

Modulation of adipose tissue function by *Philadelphus schrenkii* extract as a potential anti-obesity agent

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Received: Nov 26, 2025

Revised: Dec 8, 2025

Accepted: Dec 8, 2025

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Abstract

Plant-derived natural products, recognized for their bioactive properties and minimal side effects, have been widely explored for their potential in obesity management. Identifying plant-based agents that can modulate adipocyte function with low cytotoxicity is essential for developing safe and effective anti-obesity interventions. In this study, *Philadelphus schrenkii* (Korean mock orange) was identified as a promising candidate following an initial screening for agents that exhibit minimal cytotoxicity and reduced adipocyte differentiation, as assessed by Oil Red O staining. The anti-obesity effects of *P. schrenkii* methanol extract (PSE) were evaluated by using *in vitro* and *in vivo* models. PSE treatment significantly reduced C3H10T1/2 preadipocyte differentiation and upregulated thermogenic markers, including *Ucp1* and *Dio2*, in differentiated cells. Although PSE did not induce weight loss, alter food intake, or improve the serum metabolic profiles in a diet-induced obesity mouse model, it notably enhanced the thermogenic *Ucp1* expression in inguinal white adipose tissue (iWAT) and brown adipose tissue. It also mitigated high-fat diet-induced adiposity in iWAT, accompanied by Protein Kinase A signaling activation. These findings suggest that PSE modulates adipose tissue function by suppressing adipogenesis and promoting thermogenic gene expression without weight reduction or metabolic improvement. Based on these effects, PSE may contribute as a supportive agent to plant-based therapeutic strategies against obesity.

Keywords: obesity; thermogenesis; dietary supplements; plant extracts; metabolism

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

This research was supported by "Regional Innovation System & Education (RISE)" through the Gwangju RISE Center, funded by the Ministry of Education (MOE) and the Gwangju Metropolitan Government, Republic of Korea (2025-RISE-05-011, to M-J. P.). This work was supported by the National Research Council of Science & Technology (NST) grant by the Korea government (MSIT) (CRC21022-000).

Ethics Approval

All mouse experimental procedures were approved by the Institutional Animal Care and Use Committee of Chonnam National University (CNU IACUC-YB-2024-208).

INTRODUCTION

The global prevalence of obesity has driven the development of various treatment strategies aimed at improving weight loss and metabolic health [1]. Although these approaches have considerably advanced, existing therapies may cause adverse side effects or pose the risk of weight regain [2]. Therefore, safer, more effective, and sustainable alternatives, such as plant-based treatments, should be established to manage obesity. Plant-based natural products have gained considerable attention as novel therapeutic agents because of their bioactive properties and minimal side effects [3].

Herbal compounds are widely recognized for their therapeutic benefits, such as regulating physiological processes and supporting weight loss [4]. These properties make them suitable candidate agents for obesity treatment. Furthermore, plant-derived extracts exert beneficial responses, such as inhibiting gluconeogenic and lipogenic gene expression, reducing insulin resistance and inflammation, and protecting against oxidative liver damage [5–9]. However, no anti-obesity agent has been identified as the most effective to date.

The leaf extract of *Philadelphus schrenkii*, an East Asia native species used in traditional medicine [10], exhibits antibacterial [11], antioxidant [12], and anti-inflammatory [13] properties. Such properties are associated with metabolic benefits, including improved insulin sensitivity and reduced adipose tissue inflammation [14]. However, despite these known advantages, whether *P. schrenkii* can directly influence key mechanisms in adipose tissue to mitigate obesity remains unexplored.

One promising strategy for obesity treatment is the activation of thermogenic adipocytes, which are brown and beige fat cells, to enhance energy utilization [15]. These specialized adipocytes dissipate energy as heat through nonshivering thermogenesis, which is a process primarily mediated by uncoupling protein 1 (UCP1) [16]. This process involves the coordinated expression of proteins participating in thermogenesis and mitochondrial oxidative phosphorylation (OXPHOS) [16]. Thermogenic adipocyte activation has been associated with increased energy expenditure [17], improved glucose homeostasis [18], and lipid metabolism [18], making it a multifaceted strategy against obesity.

In this study, we investigated the anti-obesity properties of *P. schrenkii* methanol extract (PSE), selected as a candidate from a screening of 105 plant-derived samples, in adipocytes *in vitro*, as well as normal or obese mouse models *in vivo*. We examined the molecular changes associated with PSE treatment, focusing on thermogenic markers, to elucidate its possible physiological benefits.

MATERIALS AND METHODS

Preparation of plant extracts

Plant extracts were obtained from the Natural Product Central Bank at the Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea). The detailed information on the plant samples is presented in Supplementary Table S1. Each plant was dried under the shade

for 2 weeks and extracted with methyl alcohol 99.9 % (HPLC grade) as the extract solvent in an enclosed ultrasonic extractor (SDN-900H, SD-Ultrasonic, Seoul, Korea) under the following conditions: 1,500 W, 40 kHz, 120 min ultrasonication, standing per cycle, and 30 cycles. After extraction, the mixture was filtered (Qualitative Filter No. 100, Hyundai Micro, Anseong, Korea) at room temperature and dried under reduced pressure.

Cell viability and Oil Red O staining

3T3-L1 cells were cultured in DMEM with 10% fetal bovine serum (FBS) and penicillin-streptomycin (10 U/mL, 15140163, Thermo Fisher Scientific, Waltham, MA, USA) and maintained in a 5% CO₂ incubator at 37°C until confluent. 3T3-L1 preadipocytes were seeded at a density of 1×10^4 cells/well in 96-well plates to confirm the effects of plant extracts on cell viability. They were treated with 50 µg/mL plant extracts. After 24, 48, and 72 hr of incubation with the treatment, 10 µL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (Amresco, San Francisco, CA, USA) was added to each well, and the cells were incubated at 37°C for 3 hr. After the supernatant was removed, 0.04 M HCl-isopropanol was added to each well with the same volume as the media to dissolve formazan crystals. The absorbance of each well was measured at 540 nm by using the microplate reader. The optical density of the control cells (untreated) was taken as 100% viability.

