Food Sci. Biotechnol. Vol. 17, No. 4, pp. 805 ~ 808 (2008)

Food Science and Biotechnology

© The Korean Society of Food Science and Technology

Isolation of Ginsenoside Rh1 and Compound K from Fermented Ginseng and Efficacy Assessment on Systemic Anaphylactic Shock

Mi Soon Kim[1], Bin Kwon[2], Myeong Soo Park[2,3], and Geun Eog Ji[1,2*]

[1]*Department of Food and Nutrition, Research Institute of Human Ecology, Seoul National University, Seoul 151-742, Korea*
[2]*Research Center, Bifido Inc., Hongcheon, Gangwon 250-804, Korea*
[3]*Department of Hotel Culinary Art, Anyang Technical College, Anyang, Gyeonggi 430-749, Korea*

Abstract Ginsenosides are responsible for the pharmacological and biological activities of ginseng. In this study, ginsenoside Rh1 and compound K were isolated and purified from fermented ginseng substrate and their anti-allergic effects were assessed in compound 48/80-induced anaphylactic shock model. The fermented ginseng substrate was extracted by methanol and ginsenoside Rh1 and compound K were efficiently purified by preparative high performance liquid chromatography (prep HPLC). Their quality and quantity were analyzed by liquid chromatography-mass spectrometer (LC-MS) and HPLC. Ginsenoside Rh1 showed better anti-allergic effects than compound K in compound 48/80-induced anaphylactic shock model. This study suggested that fermented ginseng extracts with enriched Rh1 may be utilized as a potential biomaterial of functional food for the alleviation of allergic symptoms.

Keywords: anaphylactic shock model, compound K, ginsenoside Rh1

Introduction

Ginsenosides mainly designate a group of glycosides containing an aglycone with a dammarane skeleton (1). More than 30 ginsenosides have been identified and isolated (2). Ginsenosides, also called ginseng saponins, have been regarded as the principal components responsible for the pharmacological and biological activities of ginseng in the prevention of oxidation (3), diabetes (4), tumorigenesis (5-7), arteriosclerosis (8), and aging (9). Particularly, ginsenoside Rh1 of protopanaxatriol group had cytotoxic effects on various cancer cell lines (10-12), and anti-allergic and anti-inflammatory effects (13). Ginsenoside compound K of protopanaxadiol group also showed cytotoxicity against some tumor cells and contributed to antitumor-promoting and anti-inflammatory activities (14-16).

Most of the ginsenosides such as Rb1, Rb2, Rc, Rd, and Re are known to be absorbed into the blood stream only after they are metabolized by intestinal bacteria (17) and converted into the less-polar forms such as compound K or ginsenoside Rh1, which could reach the systemic circulation and represent pharmacological effects (18). This suggests that variations in the efficacy of ginseng among people might be due to the individual differences in intestinal microflora (19). In this study, ginsenoside Rh1 and compound K were isolated and purified from the fermented ginseng substrate and their effects were assessed in compound 48/80-induced anaphylactic shock model.

*Corresponding author: Tel: +82-2-880-8749; Fax: +82-2-884-0305
E-mail: geji@snu.ac.kr
Received December 4, 2007; Revised January 21, 2008;
Accepted January 30, 2008

Materials and Methods

Animals ICR mice (male, 6 weeks old) were obtained from EKOATECH.Com. (Pyeongtaek, Korea) and 2-3 mice were housed per cage in SPF system maintained at 21±1°C with a relative humidity of 53±15%. They were given sterilized Teklad certified irradiated global 18% protein rodent diet (Harlan Teklad, Madison, WI, USA) and filtered water. The care and treatment of the mice were in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

Reagents Ginsenoside Rh1 standard was purchased from BTGin (Chungnam, Korea). Compound K standard and the fermented ginseng substrate were donated from the Department of Pharmaceutical Science, Kyung Hee University (Seoul, Korea) and Bifido Co., Ltd. (Gangwon, Korea). High performance liquid chromatography (HPLC) grade acetonitrile, methanol, and water were purchased from J. T. Baker (Phillipsburg, NJ, USA). Compound 48/80 and disodium cromoglycate (DSCG) were acquired from Sigma-Aldrich (St. Louis, MO, USA). The test drugs were dissolved in phosphate buffer and distilled water. All other reagents used were of analytical grade.

