Article

Anti-Inflammatory Constituents from *Bidens frondosa*

Jiamei Le [1,2,†], Wenquan Lu [1,†], Xiaojuan Xiong [2], Zhijun Wu [1,*] and Wansheng Chen [1,*]

[1] Department of Pharmacy, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China; E-Mails: lejiamei0923@163.com (J.L.); lwqp@163.com (W.L.)

[2] College of Chemical and Biological Engineering, Yichun University, Jiangxi 336000, China; E-Mail: ycxxxj@163.com

[†] These authors contributed equally to this work.

[*] Authors to whom correspondence should be addressed; E-Mails: wuzhijun999@sina.com (Z.W.); chenws126@126.com or chenwansheng@smmu.edu.cn (W.C.); Tel.: +86-21-8188-6181 (W.C.); Fax: +86-21-3310-0038 (W.C.).

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Abstract: A new polyacetylene glucoside (3*E*,5*E*,11*E*)-tridecatriene-7,9-diyne-1,2,13-triol-2-*O*-β-D-glucopyranoside (**1**), a new phenylpropanoid glucoside 2′-butoxyethylconiferin (**2**), and a new flavonoid glycoside 8,3′,4′-trihydroxyflavone-7-*O*-(6″-*O*-*p*-coumaroyl)-β-D-glucopyranoside (**3**), have been isolated from *Bidens frondosa* together with fifty-three known compounds **4–56**. The structures of these compounds were established by spectroscopic methods. mainly ESIMS, 1D- and 2D-NMR spectroscopic data. and comparison with literature data. Compounds **1–34**, **36**, **39**, **43**, **47**, **51**, and **52** were tested for inhibition of nuclear factor kappa B (NF-κB) in 293-NF-κB-luciferase report cell line induced by lipopolysaccharide (LPS), and compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51** were tested for the production of TNF-α, IL-1β, IL-6, IL-10 in RAW 264.7 macrophages induced by LPS. In conclusion, the isolated compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51** exhibited significant activity in anti-inflammatory activity assays.

Keywords: *Bidens frondosa*; polyacetylene glucoside; phenylpropanoid glucoside; flavonoid glycoside; anti-inflammatory activity

1. Introduction

Bidens frondosa (L.) is an annual herbaceous plant growing widely on nutrient-rich mudsoils or muddy sandsoils at the shores of rivers and lakes, which is one of species of genus *Bidens* from the family Asteraceae. It is native to North America, and now distributed throughout China. It has attracted a great deal of attention for its wide range of biological activities, such as antibacterial [1], antioxidant [2], antimalarial [3] and antidiarrheal [4]. Previous studies reported that *B. frondosa* contained acetylene [3], polyenic D-glucosides [5,6], chalcones and aurones [2,3,7,8], flavones and terpenes [9]. However, a literature survey reveals that there are no reports on the anti-inflammatory activity of *B. frondosa*, and the research on chemical constituents of *B. frondosa* is also limited. Thus we decided to isolate chemical constituents of *B. frondosa* and to investigate its anti-inflammatory activity.

As part of an ongoing search for bioactive natural products from traditional folk medicines, we have conducted a phytochemical investigation on the EtOH extract of *B. frondosa*. This has led to the isolation of a new polyacetylene glucoside, a new phenylpropanoid glucoside, a new flavonoid glycoside and fifty-three known compounds, identified as (3*E*,5*E*,11*E*)-tridecatriene-7,9-diyne-1,2,13-triol-2-*O*-β-D-glucopyranoside (**1**), 2′-butoxyethylconiferin (**2**), 8,3′,4′-trihydroxyflavone-7-*O*-(6″-*O*-*p*-coumaroyl)-β-D-glucopyranoside (**3**), butylconiferin (**4**), 2-methoxy-4-(2-propenyl)-phenyl-β-D-glucopyranoside (**5**), 2-methoxy-4-(2′-hydroxyethyl)-phenol-1-*O*-β-D-glucopyranoside (**6**), (1′*R*,2′*R*)-guaiacyl glycerol 3′-*O*-β-D-glucopyranoside (**7**), *threo*-5-hydroxy-3,7-dimethoxyphenylpropane-8,9-diol (**8**), 3-(4-hydroxy-3-methoxyphenyl)-3-methoxypropane-1,2-diol (**9**), 3-(4-hydroxy-3-methoxy-phenyl)-propane-1,2-diol (**10**), guaiacylglycerol (**11**), ananasate (**12**), *p*-hydroxyphenyl-6-*O*-*trans*-caffeoyl-β-D-alloside (**13**), 6′-*O*-caffeoyl-*p*-hydroxyacetophenone-4-*O*-β-D-glucopyranoside (**14**), wilfordiol B (**15**), 4,5-di-*O*-caffeoylquinic acid 1-methyl ether (**16**), caffeoylcalleryanin (**17**), 1-*O*-(*E*)-caffeoyl-β-D-gentiobiose (**18**), plantasioside (**19**), okanin-4′-*O*-(6″-*O*-*p*-coumaroyl-β-D-glucopyranoside) (**20**), (−)-4′-methoxy-7-*O*-(6″-acetyl)-β-D-glucopyranosyl-8,3′-dihydroxyflavanone (**21**), (−)-4′-methoxy-7-*O*-β-D-glucopyranosyl-8,3′-dihydroxyflavanone (**22**), hesperetin-7-*O*-β-D-glucopyranoside (**23**), apigenin (**24**), 3′-hydroxyscutellarein-7-*O*-(6″-*O*-protocatechuoyl)-β-glucopyranoside (**25**), quercetin-3-*O*-glucopyranoside (**26**), 8,3′,4′-trihydroxyflavone-7-*O*-β-D-glucopyranoside (**27**), luteolin-7-*O*-glucoside (**28**), 6-hydroxyluteolin-7-*O*-glucoside (**29**), 3″-(3-hydroxy-3-methyl-glutaroyl)-ester of 6-hydroxy-luteolin-7-*O*-β-D-glucopyranoside (**30**), luteolin-7-*O*-(β-D-glucopyranosyl)-2-glucopyranoside (**31**), (*Z*)-7-*O*-β-D-glucopyranosyl-6,7,3′,4′-tetrahydroxyaurone (**32**), sulfuretin-6-*O*-β-D-glucoside (**33**), maritimetin-6-*O*-β-D-glucoside (**34**), dihydrophaseic acid (**35**), caffeic acid (**36**), isoferuloyl ethyl ester (**37**), protocatechuic acid (**38**), 1,3,5-trimethoxybenzene (**39**), vanillin (**40**), 7*R*,11*R*-phytol (**41**), 1-octacosanol (**42**), indole-3-carboxylic acid (**43**), 1*H*-indole-3-carboxadehyde (**44**), niacinamide (**45**), β-sitosterol (**46**), stigmasterol (**47**), hiziprafuran (**48**), 5-hydroxy-2-furaldehyde (**49**), α-tocopherol (**50**), 4-hydroxy-2-furaldehyde (**51**), α-tocospiro A (**52**), ethyl linoleate (**53**), methyl linolenate (**54**), tripalmitolein (**55**) and trilinolenin (**56**). Compounds **1**–**34**, **36**, **39**, **43**, **47**, **51**, and **52** were tested for anti-inflammatory activities, and several of the isolated compounds (**1**, **2**, **3**, **9**, **15**, **21**, **24** and **51**) exhibited significant activities in anti-inflammatory activity assays. Here, we report the isolation of these compounds, structure elucidation of three new compounds and anti-inflammatory activities of compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51**.

