J Life Science, 5(2): 127-132 (2013)
PRINT: ISSN 0975-1270 ONLINE: ISSN 2456-6306 DOI: 10.31901/24566306.2013/05.02.03

Evaluation of *Allium* Vegetables for Anti-Adipogenic, Anti-Cancer, and Anti-Inflammatory Activities *InVitro*

Hee Sook Park, Eun Jeong Choi, Je-Hyuk Lee and Gun-Hee Kim*

Plant Resources Research Institute, Duksung Women's University, 419 Ssangmun-dong, Dobong Gu, Seoul, 132-714, Republic of Korea

KEYWORDS Allium *Vegetables*. Anti-cancer. Anti-adipogenesis. Anti-inflammation

ABSTRACT *Allium* vegetables include onions, garlic, and leeks that are used all over the world in different cuisines. We evaluate anti-cancer, anti-obesity, anti-inflammation of nine *Allium* vegetables. Most the extracts from nine *Allium* vegetables decreased breast cancer cell viability compared to the untreated cells. The order of the anti-cell growth effects of the extracts at a concentration of 100μg/mL for 72h was as follows: *Allium tuberosum*e ≥ *Allium macrostemon*>*Allium thumbergii*. The *Allium* vegetables were able to inhibit MDA-MB-453 cancer cell proliferation. Some of the *Allium* vegetable extracts increased caspase-3 activity compared to the control. Caspase-3 activity levels in cell treated with 100μg/mL of *Allium tuberosum*, *Allium macrostemon*, and *Allium thumbergii* were 52.1, 46.2 and 41.3%, respectively. To test whether *Allium* vegetables have anti-obesity effects, we investigated the effects of vegetable extracts on differentiation of preadipocyte 3T3-L1 cells. Most of the tested extracts exhibited anti-adipogenic effects at a concentration of 100μg/mL. *Allium tuberosum*, *Allium macrostemon*, and *Allium thumbergii*, which have anti-cancer effects, also inhibited lipid accumulation by 45.6, 57.0, and 64.9 %, respectively, at a concentration of 100μg/mL. However, no effects of the vegetables on lipolysis in adipocytes were observed. For anti-inflammatory effects, no effects of all *Allium* vegetables on nitric oxide (NO) production in lipopolysaccharide (LPS)-induced macrophage cells. These findings indicate that *Allium* vegetables do not affect NO-mediated inflammatory responses. Taken together, our study demonstrated that *Allium* vegetables may be useful as a functional food material for regulating caner and lipid accumulation.

INTRODUCTION

Allium vegetables belonging to the Liliaceae family include approximately 500 species. These vegetables, such as garlic and onion, have been used to enhance the appetite and as traditional medicines (Song et al. 2009). Many studies reported that *Allium* vegetables and their constituents stimulate the immune system and have anti-cancer, anti-fungal, and anti-oxidant effects (Milner 2001a; Agarwal 1996; Milner 2001b). As interest in the pharmacologic properties of *Allium* vegetables has increased, these plants have been used as functional foods. Traditionally, *Allium* species, particularly onions (*Alliumcepa*), garlic (*Alliumsativum)*, leeks (*Allium tuberosum*), and chives (*Allium schoenoprasumum*) have been used for centuries in Asian, American, and European folk medicines for treating numerous human diseases (Rose et al. 2005). Garlic, onions, and leeks have been reported to protect against cancer (Sengupta et al. 2004). The protective anti-cancer effects appear to be related to the presence of organosulfur compounds and allyl derivatives (Bianchini and Vainio 2001).

