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SHORT COMMUNICATION

Inhibitory Activities of Pancreatic Lipase and Phosphodiesterase from Korean Medicinal Plant Extracts

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To find new pancreatic lipase (triacylgycerol acylhydrolase, EC 3.1.1.3) inhibitors from natural products, 61 medicinal plants from Korea were screened for their antilipase activity for prevention of obesity. Dried and powdered plants were extracted three times with EtOH and extracts were obtained by removal of the solvent *in vacuo*. Lipase activity was determined by measuring the hydrolysis of *p*-nitrophenyl butyrate to *p*-nitrophenol. Also, the inhibitory effect was measured on phosphodiesterase (PDE), another therapeutic target for obesity. Of the extracts tested, *Sorbus commixta* (stem, leaf) and *Viscum album* (whole plant) exhibited antilipase activity (with IC_{50} values of 29.6 μg/mL and 33.3 μg/mL, respectively) and only anti-PDE activity (IC_{50} values of 20.08 μg/mL and 35.15 μg/mL, respectively).

Keywords: pancreatic lipase; phosphodiesterase; Korean medicinal plants; *Sorbus commixta*; *Viscum album.*

INTRODUCTION

Obesity is characterized by excessive accumulation of fat and is a serious problem that is reaching epidemic proportions worldwide (James *et al*., 2004). It is closely associated with lifestyle-related diseases, such as hypertension, hyperlipidemia, arteriosclerosis and diabetes mellitus (Campbell and Mathys, 2001). Triglycerides are the high-calorie energy sources, and suppression of triglyceride absorption is one possible way of preventing obesity and obesity-related diseases (Carek and Dickerson, 1999). Pancreatic lipase is a key enzyme for triglyceride absorption in the small intestine. It is secreted from the pancreas and hydrolyses triglycerides into glycerol and free fatty acids (Lowe, 1994). Orlistat inhibits the action of gastrointestinal lipase and thus reduces absorption of dietary fat. However, it has serious side effects, such as steatorrhea, stomach pain, irregular menstrual periods and headaches (Bray, 2009). Phosphodiesterase inhibitors (PDEIs) are a class of drugs that are widely used because of their various pharmacological properties; thus they may have potential as therapeutic targets for cardiovascular disease and obesity (Rahimi *et al*., 2010; Sato *et al*., 2006; Kim *et al*., 2010). In this paper, extracts obtained from 61 traditional Korean medicinal plants were screened in pursuit of new sources of pancreatic lipase inhibitors from relatively safe and effective natural products. *In vitro*, PDE activity was also measured.

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MATERIALS AND METHODS

Plant materials. Sixty-one traditional Korean herbal medicines (Table 1) were collected from all areas of South Korea, from September 2004 to July 2007, and identified by Professor J. H. Kim, Division of Life Science, Kyungwon University, Seongnam, Korea. Voucher specimens have been deposited at the Herbarium of Diabetic Complications Research Center, Korea Institute of Oriental Medicine.

Preparation of plant extracts. Dried and powdered plant (0.2 g) was extracted three times with 4 mL of EtOH and extracts were obtained by removal of the solvent *in vacuo*. Concentrated samples were stored at −20 °C for further studies. Extracts were dissolved in DMSO at a concentration that in the total volume (3%) did not affect enzyme activity.

Chemicals. Lipase (Type II; from porcine pancreas), orlistat and *p*-nitrophenyl butyrate were purchased from Sigma Chemical Co. (St Louis, MO, USA). All reagents were of biochemical grade.

