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Mesonosides A-H, primeverose derivatives from *Mesona procumbens* suppress adipogenesis by downregulating PPAR\(\gamma\) and C/EBP\(\alpha\) in 3T3-L1 cells

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Abstract

Obesity is becoming a worldwide epidemic, especially in industrialized countries. We hereby report a methanolic extract of *Mesona procumbens* (known as Hsian-tsao in Taiwan) significantly inhibits lipid accumulation in 3T3-L1 adipocytes, and eight new primeverose derivatives, mesonosides A-H (1\(\text{e}\)–8), were isolated from the methanolic extract of *M. procumbens*. Structural elucidation of 1\(\text{e}\)–8 was established by spectroscopic methods, especially 2D NMR techniques (\(^{1}\)H–\(^{1}\)H COSY, HSQC, HMBC, and NOESY) and HRESIMS. Anti-obesity evaluation revealed that isolates 1\(\text{e}\)–5, 7, and 8 showed inhibitory effects on lipid accumulation and protein levels of adipogenic transcription factor, PPAR\(\gamma\) and C/EBP\(\alpha\) in 3T3-L1 cells. Our study suggests that *M. procumbens* extract including new primeverose isolates may be potentially used as a natural source to ameliorate fat accumulation and even obesity. **Keywords:** Anti-adipogenic, Lipid accumulation, *Mesona procumbens*, Mesonoside, PPAR\(\gamma\)

1. Introduction

Obesity has become the leading metabolic disease globally [1] and has been associated with other diseases, such as coronary heart disease, hypertension, type 2 diabetes mellitus, cancer, respiratory complications, and osteoarthritis [2]. Obesity is a condition in which adipocytes accumulate a large amount of fat and become enlarged. At the cellular level, obesity is characterized by an increase in the number and size of adipocytes differentiated from fibroblastic preadipocytes in adipose tissue [3]. Adipocyte differentiation is a complex process involving changes in cell morphology, hormone sensitivity, and gene expression [4]. The transcription factors CCAAT/enhancer binding protein (C/EBP) \(\beta\) and \(\delta\) are first induced in response to adipogenic factors and then in turn activate peroxisome proliferator-activated receptor gamma (PPAR\(\gamma\)) and C/EBP\(\alpha\) [5], which are essential for the expression of a large group of genes that produce the adipocyte phenotype [6]. An increasing number of investigations have been conducted to discover the components that may reduce the accumulation of excess body fat. More recently, eugenol diglycosides have been reported to inhibit lipid droplet accumulation in adipocytes by reducing the transcription levels of adipocyte marker genes [7].

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Mesona procumbens Hemsl., known as Hsian-tsao in Taiwan, is a common material used to prepare functional beverages in several Asia countries and is also frequently used as an ingredient in traditional Chinese medicine to treat heat shock, hypertension, diabetes, liver disease, and muscle pain [8]. Previous research reported that M. procumbens extracts possess several bioactivities, such as anti-inflammatory [9], antihypertensive [10], DNA damage protection [11], antimutagenic [12], liver fibrosis prevention [13], and renal protective [14] activities. However, the active components in M. procumbens extracts imparting these effects are not well defined.

Since obesity increases the risk of several metabolic diseases, the effective components for ameliorating excess body fat accumulation are worth investigating. To our knowledge, there is no report about the effect of M. procumbens on the regulation of adipogenesis or obesity. In the present study, we demonstrated that the methanolic extract of M. procumbens exhibited significant anti-adipogenesis activity. Here, 3T3-L1 cells were used as a cell model for adipose cell biology research, which has been well established over the course of several decades [15,16].

2. Materials and methods

2.1. General experimental procedures

Optical rotations were determined by using a P-2000 polarimeter (JASCO). Infrared (IR) spectra were recorded on a Mattson Genesis II spectrometer (Thermo). High-resolution electrospray ionization mass spectrometry (HRESIMS) data were obtained on an LCQ mass spectrometer (Thermo). High-resolution electrospray ionization mass spectrometry (HRESIMS) data were measured on a Shimadzu IT-TOF HR mass spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on Varian Unity Inova 500 MHz, or Varian VNMRS 600 MHz spectrometers. For column chromatography (CC), and silica gel 60 (70–230 and 230–400 mesh, Merck) were used. Precoated silica gel plates (Merck 60 F-254) were used for thin layer chromatography (TLC). High-performance liquid chromatography (HPLC) separations were performed on a Shimadzu LC-8A pump with a UV SPD-20A detector equipped with a 250 × 20 mm i.d. preparative Cosmosil 5C18 AR-II column (Nacalai Tesque).