Preparation of ginsenosides using prep HPLC The ginsenosides of fermented ginseng substrate were extracted by methanol. Two g of the fermented ginseng substrate were mixed with 60 mL of 80% methanol. The mixture was incubated in shaking water bath at 80°C for 1 hr. The mixture was filtered with Whatman paper No. 2. The residue was collected and extracted with 40 mL of 80% methanol, twice. The filtered extract was evaporated *in vacuo* (Speed Vacuum Concentrator 4080 C; Biotron, Bucheon, Korea) and dissolved in 100% methanol to

conduct prep HPLC or HPLC analysis. An HPLC pump (model ACME 9000; Younglin Instrument, Seoul, Korea), a reverse-phase column (21.2 mm ϕ×250 mm, HiQ Sil C18 HS-10; KYA Tech, Tokyo, Japan), an ultra violet (UV) detector (UV/VIS detector; Younglin Instrument), a fraction collector (FC203B fraction collector), and Autochro 3000 software (Gilson Inc., Middleton, WI, USA) were used for the purification of ginsenoside Rh1 and compound K. The mixture of solvent A (acetonitrile) and solvent B (water) flowed at a rate of 20 mL/min for 60 min. The gradient program was as followed; 0-10 min (35-40% A), 10-60 min (40-100% A), and UV wave was 203 nm. Finally, prep HPLC was conducted twice more to purify the collected ginsenoside Rh1 and compound K.

Qualitative and quantitative analyses of ginsenosides Each fraction of ginsenoside Rh1 and compound K collected from prep HPLC was evaporated *in vacuo* (Speed Vacuum Concentrator 4080 C; Biotron), and the residues were dissolved in methanol and filtered by syringe filter (Millex SLLHR04NL, 0.45 μm PTFE, 4-mm LH; Millipore, Bedford, MA, USA). An HPLC pump (model 526; Alltech Assoc., Deerfield, IL, USA), a reverse-phase column (5 μm, 250×4.6 mm; Apollo C18 ODS, Alltech), an evaporative light scattering detector (ELSD, model 800; Alltech) and Allchrom™ and Allchrom™ Plus software were used. The quick mixture of solvent (A) acetonitrile:solvent (B) 0.001% phosphoric acid water=92:8 flowed at a rate of 0.5 mL/min for 30 min with isocratic profile. ELSD was operated at 80°C with a nitrogen gas pressure of 2.0 bar. Quantitative analysis for ginsenoside Rh1 and compound K was obtained by comparison with standard curves. Triplicate samples were used throughout the experiment. Qualitative analysis for ginsenoside Rh1 and compound K was conducted by LC-MS. (HP-1100 HPLC, QUATTRO LC Triple Quadruple Tandem Mass Spectrometer; National Center for Inter-University Research Facilities, Seoul National University, Korea).

Systemic anaphylactic shock induced by compound 48/80 in mice Each mouse was given an intraperitoneal injection of compound 48/80 (8 mg/kg) dissolved in phosphate buffer to evoke a systemic anaphylactic reaction. Either ginsenoside, positive control group (DSCG), or 40% ethanol (negative control group) was administered by mouth 1 hr before the injection of compound 48/80. All ginsenosides were dissolved in 40% ethanol; stock concentration of ginsenosides was 25 mg/mL. Ginsenosides (0.5-10 mg/kg) were administrated to 10 mice per each concentration. DSCG (1 g/kg) dissolved in 40% ethanol was also given to 10 mice as a control. All the injection volumes were 0.2 mL per mouse. To investigate toxicity of ginsenosides, each ginsenoside (10 mg/kg) was administrated to 3 mice without compound 48/40. Mortality was monitored for 1 hr after the induction of anaphylactic shock.

Results and Discussion

Isolation of ginsenoside Rh1 and compound K from fermented ginseng Fractions of ginsenoside Rh1 and compound K were obtained from the fermented ginseng substrate by using prep HPLC (data not shown). Each

