Anti-inflammatory sesquiterpene and triterpene acids from *Mesona procumbens* Hemsley

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1 Introduction

*Mesona procumbens* Hemsley (Hsian-tsao), an annual herb belonging to the Lamiaceae family, is distributed in the tropical and subtropical regions of South Asia, such as Taiwan, Indonesia, Thailand, Vietnam, and southern China (Feng et al., 2012). This herb is conventionally used alone as a heat-clearing (Qingre) and detoxifying (Jiedu) agent or filled in a prescription of traditional Chinese medicine typically for the treatment of heat-shock, hypertension, diabetes, hepatic disease, and various inflammations, such as joint and muscle pains (Yen and Hung, 2000; Hung and Yen, 2002). Hsian-tsao (also
known as grass jelly herb) is preferably consumed as herbal tea, herbal jelly dessert (grass jelly), or dessert soup given its unique smell, refreshing taste, and medicinal benefits from the major component, polysaccharide gum, which is the most attractive target in this edible plant (Lai and Liao, 2002; Lai et al., 2003; Zhuang et al., 2010). Recent studies further revealed that the Hsian-tsao aqueous extract can stop disease progression of liver fibrosis through its apoptotic effects on the activation of hepatic stellate cells (Yeh et al., 2019). The crude polysaccharides from Hsian-tsao demonstrated a wound healing activity in the streptozotocin-induced diabetic mouse model (Fan et al., 2021). Numerous pharmacological properties, such as anti-inflammatory (Huang et al., 2012), antioxidant (Lin et al., 2018), antihypertensive (Yeh et al., 2009), antimutagenic (Yen et al., 2001), DNA damage protection (Yen et al., 2000), liver fibrosis prevention (Shyu et al., 2008), and renal protective activities, from M. procumbens extracts have been independently reported from time to time (Yang et al., 2008). In addition, some such chronic inflammations such as cardiovascular, cancer, diabetes, arthritis, pulmonary, and autoimmune diseases reported (Singh et al., 2019) can be improved by natural products for they possess some yet-unknown chemo-preventive activities (Azab et al., 2016; Huang et al., 2021).

This study aimed at investigating untargeted anti-inflammatory ingredients from Hsian-tsao rather than the known polysaccharide gum. Seven new chemical entities were identified herein, including five new triterpene acids (two 24-nor-oleanane-type triterpenes 1–2, one 24-nor-ursane-type triterpe 3, one ursane-type triterpenec acid 4, and one ursane-type seco-triterpene acid 5), one known 2a,3a,19α-trihydroxy-24-norurs-4(23),12-dien-28-oic acid (6), and one new proximadiol (cryptomeridiol)-type sesquiterpene 7 isolated from the methanolic extract of M. procumbens. Having identified their chemical structures, these compounds were subjected to biochemical assay for evaluating their anti-inflammatory capacities against the lipopolysaccharide-induced NO production in RAW264.7 macrophage cells.

2 Materials and methods

2.1 General experimental procedures

Optical rotations were determined by a JASCO P-2000 polarimeter at 25°C. Infrared (IR) spectra were recorded on a Thermo Scientific Nicolet iS5 FTIR spectrometer. The ECD spectra were measured by a JASCO J-715 spectropolarimeter. High-resolution electrospray ionization mass spectrometry (HRESIMS) data were established by a Thermo Scientific Ultimate 3000 UHPLC System with a Thermo Scientific Q Exactive™ Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer. NMR (nuclear magnetic resonance) spectra, including 1H, 13C, DEPT, 1H–1H COSY, HMBC, HSQC, and NOESY, were recorded on Varian Unity Inova 500 MHz (5 mm SWPFG/TRPFG probe) or Varian VNMRS 600 MHz spectrometers (cold probe) and the chemical shifts were referenced by deuterated solvent methanol-d4. Silica gel 60 (Merck, 70–230 and 230–400 meshes), C18 gel (Chromatorex, 40–75 mesh), Diaion HP-20 (Mitsubishi Chemical Co.), and Sephadex LH-20 (GE) were used for column chromatography. Preparative HPLC (high-performance liquid chromatography) was performed using a Shimadzu LC-8A pump and an SPD-10A VP UV detector (210 and 254 nm wavelengths) with a Cosmosil 5C18 AR-II column (250 × 20 mm, Nacalai Tesque). TLC (thin-layer chromatography) analyses were conducted on pre-coated silica gel plates (Merck, Kieselgel 60 F254, 1 mm) and sprayed with anisaldehyde–sulfuric acid reagent and then heated at 100°C.

2.2 Plant material

The whole plants of the air-dried M. procumbens Hemsley (8.0 kg) were purchased from Starsci Biotech company in September 2019. A voucher specimen (No. NRICM-20190909) was deposited in the Herbarium of Division of Chinese Materia Medica Development, NRICM, Taipei, Taiwan.

