistant directly (Yin *et al.*, 2007). Our study showed that total phenolic content of the EtOH extract was the richest than the other four extracts. Thus, we hypothesized that the EtOH extract had potential as a kind of antioxidant. Free radicals can cause oxidative damage to the human body, leading to a series of diseases, such as dementia, cancer, aging and coronary heart disease (Siriwardhana *et al.*, 2003). DPPH is an acknowledged stable radical and a scavenger for other free radicals. The DPPH assay has been regarded as one of the good method to indicate the free radicals scavenging ability of antioxidants since its simple, sensitive and reliable (Zhao *et al.*, 2014). Owning to the foresaid relationship between antioxidant activity and phenolic content, the EtOH extract revealed strong DPPH radical scavenging ability may link with its rich phenolic content. The reducing power assay is well known as an analysis method of antioxidant capacity. Many studies have found a direct correlation between reducing power and antioxidant capacity of certain plant samples (Duh, 1998; Tanaka *et al.*, 1988). Sample expresses the antioxidant ability by donating a hydrogen atom and breaking the free radical chain. For investigating the reductive ability, the ability to reduce ferric (III) iron into ferrous (II) iron was measured. Compared with the five extracts, the order of reducing power activity was consistent with that of the phenolic content. The higher reducing power activity of the EtOH extract may be due to the rich total phenolic content. The antioxidant protection on total antioxidant activity play a consolidated role of oxidative stress in mitochondrial aging processes, malaria infection and many other pathophysiological states (Kusano and Ferrari, 2008). The reduction of Mo (VI) to Mo (V) by the antioxidant compounds is used to evaluate the total antioxidant activity. This test is widely combined with other antioxidant defense assays to measure the oxidative stress. Several studies have reported that the total antioxidant activity might be derived from the amount of total phenolic content (Lee *et al.*, 2003).

NO has diverse physiological functions and also makes contribution to the immune defense against bacteria, viruses,

and other parasites; however, overproduction of NO causes a series of disease such as diabetes, arthritis, chronic inflammatory diseases and autoimmune diseases (Won *et al.*, 2001). First of all, the cytotoxicity of the five extracts on RAW264.7 cells was measured. From the result, we could conclude that there is no toxic to cell viability at test concentrations with the exception of H_2O extract. Due to NO is usually regarded as a crucial mediator in the inflammatory diseases, NO production was used to investigate the anti-inflammatory activity of the extract on RAW264.7 cells. Following stimulated by LPS, abundant NO was produced. The EtOH extract significantly exhibited NO inhibition ability, which showed that the EtOH extract had a strong anti-inflammatory activity. It has been reported the anti-inflammatory activity had a connection with the phenolic content on some level (Kassim *et al*., 2010).

The MTT assay, originally known as a colorimetric method for monitoring cell survival and proliferation, has been employed successfully to quantitate cytotoxicity (Jun *et al.*, 2014). In this assay, MTT is reduced to an insoluble formazan product by reducing oxidoreductase enzymes which exist only in living metabolically active cells (Cole, 1986). Besides, the MTT assay has the characteristics of simple, quick and economic. Therefore, it has been widely used to measure *in vitro* anti-cancer activity. Over recent years, lung cancer occupies for more solid tumor deaths than breast, prostate, pancreatic, and colorectal cancers (Chou *et al.*, 2003). In our study, the cytotoxicity on A549 cells was produced among the five extracts, and notably the H_2O extract. It may be attributed to, at least a part, to the anti-cancer compounds dissolved more easily in water.

In summary, the present study proved the antioxidant, anti-inflammatory and cytotoxicity on human lung epithelial A549 cells of Jerusalem artichoke tuber which was extracted by H_2O, MeOH, 70% MeOH, EtOH and 70% EtOH. The EtOH extract revealed the strongest antioxidant activity and anti-inflammatory activity, which may be associated with its highest level of phenolic content. Furthermore, the H_2O extract showed the most potent cytotoxicity on human lung epithelial A549 cells. However, the *in vitro* antioxidant, anti-inflammatory and cytotoxicity on human lung epithelial A 549 cells did not afford much insight into the biological mechanisms. Therefore, the antioxidant, anti-inflammatory and cytotoxicity on human lung epithelial A549 cells of Jerusalem artichoke tuber need to be investigated further studies.

Acknowledgement

This paper was supported in part by research funds of Kangwon National University (220140131).

References

Ahn, J. and J.H. Park. 2013. Effects of *Abeliophyllum distichum* Nakai flower extracts on antioxidative activities and inhibition of DNA damage. Korean J. Plant Res. 26:355-361.

Aslan, M., N. Orhan, D.D. Deliorman and F. Ergun. 2010. Hypoglycemic activity and antioxidant potential of some medicinal plants traditionally used in Turkey for diabetes. J. Ethnopharmacol. 128:384-389.

Bulkleyk, G.B. 1983. The role of oxygen free radicals in human disease processes. Surgery 94:407-411.

Cheng, H., T. Lin, K. Yu, C. Yang and C. Lin. 2003. Antioxidant and free radical scavenging activities of *Terminalia chebula*. Biol. Pharm. Bull. 26:1331-1335.

