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In Vitro Evaluation on the Antiobesity Effect of Lignans from the Flower Buds of *Magnolia denudata*

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ABSTRACT: In the present study, an attempt has been made to isolate antiobesity components from crude extracts of the flower buds of *Magnolia denudata* by CH_2Cl_2 and MeOH solvents. The crude extracts were partitioned into *n*-hexane, 85% aqueous MeOH, *n*-butanol, and water fractions. Their antiobesity effects were evaluated by measuring the effect on adipogenic differentiation using 3T3-L1 cells. Among the fractions, *n*-hexane and 85% aqueous MeOH fractions effectively reduced the lipid accumulation and the regulation of the adipogenic transcription factor. Both *n*-hexane and 85% aqueous MeOH fractions were further separated by diverse chromatographic methods to give four lignans (**A**−**D**). In comparative analysis, the presence of the lignans during adipogenic differentiation reduced the absorbance values of eluted Oil Red O solution in the order of potency **C** > **D** > **B** > **A**. Moreover, **C** and **D** effectively downregulated SREBP1, PPARγ, and C/EBPα.

KEYWORDS: *Magnolia denudata*, antiobesity effect, lignan, (+)-epimagnolin, (+)-magnolin

INTRODUCTION

Magnolia species have a disjunct distribution in east and southeastern Asia, northeastern and central America, and west Indies, and some species grow in South America. Several kinds of *Magnolia* species, such as *Magnolia salicifolia*, *Magnolia kobus*, and *Magnolia denudata*, have reportedly been used for centuries as folk medicines in Asian countries.[1] Their dried flower buds are sources of crude drugs. For example, the dried flower buds of *M. denudata* have been known as Xin Yi in traditional Chinese herbal medicine and are used to treat emphyema, nasal congestion, sinusitis, and allergic or chronic rhinitis.[2] *M. denudata* is a small deciduous tree that typically grows to 30−40 ft with a rounded spreading crown. It contains many kinds of lignans and neolignans.[2−5] Many studies have focused on its interesting biological activities, such as antiplatelet activation, cholesterol acetyltransferase and melanogenesis inhibitions, anti-inflammation, antiallergy, and apoptosis induction.[1,4−10] However, there is currently still insufficient evidence on the antiobesity effect of *M. denudata* and its components.

In the present study, to isolate antiobesity components from the flower buds of *M. denudata*, they were extracted using MeOH and CH_2Cl_2 solvents and the solvent-partitioned fractions, such as the *n*-hexane, 85% aqueous MeOH, *n*-butanol (*n*-BuOH), and water (H_2O) fractions, were prepared from their combined solvent extracts. Their antiobesity effects were evaluated by measuring the effect on adipogenic differentiation of 3T3-L1 preadipocytes. In addition, four known compounds (**A**−**D**) were isolated from the antiadipogenic-active fractions of *M. denudata* by a comparison to the reported data in the literature.[7,11−16]

MATERIALS AND METHODS

General Experimental Procedures. Optical rotation was determined on a Perkin-Elmer polarimeter 341 using a 1 cm cell. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on a Varian Mercury 300 instrument at 300 MHz for ^1H and 75 MHz for ^{13}C using standard pulse sequence programs, respectively. All chemical shifts were recorded with respect to tetramethylsilane (TMS) as an internal standard. High-performance liquid chromatography (HPLC) was performed with a Dionex P580 with a Varian 350 RI detector. All used solvents were spectroscopic-grade or were distilled from glass prior to use.

Plant Material. The dried flower buds of *M. denudata* were purchased from the Cheongyangni Oriental Herb Market in Seoul, South Korea, in May 2007. A voucher specimen (KM 32007) was deposited at the Herbarium of the Division of Marine Environment and Bioscience, Korea Maritime University, Busan, South Korea.

