Food Sci. Biotechnol. Vol. 16, No. 5, pp. 858 ~ 862 (2007)

RESEARCH NOTE

Food Science and Biotechnology

© The Korean Society of Food Science and Technology

Protective Effect of *Schizonepeta tenuifolia* Briquet Extracts on Oxidative DNA Damage in Human Leucocytes and on Hydrogen Peroxide-induced Cytotoxicity in PC12 Cells

Mi-Young Yoon, Hyun-Jin Lee[1], Bo-Bae Lee, Sang-Myeon Lee[2], Ju-Young Kim, Yongseong Kim[3], Eunju Park[1], and Hae-Ryong Park*

Department of Food Science and Biotechnology, Kyungnam University, Masan, Gyeongnam 631-701, Korea
[1]Department of Food and Nutrition, Kyungnam University, Masan, Gyeongnam 631-701, Korea
[2]Changshin High School, Masan, Gyeongnam 631-803, Korea
[3]Department of Chemistry, Kyungnam University, Masan, Gyeongnam 631-701, Korea

Abstract The present study was conducted to examine the antioxidant activities and neuroprotective effects of methanolic extracts from *Schizonepeta tenuifolia* Briquet (STE). STE (100 μg/mL) showed 43.33 μM of total phenolic content, 64.43% of radical scavenging activity, and 0.157 of reducing power. In addition, the effect of STE on H_2O_2-induced DNA damage in human leucocytes was evaluated by the comet assay, where STE was a dose dependent inhibitor of DNA damage induced by 200 μM of H_2O_2. The protective effect of STE against H_2O_2-induced oxidative damage on PC12 cells was investigated by an 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) reduction assay and lactate dehydrogenase (LDH) release assays. After 2 hr of cell exposure to H_2O_2 (500 μM), a marked reduction in cell survival was observed. However, this reduction was significantly prevented by 1-50 μg/mL of STE. Therefore, these results suggest that STE could be a new antioxidant candidate against neuronal diseases.

Keywords: *Schizonepeta tenuifolia* Briquet, antioxidant, human leucocyte, hydrogen peroxide, PC12 cell

Introduction

Oxidative stress has long been known as an inducer of neurodegenerative disorders. There is evidence that oxidative stress, including the action of free radicals, plays a key role in Alzheimer's disease (AD) and Parkinson' disease (PD) (1-5). Oxidative stress is mediated by reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anion, and hydroxyl radicals, which are generated as byproducts of normal and irregular metabolic processes that utilize molecular oxygen (6-9). ROS are known to attack proteins, deoxynucleic acid, polyunsaturated fatty acids, and lipid membranes. Thereby, these oxidative stress-induced damages disrupt cellular function and integrity (10-14). The brain and nervous system are particularly vulnerable to free radical damage for a number of reasons. For example, the membrane lipids in the brain contain high levels of polyunsaturated fatty acid, side chains, which are very prone to free radical attack. Brain tissue also consumes large quantities of total oxygen for its relatively small weight, thus contributing further to the formation of ROS. Based on this, many reports suggest that oxidative stress may play an important role in the pathogenesis of certain degenerative neurologic disorders (15).

Currently, there is no cure for neurodegenerative disorders and most available therapies focus on symptomatic treatment only. Thus, a number of researchers have attempted to prevent or diminish ROS-induced damage, and utilize natural products for treatments that prevent ROS generation and reduce neuronal damage (16). Many natural products are shown to have pharmacological applications, and potentials for chemotherapeutic use (17). Plant products are used extensively in testing due to their low toxicity and considerable medicinal value (18).

Consequently, we screened 435 varieties of herbal medicines, and determined that *Schizonepeta tenuifolia* Briquet (ST) exerted a neuroprotective effect against oxidative stress-induced cell death in PC12 cells. ST is an aromatic annual herb that reaches 60-80 cm in height. The stem is erect and quadrangular, with the upper part branching and the lower part purplish. The whole plant is covered with a shallow pubescence. Several major components and their structures has been isolated and identified from ST, such as ursolic acid, daucosterol (19), and volatile oils (menthone and pulegone) (20). Reported pharmacological effects of ST include antieczematic effects and the releasing of body aches (21), as well as anti-influenza (22) and antipruritic effects (23). However, the neuroprotective effects of ST had not been assessed until now.