2.3 Extraction, isolation, and purification

The air-dried plant of M. procumbens Hemsley (8.0 kg) was shredded into 5 mm and extracted with methanol (80 L) at 50°C thrice, and the combined extract was concentrated under reduced pressure removing methanol to obtain the methanolic crude extract. The crude extract (ca. 796.5 g) was dissolved in ddH2O (8.0 kg) and then the aqueous solution was further sequentially partitioned with n-hexane and dichloromethane (CH2Cl2) to obtain the hexane, CH2Cl2, and aqueous extracts. The CH2Cl2 extract (ca. 47.6 g) was fractionated by a C18 gel flash column (250 × 25 cm) eluting with 45% ACN to afford seven subfractions (Fr. I–VII). Fr. IV was separated by preparative HPLC on a Cosmosil 5C18 AR-II column (250 × 20 mm, flow rate: 10.0 ml/min) with 60% acetonitrile (ACN) in H2O to afford seven subfractions (Fr. IVF-1–7). Fr. IVF-4 was further purified by preparative HPLC on Cosmosil 5C18 AR-II column (250 × 20 mm, flow rate: 10.0 ml/min) with 55% ACN to afford eight subfractions (Fr.
IVE-1–8). Fr. IVE-3 was further purified again with 45% ACN (flow rate: 10.0 ml/min) to yield 3 (1.9 mg, Rf: 18.3 min). Fr. IVD was subjected to preparative HPLC with 45% ACN (flow rate: 10.0 ml/min) to yield six subfractions (Fr. IVD-1~6). Fr. IVD-4 was further repeatedly purified with 40% ACN (flow rate: 10.0 ml/min) to afford compounds 4 (2.3 mg, Rf: 17.9 min) and 7 (4.8 mg, Rf: 26.2 min). Fr. IVC was subjected to preparative HPLC with 45% ACN (flow rate: 10.0 ml/min) to divide into eight subfractions (Fr. IVC-1~5). Fr. IVC-2 was separated by a Cosmosil 5C18 AR-II column (flow rate: 10.0 ml/min) to afford 1 (1.6 mg, Rf: 31.2 min). Fr. IVC-4 was also purified by HPLC using the same RP column with 40% ACN (flow rate: 10.0 ml/min) to afford 6 (2.5 mg, Rf: 49.9 min).

### 2.4 Spectroscopic data

#### 2.4.1 Mesonaic acid D (1)

White amorphous powder; [α] +12.9 (c 0.5, MeOH); IR (KBr) νmax 3402, 2927, 1694, 1437, 1316, 1017 cm⁻¹; ¹H- (600 MHz) and ¹³C- (150 MHz) NMR spectroscopic data (methanol-d₄) are shown in Tables 1, 2, respectively.

### Table 1 ¹H-NMR spectroscopic data of 1–6 in methanol-d₄.

| No | ¹H-NMR data were recorded on 600 MHz. | ¹H-NMR data were recorded on 500 MHz. |
|---|---|---|
| No | 1° | 2° | 3° | 4° | 5° | 6° |
| 1 | 1.35 m | 1.23 dd (12.0, 12.6) | 2.14 brd (16.2) | 1.34 m | 2.39 d (18.0) | 1.38 m |
| 1.70 dd (4.8, 12.0) | 1.67 dd (6.6, 12.6) | 2.55 d (16.2) | 1.53 m | 2.58 d (18.0) | 1.71 m |
| 2 | 3.65 ddd (3.6, 4.8, 12.0) | 3.68 ddd (3.6, 4.8, 11.4) | - | 3.89 ddd (3.0, 4.8, 12.0) | - | 3.67 ddd (3.6, 4.8, 12.0) |
| 3 | 4.12 d (3.6) | 4.11 d (3.6) | - | 3.73 brd (3.0) | - | 4.13 d (3.6) |
| 5 | 2.17 dd (4.2, 9.6) | 2.22 br d (13.2) | 2.41 brd (12.0) | 1.91 m | 2.53 dd (3.0, 12.5) | 2.18 m |
| 6 | 1.46 m (2H) | 1.48 m | 1.47 m | 1.43 m (2H) | 1.53 m | 1.46 m (2H) |
| | | | | 1.62 m | 1.88 m | 1.35 m |
| 7 | 1.34 m | 1.32 m | 1.43 m | 1.23 m | 1.35 m | 1.34 m |
| 1.58 m | 1.98 td (3.0, 13.2) | 1.71 td (4.2, 12.6) | 1.74 m | 1.66 m | 1.63 m |
| 9 | 1.82 m | 2.05 m | 1.94 m | 2.76 dd (6.5, 11.5) | 1.93 m |
| 11 | 1.94 m | 2.03 m (2H) | 1.96 m | 1.99 m | 1.98 m |
| 2.02 m | 1.55 m | 2.02 m | 2.08 m | 2.14 m |
| 12 | 5.28 t (3.6) | 4.00 brd (2.4, 5.4) | 5.33 t (3.6) | 5.28 t (3.6) | 5.31 t (4.0) | 5.30 t (3.0) |
| 15 | 1.10 m | 5.02 d (5.4) | 1.06 m | 0.99 m | 1.10 m | 1.01 brd (13.8) |
| 1.80 m | 1.81 m | 1.79 td (5.4, 13.8) | 1.89 m | 1.83 td (4.8, 13.8) | |
| 16 | 1.61 m | 1.83 m | 1.50 m | 1.90 m | 1.52 m |
| 2.02 m | 2.13 d (12.6) | 2.56 td (4.2, 12.6) | 2.57 td (4.8, 13.2) | 2.28 m | 2.58 td (4.8, 13.2) |
| 18 | 2.88 dd (4.8, 14.4) | 1.88 dd (4.2, 12.6) | 2.69 brs | 2.49 brs | 2.49 brs | 2.50 brs |
| 19 | 1.08 m | 1.18 m | - | - | - | |
| 1.80 m | 1.82 dd (4.8, 13.8) | - | - | - | - |
| 20 | - | - | 1.57 m | 1.33 m | 1.50 m | 1.34 m |
| 21 | 1.15 m | 1.09 td (4.2, 13.2) | 3.90 dd (3.0, 6.0) | 1.22 m | 1.45 dd (4.5, 12.5) | 1.22 m |
| 1.48 m | 1.30 m | 1.71 m | 1.85 dd (12.0, 12.5) | 1.71 m |
| 22 | 1.57 m | 1.17 m | 1.81 m | 1.60 td (4.8, 13.8) | 3.71 dd (4.5, 12.0) | 1.61 m |
| 1.67 m | 2.09 td (4.2, 15.0) | 2.10 td (3.0, 14.4) | 1.71 m | 1.72 m |
| 23 | 4.67 brs | 4.99 brs | 1.84 d (1.8) | 1.19 s | 1.29 s | 4.68 brs |
| 5.00 brs | 4.85 brs | - | - | - | 5.01 brs |
| 24 | - | - | - | - | 1.28 s | - |
| 25 | 0.76 s | 0.66 s | 0.93 s | 1.03 s | 1.08 s | 0.78 s |
| 26 | 0.86 s | 1.17 s | 0.88 s | 0.79 s | 0.85 s | 0.84 s |
| 27 | 1.21 s | 0.74 d (7.2) | 1.36 s | 1.38 s | 1.40 s | 1.36 s |
| 28 | 1.19 m | - | - | - | - | - |
| 29 | 0.92 s | 0.94 s | 1.16 s | 1.18 s | 1.17 s | 1.19 s |
| 30 | 3.18 brs (2H) | 0.98 s | 1.16 d (6.6) | 0.92 d (6.6) | 0.98 d (6.5) | 0.92 d (6.6) |

