Acylphloroglucinol Derivatives from *Garcinia multiflora* with Anti-Inflammatory Effect in LPS-Induced RAW264.7 Macrophages

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**Abstract:** Two new acylphloroglucinol derivatives, 13,14-didehydroxygarcicowin C (1) and 13,14-didehydroxyisoxanthochymol (2), have been isolated from the stems of *Garcinia multiflora*, together with seven known compounds (3–9). The structures of new compounds 1 and 2 were elucidated by MS and extensive 1D/2D NMR spectroscopic analyses. Among the isolates, 13,14-didehydroxy-isoxanthochymol (2) and sampsonione B (3) exhibited inhibition against lipopolysaccharide (LPS)-induced NF-κB activation in macrophages at 30 µM with relative luciferase activity values (inhibitory %) of 0.75 ± 0.03 (24 ± 4%) and 0.12 ± 0.03 (88 ± 4%), respectively. Additionally, sampsonione B (3) reduced LPS-induced nitric oxide (NO) production in murine RAW264.7 macrophages and did not induce cytotoxicity against RAW 264.7 cells after 24 h treatment. Compound 3 is worth further investigation and may be expectantly developed as an anti-inflammatory *drug candidate*.

**Keywords:** *Garcinia multiflora*; Guttiferae; structure elucidation; nuclear factor κB; nitric oxide; anti-inflammatory activity

### 1. Introduction

*Garcinia multiflora* Champ. (Guttiferae) is a small evergreen tree [1], usually growing 5–15 m tall, distributed in South China, Taiwan, and Hong Kong. Fruit from this plant is edible. In Taiwan, the genus *Garcinia* is represented by three species, *Garcinia multiflora*, *Garcinia linii*, and *Garcinia subelliptica*. Xanthones [2–5], acylphloroglucinols [6–9], flavanones [10], and their derivatives are distributed in plants of the genus *Garcinia*. Multiple activities have been reported for these derivatives such as cytotoxic [3–5], anti-microbial [2], anti-inflammatory [6–8], anti-oxidant [2], and AChE enzymes inhibitory activities [10]. Aberrant inflammation is associated with many diseases such as arthritis, asthma, and cancer [11]. Among immune cells, macrophages are highly
responsive to lipopolysaccharide (LPS) and activated macrophages produce multiple pro-inflammatory molecules (such as nitric oxide (NO)). Nuclear factor κB (NF-κB) [12,13] is a transcription factor mediating inflammatory responses and known as a drug target for anti-inflammatory strategy. In our research on the anti-inflammatory constituents of Formosan plants, numerous species had been screened for inhibitory activity on LPS-induced NF-κB activation, and G. multiflora was found to be an active species. Phytochemical investigations on the stems of G. multiflora has resulted in the isolation of two new acylphloroglucinol derivatives, 13,14-didehydroxygarcicowin C (1) and 13,14-didehydroxy-isoxanthochymol (2), along with seven known compounds. We evaluated the anti-inflammatory effect of the isolated compounds in LPS-stimulated RAW264.7 macrophages and found that 13,14-didehydroxyisoxanthochymol (2) and sampsonione B (3) decreased NF-κB activity. Moreover, sampsonione B (3) inhibited the production of nitric oxide (NO) in LPS-activated macrophages. In this article, the structural elucidation of 1 and 2 and the inhibitory activity of the isolates on LPS-induced NF-κB activation are described.

2. Results and Discussion

2.1. Isolation and Structural Elucidation

Chromatographic isolation and purification of the EtOAc-soluble fraction of a MeOH extract of stems of G. multiflora on a silica gel column and preparative thin-layer chromatography (TLC) obtained two new (1 and 2) and seven known compounds (3-9) (Figure 1).

