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Anti-Inflammatory Triterpene Glycosides from the Roots of *Ilex dunniana* Levl

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**Abstract:** A new triterpene glycoside ilexdunnoside A (1) and a new sulfated triterpene derivative ilexdunnoside B (2), together with five known analogues 3–7 were isolated from the roots of *Ilex dunniana* Levl. The structures were established by NMR spectroscopic analysis and acid hydrolysis. Results of an in vivo study of the biological activity showed that 75% ethanol and n-butanol extracts of the plant displayed anti-inflammatory activities against ear edema in mice, with inhibition rates of 23.5% and 37.5%, respectively, at a dose of 50 mg/kg. Furthermore, Compounds 1, 2 and 3 exhibited moderate indirect inhibitory effects on lipopolysaccharide-induced NO production in BV2 microglial cells in vitro, with IC_{50} values of 11.60, 12.30 and 9.70 \mu M, respectively.

**Keywords:** triterpene glycosides; *Ilex dunniana* Levl; anti-inflammatory

1. Introduction

Inflammation is an important pathophysiological factor of many diseases, such as pneumonia, intestinal catarrh, rheumatoid arthritis, infectious diseases and so on. Plants from the genus *Ilex* (Aquifoliaceae) have been used in the clinic for clearing away inflammation, relieving cough, eliminating phlegm, and so on [1]. In order to search for anti-inflammatory active ingredients, we have performed systematical chemical and pharmacological investigations of *Ilex dunniana* Levl, an evergreen shrub or dungarunga widely distributed to the south of the Qinling mountains and Yangtze Valley in China. Herein we report the isolation and structural elucidation of two new triterpene glycosides, ilexdunnoside A (1) and ilexdunnoside B (2) (Figure 1), together with five known analogues 3–7 from the roots of *Ilex dunniana* Levl, as well as the anti-inflammatory activity of all the isolated compounds.
2. Results and Discussion

Ilexdunnoside A (1) was isolated as a white, amorphous powder. Its molecular formula was established as C\textsubscript{46}H\textsubscript{74}O\textsubscript{15} by HRESIMS (m/z 865.4943 [M – H]\textsuperscript{−}, calcd for 865.4955) and NMR data, implying 10 degrees of unsaturation. The \textsuperscript{1}H-NMR spectrum (see Table 1 and Figure S1) showed six tertiary methyl groups at δ\textsubscript{H} 0.86, 0.96, 1.15, 1.33, 1.39, and 1.69 (each 3H, s), a secondary methyl at δ\textsubscript{H} 1.06 (3H, d, J = 8.0 Hz), an oxymethine proton at δ\textsubscript{H} 3.32 (1H, dd, J = 11.7, 4.5 Hz), and an olefinic proton at δ\textsubscript{H} 5.53 (1H, br s). The \textsuperscript{13}C-NMR spectrum (see Table 1 and Figure S2) exhibited 46 carbon resonances, of which 30 were attributed to the aglycone. Signals at δ\textsubscript{C} 89.2 (C-3), 72.5 (C-19), 128.3 (C-12), 139.2 (C-13), and 176.9 (C-28) were assigned to an oxygenated sp\textsuperscript{3} methine, an oxygenated sp\textsuperscript{3} quaternary, a pair of typical olefinic carbons and a carboxy carbon, respectively. The aforementioned data suggested that 1 was a pentacyclic triterpenoid derivative, similar to 3β,19α-dihydroxy-urs-12-en-28-oic acid [2], with the primary difference being the presence of a D-glucuronic acid (GlcA), a D-glucose (Glc), and an n-butyl in 1. The relative configuration of the aglycone of 1 was elucidated based on the spectrum.
(Figure 2). The correlations of H-3/Me-24, H-5/Me-24, H-5/H-9, H-9/Me-27, OH-19/Me-30 confirmed that these groups were cofacial and α-oriented. Likewise, correlations of Me-23/Me-25, Me-25/Me-26, H-18/Me-26, H-18/Me-29 showed the β-orientation of these groups. The absolute configuration of the aglycone of 1 was presumed to be 3S, 5R, 8R, 9R, 10R, 14S, 17S, 18S, 19R, and 20R, by the hypothetical biogenetic pathway.