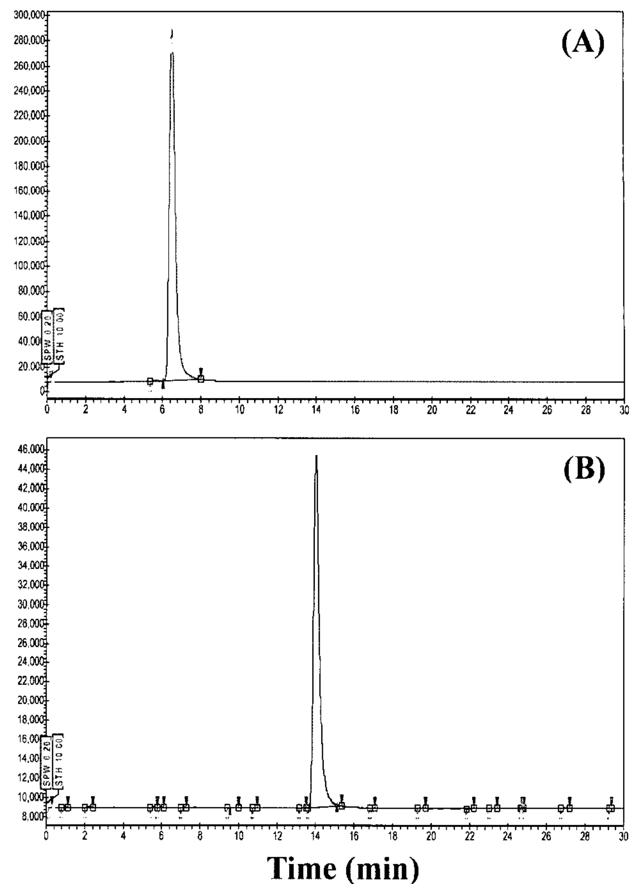

Fig. 1. HPLC profiles of the ginsenoside Rh1 and compound K purified by prep HPLC. (A) Ginsenoside Rh1; (B) compound K.

fraction of ginsenoside Rh1 and compound K was collected approximately at 9.0 and 32.0 min, respectively. The collected fractions were evaporated *in vacuo* and the residues were dissolved in methanol and filtered to estimate quality and quantity for each ginsenoside. Quantity analysis of ginsenoside Rh1 and compound K was calculated by comparison with each ginsenoside standard curve. As shown in Fig. 1, the retention times of ginsenoside Rh1 and compound K were approximately 6.0 and 15.0 min, respectively, and their purities were higher than 99%. The contents of ginsenoside Rh1 and compound K were approximately 40 and 109 mg per 1 g of the fermented ginseng substrate, and the recovery yield of the purified ginsenoside Rh1 and compound K were 43.9 and 49.5%, respectively (data not shown). Qualitative analysis for ginsenosides Rh1 and compound K was conducted by LC-MS (electronic spray ionization, ESI, positive mode). The purified Rh1 exactly corresponded to the standard Rh1 (Fig 2) with their molecular masses of 640. The standard compound K was equal to the purified compound K with the molecular masses of 624. The purified ginsenosides were also analyzed by LC-MS at ESI negative mode; the molecular weights of ginsenoside Rh1 and compound K were 622 and 638, respectively.

Efficacy assessment of ginsenoside Rh1 and compound K on systemic anaphylactic shock As shown in Table 1, an intraperitoneal injection of compound 48/80 (8 mg/kg) resulted in a fatal shock in all mice and ginsenoside

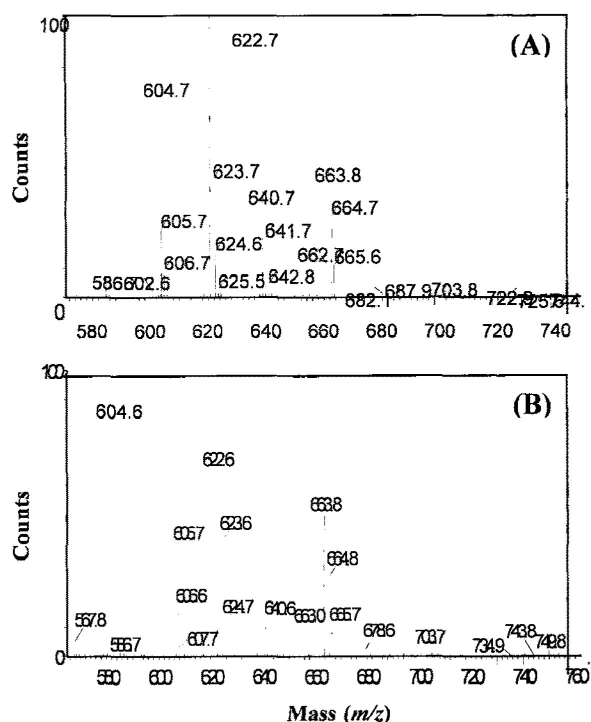

Fig. 2. LC-MS (ESI positive mode) profiles of ginsenoside Rh1. (A) Standard Rh1; (B) purified Rh1.

