Evaluation of Cytotoxicity, Carbohydrate, and Lipid Inhibitory Activity of *Codonopsis lanceolata* using Different Solvent Fractions

Hee-Ock Boo*, Jeong-Hun Park*, Seung-Mi Kim*, Sun-Hee Woo**, and Hyeon-Yong Park*[†]

*Department of Biology, College of Natural Science, Chosun University, Gwangju 61452, Korea
**Department of Crop Science, Chungbuk National University, Cheongju 28644, Korea

ABSTRACT This study was conducted to evaluate the cyto-toxicity and α-amylase, α-glucosidase, pancreatic lipase inhibition *in vitro* by different solvent fractions from the roots of *Codonopsis lanceolata*. The values of IC_{50} against Calu-6 cell showed a high effect in n-hexane fraction (10.13 μg/mL) whereas DW fraction exhibited the weakest inhibition on cell viability, having an IC_{50} value of over 1,000 μg/mL. The values of IC_{50} against HCT-116 cell showed the highest activity in n-BuOH fraction (102.01 μg/mL), followed by n-hexane fraction (145.85 μg/mL), methylene chloride fraction (332.02 μg/mL), ethyl acetate fraction (462.93 μg/mL) and DW fracion (>1,000 μg/mL). α-Amylase inhibitory activity in methylene chloride fraction and ethyl acetate fraction was found to have a higher inhibitory effect with 24.5% and 25.6% than the other fractions. The highest α-glucosidase inhibitory activity was observed from the ethyl acetate fraction extract, while the extract of DW fraction showed the lowest level of inhibitory activity at given experiment concentration. The pancreatic lipase inhibitory activity of *C. lanceolata* was found to have a higher the effect in ethyl acetate fraction. Inhibition of lipase activity of the ethyl acetate fraction and n-hexane fraction showed a relatively high, while the extract of DW fraction showed the lowest level at given experiment concentration. These results suggested that the roots of *C. lanceolata* may assist in the potential biological activity on carbohydrate, lipid Inhibitory activity and anticancer activity.

Keywords : Cytotoxicity, α-Amylase, α-Glucosidase, Pancreatic lipase, *Codonopsis lanceolata*

Codonopsis lanceolata is a perennial flowering plant belonging to the family Campanulaceae and is grown com-mercially in East Asia. The roots of *C. lanceolata* have been used as a tonic crude drug and an edible plant in Korea, and mainly contain triterpenoid saponins including codonolaside, codonolaside I-V, lancemaside A-G. Their saponins have shown anti-inflammatory effects such as bronchitis and cough, insomnia, and hypomnesia. Lancemaside A, which is a main constituent of *C. lanceolata* was reported to potently inhibit LPS-stimulated, TLR-4-linked NF-jB activation of 293-hTLR4-hemagglutinin (HA) cells (Joh *et al.,* 2010). *C. lanceolata* is well known to affect various pharmacological effects for human health and its consumption is increasing. Recently, plant and plant-derived products are treated a part of the healthcare system by applying the bioactive phytochemicals. Various chemical agents with strong apoptosis-inducing activity, but minimal toxicity have potential as anticancer drugs. As an herb, *C. lanceolata* is widely used in food preparation, but its medicinal application has not been explored yet in South Korea (Wang *et al.,* 2011). Screening of α-amylase and α-glucosidase inhibitors from medicinal plants has received much attention throughout the world. This study was designed to investigate the *in vitro* anti-cancer activity of different solvent fractions of *C. lanceolata* and to understand how the fractions act against α-amylase, α-glucosidase and lipase activity. α-Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starches from being absorbed by the body. They exert their blood glucose lowering effect through the inhibition of an enzyme such as salivary and pancreatic amylase (Frantz *et al.,* 2005). Starches are complex carbohydrates that cannot be absorbed unless they are first broken down by the digestive enzyme amylase and other secondary enzymes (Marshall *et al.,* 1975). In other words, α-amylase and α-glucosidase are enzymes involved in starch breakdown and intestinal absorption, respectively, that is, α-amylase is involved in the digestion of carbohydrates to produce simpler saccharides, whereas the α-glucosidase is involved in their absorption. Inhibition of these