2.2. Plant material

The whole plants of M. procumbens Hemsl. (8.0 kg, dry weight) were purchased from Starsci Biotech Co. Ltd. in Taoyuan and identified by Dr. Syh-Yuan Hwang of the Endemic Species Research Institute, Council of Agriculture, Taiwan. A voucher specimen (No. NRICM20190901) was deposited in the National Research Institute of Chinese Medicine, Ministry of Health and Welfare, Taipei, Taiwan.

2.3. Extraction and isolation

Air-dried M. procumbens (8.0 kg) were extracted with 100% methanol (80 L) at 50 °C three times, and the extract was concentrated under reduced pressure. The methanol extract (ca. 796.5 g, MPM) was suspended in H2O, and the suspension was partitioned successively with n-hexane and then CH2Cl2, respectively. The partition layers were concentrated under reduced pressure to obtain hexane (MPH) and CH2Cl2 (MPD) extracts. The MPD extract (ca. 47.6 g) was separated into 6 fractions (Fractions I–VI) on a C18 gel flash column (60–230 mesh, 15 × 25 cm) eluting with a solvent system of H2O/MeOH (0–100%). Fraction IV was further subjected to a silica gel flash column (60–230 mesh, 15 × 20 cm) eluting with a solvent system of CH2Cl2/acetone (5–100%) to afford eight subfractions (IVA–IVH). The IVF fraction was fractionated by preparative HPLC eluting with 60% acetonitrile (ACN) in H2O (flow rate: 10.0 mL/min) to give 7 fractions (IVF-1–IVF-7). Fraction IVF-3 was separated by HPLC eluting with 45% ACN (flow rate: 10.0 mL/min) to give compound 1 (102.3 mg, Rt: 19.8 min). Fraction IVF was separated by HPLC with 60% ACN (flow rate: 10.0 mL/min) to afford nine fractions (IVE-1–IVE-9). IVE-4 was purified by preparative HPLC eluting with 50% ACN (flow rate: 10.0 mL/min) to give 2 (76.6 mg, Rt: 19.3 min) and 3 (34.3 mg, Rt: 22.3 min). Fraction IVD was separated by HPLC with 65% ACN (flow rate: 10.0 mL/min) to afford seven fractions (IVD-1–IVD-7). The IVD-4 fraction was further purified by HPLC, repeatedly eluting with 50% ACN (flow rate: 10.0 mL/min) to give 4 (39.6 mg, Rt: 22.8 min). Compound 5 (46.6 mg, Rt: 25.1 min) was purified from fraction IVD-5 by HPLC eluting with 50% ACN (flow rate: 10.0 mL/min). Fraction IVC was separated by HPLC with 65% ACN (flow rate: 10.0 mL/min) to afford eight fractions (IVC-1–IVC-8). The IVC-7 fraction was further purified by HPLC and repeatedly eluted with 45% ACN (flow rate: 10.0 mL/min) to give 6 (0.9 mg, Rt: 82.3 min). Fraction IVB was separated by HPLC with 65% ACN (flow rate: 10.0 mL/min) elution to afford six fractions (IVB-1–IVB-6). The IVB-4 fraction was further purified by HPLC with repeated elution with 50% ACN (flow rate: 10.0 mL/min) to give 7 (23.1 mg, Rt: 38.4 min) and 8 (9.1 mg, Rt: 44.0 min).
| NO | 1<sup>a</sup> | 2<sup>a</sup> | 3<sup>a</sup> | 4<sup>a</sup> | 5<sup>a</sup> | 6<sup>a</sup> | 7<sup>a</sup> | 8<sup>a</sup> |
|----|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 1  | 4.57 d (7.5) | 4.54 d (8.0) | 4.48 d (8.0) | 4.57 d (8.0) | 4.57 d (8.0) | 4.76 d (7.8) | 4.51 d (7.8) | 4.55 d (8.0) |
| 2  | 3.61 dd (8.0, 9.5) | 3.58 m | 3.49 dd (8.0, 9.5) | 3.60 dd | 3.61 dd | 5.03 dd | 3.55 dd | 3.59 m |
| 3  | 5.36 t (9.5) | 5.34 t (9.0) | 5.19 m | 5.36 t (9.5) | 5.36 t (9.5) | 5.46 t (9.6) | 5.30 t (9.6) | 5.34 t (9.5) |
| 4  | 5.12 dd (9.5, 10.0) | 5.04 t (9.5) | 3.53 m | 5.11 t (10.0) | 5.11 t (10.0) | 5.09 t (9.6) | 5.01 t (9.6) | 5.05 dd (9.0, 9.5) |
| 5  | 3.83 m | 3.73 m | 3.51 m | 3.84 m | 3.84 m | 3.79 m | 3.71 m | 3.76 m |
| 6  | 3.20 dd (10.0, 11.0) | 3.58 m | 3.75 m | 3.69 dd | 3.67 dd | 3.57 dd | 3.59 dd | 3.59 m |
| 7  | 3.87 m (2.0, 11.0) | 4.07 dd (5.5, 11.5) | 3.88 dd (2.0, 11.0) | 3.87 dd | 3.84 dd | 3.83 dd | 3.83 dd (2.5, 11.0) |
| 8  | 5.03 dd (8.4, 10.2) | 3.55 dd (5.4, 11.4) | 3.84 dd (2.4, 11.4) | 3.57 dd (5.4, 11.4) | 3.83 dd | 3.83 dd | 3.83 dd (2.5, 11.0) |
| 9  | 3.59 m | 3.87 dd (2.5, 11.0) | 3.57 dd (5.4, 11.4) | 3.83 dd | 3.83 dd | 3.83 dd | 3.83 dd (2.5, 11.0) |