*Corresponding author
Phone: + 82-2-901-8662;
Fax: +82-2-901-8661;
E-mail: ghkim@duksung.ac.kr

The effects of *Allium* vegetables against cancer have been studied by testing individual organosulfur compounds or garlic and onion extracts. Several mechanisms were proposed to explain the cancer-preventive activities of *Allium* vegetables and related organosulfur compounds (Sengupta 2004). Rose et al. (2005) described an association between *Allium* vegetable consumption and reduced risk to cardiovascular diseases and cancer, and speculated that many of these beneficial effects are due to anti-inflammatory properties (Rose et al. 2005). Over-expression of pro-inflammatory enzymes such as inducible nitric oxide synthetase (NOS) and cyclooxygenease (COXII) are associated with numerous human diseases including cancer as well as inflammatory disorders and cardiovascular disease (CVD). Increased activity of pro-inflammatory enzymes leads to the production of pro-inflammatory mediators such as nitric oxide (NO). *Allium sativum* extracts were shown to inhibit NO production in peritoneal macrophages, rat hepatocytes, and rat aortic smooth muscle cells stimulated with lipopolysaccharide (LPS) and cytokines (Kim 2001).

High intake of plant-derived foods (i.e., vegetables, legumes, and fruits) containing flavonoids and polyphenolic compounds is directly associated with the management and prevention

of obesity, diabetes mellitus (DM), and other CVD risk factors (Hertog et al. 1993; Knekt et al. 2001; Mink et al. 2007). These properties raise the possibility that phenolic substances can attenuate obesity-related inflammatory responses and thereby exert protective effects against chronic pathologies such as CVD and DM (Hertog et al. 1993; Knekt et al. 2001; Mink et al. 2007). In the present study, the anti-cancer, anti-inflammation, and anti-adipogenic effects of extracts from *Allium* vegetables were evaluated in cell-based models.

MATERIAL AND METHODS

Plant Material

Extracts of *Allium sacculiferum (KRIB 0000777)*, *Allium senescens (KRIB 0000776)*, *Allium schoenoprasmum* var. *orientale*, *Allium macrostemon (KRIB 0001897)*, *Allium thumbergii*, *Allium tuberosum*, *Allium sativum* for *Pekinense (KRIB 0021359)*, *Allium cepa (KRIB 0021476)*, and *Allium fistulosum (KRIB 0021250)* belonging to the Liliaceae family were obtained from the Plant Extract Bank (PEB), Korea Research Institute of Bioscience and Biotechnology (KRIBB; Daejeon, Republic of Korea). All *Allium* vegetables were authenticated by the PEB, and deposited into the PEB (KRIBB). Each extract samples was diluted in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) to 100mg/mL and stored at -20°C as a stock solution.

Chemicals and Reagents

Cell culture medium and reagents including Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and trypsin-EDTA were obtained from Wellgene (Wellgene, Republic of Korea). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), LPS (*Escherichia coli* serotype 0127: B8), insulin, dexamethasone, 3-isobutyl-1-methylxanthine (IBMX), and Oil red O were purchased from Sigma-Aldrich.

Cell Culture

Human breast MDA-MB-453 cancer cells were purchased from the Korean Cell Line Bank (KCLB, Republic of Korea) and routinely maintained in RPMI 1640 (Gibco, CA, USA) supplemented with 10% FBS and antibiotics (50 U/mL of penicillin and 50μg/mL streptomycin;Gibco) at 37°C in a humidified atmosphere containing 5% CO_2. 3T3-L1 pre-adipocytes and RAW 264.7 murine macrophages were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). These cells were cultured in DMEM containing 10% FBS, penicillin (100 units/mL), and streptomycin (100 μg/mL) in a 5% CO_2 humidified incubator at 37°C.

For the adipogenesis experiment, 3T3-L1 preadipocytes were differentiated in the presence or absence of the extracts. 3T3-L1 cells were grown in a 48-well plate until 2 d post-confluence. Two post-confluence (DAY 0), the cells were treated with the induction hormone mixture (MDI; DMEM containing 10% FBS, 10 μg/mL insulin, 0.5 μM DEX, and 0.5 mM IBMX). After the induction, the medium was replaced with DMEM supplemented with 10% FBS and 10 μg/mL insulin and replenished every 2 days. Extracts from each plant part (10–100 μg/mL) were added to the medium on during 3T3-L1 cell differentiation. The cells were then incubated until a mature adipocyte state was reached on DAY 8. Lipid accumulation in the differentiated adipocytes was measured using Oil red O staining.