Table 1. $^1$H- (400 MHz) and $^{13}$C-NMR (100 MHz) spectral data of 1 and 2 in C$_5$D$_5$N (δ in ppm).

| Position | Compound 1 | Compound 2 |
|----------|------------|------------|
|          | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) |
| 1        | 38.7       | 0.84, 1.46 | 38.2       | 0.76, 1.37 |
| 2        | 26.9       | 1.85, 2.12 | 26.4       | 1.88, 1.74 |
| 3        | 89.2       | 3.32 dd (11.7, 4.5) | 88.9       | 3.23 dd (12.0, 4.5) |
| 4        | 39.4       |            | 39.0       |            |
| 5        | 55.7       | 0.80       | 55.2       | 0.71       |
| 6        | 18.6       | 1.44, 1.28 | 18.1       | 1.37, 1.21 |
| 7        | 33.4       | 1.41, 1.56 | 32.9       | 1.49, 1.31 |
| 8        | 40.4       |            | 40.0       |            |
| 9        | 47.6       | 1.74       | 47.1       | 1.65       |
| 10       | 36.8       |            | 36.4       |            |
| 11       | 23.9       | 2.00       | 23.4       | 1.89       |
| 12       | 128.3      | 5.53 br s  | 127.8      | 5.41 br s  |
| 13       | 139.2      |            | 138.7      |            |
| 14       | 42.0       |            | 41.5       |            |
| 15       | 29.1       | 2.44 m, 1.22 | 28.7       | 2.31 m, 1.13 |
| 16       | 26.0       | 3.09 m, 1.99 | 25.5       | 2.95 m, 1.97 |
| 17       | 48.6       |            | 48.1       |            |
| 18       | 54.3       | 2.90 s     | 53.9       | 2.78 s    |
| 19       | 72.5       |            | 72.0       |            |
| 20       | 42.0       | 1.33       | 41.6       | 1.25       |
| 21       | 26.6       | 2.00, 1.84 | 26.0       | 1.86, 1.99 |
| 22       | 37.6       | 2.03, 1.82 | 37.2       | 1.70, 1.89 |
| 23       | 28.0       | 1.33 s     | 27.5       | 1.17 s    |
| 24       | 16.8       | 0.96 s     | 16.3       | 0.88 s    |
| 25       | 15.5       | 0.86 s     | 15.0       | 0.72 s    |
| 26       | 17.3       | 1.15 s     | 16.8       | 1.02 s    |
| 27       | 24.5       | 1.69 s     | 24.0       | 1.58 s    |
| 28       | 176.9      |            | 176.5      |            |
| 29       | 26.9       | 1.39 s     | 26.1       | 1.29 s    |
| 30       | 16.6       | 1.06 d (8.0) | 16.1       | 0.98 d (6.4) |
| 1'       | 107.3      | 4.95 d (7.7) | 106.2      | 4.89 d (7.6) |
| 2'       | 75.3       | 4.06       | 73.4       | 4.02       |
| 3'       | 77.9       | 4.26       | 83.6       | 5.16       |
| 4'       | 73.0       | 4.48       | 72.0       | 4.40 t (9.1) |
| 5'       | 77.2       | 4.57       | 76.1       | 4.48 t (9.5) |
| 6'       | 170.3      |            | 169.3      |            |
| 1''      | 95.7       | 6.26 d (8.0) | 95.3       | 6.13 d (8.0) |
| 2''      | 73.9       | 4.20       | 71.2       | 4.10       |
| 3''      | 78.8       | 4.28       | 78.2       | 4.19       |
| 4''      | 71.1       | 4.31       | 70.6       | 4.20       |
| 5''      | 79.1       | 4.02       | 78.7       | 3.91 m     |
| 6''      | 62.2       | 4.44, 4.38 | 61.6       | 4.32, 4.27 |
| 1'''     | 64.9       | 4.26       | 64.6       | 4.19       |
| 2'''     | 30.8       | 1.56       | 30.3       | 1.48       |
| 3'''     | 19.2       | 1.30       | 18.7       | 1.22       |
| 4'''     | 13.7       | 0.75 t (7.3) | 13.2       | 0.68 t (7.1) |

