

# Acanthopanax and Platycodi Independently Prevents the Onset of High Fat Diet Induced Hyperglyceridemia and Obesity in C57BL/6 Mice

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**Abstract** Using high-fat-fed C57BL/6 mice, therapeutic effects of oral administration of aqueous extracts of *Platycodon grandiflorum* and *Acanthopanax senticosus* on obesity were evaluated by comparing body and liver weights, feed intake, adipose tissue mass, blood lipid profile, and triglyceride and total cholesterol levels in liver and feces. Obesity was successfully induced in high-fat diet group. *P. grandiflorum* and *A. senticosus* were effective in reducing food intake, serum lipid levels, adipose tissue accumulation, and weight. *P. grandiflorum* extract reduced triglyceride and cholesterol levels in liver by excluding them in feces. *A. senticosus* extract increased triglyceride level in liver but decreased in feces, and decreased total cholesterol in liver and feces, indicating active ingredient of *A. senticosus* exert antiobesity effect through mechanism different from that of *P. grandiflorum* extract. These results suggest aqueous extracts of *P. grandiflorum* and *A. senticosus* have synergistic effect for prevention of hyperglyceridemia and obesity.

Key words: Platycodon grandiflorum, Acanthopanax senticosus, hyperglyceridemia, obesity

### Introduction

Obesity is a chronic and relapsing disease characterized by increased body weight of significant magnitude (1). It is clear that obesity results from imbalance between energy intake and expenditure caused by genetic and environmental factors. Deregulation of body weight is a serious health problem associated with many chronic health conditions, such as diabetes, cardiovascular disease, hypertension, hyperlipemia, osteoarthritis, and several types of other disease including cancer, increasing the potentiality of death by 20%.

Intensive researches have been conducted to control obesity due to serious public health implications. Various therapies, such as pharmaceutical drugs and exercise, in addition to surgery, have been developed for treatment of obesity (2). These approaches, however, are not much successful in most cases. So far, the most efficient obesity treatment other than surgery is dietary intervention. In particular, antiobese dietary supplementation gives very satisfactory results when combined with low calorie diet, exercise, and behavior modification. Therefore, antiobese dietary supplementation has gained attention among health professionals as an effective means not only for treating obesity but also for preventing obesity. For this reasons, supplementary and alternative medicines such as herbal crude extracts are gaining popularity among patients with obesity (3).

Platycodi radix (Korean *Doraji*, Chinese *Jiegene*, Japanese *Kikyo*) is the root of *Platycodon grandiflorum* (PG). Platycodi radix has been used in oriental herbal medicine as an expectorant for pulmonary diseases and a remedy for respiratory disorders, such as bronchitis, tonsillitis, laryngitis, and suppurative dermatitis (4). The

major active component of Platycodi radix is saponin, which has diverse effects including anti-inflammation, anti-allergy, and anti-tumor, immune-boosting activities, and apoptosis of skin cells. Recently, Platycodi radix gained more attention due to its potentials for treating hyperlipemia, hypertension, diabetes, and obesity (5,6). Han *et al.* reported an aqueous extract of Platycodi radix significantly reduced body weight and adipose tissue weight in diet-induced obese mice. Saponins of Platycodi radix appeared to inhibit the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity (7). Further study showed that the active component responsible for anti-obese effect by inhibiting pancreatic lipase activity is platycodin D (6).

Acanthopanax senticosus, also called the "Siberian Ginseng" or "Eleutherococcus", has been well known to be prophylactic for various diseases (8). The root and stem barks of A. senticosus have traditionally been used as tonic and sedative for stress, as well as in the treatment of many chronic diseases such as diabetes mellitus, rheumatoid arthritis, chronic bronchitis, hypertension, gastric ulcer, ischemia (9, 10, 11). A. senticosus is known to have various compounds such as acanthosides, eleutherosides, senticoside, triterpenic saponin, flavon, vitamins, and minerals, which are related to various biological activities (12, 13).

A water extract of *A. senticosus* leaves increased the lipoprotein lipase activity in the adipocyte culture, suggesting its role in plasma triglyceride clearance and thereby treatment of metabolic disease (14). Oral administration of the extract prepared from cultured *A. senticosus* cells reduced the weight gain, serum LDL-cholesterol concentrations, and liver triglyceride accumulation in C57BL/6J mice with obesity induced by high-fat diets (15). Although aqueous extract of *A. senticosus* are is popular in Asian countries as herbal dietary supplement for obese people, no study has yet been

Received September 6, 2005; accepted November 9, 2005

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performed on anti-obese effect of the crude aqueous extract of *A. senticosus*, which is the most common form of dietary consumption of *A. senticosus* so far. Only a few studies reported the effects of *A. senticosus* extract from cultured cells in obese mice and lipase activity using adipocyte cells *in vitro*. Consequently, detailed action mechanism and pharmacological targets are mostly unknown.