Extraction and Isolation. The air-dried flower buds of *M. denudata* were ground to a powder and extracted for 48 h with CH_2Cl_2 (3 L × 2) and MeOH (3 L × 2), respectively. Combined crude extracts (175.2 g) were concentrated under reduced pressure and partitioned between CH_2Cl_2 and H_2O. The organic layer was further partitioned between 85% aqueous MeOH and *n*-hexane, and the aqueous layer was fractionated with *n*-BuOH and H_2O, successively. The resulting four fractions were evaporated to dryness *in vacuo*, yielding the *n*-hexane (46.0 g), 85% aqueous MeOH (92.6 g), *n*-BuOH (11.6 g), and H_2O (20.2 g) fractions, respectively.

A portion of the *n*-hexane (10.0 g) fraction was subjected to silica gel column chromatography with stepwise gradient mixtures of EtOAc and *n*-hexane (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, and 100% EtOAc in *n*-hexane) as eluents to give 16 subfractions. Further separation of the sixth subfraction (198.6 mg) by HPLC (YMC PACK-SIL, 20% EtOAc in *n*-hexane) gave compound **A** (29.3 mg). Also, further purification of the ninth fraction (121.3 mg) by HPLC (YMC ODS-A, 70% aqueous MeOH) yielded compounds **B** (24 mg) and **C** (26 mg).

A portion of the 85% aqueous MeOH (10.5 g) fraction was subjected to C_{18} reversed-phase vacuum flash chromatography using stepwise gradient mixtures of MeOH and water (50, 60, 70, 80, and 90% aqueous

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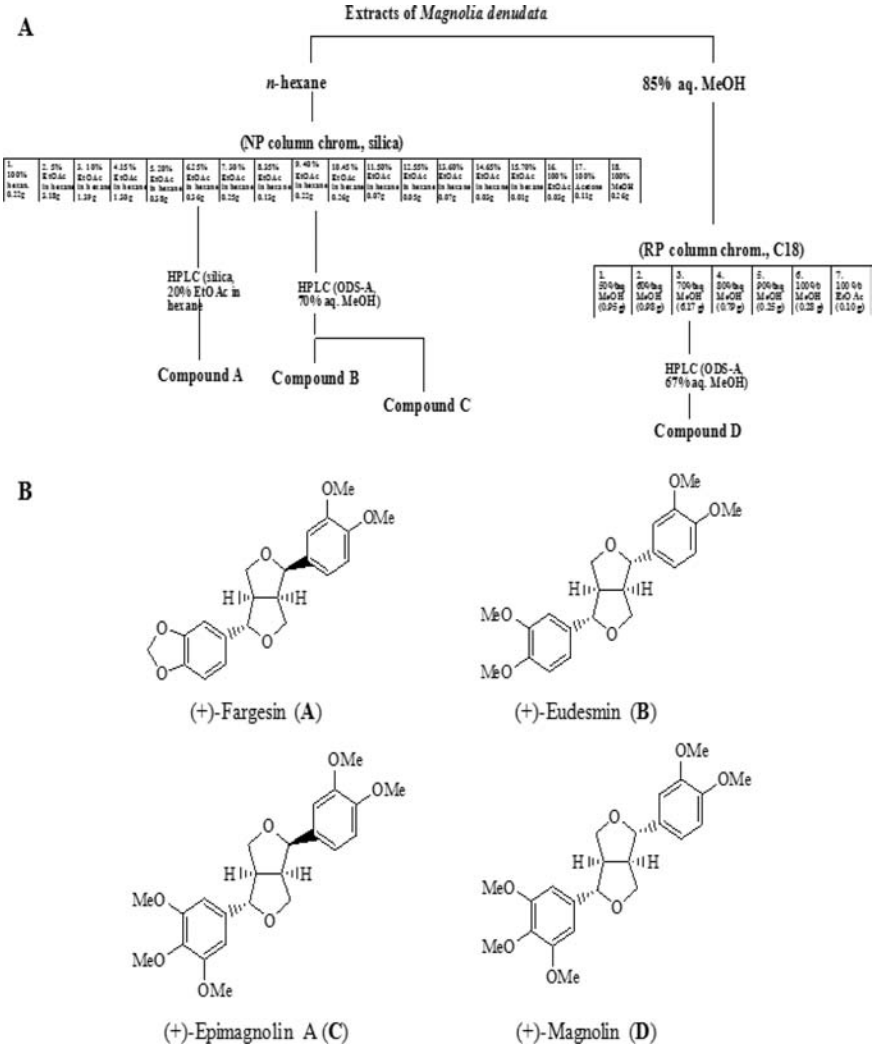

Figure 1. Basic scheme for whole isolation of compounds **A**−**D** from the flower buds of *M. denudata* and their chemical structures.

