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Eudesmane and Eremophilane Sesquiterpenes from the Fruits of *Alpinia oxyphylla* with Protective Effects against Oxidative Stress in Adipose-Derived Mesenchymal Stem Cells

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Abstract: *Alpinia oxyphylla* Miquel (Zingiberaceae) has been reported to show antioxidant, anti-inflammatory, and neuroprotective effects. In this study, two new eudesmane sesquiterpenes, 7α-hydroperoxy eudesma-3,11-diene-2-one (**1**) and 7β-hydroperoxy eudesma-3,11-diene-2-one (**2**), and a new eremophilane sesquiterpene, 3α-hydroxynootkatone (**3**), were isolated from the MeOH extract of dried fruits of *A. oxyphylla* along with eleven known sesquiterpenes (**4–14**). The structures were elucidated by the analysis of 1D/2D NMR, high-resolution electrospray ionization mass spectrometry (HRESIMS), and optical rotation data. Compounds (**1–3**, **5–14**) were evaluated for their protective effects against *tert*-butyl hydroperoxide (tBHP)-induced oxidative stress in adipose-derived mesenchymal stem cells (ADMSCs). As a result, treatment with isolated compounds, especially compounds **11** and **12**, effectively reverted the damage of tBHP on ADMSCs in a dose-dependent manner. In particular, **11** and **12** at 50 µM improved the viability of tBHP-toxified ADMSCs by 1.69 ± 0.05-fold and 1.61 ± 0.03-fold, respectively.

Keywords: *Alpinia oxyphylla*; eudesmane sesquiterpene; eremophilane sesquiterpene; adipose-derived mesenchymal stem cell; antioxidant; oxidative stress

1. Introduction

Alpinia oxyphylla Miquel (Zingiberaceae) is a flowering plant, and the genus of *Alpinia* comprises 250 species worldwide and is mainly distributed across subtropical regions [1]. It has been used both as a food [2] and a traditional herbal medicine in Korea, China, and Japan [3,4]. The fruit of *A. oxyphylla* was reported to exhibit diverse pharmacological activities, such as anti-inflammatory [5], anti-ulcer [6], anti-allergy [7], and neuroprotective effects [8]. Previous studies have revealed the presence of different classes of chemicals in *A. oxyphylla*, including monoterpenes [9], sesquiterpenes [1,4,10], diterpenoids [11], flavonoids [12], diarylheptanoids [12], and steroids [13]. Among them, sesquiterpenes, such as 12-hydroxynootkatone [14], nootkatone [4], oxyphyllanene C, and oxyphyllanene E [1], are responsible for the inhibition of nitric oxide (NO) production in interferon-γ and lipopolysaccharide-treated RAW264.7 macrophage cells. As part of our ongoing project to investigate the antioxidant constituents of herbal medicines, the chemical exploration of *A. oxyphylla* led to the identification of three novel compounds: two eudesmane sesquiterpenes—7α-hydroperoxy eudesma-3,11-diene-2-one

(**1**) and 7β-hydroperoxy eudesma-3,11-diene-2-one (**2**)—and an eremophilane sesquiterpene, 3α-hydroxynootkatone (**3**). The following known compounds were also identified as 3,4-dehydronootkatone (**4**) [15], nootkatone (**5**) [14], 7-*epi*-teucrenone (**6**) [16], teucrenone (**7**) [17], 11(12)-dien-2,9-dione (**8**) [18], 9β-hydroxynootkatone (**9**) [14], oxyphyllol B (**10**) [10], (4*R*, 5*S*, 7*R*)-13-hydroxynootkatone (**11**) [19], nootkatone-11,12-epoxide (**12**) [20], nootkatone-11,12-diol (**13**) [14], and (11*S*)-12-chloronootkaton-11-ol (**14**) by comparison with reported data [21] (Figure 1). The isolated compounds, except for **4**, due to the limited amount, were evaluated for their protective effects against *tert*-butyl hydroperoxide (tBHP)-induced oxidative stress in adipose-derived mesenchymal stem cells (ADMSCs). As a result, compounds **11** and **12** demonstrated potent cell-protective effects. tBHP is a powerful oxidizing compound that causes oxidative stress in stem cells. It results in reduced endogenous defense molecules, such as superoxide dismutase-SOD and glutathione. As a result, the cellular organelles such as mitochondria are damaged, which leads to cell death. In this study, we aimed to isolate sesquiterpenes from the dried fruits of *A. oxyphylla* and to preliminarily screen their effects of preventing the death of tBHP-toxified MSCs. Based on the results, the potent compounds will be chosen to further evaluate their effects on preserving the activity of intracellular defensive molecules in MSCs of interest in the next studies.
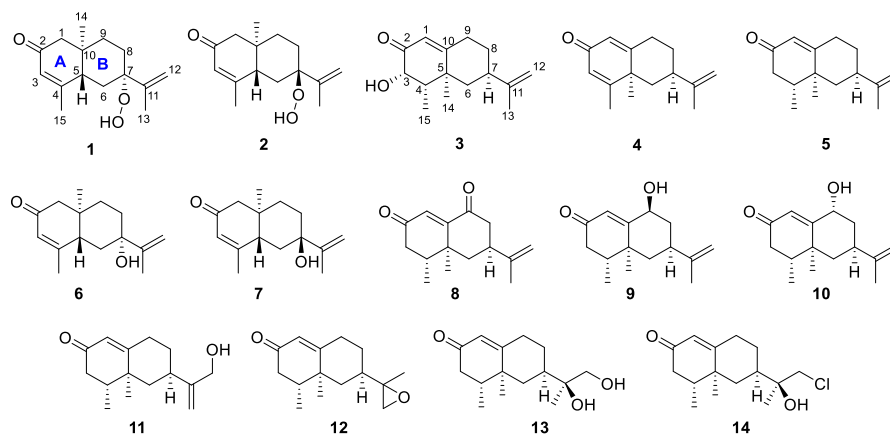