For adipocyte differentiation, 3T3-L1 preadipocytes were seeded at a density of 5×10^3 cells/well in six-well plates. After 48 hr of incubation, they were incubated with differentiation induction medium I (DMEM with 10% FBS, penicillin-streptomycin, 0.5 µg/mL insulin [I5500, Sigma-Aldrich, St. Louis, MO, USA], dexamethasone [5 µM, D4902, Sigma-Aldrich], and 3-isobutyl-1-methylxanthine [IBMX, 0.5 mM, I7018, Sigma-Aldrich]) for 72 hr and then in differentiation induction medium II (DMEM with 10% FBS, 10 U/mL penicillin-streptomycin, and 10 µg/mL insulin) for another 72 hr. The plant extracts (50 µg/mL) were added during each medium change throughout the differentiation period.

Afterward, the cells were washed twice with phosphate-buffered saline (PBS) and added with 1 mL of propylene glycol to each well for 2 min. Propylene glycol was removed, and the cells were washed again with PBS. Then, they were stained with 0.5 mL of the working solution of Oil Red O (ORO; O0625, Sigma-Aldrich) for 20 min. They were washed with 85% propylene glycol and added with 1 mL of distilled water to remove excess stain. The stained lipid droplets were visualized using a light microscope (Leica Microsystems, Wetzlar, Germany). Subsequently, the cells were rinsed twice with distilled water, and the remaining ORO dye was extracted using 0.5 mL of isopropanol for 10 min. Absorbance was determined at 500 nm by using a multi-well microplate reader (BioTek, Agilent, Santa Clara, CA, USA). Results were normalized to the optical density of the untreated control cells set as 100%.

For 10T cells, differentiation was induced using differentiation induction medium I containing DMEM, 10% FBS, 10 U/mL penicillin-streptomycin, insulin (10 µg/mL), dexamethasone (1 µM), and IBMX (0.5 mM). After 48 hr of incubation, the medium was replaced with a complete medium supplemented with insulin (10 µg/mL), and this process was repeated twice. For the analysis of 10T cell differentiation, PSE (50 µg/mL) was added during each medium

change throughout the differentiation period. For mRNA and protein analysis, PSE was added to the fully differentiated cells for 24 hr.

Animal experiments

All mouse experimental procedures were approved by the Institutional Animal Care and Use Committee of Chonnam National University (CNU IACUC-YB-2024-208). Eight-week-old C57BL/6N male mice (Damul Science, Seoul, Korea) were used in this study and given free access to food and water under a 12 hr light/dark cycle at 22°C with 55% ± 5% relative humidity. For diet-induced obesity, the mice were provided with a high-fat diet (HFD; 60% calorie fat; D12492, Research Diets, New Brunswick, NJ, USA).

PSE was prepared by dissolving the extract in 0.12% DMSO and administered orally via gavage at doses of 15 mg/kg (low concentration, PSE-L) and 30 mg/kg (high concentration, PSE-H) five times per week for 17 weeks. Food intake was manually recorded, and body weight was monitored weekly. At the time of sacrifice, the mice were euthanized by CO₂ inhalation in accordance with institutional ethical guidelines. Wet tissue weights were measured with a balance scale, and tissue samples were either snap-frozen for storage or transferred to embedding cassettes for further analysis.

Glucose tolerance test

Before GTT was conducted on the 11th week of chow diet (CD) or 14th week of HFD feeding, the mice were fasted for 16 hr and intraperitoneally injected with glucose (2 g/kg of body weight). Tail vein blood was collected to measure glucose levels at each time point by using an Accu-Check (Performa, Roche, Indianapolis, IN, USA).

Western blot analysis

Cell lysis and tissue homogenization were performed using radioimmunoprecipitation buffer (150 mM NaCl, 50 mM Tris, pH 8.0, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and 1X NP-40) with a protease inhibitor cocktail (11836153001, Sigma-Aldrich). Protein concentrations were quantified using DC Protein Assay Reagents (5000116, Bio-Rad, Hercules, CA, USA), and an equal amount of protein per sample was purified using the acetone precipitation method [19]. The resulting pellet was diluted in SDS sample buffer (60 mM Tris at pH 6.8, 25% glycerol, 0.1% bromophenol blue, 2% SDS, 2.5% 2-mercaptoethanol). Protein lysates were subjected to SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose membranes. The blots were incubated with the following antibodies: fatty acid synthase (FAS; ab22759), UCP1 (ab10983), and total OXPHOS (ab1104413, Abcam, Cambridge, UK); p-PKA substrate (9621) acetyl-coA carboxylase (ACC; 3676), p-HSL (pS660; 4126), and HSP90 (4874, Cell Signaling Technology, Danvers, MA, USA); and p-Perilipin 1 (4856, Vala Sciences, San Diego, CA, USA) as the loading control. The antibodies were prepared and diluted in accordance with the manufacturer's recommendations. Protein bands were visualized using a luminescent image analyzer (29399481 Amersham ImageQuant 800, Cytiva, Marlborough, MA, USA). Protein expression levels were quantified via densitometry analysis in ImageJ.

Histological analysis

Upon sacrifice, the adipose tissues and livers of the mice were dissected, transferred to tissue blocks, fixed in a 10% neutral buffered formalin solution, and embedded in paraffin. Then, 5 μm (interscapular brown adipose tissue or iBAT, liver) or 7 μm (inguinal white adipose tissue or iWAT, epididymal white adipose tissue or eWAT) sections were stained using hematoxylin and eosin. The adipocyte area was quantified using ImageJ.

Serum analysis

Mouse serum was examined using an Auto chemistry analyzer (Dotto 2000, MTD Medical System, Como, Italy) to measure the following variables: glucose, alanine transaminase, aspartate aminotransferase, cholesterol, and triglyceride levels.

mRNA quantification and analysis

Total RNA was isolated from frozen adipose tissues by using TRI-Solution (TS200-001, BioScience Technology, Seoul, Korea) in accordance with the manufacturer's instructions. The cDNA library of the isolated total RNA was generated using Moloney murine leukemia virus reverse transcriptase (28025021, Thermo Fisher Scientific) and oligo dT. Gene expression was analyzed through quantitative PCR by using a QuantStudio 6 real-time PCR system (4485691, Thermo Fisher Scientific). For data normalization, TATA-box binding protein (*Tbp*) mRNA was used as the reference gene. The primer sequences used in this study are listed in Supplementary Table S2.