13,14-Didehydroxygarcicowin C (1) was obtained as colorless, amorphous powder. The ESI-MS (Figure S1) displayed the quasi-molecular ion [M + H]+ at m/z 569, implying a molecular formula of C_{38}H_{49}O_{4}, which was confirmed by the HR-ESI-MS (m/z 569.36284 [M + H]+, calcd 569.36254) (Figure S2) and by the 1H, 13C, and DEPT NMR data. The presence of carbonyl groups was revealed by the bands at 1729, 1682, and 1645 cm\(^{-1}\) in the IR spectrum and was confirmed by signals at δ\(_C\) 209.0, 193.8, and 193.1 in the 13C NMR spectrum. The 1H and 13C NMR spectrum (Table 1) (Figures S3 and S4) of 1 showed signals for an acylphloroglucinol derivative based on the presence of a 2,2-dimethylbicyclo[3.3.1]nonane ring system, a benzoyl group, two isoprenyl groups, and another C_{10} unit (C-29 through C-38). The 1H NMR data of 1 were similar to those of garcicowin C (Figure 2) [14], except that the benzoyl group [δ\(_H\) 7.38 (2H, t, J = 7.5 Hz, H-13, and H-15), 7.50 (1H, t, J = 7.5 Hz, H-14), and 7.77 (2H, d, J = 7.5 Hz, H-12, and H-16)] of 1 replaced the 3,4-dihydroxybenzoyl group of garcicowin C. This was supported by the HMBC correlations observed between H-12 (δ\(_H\) 7.77) and C-10 (δ\(_C\) 193.1), C-14 (δ\(_C\) 133.0), and C-16 (δ\(_C\) 128.7). The relative configuration of 1 was deduced from the NOESY cross-peaks (Figure 3) of H-17/H-22, H-22/H-6, H\(_\alpha\)-7/H-22, H\(_\alpha\)-7/H-29, H\(_\alpha\)-29/H-35, and H-34/H-32. Consequently, H-6, the isoprenyl group at C-4, and the bond between C-8 and C-29 are on the \(\alpha\)-side, and H-34 and the prop-1-en-2-yl group at C-30 are on the \(\beta\)-side of 1. According to the data of the 1H-1H COSY (Figure S5) and NOESY (Figure S6) spectra, a computer-created 3D structure was established by applying the above-mentioned molecular modeling program with MM2 force-field calculations for energy minimization. The NOESY experiment of 1 showed selected cross-peaks as shown in the 3D drawing (Figure 4). The calculated distances between H-17/H-22 (2.248 Å), H-22/H-6 (2.304 Å), H\(_\alpha\)-7/H-29 (2.281 Å), H\(_\alpha\)-29/H-35 (2.552 Å), and H-34/H-32 (2.364 Å) are all less than 4.00 Å; this corresponds with the well-defined NOESY examined for each of the proton pairs. The absolute configuration of 1 was indicated by CD Cotton effects at 311 (Δ\(\epsilon\) + 2.1), 267 (Δ\(\epsilon\) ~ 8.5), 223 (Δ\(\epsilon\) + 5.2) nm in analogy with garcicowin C [15]. The full assignment of 13C and 1H NMR resonances was substantiated by DEPT, 1H-1H COSY, NOESY (Figure 3), HMBC (Figure 3) (Figure S7), and HSQC (Figure S8) experiments. On the basis of the above evidence, the structure of 1 was established as 13,14-didehydroxygarcicowin C.
Figure 1. The chemical structures of compounds 1–9 isolated from *Garcinia multiflora*. 
Figure 2. The chemical structure of garcicowin C.

Figure 3. Key NOESY (→) and HMBC (→) correlations of 1.

Figure 4. Selected NOESY correlations and relative configuration of 1.