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Table 1. Lipase inhibitory activity of traditional Korean medicinal plants

Scientific name	Family name	Part used	Conc. (μg/mL)	Inhibition (%)[a]	IC_{50} (μg/mL)
Abies koreana E.H.Wilson	Pinaceae	Twig, leaf	65	47.87 ± 3.10	85.41
			80	49.43 ± 1.72	
			100	51.71 ± 1.81	
Acer tataricum subsp. ginnala (Maxim.) Wesm.	Aceraceae	Fruit	20	38.80 ± 1.32	44.44
			35	45.05 ± 2.69	
			50	52.95 ± 2.20	
Actinidia polygama (Siebold & Zucc.) Maxim.	Actinidiaceae	Leaf, stem	100	6.09 ± 1.48	> 100
Agrimonia pilosa Ledeb.	Rosaceae	Whole plant	100	28.10 ± 1.0	> 100
Anemarrhena asphodeloides Bunge	Haemodoraceae	Root, stem	100	11.78 ± 4.80	> 100
Angelica decursiva (Miq.) Franch. & Sav.	Umbelliferae	Stem, leaf	100	1.91 ± 3.43	> 100
Artemisia capillaries Thunb.	Compositae	Whole plant	100	34.29 ± 2.97	> 100
Aster ageratoides Turcz.	Compositae	Whole plant	100	12.03 ± 4.27	> 100
Astilbe rubra Hook.f. & Thomson	Saxifragaceae	Root	65	42.31 ± 1.46	88.54
			80	45.25 ± 2.08	
			100	54.83 ± 0.17	
Camellia japonica subsp. rusticana	Theaceae	Stem, leaf	20	32.31 ± 1.30	48.83
			35	41.28 ± 3.03	
			50	50.82 ± 6.28	
Castanea crenata var. *dulcis*	Fagaceae	Staminate flower	20	31.52 ± 0.71	49.82
			35	40.55 ± 1.02	
			50	50.26 ± 1.22	
Castanea crenata var. *dulcis*	Fagaceae	Bark, twig	65	46.82 ± 0.19	89.39
			80	49.59 ± 1.99	
			100	50.94 ± 1.54	
Castanea crenata var. *dulcis*	Fagaceae	Leaf	65	43.76 ± 1.57	96.54
			80	47.35 ± 0.35	
			100	50.53 ± 1.92	
Catalpa bignonioides Walter	Bignoniaceae	Twig, leaf	100	29.52 ± 1.09	> 100
Celastrus orbiculatus Thunb.	Celastraceae	Stem, leaf, fruit	100	18.53 ± 2.01	> 100
Chrysanthemum indicum L.	Compositae	Stem, root	100	27.54 ± 1.12	> 100
Chrysanthemum indicum L.	Compositae	Whole plant	100	27.32 ± 3.65	> 100
Cimicifuga heracleifolia Kom.	Ranunculaceae	Whole plant	100	18.18 ± 6.26	> 100
Clematis terniflora DC.	Ranunculaceae	Stem	100	3.01 ± 5.45	> 100
Cnidium japonicum Miq.	Umbelliferae	Whole plant	100	8.06 ± 3.72	> 100
Crataegus pinnatifida Bunge	Rosaceae	Fruit	100	1.42 ± 4.55	> 100
Crataegus pinnatifida Bunge	Rosaceae	Stem, twig	100	42.50 ± 1.19	> 100
Cudrania tricuspidata (Carr.) Bur.	Moraceae	Root	100	39.08 ± 3.26	> 100
Dictamnus dasycarpus Turcz.	Rutaceae	Aerial part	100	2.36 ± 3.53	> 100
Dictamnus dasycarpus Turcz.	Rutaceae	Root	100	1.54 ± 3.50	> 100
Eleutherococcus senticosus Rupr. & Maxim.	Araliaceae	Stem	100	8.03 ± 1.27	> 100
Epimedium koreanum Nakai	Berberidaceae	Leaf, stem	100	8.71 ± 8.62	> 100
Euonymus alatus (Thunb.) Siebold	Celastraceae	Stem	100	44.67 ± 3.53	> 100
Eurya japonica Thunb.	Theaceae	Twig, leaf	100	37.35 ± 6.51	> 100
Euodia daniellii var. hupehensis (Dode) C.C.Huang	Rutaceae	Twig, leaf	100	32.76 ± 5.87	> 100
Forsythia Koreana (rehd.) Nakai	Oleaceae	Stem, leaf	100	5.36 ± 7.61	> 100
Geum aleppicum Jacq.	Rosaceae	Whole plant	100	6.40 ± 1.88	> 100
Glycyrrhiza pallidiflora Maxim.	Leguminosae	Leaf, stem	100	39.94 ± 5.30	> 100
Hemiptelea davidii (Hance) Planch.	Ulmaceae	Twig, stem, leaf	100	16.27 ± 4.51	> 100
Houttuynia cordata Thunb.	Saururaceae	Whole plant	100	3.08 ± 3.25	> 100
Ilex cornuta Lindl. & Paxton	Aquifoliaceae	Stem, leaf	100	6.08 ± 2.49	> 100
Lagerstroemia indica L.	Lythraceae	Stem, leaf	100	22.04 ± 6.74	> 100
Litsea japonica (Thunb.) Jussieu	Laurceae	Twig, leaf	100	9.0 ± 5.04	> 100
Lycopus ramosissimus var. *japonicus*	Labiatae	Whole plant	100	17.48 ± 2.31	> 100
Neillia uyekii Nakai for. Uyekii	Rosaceae	Twig, stem, leaf	100	36.55 ± 1.62	> 100
Paeonia suffruticosa Andr.	Ranunculaceae	Stem	100	18.60 ± 3.60	> 100
Patrinia scabiosaefolia Fisch. ex Link	Valerianaceae	Whole plant	100	17.34 ± 8.12	> 100
Petasites japonicas (Siebold & Zucc.) Maxim.	Compositae	Stem, leaf	100	3.86 ± 3.26	> 100
Prunella vulgaris L.	Labiatae	Leaf, stem	100	16.51 ± 10.42	> 100
Pueraria lobata (Willd.) Ohwi	Leguminosae	Stem, leaf	100	9.05 ± 3.15	> 100
Rhododendron schlippenbachii Maxim.	Ericaceae	Twig, stem, leaf	100	26.09 ± 2.06	> 100