Figure 1. Chemical structures of compounds isolated from *Alpinia oxyphylla*.

2. Results and Discussion

2.1. Structure Elucidation

Compound **1** was isolated as a yellow oil. Its molecular formula, $C_{15}H_{22}O_3$, with five degrees of unsaturation, was determined based on the high-resolution electrospray ionization mass spectrometry (HRESIMS) ion peak at m/z 251.1643 $[M + H]^+$ (calculated for $C_{15}H_{23}O_3^+$, 251.1642). The ion peak at m/z 217.1598 $[M + H - H_2O_2]^+$ [22,23] and neutral loss of 51 Da ($H_2O_2 + NH_3$) from the ammonium adduct, m/z 268.1872 $[M + NH_4]^+$ [24] are characteristic evidence for the presence of the hydroperoxy group. The ^{1}H-NMR spectrum of **1** showed three olefinic protons (δ_H 5.90 (dt, J = 2.9, 1.3 Hz, 1H), 5.37 (t, J = 1.3 Hz, 1H), 5.21 (brs, 1H)), one methine (δ_H 2.37 (m, 1H)), four methylenes (δ_H 2.41 (m, 1H)/1.54 (m, 1H), 2.30 (dd, J = 16.2, 1.3 Hz, 1H)/ 2.14 (dd, J = 16.2, 1.3 Hz, 1H), 2.19 (m, 1H)/1.78 (td, J = 14.1, 4.1 Hz, 1H), 1.53 (m, 1H)/1.42 (td, J = 13.9, 4.1 Hz, 1H), and three methyls (δ_H 0.96 (brs, 3H), 1.93 (t, J = 1.3 Hz, 3H), 1.84 (brs, 3H)). The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra displayed fifteen carbon signals, including one carbonyl group at δ_C 198.9; four quaternary carbons at δ_C 162.0, 141.5, 86.7, and 37.9; four methylenes at δ_C 54.1, 37.2, 28.7, and 26.7; an olefinic methylene at δ_C 118.5; three methyls at δ_C 22.1, 18.9 and 17.0; one methine at δ_C 44.5; and one olefinic methine at δ_C 127.2. The ^{1}H and ^{13}C-NMR spectra of **1** (Tables 1 and 2) were found to be similar to those of the known eudesmane sesquiterpene, 7-*epi*-teucrenone (**6**) [16], except for a substitution of a tertiary hydroperoxy group (δ_C 85.9) [25,26] instead of a tertiary hydroxy

group (δ_C 74.4) [16] at C-7, which was supported by the downfield shifted signal of C-7 (δ_C 86.7). Five degrees of unsaturation indicated a dicyclic ring system possesses one carbonyl group and two double bonds. The planar structure of **1** was established based on a comprehensive analysis of COSY, HSQC, and HMBC correlations, as shown in Figure 2. Key HMBC correlations from H-1 to C-2/C-5, H-3 to C-1/C-5/C-15, H-15 to C-3/C-4/C-5, and H-5 to C-15 were used to elucidate ring A. Ring B was confirmed by HMBC correlations from H-6 to C-5/C-7/C-8/C-10 and H-8 to C-10. The HMBC correlations from H-12 to C-7 and H-13 to C-7/C-11/C-12 suggested an isopropylidene group positioned at C-7. All figures of HRESIMS and 1D, 2D NMR of compound **1** were provided in supplementary material (Figures S1–S8).

Table 1. ^{1}H-NMR spectroscopic data of compounds **1**–**3**.