(Figure 5) [16], except that the benzoyl group [δΗ 7.36 (2H, t, J = 7.5 Hz, H-13 and H-15), 7.49 (1H, t, J = 7.5 Hz, H-14), and 7.72 (2H, d, J = 7.5 Hz, H-12 and H-16)] of 2 replaced the 3,4-dihydroxybenzoyl group of isoxanthochymol [16]. This was supported by the HMBC correlations observed between H-12 (δΗ 7.72) and C-10 (δC 193.8), C-14 (δC 132.9), and C-16 (δC 128.8). The relative configuration of 2 was determined by NOESY cross-peaks (Figure 6) of H-17/H-22, H-22/H-6, Hα-7/H-22, Hα-7/H-29, Hα-29/H-30, and Hβ-7/H-34. Consequently, H-6, the isoprenyl group at C-4, and the bond between C-8 and C-29 are on the α-side, and the isoprenyl group at C-6 and the isoprenyl group at C-30 are on the β-side of 2. A computer-created 3D structure (Figure 7) was established by applying the above-mentioned molecular modeling program with MM2 force-field calculations for energy minimization. The calculated distances between H-17/H-22 (2.301 Å), H-22/H-6 (2.274 Å), H-22/Hα-7 (3.109 Å), Hα-29/H-30 (2.546 Å), Hβ-7/H-34 (2.128 Å), and Hβ-7/H-24 (3.246 Å) are all less than 4.00 Å; this corresponds with the well-defined NOESY observed for each of the proton pairs. The absolute configuration of 2 was confirmed by the similar CD Cotton effects [270 (Δε + 13.6), 224 (Δε − 8.4) nm] compared with analogous benzoylphloroglucinol derivative, isoxanthochymol [16]. The structure elucidation of 2 was confirmed by 1H–1H COSY (Figure S13) and NOESY (Figure 6) (Figure S14) techniques and 13C NMR assignments were supported by DEPT, HMBC (Figure 6) (Figure S15), and HSQC (Figure S16) experiments. According to the above evidence, the structure of 2 was established as 13,14-didehydroxyisoxanthochymol.
Table 1. $^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) data for compounds 1 and 2 in CDCl$_3$.

| Position | $^1$H (f in Hz) | $^1$H (f in Hz) |
|----------|----------------|----------------|
| 1        | 170.8, C       | 170.8, C       |
| 2        | 129.1, C       | 129.1, C       |
| 3        | 193.8, C       | 193.8, C       |
| 4        | 69.2, C        | 69.2, C        |
| 5        | 46.7, C        | 46.7, C        |
| 6        | 46.4, CH       | 46.4, CH       |
| 7        | 38.1, CH$_2$   | 38.1, CH$_2$   |
| 8        | 48.1, C        | 48.1, C        |
| 9        | 209.0, C       | 209.0, C       |
| 10       | 193.1, C       | 193.1, C       |
| 11       | 137.5, C       | 137.5, C       |
| 12       | 128.7, CH      | 128.7, CH      |
| 13       | 128.3, CH      | 128.3, CH      |
| 14       | 133.0, CH      | 133.0, CH      |
| 15       | 128.3, CH      | 128.3, CH      |
| 16       | 128.7, CH      | 128.7, CH      |
| 17       | 25.3, CH$_2$   | 25.3, CH$_2$   |
| 18       | 119.8, CH      | 119.8, CH      |
| 19       | 134.6, C       | 134.6, C       |
| 20       | 26.1, CH$_3$   | 26.1, CH$_3$   |
| 21       | 18.1, CH$_3$   | 18.1, CH$_3$   |
| 22       | 26.8, CH$_3$   | 26.8, CH$_3$   |
| 23       | 22.3, CH$_3$   | 22.3, CH$_3$   |
| 24       | 29.3, CH$_2$   | 29.3, CH$_2$   |
| 25       | 124.9, CH      | 124.9, CH      |
| 26       | 133.1, C       | 133.1, C       |
| 27       | 26.0, CH$_3$   | 26.0, CH$_3$   |
| 28       | 18.2, CH$_3$   | 18.2, CH$_3$   |
| 29       | 33.2, CH$_2$   | 33.2, CH$_2$   |
| 30       | 42.8, CH       | 42.8, CH       |
| 31       | 143.5, C       | 143.5, C       |
| 32       | 113.9, CH$_2$  | 113.9, CH$_2$  |
| 33       | 20.3, CH$_3$   | 20.3, CH$_3$   |
| 34       | 79.7, CH       | 79.7, CH       |
| 35       | 121.4, CH      | 121.4, CH      |
| 36       | 141.7, C       | 141.7, C       |
| 37       | 25.7, CH$_3$   | 25.7, CH$_3$   |
| 38       | 17.8, CH$_3$   | 17.8, CH$_3$   |