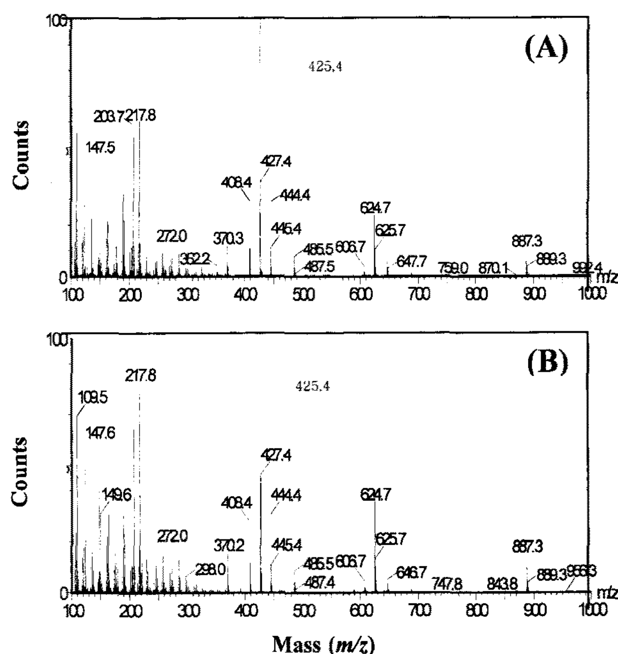

Fig. 3. LC-MS (ESI positive mode) profiles of compound K. (A) Standard compound K; (B) purified compound K.

Table 1. Effects of ginsenoside Rh1 and compound K on compound 48/80-induced systemic anaphylaxis

Group	Dose (mg/kg)	Compound 48/80 (8 mg/kg)	Mortality[1)] (%)
40% ethanol	-	+	100
DSCG	1,000	+	70
Rh1	0.5	+	80
	1	+	70
	5	+	40
	10	+	70
	10	-	0
Compound K	1	+	90
	5	+	90
	10	+	90
	10	-	0

[1)]Mortality of the mice=the number of dead mice×100/total number of experimental mice.

Rh1 pretreatment considerably reduced the overall mortality rate. The mortality of the mice pretreated with ginsenoside Rh1 at the range of 0.5-5 mg/kg was dose-dependently decreased and then increased at 10 mg/kg compared to at 5 mg/kg. The positive drug DSCG also inhibited the compound 48/80-induced systemic anaphylactic shock. The mortality of DSCG treated mice at a dose of 1 g/kg was 70%. On the other hand, compound K treatment could not decrease the mortality below 90%.

Ginsenosides have been regarded as the outstanding components responsible for the pharmacological and biological activities of ginseng. Several studies have reported that ginsenoside Rh1 and compound K represent anti-allergic and anti-inflammatory effects. Park *et al.* (13) showed that Rh1 potently inhibited histamine release from rat peritoneal mast cells and the IgE-mediated passive cutaneous anaphylaxis (PCA) reaction in mice. The inhibitory activity of Rh1 (87% inhibition at 25 mg/kg) on the PCA reaction was found to be more potent than that of DSCG (31% inhibition at 25 mg/kg). They reported that the anti-allergic action of Rh1 might have been originated from its cell membrane-stabilizing and anti-inflammatory activities. Park *et al.* (15) reported that compound K potently inhibited the production of nitric oxide (NO) and prostaglandin E2 in LPS-induced RAW 264.7 cells, with IC_{50} values of 12 and 4 μM, respectively. Compound K also reduced the expression levels of the inducible NO synthase (iNOS) and COX-2 proteins and inhibited the activation of NF-κB, a nuclear transcription factor. Compound K inhibited the NO level produced by iNOS enzyme activity in a cell-free system, but did not inhibit COX-1 and 2 activities. However, there was no study on the systemic anaphylactic shock model for ginsenoside Rh1 and compound K. Therefore, it was of interest that ginsenoside Rh1 was more effective than compound K and well known anti-allergic compound DSCG in anaphylactic shock model. This result suggests that ginsenoside Rh1 has a remarkable efficacy in the systemic anaphylactic shock model induced by compound 48/80.