MeOH and 100% MeOH) and, finally, 100% EtOAc as eluents to give six subfractions, respectively. The third subfraction (316.0 mg) was further separated by reversed-phase HPLC (YMC ODS-A, 67% aqueous MeOH) to afford compound **D** (110.4 mg).

The basic scheme for whole isolation and chemical structures of these compounds **A**, **B**, **C**, and **D** are illustrated in Figure 1.

Cell Culture and Adipocyte Differentiation. Mouse 3T3-L1 preadipocytes were grown at a density of 2×10^5 cells/well in 6-well plates to confluence in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5% CO_2. At 1 day postconfluence (designated "day 0"), cell differentiation was induced with a mixture of methylisobutylxanthine (0.5 mM), dexamethasone (0.25 μM), and insulin (5 μg/mL) in DMEM containing 10% FBS. After 48 h (day 2), the induction medium was removed and replaced with DMEM containing 10% FBS supplemented with insulin (5 μg/mL). This medium was changed every 2 days. Solvent-partitioned fractions and compounds were administered to the culture medium from day 4 to 6.

Determination of Oil Red O Staining. For Oil Red O staining, cells were washed gently twice with phosphate-buffered saline (PBS), fixed with 3.7% fresh formaldehyde in PBS for 1 h at room temperature, and stained with filtered Oil Red O solution (60% isopropanol and 40% water) for at least 1 h. After staining, the Oil Red O staining solution was removed and the plates were rinsed with the distilled water and dried. Images of lipid droplets in 3T3-L1 adipocytes were collected by an Olympus microscope (Tokyo, Japan). Finally, dye retained in the cells was eluted with isopropanol and quantified by measuring optical absorbance at 490 nm using a microplate reader (Tecan Austria GmbH, Austria).

RNA Extraction and Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis. Total RNA was isolated from 3T3-L1 adipocytes treated with/without each solvent-partitioned fraction using Trizol reagent (Invitrogen Co., Carlsbad, CA). For synthesis of cDNA, RNA (2 μg) was added to RNase-free water and oligo(dT), denaturated at 70 °C for 5 min, and cooled immediately. RNA was reverse-transcribed in a master mix containing 1× RT buffer, 1 mM dNTPs, 500 ng of oligo(dT), 140 units of M-MLV reserve transcriptase, and 40 units of RNase inhibitor at 42 °C for 60 min and at 72 °C for 5 min using an automatic Whatman thermocycler (Biometra, U.K.). The target cDNA was amplified using the following sense and antisense primers: forward 5′-TTT-TCA-AGG-GTG-CCA-GTT-TC-3′ and reverse 5′-AAT-CCT-TGG-CCC-TCT-GAG-AT-3′ for PPARγ, forward 5′-TTA-CAA-CAG-GCC-AGG-TTT-CC-3′ and reverse 5′-GGC-TGG-CGA-CAT-ACA-GTA-CA-3′ for C/EBPα, and forward 5′-AGC-CAT-GTA-CGT-AGC-CAT-CC-3′ and reverse 5′-TCC-CTC-TCA-GCT-GTG-GTG-GTG-AA-3′ for β-actin. The amplification cycles were carried out at 95 °C for 45 s, 60 °C for 1 min, and 72 °C for 45 s. After 30 cycles, the PCR products were separated by electrophoresis on 1.5% agarose gel for 30 min at 100 V. Gels were then stained with 1 mg/mL