The purpose of this study was to determine the antioxidant activity of ST and its properties as a free radical scavenger, as well as to assess its possible protective effects against oxidative stress on neurons.

Materials and Methods

Materials *Schizonepeta tenuifolia* Briquet (ST) was obtained from Kumkang Pharm Co., Ltd., Masan, Korea. Hydrogen peroxide solution (H_2O_2), 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), dimethyl

*Corresponding author: Tel: 82-55-249-2689; Fax: 82-55-249-2995
E-mail: parkhy@kyungnam.ac.kr
Received March 16, 2007; accepted May 25, 2007

sulfoxide (DMSO), 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium chloride, and potassium phosphate were obtained by Sigma Chemical Co. (St. Louis, MO, USA). Lactate dehydrogenase (LDH) release assay kit and Folin-Ciocalteu reagent were purchased by Wako Pure Chemical Industries, Ltd. (Osaka, Japan). PC12 cells were purchased from Korean Cell Line Bank (KCLB, Seoul, Korea). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were obtained from Gibco BRL (Grand Island, NY, USA). All organic solvents and other chemicals were of analytical grade or complied with the standards needed for cell culture experiments.

Preparation of methanolic extracts from ST Five g of ST were extracted with 100 mL of methanol for 2 days at room temperature and filtered through a Whatman No. 1 filter paper (Advantec, Tokyo, Japan). Methanol solvent was then removed by evaporation, and the dried methanol extract was obtained (STE). The methanol extract was then dissolved in DMSO with concentration 10 mg/mL for experiments. It was designated as STE.

Total phenolic contents (TPC) TPC of STE were determined according to the method of Gutfinger (24). STE (100 μg/mL) was mixed with 1 mL of 2% Na_2CO_3. After 3 min, 0.2 mL of 50% Folin-Ciocalteu reagent was added. After 30 min of standing, the mixture was centrifuged 13,400×g for 10 min. The absorbance of supernatant was measured with a spectrophotometer (UV-1601; Shimadzu, Tokyo, Japan) at 750 nm. TPC were expressed as gallic acid equivalents.

DPPH radical scavenging activity (RSA) The DPPH RSA of STE was determined according to the method of Lee *et al.* (25). After 0.1 mL of each concentration of STE had been mixed with 0.9 mL of 0.041 mM DPPH in ethanol for 30 min, the absorbance of the sample was measured at 517 nm by a spectrophotometer (UV-1601; Shimadzu). RSA was percentage was calculated by using the following formula:

$$\% \text{ DPPH RSA} = [1 - (\text{sample OD}/\text{control OD})] \times 100$$

Where sample OD is the absorbance in the presence of STE and control OD is the absorbance of the control reaction.

Reducing power (RP) RP of STE was determined according to the method of Oyaizu (26). STE (1 mL), sodium phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide (1 mL, 10 mg/mL) were mixed and incubated at 50°C for 20 min. Trichloroacetic acid (1 mL, 100 mg/mL) was added to the mixture and centrifuged at 13,400×g for 5 min. The supernatant (1 mL) was mixed with distilled water (1 mL) and ferric chloride (0.1 mL, 1 mg/mL), and then its absorbance was measured at 700 nm.

Preparation of human leucocytes Blood samples were obtained from 3 healthy male volunteers. Five mL of fresh whole blood was added to 5 mL of a phosphorous buffered saline (PBS) and layered onto 5 mL of Histopaque 1077. After a centrifugation for 30 min at 400×g at room temperature, the leucocytes were collected from just above the boundary with Histopaque 1077, and washed with 5 mL PBS. Finally, they were used for a comet assay or resuspended in a freezing medium (90% fetal calf serum, 10% DMSO) at 6×10^6 cells/mL. The cells were frozen to -80°C by using a Nalgene Cryo 1°C freezing container (Nalgene, Rochester, NY, USA) and stored in liquid nitrogen. The cell were thawed rapidly prior to each experiment in a water bath at 37°C.