$^{13}$,14-Didehydroxyisoxanthochymol (2) was isolated as a colorless amorphous powder with molecular formula C$_{38}$H$_{50}$O$_4$ as established by ESI-MS (Figure S9) and HR-ESI-MS (Figure S10), revealing an [M + H]$^+$ ion at m/z 571.3782 (calcld for C$_{38}$H$_{51}$O$_4$, 571.37819). The presence of carbonyl groups was revealed by the bands at 1724, 1675, and 1637 cm$^{-1}$ in the IR spectrum and was confirmed by signals at $^3$C 207.1, 193.8, and 193.7 in the $^{13}$C NMR spectrum. The $^1$H- and $^{13}$C NMR spectrum (Table 1) (Figures S11 and S12) of 2 showed signals for an acylphloroglucinol derivative based on the presence of a 2,2-dimethylbicyclo[3.3.1]nonane ring system, a benzoyl group, three isoprenyl groups, and another C$_5$ unit (C-29 through C-33). The NMR data of 2 was similar to those of isoxanthochymol (Figure 5) [16], except that the benzoyl group [$^3$H 7.36 (2H, t, $J$ = 7.5 Hz, H-13 and H-15), 7.49 (1H, t,
$J = 7.5 \text{ Hz}, \text{H-14}, \text{and} 7.72 \text{ (2H, d, } J = 7.5 \text{ Hz, H-12 and H-16)}$ of 2 replaced the 3,4-dihydroxybenzoyl group of isoxanthochymol [16]. This was supported by the HMBC correlations observed between H-12 ($\delta_{\text{H}} 7.72$) and C-10 ($\delta_{\text{C}} 193.8$), C-14 ($\delta_{\text{C}} 132.9$), and C-16 ($\delta_{\text{C}} 128.8$). The relative configuration of 2 was determined by NOESY cross-peaks (Figure 6) of H-17/H-22, H-22/H-6, H$_{\alpha}$-7/H-22, H$_{\alpha}$-7/H-29, H$_{\alpha}$-29/H-30, and H$_{\beta}$-7/H-34. Consequently, H-6, the isoprenyl group at C-4, and the bond between C-8 and C-29 are on the $\alpha$-side, and the isoprenyl group at C-6 and the isoprenyl group at C-30 are on the $\beta$-side of 2. A computer-created 3D structure (Figure 7) was established by applying the above-mentioned molecular modeling program with MM2 force-field calculations for energy minimization. The calculated distances between H-17/H-22 (2.301 Å), H-22/H-6 (2.274 Å), H-22/H$_{\alpha}$-7 (3.109 Å), H$_{\alpha}$-29/H-30 (2.546 Å), H$_{\beta}$-7/H-34 (2.128 Å), and H$_{\beta}$-7/H-24 (3.246 Å) are all less than 4.00 Å; this corresponds with the well-defined NOESY observed for each of the proton pairs. The absolute configuration of 2 was confirmed by the similar CD Cotton effects [270 ($\Delta\varepsilon + 13.6$), 224 ($\Delta\varepsilon − 8.4$) nm] compared with analogous benzoylphloroglucinol derivative, isoxanthochymol [16]. The structure elucidation of 2 was confirmed by $^{1}H-^{1}H$ COSY (Figure S13) and NOESY (Figure 6) (Figure S14) techniques and $^{13}$C NMR assignments were supported by DEPT, HMBC (Figure 6) (Figure S15), and HSQC (Figure S16) experiments. According to the above evidence, the structure of 2 was established as 13,14-didehydroxyisoxanthochymol.