(Continues)

Table 1. (Continued)

Scientific name	Family name	Part used	Conc. (μg/mL)	Inhibition (%)[a]	IC_{50} (μg/mL)
Rhus javanica L.	Anacardiaceae	Twig, stem, leaf	100	6.75 ± 3.25	> 100
Saururus chinensis Baill.	Saururaceae	Leaf, stem	65	49.18 ± 1.85	81.05
			80	49.89 ± 2.24	
			100	51.18 ± 1.02	
Schisandra chinensis Baillon	Magnoliaceae	Stem	100	11.94 ± 1.02	> 100
Sedum kamtschaticum Fisch. &Mey.	Crassulaceae	Root	65	43.97 ± 2.01	87.14
			80	48.70 ± 2.40	
			100	52.99 ± 0.92	
Sophora flavescens Aiton	Leguminosae	Root	100	48.63 ± 3.59	> 100
Sorbus commixta Hedl.	Rosaceae	Stem, leaf	20	46.45 ± 1.75	29.62
			35	53.1 ± 2.41	
			50	55.23 ± 0.84	
Stephanandra incisa (Thunb.) Zabel	Rosaceae	Twig, stem, leaf	100	27.79 ± 2.0	> 100
Styrax obassia Siebold & Zucc.	Styracaceae	Twig, stem	100	11.46 ± 1.63	> 100
Teucrium viscidum Blume	Labiatae	Whole plant	100	15.92 ± 6.25	> 100
Viola takesimana Nakai	Violaceae	Whole plant	100	12.59 ± 10.56	> 100
Viscum album L.	Loranthaceae	Whole plant	10	31.06 ± 1.79	33.32
			20	41.71 ± 1.57	
			35	50.55 ± 1.32	
Vitex rotundifolia L.f.	Verbenaceae	Stem	100	10.78 ± 1.11	> 100
Vitex rotundifolia L.f.	Verbenaceae	Leaf	100	7.52 ± 3.57	> 100
Weigela subsessilis L.H. Bailey	Caprifoliaceae	Twig, stem, leaf	100	0.74 ± 5.37	> 100
Xanthium strumarium L.	Compositae	Whole plant	100	8.92 ± 7.93	> 100
Orlistat (positive control)			0.025	18.03 ± 2.98	0.076
			0.05	35.64 ± 1.25	(0.154 μM)
			0.1	63.68 ± 1.41	

[a]Results are the mean ± SD (n = 4).