Statistical analysis

All data reported in this study were expressed as the mean \pm the S.E.M. and analyzed from individually collected biological samples. One-way ANOVA was performed when repeated measurements were conducted over an extended period. Statistical significance across experimental groups was examined via Student's *t*-test. Data were analyzed and visualized using GraphPad Prism (version 9.3.1, GraphPad Software, San Diego, CA, USA).

RESULTS

In vitro screening of plant extracts

A total of 105 plant samples obtained from the Natural Product Central Bank at the Korea Research Institute of Bioscience and Biotechnology were extracted in methanol and screened for their cytotoxic and anti-adipogenic effects on 3T3-L1 cells (Fig. 1A). The cells were treated with the extracts derived from various plant parts and species to assess cytotoxicity (Fig. 1B, Supplementary Fig. S1A). The cell viability assay indicated that the cells survived in most leaf extracts, while some, particularly those from *Alangium platanifolium* (*A. platanifolium*) collected at two different periods, significantly reduced cell viability (Fig. 1B, Supplementary Fig. S1A), indicating their cytotoxic effects. ORO staining analysis revealed that lipid accumulation, a hallmark of fully differentiated adipocytes, was markedly reduced in 3T3-L1 cells treated with

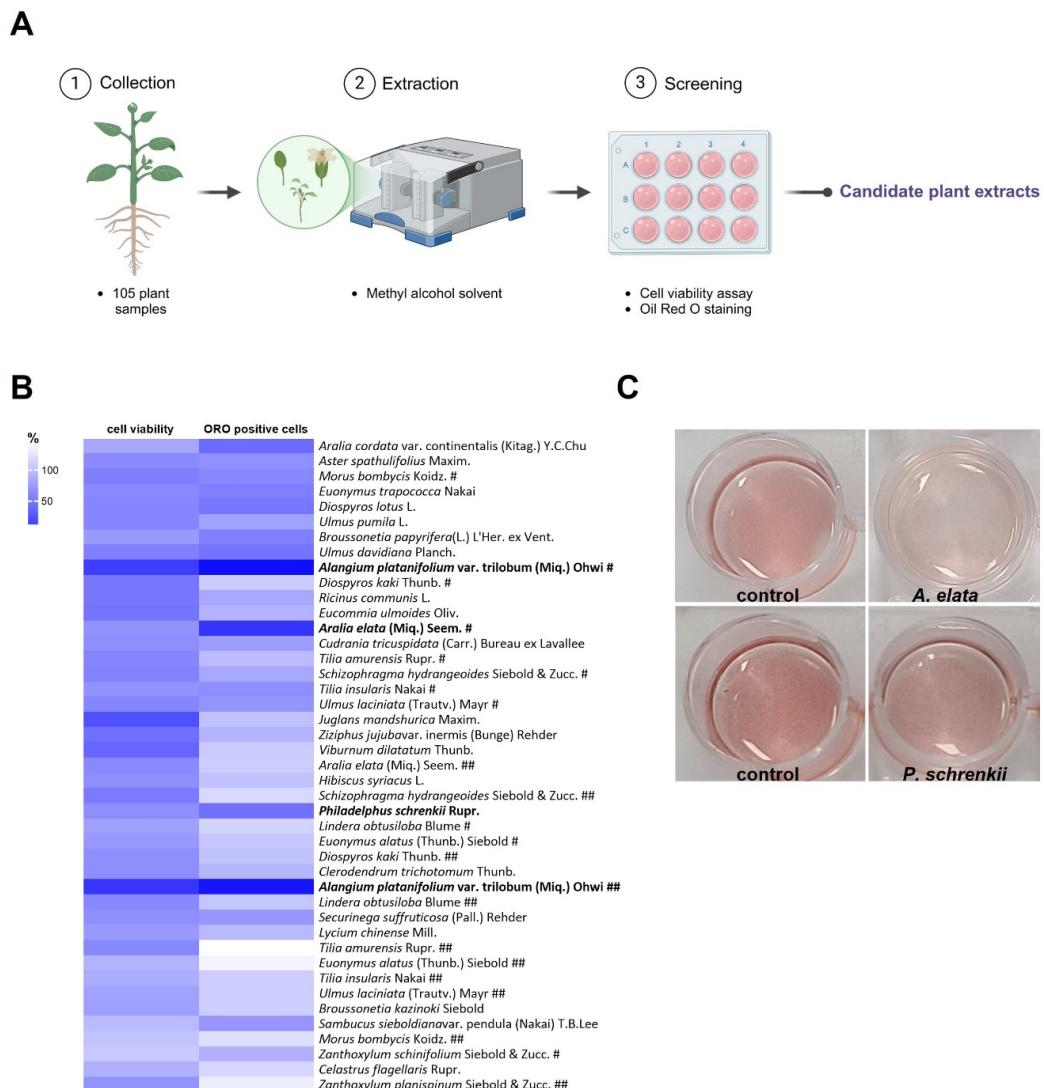


Fig. 1. Screening of plant extracts for evaluation via cell-based assays. (A) Schematic showing the selection methods of candidate plant extracts. (B) Heatmap of percent (%) cell viability and Oil Red O-positive cells in differentiated 3T3-L1 cells treated with the extracts of different plant species (leaf, n = 43). Duplicate species of the same plant parts were collected at different periods (i.e., year of collection; #: early collection, ##: later collection). Data are presented as mean values (C) Oil Red O staining images of the plant species used for screening.

A. platanifolium, *Aralia elata*, or *P. schrenkii* extract (Fig. 1B and C, Supplementary Fig. S1B). On the basis of the cytotoxicity and ORO staining results, *A. platanifolium* extract was excluded, and only *P. schrenkii* leaf extract was selected as a candidate for further investigation into its anti-obesity potential. Although *A. elata* leaf extracts exhibited low cytotoxicity and markedly reduced lipid droplets (Fig. 1B and C), this species was also excluded from subsequent analyses because of a previous publication describing its anti-obesity effects [20].