	1	**2**	**3**
Position	δ_H, mult (*J* in Hz).	δ_H, mult (*J* in Hz)	δ_H, mult (*J* in Hz)
1α	2.30, dd (16.2, 1.3)	2.26, s	5.84, d (1.5)
1β	2.14, dd (16.2, 1.3)		
2			
3	5.90, dt (2.9, 1.3)	5.90, dq (2.9, 1.4)	4.39, dd (5.3, 2.2)
4			2.03, dd (7.1, 5.3)
5	2.37, m	2.83, ddq (13.2, 2.9, 1.4)	
6α	1.54, m	1.55, dd (14.0, 13.2)	1.76, m
6β	2.41, m	2.34, dt (14.0, 2.9)	1.81, t (12.3)
7			2.59, m
8α	1.78, td (14.1, 4.1)	1.71, m	2.07, m
8β	2.19, m	1.91, m	1.32, dd (13.1, 4.5)
9α	1.53, m	1.34, m	2.55, tdd (12.9, 4.5, 1.5)
9β	1.42, td (13.9, 4.1)	1.72, m	2.29, ddd (12.9, 4.5, 2.7)
10			
11			
12a	5.37, t (1.3)	5.08, brs	4.75, t (1.5)
12b	5.21, brs	5.04, t (1.4)	4.72, brs
13	1.84, brs	1.84, m	1.72, brs
14	0.96, brs	0.89, brs	1.28, brs
15	1.93, t (1.3)	1.91, t (1.4)	0.92, d (7.1)
OH			3.26, d (2.2)

The ^{1}H-NMR data were acquired using CDCl$_3$ at 400 MHz.

Table 2. ^{13}C-NMR spectroscopic data of compounds **1**–**3**.

	1	**2**	**3**
Position	δ_C, type	δ_C, type	δ_C, type
1	54.1, CH$_2$	54.0, CH$_2$	119.6, CH
2	198.9, C	199.5, C	199.8, C
3	127.2, CH	127.1, CH	73.4, CH
4	162.0, C	163.7, C	44.0, CH
5	44.5, CH	42.4, CH	41.3, C
6	28.7, CH$_2$	28.5, CH$_2$	43.7, CH$_2$
7	86.7, C	85.1, C	40.1, CH
8	26.7, CH$_2$	27.2, CH$_2$	34.6, CH$_2$
9	37.2, CH$_2$	35.2, CH$_2$	33.1, CH$_2$
10	37.9, C	37.2, C	170.7, C
11	141.5, C	147.8, C	148.3, C
12	118.5, CH$_2$	112.5, CH$_2$	109.9, CH$_2$
13	18.9, CH$_3$	18.9, CH$_3$	21.1, CH$_3$
14	22.1, CH$_3$	22.0, CH$_3$	9.0, CH$_3$
15	17.0, CH$_3$	16.2, CH$_3$	22.7, CH$_3$

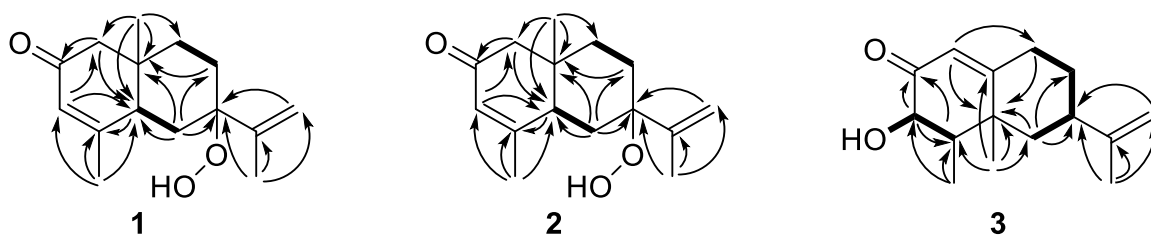The ^{13}C-NMR data were acquired using CDCl$_3$ at 100 MHz.

Figure 2. Key HMBC (H→C) and COSY (━) correlations of **1**–**3**.

The relative configuration of **1** was determined through the NOESY experiment (Figure 3). The NOE correlations between H-6β and H-12b as well as H-6α and H$_3$-14 indicated that both the C-7 hydroperoxide and C-14 methyl groups were α-positioned. In addition, the NOE correlation between H-5 and H-12b suggested a β-oriented proton at C-5. The optical rotation was obtained to determine the absolute configuration of **1**; a weak negative specific rotation, $[\alpha]_D^{25}$: −1.2 (*c* 0.25, CH$_3$OH), was observed, indicating the presence of a racemic mixture. Based on these observations, the structure of **1** was elucidated as 7α-hydroperoxy eudesma-3,11-diene-2-one.
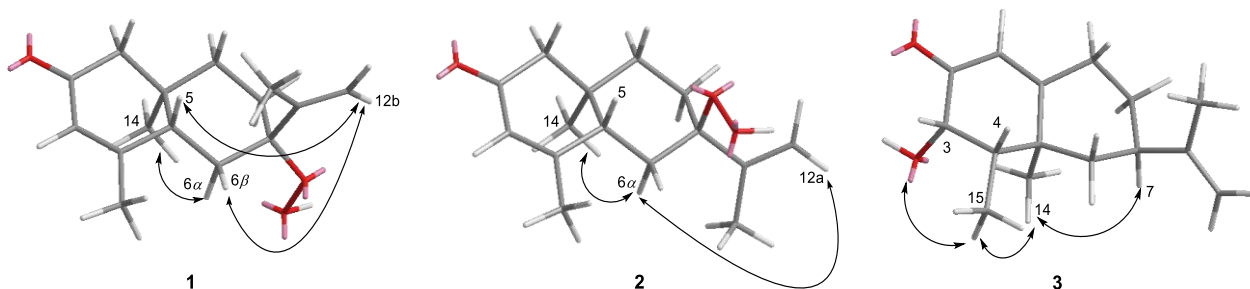

Figure 3. Key NOESY (H↔H) correlations of **1**–**3**.