![Figure 5. The chemical structure of isoxanthochymol.](image)

![Figure 6. Key NOESY (→) and HMBC (→) correlations of 2.](image)
2.2. Structure Identification of the Known Isolates

The known isolated compounds were easily identified by a comparison of spectroscopic and physical data (1H NMR, UV, MS, [α]D, and IR) with corresponding authentic samples or literature values, and this included two acylphloroglucinol derivatives, sampsonione B (3) [17] and garcinol (4) [16], a biphenyl derivative, 3-hydroxy-5-methoxybiphenyl (5) [18], a ferulic acid ester derivative, 1,24-tetracosanediol diferulate (6) [19], a triterpene, friedelan-3-one (7) [20], and a mixture of steroids, β-sitostenone (8) [21] and stigmasta-4,22-dien-3-one (9) [22].

2.3. Biological Studies

NF-κB [12,13] plays an essential role in inflammatory responses. We previously established an LPS-responsive macrophage cell clone (RAW264.7/Luc-P1), in which NF-κB activity correlates with the luciferase gene expression [23]. The RAW 264.7/Luc-P1 cells allowed us to successfully identify NF-κB-suppressing compounds such as fisetin and methyl isornate [24,25]. Therefore, we applied this system to measure the effects of isolated compounds on NF-κB activity, and their inhibitory activities (with inhibitory percentages) are summarized in Table 2. 13,14-Didehydroxysaxantho-chymol (2) and sampsonione B (3) show significant inhibition of LPS-stimulated NF-κB activity (Figure 8A,B). Moreover, 13,14-didehydroxyaxanthochymol (2) and sampsonione B (3) did not induce cytotoxicity against RAW264.7/Luc-P1 cells after 24 h treatment (Figure 8C,D).

LPS-mediated NF-κB activation results in upregulation of pro-inflammatory molecules, such as NO, in macrophages [12,26]. Thus, NO generation is a hallmark of inflammatory responses. Our study further evaluated the potential anti-inflammatory compounds, 13,14-didehydroxy-axanthochymol (2) and sampsonione B (3) on NO production. The result showed that 13,14-didehydroxyaxanthochymol (2) did not obviously affect LPS-induced NO generation in RAW264.7 macrophages and did not display cytotoxicity against RAW 264.7 cells after 24 h treatment (Figure 9A,C). In contrast, sampsonione B (3) could suppress LPS-induced NO generation in a concentration-dependent manner (Figure 9B) without causing significant cytotoxicity (Figure 9D).

It is observed that the inhibition on NO production is in a close correlation with NF-κB activation (e.g. compound 3 in Figures 8B and 9B). Thus, compound 3 may be involved in NF-κB-dependent NO regulation.
Figure 8. The compounds 2 and 3 inhibit NF-κB activation in lipopolysaccharide (LPS)-induced RAW 264.7/Luc-P1 cells. The luciferase activities of 13,14-didehydroxyisoxanthochymol (2) (A) and sampsonione B (3) (B) in LPS-stimulated RAW 264.7/Luc-P1 macrophages were observed. Andrographolide is the positive control. The cell viability of RAW 264.7/Luc-P1 cells incubated with 13,14-didehydroxyisoxanthochymol (2) (C) or sampsonione B (3) (D) for 24 h was measured using MTT assay. * indicates significant difference vs. LPS-treated vehicle control (p < 0.05).

Figure 9. The effects of compounds 2 and 3 on nitric oxide (NO) production in LPS-induced RAW 264.7 macrophages. The effects of 13,14-didehydroxyisoxanthochymol (2) (A) and sampsonione B (3) (B) in LPS-treated RAW 264.7 macrophages were detected by Griess reagent. Andrographolide is the positive control. The cell viability of RAW 264.7 cells incubated with 13,14-didehydroxyisoxanthochymol (2) (C) or sampsonione B (3) (D) for 24 h was measured using MTT assay. * indicates significant difference vs. LPS-treated vehicle control (p < 0.05).
Table 2. The effects of compounds 1–7 from the stems of *Garcinia multiflora* on NF-κB activation in RAW 264.7/Luc-P1 cells.