two enzymes would result in a lower blood glucose levels after a rich carbohydrate diet. Acting as a key enzyme for carbohydrate digestion is intestinal α-glucosidase, a glucosidase secreted in the epithelium of the small intestine. α-Glucosidase has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality that occurs in NIDDM (Non-insulin dependent diabetes mellitus) (Kim *et al.,* 2005). α-Glucosidase inhibitors such as acarbose, miglitol, and voglibose are known to reduce postprandial hyperglycemia primarily by interfering with the activity of carbohydrate-digesting enzymes and delaying glucose absorption. Weight loss and weight maintenance are the important goals of obesity treatment which can be done by several ways including the use of lipase inhibitors. Recently, lipase inhibitors from plants such as saponins, polyphenolic compounds and terpenes have garnered increasing attention since they showed sufficient activity (Rahul and Kamlesh, 2007). In order to search for the new sources of lipase inhibitors, the present study investigated the lipase inhibitory activity of *C. lanceolata*. This study was also designed to determine the effect of cytotoxicity in the different solvent fractions of *C. lanceolata* extract. Recent interests in the study of functional plants have focused on their potential benefits to human health. The functional plants have been used as traditional medicine, but scientific evaluation is still lacking in this regard. Therefore, in this study, α-amylase, α-glucosidase, pancreatic lipase inhibitory activities were screened *in vitro*, and cytotoxicity was evaluated against human cancer cell lines (Calu-6 and HCT-116) from solvent fractions of *C. lanceolata*.

MATERIALS AND METHODS

Samples preparation

Roots of *C. lanceolata* plant were freeze dried and then ground into a fine powder. Each plant powder was stored at -20°C for further experiments. The freeze dried powder was immersed in 70% methanol and the filtrate was collected for three times with constant stirring of the mixture at every 24 hrs interval of a 72 hrs total collection period. The extract was then concentrated under reduced pressure at 45°C using a vacuum rotary evaporator. The concentrated extract was partitioned between hexane and water. The aqueous layer further fractionated with methyl chloride, ethyl acetate and butyl alcohol. Four

solvent fractions (hexane, methyl chloride, ethyl acetate and butyl alcohol) were collected and concentrated using vacuum rotary evaporator.

Cytotoxicity measurement using the MTT assay

The cytotoxicity of *C. lanceolata* sample was assayed using human cancer cell lines, Calu-6 for human pulmonary carcinoma and HCT-116 for human colorectal carcinoma. The cell lines were purchased from Korea Cell Line Bank (KCLB) for MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The cells plated on 96 well plates at a concentration of 3×10^4 cells/mL. The cells were incubated for 24 hrs in RPMI-1640 medium at 37°C under 5% CO_2 in a humidified incubator, and treated with 2 μL of various concentrations (50, 100, 200, 400, 800 and 1000μg mL^{-1}) of extracts. After the incubation for 48 hr, the cells were washed twice with phosphate buffer solution (PBS). MTT solution at 5mg/mL was dissolved in 1mL of PBS, and 10μL of it was added to each of the 96 wells. After the reaction for 4 hr, the solution in each well containing media, unbound MTT and dead cells were removed by suction and 100 μL of DMSO was added to each well. The plates were shaken for 15 minutes using plate shaker, and the absorbance was recorded using an ELISA reader (Bio-Rad model 550, USA) at a wavelength 540 nm. The viability of the treatment was determined as percentage of viability compared to untreated cell, and the values were then used to iteratively calculate the concentration of plant extracts required to cause a 50% reduction (IC_{50}) in growth for each cell lines.

Measurement of α-amylase inhibitory activity *in vitro*

The α-amylase inhibitory assay was adapted and modified from Satoyama *et al.* (1998) and Oh *et al.* (2010). An identical volume of 2% starch solution (0.1 M citric acid; pH 6.0) and 3.2% agar solution (0.1 M citric acid; pH 6.0) was mixed at 60°C water bath, and then substrate plate was made by the addition, and cooling of 100 μL in each well of microplate. After incubation at 37°C for 10 min, 25 μL of α-amylase (10 unit /mL) and 25 μL of each plant extract were added in each well and followed by a 120 min reaction at 37°C. The α-amylase activity was determined by measuring the absorbance of the mixture at 655 nm, using a spectrophotometer (Biochrom Co., England). The α-amylase activity (inhibition rate percent) was calculated using the following equation:

α-amylase inhibitory activity (%)

$$= [1-(A_i-A_f)/(B_i-B_f)] \times 100$$

A_i-A_f : the absorbance of reaction solution before and after
　　　　reaction

B_i-B_f : the absorbance of the blank test before and after
　　　　reaction

Measurement of α-glucosidase inhibitory activity *in vitro*

The α-glucosidase inhibitory assay of the extracts was adapted as described by Kim *et al.* (2011) and Wang *et al.* (2006) with slight modification. The sample extract of 20 mg/mL concentration and 720 μL of 0.2 M potassium phosphate buffer (pH 6.8) containing 100 μL of α-glucosidase solution (0.15 U/mL) were mixed. After measuring the absorbance of the mixture at 405 nm and keeping at room temperature for 5 min, 100 μL of 5 mM 4-nitrophenyl-α-D-glucopyranoside solution was added, more reacted at room temperature for 10 min, and then the absorbance reading of the reaction mixtures was recorded at 405 nm. The α-glucosidase inhibitory activity was calculated from the change in absorbance and was expressed as inhibition %.

α- glucosidase inhibitory activity (%)

$$= [(Control_{405} - Extract_{405})] / Control_{405} \times 100$$

Measurement of pancreatic lipase inhibitory activity *in vitro*

The inhibitory activity against pancreatic lipase was measured using p-nitrophenyl butyrate (p-NPB) as a substrate with a modified method from Zhang *et al.* (2008). The pancreatic lipase stock solution was prepared to a concentration of 1 mg/mL in 0.1 mM potassium phosphate (pH 6.0), and then was stored at -20°C. The assay mixture contained 25 μL of the extracts and 25 μL of the pancreatic lipase solution was added in 950 μL of 0.1 mM potassium phosphate (pH 7.2, 0.1% Tween 80), after incubation at 30°C for 1 hour, and then was reacted at 37°C for 5 min with 1 μL of p-NPB. The lipase inhibitory activity was determined by measuring the absorbance at 405 nm, and was calculated using the following equation:

Lipase inhibitory activity (%) = $[1-(B-C) / A] \times 100$

A : Absorbance of reaction solution without the sample
　　extract
B : Absorbance of reaction solution with the sample extract
C : Absorbance of reaction solution without the enzyme

Data analysis

The statistical analysis was performed using the procedures of the Statistical Analysis System (SAS version 9.1). The ANOVA procedure followed by Duncan test was used to determine the significant difference (p < 0.05) between treatment means.

RESULTS AND DISCUSSION

Cytotoxicity

The cytotoxicity of *C. lanceolata* on two human cancer cell lines were evaluated using the MTT assay. When cells were treated for 48 hrs with various concentrations (50, 100, 200, 400, 800 and 1000 μg/mL) of different solvent fractions, the rate of cell survival progressively decreased in a dose-dependent manner. The cytotoxicity evaluation against human cancer cell lines of different solvent fractions from roots of *C. lanceolata* are shown in Table 1. Hexane fraction at 50 μg/mL exhibited a pronounced cytotoxic effect (46.37%) on Calu-6 cell comparable to that of the ethyl acetate fraction (83.54%) and DW fraction (95.89%) at the same concentration. The cytotoxicity on HCT-116 cell at 50 μg/mL showed a high effect in n-hexane fraction (60.63%) and methylene chloride fraction (60.37%). The values of IC$_{50}$ (concentration causing 50% cell death) against Calu-6 cell showed a high effect in n-hexane fraction (10.13 μg/mL) whereas DW fraction exhibited the weakest inhibition on cell viability, having an IC$_{50}$ value of over 1,000 μg/mL. The values of IC$_{50}$ against HCT-116 cell showed the highest activity in n-BuOH fraction (102.01 μg/mL), followed by n-hexane fraction (145.85 μg/mL), methylene chloride fraction (332.02 μg/mL), ethyl acetate fraction (462.93 μg/mL) and DW fracion (>1,000 μg/mL). Numerous compounds found in plants with anticancer properties are such as alkaloids, phenyl-propanoids, and terpenoids (Kintzios, 2006; Park *et al.,* 2008; Yan-Wei *et al.,* 2009; Vijayarathna and Sasidharan, 2012). Wang *et al.* (2011) investigated the effects of *C. lanceolata* extract on cancer cells and the molecular mechanism by which it induces apoptosis in human HT-29 colon cancer cells. It is well known that chemicals and medicinal plant medicines may

Table 1. Cytotoxicity of different solvent fractions of *Codonopsis lanceolata* on two human cancer cell lines.