2. Results and Discussion

The 80% EtOH extract of air-dried *B. frondosa* was suspended in H_2O and partitioned successively with petroleum ether, EtOAc and *n*-BuOH, respectively. The petroleum ether, EtOAc and *n*-BuOH fractions were then purified repeatedly by column chromatography with silica gel, Sephadex LH-20 and ODS C18 to yield compounds **1–56**.

2.1. Structural Elucidation of Isolated Compounds

Compound **1** was isolated as a brownish amorphous powder. The positive HR-ESIMS showed a pseudo-molecular ion at *m/z* 403.1371 [M + Na]$^+$ (calcd. for $C_{19}H_{24}O_8Na$, 403.1369), consistent with the formula $C_{19}H_{24}O_8$, indicating the presence of eight degrees of unsaturation. The IR spectrum showed absorptions due to hydroxy (3362 cm^{-1}) and olefinic bond (1658, 1632 cm^{-1}) moieties. The ^{13}C-NMR spectrum exhibited 19 carbon resonances, classified into four quaternary carbons, twelve methines including five oxygen-substituted carbons, and three oxygen-substituted methylenes. By analysis of degrees of unsaturation, four quaternary carbon signals at δ_C 81.9, 81.5, 76.2, 73.8 were ascribed to two acetylene bonds. The signals at δ_C 149.5, 145.5, 137.9, 129.8, 109.1, 106.4 were ascribed to three double bonds. The signals at δ_C 102.7, 77.1, 76.8, 74.0, 70.2, 61.0 were assigned to a glucopyranosyl unit. In the ^{1}H-NMR spectrum, the signals at δ_H 6.81 (1H, dd, *J* = 15.6, 10.8 Hz), 6.48 (1H, ddd, *J* = 16.2, 9.0, 8.4 Hz), 6.41 (1H, dd, *J* = 15.6, 10.8 Hz), 6.00 (1H, dd, *J* = 15.6, 5.4 Hz), 5.88 (1H, d, *J* = 15.6 Hz), 5.86 (1H, d, *J* = 16.2 Hz) implied the presence of three conjugated double bonds with *E* geometry as deduced from the coupling constants [10]. The ^{1}H- and ^{13}C-NMR spectra of **1** were quite similar to those of (2*E*,8*E*,10*E*)-tridecatriene-4,6-diyne-1,12,13-triol-1-*O*-β-D-glucopyranoside (**1a**) [11] (Figure S1). A comparison of its spectrum with **1a** showed that the glucopyranosyl unit was linked at C-2 in compound **1** instead of C-1 in compound **1a**. HMBC correlation from H-1′ (δ_H 4.27, d, *J* = 7.8 Hz) to C-2 (δ_C 79.9) confirmed the glucopyranosyl unit was linked at C-2. The configuration of C-2 remained undetermined. Therefore, the structure of **1** was elucidated as (3*E*,5*E*,11*E*)-tridecatriene-7,9-diyne-1,2,13-triol-2-*O*-β-D-glucopyranoside (Figure 1).
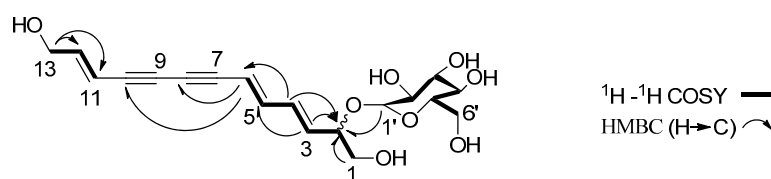

Figure 1. Key ^{1}H-^{1}H COSY and HMBC (H→C) correlations of compound **1**.