Effects of *Philadelphus schrenkii* extract on cultured adipocytes

To further confirm the anti-adipogenic effects of PSE *in vitro*, we treated C3H10T1/2 (10T) cells with 5 µg/mL (PSE-L) or 50 µg/mL (PSE-H) of PSE during differentiation. The PSE treatment reduced the lipid droplet formation in 10T cells, suggesting that adipogenesis was

inhibited (Fig. 2A). The PSE-H treatment in fully differentiated 10T cells for 24 hr increased the expression of *Ucp1* and *Dio2*, genes involved in thermogenesis (Fig. 2B). The UCP1 protein levels were also upregulated by PSE-H, while no changes were observed in the lipogenic proteins FAS and ACC or in OXPHOS proteins (Fig. 2C). These results suggest that PSE exerts anti-adipogenic and thermogenic effects on cultured adipocytes.

Philadelphus schrenkii methanol extract stimulates thermogenic gene expression in chow diet-fed mice

To reveal the effects of PSE treatment on adipocytes *in vivo*, we treated the CD-fed mice with 15 mg/kg (PSE-L) or 30 mg/kg (PSE-H) PSE via oral gavage for 17 weeks. Both treatment doses of PSE did not cause significant differences in body weight, food intake, and tissue weights compared with those of the control mice (Fig. 3A and B, Supplementary Figs. S2A and S2B). The expression levels of thermogenic genes, such as *Dio2* and *Ppargc1a* mRNA, in the iBAT, the primary site of non-shivering thermogenesis, significantly increased (Fig. 3C). This increase was accompanied by the upregulation of UCP1 and OXPHOS protein levels, which increased by approximately 400% and 270% in the high-dose group compared with those in the control group, respectively (Fig. 3C and D). In iWAT, another site of thermogenic activation through the formation of beige adipocytes, PSE-L increased the *Ucp1* expression,

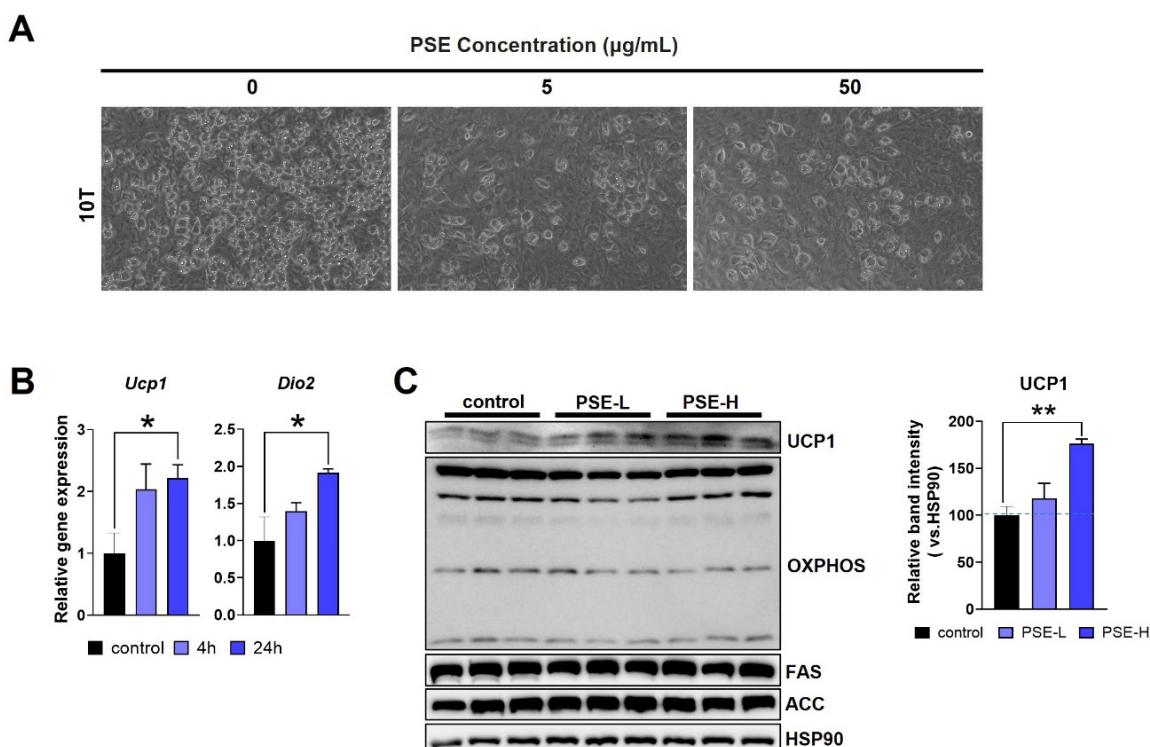


Fig. 2. Assessment of the effects of PSE *in vitro*. (A) Comparison of the morphological characteristics of 10T cells treated with varying PSE concentrations during differentiation ($n = 3$ per group). (B) Gene expression levels of *Ucp1* and *Dio2* normalized to *Tbp* in 10T cells treated with high-dose PSE for 4 or 24 hr. (C) Immunoblots (left panel) of 10T cells treated with PSE for 24 hr, with HSP90 as a loading control. Densitometry analysis (right panel) of UCP1 in 10T. All values are presented as the mean \pm the S.E.M. Student's *t*-test was used for the statistical analysis. Asterisks indicate significant differences: * $p < 0.05$ and ** $p < 0.01$. PSE, *Philadelphus schrenkii* methanol extract; UCP1, uncoupling protein 1; OXPHOS, oxidative phosphorylation; FAS, fatty acid synthase; ACC, acetyl-coA carboxylase.