<sup>a</sup> Recorded at 500 MHz.
<sup>b</sup> Recorded at 600 MHz.
2.3.1. Mesonoside A (I)

Colorless syrup; \( \delta_{1}^{25}{ }^1H=0.45 \) ppm (d, 6 MeOH); UV \( \lambda_{\text{max}} \) (MeOH) \( \log \varepsilon \) 273 (2.96), 229 (4.13) nm; IR (KBr) \( \nu_{\text{max}} \) 3414, 2932, 2865, 1731, 1274, 1047 cm\(^{-1}\); \(^1H\) and \(^13C\)-NMR spectroscopic data (methanol-\( d_4 \)) are shown in Tables 1 and 2, respectively; HRESIMS \( m/z \) 591.2419 [M + Na]\(^+\) (calcd. for C\(_{28}\)H\(_{42}\)O\(_{13}\)Na, 591.2412).

2.3.2. Mesonoside B (II)

Colorless syrup; \( \delta_{1}^{25}{ }^1H=0.72 \) ppm (d, 6 MeOH); UV \( \lambda_{\text{max}} \) (MeOH) \( \log \varepsilon \) 273 (3.01), 229 (4.06) nm; IR (KBr) \( \nu_{\text{max}} \) 3419, 2932, 2860, 1734, 1277, 1039 cm\(^{-1}\); \(^1H\) and \(^13C\)-NMR spectroscopic data (methanol-\( d_4 \)) are shown in Tables 1 and 2, respectively; HRESIMS \( m/z \) 633.2504 [M + Na]\(^+\) (calcd. for C\(_{30}\)H\(_{42}\)O\(_{13}\)Na, 633.2518).

2.3.3. Mesonoside C (III)

Colorless syrup; \( \delta_{1}^{25}{ }^1H=-3.24 \) ppm (d, 6 MeOH); UV \( \lambda_{\text{max}} \) (MeOH) \( \log \varepsilon \) 273 (3.10), 229 (4.04) nm; IR (KBr) \( \nu_{\text{max}} \) 3441, 2930, 2864, 1726, 1247, 1042 cm\(^{-1}\); \(^1H\) and \(^13C\)-NMR spectroscopic data (methanol-\( d_4 \)) are shown in Tables 1 and 2, respectively; HRESIMS \( m/z \) 633.2501 [M + Na]\(^+\) (calcd. for C\(_{30}\)H\(_{42}\)O\(_{13}\)Na, 633.2518).

2.3.4. Mesonoside D (IV)

Colorless syrup; \( \delta_{1}^{25}{ }^1H=-0.68 \) ppm (d, 6 MeOH); UV \( \lambda_{\text{max}} \) (MeOH) \( \log \varepsilon \) 273 (3.04), 229 (4.00) nm; IR (KBr) \( \nu_{\text{max}} \) 3456, 2932, 2863, 1729, 1277, 1039 cm\(^{-1}\); \(^1H\) and \(^13C\)-NMR spectroscopic data (methanol-\( d_4 \)) are shown in Tables 1 and 2, respectively; HRESIMS \( m/z \) 633.2500 [M + Na]\(^+\) (calcd. for C\(_{30}\)H\(_{42}\)O\(_{13}\)Na, 633.2518).
2.3.5. Mesonoside E (5)

Colorless syrup; $\delta^1H$ 6.56 (c 0.6, MeOH); UV $\lambda_{max}$ (MeOH) (log $\epsilon$) 273 (3.07), 229 (4.07) nm; IR (KBr) $\nu_{max}$ 3468, 2932, 2858, 1741, 1274, 1037 cm$^{-1}$; $^1H$ and $^{13}C$-NMR spectroscopic data (methanol-d$_4$) are shown in Tables 1 and 2, respectively; HRESIMS $m/z$ 633.2507 $[M + Na]^+$ (calcd. for C$_{30}$H$_{42}$O$_{13}$Na, 633.2518).