In this study, the effect of aqueous extracts of *A. senticosus* on obesity of diet induced obese mice was studied comparatively with aqueous extracts of *P. grandiflorum*. The C57BL/6 mice strain was chosen to study the effects of *A. senticosus* and *P. grandiflorum* in obesity, because it provides an excellent animal model for study of the mechanism and treatment of obesity.

### Materials and Methods

**Materials** Aqueous extracts (40 and 20%, respectively; w/w) of *A. senticosus* and *P. grandiflorum* were purchased from an herbal processing company (Sung-lim, Chonbuk, Korea) and kept at 4°C until use.

Animals and diets Four-week-old male C57BL/6 mice were purchased from the Semdaco Biokorea Co. (Kyungki-do, Korea) and housed for 1 week before random assignment into experimental groups. Twelve mice were housed in each cage with a 12-h light: 12-h dark cycle and free access to food and water. Temperature and humidity were controlled at 22±1°C and 55±5%. respectively. During the acclimatization period, each mouse was raised on a regular diet (AIN 76A rodent purified diet) ad libitum. After adaptation to the lighting conditions for 1 week, healthy C57BL/6 mice were randomly divided into four groups (12 animals each): regular diet control (RD), high-fat diet control (HFD), high-fat diet plus A. senticosus (HFD+AS), and high-fat diet plus P. grandiflorum (HFD+PG). The compositions of regular diet and high-fat diet (HFD, containing 40% fat and 0.5% cholesterol (w/w) are shown in Table 1. The control mice continued to receive either a regular diet or

Table 1. Composition of diets

	Regular diet (g/kg diet)	High fat diet (g/kg diet)
Ingredients		
Casein	200	200
DL-Methionine	3	3
Corn Starch	150	150
Sucrose	500	145
Cellulose	50	50
Corn oil	50	50
Shortening	-	350
AIN76 Mineral Mixture	35	35
AIN76 Vitamin Mixture	10	10
Choline bitartrate	2	2
Cholesterol	-	5
Total(g)	1,000	1,000

Regular diet: AIN76 rodent purified diet

high-fat diet. The supplementation group mice were orally fed a high-fat diet plus 0.5 ml aqueous solution containing 5 mg *Platycodi radix* extraction powder (HFD+PG) or 0.5 ml aqueous solution containing 2.5 mg *Acanthopanax* extraction powder (HFD+AS) for 12 weeks. The food consumption and body weight were measured periodically. Mice were sacrificed at 12 weeks of test period, sera were drawn, and fat tissue and liver were harvested as described below.

Serum and tissue samples Blood, liver, and feces were collected at 12 weeks (the last day of the experiment). After 9-hr fasting, blood samples were drawn from the retro-orbital sinus under anesthesia and placed at room temperature for clotting. Serum was separated from the blood by centrifugation at 1,500 g for 15 min at 4°C. The collected serum was stored at -70°C until analysis of lipids. The liver and fat tissue (white fat: periepididymis, mesenterium, retroperitonium, and ventral subcutaneous, brown fat: interscapular) were quickly removed and weighed, and separately stored at -70°C until analysis of lipids. The fecal samples were collected 24 hr prior to animal sacrifice, freeze-dried, weighed, and kept at -70°C until analysis of lipids.

**Analysis of lipids** The concentrations of total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride in serum were measured using biochemical autoanalyser (Hitachi 7600-110, Autoanalyzer, Tokyo, Japan). Total cholesterol and triglyceride concentrations are based on enzymatic colorimetric test, and HDL-C and LDL-C concentrations are based on the selective solubilizing effect of proprietary detergent to the different lipoproteins. Extraction of liver followed Jeon & Park methods (16). Briefly, the frozen liver sample (1 g) was homogenized in PBS using a Potter-Elvehjem homogenizer (EYELA MDC-NR. Tokyo Rijakikai, Tokyo, Japan) at 9 strokes. The total volume of each homogenate was adjusted to 10 ml using 2-isopropanol. Triglyceride and total cholesterol were extracted at 4°C for 24 hr, and the homogenate was centrifuged for 10 min at 500 × g. The supernatant obtained and total cholesterol and triglyceride were measured using biochemical autoanalyzer (Hitachi 7600-110, Autoanalyzer).