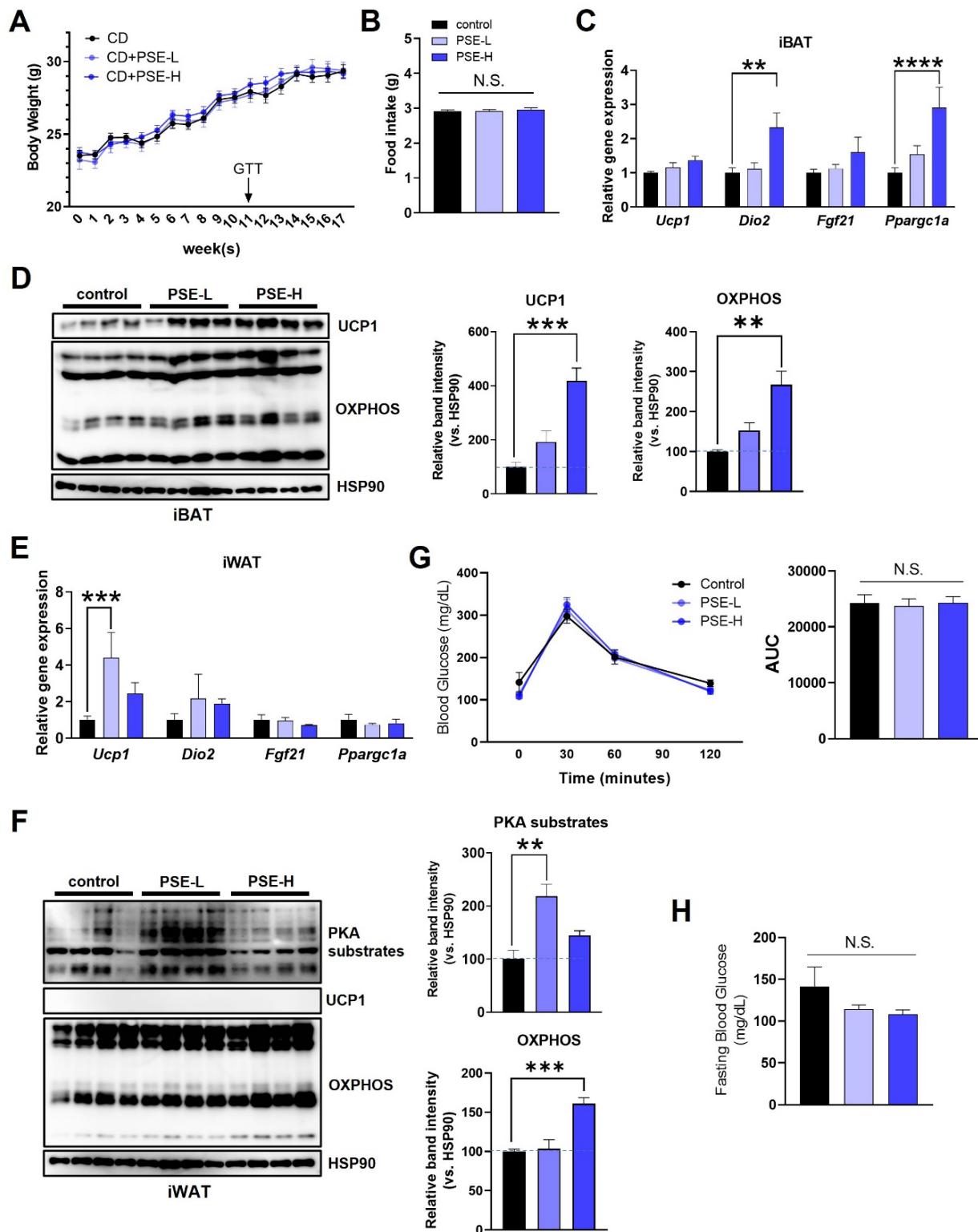


Fig. 3. Activation of thermogenic expression by PSE in chow diet-fed mice. (A) Body weight of the mice orally injected with the vehicle or PSE for 17 weeks (n = 10 vs. 9 vs. 9; CD vs. CD + PSE-L vs. CD + PSE-H). (B) Daily food intake of the mice. (C) Thermogenic gene expression levels normalized to *Tbp* in the iBAT. (D) Immunoblots (left panel) and densitometry analysis (right panel) in iBAT (n = 4 per group), with HSP90 used as a loading control. (E) Thermogenic gene expression levels normalized to *Tbp* in iWAT. (F) Immunoblots (top) and densitometry analysis (bottom) in iWAT (n = 4 per group), with HSP90 used as a loading control. (G) Glucose tolerance test (top) and area under the curve (AUC; bottom). (H) Fasting blood glucose levels of chow diet-fed mice. All values are presented as the mean \pm the S.E.M. One-way ANOVA (Figs. B, C, D, E, F, H) and two-way ANOVA (Figs. A, G) were used for the statistical analysis. Asterisks indicate significant differences: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. CD, chow diet; PSE, *Philadelphia schrenkii* methanol extract; N.S., not significant; iBAT, interscapular brown adipose tissue; UCP1, uncoupling protein 1; OXPHOS, oxidative phosphorylation; iWAT, inguinal white adipose tissue.

although UCP1 levels were undetectable (Fig. 3E and F). The activity of protein kinase A (PKA), a key factor in the classical thermogenic pathway determined by PKA substrate phosphorylation, and the protein level of OXPHOS increased in PSE-administered mice (Fig. 3F). In eWAT, the levels of proteins involved in lipolysis (p-perilipin and p-HSL) or lipogenesis (ACC and FAS) did not significantly change (Supplementary Fig. S2C). Despite the enhanced thermogenic factors, no histological differences, glucose tolerance, or fasting blood glucose changes were observed between the groups (Fig. 3G and H, Supplementary Fig. S2D). These results suggest that PSE upregulates factors related to thermogenesis in mice fed a CD.