Treatment of human leucocytes Leucocytes (2×10^4 cell/mL) were incubated with STE dissolved in PBS and diluted to concentrations of 0, 1, 5, and 10 μg/mL for 30 min at 37°C in a dark incubator. For an oxidative stimulus they were then resuspended in PBS with 200 μM H_2O_2 for 5 min on ice. After each treatment, the samples were washed with PBS. All the experiments were repeated 3 times with leucocytes from each of 3 donors on a separate day. One % DMSO without oxidative stimulus was treated for negative control.

Determination of DNA damage (Comet assay) The alkaline comet assay was conducted according to Singh *et al.* (27) with a little modification. The cell suspension was mixed with 75 μL of 0.5% low melting agarose (LMA), and added to the slides precoated with 1% normal melting agarose. After a solidification of the agarose, the slides were covered with another 75 μL of 0.5% LMA, and then they were immersed in a lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, and 1% sodium laurylasarcosine; 1% Triton X-100 and 10% DMSO) for 1 hr at 4°C. The slides were then placed into an electrophoresis tank containing 300 mM NaOH and 10 mM Na_2EDTA (pH 13.0) for 40 min for a DNA extrapolation. For an electrophoresis of the DNA, an electric current of 25 V/300 mA was applied for 20 min at 4°C. The slides were washed 3 times with a neutralizing buffer (0.4 M Tris, pH 7.5) for 5 min at 4°C, and then they were treated with ethanol for another 5 min before a staining with 50 μL of ethidium bromide (20 μg/mL). Measurements were made by an image analysis (Kinetic Imaging, Komet 5.0, UK) and a fluorescence microscope (Leica DM LB, Wetzlar, Germany), for determining the percentage of the fluorescence in the tail (tail intensity, TI; 50 cells from each of 2 replicate slides). Cell viability which was measured by the trypan blue exclusion test was above 95% for all the treatments.

Cell culture and treatments Rat pheochromocytoma PC12 cells were maintained in DMEM supplemented with 10% FBS, 5% horse serum (HS), 100 U/mL penicillin, 100 μg/mL streptomycin, and 3.7 g/mL $NaHCO_3$. PC12 cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2. In all experiments, cells were treated with STE before H_2O_2-stress for the indicated times. STE were dissolved in DMSO and added to the culture medium so that the final concentration of DMSO was less than 1%.

MTT reduction assay for cell survival Cell viability was measured with blue formazan that was metabolized from colorless MTT by mitochondrial dehydrogenases,

which are active only in live cells. PC12 cells were preincubated in 96-well plates at a density of 1×10^5 cells/mL for 24 hr. Cells with various concentrations of STE were treated with H_2O_2 for 2 hr. After incubation, MTT reagent (5 mg/mL) was added to each of the wells, and the plate was incubated for an additional about 2 hr at 37°C. The intracellular formazan product was dissolved in 100 μL of DMSO. The absorbency of each well was then measured at 540 nm using the enzyme-linked immunosorbent assay (ELISA) reader (model 680; Bio-Rad Lab., Hercules, CA, USA), and the percentage viability was calculated.

Lactate dehydrogenase release assay Cytotoxicity was determined by measuring the release of LDH. PC12 cells with various concentrations of STE were treated with H_2O_2 for 2 hr and the supernatant was used to assay LDH activity. The reaction was initiated by mixing 50 μL of cell-free supernatant with potassium phosphate buffer containing nicotinamide adenine dinucleotide (NADH) and sodium pyruvate in a final volume of 100 μL to 96-well plate. The absorbance of sample was read at 490 nm (model 680; Bio-Rad Lab.). Data were normalized to the activity of LDH released from H_2O_2-treated cells (obtained separate plating).

Observation of morphologic changes PC12 cells were seeded at 1×10^5 cells/well in 6-well plate and incubated overnight in a humidified atmosphere of 5% CO_2 in air at 37°C. Cells were pretreated with various concentrations of STE. After incubation for 30 min, cells were treated with 500 μM H_2O_2 for 2 hr. The cellular morphology was observed using phase-contrast microscope (Nikon, Tokyo, Japan). Photographs were taken at a magnification of 100×.