* Multiplicity is not clear for some signals due to overlapping.
In the $^1$H-NMR spectrum, the sugar portion showed two anomic proton signals at $\delta_H$ 4.95 (1H, d, $J = 7.7$ Hz) and $\delta_H$ 6.26 (1H, d, $J = 8.0$ Hz), with corresponding anomic carbons at $\delta_C$ 107.3 and 95.7, respectively. The sugar units were confirmed to be D-glucuronic acid (GlcA) and D-glucose (Glc) by GC analysis of their chiral derivatives after the hydrolysis of 1 with 2 N CF$_3$COOH. The $^1$H-$^1$H COSY and HSQC spectra revealed the presence of an n-butyl in 1, while HMBC correlations from H-1‴ to C-6‴ (170.3) (Figure 3), indicated the attachment of the n-butyl to C-6‴. Relative large coupling constants of $^3$$J_{H1‴-H2‴}$ (7.7 Hz) and $^3$$J_{H1‴-H2‴}$ (8.0 Hz) of (6‴-BuO) GlcA and Glc in their pyranose form indicated the $\beta$-anomeric orientation of them. HMBC correlations from H-1′ to C-3 ($\delta_C$ 89.2), and from H-1‴ to C-28 ($\delta_C$ 176.9) indicated that the anomeric carbons of (6‴-BuO) GlcA and Glc were connected to C-3 and C-28 of the aglycone, respectively. Thus, compound 1 was identified as 3$\beta$-(6-O-n-butyl-D-glucuronopyranosyl)-19$\alpha$-hydroxy-urs-12-en-28-oic acid 28-O-$\beta$-D-glucopyranoside, and named ilexduumnoside A.

Ilexduumnoside B (2) was obtained as a white, amorphous powder. It had the molecular formula of C$_{46}$H$_{74}$O$_{18}$S according to the deprotonated molecule at $m/z$ 945.4538 [M − H]$^-$ (calcd for 945.4523) in the HRESIMS, suggesting that there was a sulfate group in the molecule. The $^1$H- and $^{13}$C-NMR spectra showed the characteristic resonances of a 3$\beta$,19$\alpha$-dihydroxy-urs-12-en-28-oic acid skeleton, similar to those of 1, the only difference being in the sugar portion of 2. The NMR data showed the presence of an unusual D-(3-O-sulphonyl-6-O-n-butyl)-glucuronopyranosyl unit in 2 instead of the D-(6-O-n-butyl)-glucuronopyranosyl moiety in 1, as supported by the downfield shifts of the H-3′ (δ$_H$ 5.16) and C-3′ ($\delta_C$ 83.6) of 2 compared with H-3′ (δ$_H$ 4.26) and C-3′ ($\delta_C$ 77.9) of 1. Acid hydrolysis of 2, followed by treatment with BaCl$_2$, obtained a white precipitate, also demonstrating the presence of a sulfate group. On the basis of the NOESY spectrum, the relative configuration of 2 was identical to that of 1. Using the hypothetical biogenetic pathway permitted the assignment of the absolute configuration of 2 as 3S, 5R, 8R, 9R, 10R, 14S, 17S, 18S, 19R and 20R. Therefore, the structure of compound 2 was elucidated as 3$\beta$-(3-O-sulphonyl-6-O-n-butyl-D-glucuronopyranosyl)-19$\alpha$-hydroxy-urs-12-en-28-oic acid 28-O-$\beta$-D-glucopyranoside, and named ilexduumnoside B.
The five known compounds were identified as hylonoside II (3), ilexpublesnin H (4), ilexpublesnin I (5), ilexoside XXXV (6), ilekudinoside F (7), based on the analysis and comparison of their experimental physical and spectroscopic data with literature values [3–6]. Compounds 1–7 are triterpene glycosides, of which compounds 4 and 5 belong to the sulfated triterpene glycoside class of compounds.

Microglial cells, which are regarded as the most important immune cells in the central nervous system (CNS), are activated by brain injuries. Following activation by bacterial toxins, microglial cells secrete a wide range of inflammatory mediators, such as nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and prostanoids [7]. Nitric oxide (NO) plays an important role in the inflammatory process, and an inhibitor of NO production may be considered as a potential anti-inflammatory agent [8]. NO is a physiological messenger that triggers a variety of actions in several systems [9]. It can modulate the release of various inflammatory mediators from a wide range of cells participating in inflammatory responses. Because of its anti-inflammatory properties and cytoprotective effects, adjunctive NO has been considered a plausible means for improving the anti-inflammatory activity. In recent years, NO-releasing drugs have been developed, usually as derivatives of other drugs, which exhibit very powerful anti-inflammatory effects [10–13].