Pancreatic lipase inhibitory activity. Determination of pancreatic lipase activity was modified from that of Kim *et al.* (2005). Briefly, an enzyme buffer was prepared by the addition of 6 μL a solution of porcine pancreatic lipase (Sigma) [2.5 mg/mL in 10 mM MOPS (morpholinepropanesulphonic acid) and 1 mM EDTA, pH 6.8] to 169 μL of Tris buffer (100 mM Tris-HC1 and 5 mM $CaCl_2$, pH 7.0). Then, either 20 μL of the compound at the test concentration, or orlistat (Sigma), was mixed with 175 μL of the enzyme buffer and incubated for 15 min at 37 °C with 5 μL of the substrate solution [10 mM p-NPB (*p*-nitrophenyl butyrate) in dimethyl formamide]. The enzymatic reactions were allowed to proceed for 30 min at 37 °C. Lipase activity was determined by measuring the hydrolysis of p-NPB into p-nitrophenol. Increase in light absorption at 405 nm was measured using a plate reader (Bio-Tek, Synergy HT, USA). Inhibition of lipase activity was expressed as the percentage decrease in OD when porcine pancreatic lipase was incubated with the test compounds. Lipase inhibition (%) was calculated according the following formula:

$$\text{Inhibition } (\%) = 100 - \left(\frac{B-b}{A-a} \times 100\right)$$

where A is the activity without inhibitor, a the negative control without inhibitor, B the activity with inhibitor and b is the negative control with inhibitor. The results were expressed as an average ($n = 4$).

Phosphodiesterase (PDE) activity assay. The PDE activity was assayed using the PDE-GloTM phosphodiesterase activity kit (Promega Corp., WI, USA) according to the manufacturer's instructions. The results were expressed as an average ($n = 3$) (Kim *et al.*, 2010).

Statistical analysis. Unpaired Student's *t*-tests were performed and data are expressed as the mean ± SEM of multiple experiments (Prism Software, Graph Pad, San Diego, CA, USA). A difference in the mean values of $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Pancreatic lipase inhibition is one of the most widely studied mechanisms used to determine the potential efficacy of natural products as antiobesity drugs (Kim and Kang, 2005; Kim *et al.*, 2007; Rahimi *et al.*, 2010). The results of inhibitory activity of 61 Korean medicinal plants on pancreatic lipase have been summarized in Table 1. Lipase inhibition is expressed as the percentage (%) and IC_{50} value (the concentration required to inhibit a lipase activity by 50%). Of the extracts tested, 11 showed an IC_{50} of less than 100 μg/mL, and of the 11 extracts, five exhibited an IC_{50} of less than 50 μg/mL. *Sorbus commixta* (stem, leaf) showed the strongest inhibitory activity on lipase with an IC_{50} value of 29.62 μg/mL. Those of *Viscum album* (whole plant), *Acer ginnala* (fruit), *Camellia japonica* (stem, leaf) and

Castanea crenata (staminate flower) exhibited the inhibitory effect with an IC_{50} value of 30–50 μg/mL.