2.3.6. Mesonoside F (6)

Colorless syrup; $\delta^1H$ 6.85 (c 0.6, MeOH); UV $\lambda_{max}$ (MeOH) (log $\epsilon$) 273 (3.01), 229 (4.06) nm; IR (KBr) $\nu_{max}$ 3421, 2932, 2858, 1734, 1274, 1042 cm$^{-1}$; $^1H$ and $^{13}C$-NMR spectroscopic data (methanol-d$_4$) are shown in Tables 1 and 2, respectively; HRESIMS $m/z$ 675.2623 $[M + Na]^+$ (calcd. for C$_{32}$H$_{44}$O$_{14}$Na, 675.2623).

2.3.7. Mesonoside G (7)

Colorless syrup; $\delta^1H$ 6.54 (c 0.6, MeOH); UV $\lambda_{max}$ (MeOH) (log $\epsilon$) 273 (2.93), 229 (3.94) nm; IR (KBr) $\nu_{max}$ 3473, 2935, 2860, 1756, 1245, 1035 cm$^{-1}$; $^1H$ and $^{13}C$-NMR spectroscopic data (methanol-d$_4$) are shown in Tables 1 and 2, respectively; HRESIMS $m/z$ 675.2631 $[M + Na]^+$ (calcd. for C$_{32}$H$_{44}$O$_{14}$Na, 675.2623).

2.3.8. Mesonoside H (8)

Colorless syrup; $\delta^1H$ 6.38 (c 0.6, MeOH); UV $\lambda_{max}$ (MeOH) (log $\epsilon$) 273 (3.04), 229 (4.04) nm; IR (KBr) $\nu_{max}$ 3434, 2932, 2860, 1729, 1252, 1042 cm$^{-1}$; $^1H$ and $^{13}C$-NMR spectroscopic data (methanol-d$_4$) are shown in Tables 1 and 2, respectively; HRESIMS $m/z$ 675.2603 $[M + Na]^+$ (calcd. for C$_{32}$H$_{44}$O$_{14}$Na, 675.2623).

2.4. Acid hydrolysis of glycosides

Each isolated compound (1.0 mg) was treated with 2 N methanolic HCl (2 mL) under reflux at 90 °C for 1 h. Each mixture was extracted with CH$_2$Cl$_2$ to afford the aglycone portion, and the aqueous layer was neutralized with Na$_2$CO$_3$ and treated with Na$_2$CO$_3$ and triethylamine in ethylformamide (3 mL each) were added to the filtrate, and the mixture was stirred at room temperature. After the reaction mixture was dried, the mixture was washed twice with PBS pH 7.4 and then fixed with 10% neutral buffered formalin for at least 20 min at room temperature. After fixation, the cells were washed twice with PBS and stained with Oil red O working solution for 60 min. Images of stained cells were photographed and then eluted with stained Oil red O by adding 100% isopropanol. The optical density (OD) was measured at 510 nm in a spectrophotometer (Thermo Multiskan Spectrum).

2.7. Western blot assay

Cells were lysed in RIPA buffer with phenylmethylsulfonyl fluoride (PMSE; Beyotime Biotechnology, Jiangsu, China). Protein concentration was determined using a Bio-Rad protein assay system (Bio-Rad, Hercules, CA, USA). Equivalent amounts of proteins were separated by SDS–PAGE and then transferred to PVDF membranes (Bio-Rad). After being blocked in TBS containing 5% nonfat milk, the...
**Fig. 1.** Chemical structure of compounds 1–8 isolated from *M. procumbens.*

|   | R₁ | R₂ | R₃ | R₄ | R₅ |
|---|----|----|----|----|----|
| 1 | H  | Ac | H  | H  | H  |
| 2 | H  | Ac | Ac | H  | H  |
| 3 | H  | H  | Ac | Ac | H  |
| 4 | H  | Ac | H  | Ac | H  |
| 5 | H  | Ac | H  | H  | Ac |
| 6 | Ac | Ac | Ac | H  | H  |
| 7 | H  | Ac | Ac | Ac | H  |
| 8 | H  | Ac | Ac | H  | Ac |

**Fig. 2.** Effects of *M. procumbens* extracts on the cytotoxicity and adipogenesis of 3T3-L1 cells. (A) Cell viability of the MPM, MPH, MPD, and MPW fractions. Lipid accumulation was visualized (B) and quantified (C) by Oil Red O staining. Cells were photographed at 100 × magnification, and morphological changes were assessed based on lipid accumulation with or without the extracts (25 and 50 µg/mL) on d 8. The OD value of each sample at 510 nm was measured using an ELISA reader. Values are presented as the mean ± SD (n = 3). The values with different letters indicate significant differences as determined by Dunnett’s method. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 relative to the control group.
membranes were incubated with primary antibodies (1:1000 dilution) at 4 °C for 12 h and then with horseradish peroxidase-conjugated secondary antibody (1:5000 dilution). Signals were detected on X-ray film using an ECL detection system (Pierce, Rockford, IL, USA). The relative protein levels were calculated based on β-actin as the loading control.