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2.4.2 Mesonaic acid E (2)

White amorphous powder; [α] +19.7 (c 0.5, MeOH); IR (KBr) \( \nu_{\text{max}} \) 3399, 2930, 1744, 1390, 1245, 1012 cm\(^{-1}\); \(^1\)H- \( (600 MHz) \) and \(^{13}\)C- \( (150 MHz) \) NMR spectroscopic data (methanol-\( d_4 \)) are shown in Tables 1, 2, respectively; HRESIMS \( m/z \) 469.2965 [M – H]\(^-\) (calcd. for C\(_{29}\)H\(_{41}\)O\(_5\), 469.2949).

2.4.3 Mesonaic acid F (3)

White amorphous powder; [α] +25.1 (c 0.5, MeOH); IR (KBr) \( \nu_{\text{max}} \) 3369, 2936, 1712, 1630, 1385, 1170, 1017 cm\(^{-1}\); \(^1\)H- \( (600 MHz) \) and \(^{13}\)C- \( (150 MHz) \) NMR spectroscopic data (methanol-\( d_4 \)) are shown in Tables 1, 2, respectively; HRESIMS \( m/z \) 485.2919 [M – H]\(^-\) (calcd. for C\(_{29}\)H\(_{41}\)O\(_6\), 485.2898).

2.4.4 Mesonaic acid G (4)

White amorphous powder; [α] +23.4 (c 0.5, MeOH); IR (KBr) \( \nu_{\text{max}} \) 3402, 2927, 1687, 1235, 1022 cm\(^{-1}\); \(^1\)H- \( (500 MHz) \) and \(^{13}\)C- \( (125 MHz) \) NMR spectroscopic data (methanol-\( d_4 \)) are shown in Tables 1, 2, respectively; HRESIMS \( m/z \) 517.3176 [M – H]\(^-\) (calcd. for C\(_{30}\)H\(_{45}\)O\(_7\), 517.3160).

2.4.5 Mesonaic acid H (5)

White amorphous powder; [α] +27.3 (c 0.5, MeOH); IR (KBr) \( \nu_{\text{max}} \) 3414, 2952, 1707, 1462, 1163, 1027 cm\(^{-1}\); \(^1\)H- \( (500 MHz) \) and \(^{13}\)C- \( (125 MHz) \) NMR spectroscopic data (methanol-\( d_4 \)) are shown in Tables 1, 2, respectively; HRESIMS \( m/z \) 533.3124 [M – H]\(^-\) (calcd. for C\(_{30}\)H\(_{45}\)O\(_8\), 533.3109).

### TABLE 2 \(^{13}\)C-NMR spectroscopic data of 1–6 in methanol-\( d_4 \).

| No | 1\(^a\) | 2\(^a\) | 3\(^a\) | 4\(^a\) | 5\(^b\) | 6\(^a\) |
|----|-------|-------|-------|-------|-------|-------|
| 1  | 42.0  | CH\(_2\) | 42.1  | CH\(_2\) | 51.8  | CH\(_2\) |
| 2  | 68.7  | CH     | 68.8  | CH     | 194.2 | qC   |
| 3  | 75.6  | CH     | 75.4  | CH     | 143.9 | qC   |
| 4  | 151.0 | qC     | 150.5 | qC     | 131.6 | qC   |
| 5  | 44.4  | CH     | 45.1  | CH     | 48.5  | CH   |
| 6  | 20.0  | CH\(_2\)| 19.9  | CH\(_2\)| 20.5  | CH\(_2\)|
| 7  | 31.1  | CH\(_2\)| 37.0  | CH\(_2\)| 32.0  | CH\(_2\)|
| 8  | 39.3  | qC     | 39.5  | qC     | 39.1  | qC   |
| 9  | 45.0  | CH     | 43.2  | CH     | 43.4  | CH   |
| 10 | 37.3  | qC     | 36.9  | qC     | 40.9  | qC   |
| 11 | 23.9  | CH\(_2\)| 28.9  | CH\(_2\)| 23.4  | CH\(_2\)|
| 12 | 122.4 | CH     | 73.0  | CH     | 128.0 | CH   |
| 13 | 144.1 | qC     | 33.8  | qC     | 138.2 | qC   |
| 14 | 41.8  | qC     | 36.1  | qC     | 41.6  | qC   |
| 15 | 27.3  | CH\(_2\)| 78.0  | CH\(_2\)| 28.4  | CH\(_2\)|
| 16 | 22.6  | CH\(_2\)| 33.0  | CH\(_2\)| 27.6  | CH\(_2\)|
| 17 | 46.5  | qC     | 45.2  | qC     | 47.2  | qC   |
| 18 | 40.7  | CH     | 39.2  | CH     | 54.1  | CH   |
| 19 | 39.9  | CH\(_2\)| 41.0  | CH\(_2\)| 75.2  | CH\(_2\)|
| 20 | 35.4  | qC     | 29.5  | qC     | 41.8  | CH   |
| 21 | 27.9  | CH\(_2\)| 34.2  | CH\(_2\)| 73.1  | CH\(_2\)|
| 22 | 31.7  | CH\(_2\)| 27.7  | CH\(_2\)| 43.4  | CH\(_2\)|
| 23 | 109.4 | CH\(_2\)| 109.0 | CH\(_2\)| 11.9  | CH   |
| 24 |      |        |       |        |       | 178.5 | qC   |
| 25 | 13.0  | CH\(_2\)| 13.5  | CH\(_2\)| 13.0  | CH\(_2\)|
| 26 | 16.4  | CH\(_2\)| 20.0  | CH\(_2\)| 16.3  | CH\(_2\)|
| 27 | 25.1  | CH\(_2\)| 15.7  | CH\(_2\)| 22.5  | CH\(_2\)|
| 28 | 180.3 | qC     | 182.3 | qC     | 180.1 | qC   |
| 29 | 18.1  | CH\(_2\)| 32.0  | CH\(_2\)| 25.1  | CH\(_2\)|
| 30 | 73.0  | CH\(_2\)| 22.7  | CH\(_2\)| 12.5  | CH   |