Cell Viability Assay

MDA-MB-453 and RAW 264.3 cell proliferation was measured using an MTT assay. Cells were plated in a 96-well flat-bottom tissue culture plate and incubated for 24, 48, and 72 h. Plated cells were exposed to each extract at a concentration ranging from 5 to 200 μg/mL. After incubating for 24, 48, and 72 h, the plated cells were incubated with MTT (0.5 mg/mL final concentration) for 4 h at 37°C. After discarding all medium from the plates, 100 μL of DMSO were added to each well. The plates were incubated for 5 min at room temperature with shaking until the formazan crystals were completely dissolved. Absorbance of the wells was measured at 540 nm with a UV/VIS spectrophotometric plate reader (Emax; Molecular Devices Co., CA, USA). Viability was defined as the ratio (expressed as a percentage) of the absorbance of the treated cells to that of the untreated cells.

Measurement of Caspase-3 Activity

MDA-MB-453 cells were plated at a density of 7.5×10^5 cells/well in a6-well plate tissue cul-

ture plate and incubated for 24 h. The plated cells were then exposed to each extract at a concentration of 100 μg/mL for 72 h. For the caspase-3 activity analysis, cells were collected by trypsinization and lysed with lysis buffer (25 mM HEPES, pH 7.4; 2.5 mM CHAPS, and 2.5 mM DTT). Next, a 10 μL sample of the lysate was transferred to wells in a 96-well flat-bottom plate and 980μL of reaction solution (20 mM HEPES, pH 7.4; 0.1% CHAPS, 5 mM DTT, and 2 mM EDTA) were added. A peptide with the caspase-3 target motif DEVD bound to the chromophore *p*-nitroanilide was added and the plate was incubated for 1 h. The intensity of the developed color was measuredat 405 nm witha UV/VIS spectrophotometric plate reader (Emax; Molecular Devices).

Oil Red O Staining

Lipid accumulation in differentiated adipocytes was evaluated with Oil red O staining. For this, the cells were gently washed with PBS and fixedas 3.7% paraformaldehyde for 10 min. An Oil red O staining solution (3: 2 mixture of a 0.2% oil red O-isopropanol solution and water) was added to the cells that were then incubated at room temperature for 1 h and subsequently washed with deionized water. To quantify Oil red O uptake, the cells were resolved with isopropanol and their absorbance was measured at 510 nm using a spectrophotometer (M2 microplate reader, Molecular Devices).

Lipolysis Assay

Differentiated 3T3-L1 adipocytes were incubated with indicated concentrations of the extracts for 72 h. The medium was then removed and heated to 70°C for 10 min to inactivate any enzymes released by the cells. Next, themedium was analyzedfor free glycerol using a glycerol reagent (Sigma-Aldrich) according to the manufacturer's instruction.To compare free glycerol contents, the concentrations for each sample werecorrected using total protein levels determined by a Bradford protein assay.

Nitrite Assay

Macrophages were plated at a density of 2×10^5 cells/well in a 96-well culture plate and incubated for 3–4 h in a 5% CO_2 humidified incubator at 37°C.The cells were then treated with LPS (1 μg/mL) to stimulate nitrite production and the extracts at the indicated concentrationsfor 24 h. LPS-stimulated nitrite production inthe macrophages was measured according to the Griess reaction. Briefly, 100 μL of each supernatant of cell was mixed with 100 μL of Griess reagent (1% sulfanilamide with 5% phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride in distilled water). Absorbance of the mixture was measured with a microplate reader (Emax, Molecular Devices) at 540 nm. For this experiment, 10 μM of N-monomethyl arginine (L-NMMA), an iNOS inhibitor, was used as a positive control as previously described (Sutherland et al. 2001).

Statistical Analysis

The data are presented as the mean ±standard deviation (S.D.; n>3). Statistical differences were evaluated using an unpaired Student's t-test. *P*-values < 0.05were considered to be significant.