Cell line	Solvent	Cell viability (% of control)						
		Concentration (μg/mL)						
		50	100	200	400	800	1000	IC$_{50}$ (μg/mL)
Calu-6	n-Hexane	46.37±4.85[c]	43.80±8.28[c]	32.42±4.29[d]	19.65±4.48[d]	19.01±0.55[c]	17.26±3.42[c]	10.13
	Methylene Chloride	71.71±4.71[b]	71.50±5.93[b]	77.41±2.85[b]	48.29±1.26[c]	40.31±0.90[b]	37.10±4.82[b]	393.27
	Ethyl Acetate	83.54±6.90[ab]	81.83±9.36[b]	85.49±1.10[b]	65.14±4.98[b]	41.14±2.25[b]	40.23±2.62[b]	609.27
	n-BuOH	72.40±7.78[b]	70.03±9.26[b]	51.24±8.71[c]	28.88±5.21[d]	19.58±1.07[c]	18.99±3.34[c]	344.10
	D.W	95.89±9.81[a]	99.56±3.9[a]	97.75±4.68[a]	98.14±8.76[a]	90.32±4.44[a]	90.52±2.66[a]	1000<
HCT-116	n-Hexane	60.63±4.44[b]	59.87±3.12[c]	44.45±6.37[c]	27.64±6.25[c]	5.62±0.09[c]	4.66±2.95[c]	145.85
	Methylene Chloride	60.37±7.10[b]	61.06±7.18[c]	56.97±1.59[b]	49.44±0.17[b]	39.40±1.71[b]	37.75±5.80[b]	332.02
	Ethyl Acetate	88.91±3.32[a]	86.31±8.73[b]	65.05±3.92[b]	55.91±1.69[b]	34.00±5.60[b]	8.46±0.68[c]	462.93
	n-BuOH	66.48±3.32[b]	51.61±5.19[c]	24.81±3.01[d]	20.81±2.29[c]	7.11±0.53[c]	6.73±0.58[c]	102.01
	D.W	93.42±2.06[a]	99.70±1.47[a]	94.82±6.18[a]	98.90±8.72[a]	94.99±6.33[a]	89.28±3.71[a]	1000<

[z]Data represent the mean values±SE of three independent experiments. Means with the same letter in column are not significantly different at p<0.05 level by Duncan's multiple range test.
Calu-6: human pulmonary carcinoma cell, HCT-116: human colorectal carcinoma cell.

produce toxic effects. These results suggest that different solvent fractions from *C. lanceolata* can be used as a source of cytotoxic agent.

α–Amylase inhibition

Inhibition of α-amylase by different solvent fractions from *C. lanceolata* are shown in Fig. 1. α-Amylase inhibitory activity in methylene chloride and ethyl acetate fractions was found to have a higher inhibitory effect with 24.5% and 25.6% than the other fractions. α-Amylase inhibitory activity in n-hexane fraction, n-BuOH fraction and DW fraction showed to appear significantly lower. α-Amylase and α-glucosidase inhibitors have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with type 2 diabetes mellitus. Inhibition of these enzymes delays carbohydrate digestion and prolong the overall carbohydrates digestion time causing a reduction in the rate of glucose absorption and consequently reducing postprandial plasma glucose rise (Kimura *et al.*, 2006). The α-amylase inhibitory effects of *C. lanceolata* may be attributed to the presence of phytochemicals. This also explains the reason behind the effective inhibitory activity displayed by the water extract towards the enzyme when compared to that exhibited by methanol extract. α-Amylase is an enzyme found in the salivary, intestinal mucosal and pancreatic secretions, functioning in the breakdown of the α-1-4-glycosidic bonds in
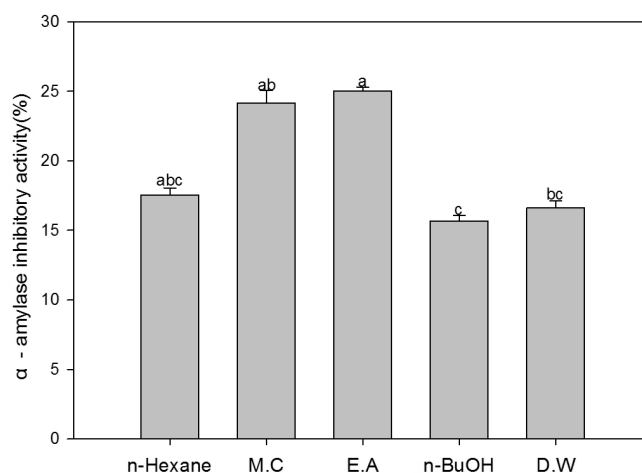