Compound **2** was isolated as a white powder. Its molecular formula, C$_{15}$H$_{22}$O$_3$, with five degrees of unsaturation, was confirmed based on the HRESIMS ion peak at *m/z* 251.1639 [M + H]$^+$ (calcd for C$_{15}$H$_{23}$O$_3^+$, 251.1642) (Figure S16). The fragmentation pattern showed the same pattern as mentioned in **1** to confirm the presence of a hydroperoxy group. The comparison of the ^{1}H and ^{13}C-NMR spectroscopic data revealed that the planar structure of **2** was identical to that of **1**, which was further supported by the analysis of the 2D NMR spectra, as shown in Figure 2 (Figures S9–S15). The relative configurations of a hydroperoxy group at C-7 in the eudesmane sesquiterpene were determined using ^{13}C-NMR chemical shift differences at C-11 and C-12 [16,17] as well as NOESY spectrum. The resonances for C-11 and C-12 in **2** were downfield and upfield shifted, respectively, both by 6 ppm, compared to those of **1**, suggesting that the relative configurations at C-7 in **2** were different from those in **1**. The H-6α was observed as dd with relatively large coupling constants of 14.0 Hz ($^2J_{\text{H-6α, H-6β}}$) and 13.2 Hz ($^3J_{\text{H-5, H-6α}}$), which indicated H-6α and H-6β are axial and equatorial orientations, respectively. The NOE correlations between H-6α and H-14, as well as between H-6α and H-12a revealed that an isopropylidene group at C-7 of **2** was positioned on the same side with a methyl group (H$_3$-14). Again, to determine the absolute configuration of **2**, the optical rotation was obtained, and a weak positive specific rotation, $[\alpha]_D^{25}$: +3.16 (*c* 0.19, CH$_3$OH), was observed, concluding the presence of a racemic mixture. Based on these observations, the structure of **2** was elucidated as 7β-hydroperoxy eudesma-3,11-diene-2-one, a 7-epimer of **1**.

Compound **3** was isolated as a white powder. Its molecular formula, C$_{15}$H$_{22}$O$_2$, with five degrees of unsaturation, was determined based on the HRESIMS ion peak at *m/z* 235.1692 [M + H]$^+$ (calcd for C$_{15}$H$_{23}$O$_2^+$, 235.1693) (Figure S24). The ^{1}H-NMR spectroscopic data (Table 1) indicated three olefinic protons (δ_H 5.84 (d, *J* = 1.5 Hz, 1H), 4.75 (t, *J* = 1.5 Hz,

1H), 4.72 (brs, 1H)), three methyls (δ_H 1.72 (brs, 3H), 1.28 (brs, 3H), 0.92 (d, J = 7.1 Hz, 3H)), three methylenes (δ_H 1.81 (t, J = 12.3 Hz, 1H)/1.76 (m, 1H), 2.07 (m, 1H)/1.32 (dd, 13.1, 4.4 Hz, 1H), 2.55 (tdd, J = 12.9, 4.5, 1.5 Hz, 1H)/2.29 (tdd, J = 12.9, 4.5, 2.7 Hz, 1H)), and three methines (including oxygenated methine) at δ_H 4.39 (dd, J = 5.3, 2.2 Hz, 1H), 2.59 (m, 1H) and 2.03 (dq, J = 6.1, 5.3 Hz, 1H). The ^{13}C-NMR spectrum showed fifteen carbon signals: three methyls (δ_C 22.7, 21.1, and 9.0), three methylenes (δ_C 43.7, 34.6, and 33.1), one olefinic methylene δ_C 109.9, three methines (δ_C 73.4, 44.0, and 40.1), one olefinic methine (δ_C 119.6), three quaternary carbons (δ_C 170.7, 148.3, and 41.3), and one carbonyl (δ_C 199.8). Based on the ^{1}H and ^{13}C-NMR spectral analyses of **3** (Figures S17 and S18, Table 1), the structure was similar to that of an eremophilane sesquiterpene, nootkatone (**5**), except for the presence of an additional hydroxy group at C-3 in **3** [27]. Analysis of the COSY, HSQC, and HMBC spectroscopic data (Figures S20–S22) revealed the connectivity of the structure. The key HMBC correlations were as follows: from H-1 to C-5/C-9, H-3 to C-2/C-4, H-15 to C-3/C-4, H-4 to C-2, H-14 to C-4/C-5/C-6/C-10, H-6 to C-5/C-7/C-8, and H-9 to C-5, confirming the core planar structure of **3**. An isopropylidene group at C-7 was confirmed based on the HMBC correlations from H-12 to C-7 and H-13 to C-7/C-11/C-12. The relative configuration of **3** was assigned by NOE correlations, as shown in Figure 3 (Figure S23). The NOE correlations between H-7/H$_3$-14, H$_3$-14/H$_3$-15, and H$_3$-15/OH-3 suggested the α-orientation of the H-7, H$_3$-14, H$_3$-15, and β-orientation of H-3, which is further supported by the absence of NOE correlation between OH-3 and H-4. The absolute configuration of **3** was determined as 3*S*, 4*S*, 5*S*, 7*R* by comparing the optical rotation value, $[\alpha]_D^{25}$: +6.18 (*c* 0.15, CH$_3$OH), with that of (4*R*,5*S*,7*R*)-nootkatone (**5**) [27]. Accordingly, the structure of **3** was elucidated as (3*S*,4*S*,5*S*,7*R*)-3α-hydroxynootkatone.