RESULTS AND DISCUSSION

Anti-cancer Effects of Allium Vegetables

Proliferation of the MDA-MB-453 cellswas measured using an MTT assay after treatment with 100μg/mL of *Allium* vegetable extracts for 72 h (Fig. 1A). Most of the extracts decreased cell viability compared to the untreated cells except for *Allium fistulosum*. The order of the antiproliferation effects of the extractsat a concentration of 100μg/mL for 72 h was as follows: *Allium tuberosum* ≥*Allium macrostemon* >*Allium thumbergii*. The growth of cells exposed to *Allium tuberosum* decreased by 50.6% after treatment with 100 μg/mL for 72 h. Whereas the growth of cells treated with *Allium fistulosum* were increased by 5.68%. Since we found that all but one *Allium* vegetable extract were able to inhibit MDA-MB-453 cancer cell proliferation, we next assessed the induction of apoptosis by measuring the effect of the extracts on caspase-3 activity.This activity, an early biomarker of apoptosis, was measured in MDA-MB-453 cells after 48 h of exposure to the *Allium* vegetables. Some of the extracts increased caspase-3 activity compared to the control (Fig. 1B).

Caspase-3 activity levels in cell treated with 100μg/mL of *Allium tuberosum*, *Allium*

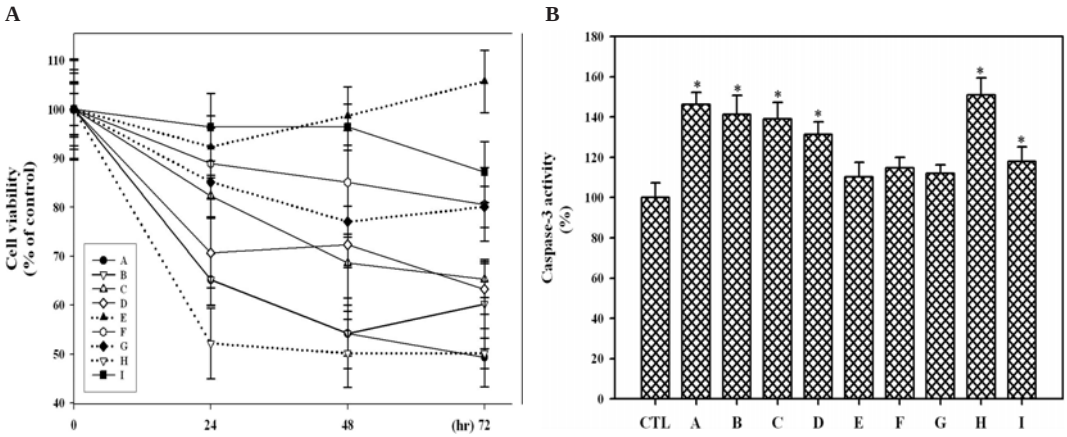

Fig.1. Effects of *Allium* vegetable extracts on the proliferation and caspase-3 activity of MDA-MB-453 cells.
A: *Allium tuberosum*, B: *Allium thumbergii*, C: *Allium sacculiferum*, D: *Allium senescens*, E: *Allium fistulosum*, F: *Allium cepa*, G: *Allium schoenoprasmum* var. *orientale*, H: *Allium macrostemon*, I: *Allium sativum* for. *Pekinense*

macrostemon, and *Allium thumbergii* were 52.1, 46.2 and 41.3%, respectively. These results indicated that treatment with *Allium* vegetables decreased the proliferation of breast cancer cells by regulating caspase-3 activity.

Anti-adipogenic Effects of Allium Vegetables

To determinewhether *Allium* vegetables have anti-obesity effects, we investigated the effects of vegetable extracts on differentiation of preadipocyte 3T3-L1 cells. Most of the tested extracts exhibited anti-adipogenic effects at a concentration of 100μg/mL (Table 1). Specially, 100μg/mL of *Allium tuberosum*, *Allium macrostemon*, and *Allium thumbergii*, which have anticancer effects, inhibited lipid accumulation by 45.6, 57.0 and 64.9%, respectively. Among the different vegetables, green onion (*Allium fistulosum*) extract reduces adipocyte size, fat accumulation, and serum lipid concentrations by down-regulation of the expression of genes involved in lipogenesis in the adipose tissue of obese mice fed a high-fat diet (Sung et al. 2011).