Extraction of fecal cholesterol and triglyceride followed the Lee methods (17). Fecal cholesterol and triglyceride were extracted three times from the powered freeze-dried mouse feces (1 g) using hot ethanol ( $50^{\circ}$ C) with mixing. Extracted lipids were resolved in  $10 \times$  methanol, and the suspension was centrifuged for 10 min at  $500 \times \text{g}$ . The supernatant was analyzed by enzymatic measurement of triglyceride and total cholesterol using biochemical autoanalyzer (Hitachi 7600-110, Autoanalyzer).

**Statistical analysis** All measurements were expressed as the means  $\pm$  SEM. The data from the control group and experimental groups were analyzed by analysis of variance (ANOVA) followed by Duncan's multiple range t-test. *P*-values < 0.05 were considered significant.

# Results

Body weight change and feed efficiency To study the

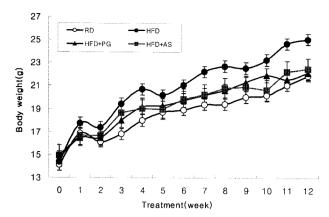


Fig. 1. Body weight change of mice during the 12-week period. Values represent the mean±SEM (n=12). RD, mice consuming regular diet; HFD, high fat diet; HFD+PG, mice fed high fat diet plus daily, 0.5 ml aqueous solution containing 5 mg Platycodi radix extraction powder; HFD+AS, 0.5 ml aqueous solution containing 2.5 mg Acanthopanax extraction powder.

effect of aqueous extracts of A. senticosus and P. grandiflorum on obesity, C57BL/6 mice were fed high-fat diet with aqueous extract of A. senticosus or P. grandiflorum. Following 12 weeks of high-fat diet. C57BL/6 mice with high-fat diet group showed faster weight gain and heavier body weight than the regular diet group, with 20% more body weight at the end of 12-week trial (Fig. 1). Body weight gains in regular diet and high fat diet control groups at the end of 12-week trial were 7.8  $\pm 0.4$  and  $10.7\pm 0.7$  g, respectively (Table 2). However, the test animals administered with either aqueous extract of A. senticosus or P. grandiflorum showed significantly less weight gain compared to the high-fat diet group (P < 0.05). Subjects fed the high-fat diet plus either P. grandiflorum or A. senticosus showed a weight gain of 7.5  $\pm$ 0.4 and 7.9±0.5 g, respectively, which are very close to that of regular diet group (Fig. 1 and Table 2). These increases are significantly lower than that for the high-fat diet control group in spite of the continued and prolonged access to the high-fat diet, implying the strong anti-obese effect of aqueous extracts of A. senticosus and P. grandiflorum.

Along with body weight change, decreasing tendency in the food intake of test animals administered with either aqueous extract of A. senticosus or P. grandiflorum was observed as compared to the high-fat diet group (Table 2). Feed efficiency, calculated by weight gain divided by total food intake during the 12-week period, was compared to determine the relationship between food intake and weight gain. In addition to the reduction of food intake, feed efficiency in mice fed HFD plus A. senticosus or P. grandiflorum was lower than that of the HFD control (Table 2).

Liver weight and fat mass Feeding high-fat diet significantly increased liver weight, as similarly observed with the body weight (Table 3). The diet-induced increase of liver weight returned to the normal level as in the regular diet group by administration of A. senticosus or P. grandiflorum. In terms of total fat mass, white and brown adipose tissues of HFD mice increased by 17 and 14%, respectively, compared to that of regular diet group. Particularly, accumulation of fat was remarkable in epididymal and ventrosubcutaneous white adipose tissues, demonstrating up to 50 and 108% increases, respectively, compared to the regular diet control. Administration of A. senticosus and P. grandiflorum, however, resulted in the reduction of white fat deposition, almost to the same level as in the regular diet group in all tested adipose tissue, epididymal fat, mesentric fat, ventrosubcutaneous fat, and

Table 2. Effects of the aqueous extract of Platycodon grandiflorum and Acanthopanax senticosus on weight gain, food intake, and feed efficiency