***Philadelphus schrenkii* methanol extract promotes the expression of thermogenic and mitochondrial markers in high-fat diet-fed mice**

Based on the anti-adipogenic and thermogenic effects of PSE, we investigated whether PSE treatment could benefit the diet-induced obese mice. Mice were fed with HFD for 16 weeks with or without PSE treatment. In the PSE-treated group, thermogenic- and OXPHOS-related genes, including *Fgf21*, *Ppargc1a*, *Ndufv1*, *Sdha*, *Cox5b*, and *Atp5b*, were upregulated in iBAT compared with those in the HFD-only group (Fig. 4A and B). UCP1 levels were significantly upregulated in the high-dose PSE-treated group (Fig. 4C). In iWAT, low-dose PSE increased UCP1 levels, while both doses increased PKA activities (Fig. 4D). The number of smaller adipocytes in iWAT significantly increased in the high-dose group, indicating that adiposity was reduced (Fig. 4E and F). Despite these improvements, body weight, fat and liver tissue weights, food intake, glucose tolerance, fasting blood glucose, serum triglyceride, or serum cholesterol levels did not significantly differ (Fig. 4G, H, I, and J, Supplementary Fig. S3A, S3B, and S3C). The histological analyses of iBAT, eWAT, and liver also revealed no notable differences between the groups (Supplementary Fig. S3D). Furthermore, there were no differences in the levels of liver damage markers such as ALT and AST between groups (Supplementary Fig. S3C). Overall, the upregulation of thermogenic UCP1 and reduction in iWAT adiposity suggest that PSE may be a potential candidate for targeting adipose tissue function in obesity management.

DISCUSSION

The development of effective obesity treatments remains a significant challenge due to the limitations of existing pharmacological and surgical options [1, 2]. For instance, the use of GLP-1 analogs, such as liraglutide and semaglutide, as weight-loss drugs is controversial for their associated increased risk of pancreatitis, gastrointestinal adverse effects, and psychiatric problems [21–23]. Bariatric surgery, which is an effective long-term treatment for severe obesity, involves invasive procedures that pose risks such as bleeding and infections [2]. Therefore, in the present study, we sought to explore a plant-based alternative with anti-obesity potential. Leaf extracts from various plant species are known to harbor bioactive compounds with therapeutic effects against obesity [3, 24–26]. Most of them, including *A. elata* leaf extracts [20], have been reported to target lipid metabolism or inhibit pancreatic lipase activity [3,