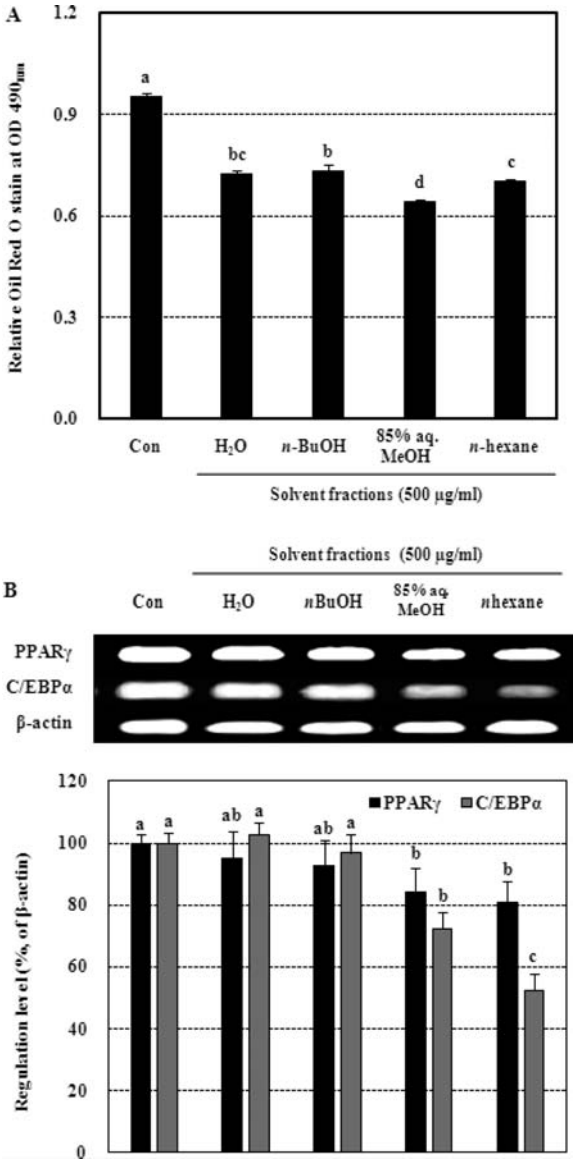

Figure 2. Effect of each solvent-partitioned fraction on adipogenic differentiation. Confluent 3T3-L1 preadipocytes were differentiated into adipocytes for 6 days, and the cells were treated with solvent-partitioned fractions in 6-well plates at the end of the differentiation period for 2 days (from day 4 to 6). (A) Lipid droplets were fixed with 3.7% fresh formaldehyde and stained with Oil Red O staining solution. The dye retained in the cells was eluted with isopropanol and quantified by measuring the optical absorbance at 490 nm. (B) Expression levels of PPARγ and C/EBPα were determined by RT-PCR analysis. Con: fully differentiated control adipocytes for 6 days (0.5 mM methylisobutylxanthine, 0.25 μM dexamethasone, and 5 μg/mL insulin). (a−d) Means with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

ethidium bromide visualized by ultraviolet (UV) light using AlphaEase gel image analysis software (Alpha Innotech, San Leandro, CA).

Western Blot Analysis. Western blotting was performed according to standard procedures. Briefly, cells were lysed in RIPA lysis buffer (Sigma-Aldrich Corp., St. Louis, MO) at 4 °C for 30 min. Cell lysates (35 μg) were separated by 12% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE), transferred onto a polyvinylidene fluoride membrane (Amersham Pharmacia Biotech., U.K.), blocked with 5% skim milk, and hybridized with primary antibodies (diluted 1:1000). After incubation with horseradish-peroxidase-conjugated secondary antibody at room temperature, immunereactive proteins were detected using a chemiluminescece ECL assay kit (Amersham Pharmacia Biosciences, U.K.) according to the instructions of the manufacturer. Western blot bands were visualized using a LAS3000 Luminescent image analyzer (Fujifilm Life Science, Tokyo, Japan).

Statistical Analysis. The data were presented as the mean ± standard deviation (SD). Differences between the means of the individual groups were analyzed using the analysis of variance (ANOVA) procedure of Statistical Analysis System, SAS, version 9.1 (SAS Institute, Cary, NC), with Duncan's multiple range tests. The significance of differences was defined at the $p < 0.05$ level.