Group	Initial(g)	Final(g)	Weight gain (g/12wk)	Food intake (g/12wk)	Feed efficiency (× 10 <sup>-3</sup> )
RD	14.1±0.3	21.9±0.4	7.8±0.4	247	3.15
HFD	$14.4 \pm 0.6$	$25.1 \pm 0.7^{\dagger}$	$10.7 \pm 0.7^{\dagger}$	238	4.5 <sup>†</sup>
HFD+PG	$14.7 \pm 0.4$	$22.2\pm0.4^*$	$7.5\pm0.4^*$	198	3.8*
HFD+AS	14.9±0.3	22.5±0.5*	$7.9\pm0.5^*$	189	4.0*

Values represent the mean±SEM (n=12)

Feed efficiency = [weight gain(g/12wk)]/[food intake(g/12wk)]

Table 3. Effects of the aqueous extract of Platycodon grandiflorum and Acanthopanax senticosus on liver weight and adipose tissue accumulation

A TROUTS	liver wt(mg) —	white adipose tissue(mg)				brown adipose
	/body wt(g)	epididymal fat	mesentric fat	ventro-subcutaneous fat	retro-peritoneal fat	tissue (mg) Interscapular fat
RD	49.7±5.3	342.3±77.2	145.3±28.3	211.7±47.8	118.0±19.5	65.0±6.1
HFD	54.5±3.5 <sup>†</sup>	514.7±128 <sup>†</sup>	167.0±29.9	$441.6 \pm 79.6^{\dagger}$	224.3±67.9 <sup>†</sup>	89.1±14.7
HFD+PG	47.1±3.1*	259.4±71.2*	127.2±7.40*	$202.6\pm42.0^{*}$	115.8±24.4*	$75.8 \pm 18.3$
HFD+AS	51.8±4.3	265.5±31.6*	$121.8\pm7.40^*$	213.8±39.9*	92.5±25.6*	$81.0 \pm 14.5$

The liver and total fat (white fat: periepididymis, mesunterium, retroperitonium and ventral subcutaneous, brown fat: interscapular) were collected at the last day of the experiment. Values represent the mean±SEM (n=12).

\*P<0.05 vs. RD

<sup>&</sup>lt;sup>†</sup>P<0.05 vs. RD \*P<0.05 vs. HFD

<sup>\*</sup>P<0.05 vs. HFD.

Table 4. Effects of the aqueous extract of *Platycodon* grandiflorum and *Acanthopanax senticosus* on lipid profiles in serum

	Lip	HDL/			
Group	Total cholesterol	Triglyceride	HDL	LDL	LDL/
RD	94.3±3.1	70.5±3.1	54.0±2.2	24.2±2.5	2.23
HFD	132.0±4.6 <sup>†</sup>	91.5±8.1 <sup>†</sup>	$76.5{\pm}3.2^{\dagger}$	35.2±3.1 <sup>†</sup>	2.17
HFD+PG	124.8±3.3	$60.0\pm6.5^*$	79.0±2.8	32.5±2.2	2.43
HFG+AS	123.8±4.1	65.0±7.1*	80.8±3.1	$33.3\pm2.8$	2.40

Blood for serum lipid levels was collected at the 12 weeks (the last day of the experiment). Values represent the mean±SEM (n=12). 
†P<0.05 vs. RD

retroperitoneal fat (P<0.05). However, A. senticosus and P. grandiflorum administrations had no significant effects on brown fat mass.

**Lipid levels in serum** The effects of aqueous extracts of *A. senticosus* and *P. grandiflorum* on obesity were studied by measuring serum lipid profiles in C57BL/6 mice after 12 weeks of test period (Table 4). Serum total cholesterol concentrations were significantly higher in mice fed highfat diet than in the regular diet group: 94.3±3.1 and 132.0±4.6 mg/dl in regular diet and high-fat diet groups, respectively (*P*<0.05). Increment of total cholesterol, however, was slightly reduced in animals fed either aqueous extract of *A. senticosus* or *P. grandiflorum* in addition to high-fat diet.

Similarly, triglyceride levels in the serum were remarkably higher in mice fed high-fat diet than the regular diet group. However, the supplementation of aqueous extracts of *A. senticosus* and *P. grandiflorum* in addition to HFD reduced the level of triglyceride significantly, resulting in even lower concentration than that of regular diet: 70.5±3.1, 91.5±8.1, 60.0±6.5, and 65.0±7.1 mg/dl in regular diet, high-fat diet, high-fat diet plus *P. grandiflorum*, and high-fat diet plus *A. senticosus* groups, respectively (*P*<0.05).