2.2. Biological Activities of Isolated Compounds on ADMSCs

In this study, we tested the protective effects of compounds **1–3** and **5–14** on oxidative stress-induced ADMSCs. The activity of compound **4** could not be evaluated due to amount limitations. *Tert*-butyl hydroperoxide (tBHP) was used as an inducer of ADMSC death by generating excessive intracellular reactive oxygen species and reducing the activity of endogenous defensive molecules, such as superoxide dismutase-SOD and glutathione [28]. It was found that tBHP triggered ADMSC death dose-dependently (Figure 4). Particularly, the relative viability of ADMSCs after exposure to 0, 50, 75, 100, and 150 μM tBHP was 100.0 ± 3.3%, 103.3 ± 6.6%, 88.7 ± 6.4%, 53.8 ± 8.6% ($p < 0.0001$), and 25.1 ± 1.3% ($p < 0.0001$), respectively. To investigate the effects of the isolated compounds, 100 μM tBHP was chosen to induce oxidative stress in ADMSCs. Figure 5 shows the relative cell viability of the control (only tBHP treatment) and treated groups (co-treatment with tBHP and compounds). Interestingly, compounds **8** to **13** effectively prevented oxidative stress-induced ADMSC death, as indicated by a minimum 1.4-fold improvement in cell viability at 50 μM. Among these, compounds **11** and **12** exhibited the highest protective effects, which were improved by 1.69 ± 0.05-fold ($p < 0.0001$) and 1.61 ± 0.03-fold ($p < 0.0001$), respectively, at 50 μM. Meanwhile, compounds **3** and **6** only slightly inhibited the toxic effects of tBHP on ADMSCs, with 1.21 ± 0.14-fold and 1.20 ± 0.12-fold higher cell viability at 50 μM, respectively. We found that all the remaining compounds were ineffective in recovering ADMSCs from chemically induced oxidative stress at all tested concentrations. Further analysis would be necessary to investigate the relationship between structure and its effect.
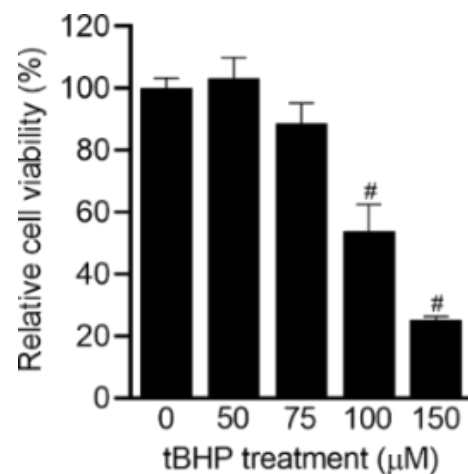
Figure 4. Effect of *tert*-butyl hydroperoxide (tBHP) on the viability of ADMSCs, evaluated by a CCK-8 assay (*n* = 5). The cell viability test was performed 24 h after treatment. # $p < 0.0001$.
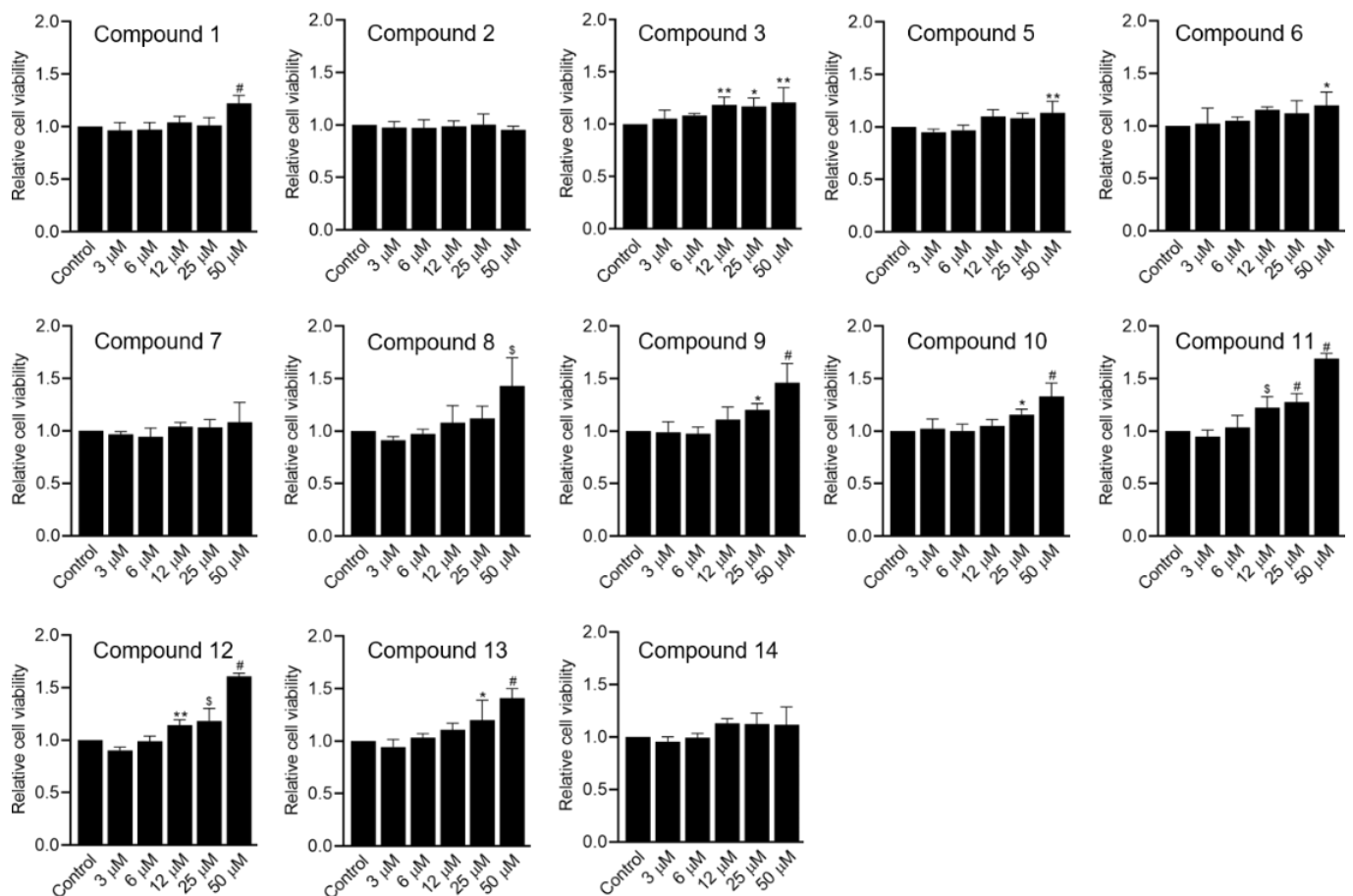