Cheng, Y., W.G. Zhou, C.F. Gao, K. Lan, Y. Gao and Q.Y. Wu. 2009. Biodiesel production from Jerusalem artichoke (*Helianthus tuberosus L.*) tuber by heterotrophic microalgae *Chlorella protothecoides*. J. Chem. Technol. Biot. 84:777-781.

Chou, J.Y., S.Y. Lai, S.L. Pan, G.M. Jow, J.W. Chern and J. H. Guh. 2003. Investigation of anticancer mechanism of thiadiazole-based compound in human non-small cell lung cancer A549 cells. Biochem. Pharmacol. 66:115-124.

Cole, S.P.C. 1986. Rapid chemosensitivity testing of human lung tumor cells using the MTT assay. Cancer Chemoth. Pharm. 17:259-263.

Dufour, D., A. Pichette, V. Mshvidadze, M.E. Brasette-Hebert, S. Lavoie, A. Longtin, C. Laprise and J. Legault. 2007. Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Ledum groenlandicum* Retzius. J. Ethnopharmacol. 111:22-28.

Duh, P. 1998. Antioxidant activity of burdock (*Arctium lappa* Linne): its scavenging effect on free-radical and active oxygen. J. Am. Oil Chem. Soc. 75:455-461.

Finkel, T. and N. J. Holbrook. 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408:239-247.

Hu, J.E., Y.G. Du, X.F. Bai and C.Y. Tang. 2006. Method for producing high-quality inulin by using jerusalem artichoke as raw material. China. CN 200610046049.

Jeong, H.J., J.S. Kim, Y.J. Sa, M.O. Kim, J.F. Yang and M.J. Kim. 2011. Antioxidant activity and α-glucosidase inhibitory effect of Jerusalem artichoke (*Helianthus tuberosus*) methanol extracts by heat treatment conditions. Korean J. Medicinal Crop Sci. 19:257-263.

Jun, N.J., S.C. Kim, E.Y. Song, K.C. Jang, D.S. Lee and S.K. Cho. 2014. Isolation of anticancer compounds from *Peucedanum-japonicum* thumb. roots. Korean J. Plant Res. 27:215-222.

Kassim, M., M. Achoul, M.R. Mustafa, M.A. Mohd and K.M. Yusoff. 2010. Ellagic acid, phenolic acids, and flavonoids in Malaysian honey extracts demonstrate *in vitro* anti-inflammatory activity. Nutr. Res. 30:650-659.

Kim, K.S. and K.S. Roh. 2013. Comparative study of antioxidant activity of apple and pear peel. Korean J. Plant Res. 26:347-354.

Kusano, C. and B. Ferrari. 2008. Total antioxidant capacity: a biomarker in biomedical and nutritional studies. J. Cell Mol. Biol. 7:1-15.

Lee, K.W., Y.J. Kim, D. Kim, H.J. Lee and C.Y. Lee. 2003. Major phenolics in apple and their contribution to the total antioxidant capacity. J. Agr. Food Chem. 51:6516-6520.

Lee, Y.J., M.G. Lee, S.Y. Yu, W.B. Yoon and O.H. Lee. 2014. Changes in physicochemical characteristics and antioxidant activities of Jerusalem artichoke tea infusions resulting from different production processes. Food Sci. Biotechnol. 23:1885-1992.

Luthria, D.L. 2008. Influence of experimental conditions on the extraction of phenolic compounds from parsley (*Petroselinum crispum*) flakes using a pressurized liquid extractor. Food Chem. 107:745-752.

Pietta, P. 2000. Flavonoids as antioxidants. J. Nat. Prod. 63:1035-1042.

Siriwardhana, N., K. Lee, Y. Jeon, S. Kim and J. Haw. 2003. Antioxidant activity of *Hizikia fusiformis* on reactive oxygen species scavenging and lipid peroxidation inhibition. Food Sci. Technol. Int. 9:339-346.

Tanaka, M., C. Kuei, Y. Nagashima and T. Taguchi.1988. Application of antioxidative maillard reaction products from histidine and glucose to sardine products. Nippon Suisan Gakk. 54:1409-1414.

Won, G.S., H.O. Pae, G.S, Oh, K.Y. Chai, T.O. Kwon, Y.G. Yun, N.Y. Kim and H.T. Chung. 2001. Inhibitory effects of methanol extract of *Cyperusrotundus* rhizomes on nitric oxide and superoxide productions by murine macrophage cell line, RAW 264.7 cells. J. Ethnopharmacol. 76:59-64.

Yin, J., G. Kwon and M.H. Wang. 2007. The antioxidant and cytotoxic activities of *Sonchus oleraceus* L. extracts. Nutr. Res. Pract. 1:189-194.

Zhao, Y., S. Du, H. Wang and M. Cai. 2014. *In vitro* antioxidant activity of extracts from common legumes. Food Chem. 152:462-466.

(Received 19 January 2015 ; Revised 1 April 2015 ; Accepted 11 May 2015)