Compound **2** was isolated as a white amorphous powder. The negative HR-ESIMS showed a pseudo-molecular ion at *m/z* 477.1917 [M + Cl]$^-$ (calcd for $C_{22}H_{34}O_9Cl$, 477.1891), consistent with the formula $C_{22}H_{34}O_9$, indicating the presence of six degrees of unsaturation. The ^{13}C-NMR spectrum exhibited 22 carbon resonances, classified into three quaternary carbons, ten methines including four oxygen-substituted carbons, seven methylenes including five oxygen-substituted carbons, one methyl group and one methoxyl group. By analysis of degrees of unsaturation, the signals at δ_C 133.3, 125.9 were ascribed to a double bond and the signals at δ_C 150.9, 147.8, 133.4, 120.9, 117.9, 111.4 were

ascribed to an aromatic ring. The DEPT spectrum showed signals at δ_C 71.2, 70.5 and 72.1, 32.8, 20.3, 14.2 that were ascribed to a *-O-C-1′-C-2′-O-* diether chain and a terminal *-O-C-1″-C-2″-C-3″-C-4″* chain, respectively The signals at δ_C 102.7, 78.2, 77.8, 74.9, 71.3, 62.5 were assigned to a glucopyranosyl unit. In the ^{1}H-NMR spectrum, the signals at δ_H 6.54 (1H, d, *J* = 15.6 Hz), 6.20 (1H, dd, *J* = 15.6, 6.0 Hz) implied the presence of a conjugated double bond with *E* geometry as deduced from the coupling constants. The signals at δ_H 7.06 (1H, d, *J* = 8.4 Hz), 7.03 (1H, d, *J* = 1.8 Hz), 6.90 (1H, dd, *J* = 8.4, 1.8 Hz) showed an ABX aromatic ring spin system and implied the presence of 1,3,4-trisubstituted aromatic ring [10]. The ^{1}H- and ^{13}C-NMR spectra of **2** were quite similar to those of ethylconiferin (**2a**) [12] (Figure S1). Detailed comparison of the NMR data of **2** with **2a** indicated that there was an additional butoxy group in compound **2**. In the HMBC spectrum of **2**, a correlation from H-2′ (δ_H 3.56) to the C-1″ (δ_C 72.1) position of the butoxy was observed, which suggested the butoxy was linked at C-2′. The structure of **2** was established on the basis of 1D- and 2D-NMR spectra (^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC). Accordingly, the structure of compound **2** was elucidated as 2′-butoxyethylconiferin (Figure 2). In addition, we have found compound **2** was present in a crude extract before contact with butanol, which was tested by HPLC (Figure S24). From HR-ESIMS, 1D- and 2D-NMR spectroscopic data (Figure S9–S15) and the HPLC profile, we think that compound **2** is a natural chemical constituent and not an isolation artefact.
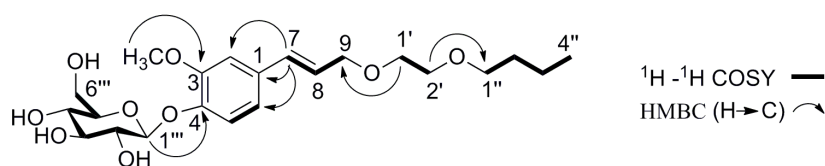

Figure 2. Key ^{1}H-^{1}H COSY and HMBC (H→C) correlations of compound **2**.

Compound **3** was isolated as a brownish-yellow amorphous powder. The negative HR-ESIMS showed a pseudo-molecular ion at *m/z* 593.1316 [M − H]⁻ (calcd for $C_{30}H_{25}O_{13}$, 593.1295), consistent with the formula $C_{30}H_{26}O_{13}$, indicating the presence of 18 degrees of unsaturation. The IR spectrum showed absorptions due to hydroxy (3405 cm⁻¹), aromatic ring (1513, 1443 cm⁻¹) and olefinic bonds (1688, 1649 cm⁻¹). The ^{13}C-NMR spectrum exhibited 30 carbon resonances, classified into twelve quaternary carbons, seventeen methines including four oxygen-substituted carbons, one oxygen-substituted methylenes. The signals at δ_C 182.6, 154.4, 152.3, 145.8, 132.8, 117.4, 114.4, 113.2, 112.0 were ascribed to a flavonoids skeleton. The signals at δ_C 166.7, 160.2, 145.2, 130.6, 125.3, 116.1, 114.2 were ascribed to a *p*-coumaroyl group. The signals at δ_C 101.4, 75.8, 74.4, 73.4, 70.2, 63.5 were assigned to a glucopyranosyl unit. In the ^{1}H-NMR spectrum, the signals at δ_H 7.54 (1H, d, *J* = 16.2 Hz) and 6.39 (1H, d, *J* = 16.2 Hz) implied the presence of a conjugated double bond with *E* geometry as deduced from the coupling constants. The signals at δ_H 7.44 (1H, d, *J* = 1.8 Hz), 7.33 (1H, dd, *J* = 7.8, 1.8 Hz) and 6.85 (1H, d, *J* = 7.8 Hz) showed an aromatic ring ABX spin system and implied the presence of a 1,3,4-trisubstituted aromatic ring. The signals at δ_H 7.53 (2H, d, *J* = 9.0 Hz) and 6.79 (2H, d, *J* = 9.0 Hz) implied the presence of a 1,4-disubstituted aromatic ring [10]. A comparison of its spectrum with that of 8,3′,4′-trihydroxyflavone-7-*O*-β-D-glucopyranoside (**27**), showed that the obvious difference in **3** was an additional *p*-coumaroyl [13] (Figure S1). HMBC correlation from H-6″ (δ_H 4.44, 4.21) to C-9‴ (δ_C 166.7) suggested the *p*-coumaroyl group was linked at C-6″. Accordingly,