Figure 5. Effect of the isolated compounds on the viability of tBHP-damaged ADMSCs, evaluated by a CCK-8 assay (*n* = 5). ADMSCs were treated with a medium containing 100 μM tBHP and the isolated compounds at various concentrations. Cells only treated with 100 μM tBHP were used as controls. The cell viability test was performed at 24 h after treatment. The data represent two independent experiments. * $p < 0.05$, ** $p < 0.01$, \$ $p < 0.001$, # $p < 0.0001$.

A. oxyphylla is commonly used for its antioxidant, anti-inflammatory, and neuroprotective effects, but the effects of its active compounds remain to be explored [29–32]. In the present study, we successfully proved the protective effects of 13 isolated sesquiterpenes on the viability of ADMSCs exposed to tBHP-induced oxidative stress. In neurodegenerative

diseases, neuronal stem cells experience a high level of oxidative stress, which impairs their regenerative capacity [33]. Although we could not isolate neuronal stem cells for the test, our results provide clear evidence for the prevention of oxidative stress in ADMSCs using several potent isolated sesquiterpenes. Future studies would be required to investigate the impact of these compounds in in vivo neurodegenerative disease models.

3. Materials and Methods

3.1. General Procedures

Optical rotation was measured using a JASCO P-2000 polarimeter (Tokyo, Japan). The NMR spectra were acquired on a 400 MHz Agilent NMR spectrometer (DD2, Santa Clara, CA, USA) using $CDCl_3$. The HRESIMS was performed on an Agilent 6220 Accurate-Mass TOF LC/MS system. Silica gel (230–400 mesh, Merck KGaA, 64271 Darmstadt, Germany) and RP-18 (YMC gel ODS-A, 12 nm, S-150 μm, YMC Co., Ltd., Kyoto 600-8106, Japan). Thin Layer Chromatography (TLC) analysis was performed on silica gel 60 F254 (0.2 mm thickness, Merck KGaA, 64271 Darmstadt, Germany) and RP-18 F 254$_S$ (Merck KGaA, 64271 Darmstadt, Germany) plates by visualization under UV light at 254 nm, 365 nm, and 10% (*v/v*) of sulfuric acid followed by heating. Preparative HPLC was performed using an Acme 9000 system (Young Lin, Anyang, Korea) equipped with a YMC-Pack Pro C18 column (5 μm, 250 × 20 mm i.d.).

3.2. Plant Materials

The dried fruits of *A. oxyphylla* were purchased from the Insan Oriental Herbal Market in Seoul, Korea. A voucher specimen (No. EA323) was deposited at the Natural Product Chemistry Laboratory at Ewha Womans University, Seoul, Korea.

3.3. Extraction and Isolation

The dried fruit of *A. oxyphylla* (3 kg) was extracted with 100% MeOH three times for 48h at room temperature. The resultant extract after removing solvent was dissolved in H_2O then fractionated with hexanes, methylene chloride (MC), EtOAc, and *n*-BuOH, sequentially. The hexane-soluble fraction (94 g) was subjected to a silica gel column using hexanes:EtOAc (99:1 to 50:50) as a step of the gradient solvent system to yield 19 subfractions (Fr. A1–A19). Subfraction Fr. A8 (13.9 g) was chromatographed