\(^{13}\)C- and DEPT NMR data were recorded on 150 MHz.

\(^{13}\)C- and DEPT NMR data were recorded on 125 MHz.
2.4.6 2α,3α,19α-Trihydroxy-24-norursa-4(23),12-dien-28-oic acid (6)

White amorphous powder; [α] +21.8 (c 0.5, MeOH); IR (KBr) \( \nu_{\text{max}} \) 3470, 2947, 1697, 1459, 1247, 1039 cm\(^{-1}\); \(^1\)H- (600 MHz) and \(^{13}\)C- (150 MHz) NMR spectroscopic data (methanol-\(d_4\)) are shown in Tables 1, 2, respectively; HRESIMS \( m/z \) 495.3072 [M + Na]\(^+\) (calcd. for C\(_{29}\)H\(_{44}\)O\(_5\)Na, 495.3081).

2.4.7 Mesoeudesmol B (7)

Colorless oil; [α] +9.1 (c 0.5, MeOH); IR (KBr) \( \nu_{\text{max}} \) 3389, 2932, 1716, 1455, 1388, 1279, 1116, 1025 cm\(^{-1}\); UV \( \lambda_{\text{max}} \) (MeOH) (log \( \varepsilon \)) 273 (2.98), 229 (3.82) nm; \(^1\)H- (600 MHz) and \(^{13}\)C- (150 MHz) NMR spectroscopic data (methanol-\(d_4\)) are shown in Table 3; HRESIMS \( m/z \) 399.2148 [M + Na]\(^+\) (calcd. for C\(_{22}\)H\(_{32}\)O\(_5\)Na, 399.2142).

2.5 Dimolybdenum tetraacetate [Mo\(_2\)(OAc)\(_4\)]-modified circular dichroism analysis

The determination of the absolute configuration of cyclic and acyclic vic-diols was achieved by employing a transition metal chelate reagent, dimolybdenum tetraacetate [Mo\(_2\)(OAc)\(_4\)]. Compound 7 was directly dissolved in a solution of Mo\(_2\)(OAc)\(_4\) complex in DMSO in a molar ratio of Mo\(_2\)(OAc)\(_4\)/compound of about 1:0.3–1:0.7, and the mixture was subsequently measured for the induced CD spectra without the preparation and isolation of the complexes.

2.6 Cell culture and viability assay

The RAW264.7 mouse macrophages were purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cells were cultured in DMEM supplemented with 10% heat-inactivated FBS in a 5% CO\(_2\) humidified incubator at 37°C. For viability assay, RAW264.7 cells were pretreated with various concentrations of the isolated compounds (0, 5, 10, and 20 \( \mu \)M) 1 h prior to LPS (1 \( \mu \)g/ml) stimulation. After 24 h treatment, cell viability was determined by Cell Counting Kit-8 (Dojindo, Rockville, MD, United States) according to the manufacturer's instructions.

2.7 NO releasing inhibition assay

Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water) was used to determine NO production. RAW264.7 cells were pretreated in the same manner described in cell viability assay. 100 \( \mu \)L of supernatant of each pretreated cell solution was transferred to a new microtiter plate, and each supernatant was mixed with 100 \( \mu \)L of Griess reagent. The microtiter plate was left at room temperature for 10 min for color development, and each solution was measured by a microplate reader at UV 540 nm. All experiments were performed in triplicate.
2.8 Western blot analysis

RAW264.7 cells were pretreated with compound 7 (0, 5, 10, or 20 μM) or quercetin (25 μM, Sigma-Aldrich) for 1 h and then stimulated with LPS for an additional 24 h. The cells were lysed in radioimmunoprecipitation assay (RIPA) lysis buffer and protein concentration was determined using the Bradford assay (Bio-Rad Laboratories, Munich, Germany). The protein lysates were separated by SDS-PAGE and then transferred to a PVDF membrane. The blot was blocked with TBS containing 5% nonfat milk for 1 h at room temperature and then incubated with primary antibodies to iNOS, COX-2 or β-actin at 4°C overnight. The blots were washed three times with 0.1% TBST (0.1% Tween 20 in TBS) and then incubated with a peroxidase-conjugated secondary antibody for 1 h at room temperature. After washing, the protein bands were detected using an ECL reagent and X-ray film.

2.9 Determination of IL-6 and TNF-α levels

The measurement of the IL-6 and TNF-α levels was performed using commercial ELISA kits (RAB0308-1KT and RAB0477-1KT (Sigma Chemical Co., St. Louis, MO, United States), respectively) according to the manufacturer’s instructions. Briefly, samples and standards were added to antibody-coated 96 wells and were incubated for 2.5 h at room temperature. After incubation, wells were washed with wash buffer four times, and then detection antibody was added and incubated for another 1 h at room temperature. After washing, 3,3′,5,5′-tetramethylbenzidine (TMB) substrate was added and incubated in the dark for 30 min at room temperature followed by adding stop solution and reading absorbance at 450 nm immediately.

2.10 Statistical analysis

Statistical analyses were performed using SPSS (SPSS, Chicago, IL, United States). Data are expressed as the mean ± standard deviations. Statistical significance was determined using one-way ANOVA analysis followed by Tukey’s test. p-values < 0.05 were considered statistically significant.