compound **3** was elucidated as 8,3′,4′-trihydroxyflavone-7-*O*-(6″-*O*-*p*-coumaroyl)-β-D-glucopyranoside (Figure 3).
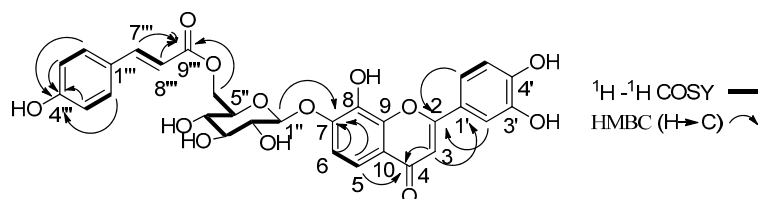

Figure 3. Key ¹H-¹H COSY and HMBC (H→C) correlations of compound **3**.

In addition to three new glucosides **1**–**3**, fifty-three known compounds, identified as butylconiferin (**4**) [14], 2-methoxy-4-(2-propenyl)-phenyl-β-D-glucopyranoside (**5**) [15], 2-methoxy-4-(2′-hydroxy-ethyl)-phenol-1-*O*-β-D-glucopyranoside (**6**) [16], (1′*R*,2′*R*)-guaiacyl glycerol 3′-*O*-β-D-glucopyranoside (**7**) [17], *threo*-5-hydroxy-3,7-dimethoxyphenylpropane-8,9-diol (**8**) [18], 3-(4-hydroxy-3-methoxy-phenyl)-3-methoxypropane-1,2-diol (**9**) [19], 3-(4-hydroxy-3-methoxyphenyl)-propane-1,2-diol (**10**) [20], guaiacylglycerol (**11**) [21], ananasate (**12**) [22], *p*-hydroxyphenyl-6-*O*-trans-caffeoyl-β-D-alloside (**13**) [23], 6′-*O*-caffeoyl-*p*-hydroxyacetophenone-4-*O*-β-D-glucopyranoside (**14**) [24], wilfordiol B (**15**) [25], 4,5-di-*O*-caffeoylquinic acid 1-methyl ether (**16**) [26], caffeoylcalleryanin (**17**) [27], 1-*O*-(*E*)-caffeoyl-β-D-gentiobiose (**18**) [28], plantasioside (**19**) [29], okanin-4′-*O*-(6″-*O*-*p*-coumaroyl-β-D-glucopyranoside) (**20**) [8], (-)-4′-methoxy-7-*O*-(6″-acetyl)-β-D-glucopyranosyl-8,3′-dihydroxy-flavanone (**21**), (−)-4′-methoxy-7-*O*-β-D-glucopyranosyl-8,3′-dihydroxyflavanone (**22**) [30], hesperetin-7-*O*-β-D-glucopyranoside (**23**) [31], apigenin (**24**) [32], 3′-hydroxyscutellarein-7-*O*-(6″-*O*-proto-catechuoyl)-β-glucopyranoside (**25**) [33], quercetin-3-*O*-glucopyranoside (**26**) [34], 8,3′,4′-trihydroxyflavone-7-*O*-β-D-glucopyranoside (**27**) [13], luteolin-7-*O*-glucoside (**28**), 6-hydroxyluteolin-7-*O*-glucoside (**29**) [35], 3″-(3-hydroxy-3-methylglutaroyl)-ester of 6-hydroxyluteolin-7-*O*-β-D-glucopyranoside (**30**) [36], luteolin-7-*O*-(β-D-glucopyranosyl)-2-glucopyranoside (**31**) [37], (*Z*)-7-*O*-β-D-glucopyranosyl-6,7,3′,4′-tetrahydroxyaurone (**32**) [38], sulfuretin-6-*O*-β-D-glucoside (**33**), maritimetin-6-*O*-β-D-glucoside (**34**) [39], dihydrophaseic acid (**35**) [40], caffeic acid (**36**), protocatechuic acid (**38**) [41], isoferuloyl ethylester (**37**) [42], 1,3,5-trimethoxybenzene (**39**) [43], vanillin (**40**) [44], 7*R*,11*R*-phytol (**41**) [45], 1-octacosanol (**42**) [46], indole-3-carboxylic acid (**43**) [47], 1*H*-indole-3-carboxadehyde (**44**) [48], niacinamide (**45**) [49], β-sitosterol (**46**) [50], stigmasterol (**47**) [51], hiziprafuran (**48**) [52], 5-hydroxy-2-furaldehyde (**49**) [53], α-tocopherol (**50**) [54], 4-hydroxy-2-furaldehyde (**51**) [55], α-tocospiro A (**52**) [56], ethyl linoleate (**53**), methyl linolenate (**54**) [57], tripalmitolein (**55**) [58], trilinolenin (**56**) [59] were isolated from *B. frondosa*. These compounds were identified by spectral analysis, and we found their spectral data were consistent with spectroscopic data reported in the corresponding literature.