over a silica gel column and eluted with hexanes:acetone (99:1 to 98:2) to afford 17 fractions (Fr. B1–B17). Reversed-phase (RP) C_{18} column chromatography (CC) was carried out with Fr. B7 (5.3 g) using MeOH:H_2O (2:1 to 3:1) to yield 15 subfractions (Fr. C1–C15). Subfraction Fr. C9 (2.97 g) was chromatographed over a silica gel column eluted with hexanes:EtOAc (99:1 to 0:100) to obtain 25 subfractions (Fr. D1–D25). The combined subfraction Fr. D7–D9 (1.40 g) was fractionated over a silica gel column by elution with hexanes:EtOAc (98:2 to 90:10) to yield compound **5** (1.45 mg). Fr. D13–D15 (125.63 mg), eluted with hexanes:EtOAc (99:1 to 60:30), was chromatographed using a silica gel column to obtain 13 fractions (Fr. E1–E13). Fr. E6 (70.20 mg) was subjected to RP-C18 CC using MeOH:H_2O (2:1) to give thirteen subfractions (Fr. F1-F13), and compound **3** (1.46 mg) was obtained from Fr. F7. Subfraction Fr. F8 (30.60 mg) was further fractionated using RP-C18 CC and eluted with MeOH:H_2O (1:1 to 2:1) to isolate compound **8** (16.14 mg). The combined subfraction Fr. D16–D17 (110.20 mg) was subjected to a silica gel column by elution with hexanes:EtOAc (99:1 to 80:20) to afford 12 subfractions (Fr. G1–G12). The combined subfraction Fr. G8–G9 (22 mg) was chromatographed over an RP-C18 column and eluted with MeOH:H_2O (1:1 to 2:1) to afford 10 fractions (Fr. H1–H10). Compound **13** (2.56 mg) was isolated after RP-C18 chromatography of the combined subfraction Fr. H4–H5 (10.20 mg) by elution with MeOH:H_2O (1:1 to 2:1) to afford 8 fractions Fr. I1–I8. Subfraction Fr. I4 (1.29 mg) was purified using RP-C18 HPLC with MeOH:H_2O (9:7) as the mobile phase to obtain compound **12** (1.25 mg). The combined subfraction Fr. G10–G11 (16.77 mg) was chromatographed over an RP-C18 open column using MeOH:H_2O (1:1 to 2:1) to yield compounds **4** (0.94 mg) and **14** (0.80 mg). The combined subfraction D19–D20

(2.97 g) was chromatographed over an RP-C18 column by elution with MeOH:H_2O (4:1 to 1:0) to obtain 11 subfractions (Fr. L1–L11). Along with these eleven fractions (Fr. L1–L11), **2** (19.04 mg) and **9** (20.22 mg) were purified using an RP-C18 column with MeOH:H_2O (2:1 to 1:0) from the combined subfraction Fr. L3–L4 (1.54 g). Compound **1** (5.00 mg) was separated from subfraction Fr. M4 with RP-C18 HPLC using MeOH:H_2O (2:1, 0.1% acetic acid in MeOH) as the mobile phase. Subfraction Fr. L5 (1.37 g) was fractionated with RP-C18 CC with MeOH:H_2O (3:1) as the eluent to afford **10** (80.15 mg). Subfraction Fr. D21 (109.42 mg) was chromatographed over an RP-C18 column and eluted with MeOH:H_2O (1:1 to 1:0) to afford 19 fractions (Fr. K1–K19), including compound **11** (12.74 mg). Subfraction Fr. K7 (13.02 mg) was subjected to RP-C18 CC and eluted with MeOH:H_2O (2:1 to 1:0) to isolate compound **6** (7.14 mg). We separated compound **7** (0.90 mg) using RP-C18 HPLC with MeOH:H_2O (3:1) as a solvent system from subfraction Fr. K13.