RESULTS

Effects of Solvent-Partitioned Fractions on Intracellular Lipid Accumulation and Adipogenic-Specific Gene Expression. The extracts of *M. denudata* were solvent-partitioned to yield four fractions, *n*-hexane, 85% aqueous MeOH, *n*-BuOH, and water. Effects of these four fractions on the induction of terminal differentiation markers were measured at the end of adipocyte differentiation (day 6) (Figure 2). Their cytotoxicity levels to 3T3L1 cells were evaluated by the MTT assay. No significant toxic effects were observed on the cells treated with solvent-partitioned fractions up to a concentration of 1 mg/mL (data was not shown). Therefore, the experiments were carried out up to a concentration of 500 μg/mL. Also, their effects on intracellular lipid accumulation in 3T3-L1 adipocytes were quantified by Oil Red O staining. The absorbance values of the eluted Oil Red O solution in adipocytes indicate a quantitative analysis of neutral lipid content related to lipid droplet accumulation in the cytoplasm. Therefore, the fully differentiated adipocytes were stained with Oil Red O staining solution, and the absorbance value of the eluted Oil Red O solution was compared to a control at 490 nm. All solvent-partitioned fractions, *n*-hexane, 85% aqueous MeOH, *n*-BuOH, and H_2O fractions, significantly reduced lipid accumulation levels ($p < 0.05$) (Figure 2A). Among them, the reduction was more effective in the presence of *n*-hexane and 85% aqueous MeOH fractions.

In addition, RT-PCR analysis was conducted to investigate the effect of each solvent-partitioned fraction on the expression of adipogenic transcription factors, peroxisome proliferator-activated receptor-γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα) (Figure 2B). Among them, treatment with *n*-hexane and 85% aqueous MeOH fractions effectively reduced the size and intensity of the lytic zone on the regulation of PPARγ and C/EBPα. These results indicate that both *n*-hexane and 85% aqueous MeOH fractions may effectively block adipogenic differentiation of 3T3-L1 preadipocytes via the suppression of intracellular lipid accumulation.

Structural Identification of Chemical Components from the Flower Buds of *M. denudata*. To identify the potential components to suppress lipid accumulation, further separation of *n*-hexane and 85% aqueous MeOH fractions was carried out on the basis of the results, as shown in Figure 2. Four known lignans, (+)-fargesin (**A**), (+)-eudesmin (**B**), (+)-epimagnolin A (**C**), and (+)-magnolin (**D**), were isolated, and their chemical structures were determined by a combination of 2D NMR spectroscopy and a comparison to the reported data in the literature[7,11−16] (Figure 1).

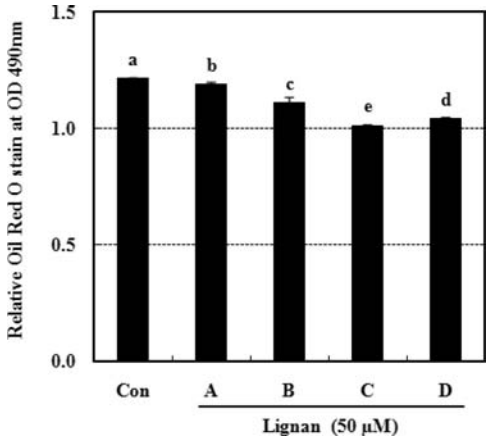

Figure 3. Effect of lignans isolated from the flower buds of *M. denudata* on intracellular lipid accumulation during adipogenic differentiation. Confluent 3T3-L1 preadipocytes were differentiated into adipocytes for 6 days, and the cells were treated with lignans in 6-well plates at the end of the differentiation period for 2 days (from day 4 to 6). The lipid droplets were fixed with 3.7% fresh formaldehyde and stained with Oil Red O staining solution. The dye retained in the cells was eluted with isopropanol and quantified by measuring optical absorbance at 490 nm. Con, fully differentiated control adipocytes (0.5 mM methylisobutylxanthine, 0.25 μM dexamethasone, and 5 μg/mL insulin); **A**, (+)-fargesin; **B**, (+)-eudesmin; **C**, (+)-epimagnolin A; and **D**, (+)-magnolin. (a−e) Means with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