Triglyceride (TG) degradation in lipid droplet and release of glycerol are potential therapeutic target for combating obesity. To determine whether *Allium* vegetable extracts exert lipolytic effects on 3T3-L1 adipocytes, the mature adipocyte was treated with indicated concentration of extract. Glycerol levels in the medium

Table1: Effects of *Allium* vegetable extracts on intracellular lipid accumulation and free glycerol release of 3T3-L1adipocytes

Allium vegetables	Intracellular lipid accumulation(% of MDI control)[a]			Free glycerol release (% of MDI control)[b]		
	10μg/ml	50μg/ml	100μg/ml	10μg/ml	50μg/ml	100μg/ml
A	92.0 ± 3.2	82.2 ± 9.5*	56.4 ± 2.7*	101.8 ± 3.0	97.2 ± 16.0	74.6 ± 9.2*
B	94.5 ± 5.3	70.5 ± 2.6*	35.1 ± 1.7*	114.6 ± 13.0	101.4 ± 8.1	96.9 ± 4.6
C	104.2 ± 8.4	69.7 ± 6.5*	39.9 ± 2.4*	108.4 ± 3.2	114.1 ± 23.2	98.3 ± 14.3
D	103.4 ± 25.9	104.3 ± 8.2	55.3 ± 2.1*	74.3 ± 5.8*	69.6 ± 16.2*	86.2 ± 11.1
E	77.9 ± 7.9	59.7 ± 12.6*	36.7 ± 6.3*	108.3 ± 12.5	80.5 ± 14.9	84.0 ± 27.8
F	75.5 ± 6.5	63.2 ± 1.4*	38.4 ± 2.6*	88.5 ± 3.7	98.1 ± 6.3	89.7 ± 17.9
G	93.1 ± 6.9	93.9 ± 5.9	53.8 ± 5.5*	91.9 ± 15.1	79.1 ± 5.4	61.3 ± 10.6*
H	95.4 ± 4.9	77.6 ± 10.0*	43.0 ± 2.9*	94.8 ± 11.5	84.9 ± 13.3	79.2 ± 10.1
I	98.5 ± 9.3	90.9 ± 5.3	86.6 ± 6.2*	103.6 ± 4.8	111.8 ± 5.8	101.0 ± 10.4

A: *Allium tuberosum*, B: *Allium thumbergii*, C: *Allium sacculiferum*, D: *Allium senescens*, E: *Allium fistulosum*, F: *Allium cepa*, G: *Allium schoenoprasmum* var. *orientale*, H: *Allium macrostemon*, I: *Allium sativum* for.*Pekinense*
[a]Intracellular lipid accumulation of the MDI-treated control was 100% ± 4.1%
[b]Free glycerol release values are expressed as a percentage of the MDI-treated group (control).
*$p < 0.05$ versus the control (MDI-treated group)

after treatment with the extracts were measured. As shown Table 1, most of the *Allium* vegetables decreased glycerol secretion. *Allium tuberosum* significantly decreased free glycerol release in a dose-dependent manner ($p<0.05$). Anti-adipogenic effects of *Allium* vegetables are needed further studies including mechanisms studies of anti-adipogenesis and TG degradation on adipocytes.

Adipose tissues secreted estrogen, adiponectin, leptin, and myriad of less well-characterized epithelial cell mitogens that may also stimulate breast tumor development (Bernstein and Ross 1993; Jones et al. 1997). Jones et al. (1997) suggested that obesity-enhanced endocrine signaling increases breast cancer risk (Jones et al. 1997). Some studies have identified key roles of estrogen-relatedreceptor α (ERRá) in regulating mitochondrial biogenesis, fatty acid oxidation, and oxidative phosphorylation (Schreiber et al. 2004; Vega and Kelly 1997). ERRα knock-out mice display significant reductions of peripheral fat deposits and resistance to high-fat diet-induced obesity (Luo et al. 2003). Therefore, recent studies in 3T3-L1 cells have showed that ERRα can stimulate adipogenesis by enhancing triglyceride (TG) accumulation and elevating the expression of several adipogenic marker genes (Ijichi et al. 2007). Dapeng et al. (2001) determined that ERRα promotes both TG accumulation and adipogenesis (Dapeng et al. 2001). Thus, anti-adipogenic effects might not irrelevant to estrogen-related receptors. However, there is no reason for which breast cancer associated with obesity. And the evidence for supporting anti-cancer and anti-obesity effects of *Allium vegetables* is exiguous.