Statistical analysis All data are the means of 3 determinations and the data were analyzed using the SPSS package for Windows (Version 11.5; Chicago, IL, USA). The data were evaluated by one-way analysis of variance (ANOVA) followed by Scheffe's test. p-Value of less than 0.05 was considered as significant.

Results and Discussion

Antioxidant activity of STE Polyphenols compounds in fruits and vegetables are known to play important roles in preventing degenerative diseases when consumed as part of a daily diet (28, 29). TPC of extracts of STE was determined using the standard curve (R^2=0.9920) for gallic acid. TPC of STE (100 μg/mL) was 43.33 μM GAE, which is lower than that (125.93 μM) of *Psoralea corylifolia* extract (30) but higher than that (35.6 μM) of defatted sesame meal extract (31).

Radical scavengers were evaluated by their reactivity toward a stable free radical DPPH. Free radicals are produced continuously in cells, causing oxidative damage to biomolecules. This process is held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged lipids and proteins (32). The organic compound DPPH is a radical in which there is an unpaired/odd electron located on one of the nitrogen atoms. The DPPH RSA of STE was also increased with amount of STE (Table 1). For example, RSA of 5 and 100 μg STE were 4.42 and 64.43%, respectively.

Table 1. DPPH radical scavenging activity (RSA), and reducing power (RP) of extract of *Schizonepeta tenuifolia* Briquet[1)]

	Sample amount (μg)			
	5	10	50	100
DPPH (RSA %)	4.42±0.31	9.12±0.11	46.20±0.43	64.43±0.11
RP (OD)	0.072±0.001	0.080±0.002	0.117±0.001	0.157±0.001

[1)]All measurements were done in triplicate.

The power of certain antioxidants is associated with their reducing power (RP) (33). Duh (34) reported that the reducing properties of antioxidants are generally associated with the presence of reductants. The RP of STE also showed amount dependent increase (Table 1). RP at the amount of 5, 10, 50, and 100 μg STE were 0.072, 0.080, 0.117, and 0.157, respectively.

There were few reports about chemical compositions of ST, while phenolic compounds such as 5, 7-dihydroxy-6, 4'-dimethoxyflavone and 5, 7- dihydroxy-6, 3', 4'-trimethoxyflavone were recently identified from spikes of ST (35). The significant antioxidant activities of STE might be due to the synergistic effect of various phenolic compounds.

Effect of STE on oxidative DNA damage to human leucocytes These results are shown visually in Fig. 1A, which represents the comet image of the results of each treatment in human leucocytes. Two hundred μM of H_2O_2 significantly induced DNA strand breaks (33.7±3.6% fluorescence in tail) in comparison to the untreated control

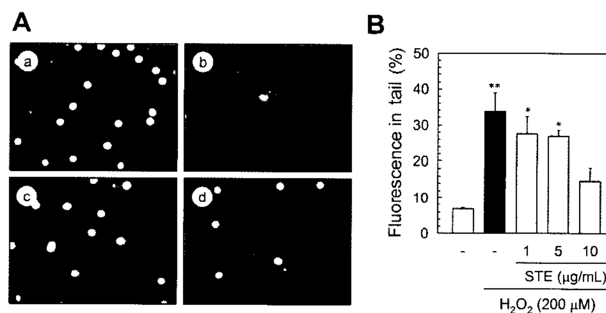

Fig. 1. Effect of supplementation *in vitro* with different concentrations of STE on 200 μM H_2O_2-induced human leucocytes DNA damage by comet assay. A. Comet images of human leucocytes. (a) normal control, (b) 200 μM H_2O_2-treated positive control, (c) leucocytes treated with 5 μg/mL of STE+200 μM H_2O_2, (d) leucocytes treated with 10 μg/mL of STE+200 μM H_2O_2. B. Values are mean with standard deviation of triplicate experiments with leucocytes from each of different donors and the mean values of DNA damage (tail intensity) from each treatment were compared using one-way ANOVA followed by Scheffe's test. Values not sharing the same letter are significantly different from one another. Significant vs. control of untreated cells (*p<0.05; **p<0.01).