The systemic anaphylactic shock studies were also conducted on various natural substances other than ginsenosides. Jiang *et al.* (20) investigated the anti-allergic effect of an ethanol extract from *Moutan cortex* on immediate allergic reactions. The *M. cortex* extract (30, 100 mg/kg) dose-dependently inhibited systemic anaphylactic shock induced by compound 48/80 in mice. The 30 mg/kg dose of *M. cortex* extract presented 50% mortality rate. Kim *et al.* (21) investigated the effect of aqueous extract of *Dracocephalum argunense* FISCH (Labiatae) (DAAE) on the mast cell-mediated allergic model and studied its

possible mechanisms of action. When DAAE was anally administered at concentrations ranging from 1 μg-1 mg/g, the mortality with compound 48/80 was dose-dependently reduced. DAAE completely inhibited the compound 48/80-induced fatal shock at 1 mg/g. Their findings provide evidence that DAAE inhibits mast cell derived allergic response, and involvement of cAMP for histamine release and p38 MAPK for pro-inflammatory cytokine secretion in these effects. When DAAE was intraperitoneally administered at concentrations ranging from 0.01 to 1 g/kg BW for 1 hr, the mortality induced by compound 48/80 was dose-dependently reduced (22). DAAE (0.5 g/kg) completely inhibited the fatal shock induced by compound 48/80. In addition, the mortality of the mice administered DAAE (1 g/kg) at 5, 10, 20, or 30 min after compound 48/80 injection was increased time dependently. Jeong *et al.* (23) investigated the effect of cell cultured Siberian ginseng (SG) by oral administration in mast cell-mediated allergic reactions. When the SG was orally administrated at concentrations ranging from 0.01 to 1 g/kg for 1 hr, the mortality of the mice with compound 48/80 was dose-dependently reduced. SG inhibited systemic allergy with the dose of 1 g/kg by 25% and SG (1 g/kg) also inhibited PCA reaction by 51%.

The studies described above reported 20-50% mortality at doses of 10-100 mg/kg natural substances. On the other hand, our study showed that a dose of ginsenoside Rh1 at 40% mortality was 5 mg/kg. Taken together, the present study showed that ginsenoside Rh1 and compound K were successfully isolated and purified from the fermented ginseng substrate. Ginsenoside Rh1 showed a promising anti-allergic effect in compound 48/80-induced anaphylactic shock model. Consequently, fermented ginseng extracts containing enriched ginsenoside Rh1 may be utilized as a functional bio-material for the alleviation of allergic symptoms (24,25).

Acknowledgments

This work was supported by the Small & Medium Business Administration and the Ministry of Science and Technology (MI-0302-00-0098), Korea.