*(+)-Fargesin (**A**).* White powder mp. $[\alpha]_D^{20}$ +112.7 (*c* 1.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.93−6.76 (6H, m, Ar−H), 5.95 (2H, s, $-OCH_2O-$), 4.87 (1H, d, J = 5.5 Hz, H-2), 4.42 (1H, d, J = 7.2 Hz, H-6), 4.12 (1H, brd, J = 9.6 Hz, H-4eq), 3.92 (3H, s, −OMe), 3.89 (3H, s, −OMe), 3.88−3.77 (2H, m, H-4ax, H-8eq), 3.40−3.22 (2H, H-1, H-8ax), 2.89 (1H, m, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 148.7 (C-3″), 147.8 (C-3′), 147.8 (C-4″), 147.0 (C-4′), 135.0 (C-1′), 130.8 (C-1″), 119.5 (C-6′), 117.6 (C-6″), 110.9 (C-5″), 108.8 (C-2″), 108.1 (C-5′), 106.5 (C-2′), 101.0 ($-OCH_2O-$), 87.6 (C-6), 82.0 (C-2), 71.0 (C-4), 69.7 (C-8), 55.9 (2× −OMe), 54.6 (C-5), 50.2 (C-1).

*(+)-Eudesmin (**B**).* Viscous oil. $[\alpha]_D^{20}$ +37.2 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.89−6.83 (6H, m, Ar−H), 4.75 (2H, d, J = 4.2 Hz, H-2, H-6), 4.25 (2H, dd, J = 6.9 Hz, 9.1, H-4eq, H-8eq), 3.91−3.85 (2H, m, H-4ax, H-8ax), 3.89 (6H, s, 2× −OMe), 3.87 (6H, s, 2× −OMe) 3.12 (2H, m, H-1, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 148.9 (C-3″, C-3′), 148.3 (C-4″, C-4′), 133.3 (C-1″, C-1′), 118.1 (C-6″, C-6′), 110.9 (C-5″, C-5′), 109.1 (C-2′, C-2″), 85.7 (C-6, C-2), 71.7 (C-8, C-4), 56.0 (2× −OMe), 55.9 (2× −OMe), 55.9 (−OMe), 54.2 (C-5, C-1).

*(+)-Epimagnolin A (**C**).* Viscous oil. $[\alpha]_D^{20}$ +110.6 (*c* 0.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.91 (1H, s, H-2′), 6.83 (2H, s, H-6′, H-5′), 6.56 (2H, s, H-2″, H-6″), 4.85 (1H, d, J = 5.6 Hz, H-2), 4.41 (1H, d, J = 6.8 Hz, H-6), 4.13 (1H, d, J = 9.4 Hz, H-4ax), 3.89 (3H, s, −OMe), 3.86 (3H, s, −OMe), 3.85 (6H, s, 2× −OMe), 3.85 (2H, m, H-4eq, H-8eq), 3.81 (3H, s, −OMe), 3.33 (2H, m, H-1, H-8ax), 2.92 (1H, m, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 153.1 (C-3″, C-5″), 148.6 (C-3′), 147.7 (C-4′), 137.3 (C-4″), 136.6 (C-1″), 130.7 (C-1′), 117.5 (C-6′), 110.9 (C-5′), 108.8 (C-2′), 102.8 (C-2″, C-6″), 87.7 (C-6), 81.9 (C-2), 71.0 (C-4), 69.8 (C-8), 60.8 (OMe), 56.1 (2× −OMe), 55.9 (2× −OMe), 54.6 (C-5), 50.1 (C-1).