(6.9±0.1% fluorescence in tail). Pretreatment of the cells for 30 min with STE significantly reduced the genotoxicity of H_2O_2 measured by DNA strand breakage (Fig. 1B). This increase of the DNA damage induced by H_2O_2 was significantly inhibited in a dose dependent manner by a pretreatment of the cells for 30 min with STE at concentrations of 1, 5, and 10 μg/mL in PBS. The protective effect of the STE increased significantly at 10 μg/mL by 56.5% when compared to that of the positive control.

STE protection of PC12 cells against H_2O_2-induced cytotoxicity To characterize the effects of STE on cell viability in H_2O_2-stressed PC12 cells, the cells were incubated with STE and 500 μM of H_2O_2, and morphological alterations were verified via a phase-contrast microscope. As shown in Fig. 2, the PC12 cells had round cell bodies with clear edges and fine dendritic networks. However, after 2 hr of exposure to 500 μM of H_2O_2, many cells showed cytoplasmic shrinkage, and either detached from each other or floated in the medium. In contrast, the cultures exposed to H_2O_2 in the presence of STE appeared remarkably preserved, indicating that STE offered protection to the H_2O_2-stressed PC12 cells.

Next, we attempted to determine the neuronal protection effects of STE via an MTT reduction assay and a cytoplasmic LDH release assay. As shown in Fig. 3A, PC12 cells treated for 2 hr with 500 μM of H_2O_2 had reductions in cell viability of 44.2% compared with the control. However, after 2 hr of exposure to 500 μM of H_2O_2 with various concentrations of STE, the cell viability increased from 10 to 40% when compared to the viability in the H_2O_2-stressed PC12 cells (Fig. 3A). To further investigate the protective effects of STE we performed the LDH assay, another indicator of cell toxicity. Here, the PC12 cells were exposed to various concentrations of STE and H_2O_2 for 2 hr. As expected, STE reduced cell damage in a dose-dependent manner, as was evident by a 10-50% decrease in LDH release from the H_2O_2-stressed PC12 cells (Fig. 3B).

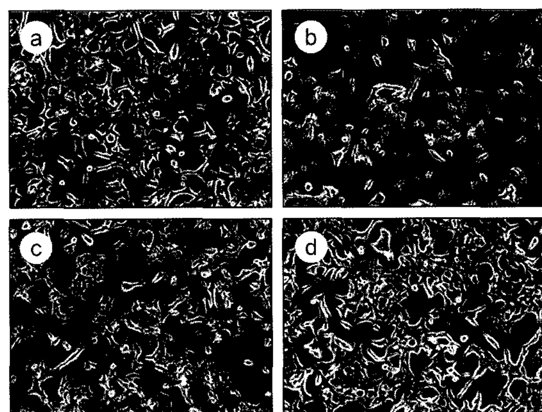

Fig. 2. Effect of STE on H_2O_2-induced morphological alterations of PC12 cells. (A) normal control, (B) 500 μM H_2O_2-treated positive control, (C) PC12 cells treated with 10 μg/mL of STE+ 500 μM H_2O_2, (D) PC12 cells treated with 50 μg/mL of STE+500 μM H_2O_2. Photographs were taken with a phase-contrast microscope at 100x magnification. The results are representative of 2 independent experiments.