2.2. Anti-Inflammatory Activity

Compounds **1**–**34**, **36**, **39**, **43**, **47**, **51**, and **52** were evaluated for their anti-inflammatory activities in a luciferase assay (Table S1). Compared with the cell group, the luciferase activity of the LPS group was significantly enhanced, which indicated that the inflammatory cell model induced by LPS was constructed successfully. Then we have found the luciferase activity of compounds (**1**, **2**, **3**, **9**, **15**, **21**, **24** and **51**) group were significantly decreased by comparing with the LPS group, which showed that

they had significant inhibitory effect on NF-κB activity. In addition, the luciferase activity decreased with the increase of sample concentration, which indicated that the inhibition of NF-κB activity was dose-dependent. In conclusion, compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51** showed significant inhibitory effect on NF-κB in 293-NF-κB-luciferase report cell line induced by LPS (Figure 4). The effects of compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51** on the inflammatory response were investigated further. The anti-inflammatory effects were evaluated by investigating the inhibitory activity of the compounds on the production of TNF-α, IL-1β, IL-6, and IL-10 in RAW 264.7 macrophages induced by LPS. For all assays, ibuprofen was used as a positive control. We have found the content of inflammatory cytokines TNF-α, IL-6, IL-1, IL-10 of the LPS group were significantly increased by comparing with the experimental results of the Cell group, which indicated that the monocyte RAW264.7 induced by LPS was constructed successfully. Compared with the LPS group, compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51** exhibited significant inhibitory activity on the production of above inflammation factors tested *in vitro* at concentrations of 1, 10 and 100 μg/mL, and the inhibition activity was dose-dependent (Figures 5–8).
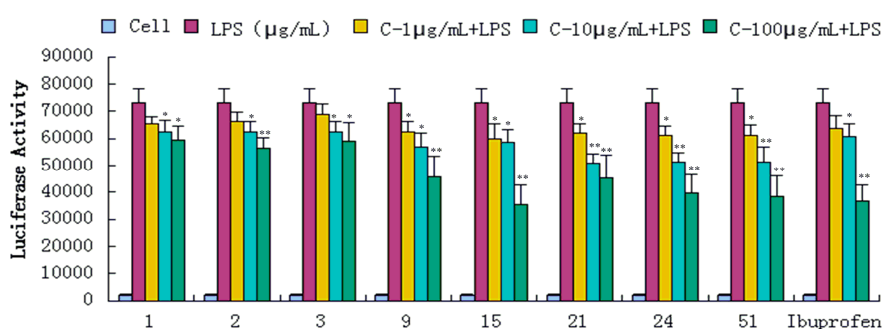

Figure 4. Inhibitory effects of compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51** (1, 10, 100 μg/mL) on NF-κB in luciferase activity assay. Data are expressed as mean ± S.E.M. of three independent experiments. Cell: cultures were not exposed to lipopolysaccharide (LPS); LPS: cultures were subjected to LPS; LPS + Drug: Compounds were added to the cultures during LPS; Positive control: LPS + Ibuprofen. * $p < 0.05$ *vs.* LPS; ** $p < 0.01$ *vs.* LPS.
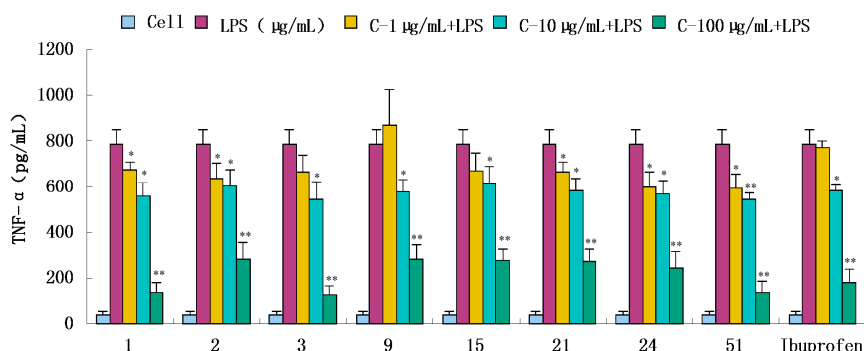

Figure 5. Inhibitory effects of compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51** (1, 10, 100 μg/mL) on TNF-α production stimulated by LPS (10 μg/mL) in RAW 264.7 cells (mouse leukemic monocyte macrophage cell line). Data are expressed as mean ± S.E.M. of three independent experiments. Cell: cultures were not exposed to lipopolysaccharide (LPS); LPS: cultures were subjected to LPS; LPS + Drug: Compounds were added to the cultures during LPS; Positive control: LPS + Ibuprofen. * $p < 0.05$ *vs.* LPS; ** $p < 0.01$ *vs.* LPS.
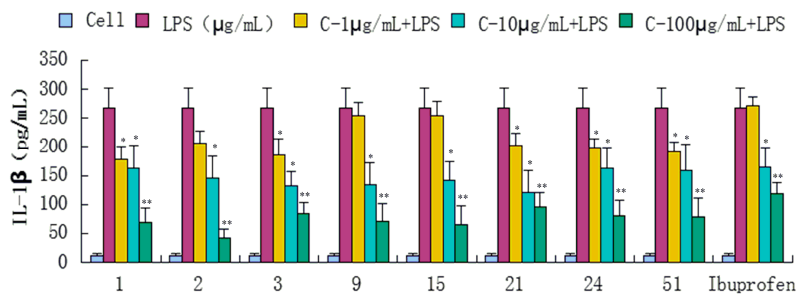
Figure 6. Inhibitory effects of compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51** (1, 10, 100 μg/mL) on IL-1β production stimulated by LPS (10 μg/mL) in RAW 264.7 cells (mouse leukemic monocyte macrophage cell line). Data are expressed as mean ± S.E.M. of three independent experiments. Cell: cultures were not exposed to lipopolysaccharide (LPS); LPS: cultures were subjected to LPS; LPS + Drug: Compounds were added to the cultures during LPS; Positive control: LPS + Ibuprofen. * $p < 0.05$ *vs.* LPS; ** $p < 0.01$ *vs.* LPS.