Fig. 1. Inhibition of α-amylase activity using different solvent fractions of *Codonopsis lanceolata*. Means with the same letter in column are not significantly different at p<0.05 level by Duncan's multiple range test. The bars represent the standard error. M.C: methylene chloride. E.A: ethyl acetate, n-BuOH: n-butyl alcohol, D.W: distilled water.

starch. Thus, this enzyme increases the bioavailability of glucose in the blood. Our study demonstrates an appreciable α-amylase inhibitory activity according to different solvent fractions. In particular, results suggests that the methylene chloride and ethyl acetate fractions act effectively as α-amylase inhibitors leading to a reduction in starch hydrolysis and hence eventually to lowered glucose levels.

α-Glucosidase inhibition

The measurement results of α-glucosidase inhibitory activity by different solvent fractions from *C. lanceolata* are shown in Fig. 2. The ethyl acetate fraction extract had the highest α-glucosidase inhibitory activity, while the extract of DW fraction showed the lowest level of α-glucosidase inhibitory activity at a given experiment concentration. Depending on the extraction solvent fraction, it was observed that there was a significant difference in α-glucosidase inhibitory activity. α-Glucosidase, a key enzyme for carbohydrate digestion, has been recognized as a therapeutic target for modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality to occur in type 2 diabetes mellitus (Vadivelan *et al.,* 2012). Many natural resources have been investigated with respect to suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine (Hara and Honda, 1990). α-Amylase catalyses the hydrolysis of a-1, 4-glucosidic linkages of starch, glycogen and various oligosaccharides and α-glucosidase further breaks down the disaccharides into simpler sugars, readily available for the intestinal absorption. The inhibition of their activity, in the digestive tract of humans, is considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by these enzymes (He *et al.,* 2006). Jung *et al.* (2006) reported the α-glucosidase inhibitory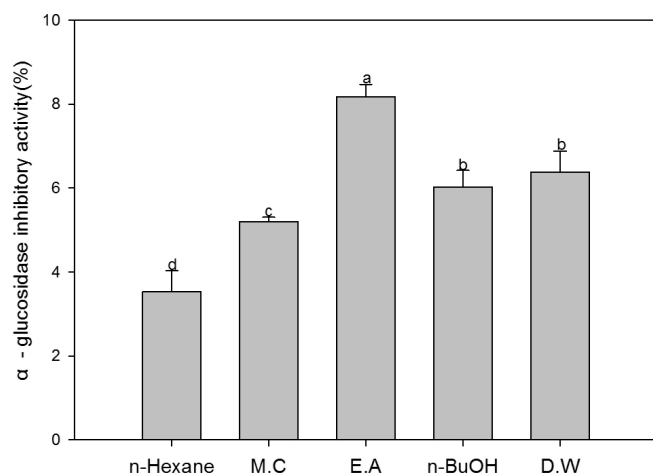 activity of tangshenoside I and adenosine isolated from the roots of *C. lanceolata.* Our present research suggest that the presence of potential functional compounds of *C. lanceolata* may have a potentially important role in managing carbohydrate digesting enzymes via the inhibition of α-glucosidase enzyme activity.