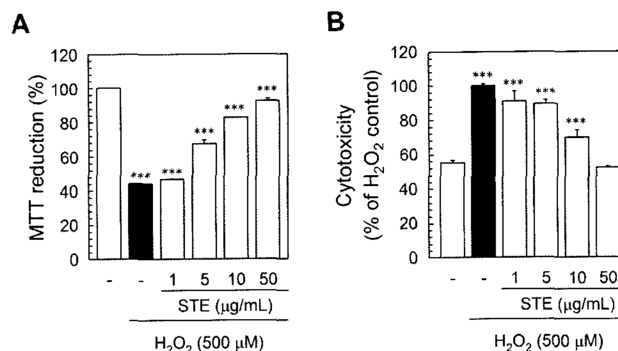

Fig. 3. Cell viability effect of STE on H_2O_2-induced cytotoxicity in PC12 cell system. (A) MTT reduction assay, (B) LDH release assay. PC12 cells were pretreated for 30 min with various concentrations. The cells were then treated with 500 mM H_2O_2 for 2 hr. After a MTT assay, the MTT reduction rate was calculated by setting each of control survivals. As the results of LDH release assay, data were normalized to the activity of LDH released from H_2O_2-treated cells (100%) (obtained separate plating). Data represent the relative values±SD of 3 independent experiments. ***Significant vs. control of untreated cells ($p<0.001$).

Thus, these results demonstrate that STE protected PC12 cells from oxidative stress.

Acknowledgments

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (No. R01-2006-000-11361-0).

References

1. Floyd RA. Antioxidants, oxidative stress, and degenerative neurological disorders. Exp. Biol. Med. 222: 236-245 (1999)
2. Behl C. Alzheimer's disease and oxidative stress: Implications for novel therapeutic approaches. Prog. Neurobiol. 57: 301-323 (1999)
3. Olanow CW, Tatton WG. Etiology and pathogenesis of Parkinson's disease. Annu. Rev. Neurosci. 22: 123-144 (1999)
4. Chai Y, Niu L, Sun XL, Ding JH, Hu G. Iptakalim protects PC12 cell against H_2O_2-induced oxidative injury via opening mitochondrial ATP-sensitive potassium channel. Biochem. Bioph. Res. Co. 350: 307-314 (2006)
5. Heo HJ, Lee CY. Strawberry and its anthocyanins reduce oxidative stress-induced apoptosis in PC12 cells. J. Agr. Food Chem. 53: 1984-1989 (2005)
6. Lee EJ, Kim KS, Jung HY, Kim DH, Jang HD. Antioxidant activities of garlic (*Allum sativum* L.) with growing districts. Food Sci. Biotechnol. 14: 123-130 (2005)
7. Shim S, Chung J, Lee J, Hwang K, Sone J, Hong B, Cho H, Jun W. Hepatopotective effects of black rice on superoxide anion radicals in HepG2 cells. Food Sci. Biotechnol. 15: 993-996 (2006)
8. Hou RC, Huang HM, Tzen JT, Jeng KC. Protective effects of sesamin and sesamolin on hypoxic neuronal and PC12 cells. J. Neurosci. Res. 74: 123-133 (2003)
9. Shang YZ, Qin BW, Cheng JJ, Miao H. Prevention of oxidative injury by flavonoids from stems and leaves of *Scutellaria Baicalensis Georgi* in PC12 cells. Phytother. Res. 20: 53-57 (2006)
10. Guan S, Bao YM, Jiang B, An LJ. Protective effect of protocatechuic acid from *Alpinia oxyphylla* on hydrogen peroxide-induced oxidative PC12 cell death. Eur. J. Pharmacol. 538: 73-79 (2006)
11. Zhang HJ, Tang XC. Huperzine B, a novel acetylcholinesterase