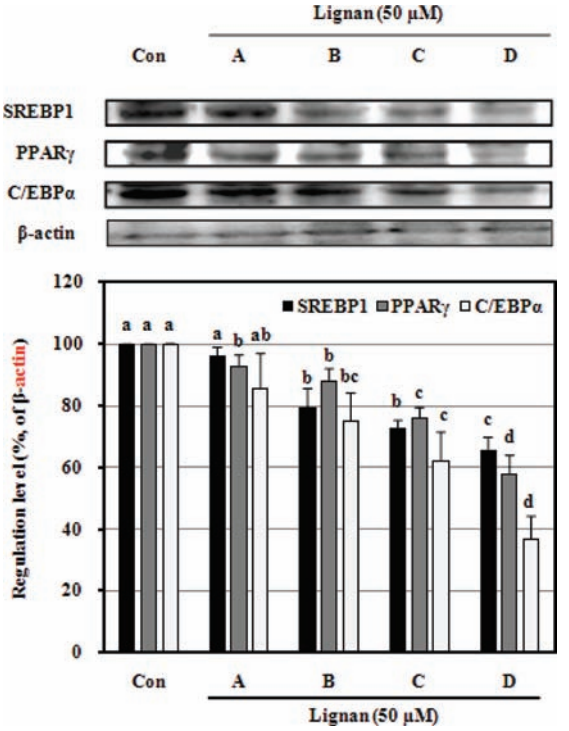

Figure 4. Effect of lignans isolated from the flower buds of *M. denudata* on the regulation of adipogenic transcription factors. Confluent 3T3-L1 preadipocytes were differentiated into adipocytes for 6 days, and the cells were treated with lignans in 6-well plates at the end of the differentiation period for 2 days (from day 4 to 6). The expression levels of adipogenic transcription factors, such as SREBP1, PPARγ, and C/EBPα, were determined by western blot analysis. Con, fully differentiated control adipocytes for 6 days (0.5 mM methylisobutylxanthine, 0.25 μM dexamethasone, and 5 μg/mL insulin); **A**, (+)-fargesin; **B**, (+)-eudesmin; **C**, (+)-epimagnolin A; and **D**, (+)-magnolin. (a−d) Means with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

*(+)-Magnolin (**D**).* Viscous oil. $[\alpha]_D^{20}$ +53.4 (*c* 3.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.88−6.83 (3H, m, Ar−H), 6.55 (2H, s, Ar−H), 4.75−4.72 (2H, m, H-2, H-6), 4.30−4.22 (2H, m, H-4eq, H-8eq), 3.93−3.86 (2H, m, H-4ax, H-8ax), 3.88 (3H, s, −OMe), 3.86 (9H, s, 3× −OMe), 3.82 (3H, s, −OMe), 3.10 (2H, m, H-1, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 153.4 (C-3″, C-5″), 149.2 (C-4′), 148.6 (C-3′), 137.4 (C-4″), 136.9 (C-1″), 133.5 (C-1′), 118.3 (C-6′), 111.1 (C-5′), 109.2 (C-2′), 102.8 (C-2″, C-6″), 86.0 (C-2), 85.7 (C-6), 71.9 (C-4), 71.8 (C-8), 60.8 (−OMe), 56.2 (2× −OMe), 55.9 (2× −OMe), 54.4 (C-1), 54.1 (C-5).

Effect of Lignans on Intracellular Lipid Accumulation. To evaluate the antiadipogenic effect of lignans **A**, **B**, **C**, and **D** isolated from the flower buds of *M. denudata*, 3T3-L1 preadipocytes were differentiated into adipocytes for 6 days and the adipocytes were treated with lignans **A**, **B**, **C**, and **D** at the end of the differentiation period. Before the intracellular level of lipid accumulation was measured, cytotoxic levels of lignans **A**, **B**, **C**, and **D** were evaluated by the MTT assay. No significant toxic effect was observed on the cells treated with these lignans up to a concentration of 100 μM (data not shown). Therefore, the experiments were carried out at a concentration of 50 μM. Lipid accumulation levels were quantified by measuring Oil Red O staining (Figure 3). All lignan compounds attenuated lipid

accumulation during adipogenic differentiation. Comparative analysis indicated that the presence of lignans **A**, **B**, **C**, and **D** during differentiation reduced the absorbance values in the order of potency **C** > **D** > **B** > **A** at a concentration of 50 μM ($p < 0.05$).