Pancreatic lipase inhibition

The inhibitory effect of different solvent fractions from *C. lanceolata* against pancreatic lipase was investigated that are shown in Fig. 3. The pancreatic lipase inhibitory activity of *C. lanceolata* was found to become a higher effect in ethyl acetate fraction. Inhibition of lipase activity of the ethyl acetate fraction and n-hexane fraction showed a relatively high, while the extract of DW fraction showed the lowest level at given experiment concentration. Obesity is an increasingly serious global problem, not only for the harm it causes in its own right, but also due to the associated health threats, especially type 2 diabetes, systemic hypertension, cardiovascular disease, certain cancers, asthma, and sleep apnea (Kopelman, 2000; Finer, 2006). Many edible plants present an exciting opportunity for the development of newer therapeutics for biologically active antihyperlipidaemic agents from natural resources, especially the reduction of fat digestion and absorption (Kurihara *et al.,* 2006; Zhang *et al.,* 2008; Moller *et al.,* 2009). Pancreatic lipase inhibitors which help to limit intestinal fat absorption at the initial stage, have
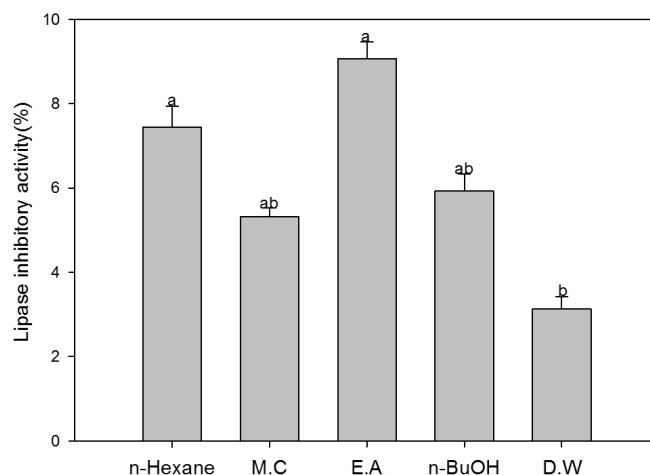

Fig. 2. Inhibition of α-glucosidase activity using different solvent fractions of *Codonopsis lanceolata.* Means with the same letter in column are not significantly different at p<0.05 level by Duncan's multiple range test. The bars represent the standard error. M.C: methylene chloride. E.A: ethyl acetate, n-BuOH: n-butyl alcohol, D.W: distilled water.

Fig. 3. Inhibition of lipase activity using different solvent fractions of *Codonopsis lanceolata.* Means with the same letter in column are not significantly different at p<0.05 level by Duncan's multiple range test. The bars represent the standard error. M.C: methylene chloride. E.A: ethyl acetate, n-BuOH: n-butyl alcohol, D.W: distilled water.

been proved as useful medications for the treatment of hyperlipidaemia and a great promise as anti-obesity agents (Sharma *et al.,* 2005). The mechanism of lipase inhibitors is to block the absorption of fat by inhibiting lipase in the gastrointestinal tract (Davidson, *et al.,* 1999). Consequently, the root of *C. lanceolata* in this study may have great potential as dietary supplements or nutraceutical foods for lipase inhibitory effect with antihyperlipidaemic properties.

ACKNOWLEDGEMENT

REFERENCES

Davidson, M. H., J. Hauptman, M. DiGirolamo, J. P. Foreyt, C. H. Halsted, and D. Heber. 1999. Weight control and risk factor reduction in obese subjects treated for 2 years with orlistat: A Randomized Controlled Trial. JAMA. 281 : 235-242.

Frantz, S., L. Calvillo, J. Tillmanns, I. Elbing, C. Dienesch, H. Bischoff, G. Ertl, and J. Bauersachs. 2005. Repetitive postprandial hyperglycemia increases cardiac ischemia/reperfusion injury: prevention by the alpha-glucosidase inhibitor acarbose. FASEB J. 19 : 591-593.

Hara, Y. and M. Honda. 1990. The inhibition of a-amylase by tea polypphenols. Agri. Biol. Chem. 54(8) : 1939-1945.

He, Q., Y. Lv, and K. Yao. 2006. Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin and lipase. Food Chem. 101 : 1178-1182.

Joh, E. H., I. A. Lee, S. J. Han, S. J. Chae, and D. H. Kim. 2010. Lancemaside A ameliorates colitis by inhibiting NF-jB activation in TNBS-induced colitis mice. Int. J. Colorectal Dis. 25 : 545-551.

Jung, S. W., A. J. Han, H. J. Hong, M. G. Choung, K. S. Kim, and S. H. Park. 2006. α-Glucosidase inhibitors from the roots of *Codonopsis lanceolata* Trautv. Agric. Chem. Biotechnol. 49(4) : 162-164.

Kim, H. Y., S. H. Lim, Y. H. Park, H. J. Ham, K. J. Lee, D. S. Park, K. H. Kim, and S. M. Kim. 2011. Screening of α-amylase, α-glucosidase and lipase inhibitory activity with Gangwon-do wild plants extracts. J. Korean Soc. Food Sci. Nutr. 40(2) : 308-315.