References

1. Hasegawa H. Proof of the mysterious efficacy of ginseng: Basic and clinical trials: Metabolic activation of ginsenoside: Deglycosylation by intestinal bacteria and esterification with fatty acid. J. Pharmacol. Sci. 95: 153-157 (2004)
2. Chi H, Lee BH, Park MS, Ji GE. Differential transformation of ginsenosides from *Panax ginseng* by lactic acid bacteria. J. Microbiol. Biotechn. 16: 1629-1633 (2006)
3. Huong NT, Matsumoto K, Kasai R, Yamasaki K, Watanabe H. *In vitro* antioxidant activity of Vietnamese ginseng saponin and its component. Biol. Pharm. Bull. 21: 978-981 (1998)
4. Attele AS, Zhou YP, Xie JT, Wu JA, Zhang L, Dey L, Pugh W, Rue PA, Polonsky KS, Yuan CS. Antidiabetic effects of *Panax ginseng* berry extract and the identification of an effective component. Diabetes 51: 1851-1858 (2002)
5. Sato K, Mochizuki M, Saiki I, Yoo YC, Samukawa K, Azuma I. Inhibition of tumor angiogenesis and metastasis by a saponin of *Panax ginseng*, ginsenoside-Rb2. Biol. Pharm. Bull. 17: 635-639 (1994)
6. Mochizuki M, Yoo YC, Matsuzawa K, Sato K, Saiki I, Tono-oka S, Samukawa K, Azuma I. Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb2, 20(R)-, and 20(S)-ginsenoside-Rg3, of red ginseng. Biol. Pharm. Bull. 18: 1197-1202 (1995)
7. Wakabayashi C, Murakami K, Hasegawa H, Murata J, Saiki I. An intestinal bacterial metabolite of ginseng protopanaxadiol saponins has the ability to induce apoptosis in tumor cells. Biochem. Bioph. Res. Co. 246: 725-730 (1998)
8. Zhang XM, Qu SC, Sui DY, Yu XF, Lv ZZ. Effects of ginsenoside-Rb on blood lipid metabolism and anti-oxidation in hyperlipidemia rats. Zhongguo Zhong Yao Za Zhi. 29: 1085-1088 (2004)
9. Cheng Y, Shen LH, Zhang JT. Anti-amnestic and anti-aging effects of ginsenoside Rg1 and Rb1 and its mechanism of action. Acta Pharmacol. Sinic. 26: 143-149 (2005)
10. Odashima S, Ohta T, Kohno H, Matsuda T, Kitagawa I, Abe H, Arichi S. Control of phenotypic expression of cultured B16 melanoma cells by plant glycosides. Cancer Res. 45: 2781-2784 (1985)
11. Ota T, Fujikawa-yamamoto K, Zong Z, Yamazaki M, Odashirna S, Kitagawa I, Abe H, Arichi S. Plant-glycoside modulation of cell surface related to control of differentiation in cultured B16 melanoma cells. Cancer Res. 47: 3863-3867 (1987)
12. Park MT, Cha HJ, Jeong JW, Lee HY, Kim SI, Back NI, Kim OH, Kim KW. Anti-invasive activity of ginsenoside Rh1 and Rh2 in the HT1080 cells. J. Ginseng Res. 22: 216-221 (1998)
13. Park EK, Choo MK, Han MJ, Kim DH. Ginsenoside Rh1 possesses antiallergic and anti-inflammatory activities. Int. Arch. Allergy Imm. 133: 113-120 (2004)
14. Shin JE, Park EK, Kim EJ, Hong YH, Lee KT, Kim DH. Cytotoxicity of compound K (IH-901) and ginsenoside Rh2, main biotransformants of ginseng saponins by bifidobacteria, against some tumor cells. J. Ginseng Res. 27: 129-134 (2003)
15. Park EK, Shin YW, Lee HU, Kim SS, Lee YC, Lee BY, Kim DH. Inhibitory effect of ginsenoside Rb1 and compound K on NO and prostaglandin E2 biosyntheses of RAW264.7 cells induced by lipopolysaccharide. Biol. Pharm. Bull. 28: 652-656 (2005)
16. Lee JY, Shin JW, Chun KS, Park KK, Chung WY, Bang YJ, Sung JH, Surh YJ. Antitumor promotional effects of a novel intestinal bacterial metabolite (IH-901) derived from the protopanaxadiol-type ginsenosides in mouse skin. Carcinogenesis 26: 359-367 (2005)
17. Akao T, Kida H, Kanaoka M, Hattori M, Kobashi K. Intestinal bacterial hydrolysis is required for the appearance of compound K in rat plasma after oral administration of ginsenoside Rb1 from *Panax ginseng*. J. Pharm. Phamacol. 50: 1155-1160 (1998)
18. Tawab MA, Bahr U, Karas M, Wurglics M, Schubert-Zsilavecz M. Degradation of ginsesnosides in humans after oral administration. Drug Metab. Dispos. 31: 1065-1071 (2003)
19. Hasegawa H, Uchiyama M. Antimetastatic efficacy of orally administered ginsenoside Rb1 in dependence on intestinal bacterial hydrolyzing potential and significance of treatment with an active bacterial metabolite. Planta Med. 64: 696-700 (1998)
20. Jiang S, Nakano Y, Yatsuzuka R, Ono R, Kamei C. Inhibitory effects of *Moutan cortex* on immediate allergic reactions. Biol. Pharm. Bull. 30: 1707-1710 (2007)
21. Kim SH, Kim SH, Kim SH, Moon JY, Park WH, Kim CH, Shin TY. Action of *Dracocephalum argunense* on mast cell-mediated allergy model. Biol. Pharm. Bull. 29: 494-498 (2006)
22. Kim SH, Shin TY. Effect of *Dracocephalum argunense* on mast-cell-mediated hypersensitivity. Int. Arch. Allergy Imm. 139: 87-95 (2006)
23. Jeong HJ, Koo HN, Myung NI, Shin MK, Kim JW, Kim DK, Kim KS, Kim HM, Lee YM. Inhibitory effects of mast cell-mediated allergic reactions by cell cultured Siberian ginseng. Immunopharm. Immunot. 23: 107-117 (2001)
24. Kim JY, Suh JW, Ji GE. Evaluation of *S*-adenosyl-L-methionine production by *Bifidobacterium bifidum* BGN4. Food Sci. Biotechnol. 17: 184-187 (2008)
25. Yang SO, Ji GE. Inhibitory effects of *Ulmus parvifolia* and *Liriope platyphylla Wang et Tang* on histamine release from rat peritoneal mast cells. Food Sci. Biotechnol. 15: 363-368 (2006)