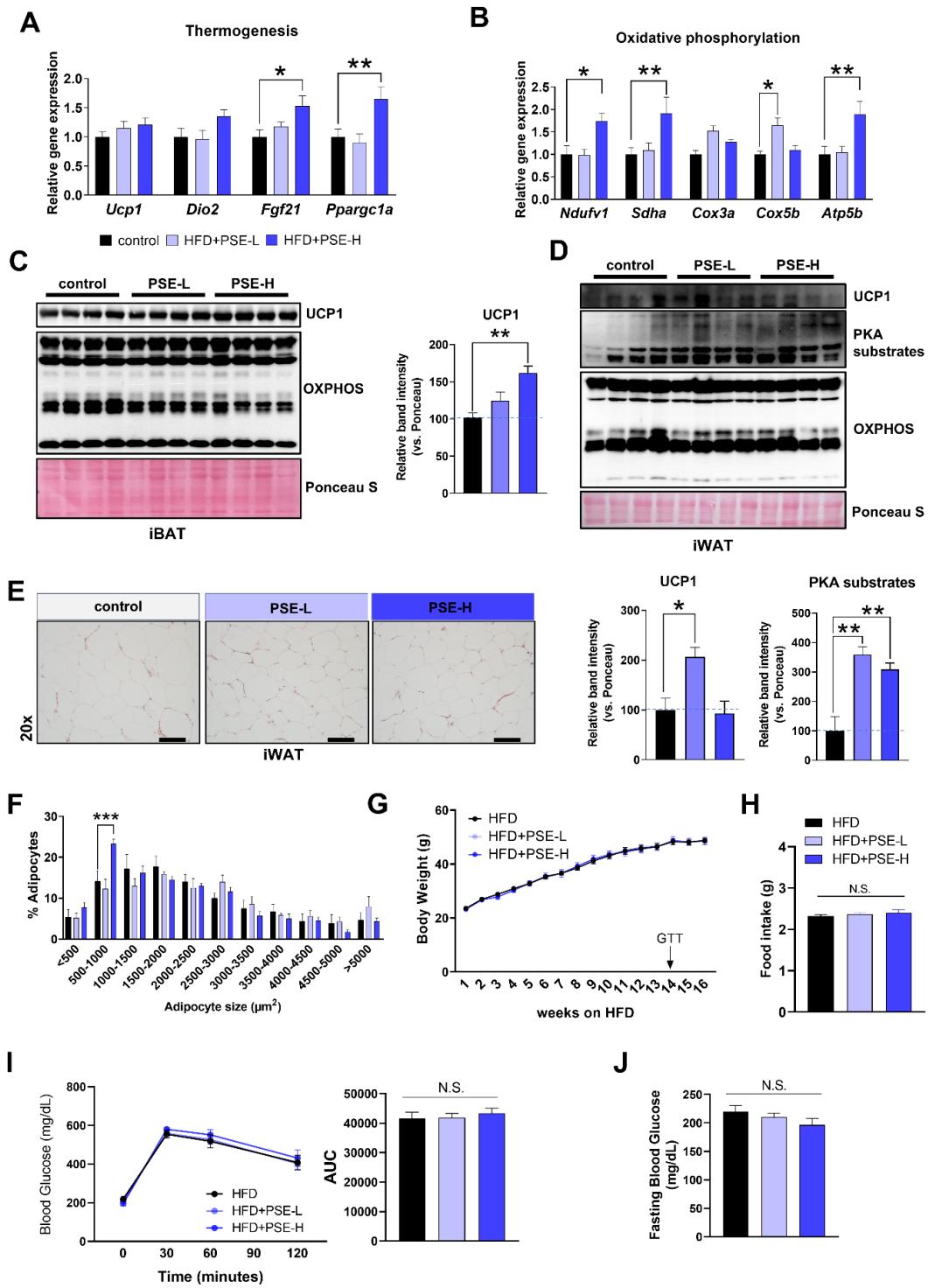


Fig. 4. Metabolic marker expression promoted by PSE without inducing weight loss. (A) Gene expression levels of thermogenesis- and (B) oxidative phosphorylation-related markers normalized to *Tbp* in iBAT (n = 5 per group). (C) Immunoblots (left panel) and densitometry analysis (right panel) of UCP1 in iBAT (n = 4 per group). Ponceau-stained blot was used as a reference control. (D) Immunoblots (top) and densitometry analysis (bottom) in iWAT (n = 4 per group). Ponceau-stained blot was used as a reference control. (E) Representative H&E staining images and adipocyte quantification data (F) of the iWAT collected from HFD-fed mice. Scale bar = 100 μm , 20 \times magnification. (G) Body weight of the mice orally injected with the vehicle or PSE and fed with HFD for 16 weeks (n = 10 vs. 10 vs. 9; HFD vs. HFD + PSE-L vs. HFD + PSE-H). (H) Daily food intake of the mice. (I) Glucose tolerance test (left panel) and area under the curve (AUC; right). (J) Fasting blood glucose levels of HFD-fed mice. All values are presented as the mean \pm the S.E.M. One-way ANOVA (Figs. A, C, D, F, H, J) and two-way ANOVA (Figs. G, H) were used for statistical analysis. Asterisks indicate significant differences: *p<0.05, **p<0.01, and ***p<0.001. HFD, high-fat diet; PSE, *Philadelphia schrenkii* methanol extract; iBAT, interscapular brown adipose tissue; UCP1, uncoupling protein 1; OXPHOS, oxidative phosphorylation; iWAT, inguinal white adipose tissue; N.S., not significant.

4]. This study highlights the novel mechanism of action of PSE, derived from the methanolic extraction of *P. schrenkii* leaves, through its ability to enhance the expression of thermogenesis-related proteins. The safety of PSE was also confirmed through cytotoxicity assays and unaltered ALT and AST levels in mice, proving its suitability as a supportive anti-obesity agent.

Our results demonstrated that the oral administration of PSE significantly increased the expression of thermogenic UCP1 in 10T cells and adipose tissues of PSE-treated mice. Notably, PKA substrate phosphorylation was enhanced in iWAT, accompanied by an increase in smaller adipocytes, indicating a potential enhancement of the lipolytic pathway. Adipose thermogenesis is primarily attributed to the abundant mitochondria and the expression of UCP1 in brown and beige adipocytes [15]. Therefore, compounds that activate this pathway may cause therapeutic effects by promoting fat utilization. Despite the observed changes, PSE administration did not significantly affect body weight or serum parameters such as glucose, triglyceride, and cholesterol in HFD-fed mice. This outcome is consistent with growing evidence showing that UCP1 induction alone is inadequate to increase thermogenic output, especially if not augmented by activated electron transport chain activity [27]. In addition, UCP1-independent thermogenic pathways also contribute substantially to brown and beige fat thermogenesis [28–30], and without activation of these parallel processes, upregulation of UCP1 is unlikely to translate into measurable physiological benefits. These findings suggested that the effects of PSE were insufficient to induce weight loss, although it enhanced the adipose tissue function, as evidenced by the increased thermogenic UCP1 expression, PKA signaling activation, and reduction in iWAT adiposity. Future studies should investigate combinatorial approaches or extended treatment durations of PSE to enhance its efficacy. It should also be noted that the composition of plant-derived compounds is influenced by the extraction method, with solvent polarity determining the yield of phytochemicals and antioxidants that are most efficiently recovered [31]. Different extraction approaches can yield extracts with distinct biological actions. Methanol extraction, for example, typically enriches semi-polar phytochemicals such as flavonoids and phenolic acids, whereas water extraction favors more polar constituents like polysaccharides and glycosides [32]. Because PSE is a complex extract, isolating and identifying its active components will also be important for elucidating the mechanisms underlying its thermogenic effects. This will allow for the development of possibly more potent therapeutic agents.

Nonetheless, given its safety profile and ability to regulate thermogenic UCP1 expression in adipose tissues, PSE holds promise as a supportive component of plant-based obesity therapies. Although its standalone effects may be modest, PSE could complement existing treatments by enhancing thermogenic protein expression and reducing adiposity.

SUPPLEMENTARY MATERIALS

Supplementary materials are only available online from: <https://doi.org/10.12729/jbtr.2025.26.4.145>.

REFERENCES

1. Wyatt HR. Update on treatment strategies for obesity. *J Clin Endocrinol Metab* 2013;98:1299-1306.
2. Williams DM, Nawaz A, Evans M. Drug therapy in obesity: a review of current and emerging treatments. *Diabetes Ther* 2020;11:1199-1216.
3. Karri S, Sharma S, Hatware K, Patil K. Natural anti-obesity agents and their therapeutic role in management of obesity: a future trend perspective. *Biomed Pharmacother* 2019;110:224-238.
4. Vermaak I, Viljoen AM, Hamman JH. Natural products in anti-obesity therapy. *Nat Prod Rep* 2011;28:1493-1533.
5. Akanda MR, Tae HJ, Kim IS, Ahn D, Tian W, Islam A, Nam HH, Choo BK, Park BY. Hepatoprotective role of hydrangea macrophylla against sodium arsenite-induced mitochondrial-dependent oxidative stress via the inhibition of MAPK/caspase-3 pathways. *Int J Mol Sci* 2017;18:1482.
6. Bumke-Vogt C, Osterhoff MA, Borchert A, Guzman-Perez V, Sarem Z, Birkenfeld AL, Bähr V, Pfeiffer AFH. The flavones apigenin and luteolin induce FOXO1 translocation but inhibit gluconeogenic and lipogenic gene expression in human cells. *PLOS ONE* 2014;9:e104321.
7. Kulabas SS, Ipek H, Tufekci AR, Arslan S, Demirtas I, Ekren R, Sezerman U, Tumer TB. Ameliorative potential of in *Lavandula stoechas* metabolic syndrome via multitarget interactions. *J Ethnopharmacol* 2018;223:88-98.
8. Lorizola IM, Furlan CPB, Portovedo M, Milanski M, Botelho PB, Bezerra RMN, Sumere BR, Rostagno MA, Capitani CD. Beet stalks and leaves (*Beta vulgaris* L.) protect against high-fat diet-induced oxidative damage in the liver in mice. *Nutrients* 2018;10:872.
9. Narasimhan A, Chinnaiyan M, Karundevi B. Ferulic acid exerts its antidiabetic effect by modulating insulin-signalling molecules in the liver of high-fat diet and fructose-induced type-2 diabetic adult male rat. *Appl Physiol Nutr Metab* 2015;40:769-781.
10. Kim YM, Baasanmunkh S, Song JH, Yang S, Son DC, Choi HJ. A taxonomic revision of *Philadelphus* (Hydrangeaceae) in South Korea. *J Asia-Pac Biodivers* 2022;15:442-449.
11. Lee CK, Kim H, Moon KH, Shin KH. Screening and isolation of antibiotic resistance inhibitors from herb materials-resistance inhibition of volatile components of Korean aromatic herbs. *Arch Pharm Res* 1998;21:62-66.
12. Kim J, Kim JH, Bang SI, Shin H, Cho EJ, Lee S. Antioxidant activity of edible sprouts and phytosterol contents by HPLC/UV analysis. *Hortic Environ Biotechnol* 2022;63:769-778.
13. Bae SH, Kang MJ, Kwon IJ, Son SH, Do Kim G. Anti-inflammatory activity of *Philadelphus schrenkii* methanol extract in lipopolysaccharide-stimulated RAW 264.7 cells. *Korean J Medicinal Crop Sci* 2024;32:121-135.
14. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. *Arch Med Sci* 2017;13:851-863.
15. Shamsi F, Wang CH, Tseng YH. The evolving view of thermogenic adipocytes: ontogeny, niche and function. *Nat Rev Endocrinol* 2021;17:726-744.
16. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physi-*