Figure 7. Inhibitory effects of compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51** (1, 10, 100 μg/mL) on IL-6 production stimulated by LPS (10 μg/mL) in RAW 264.7 cells (mouse leukemic monocyte macrophage cell line). Data are expressed as mean ± S.E.M. of three independent experiments. Cell: cultures were not exposed to lipopolysaccharide (LPS); LPS: cultures were subjected to LPS; LPS + Drug: Compounds were added to the cultures during LPS; Positive control: LPS + Ibuprofen. * $p < 0.05$ *vs.* LPS; ** $p < 0.01$ *vs.* LPS.
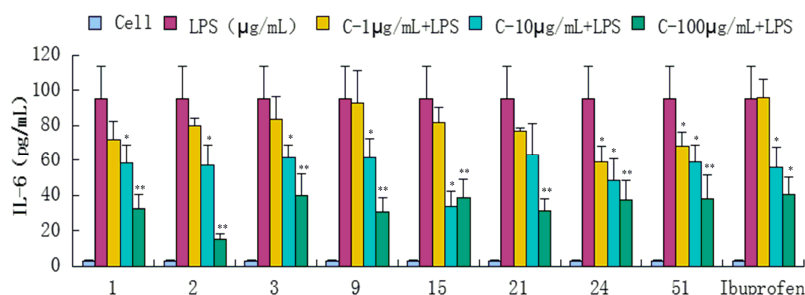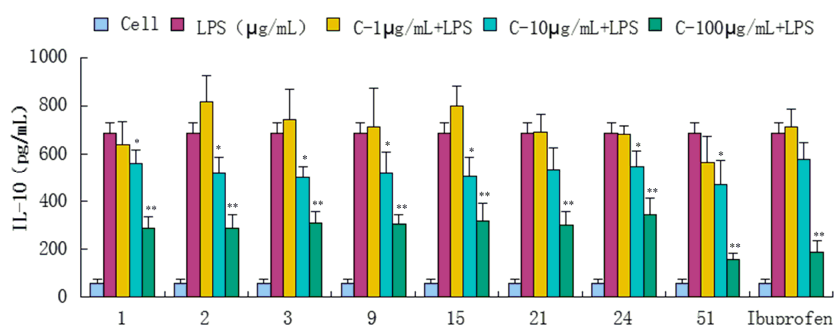

Figure 8. Inhibitory effects of compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51** (1, 10, 100 μg/mL) on IL-10 production stimulated by LPS (10 μg/mL) in RAW 264.7 cells (mouse leukemic monocyte macrophage cell line). Data are expressed as mean ± S.E.M. of three independent experiments. Cell: cultures were not exposed to lipopolysaccharide (LPS); LPS: cultures were subjected to LPS; LPS + Drug: Compounds were added to the cultures during LPS; Positive control: LPS + Ibuprofen. * $p < 0.05$ *vs.* LPS; ** $p < 0.01$ *vs.* LPS.

3. Experimental Section

3.1. General Procedures

Optical rotations were measured on a Perkin-Elmer 341 Polarimeter (Perkin Elmer, Fremont, CA, USA). IR analyses were performed with a NEXUS 470 FT-IR spectrometer (Thermo-Nicolet, Madison, WI, USA). UV spectra were recorded on Shimadzu UV/VIS-240 recording spectrophotometer (Shimadzu, Tokyo, Japan). 1D- and 2D-NMR spectra were obtained on a Bruker Avance 600 NMR spectrometer (Bruker, Karlsruhe, Germany). HR-ESIMS were acquired on an Agilent 6220 TOF LC-MS instrument (Agilent, Santa Clara, CA, USA). Column chromatography was performed by using silica gel (100–200 and 200–300 mesh; Yantai Jiangyou Silica Gel Development Co. Ltd., Yantai, China), ODS (50 μm; YMC, Wilmington, NC, USA), MCI GEL (75–150 μm; Mitsubishi Chemical Corporation, Tokyo, Japan), Sephadex LH-20 (40–70 μm; Pharmacia Company, Uppsala, Sweden). Semi-preparative HPLC isolation was achieved with an Agilent 1200 instrument (Agilent, Santa Clara, CA, USA) equipped with a refractive index detector (RID), using a C18 column (250 mm × 10 mm × 5 μm, YMC) and eluting with MeOH–H_2O at 2.0 mL/min. Precoated silica gel GF$_{254}$ and HF$_{254}$ plates were used for TLC, and Zones were visualized under UV light (254 nm and 365 nm) or by spraying with 10% H_2SO_4–EtOH followed by heating.

3.2. Plant Material

The plant of *B. Frondosa* was collected from Jiujiang, Jiangxi Province, during July 2013, and identified by Ceming Tan (Jiujiang Forest Herbarium, Jiangxi, China). A voucher specimen (No. 20130729) was deposited at the Department of Pharmacognosy of the Second Military Medical University.

3.3. Extraction and Isolation

The air-dried aerial parts of *B. frondosa* (10 kg) were extracted three times with 80% EtOH (100 L) under reflux. After removal of the solvent by evaporation under vacuum, the residue was suspended in water (10 L) and then successively partitioned with petroleum ether, EtOAc and *n*-BuOH (3 × 15 L), respectively.