2.8. Statistical analysis

The results of this investigation are the means ± SD of three independent experiments. One-way ANOVA followed by Dunnett’s t-test was used to analyze the differences between samples at different doses. The statistical analysis was performed with GraphPad Prism 7.0 software (GraphPad, La Jolla, CA). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 relative to the control-treated group.

3. Results and discussion

3.1. Extraction, fractionation, and purification of the extract from M. procumbens

The methanolic extract of the whole M. procumbens plant (MPM, 796.5 g/8 kg, 9.95%) was suspended in H2O and partitioned with n-hexane and then CH2Cl2 successively to yield two organic layers (MPH and MPD) and one H2O layer (MPW). The CH2Cl2 extract (MPD, 5.98%) was chromatographically separated on a C18 gel flash column followed by a silica gel flash column. The highest-yielding fractions were further subjected to preparative HPLC using a reversed-phase (ODS) column to yield eight new primeverose derivatives, mesonosides A-H (Fig. 1). These structures were determined by spectroscopic data, especially NMR and HRMS data. All isolated compounds 1–8 were screened for anti-adipogenesis activity and further demonstrated the relationship of adipogenesis protein expression.

3.2. Effect of M. procumbens extracts on intracellular lipid accumulation in 3T3-L1 cells

Four layers (MPM, MPH, MPD, and MPW) yielded from M. procumbens extracts were examined the cytotoxic effect on 3T3-L1 preadipocytes, which treated with 25 or 50 μg/mL of samples for 48 h, and cell viability was measured by CCK-8 assay. As shown in Fig. 2A, 50 μg/mL of MPH caused severe cell death, while the others showed little to no cytotoxic effect on 3T3-L1 preadipocytes. Next, 3T3-L1 cells were treated with 25 or 50 μg/mL of samples, and lipid accumulation of cells was measured using Oil Red O staining. As shown in Fig. 2B and C, samples exerted differential inhibitory effects on the inhibition of lipid accumulation in 3T3-L1 cells. Among these fractions, MPD showed the best activity in blocking lipid accumulation and had no obvious cytotoxic effect in 3T3-L1 cells.