3 Results and discussion

The methanolic extract of M. procumbens was dried and resuspended in H2O; this aqueous solution was extracted by n-hexane and CH2Cl2 to give rise to two organic layers. The CH2Cl2 portion was subjected to chromatography by a flash column and preparative RP-HPLC to afford five new and one known triterpenic acids (1–7), together with one brand new sesquiterpene (7) (Figure 1). All pure components (1–7) were then evaluated for their anti-inflammatory activities using an in vitro LPS-stimulated murine macrophage model.

3.1 Structural elucidation of the isolated compounds

Compound 1 ([α] +12.9, c = 0.5, MeOH), a colorless amorphous powder, has a molecular formula of C29H44O5 with 8 degrees of unsaturation (DOU) deduced from the sodiated HRESIMS pseudo-ion at m/z 495.3097 [M + Na]+ (calcd. for C29H44O5Na, 495.3081). In 1H-NMR spectrum (Table 1), four tertiary methyl signals (δH 0.76, 0.86, 0.92, and 1.21), one olefinic methylene signal (δH 4.67 brs and 5.00 brs), and one olefinic methine signal (δH 5.28 t, J = 3.6 Hz) were observed. Based on 13C-NMR and DEPTs spectra (Table 2), the carbon signals of the above two olefinic methylene at δC 73.0 and one olefinic methine at δC 109.4, 6 methines (two oxyethylene at δC 68.7 and 75.6 as well as one olefinic methine at δC 122.4), and 8 quaternary carbons (two sp2 quaternary carbons at δC 144.1 and 151.0 and one carboxylic acid group at δC 180.3). The above two olefins and one carboxyl group accounted for three DOU, and the remaining five constrained 1 to a pentacyclic structure. The assignments of 1H- and 13C-NMR spectroscopic data were completed by a combination of HSQC, 1H–1H COSY, and HMBC experiments. By comparison of carbon signals of 1 with that of oleanolic acid along with the analysis of the COSY and HMBC correlations of 1 altogether determined the carbon skeleton of 1, an oleanane-type nortriterpenoid (Kashiwada et al., 1998). In Figure 2, the COSY correlations of H-2 (δH 3.65 ddd, J = 3.6, 4.8, 12.0 Hz)/H-3 (δH 4.12 d, J = 3.6 Hz) confirmed that the attachments of these two hydroxyls are at C-2 and C-3, whereas the HMBC correlations from H2-30 (δH 3.18 brs) to C-19 (δC 39.9), C-20 (δC 35.4), and C-21 (δC 27.9) revealed a primary alcohol at C-30. Furthermore, an exo-4 (23)-double bond was verified according to the HMBC correlations of oleanolic methine H2-23 to C-3 (δC 75.6), C-4 (δC 151.0), and C-5 (δC 44.4); the tri-substituted Δ2 double bond was confirmed by the COSY correlations of H-9 (δH 1.82 m)/H-11 (δH 1.94 and 2.02 m)/H-12 (δH 5.28 t, J = 3.6 Hz) and by the HMBC correlations from H-18 (δH 2.88 dd, J = 4.8 and 14.4 Hz) to C-12 (δC 122.4), C-13 (δC 144.1), and C-14 (δC 41.8). The spectroscopic evidence altogether indicated that 1 is a new 24-noroleanane-type triterpene acid.

The pentacyclic moiety of oleanane-type triterpenoids is rigid, showing the configurations with those of regular oleanane-type triterpenoids based on NOESY experiment, which provides clear assignments. The NOESY correlations of H-5/H-9, H-9/CH3-27, CH3-27/Hα-19, and Hα-19/Hβ-30 indicated α-orientations, whereas those of CH3-25, CH3-26, H-18, and CH3-29 presented β-directions with regard to H-18/CH3-25, CH3-25/CH3-26, and H-18/CH3-29 (Figure 3).
Meanwhile, the NOESY correlations of H-2/H-3 and H-2/CH3-25 and the small proton constant of H-3 ($\gamma_{H-2,H-3} = 3.6$ Hz) agreed with the α-oriented OH-2 and OH-3 within a cis-relationship. As a result, the chemical structure of mesonal acid D (1) was established as 2α,3α,30-trihydroxy-24-norolean-4(23),12-dien-28-oic acid.

Mesonal acid E (2) was white amorphous powders with $[\alpha]_D +19.7$ (c 0.5, MeOH). The molecular formula C$_{29}$H$_{42}$O$_5$ on par with 9 DOU was deduced based on a quasi-molecular ion at $m/z$ 469.2965 [M – H]– (calcd. for C$_{29}$H$_{41}$O$_5$, 469.2949) in the HRESIMS experiment. The characteristic 1H- and 13C-NMR signals (Tables 1, 2), four tertiary methyls (δ$_{H}$ 0.66 s/δ$_{C}$ 13.5; δ$_{H}$ 0.94 s/δ$_{C}$ 32.0; δ$_{H}$ 0.98 s/δ$_{C}$ 22.7; δ$_{H}$ 1.17 s/δ$_{C}$ 20.0), two oxymethines C-2 (δ$_{H}$ 3.68 ddd, $J = 3.6$, 4.8, 11.4 Hz/δ$_{C}$ 68.8) and C-3 (δ$_{H}$ 4.11 d, $J = 3.6$ Hz/δ$_{C}$ 75.4), one exocyclic methylene involving C-4 (δ$_{H}$ 150.5) and C-23 (δ$_{H}$ 4.85 brs and 4.99 brs/δ$_{C}$ 109.0), and one COOH-28 (δ$_{C}$ 180.3) suggested that compound 2 is a derivative of 24-nor-oleane triterpene acid possessing the same 2,3-diol ring A as 1. Deduction of DOU from the 4 (23)-double bond, COOH-28, and the pentacyclic ring moiety, there are two DOU unassigned. After a detailed comparison of 1H-, 13C-NMR, and DEPTs spectra, we found that a tri-substituted Δ12 double bond and the primary alcohol of C-30 disappeared, while two oxymethines (δ$_{H}$ 4.00 dd, $J = 2.4$ and 5.4 Hz/δ$_{C}$ 73.0 and δ$_{H}$ 5.02 d, $J = 5.4$ Hz/δ$_{C}$ 78.0), one aliphatic methylene (δ$_{H}$ 0.74 d, $J = 7.2$ Hz/δ$_{C}$ 15.7), and one aliphatic quaternary carbon (δ$_{C}$ 33.8) emerged. By a combination of the COSY correlations of H-9/H$_{2}$-11/H-12 (indicating that olefinic methine C-12 converts to an
oxymethine) and the HMBC correlations from another oxymethine H-15 to C-12, one DOU was contributed to an oxygen-bridge between C-12 and C-15. The remaining DOU is resulted from a cyclopropane unit assembled by one aliphatic methylene C-27 and two aliphatic quaternary carbons C-13 and C-14, which were confirmed by the HMBC correlations (Figure 2) of H-18 (δ_H 1.88 dd, J = 4.2 and 12.6 Hz)/C-12, C-13, C-17 (δ_C 45.2), C-27, and COOH-28 and H2-26 (δ_H 1.17 s)/C-7 (δ_C 37.0), C-8 (δ_C 39.5), C-9 (δ_C 43.2), and C-14 (δ_C 36.1).