In order to identify the biological activity, an in vivo anti-inflammatory model were used, the results showed that the 75% ethanol and n-butanol extracts of the plant exhibited anti-inflammatory activities against ear edema in vivo, with inhibition rates of 23.5% and 37.5%, respectively, at a dose of 50 mg/kg (as shown in Table 2). Furthermore, as shown in Table 3, in vitro results showed that compounds 1–7 obtained from the n-butanol extract were tested for their inhibitory effects on lipopolysaccharide-induced NO production in mouse microglial cells. Compounds 1–3 displayed moderate indirect anti-inflammatory activity, in which, compound 3 showed cytotoxic activity against microglial cell in vitro (Table 4), so the indirect anti-inflammatory activity of 3 partly may be caused by the cytotoxic activity. The other compounds exhibited weak activities for the inhibition of NO production, and did not show cytotoxic activity.

### Table 2. Effects of the plant extracts on ear edema induced by croton oil in mice.

| Extracts          | Dose (mg/kg) | Edema Degree (mg) | Inhibition Rate (%) |
|-------------------|--------------|-------------------|--------------------|
| 75% Ethanol extract | 50.0         | 15.31 ± 2.01 *    | 23.5               |
| n-Butanol extract  | 50.0         | 12.50 ± 1.50 **   | 37.5               |
| Dexamethasone a    | 1.0          | 5.80 ± 0.90 **    | 71.0               |
| Control group      | -            | 20.01 ± 2.31      | -                  |

* Positive control; *p < 0.05 vs. control group; **p < 0.01 vs. control group.

### Table 3. Inhibitory effects of compounds against LPS-induced NO production in mouse BV2 microglial cells (n = 3).

| Compounds | IC50 (µM) | Compounds | IC50 (µM) |
|-----------|-----------|-----------|-----------|
| 1         | 11.60 ± 0.89 | 5         | 50.7 ± 3.25 |
| 2         | 12.30 ± 1.21 | 6         | 22.3 ± 2.23 |
| 3         | 9.70 ± 0.86  | 7         | 55.2 ± 3.26 |
| 4         | 33.5 ± 2.11  | dexamethasone a | 0.03       |

* Positive control.
Table 4. The cytotoxic activity of compounds on BV2 microglial cells (n = 3).

| Compounds | Cell Viability |
|-----------|---------------|
|           | 5.0 µM | 10.0 µM | 20.0 µM | 40.0 µM | 80.0 µM |
| 1         | 99.74 ± 1.21 | 99.28 ± 1.32 | 99.35 ± 1.26 | 99.62 ± 1.89 | 99.70 ± 1.36 |
| 2         | 98.32 ± 2.05 | 98.25 ± 1.87 | 98.16 ± 1.25 | 98.56 ± 1.37 | 98.26 ± 1.88 |
| 3         | 91.74 ± 1.11 * | 90.28 ± 1.82 * | 89.35 ± 1.35 * | 88.62 ± 1.21 * | 88.50 ± 1.32 * |
| 4         | 99.36 ± 1.31 | 99.37 ± 1.21 | 99.67 ± 1.32 | 99.57 ± 1.53 | 99.33 ± 1.30 |
| 5         | 98.21 ± 1.93 | 99.33 ± 1.54 | 99.32 ± 1.32 | 99.21 ± 1.66 | 99.13 ± 1.28 |
| 6         | 102.21 ± 1.03 | 102.28 ± 1.56 | 103.35 ± 1.58 | 103.26 ± 1.28 | 103.22 ± 1.89 |
| 7         | 101.55 ± 1.42 | 101.68 ± 1.36 | 101.76 ± 1.47 | 101.38 ± 1.58 | 101.68 ± 1.38 |
| Control group | 100.00 ± 1.51 | 100.00 ± 1.26 | 100.00 ± 1.56 | 100.00 ± 1.78 | 100.00 ± 1.55 |
| Dexamethasone a | 101.02 ± 1.01 | 99.98 ± 1.32 | 100.25 ± 1.67 | 101.37 ± 1.83 | 99.78 ± 1.63 |

a Positive control; * p < 0.05 vs. control group.