| Compounds a                                                                 | Relative Luciferase Activity | Inhibition (%) e   |
|-----------------------------------------------------------------------------|------------------------------|-------------------|
|                                                                             | Mean ± SD d                  | Mean ± SD d       |
| 13,14-Didehydroxygarcicowin C (1)                                            | 0.78 ± 0.11                  | 21 ± 11           |
| 13,14-Didehydroxyisoxanthochymol (2)                                         | 0.75 ± 0.03 *                | 24 ± 4 *          |
| Sampsonione B (3)                                                           | 0.12 ± 0.03 *                | 88 ± 4 *          |
| Garcinol (4)                                                                | 1.23 ± 0.21                  |                   |
| 3-Hydroxy-5-methoxybiphenyl (5)                                             | 0.85 ± 0.06                  | 14 ± 3            |
| 1,24-Tetracosanediol diferulate (6)                                          | 0.96 ± 0.08                  | 3 ± 10            |
| Friedelan-3-one (7)                                                         | 1.04 ± 0.20                  |                   |
| LPS-treated vehicle control b                                               | 0.94 ± 0.09                  | 5 ± 9             |
| Andrographolide c                                                           | 0.31 ± 0.05 *                | 70 ± 5 *          |

a Compounds 1–7: 30 μM. b Vehicle control: 0.1% DMSO. c Andrographolide (30 μM) is the positive control. d Data are displayed as the mean ± SD from three independent experiments. e * indicates significant difference versus lipopolysaccharide (LPS) (1 μg/mL)-treated vehicle control (*p < 0.05). e Inhibition (%) = [1 − luciferase activity (compounds)/luciferase activity (LPS-treated control)] × 100.

3. Experimental Section

3.1. General Procedures

Ultraviolet (UV) spectra were measured on a Jasco UV-240 spectrophotometer (Jasco Co., Hachioji, Japan). Optical rotations were measured using a Jasco DIP-370 polarimeter (Jasco Co., Hachioji, Japan) in MeOH. CD spectra were obtained on a Jasco J-815 spectropolarimeter (Jasco Co., Hachioji, Japan). Infrared (IR) spectra (neat or KBr) were determined on a Perkin Elmer 2000 FT-IR spectrometer (Perkin-Elmer Corp., Waltham, MA, USA). Nuclear magnetic resonance (NMR) spectra, including nuclear Overhauser effect spectrometry (NOESY), correlation spectroscopy (COSY), heteronuclear single-quantum coherence (HSQC), and heteronuclear multiple-bond correlation (HMBC) experiments, were measured on a Varian Inova 500 spectrometer (Varian, Palo Alto, CA, USA) operating at 125 MHz (13C) and 500 MHz (1H), respectively, with chemical shifts given in ppm (δ) using tetramethylsilane (TMS) as an internal standard. Electrospray ionization (ESI) and high-resolution electrospray ionization (HRESI)-mass spectra were determined on a Bruker APEX II mass spectrometer (Bruker, Billerica, MA, USA). Silica gel 60 F-254 (Merck, Darmstadt, Germany) was used for preparative thin-layer chromatography (PTLC) and thin-layer chromatography (TLC). Silica gel (70–230 mesh and 230–400 mesh, Merck) was used for column chromatography (CC).

3.2. Plant Material

The stems of *G. multiflora* were collected from Mudan, Pingtung County, Taiwan, in December 2012 and identified by Dr. M. H. Yen (School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Taiwan). A voucher specimen (GM-201212) was deposited in the Faculty of Pharmacy, School of Pharmaceutical Sciences, National Yang-Ming University, Taipei, Taiwan.