3.3. Structural elucidation of the isolated compounds

Compound 1 was obtained as a colorless syrup. Its molecular formula, C28H40O12, was established on the basis of the quasimolecular ion peak at m/z 591.2419 [M + Na]+ (calcd. for C28H40O12Na, 591.2412) in the HRESIMS. The IR and UV spectra displayed absorption bands for hydroxyl (3414 cm⁻¹), carbonyl (1731 cm⁻¹) and aromatic ester groups (1731 cm⁻¹ and 273 nm). The 1H-NMR spectrum (Table 1) of 1 exhibited signals for one triplet methyl at δH 0.92 (J = 7.0 Hz); one singlet methyl at δH 1.95; eight olefinic protons at 5.16 (ddd, J = 10.5, 1.5, 1.0 Hz), 5.29 (ddd, J = 17.0, 1.5, 1.0 Hz), 5.92 (ddd, J = 17.5, 10.5, 7.0 Hz), 7.49 (2H, m), 7.63 (1H, m), and 8.02 (2H, m). The 13C-NMR spectrum (Table 2) showed 28 signals and when combined with the DEPT experiment showed that compound 1 contains a benzoate group (δC 166.1, 133.0, 129.7, 129.3, 129.3, 128.2, and 128.2), one acetoxy group (δC 19.2 and 170.3), two anomeric carbons (δC 101.9 and 103.8), eight olefinic protons at 5.16 (ddd, J = 10.5, 1.5, 1.0 Hz), 5.29 (ddd, J = 17.0, 1.5, 1.0 Hz), 5.92 (ddd, J = 17.5, 10.5, 7.0 Hz), 7.49 (2H, m), 7.63 (1H, m), and 8.02 (2H, m). The 13C-NMR (Table 2) spectrum showed 28 signals and when combined with the DEPT experiment showed that compound 1 contains a benzoate group (δC 166.1, 133.0, 129.7, 129.3, 129.3, 128.2, and 128.2), one acetoxy group (δC 19.2 and 170.3), two anomeric carbons (δC 101.9 and 103.8), eight olefinic protons at 5.16 (ddd, J = 10.5, 1.5, 1.0 Hz), 5.29 (ddd, J = 17.0, 1.5, 1.0 Hz), 5.92 (ddd, J = 17.5, 10.5, 7.0 Hz), 7.49 (2H, m), 7.63 (1H, m), and 8.02 (2H, m). The 13C-NMR (Table 2) spectrum showed 28 signals and when combined with the DEPT experiment showed that compound 1 contains a benzoate group (δC 166.1, 133.0, 129.7, 129.3, 129.3, 128.2, and 128.2), one acetoxy group (δC 19.2 and 170.3), two anomeric carbons (δC 101.9 and 103.8), eight olefinic protons at 5.16 (ddd, J = 10.5, 1.5, 1.0 Hz), 5.29 (ddd, J = 17.0, 1.5, 1.0 Hz), 5.92 (ddd, J = 17.5, 10.5, 7.0 Hz), 7.49 (2H, m), 7.63 (1H, m), and 8.02 (2H, m).
together with nine oxygenated carbons, indicated that compound 1 possesses a disaccharide moiety. Furthermore, detailed analyses of the $^1$H and $^{13}$C NMR spectroscopic data with the aid of $^1$H–$^1$H COSY and HMBC correlation (Fig. 3) suggested that the disaccharide moiety should be asprimeverose, including a glucopyranosyl group [$\delta_{C}$ 101.9 (C-1), 72.1 (C-2), 75.9 (C-3), 69.3 (C-4), 72.9 (C-5) and 67.5 (C-6)] and a xylopyranosyl group [$\delta_{C}$ 103.8 (C-1'), 73.4 (C-2'), 76.1 (C-3'), 69.8 (C-4') and 65.5 (C-5')] [16,17]. Moreover, the configurations of H-1 and H-1' were assigned to be $\beta$-forms by the coupling constant (glu: $J = 7.5$ Hz and xyl: $J = 7.5$ Hz) and comparison of $^{13}$C data with literature [17,18]. The $^1$H–$^1$H COSY correlations of H-1''/H-2''/H-3''/H-4''/H-5''/H-6''/H-7''/H-8'' and H-1''/H-2''/H-3''/H-4''/H-5''/H-6''/H-7'' in addition to the key HMBC correlations of H-3-1 [($\delta_{H}$ 5.36)/$\delta_{C}$ 166.1 and H-1 [($\delta_{H}$ 4.57) with $\delta_{C}$ 81.7 (C-3')], suggested that oct-1-en-3-ol was attached to C-1 and that benzoic acid was located at C-3. The acetoxy group was linked at C-4 by the HMBC correlation of H-4 ($\delta_{H}$ 5.11) with $\delta_{C}$ 170.7. In $\delta_{H}$ 4.71 in 4, two acetyl groups were assigned at C-1 and C-3' based on the HMBC correlations of H-4 ($\delta_{H}$ 5.11)/$\delta_{C}$ 170.2 and H-3' ($\delta_{H}$ 4.89)/$\delta_{C}$ 171.2. On the basis of the above evidence, compounds 3 and 4 were identified as 1-(R)-oct-1-en-3-yl-3-O-benzoyl-2',3'-O-diacyethyl-$\beta$-D-primeveroside (mesonoside C) and 1-(R)-oct-1-en-3-yl-3-O-benzoyl-4',3'-O-diacyethyl-$\beta$-D-primeveroside (mesonoside D), respectively.

Compound 5 has a quasimolecular ion peak at $m/z$ 633.2507 $[\text{M} + \text{Na}]^+$, consistent with the molecular formula C$_{30}$H$_{42}$O$_{13}$ from analysis of its HRESIMS. The NMR data (Tables 1 and 2) were very similar to those of 4, except for the proton resonance at $\delta_{H}$ 4.89 (H-3') in 4 was shifed upfield to $\delta_{H}$ 3.57 in 5, while the proton of H-4' in 4 downfield to $\delta_{H}$ 4.71 in 5. The findings indicated the acetyl group was shifted from C-3' in 4 to C-4' in 5, and that was further confirmed by the HMBC correlation of H-4'/$\delta_{C}$ 170.8. Taken together, the above evidence determined that the structure of mesonoside E (5) was 1-(R)-oct-1-en-3-yl-3-O-benzoyl-4',3'-O-diacyethyl-$\beta$-D-primeveroside.

Compound 6 was obtained as a colorless syrup and possessed a molecular formula of C$_{30}$H$_{42}$O$_{13}$ by its sodiated quasimolecular ion peak at $m/z$ 675.2639 $[\text{M} + \text{Na}]^+$ (calcld. for C$_{30}$H$_{42}$O$_{13}$Na, 675.2623) in the HRESIMS. The $^1$H and $^{13}$C-NMR spectra (Tables 1 and 2) of 6 exhibited the presence of three acetyl groups ($\delta_{H}$ 1.90, 1.91, 2.12; $\delta_{C}$ 169.5, 169.7, 170.6, 19.1, 19.2, 19.8), a benzoate group ($\delta_{C}$ 165.7, 129.0, 129.3 x 2, 128.2 x 2, 133.3), two anomeric signals [$\delta_{H}$ 4.76 (d, $J = 7.8$ Hz), $\delta_{C}$ 99.5, glu-1; 4.41 (d, $J = 7.8$ Hz), 101.3, xyl-1], and a 1-oct-1-en-3-yl group [characteristic signals of one triplet methyl at $\delta_{H}$ 0.89 ($J = 6.6$ Hz) and 5.12 (ddd, $J = 17.4, 1.5, 1.0$ Hz), 5.24 (ddd, $J = 10.8, 1.5, 1.0$ Hz), 5.85 (ddd, $J = 17.0, 10.5, 7.0$ Hz), and 4.10 (ddd, $J = 6.0, 6.5, 12.5$ Hz)]. The $^1$H and $^{13}$C NMR data (Tables 1 and 2) were very similar to those of 2, except for the addition of an acetyl group ($\delta_{H}$ 2.07; $\delta_{C}$ 170.0, 19.6). Furthermore,
the acetyl group was assigned at C-2 ($\delta_C$ 71.9) by HMBC correlation between H-2 [J = 0.89, dd (f = 9.5, 8.0 Hz)] and $\delta_C$ 170.0. Consequently, 6 was deduced to be 1-(R)-oct-1-en-3-yl-3-O-benzoyl-2,4,2,0-triacetyl-$\beta$-D-primeveroside and named mesonoside F.