The petroleum ether-soluble part (279 g) was fractionated by silica gel CC and and eluted with petroleum ether–ethyl acetate in increasing polarity (100:1→10:1) to yield six fractions (Fr.1–Fr.6). Fr.1 (42 g) was subjected to repeated silica gel CC using a step gradient of petroleum ether–EtOAc (80:1→60:1→40:1→20:1→10:1) and further purified by Sephadex LH-20 column eluted with CH_2Cl_2–MeOH (1:1) to afford compounds **55** (85 mg). Fr.2 (50 g) was chromatographed on silica gel column and eluted with increasing polarities of petroleum ether–EtOAc (60:1→30:1→10:1) to give four subfractions (Fr.2-1–Fr.2-4). Fr.2-1 (5 g) was chromatographed on silica gel CC using a step gradient of petroleum ether–EtOAc (50:1→20:1→10:1) and finally purified by Sephadex LH-20 column eluted with CH_2Cl_2–MeOH (1:1) to yield compound **53** (196 mg). Fr.2-2 (1.4 g) and Fr.2-4 (2 g) was respectively separated by Sephadex LH-20 column eluted with CH_2Cl_2–MeOH (1:1) to yield compound **56** (500 mg). Fr.3 (43 g) was subjected to repeated silica gel CC using a step gradient of petroleum ether–EtOAc (40:1→30:1→20:1→10:1→5:1) and further purified by Sephadex LH-20

column eluted with CH$_2$Cl$_2$–MeOH (1:1) to yield compounds **54** (450 mg). Fr.4 (40 g) was subjected to repeated silica gel CC using a step gradient of petroleum ether–EtOAc (30:1→20:1→10:1→5:1→1:1) and further purified by Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (1:1) to yield compounds **42** (2 mg). Fr.5 (34 g) was subjected to repeated Silica gel CC using a step gradient of petroleum ether–EtOAc (20:1→10:1→5:1→1:1) and further purified by Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (1:1) to afford compounds **41** (27 mg).

The EtOAc-soluble part (214 g) was fractionated by silica gel CC eluted with CH$_2$Cl$_2$–MeOH (50:1→4:1) to give eight fractions (Fr.7–Fr.14). Fr.7 (30 g) was subjected to repeated silica gel CC eluted with CH$_2$Cl$_2$-MeOH (40:1→20:1→10:1→4:1) and further purified by Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (1:1) to afford compounds **47** (8 mg), **50** (3 mg) and **52** (10 mg). Fr.8 (29 g) was subjected to silica gel CC eluted with CH$_2$Cl$_2$–MeOH (30:1→20:1→10:1→4:1), further separated by ODS CC eluted with MeOH–H$_2$O (40%→60%→80%) and finally purified by Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (1:1) to afford compounds **46** (100 mg), **48** (23 mg) and **49** (19 mg). Fr.9 (23 g) was subjected to silica gel CC eluted with CH$_2$Cl$_2$–MeOH (20:1→10:1→4:1), further separated by ODS CC eluted with MeOH–H$_2$O (40%→60%→80%) and finally purified by Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (1:1) to afford compounds **43** (12 mg) and **44** (3 mg). Fr.10 (25 g) was subjected to silica gel CC eluted with CH$_2$Cl$_2$–MeOH (20:1→10:1→4:1→1:1) and finally purified by Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (1:1) to afford compounds **45** (10 mg) and **51** (4 mg). Fr.11 (24 g) was subjected to ODS CC eluted with MeOH–H$_2$O (40%→60%→80%) and finally purified by Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (1:1) to afford compound **40** (17 mg). Fr.12 (15 g) was subjected to repeated ODS CC eluted with MeOH–H$_2$O (40%→60%→80%) and finally purified by Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (1:1) to afford compounds **35** (14 mg) and **39** (6 mg). Fr.13 (23 g) was subjected to ODS CC eluted with MeOH–H$_2$O (40%→60%→80%) , further separated by silica gel CC eluted with CH$_2$Cl$_2$-MeOH (10:1→4:1→1:1) and finally purified by Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (1:1) to afford compounds **36** (74 mg) and **38** (574 mg). Fr.14 (17 g) was subjected to ODS CC eluted with MeOH–H$_2$O (50%→70%→90%) and finally purified by Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (1:1) to afford compound **37** (3 mg).

The *n*-BuOH-soluble part (325 g) was fractionated by silica gel CC eluted with CH$_2$Cl$_2$–MeOH (20:1→2:1) to give five fractions (Fr.15–Fr.19). Fr.15 (45 g) was subjected to ODS column with a step gradient elution (50%→80%, MeOH in H$_2$O) to afford two subfractions (Fr.15-1–Fr.15-2). Fr.15-1 (15 g) was purified by semi-preparative HPLC (30% MeOH–H$_2$O, 2 mL/min) to give compounds **2** (2 mg), **4** (5 mg), **7** (2 mg) and **13** (3 mg). Fr.15-2 (20 g) was purified by Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (1:1) to afford compounds **5** (595 mg), **6** (5 mg) and **28** (12 mg). Fr.16 (60 g) was subjected to MCI column with a step gradient elution (50%→70%→90%, MeOH in H$_2$O) to afford three subfractions (Fr.16-1–Fr.16-3). Fr.16-1 (18 g) was purified by semi-preparative HPLC (25% MeOH–H$_2$O, 2 mL/min) to give compounds **8** (13 mg), **9** (2 mg) and **10** (4 mg). Fr.16-2 (20 g) was purified by Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (1:1) to give compounds **11** (5 mg) and 1**2** (7 mg). Fr.16-3 (10 g) was purified by semi-preparative HPLC (40% MeOH–H$_2$O, 2 mL/min) to give compounds **1** (4 mg) and **33** (4 mg). Fr.17 (65 g) was subjected to ODS column with a step gradient elution (5%–20%–50%–70%–90%, MeOH in H$_2$O) to afford two subfractions (Fr.17-1–Fr.17-2). Fr.17-1 (28 g) was purified by semi-preparative HPLC (25% MeOH–H$_2$O, 2 mL/min)