3.3. Extraction and Isolation

The dried stems of *G. multiflora* (5.4 kg) were extracted three times with MeOH (10 L each) for 3 days. The MeOH extracts were concentrated under reduced pressure at 35 °C, and the residue (211 g) was partitioned between EtOAc and H2O (1:1). The EtOAc layer was concentrated to give a residue (fraction A, 118 g). The water layer was further extracted with n-BuOH, and the water-soluble part (fraction C, 41 g) and the n-BuOH-soluble part (fraction B, 45 g) were separated. Fraction A (118 g) was separated on silica gel (70–230 mesh, 5.2 kg), eluting with CH2Cl2, gradually increasing the polarity with MeOH to give 13 fractions: A1 (2 L, CH2Cl2), A2 (2 L, CH2Cl2/MeOH, 90:1), A3 (2 L, CH2Cl2/MeOH, 80:1), A4 (1 L, CH2Cl2/MeOH, 50:1), A5 (1 L, CH2Cl2/MeOH, 40:1), A6 (2 L, CH2Cl2/MeOH, 30:1), A7 (2 L, CH2Cl2/MeOH, 20:1), A8 (5 L, CH2Cl2/MeOH, 10:1),
A9 (7 L, CH$_2$Cl$_2$/MeOH, 5:1), A10 (2 L, CH$_2$Cl$_2$/MeOH, 4:1), A11 (4 L, CH$_2$Cl$_2$/MeOH, 2:1), A12 (5 L, CH$_2$Cl$_2$/MeOH, 1:1), and A13 (5 L, MeOH).

Fraction A2 (5.6 g) was chromatographed further on silica gel (70–230 mesh, 250 g) eluting with n-hexane/acetone (20:1–0:1) to give 10 fractions (each 1.2 L, A2-1–A2-10). Compound 7 (7.2 mg) was yielded from fraction A2-1 (85 mg) by recrystallization with n-hexane/EtOAc. Compounds 8 and 9 (12.2 mg) were obtained from fraction A2-2 (358 mg) by recrystallization with n-hexane/EtOAc.

Fraction A2-4 (126 mg) was purified further by preparative TLC (silica gel, CH$_2$Cl$_2$) to obtain 1 (5.2 mg) and 2 (2.3 mg). Part (168 mg) of fraction A2-5 was purified by preparative TLC (silica gel, n-hexane/EtOAc, 6:1) to obtain 3 (2.2 mg). Part (120 mg) of fraction A2-7 was purified by preparative TLC (silica gel, n-hexane/EtOAc, 3:1) to afford 5 (2.2 mg). Part (89 mg) of fraction A2-9 was purified by preparative TLC (silica gel, CHCl$_3$) to obtain 6 (2.5 mg).

Fraction A3 (4.5 g) was chromatographed further on silica gel (230–400 mesh, 205 g) eluting with n-hexane/EtOAc (15:1–0:1) to give 7 fractions (each 1 L, A3-1–A3-7). Part (172 mg) of fraction A3-5 was purified by preparative TLC (silica gel, n-hexane/acetone, 2:1) to afford 4 (6.2 mg).

13,14-didehydroxygarcicowin C (1): amorphous powder; [α]$_{D}^{25}$ = −68.6 (c 0.18, CHCl$_3$); CD (MeOH, ∆ε): 311 (+2.1), 267 (−8.5), 223 (+5.2) nm; UV (MeOH): λ$_{max}$ (log ε) = 250 (3.90), 273 (sh, 3.77) nm; IR (KBr): υ$_{max}$ = 1729 (C=O), 1682 (C=O), 1645 (C=O) cm$^{-1}$; ESI-MS: m/z = 569 [M + H]$^+$; HR-ESI-MS: m/z = 569.36284 [M + H]$^+$ (calcd for C$_{38}$H$_{49}$O$_4$, 569.36254); $^1$H and $^{13}$C NMR data: see Table 1.