Similar to 6, compounds 7 and 8 also possessed similar 1D and 2D NMR, UV, IR, and HRESIMS spectra. The HRESIMS of 7 and 8 suggested a molecular formula of C$_{30}$H$_{42}$O$_{13}$ based on its sodiated quasimolecular ion peak at m/z 675.2631 in 7 and 675.2603 in 8 ([M + Na]$^+$, calcld. for C$_{32}$H$_{44}$O$_{14}$Na, 675.2623). The IR spectra displayed absorption bands for hydroxyl, carbonyl, and aromatic groups, and the UV spectra showed absorption maxima at 273 and 229 nm. The $^1$H and $^{13}$C-NMR spectroscopic data of compounds 7 and 8 (Tables 1 and 2) suggested that their structures were similar to 6 which belong to 1-(R)-oct-1-en-3-yl-3-O-benzoyl primeverose derivatives with three acetyl groups. Detailed analysis of the HMBC correlations revealed that three acetyl groups were located at C-4, C-2' and C-3' in 7 but located at C-4, C-2' and C-4' in 8. Therefore, mesonosides G (7) and H (8) were identified as 1-(R)-oct-1-en-3-yl-3-O-benzoyl-4,2,0,3'-O-triacetyl-$\beta$-D-primeveroside and 1-(R)-oct-1-en-3-yl-3-O-benzoyl-4,2,0,4'-O-triacetyl-$\beta$-D-primeveroside, respectively.

3.4. Identification of the isolated compounds from MPD on HPLC fingerprint profile

We studied the HPLC fingerprints of the bioactive MPD layer from M. procumbens and analyzed them under the following conditions: The mobile phase consisted of water containing 0.1% phosphoric acid
and ACN using a gradient program of 5–45% ACN from 0 to 25 min, 45–45% ACN from 20 to 40 min, 45–80% ACN from 40 to 55 min and 80–100% ACN from 55 to 65 min; separation was achieved on a Cosmosil 5C18-AR-II column (5 μm, 250 mm × 4.6 mm i.d.) with a flow rate of 1.0 mL/min at 35 °C; real-time UV absorption was detected at 254 nm. Seven isolated compounds were successfully recognized in the HPLC fingerprints of MPD layer, and their retention times (RTs) were 33.82 min (1), 42.97 min (2), 44.24 min (3), 45.52 min (4), 46.11 min (5), 49.76 min (7), and 50.23 min (8), respectively (Supplementary Fig. S1). Due to the most yielded amounts together with high resolution and well separation around its corresponding peak in HPLC fingerprints of MPD layer, 1 could be served as the target compound for the quality control of *M. procumbens*.

### 3.5. Effects of compounds 1–8 on intracellular lipid accumulation in 3T3-L1 cells

The cell survival assay showed that all compounds (1–8) exhibited little or no cytotoxic effects in 3T3-L1 preadipocytes at 20 or 40 μM (Fig. 4A). We next examined the effects of compounds 1–8 on intracellular lipid accumulation in 3T3-L1 adipocytes.

![Figure 5](image_url)

**Fig. 5.** Compounds 1–5, 7, and 8 inhibited the expression of PPARγ and C/EBPα in 3T3-L1 cells. 3T3-L1 cells (1.5 × 10^5 cells/6-cm dish) were treated with compounds 1–5, 7, and 8 (40 μM) for 8 d. Curcumin (Cur, 15 μM) was the positive control. The total protein of each group was extracted, and the expression of PPARγ and C/EBPα was determined by western blotting. The relative levels of PPARγ and C/EBPα were quantified by normalizing with the β-actin levels. Values with different letters indicate significant differences as determined by Dunnett's method. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 relative to the control group.
The staining of lipid droplets with Oil Red O solution showed that compounds 1–8 inhibited lipid droplet accumulation from 8.2 to 18.7% at 20 μM and 24.3–42.0% at 40 μM in the adipocytes (Fig. 4B and C). Notably, compounds 1–8 all showed a significant decrease in lipid accumulation in a dose-dependent manner in 3T3-L1 cells. Among these isolates, compound 3 showed the most inhibition in 3T3-L1 cells.