In terms of stereochemistry, the chiral centers at ring junctions of 2 are carried on as those at compound 1; The α-side of the cyclopropane unit (C-13, C-14, and C-27) which formed via a si-attack by α-oriented CH3-27 were supported by the NOESY correlations (H-2/H-3 and H-2/CH3-25) as well as the small proton constant of H-3 (J = 3.6 Hz). The configurations of the oxygen bridge heads, C-12 and C-15, were determined to be C29H42O6 (9 DOU) by the given HRESIMS molecular ion at m/z 545.2919 [M-H]− (calcd. for C29H41O6, 517.3160). The 1H- and 13C-NMR (Tables 1, 2), and HSQC spectra of compound 3 was clearly elucidated as 3,19α,21α-trihydroxy-2-oxo-24-norurs-3,12-dien-28-oic acid.

Mesonaic acid F (3) was obtained as a white powder with specific rotation [α] +25.1 (c 0.5, MeOH). Its IR spectrum showed the presence of a hydroxyl group (3369 cm−1), a carboxyl group (1712 cm−1), a conjugated carbonyl group (1630 cm−1), a conjugated carbonyl group, one tri-substituted double bond, and one carboxylic acid, reconciling these functional groups and pentacyclic fused ring system with the 9 DOU. The COSY correlations of H-9/H2-11/H-12 and the HMBC correlations from a junction proton H-18 (δ_H 2.69 brs) to C-12, C-13, C-14, C-17 and C-28 together made a tri-substituted double bond at the Δ19 position clear; the 1H–1H COSY correlations of CH3-27/ H-28-OH-21/CH2-22 alongside the HMBC correlations of CH3-29/ C-18, C-19, C-20 illustrated that 3 is a derivative of ursane-type triterpenoids. The most difficult part is that the α-hydroxy-β-methyl substitution in the α,β-unsaturated conjugated carbonyl part in ring A is deduced by the HMBC correlations of CH3-23 to C-2 and C-3 positions were amended for the con

![FIGURE 3](attachment:image.png)

Main NOESY correlations of triterpene acids 1–5.
amorphous powders and had a molecular formula of C29H44O5 determined by sodiated quasimolecular ion at m/z 495.3072 [M + Na]+ (calcld. for C29H44O5Na, 495.3081) in the HREIMS experiment. The IR, 1H, and 13C-NMR spectroscopic data are very similar to compound 4. Detailed analysis of their data, compound 6 possesses an exocyclic double bond (δ61 4.68 brs, 5.01 brs; δ5 151.1, 109.3), which replaced a carboxyl group and a singlet methyl in 4. The complete chemical structure and NMR assignment (Tables 1, 2) were further established and confirmed by 1D and 2D NMR to be identified as 2a,3a,19α-trihydroxy-24-norursa-4 (23),12-dien-28-oic acid (Jiang et al., 2005).