However, no significant differences were observed in HDL-cholesterol levels of the HFD control and HFD group with *A. senticosus* or *P. grandiflorum*, although the ratios of HDL/LDL were slightly higher in HFD group with *A. senticosus* or *P. grandiflorum* due to the decrement of LDL-cholesterol level.

**Lipid levels in liver and feces** Triglyceride levels in liver and feces were measured to track any changes in the liver triglyceride level and fecal excretion after feeding aqueous extract of *A. senticosus* or *P. grandiflorum* (Fig. 2). Feeding high-fat diet increased liver triglyceride up to twofold compared to the control, while fecal extraction remained almost the same, implying high-fat diet causes increment of liver triglyceride. Interestingly, feeding aqueous extract of *P. grandiflorum* once again restored the normal level of liver triglyceride, while significantly increased fecal triglyceride concentration, 1.5-fold compared to high-fat diet only. In contrast, administration of *A. senticosus* extract increased the liver triglyceride level, but decreased fecal triglyceride level compared to the HFD

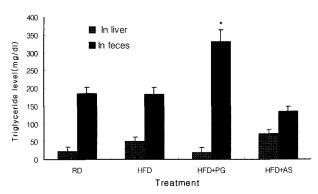


Fig 2. Effects of the aqueous extract of *Platycodon grandiflorum* and *Acanthopanax senticosus* on the triglyceride levels in liver and feces. Feces and liver were collected at the last day of the experiment. Each bar represent the mean±SEM (n=12). \*P<0.05 vs. HFD.

control.

In addition to triglyceride, total cholesterol levels in liver and feces were also measured after feeding aqueous extract of *A. senticosus* or *P. grandiflorum* (Fig. 3). High-fat diet slightly increased the total cholesterol level in the liver but not in feces. Feeding aqueous extract of *P. grandiflorum*, however, significantly reduced the liver total cholesterol, even to less than one third of the regular diet group level. Fecal cholesterol level, however, increased slightly. Administration of *A. senticosus* extract decreased the liver cholesterol level slightly, while increased fecal excretion of total cholesterol slightly compared to the control.

# Discussion

Obesity is one of the fastest growing major diseases in developed countries today (18). The prevalence of obesity in the United States essentially doubled from 15% in 1980 to 30% in 2000 (19,20), and recently surpassed smoking as the number one cause of death in the United States (21). Currently, more than half of the U.S. population is overweight, and one-third of the American population is considered obese (22). This increased prevalence is accompanied by a life economic burden. It has been

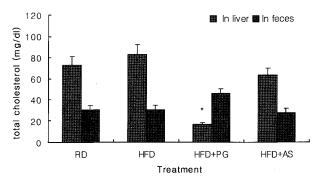


Fig 3. Effects of the aqueous extract of *Platycodon grandiflorum* and *Acanthopanax senticosus* on total cholesterol levels in liver and feces. Feces and liver were collected at the last day of the experiment. Each bar represent the mean±SEM (n=12). \*P<0.05 vs. HFD

<sup>\*</sup>P<0.05 vs. HFD.

estimated that obesity accounts for \$100 billion dollars in health care expenses per year and is responsible for 5.7% of US health care costs (23).

Intensive researches have been conducted to control obesity by drug therapy due to serious risk to public health. Large numbers of patients, however, are not suitable candidates for drug therapy either due to lack of efficacy or poor safety performance and resultant side effects including diarrhea, anorexia, sleep disturbance, migraine, indigestion, dystrophia, cataracts, neuropathy, as well as abuse potential (24, 25). Weight loss with exercise is ineffective in many cases, because lifestyle changes in exercise and behavior are very difficult. As an alternative, traditional herbal medicines have received more acceptance than prescription drugs in many cultures in spite of insufficient data.

This research was designed to compare the therapeutic effect of oral administration of the aqueous extracts prepared from P. grandiflorum and A. senticosus on obesity using high-fat fed C57BL/6 mice. Studies on the antiobesity effect of P. grandiflorum and A. senticosus have only recently been performed, although they have been traditionally used as expectorants and remedies for various diseases. Several studies showed that Platycodi radix affects lipid metabolism in high-fat diet induced obese mice (5, 6, 7, 26). For Acanthopanax, however, antiobesity effect has only been reported very recently and needs to be demonstrated (15). Therefore, the anti-obesity effect of the aqueous extracts prepared from P. grandiflorum and A. senticosus were measured by comparing the weights of body and liver, feed intake, adipose tissue mass, levels of triglyceride and total cholesterol levels in the liver and feces.