(5S,7S*,10R*)-7α-Hydroperoxy eudesma-3,11-diene-2-one (**1**):* yellow oil; $[\alpha]_D^{25}$: −1.2 (*c* 0.25, CH_3OH); ^1H and ^{13}C-NMR, see Tables 1 and 2; HRESIMS $[M + H]^+$ *m/z* 251.1643 ($[M + H]^+$ calcd for $C_{15}H_{23}O_3^+$, 251.1642).

(5S,7R*,10R*)-7β-Hydroperoxy eudesma-3,11-diene-2-one (**2**):* white powder; $[\alpha]_D^{25}$: +3.16 (*c* 0.19, CH_3OH); ^1H and ^{13}C-NMR, see Tables 1 and 2; HRESIMS $[M + H]^+$ *m/z* 251.1639 ($[M + H]^+$ calcd for $C_{15}H_{23}O_3^+$, 251.1642)

*(3S,4S,5S,7R)-3α-Hydroxynootkatone (**3**):* white powder; $[\alpha]_D^{25}$: +6.18 (*c* 0.15, CH_3OH); ^1H and ^{13}C-NMR, see Tables 1 and 2; HRESIMS $[M + H]^+$ *m/z* 235.1692 (calcd for $C_{15}H_{23}O_2^+$, 235.1693).

3.4. Culture of Mouse ADMSCs

Experiments on animals were performed according to the national guidelines and with the approval of the Institutional Ethical Committee, Yeungnam University, Republic of Korea. ADMSCs were obtained from C57BL/6 mice (male, 8-week age; Samtako, Republic of Korea). The cells were carefully characterized using specific surface markers and differentiation capacities, according to previous studies [34]. ADMSCs were cultured in MEM-α supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) and 1% penicillin/streptomycin 100X (Hyclone Laboratories, South Logan, UT, USA). The cells were used within passages 3–5.

3.5. Effect of Isolated Compounds on the Viability of tBHP-Damaged ADMSCs

The stock solution of each compound was prepared in advance by dissolving it in cell culture-grade DMSO (Sigma-Aldrich). To investigate the effect of the compounds, ADMSCs were chemically induced with tBHP (Tokyo Chemical Industry, Tokyo, Japan), and cell viability was measured using a cell counting kit 8 (CCK-8; Dojindo, Molecular Technologies Inc., Rockville, MD, USA). Briefly, ADMSCs were trypsinized and cultured in 96-well plates at a density of 5000 cells/well. The following day, the cells were incubated in a medium containing 100 μM tBHP and the compounds at various concentrations (3–50 μM) for 24 h in a CO_2 incubator at 37 °C. The cells were then washed twice using phosphate-buffered saline (pH = 7.4; Hyclone Laboratories) and incubated in a medium (120 μl/well) containing 5% CCK-8 reagent for 2 h at 37 °C. The absorbance of the supernatant in a 96-well plate was measured at 450 nm using a plate spectrophotometer (Spark 10M; Tecan, Männedorf, Switzerland). Relative cell viability was calculated by comparing the absorbance of the control and treated cells. The data were analyzed using the GraphPad Prism software version 8.4.2.

4. Conclusions

In this study, three new and eleven known sesquiterpenes were isolated from the dried fruits of *Alpinia oxyphylla*. Their structures were elucidated and identified by NMR spectroscopic methods. In the screening for biological activities on adipose-derived mesenchymal stem cells (ADMSCs) of the isolates **1–3**, **5–14,** compounds **8** to **13** effectively prevented oxidative stress-induced ADMSC death. Among these, compounds **11** and **12** showed the

highest protective effect. These results supported that *A. oxyphylla* fruit-derived sesquiter-penoids and active compounds enriched extract can be applied to regenerative medicine therapies by prevention of oxidative stress in ADMSCs, which can be differentiated into neurons, osteoblasts, pancreatic cells, etc. Further examination using in vivo degenerative disease models is required in future studies.

Supplementary Materials: The following are available online.

Author Contributions: P.T. and Y.J.L. (Yoo Jin Lee) contributed equally to this work. J.-H.J., J.-W.N., H.C. and E.K.S. conceived and designed the experiments; T.T.N., P.T. and Y.J.L. (Yoo Jin Lee) performed the experiments and analyzed the data; D.P., H.L., S.H. and Y.J.L. (Yeon Jin Lee) validated the data; A.-R.H. and H.C. contributed reagents/materials/analysis tools; T.T.N., P.T., H.C., J.-H.J., J.-W.N. and E.K.S. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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Sample Availability: Not applicable.

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