Kim, Y. M., Y. K. Jeong, M. H. Wang, W. Y. Lee, and H. I. Rhee. 2005. Inhibitory effect of pine extract on α-glucosidase activity and postprandial hyperglycemia. Nutrition. 21 : 756-761.

Kimura, Y., Y. Araki, A. Takenaka, and K. Igarashi. 2006. Protective effect of dietary nasunin and parapect induced oxidative stress in rat. Biosci. Biotechnol. Biochem. 63 : 799-804.

Kintzios, E. 2006. Terrestrial plant-derived anticancer agents and plant species used in anticancer research. Crit. Rev. Plant Sci. 25 : 79-113.

Kurihara, H., H. Shibata, Y. Fukui, Y. Kiso, J. K. Xu, X. S. Yao, and H. Fukami. 2006. Evaluation of the hypolipemic property of Camellia sinensis var. ptilophylla on postprandial hyper-triglyceridemia. J. Agric. Food Chem. 54 : 4977-4981.

Marshall, J. J. and C. M. Lauda. 1975. Purification and properties of phaseolamin, an inhibitor of alpha-amylase, from the kidney bean, *Phaseolus vulgaris*. J Biol Chem. 250 : 8030-8037.

Moller, N. P., N. Roos, and J. Schrezenmeir. 2009. Lipase inhibitory activity in alcohol extracts of worldwide occurring plants and propolis. Phytother. Res. 23 : 585-586.

Oh, S. J., J. H. Lee, K. S. Ko, D. B. Shin, and S. C. Koh. 2010. Antioxidative activity, including inhibitory activities of ACE, APN and α-amylase, in *Theaceae* plants native to Jeju Island. Korean J. Plant Res. 23(5) : 406-414.

Park, H. J., M. J. Kim, E. Ha, and J.H. Chung. 2008. Apoptotic effect of hesperidin through caspase 3 activation in human colon cancer cells, SNU-C4. Phytomedicine. 15 : 147-151.

Rahul, B. B. and K. B. Kamlesh. 2007. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discov. Today. 12 : 879-889.

Satoyama, T., T. Hara, M. Murata, and Y. Fujio. 1998. A simple assay method for α-amylase using microplates. Nippon Nogei-kagaku Kaishi. 72 : 933-936.

Sharma, N., V. K. Sharma, and S. Y. Seo. 2005. Screening of some medicinal plants for anti-lipase activity. J. Ethnopharmacol. 97 : 453-456.

Vadivelan, R., S. P. Dhanabal, A. Wadhawani, and K. Elango. 2012. α-Glucosidase and α-amylase inhibitory activities of *Raphanus sativus* Linn. IJPSR. 3(9) : 3186-3188.

Vijayarathna, S. and S. Sasidharan. 2012. Cytotoxicity of methanol extracts of *Elaeis guineensis* on MCF-7 and Vero cell lines. Asian Pac. J. Trop Biomed. 2(10) : 826-829.

Wang, H. Z., C. H. Chang, C. P. Lin, and M. C. Tsai. 2006. Using MTT viability assay to test the cytotoxicity of antibiotics and steroid to cultured porcine corneal endothelial cells. J. Ocular Pharm Therapeutics 12 : 35-43.

Wang, L., M. L. Xu, J. H. u, S. K. Rasmussen, and M. H. Wang. 2011. Codonopsis lanceolata extract induces G0/G1 arrest and apoptosis in human colon tumor HT-29 cells − Involvement of ROS generation and polyamine depletion. Food Chem. toxicol. 49 : 149-154.

Yan-Wei, H., L. Chun-Yu, D. Chong-Min, W. Wen-Qian, and G. Zhen-Lun. 2009. Induction of apoptosis in human hepatocarcinoma SMMC-7721 cells *in vitro* by flavonoids from Astragalus complanatus. J. Ethnopharmacol. 123 : 293-301.

Zhang, J., M. J. Kang, M. J. Kim, M. E. Kim, J. H. Song, Y. M. Lee, and J. I. Kim. 2008. Pancreatic lipase inhibitory activity of *taraxacum officinale in vitro* and *in vivo*. Nutr. Res. Pract. 2(4) : 200-203.