ol Rev 2004;84:277-359.

17. Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schärt G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerbäck S, Schrauwen P, Spiegelman BM. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 2012;150:366-376.
18. Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K, Kaul MG, Tromsdorf UI, Weller H, Waurisch C, Eychmüller A, Gordts PLSM, Rinniger F, Bruegelmann K, Freund B, Nielsen P, Merkel M, Heeren J. Brown adipose tissue activity controls triglyceride clearance. *Nat Med* 2011;17:200-205.
19. Park MJ, Lee CM, Kim D. An easy method for the clear detection of beige fat UCP1 by Western blotting. *Adipocyte* 2019;8:357-361.
20. Hwang KA, Hwang YJ, Kim GR, Choe JS. Extracts from *Aralia elata (Miq)* Seem alleviate hepatosteatosis via improving hepatic insulin sensitivity. *BMC Complement Altern Med* 2015;15:347.
21. Chen W, Cai P, Zou W, Fu Z. Psychiatric adverse events associated with GLP-1 receptor agonists: a real-world pharmacovigilance study based on the FDA Adverse Event Reporting System database. *Front Endocrinol* 2024;15:1330936.
22. Sodhi M, Rezaeianzadeh R, Kezouh A, Etminan M. Risk of gastrointestinal adverse events associated with glucagon-like peptide-1 receptor agonists for weight loss. *J Am Med Assoc* 2023;330:1795-1797.
23. Trujillo JM, Nuffer W, Smith BA. GLP-1 receptor agonists: an updated review of head-to-head clinical studies. *Ther Adv Endocrinol Metab* 2021;12:2042018821997320.
24. Han HS, Lee HH, Gil HS, Chung KS, Kim JK, Kim DH, Yoon J, Chung EK, Lee JK, Yang WM, Shin YK, Ahn HS, Lee SH, Lee KT. Standardized hot water extract from the leaves of *Hydrangea serrata* (Thunb.) Ser. alleviates obesity via the AMPK pathway and modulation of the gut microbiota composition in high fat diet-induced obese mice. *Food Funct* 2021;12:2672-2685.
25. Rahman HA, Sahib NG, Saari N, Abas F, Ismail A, Mumtaz MW, Hamid AA. Anti-obesity effect of ethanolic extract from *Cosmos caudatus* Kunth leaf in lean rats fed a high fat diet. *BMC Complement Altern Med* 2017;17:122.
26. Seo DB, Jeong HW, Kim YJ, Kim S, Kim J, Lee JH, Joo K, Choi JK, Shin SS, Lee SJ. Fermented green tea extract exhibits hypolipidaemic effects through the inhibition of pancreatic lipase and promotion of energy expenditure. *Br J Nutr* 2017;117:177-186.
27. Park MJ, Lee J, Matienzo ME, Lim S, Kim K, Lee CM, Kim D. Delivery of thermogenic genes to metabolic tissues: effects on body weight and glucose tolerance. *Trans Res* 2025;283:47-55.
28. Liu X, He A, Lu D, Hu D, Tan M, Abere A, Goodarzi P, Ahmad B, Kleiboeker B, Finck BN, Zayed M, Funai K, Brestoff JR, Javaheri A, Weisensee P, Mittendorfer B, Hsu FF, Van Veldhoven PP, Razani B, Semenkovich CF, Lodhi IJ. Peroxisomal metabolism of branched fatty acids regulates energy homeostasis. *Nature* 2025;646:1223-1231.
29. Kazak L, Chouchani ET, Jedrychowski MP, Erickson BK, Shinoda K, Cohen P, Vetrivelan R, Lu GZ, Laznik-Bogoslavski D, Hasenfuss SC, Kajimura S, Gygi SP, Spiegelman BM. A cre-

atine-driven substrate cycle enhances energy expenditure and thermogenesis in beige fat. *Cell* 2015;163:643-655.

30. Ikeda K, Kang Q, Yoneshiro T, Camporez JP, Maki H, Homma M, Shinoda K, Chen Y, Lu X, Maretich P, Tajima K, Ajuwon KM, Soga T, Kajimura S. UCP1-independent signaling involving SERCA2b-mediated calcium cycling regulates beige fat thermogenesis and systemic glucose homeostasis. *Nat Med* 2017;23:1454-1465.
31. Zaman MAK, Azzeme A, Ramli SN, Shaharuddin NA, Ahmad S, Abdullah SNA. Solvent extraction and its effect on phytochemical yield and antioxidant capacity of woody medicinal plant, *Polyalthia bullata*. *BioResources* 2020;15:9555-9568.
32. Do QD, Angkawijaya AE, Nguyen PLT, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J Food Drug Anal* 2014;22:296-302.