Anti-inflammatory Effects of Allium Vegetables

The antioxidant effects of flavonoid have been studied by evaluating reactive oxygen species (ROS) scavenging activitiesand iron ion chelating properties (Morel et al. 1993). Additionally, their ability to inhibit lipid oxidation has been widely investigated in chemical and *in vitro* cellular systems. NO, an important inflammatory mediator, is also involved in the pathophysiology of inflammatory joint disease and plays a key role in cartilage catabolism mediated by inflammation (Goldring et al. 2011). In addition, NO synthesis by iNOS is stimulated in various animal cells and tissues by activating factors such as LPS or cytokines (Nathan 1992). NO participates in various biological processes, including inflammation and immunoregulation, associated with rheumatoid arthritis (RA). Therefore, inhibition of NO production by iNOS may have potential therapeutic value for treating inflammation and RA.

Eight *Allium* vegetables extracts did not affect cell viability within tested concentration. To investigate the anti-inflammatory effect of *Allium* vegetables, we examined whether extracts could modulate NO synthesis in LPS-stimulated cultures of macrophages cells (Table 2). Compared to the control, none of the extracts were able to inhibit NO production in the LPS-treated macrophages. *Allium tuberosum*, *Allium macrostemon*, and *Allium thumbergii*, which were shown to have anti-cancer and anti-adipogenic effects, did not affect NO production in the RAW264.7 cells. Results of this experiment indicated that *Allium* vegetables do not modulate

Table 2: Effects of *Allium* vegetables on the viability and nitric oxide (NO) production of lipopolysaccharide (LPS)-stimulated macrophages

Allium vegetables	*CellViability(Control of %)*[a]		*NO production (Control of %)*[b]	
	50μg/ml	*100μg/ml*	*50μg/ml*	*100μg/ml*
A	104.6 ± 4.1	106.5 ± 3.0	96.6 ± 4.2	89.5 ± 5.3
B	104.6 ± 8.8	109.4 ± 4.6	100.1 ± 2.9	101.5 ± 0.9
C	111.1 ± 5.0	111.4 ± 5.5	98.9 ± 1.6	94.2 ± 4.2
D	106.7 ± 5.2	111.8 ± 2.1	96.0 ± 9.0	82.6 ± 4.0
E	111.2 ± 5.4	100.1 ± 6.9	89.8 ± 3.7	88.8 ± 3.7
F	109.1 ± 3.8	112.8 ± 3.5	105.6 ± 3.7	97.8 ± 6.5
G	109.4 ± 3.6	109.8 ± 2.3	95.6 ± 4.8	94.4 ± 4.9
H	102.3 ± 1.2	106.8 ± 2.3	106.6 ± 6.8	94.0 ± 5.7
I	111.4 ± 1.9	114.0 ± 3.9	96.4 ± 6.7	91.7 ± 4.0

A: *Allium tuberosum*, B: *Allium thumbergii*, C: *Allium sacculiferum*, D: *Allium senescens*, E: *Allium fistulosum*, F: *Allium cepa*, G: *Allium schoenoprasmum* var. *orientale*, H: *Allium macrostemon*, I: *Allium sativum* for. *Pekinense*

[a]Viability is expressed as a percentage for treated cells to untreated cells.

[b]Nitric oxide (NO) production values are expressed as a percentage of the LPS-stimulated group (control).

NO-mediated inflammatory responses. Taken together, our study demonstrated that *Allium* vegetables may be a useful as functional food candidate for management of cancer and obesity.

ACKNOWLEDGEMENTS

This study is supported by National Research Foundation of Korea (NRF) grant (no. 2011-0017017) funded by the Republic of Korea government [MEST] in part from the Priority Research Centers Programs through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0031386).

REFERENCES

Agarwal KC 1996. Therapeutic actions of garlic constituents. *Med Res Rev,* 16: 111-124.