Previous study shows that 200 μg/mL of *Eriochloa villosa, Orixa japonica* (81.3%) and *Setaria italica* (80.3%) extracts exhibit antilipase activity greater than 80% *in vitro* (Sharma *et al.,* 2005). In this study, *Sorbus commixta* (stem, leaf) extracts (IC_{50} = 29.62 μg/mL) showed a stronger inhibitory effect than those of these extracts on antilipase activity. However, it was not more effective than orlistat (IC_{50} = 0.076 μg/mL), a positive control. Orlistat, one of the clinically approved drugs for obesity treatment, has been shown to act by inhibition of pancreatic lipase. Although it is one of the best-selling drugs worldwide, it has serious side effects (Kim *et al.*, 2007). In this study, *Sorbus commixta* and *Viscum album* showed inhibitory effects on PDE activity in dose-dependent manners (Fig. 1). *Sorbus commixta* shows strong inhibitory activity against protein tyrosine phosphatase 1B (PTP1B), an attractive target for the development of new drugs for type 2 diabetes (Ahmed *et al.,* 2008; Na *et al.,* 2009). Furthermore, it has also antioxidative activity (Bae *et al*., 2007). *Viscum album* (mistletoe), which is distributed in Korea and other East-Asian countries, has long been recognized as a therapeutic herb. It has been traditionally used as a sedative, analgesic, antispasmolytic, cardiotonic and anticancer agent (Lyu and Park, 2006). *Acer ginnala* shows the inhibitory effect on rat lens aldose reductase activity *in vitro* (Kim and Oh, 1999). Saponins isolated from *Camellia japonica* leaves have been shown to have antifungal activity and antifeedant activity and tannins from *Camellia japonica* leaves have an inhibitory effect on HIV-1 protease (Onodera *et al*., 2006). Flower extracts of *Castanea crenata* are reported to have antimicrobial activity (Lee *et al*., 1999).

To date, *Sorbus commixta* (twig, leaf) extracts have not been reported to have lipase and PDE inhibitory activities. Thus, it is worthwhile to further investigate these extracts for their potential pharmacological effect in obesity *in vivo* and attempts should be made to purify their active components to be used as safer and cheaper therapeutic agents in future.

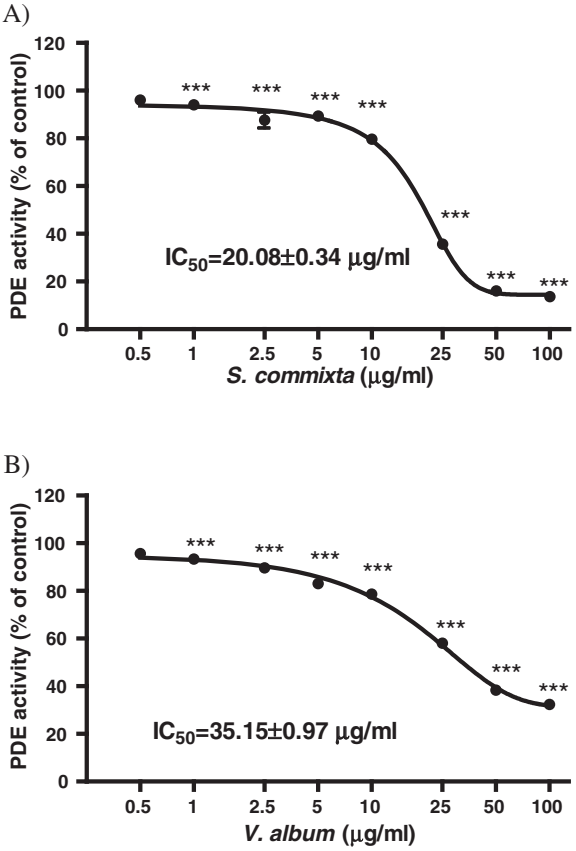

Figure 1. Inhibitory effects of *S. commixta.* and *V. album* on PDE activity. (A) *S. commixta.* Data are expressed as the mean ± SEM (n = 3). ***$p < 0.001$ vs. untreated group. (B) *V. album.* Data are expressed as the mean ± SEM (n = 3). ***$p < 0.001$ vs. untreated group.

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Conflict of Interest

The authors report no conflict of interest.

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