3. Experimental Section

3.1. General Information

Optical rotations were obtained on a P2000 automatic digital polarimeter (JASCO, Tokyo, Japan). NMR spectra were measured on a Mercury-400 spectrometer (Varian, Palo Alto, CA, USA). HRESIMS spectra were acquired with an Agilent Technologies 6250 Accurate-Mass Q-TOF LC/MS spectrometer (Agilent, Santa Clara, CA, USA). The MPLC system (Biotage, Uppsala, Sweden) was equipped with an YMC-Pack ODS-A column (500 mm × 50 mm, 50 µm, YMC, Tokyo, Japan). Column chromatography was conducted with MCI GEL CHP20P resin (75–150 µm, Mitsubishi, Tokyo, Japan) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). TLC was carried out with glass precoated with silica gel GF 254. BV2 microglial cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). A microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) was used for the cytotoxicity assays.

3.2. Plant Material

The roots of Ilex dunniana Levl were collected in Mount Emei, Sichuan Province, People’s Republic of China, in August 2015, and were identified by Associate Prof. Xiao-Zhong Chen from Heilongjiang University of Chinese Medicine. A voucher specimen (ID-g-20150828) was deposited at the herbarium of the Jiamusi College, Heilongjiang University of Chinese Medicine.

3.3. Extraction and Isolation

The air-dried, powdered root of Ilex dunniana Levl (5 kg) were extracted with 75% EtOH (10 L × 1 h × 3). A dried extract (800 g), obtained after concentration in vacuo, was suspended in H2O, and partitioned with petroleum ether (60–90 °C), EtOAc, and n-BuOH, successively. After the solvent was removed, the n-BuOH extracts (60 g) was subjected to MCI GEL CHP20P resin (75–150 µm) eluting with H2O, 50% EtOH, and 95% EtOH. The 50% EtOH eluate (20 g) was separated by MPLC (5–100% MeOH-H2O, 80 mL/min, 6 h) to obtain 30 fractions (Fr. 1–Fr. 30). Fraction 5 (1.0 g) was chromatographed over Sephadex LH-20 using MeOH, to give compound 7 (12.0 mg). Fraction 6 (2.0 g) was subjected to Sephadex LH-20 eluting with MeOH, to obtain compound 3 (8.0 mg). The separation of fraction 9 (1.0 g) was achieved by Sephadex LH-20 using MeOH as the eluent, to afford 4 (5.0 mg) and 5 (6.3 mg). Fractions 10 (3.5 g) and 15 (1.3 g) were also purified by Sephadex LH-20 eluting with MeOH, to give compounds 1 (7.5 mg), 2 (9.5 mg), and 6 (5.0 mg), respectively.
3.4. Product Characterization

**Ilexdunnoside A (1):** white, amorphous power; $\alpha_{25}^D + 38.1$ (c 0.1, CH$_3$OH); $^1$H-NMR (C$_5$D$_5$N, 400 MHz) and $^{13}$C-NMR (C$_5$D$_5$N, 100 MHz) see Table 1; HRESIMS $m/z$ 865.4943 [M − H]$^-$ (calcd. for 865.4955, C$_{46}$H$_{73}$O$_{15}$).

**Ilexdunnoside B (2):** white, amorphous power; $\alpha_{25}^D + 56.1$ (c 0.2, CH$_3$OH); $^1$H-NMR (C$_5$D$_5$N, 400 MHz) and $^{13}$C-NMR (C$_5$D$_5$N, 100 MHz) see Table 1; HRESIMS $m/z$ 945.4538 [M − H]-$^-$ (calcd. for 945.4523, C$_{46}$H$_{73}$O$_{18}$S).

3.5. Acid Hydrolysis of Saponins

Each saponin (1.0 mg) was refluxed with 2 N aqueous CF$_3$COOH (10 mL) at 100 °C for 2 h. The reaction mixture was partitioned between H$_2$O (10 mL) and CH$_2$Cl$_2$ (3 × 4 mL). The CH$_2$Cl$_2$ layer was washed with H$_2$O and evaporated to obtain the aglycone. After evaporation in vacuo and removing the acid by adding MeOH, the aqueous extracts were analyzed by GC. Thus, the absolute configuration of the crude sugar was demonstrated as described in the previous papers [14,15].