Bernstein L, Ross R 1993. Endogenous hormones and breast cancer risk. *Epidemiol Rev,* 15: 48-65.

Bianchini F, Vainio H 2001. *Allium* vegetables and organosulfur compounds: Do they help prevent cancer? *Environ Health Perspect*, 109: 893-902.

Dapeng Ju, Jingjing He, Lili Zhao et al. 2012. Estrogen related receptor α-induced adipogenesis is PGC-1β-dependent. *Mol Biol Rep,* 39: 3343-3354.

Goldring MB, Otero M, Plumb DA et al. 2011. Roles of inflammatory and anabolic cytokines in cartilage metabolism: signals and multiple effectors converge upon MMP-13 regulation. *Osteoarthritis*, 21: 202-220.

Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. *Lancet*, 342: 1007–1011.

Ijichi N, Ikeda K, Horie-Inoue K et al. 2007. Estrogen-related receptor alpha modulates the expression of adipogenesis-related genes during adipocyte differentiation. *Biochem Biophys Res Commun*, 358: 813-818.

Jones B, Kasi S, Curnen M et al. 1997. Severe obesity as an explanatory factor for the black/white difference in stage at diagnosis of breast cancer. *Am J Epidemiol*, 146: 394-404.

Kim KM, Chun SB, Koo MS et al. 2001. Differential regulation of NO availability from macrophages and endothelial cells by the garlic component S-allyl cysteine. *Free Radical Biol Med*, 30: 747-756.

Knekt P, Kumpulainen J, Jarvinen R et al. 2002. Flavonoid intake and risk of chronic diseases. *Am J ClinNutr*, 76: 560-568.

Luo JM, Sladek R, Carrier J et al. 2003. Reduced fat mass in mice lacking orphan nuclear receptor estrogen-related receptor alpha. *Mol Cell Biol*, 23: 7947-7956.

Milner JA 2001a. A historical perspective on garlic and cancer. *J Nutr*, 131(3s): 1027S-31S.

Milner JA 2001b. Mechanisms by which garlic and allyl sulfur compounds suppress carcinogen bioactivation. Garlic and carcinogenesis. *Adv Exp Med Biol*, 492: 69-81.

Mink PJ, Scrafford CG, Barraj LM et al. 2007. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. *Am J Clin Nutr*, 85: 895-909.

Morel I, Lescoat G, Cogrel P et al. 1993.Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. *Biochem Pharmacol*, 45: 13-19.

Nathan C 1992. Nitric oxide as a secretory product of mammalian cells. *FASEB J*, 6: 3051-3064.

Rose P, Whiteman M, Moore PK, Zhu YZ 2005. Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus Allium: the chemistry of potential therapeutic agents. *Nat Prod Rep*, 22: 351-368.

Schreiber SN, Emter R, Hock MB et al. 2004. The estrogenrelated receptor alpha (ERR alpha) functions in PPAR gamma coactivator 1 alpha (PGC-1 alpha)-induced mitochondrial biogenesis. *Proc Natl Acad Sci USA*, 101: 6472-6477.

Sengupta A, Ghosh S, Bhattacharjee S 2004. *Allium* vegetables in cancer prevention: An overview. *Asian Pac J Cancer Prev*, 5: 237-245.

Song HP, Shim SL, Jung IS et al. 2009. Analysis of volatile organosulfur compounds in Korean *Allium* species. *Korean J Food Preserv*, 16: 929-937.

Sung YY, Yoon T, Kim SJ, Yang WK, Kim HK 2011. Anti-obesity activity of *Allium fistulosum* L. extract by down-regulation of the expression of lipogenic genes in high-fat diet-induced obese mice. *Mol Med Report*, 4: 431-435.

Sutherland H, Khundkar R, Zolle O et al. 2001. A Fluorescence-based method for measuring nitric oxide in extracts of skeletal muscle. *Nitric Oxide*, 5: 475-481.

Vega RB, Kelly DP 1997. A role for estrogen-related receptor alpha in the control of mitochondrial fatty acid beta-oxidation during brown adipocyte differentiation. *J Biol Chem,* 272: 31693-31699.