Compound 7 was collected as an oil sample ([α] +9.1, c 0.5 in MeOH) with a molecular formula C30H46O8 (8 DOU) determined by the HREIMS ion at m/z 533.2148 [M + Na]+ (calcld. for C30H46O8Na, 533.2142). According to 13C-NMR and DEPT spectra (Table 3), the 22 carbons can be divided into three methyls, six methylenes (one oxymethylene at δc 67.8), seven methines [including one oxymethine at δc 69.3 and five sp2 methines at δc 128.1 (2), 129.0 (2), and 132.7], and five quaternary carbons (two oxygenated ones at δc 72.0 and 74.0, one sp2 quaternary carbon at δc 130.4, and one ester carbonyl at δc 166.0). In the 1H-NMR spectrum (Table 3), three methyl singlets (δH 1.02 s, 1.12 s, and 1.19 brs), one oxygenated methylene [δH 3.46 (2H)] and one oxygenated methine [δH 5.18 (2H), δH 4.2, 7.8, and 12.0 Hz] together with the multiple peaks of mono-substituted benzene protons (δH 7.45–7.98) distributed spread from upfield to downfield. Combining the information, compound 7 was reasoned to have a double-ring moiety in addition to a benzene and an ester carbonyl portion. A comprehensive analysis of 1H–1H COSY and HMBC spectra, an eudesmane-type sesquiterpene (Evans et al., 1982) was put forward by incorporating two COSY fragments (H-2′-H-3′, H-5′-H-6′), the correlations of HMBC at CH3-14/C-1, C-5, C-9, and C-10 and CH3-15/C-3, C-4, and C-5. Moreover, the attachments of an O-benzyol (OBz) group and a propane-1,2-diol group were confirmed to be at C-2 and C-7 by the HMBC correlations: H-2, H-2′, and H-3′ to ester carbonyl C-1′; H-12 and H-13 to C-7 and C-11 (Figure 4A). Concerning the stereochemistry of 7, it was concluded to be a regular eudesmane-type sesquiterpene with anti-relationships of α-oriented H-5 and β-oriented CH-14. Furthermore, both the cross peaks of NOESY correlations for H-2′ and H-14, H-7′ and H-14, and H-15 and H-14 suggested the same sign at around 400 nm. However, the conformation of C-11 was not unambiguous because it cannot be differentiated directly by the NOESY correlations of H-7′/C-3′, H-9′/C-3′, H-12′/C-14′, and H-15′/C-14′. For this reason, the dimolybdenate tetra-acetate [MoO4(OAc)4]-modified CD analysis was applied to resolve the stereo assignment of the acyclic propane-1,2-diol group, a prim, sec-glycol (Fredk et al., 1997). As in many vic-glycols with a rigid conformation, one can follow the “helicity rule” to interpret the CD curves, whereby a positive (negative) torsional angle in the (HO)–C–(OH) moiety should lead to a positive (negative) Cotton effect of 300 nm. In Figure 4C, a negative CD band at 300 nm is obvious and accompanied by a second Cotton effect of the same sign at around 400 nm corresponding to a negative torsional angle in the (HO)–C–(OH) moiety. A Newman projection for the propane-1,2-diol moiety in chelation with MoO4(OAc)4 demonstrated a counterclockwise (a negative torsional angle) relationship from compound 5 to compound 6.

Concerning the stereochemistry of 5, it was concluded to be a regular eudesmane-type sesquiterpene with anti-relationships of α-oriented H-5 and β-oriented CH-14. Furthermore, both the cross peaks of NOESY correlations for H-2′ and H-14, H-7′ and H-14, and H-15 and H-14 suggested the same sign at around 400 nm. However, the conformation of C-11 was not unambiguous because it cannot be differentiated directly by the NOESY correlations of H-7′/C-3′, H-9′/C-3′, H-12′/C-14′, and H-15′/C-14′. For this reason, the dimolybdenate tetra-acetate [MoO4(OAc)4]-modified CD analysis was applied to resolve the stereo assignment of the acyclic propane-1,2-diol group, a prim, sec-glycol (Fredk et al., 1997). As in many vic-glycols with a rigid conformation, one can follow the “helicity rule” to interpret the CD curves, whereby a positive (negative) torsional angle in the (HO)–C–(OH) moiety should lead to a positive (negative) Cotton effect of 300 nm. In Figure 4C, a negative CD band at 300 nm is obvious and accompanied by a second Cotton effect of the same sign at around 400 nm corresponding to a negative torsional angle in the (HO)–C–(OH) moiety. A Newman projection for the propane-1,2-diol moiety in chelation with MoO4(OAc)4 demonstrated a counterclockwise (a negative torsional angle) relationship from compound 5 to compound 6.

Concerning the stereochemistry of 5, it was concluded to be a regular eudesmane-type sesquiterpene with anti-relationships of α-oriented H-5 and β-oriented CH-14. Furthermore, both the cross peaks of NOESY correlations for H-2′ and H-14, H-7′ and H-14, and H-15 and H-14 suggested the same sign at around 400 nm. However, the conformation of C-11 was not unambiguous because it cannot be differentiated directly by the NOESY correlations of H-7′/C-3′, H-9′/C-3′, H-12′/C-14′, and H-15′/C-14′. For this reason, the dimolybdenate tetra-acetate [MoO4(OAc)4]-modified CD analysis was applied to resolve the stereo assignment of the acyclic propane-1,2-diol group, a prim, sec-glycol (Fredk et al., 1997). As in many vic-glycols with a rigid conformation, one can follow the “helicity rule” to interpret the CD curves, whereby a positive (negative) torsional angle in the (HO)–C–(OH) moiety should lead to a positive (negative) Cotton effect of 300 nm. In Figure 4C, a negative CD band at 300 nm is obvious and accompanied by a second Cotton effect of the same sign at around 400 nm corresponding to a negative torsional angle in the (HO)–C–(OH) moiety. A Newman projection for the propane-1,2-diol moiety in chelation with MoO4(OAc)4 demonstrated a counterclockwise (a negative torsional angle) relationship from compound 5 to compound 6.
Finally, compound 7 was clearly identified as 2-\textit{O}-benzoyl-proximadiol, named mesoeudesmol B.

3.2 Effects of compounds 1‒7 on RAW264.7 macrophage cell viability and NO production

\textit{M. procumbens} extracts have been shown with pharmacological potentialities for many inflammation-associated disorders (Huang et al., 2012; Huang et al., 2021). Given the isolated compounds 1‒7 from \textit{M. procumbens}, we examined these isolates to see whether they similarly exhibit anti-inflammatory activities by using the LPS-induced RAW 264.7 macrophage cell model. As shown in Table 4, mesonoiac acids D (1) and E (2), 2\textalpha,3\textalpha,19\textalpha-trihydroxy-24-norursa-4(23),12-dien-28-oic acid (6), and mesoeudesmol B (7) show lower EC_{50} values than the positive control, quercetin (Li et al., 2016), suggesting that these four compounds possess better anti-inflammatory activity than those previously reported. Among them, mesoeudesmol B (7), a proximadiol derivative, displayed the highest inhibition activity on NO production with an EC_{50} value of 12.88 ± 0.23 \textmu M than that of quercetin (an EC_{50} value of 24.12 ± 0.21 \textmu M). Furthermore, all these seven compounds (at a concentration of 30 \textmu M) showed an approximate 100% survival rate in cell viability assay, suggesting that they are, in general, safe with no major cytotoxicity (to RAW264.7 cells).