3.6. Detection of the Sulfate Group

Each compound (2–3 mg) was refluxed with 10% HCl (4 mL) for 4 h. After that the reaction mixture was extracted with Et$_2$O. Then, an aliquot of the aqueous layer of each sample was treated with 70% BaCl$_2$ to yield a white precipitate (BaSO$_4$) [16].

3.7. In Vitro Anti-Inflammatory Activity Assays

BV2 microglial cells were maintained in RPMI1640 medium at 37 °C in 5% CO$_2$. The cells were placed in 48-well plates and preincubated for 24 h, treated with tested triterpene glycosides dissolved in DMSO at various final concentrations (5.0, 10.0, 20.0, 40.0, 80.0 μM) in triplicate for 1 h, and continuously incubated with LPS (1 μg/mL) for 24 h. Dexamethasone (10$^{-6}$ M) was used as the positive control. From each well, the supernatants (100 μL) were mixed with an equal amount of Griess reagent at room temperature for 20 min. The concentration of NO$_2^-$ was measured for the amount of NO by a microplate reader at 570 nm, using sodium nitrite as the standard to calculate the concentration of the nitrite [17,18].

3.8. Cytotoxicity Assays

Cell viabilities were measured using the MTT assay. Briefly, BV2 microglial cells were seeded in 96-well plates at concentrations of 1 × 10$^5$ cells per well. After incubation for 2 h, the cells were incubated with compounds for 24 h and then washed with PBS three times. Following the washing step, 200 μL of RPMI 1640 medium containing 0.5 mg/mL MTT were added to each well, the cells were then incubated at 37 °C for another 4 h. Finally, the culture medium was removed, and the formazan crystal was dissolved by adding 150 μL of DMSO. Absorbances at 570 nm were measured using a microplate reader.

3.9. In Vivo Anti-Inflammatory Assays

On the basis of the inhibition of croton oil-induced ear edema in mice, the topical anti-inflammatory activity of the test compounds was evaluated. Animal experiments were performed in accordance to the Institutional Guidelines for Animal Care and Use of the Chinese Academy of Medical Sciences and Peking Union Medical College. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Chinese Academy of Medical Sciences and Peking Union Medical College (permit number: 002973). The croton oil (Croton tiglium L., seed oil) was purchased from Sigma-Aldrich (St. Louis, MO, USA). In ICR male mice (18–20 g, n = 10 per group), croton oil (0.4 mg) was mixed with acetone (1 mL) to be applied to the left ear (10 μL each side) topically to induce ear edema. The animals were administered with candidate extracts (50 mg/kg) for 1 h, followed by
treatment with croton oil for 4 h. After the application of croton oil, the mice were euthanized using sodium pentobarbital, and ear tissues (8 mm diameter punches) were collected for the measurement of the weight of ear patches (left and right). Based on the weight difference between two plugs (8 mm diameter) of the treated (left) and untreated (right) ears, the edematous response was measured. The percentage reduction in edema in treated mice compared with control mice is calculated for the expression of anti-inflammatory activity. Dexamethasone was used as the positive control [18,19].

4. Conclusions

The results of croton-oil ear inflammation in mice showed that 75% ethanol and n-butanol extracts of powdered root of Ilex dunniana Levl exerted anti-inflammatory activity in vivo against ear edema. Bioactivity-guided isolation of the n-butanol extract yielded two new triterpene glycoside 1 and 2 together with five known ones 3–7. Compounds 1–7 belong to the triterpene glycosides, of which compounds 4 and 5 belong to sulfated triterpene glycosides. Furthermore, in vitro anti-inflammatory activity showed that compounds 1, 2 and 3 obtained from the n-butanol extract exhibited moderate indirect anti-inflammatory activity on LPS-induced proinflammatory factors production in BV2 microglial cells.

Supplementary Materials: The following are available online at http://www.mdpi.com/1420-3049/22/7/1206/s1.

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Conflicts of Interest: The authors declare no conflict of interest.

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**Sample Availability:** Samples of the compounds 1–7 are available from the authors.

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