13,14-didehydroxyisoxanthochymol (2): amorphous powder; [α]$_{D}^{25}$ = +205.7 (c 0.15, CHCl$_3$); CD (MeOH, ∆ε): 270 (+13.6), 224 (−8.4) nm; UV (MeOH): λ$_{max}$ (log ε) = 203 (4.16), 249 (3.96), 276 (sh, 4.02) nm; IR (KBr): υ$_{max}$ = 1724 (C=O), 1675 (C=O), 1637 (C=O) cm$^{-1}$; ESI-MS: m/z = 571 [M + H]$^+$; HR-ESI-MS: m/z = 571.37822 [M + H]$^+$ (calcd for C$_{38}$H$_{51}$O$_4$, 571.37819); $^1$H and $^{13}$C NMR data: see Table 1.

### 3.4. Biological Assay

The effect of the isolates on LPS-induced NF-κB activation in RAW 264.7/Luc-P1 macrophage was assessed by determining the luminescence resulted from luciferase activity in a concentration-dependent manner. The purity of the tested compounds was >98% as identified by MS and NMR.

#### 3.4.1. Cells and Culture Medium

The RAW 264.7/Luc-P1 cell is an LPS-responsive cell line with an integrated reporter gene (pELAM1-Luc) [23]. The murine RAW 264.7 macrophage and RAW 264.7/Luc-P1 cells were cultured and originated conditions as described previously [23,24].

#### 3.4.2. Luciferase Reporter Assay

The RAW 264.7/Luc-P1 cells (1.5 × 10$^5$ cells in 24-well plates) were treated with pure compounds, the positive control (30 µM andrographolide) or vehicle (0.1% DMSO) for 1 h and then LPS (10 ng/mL) for 23 h. The treated cells were then collected and assessed using luciferase assays (Promega, Madison, WI, USA) as described previously [25].

#### 3.4.3. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) Assay

RAW 264.7/Luc-P1 cells or RAW 264.7 cells (10$^4$ cells in 96-well plates) were treated with 13,14-didehydroxyisoxanthochymol (2), sampsonione B (3) and 0.1% DMSO for 24 h. MTT assays were performed as described previously [25].

#### 3.4.4. Nitric Oxide (NO) Production

The RAW 264.7 cells (4 × 10$^4$ cells in 96-well plates) were treated with 13,14-didehydroxyisoxanthochymol (2), sampsonione B (3) and 0.1% DMSO for 1 h and then incubated with LPS (1µg/mL) for 23 h. The 100 µL of cell culture medium with an equal volume of Griess reagent (0.1% naphthylethylene diamine dihydrochloride and 1% sulfanilamide in 2.5% phosphoric acid) in a
96-well plate was incubated for 10 min. The absorbance at 550 nm was measured by using a Model 680 Microplate Reader (Bio-rad, Hercules, CA, USA). The level of NO production was calculated from sodium nitrite (NaNO₂) standard curve [27].

3.4.5. Statistical Analysis

The data are displayed as mean ± SD from three independent experiments. Statistical analysis was performed using Student’s t test. Differences were considered as statistically significant when \( p < 0.05 \).

4. Conclusions

Our research on the phytochemical investigation of *G. multiflora* has led to the isolation of two new (1, 2) and seven known (3–9) compounds. The structures of these isolates were established by spectroscopic data. Based on the results of our bioactivity assays, among the isolates, 13,14-didehydroxyisoxanthochymol (2) and sampsonione B (3) exhibited inhibition against lipopolysaccharide (LPS)-induced NF-κB activation in macrophages at 30 µM with relative luciferase activity values of 0.75 ± 0.03 and 0.12 ± 0.03, respectively. Furthermore, sampsonione B (3) showed LPS-induced NO generation in concentration dependent manner. Thus, our research suggests *G. multiflora* and its isolated compound (especially 3) are worth further study and may be expectantly developed as the candidates for the prevention or treatment of diverse inflammatory diseases.

**Supplementary Materials**: Supplementary materials are available online, Figures S1–S8: MS, 1D, and 2D-NMR spectra for 13,14-didehydroxygarcicowin C (1), Figures S9–S16: MS, 1D, and 2D-NMR spectra for 13,14-didehydroxyisoxanthochymol (2).

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

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Sample Availability: Samples of the compounds are available from the authors.