C57BL/6 mice were fed high-fat diet to induce obesity was successfully induced in the group during the 12-week trial. However, oral feeding of P. grandiflorum and A. senticosus extracts resulted in significantly lower weight gain than the HFD group in spite of continued and prolonged access to the high-fat diet. Oral administration of P. grandiflorum and A. senticosus extracts decreased not only feed efficiency but also food intake, and the decrease was more significant in P. grandiflorum extract group (Fig. 1, Table 2). It is possible that gastric emptying time was delayed by the abundant amount of fibrous component in the P. grandiflorum extract, which promoted satiety and reduced food intake, consistent with the previous report (27). This suggests that P. grandiflorum extract can be developed as a slimming beverage, despite an increase in food intake or reduction of physical activity. In terms of blood lipid, Zhao et al. (26) reported that addition of P. grandiflorum to high-fat diet decreased serum TG, TC, and LDL-C and increased HDL-C in mice. In our study, the concentrations of serum TG, TC, and LDL-C decreased significantly, while those of HDL-C remained almost unchanged, suggesting that the cholesterol-lowering effect might be exclusively due to the reduction of LDL-C (Table 4). Measuring fat accumulation is another important parameter for antiobesity, because obesity is associated with abnormal fat accumulation. In this experiment, oral administrations of P. grandiflorum and A. senticosus extracts resulted in significantly lower amounts white adipose tissue, epididymal fat, ventrosubcutaneous fat.

These results are in agreement with the result of other study that aqueous extract of *P. grandiflorum* reduced the small intestinal absorption of dietary fat by inhibiting pancreatic lipase activity (6).

Next, we compared the lipid profiles of the liver and feces to evaluate whether the aqueous extracts of P. grandiflorum and A. senticosus function through similar mechanisms. Oral administration of P. grandiflorum extract reduced both TG and TC levels in the liver, while increased lipid level in the feces (Figs. 2 and 3). A. senticosus extract, however, increased triglyceride level only in the liver, while decreased TG levels in the feces and TC levels in the liver and feces. Cholesterol metabolism is controlled by feedback action of the bile salt and homeostasis in the body. Cholesterol is synthesized in the liver, transported as a complex with bile salts, and absorbed into the blood in small intestine, while bile salts return to the liver (28). In this feedback pathway, supplementation of P. grandiflorum could stimulate transportation of cholesterol-bile salt complex into fecal excretion after binding with the fiber of  $\hat{P}$ . grandiflorum (28, 29). Thereby decreasing the levels of cholesterol available for synthesis of bile salt and cholesterol in the liver.

In this study, oral administrations of *P. grandiflorum* and *A. senticosus* resulted in the antiobese effect. They both reduced lipid levels in the serum and adipose tissue accumulation, which is accompanied by weight loss. *P. grandiflorum* extract appeared to reduce food consumption, inhibit small intestinal absorption of dietary fat, stimulation of bile salt secretion, resulting in lipid regulation and weight loss. *A. senticosus* extract also resulted in weight loss, but showed lipid changes different from those of *P. grandiflorum* extract. The active ingredient of *A. senticosus* appeared to exert antiobesity effect through a different mechanism compared to *P. grandiflorum* extract, thus requiring further work to clarify the antiobesity mechanisms of *A. senticosus*.

Aqueous extracts of P. grandiflorum and A. senticosus can be developed as dietary supplements for obese people. Reduction of feed efficiency in mice suggests P. grandiflorum and A. senticosus could be used to produce beverages that allow weight loss, in spite of the increased food intake or reduced physical activity. Furthermore, results suggest the different mechanisms of antiobese components of P. grandiflorum and A. senticosus can have synergistic effect on antiobesity when taken together. Herbal extracts have attractions as alternative treatments to obese patients; they are viewed as being natural and thus assumed by patients to be safer than prescription drugs, and there is no perceived need for professional assistance with these approaches. Therefore, herbal extracts such as P. grandiflorum and A. senticosus can be very effective in preventing chronic and relapsing lifetime diseases such as obesity.

# Acknowledgments

This research was supported by Research Center for Industrial Development of BioFood Materials in Chonbuk National University, Jeonju, Korea. The center is designated as a Regional Research Center appointed by the Ministry of Commerce, Industry and Energy

(MOCIE), Jeollabuk-do Provincial Government and Chonbuk National University.

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