Effect of Lignans on the Expression of Adipogenic-Specific Protein. Further, effects of lignans **A**, **B**, **C**, and **D** on the expression of key transcription factors, such as SREBP1, PPARγ, and C/EBPα, were evaluated using western blotting analysis (Figure 4). In comparison to adipocytes differentiated without sample treatment, the presence of lignans **A**, **B**, **C**, and **D** during adipogenic differentiation resulted in reduced expression levels of all three factors. Among lignans, compounds **C** and **D** effectively attenuated the expression levels of SREBP1, PPARγ, and C/EBPα protein.

DISCUSSION

Obesity is a significant health problem with increasing prevalence and has been recognized as the pathogenesis of various chronic diseases, such as type-2 diabetes, hypertension, cancer, cardiac injury, and heart disease.[17,18] Recently, controlling obesity or weight has become a major target of research, and concentrated attention has been forced on exploring new components from natural resources. It has been known that adipocytes play an important role in modulating adipose mass and obesity in accordance with lipid homeostasis, energy balance, and secreting of various transcription factors.[19,20] Obesity is initiated pursuant to hypertrophy of adipose tissue as well as adipocyte differentiation of preadipocytes. Therefore, a number of studies have been assessed for adipogenic differentiation and gene regulation in several stages associated with obesity and have reported the relationship between the occurrence of obesity and adipocyte differentiation or lipid accumulation.[21] 3T3-L1 adipocytes have served as a useful *in vitro* model to assess antiobesity activity, because 3T3-L1 cells undergo an adipogenic differentiation by treatment with a cocktail, including dexamethasone, methylisobutylxanthine, and insulin.[19−21]

In the present study, we attempted to explore the antiadipogenic effect of flower buds of *M. denudata* and evaluate its potential component as a key element that can attenuate adipogenic differentiation in 3T3-L1 adipocytes. Antiadipogenic components of *M. denudata* were traced by activity-guided separation. As a primary screening, solvent-partitioned fractions from crude extracts of *M. denudata* were evaluated on lipid accumulation in 3T3-L1 adipocytes.

The 85% aqueous MeOH and *n*-hexane fractions have indicated the most noticeable effect on the suppression of lipid accumulation and adipogenic-specific gene expression (Figure 2). On the basis of these results, further separation from these two fractions led to the isolation of four lignans, (+)-fargesin (**A**), (+)-eudesmin (**B**), (+)-epimagnolin A (**C**), and (+)-magnolin (**D**). The lignans, applied to adipocytes, significantly reduced lipid accumulation in adipocytes with a decrease of the key adipogenic marker proteins SREBP1, PPARγ, and C/EBPα, which are secreted in proportion to triglyceride stores. The transcription factors SREBP1, PPARγ, and C/EBPα are able to control differentiation of preadipocytes into adipocytes, leading to the elevation of adipocyte-specific gene expression and secretion of adipokines, as well as acceleration of adipose and glucose metabolism.[22−24] In comparative analysis, lignans showed an antiadipogenic effect during differentiation in the order of potency **C** > **D** > **B** > **A**, which is in agreement with the number of methoxy groups in a molecule, indicating that these inhibitory effects may be structurally closely associated with the position and number of methoxy groups that exist in lignans. Although a direct relationship to structure and bioactivity in the lignan ring system has not been reported until now, it has been well-known that bioactivity and functionalities including a methoxy group in many compounds are closely related.[25,26] These results might be able to suggest the possible neutraceutical value of lignans to treat obesity. In humans, however, it has been suggested that regional adipocyte hyperplasia can be caused and body fat distribution is regulated partly via differences in adipogenesis.[27,28] In addition, most metabolic problems associated with obesity in humans have been reported to arise from the lack of adipocyte precursor cells or from the inability to become mature adipocytes.[29] Therefore, the mechanistic process related with obesity looks much more complicated than 3T3-L1 adipocyte differentiation.

In conclusion, our results demonstrated that the flower buds of *M. denudata* and its isolated lignan components have the potential to affect the suppression of adipogenic differentiation. Among the isolated lignans, compounds **C** and **D**, (+)-epimagnolin A and (+)-magnolin, revealed a much stronger suppressive effect on adipogenic differentiation than other compounds, and these results further suggest that they hold great promise for the regulation of lipid accumulation through the effects on preadipocyte differentiation.

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