inhibitor, attenuates hydrogen peroxide induced injury in PC12 cells. Neurosci. Lett. 292: 41-44 (2000)
12. Choi YM, Ku JB, Chang HB, Lee J. Antioxidant activities and total phenolics of ethanol extracts from several edible mushrooms produced in Korea. Food Sci. Biotechnol. 14: 700-703 (2005)
13. Yang Y, Wang J, Xu C, Pan H, Zhang Z. Maltol inhibits apoptosis of human neuroblastoma cells induced by hydrogen peroxide. J. Biochem. Mol. Biol. 39: 145-149 (2006)
14. Rao AV, Balachandran B. Role of oxidative stress and antioxidants in neurodegenerative diseases. Nutr. Neurosci. 5: 291-309 (2002)
15. Benedi J, Arroyo R, Romero C, Martin-Aragon S, Villar AM. Antioxidant properties and protective effects of a standardized extract of *Hypericum perforatum* on hydrogen peroxide-induced oxidative damage in PC12 cells. Life Sci. 75: 1263-1276 (2004)
16. Gouaze V, Yu JY, Bleicher RJ, Han TY, Liu YY, Wang H, Gottesman MM, Bitterman A, Giuliano AE, Cabot MC. Overexpression of glucosylceramide synthase and P-glycoprotein in cancer cells selected for resistance to natural product chemotherapy. Mol. Cancer Ther. 3: 633-639 (2004)
17. Kodach LL, Bos CL, Duran N, Peppelenbosch MP, Ferreira CV, Hardwick JCH. Violacein synergistically increases 5-fluorouracil cytotoxicity, induces apoptosis, and inhibits Akt-mediated signal transduction in human colorectal cancer cells. Carcinogenesis 27: 508-516 (2006)
18. Fung D, Lau CB. *Schizonepeta tenuifolia*: Chemistry, pharmacology, and clinical applications. J. Clin. Pharmacol. 42: 30-36 (2002)
19. Zhang L, Feng Y, Ding A. The research on the chemical components of *Schizonepeta tenuifolia* Briq. Zhong Yao Cai 24: 183-184 (2001)
20. Lin R, Tian J, Huang G, Li T, Li F. Analysis of menthol in three traditional Chinese medicinal herbs and their compound formulation by GC-MS. Biomed. Chromatogr. 16: 229-233 (2002)
21. Kirby AJ, Schmidt RJ. The antioxidant activity of Chinese herbs for eczema and of placebo herbs-I. J. Ethnopharmacol. 56: 103-108 (1997)
22. Wang X, Jia W, Zhao A, Wang X. Anti-influenza agents from plants and traditional Chinese medicine. Phytother. Res. 20: 335-341 (2006)
23. Tohda C, Kakihara Y, Komatsu K, Kuraishi Y. Inhibitory effects of methanol extracts of herbal medicines on substance P-induced itch-scratch response. Biol. Pharm. Bull. 23: 599-601 (2000)
24. Gutfinger T. Polyphenol in olive oils. J. Am. Oil Chem. Soc. 58: 996-998 (1981)
25. Lee SC, Kim JH, Jeong SM, Kim DR, Ha, JU, Nam KC, Ahn DU. Effect of far-infrared radiation on the antioxidant activity of rice hulls. J. Agr. Food Chem. 51: 4400-4403 (2003)
26. Oyaizu M. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jap. J. Nutr. 44: 307-315 (1986)
27. Singh PN, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175: 184-191 (1988)
28. Jung S, Lee N, Kim SJ, Han D. Screening of tyrosinase inhibitor from plants. Korean J. Food Sci. Technol. 27: 891-896 (1995)
29. Arnous A, Makris DP, Kefakas P. Effect of principal polyphenolic components in relation antioxidant characteristics of aged red winds. J. Agr. Food Chem. 49: 5736-5742 (2001)
30. Yoon MY, Lee BB, Kim JY, Kim Y, Park E, Lee SC, Park HR. Antioxidant activity and neuroprotective effect of *Psoralea corylifolia* Linne extracts. Korean J. Pharmacogn. 38: 84-89 (2007)
31. Jeong SM, Kim SY, Kim DR, Nam KC, Ahn DU, Lee SC. Effect of seed roasting conditions on the antioxidant activity of defatted sesame meal extracts. J. Food Sci. 69: 377-381 (2004)
32. Grune T, Davies KJ. Breakdown of oxidized proteins as a part of secondary antioxidant defenses in mammalian cells. Biofactors 6: 165-172 (1997)
33. Jayaprakasha GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. J. Food Sci. 73: 285-290 (2001)
34. Duh PD. Antioxidant activity of budrock (*Arctium lappa* L.): Its scavenging effect on free radical and active oxygen. J. Am. Oil Chem. Soc. 75: 455-461 (1998)
35. Zhang YH, Zhou L, Shi RB, Guo YJ, Dong Y. Studies on chemical constituents in spikes *of Schizonepeta tenuifolia*. Zhongguo Zhong Yao Za Zhi 31: 1247-1249 (2006)