3.3 Effects of compound 7 on the protein expression of iNOS and COX-2

To better understand the anti-inflammatory mechanism of compound 7, we examined two key inflammation-mediated proteins in LPS-induced RAW264.7 cells, iNOS and COX-2 at the protein level (Murakami and Ohigashi, 2007). In terms of the expression level of iNOS and COX-2 shown in Figure 5A, the LPS-treated cells display a higher level as opposed to the non-

![FIGURE 4](image)

2D correlations and CD spectrum of 7. (A) Key HMBC and \textsuperscript{1}H‒\textsuperscript{1}H COSY correlations. (B) Main NOESY correlations. (C) CD spectrum of 7 with Mo\textsubscript{2}(OAc)\textsubscript{4} in DMSO with the inherent CDs subtracted.

| Compound | EC\textsubscript{50} (\textmu M)* | Cell viability (%)b |
|----------|-----------------|------------------|
| 1        | 20.34 ± 0.61    | 102.57 ± 0.29    |
| 2        | 21.21 ± 0.52    | 101.43 ± 0.14    |
| 3        | >30             | 103.31 ± 0.37    |
| 4        | >30             | 99.41 ± 0.50     |
| 5        | >30             | 100.94 ± 0.42    |
| 6        | 20.23 ± 0.12    | 101.52 ± 0.16    |
| 7        | 12.88 ± 0.23    | 102.56 ± 0.32    |
| Quercetin | 24.12 ± 0.21    | 100.41 ± 0.53    |

*aCells were treated with LPS (1 \textmu g) in combination with the test compound for 24 h.

*bCell viability was measured in the presence of 30 \textmu M compound using the CCK-8 assay.

*cQuercetin was used as a positive control.

OH-12 to OH-11. Finally, compound 7 was clearly identified as 2-\textit{O}-benzoyl-proximadiol, named mesoeudesmol B.
treated cells, which show a low expression level. In addition, the protein level of the LPS-induced iNOS and COX-2 is negatively proportional to the addition of mesoeudesmol B (7) (5–20 μM) into the LPS-treated cells in a dose-dependent manner. Notably, 10 μM of 7 exhibits the lowest iNOS/COX-2 than 25 μM of quercetin does; moreover, the protein level of iNOS and COX-2 in the LPS-treated cells with the addition of 7 (20 μM) is nothing more than that in the non-treated cells, confirming that sesquiterpene 7 possesses stronger inhibitory activity than quercetin.

3.4 Effects of compound 7 on the secretion of cytokines TNF-α and IL-6

Given that TNF-α and IL-6 are major pro-inflammatory cytokines, they were further measured by ELISA in the LPS-stimulated murine macrophages. As shown in Figure 5B, it shows a dose-dependent suppression of the LPS-induced secretion of cytokines TNF-α and IL-6 with the addition of compound 7, which shows a similar trend to that of the LPS-induced expression of iNOS and COX-2. Likewise, 10 μM of 7 exhibits a lower level of TNF-α and IL-6 than 25 μM of quercetin does; the level of TNF-α and IL-6 in the LPS-treated cells with the addition of 7 (20 μM) is close to the basal one, once again confirming that 7 is a stronger inflammatory inhibitor than quercetin.

4 Conclusion

Several lines of evidence have underscored that the phenolics (i.e. kaempferol and caffeic acid), triterpenoids (i.e. oleanolic acid and ursolic acid), and polysaccharides in *M. procumbens* Hemesley are functional ingredients with strong antioxidant and anti-inflammatory properties, thus making Hsian-tsao a superb heat-clearing (Qingre) and detoxifying (Jiedu) herb. Having gone through rigorous and comprehensive interrogations against the literature and chemical databases, the five triterpene acids (1–5) and one sesquiterpene (7) discovered herein are new chemical entities; mesoeudesmol B (7) featuring a proximadiol core structure, in particular, is the first of its kind isolated from the extract of *M. procumbens* Hemesley. Of them, three triterpene acids (1, 2, and 6)
showed strong anti-inflammatory activities with the EC_{50} values of 20.3, 21.1, and 20.2 μM comparable to quercetin (EC_{50} values of 24.1 μM) and 15 triterpene acids isolated from our previous study, all presenting strong and promising anti-inflammatory potentials (Huang et al., 2021).

Of them, mesoeudesmol B (7), a 2-OBz proximadiol, outperformed others with an EC_{50} value of 12.9 μM 2-fold higher than quercetin in terms of anti-inflammation. Proximole (cryptomeridiol, a eudesmane sesquiterpenoid) is a well-known Egyptian folk medicine effective as a renal antispasmodic and diuretic agent extracted from the desert weed Cymbopogon proximus Stapf (Gramineae) but also from other species, such as Dysphania graveolens (Gui et al., 2020) and Chenopodium vulvaria (Lockley et al., 1982). This active sesquiterpene is best known for its high antidiabetic activity, whereas the C. proximus extracts are more versatile exhibiting multiple bioactivities, including antihypertensive activity and relaxation of the smooth muscle fibers (El-Nezhawy et al., 2014).

We believe that mesoeudesmol B (7) is the dominant agent in M. procumbens. The anti-inflammation mechanism of 7 is that it suppresses inflammation-mediated proteins (iNOS and COX-2) and pro-inflammatory cytokines (TNF-α and IL-6), thereby underscoring Hsian-tsao (the grass jelly herb) a superb medicinal herb worth of further studies for advanced pharmacologic applications and biosynthetic diversifications.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

H-TH: performing the experiments of the isolation and bioassay and writing the article. I-WL: elucidating the chemical structures and writing the article. G-YL and Y-CS: analyzing and interpreted the bioassay data and writing the article. T-LL: advising on the experiment and revising the article. Y-CL and H-CH: analyzed and interpreted the NMR data. C-CL: conceiving and designing the experiments and editing the article. Y-HK and K-TL: funding acquisition. All authors listed approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2022.1003356/full#supplementary-material

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