3.6. Effect of the isolated compounds on key adipogenic gene regulators, PPARγ and C/EBPα protein levels, in 3T3-L1 cells

In mammalian cells, PPARγ and C/EBPα are the main regulators of adipogenesis and have been shown to have a broad overlap in their transcriptional targets [1]. To further investigate the underlying mechanism by which compounds 1–5, 7, and 8 attenuate lipid accumulation in 3T3-L1 cells, we examined the protein levels of critical regulators of adipogenesis, PPARγ and C/EBPα. After treatment with 40 μM of compounds 1–5, 7, and 8 for 8 d, total protein was extracted, and Western blot analysis was used to detect PPARγ and C/EBPα protein levels. As shown in Fig. 5, the PPARγ and C/EBPα levels were significantly reduced in cells treated with compounds 1–5, 7, and 8 compared to control cells. These results indicate that compounds 1–5, 7, and 8 may suppress lipid accumulation in 3T3-L1 cells by decreasing the protein levels of PPARγ and C/EBPα, critical adipogenic gene regulators. Notably, 3 exhibits the most inhibition of lipid accumulation, not only in 3T3-L1 cells, but also in the protein levels of PPARγ and C/EBPα.

Our results are consistent with a recent study that eugenol diglycosides inhibited lipid droplet accumulation in adipocytes by reducing the transcription levels of adipocyte marker genes (Fabp4, PPARγ, C/EBPβ, Adipsin, and Adipoq) [7]. Here, we demonstrated the anti-adipogenic bioactivity of M. procumbens and further isolation and identification of eight new primeverose derivatives (1–8) from a methanolic extract of M. procumbens. Furthermore, the anti-adipogenic effect of the isolated compounds (1–5, 7, and 8) may occur via the inhibition of PPARγ and C/EBPα protein levels. Our studies provide scientific evidence to support this plant serving to reduce the level of intracellular lipid droplet accumulation.

4. Conclusion

In the report, we have isolated and identified eight new primeverose derivatives from the methanolic extract of M. procumbens. Those isolates (1–5, 7, and 8), which possessed primeverose unit and substituted with benzoyl, acetyl, and oct-1-en-3-ol groups, showed the inhibitory effect on lipid accumulation in 3T3-L1 cells by suppressing expression the PPARγ and C/EBPα protein levels of the adipogenic transcription factors. The findings provide a potential therapeutic strategy to use primeverose derivatives the major components isolated from M. procumbens on the treatment of excessive adipogenesis in obesity.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

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Appendix. Supplementary data

Fig. S1 Identification of the active compounds 1–5, 7, and 8 in the HPLC fingerprint of the MPD layer.

Fig. S2 $^1$H-NMR spectrum of mesonoside A (1) (500 MHz in methanol-$d_4$).
Fig. S3 $^{13}$C-NMR spectrum of mesonoside A (1) (125 MHz in methanol-d$_4$).

Fig. S4 $^1$H-NMR spectrum of mesonoside B (2) (500 MHz in methanol-d$_4$).
Fig. S5 $^{13}$C-NMR spectrum of mesonoside B (2) (125 MHz in methanol-d$_4$).

Fig. S6 $^1$H-NMR spectrum of mesonoside C (3) (500 MHz in methanol-d$_4$).
Fig. S7 $^{13}$C-NMR spectrum of mesonoside C (3) (125 MHz in methanol-d$_4$).

Fig. S8 $^1$H-NMR spectrum of mesonoside D (4) (500 MHz in methanol-d$_4$).
Fig. S9 $^{13}$C-NMR spectrum of mesonoside D (4) (125 MHz in methanol-d$_4$).

Fig. S10 $^1$H-NMR spectrum of mesonoside E (5) (500 MHz in methanol-d$_4$).
Fig. S11 $^{13}$C-NMR spectrum of mesonoside E (5) (125 MHz in methanol-d$_4$).

Fig. S12 $^1$H-NMR spectrum of mesonoside F (6) (600 MHz in methanol-d$_4$).
Fig. S13 $^{13}$C-NMR spectrum of mesonoside F (6) (150 MHz in methanol-\textit{d}_4).

Fig. S14 $^1$H-NMR spectrum of mesonoside G (7) (600 MHz in methanol-\textit{d}_4).
Fig. S15 $^{13}$C-NMR spectrum of mesonoside G (7) (150 MHz in methanol-d$_4$).

Fig. S16 $^1$H-NMR spectrum of mesonoside H (8) (500 MHz in methanol-d$_4$).
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