to give compounds **18** (20 mg), **19** (20 mg), **21** (23 mg) and **22** (7 mg). Fr.17-2 (29 g) was purified by semi-preparative HPLC (35% MeOH–H$_2$O, 2 mL/min) to give compounds **14 (**8 mg) and **20** (6 mg). Fr.18 (58 g) was subjected to MCI GEL column with a step gradient elution (5%–20%–50%–70%–90%, MeOH in H$_2$O) to afford two subfractions (Fr.18-1–Fr.18-2). Fr.18-1 (21 g) was purified by semi-preparative HPLC (30% MeOH–H$_2$O, 2 mL/min) to give compounds **23** (4 mg), **26** (5 mg), **32** (20 mg) and **34** (32 mg). Fr.18-2 (25 g) was purified by semi-preparative HPLC (60% MeOH–H$_2$O, 2 mL/min) to give compounds **15** (57 mg), **16** (8 mg) and **24** (3 mg). Fr.19 (58 g) was subjected to ODS column with a step gradient elution (5%–20%–50%–70%–100%, MeOH in H$_2$O) to afford two subfractions (Fr.19-1–Fr.19-2). Fr.19-1 (22 g) was purified by semi-preparative HPLC (30% MeOH–H$_2$O, 2 mL/min) to give compounds **3** (15 mg), **27** (14 mg), **29** (5 mg) and **30** (5 mg). Fr.19-2 (21 g) was purified by semi-preparative HPLC (65% MeOH-H$_2$O, 2 mL/min) to give compounds **17** (11 mg), **25** (15 mg) and **31** (11 mg).

3.4. Luciferase Assay

The NF-κB 293 cells were cultured in a DMEM medium supplemented with 10% fetal bovine serum (FBS). The cells were pretreated with these forty compounds at concentrations of 1, 10 and 100 μg/mL for 4 h and then stimulated with 10 μg/mL lipopolysaccharide (LPS) for 24 h. The cells were rinsed twice with phosphate-buffered saline (PBS, pH 7.4) and lysed with passive lysis buffer (Promega, Madison, WI, USA). Then inhibitory effect on NF-κB was analyzed using the luciferase assay system (Promega) according to the manufacturer′s instructions [60].

3.5. Measurement of TNF-α, IL-1β, IL-6, and IL-10

The cells were cultured in serum-free medium for 8 h and then incubated in medium containing 1, 10 and 100 μg/mL of compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51** for 2 h. The cells were then treated with 10 μg/mL of LPS for 24 h. Ibuprofen (1, 10 and 100 μg/mL) was used as a positive control. The supernatants of cell culture were harvested and centrifuged at 3000× *g* at 4 °C for 2 min for the analysis of TNF-α, IL-1β, IL-6, and IL-10. Enzyme-linked immunosorbent assays for detecting the cytokines in the supernatants were carried out according to the instructions provided by the manufacturer. Finally, the standard provided with the kits was used to quantify each cytokine in the supernatants [61].

3.6. Characterization of Compounds

(3E,5E,11E)-Tridecatriene-7,9-diyne-1,2,13-triol-2-O-β-D-glucopyranoside (**1**): brownish amorphous powder; $[\alpha]_D^{20}$ −16.25 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 339, 317, 298, 268, 253 nm; IR (KBr) ν_{max} 3362, 3005, 2922, 2852, 2025, 1658, 1632, 1467, 1423, 1411, 1383, 1075, 812 cm^{-1}; ^{1}H-NMR (DMSO-*d$_6$*, 600 MHz): δ 6.81 (1H, dd, *J* = 15.6, 10.8 Hz, H-5), 6.48 (1H, ddd, *J* = 16.2, 9.0, 8.4 Hz, H-12), 6.41 (1H, dd, *J* = 15.6, 10.8 Hz, H-4), 6.00 (1H, dd, *J* = 15.6, 5.4 Hz, H-3), 5.88 (1H, d, *J* = 15.6 Hz, H-6), 5.86 (1H, d, *J* = 16.2Hz, H-11), 4.27 (1H, d, *J* = 7.8 Hz, H-1′), 4.22 (1H, d, *J* = 4.8 Hz, H-2), 4.05 (2H, dd, *J* = 4.2, 2.4 Hz, H-13), 3.62 (1H, d, *J* = 10.2 Hz, H-6′α), 3.45 (2H, m, H-1), 3.43 (1H, m, H-6′β), 3.14 (1H, m, H-5′), 3.05 (1H, m, H-3′), 3.04 (1H, m, H-4′), 2.97 (1H, m, H-2′).

¹³C-NMR (DMSO-d_6, 150 MHz) : δ 63.7 (C-1), 79.9 (C-2), 137.9 (C-3), 129.8 (C-4), 145.5 (C-5), 109.1 (C-6), 81.5 (C-7), 76.2 (C-8), 81.9 (C-9), 73.8 (C-10), 106.4 (C-11), 149.5 (C-12), 61.2 (C-13), 102.7 (C-1′), 74.0 (C-2′), 77.1 (C-3′), 70.2 (C-4′), 76.8 (C-5′), 61.0 (C-6′). HR-ESIMS: *m/z* 403.1371 [M + Na]⁺ (calcd. for $C_{19}H_{24}O_8Na$, 403.1369).

2′-Butoxyethylconiferin (**2**): white amorphous powder; $[\alpha]_D^{20}$ −27.22 (*c* 0.06, MeOH); UV(MeOH) λ_{max} (log ε) 259, 202 nm; IR (KBr) ν_{max} 3438, 2922, 2853, 2026, 1725, 1710, 1630, 1512, 1462, 13834, 1127 cm⁻¹; ¹H-NMR (CD₃OD, 600 MHz): δ 7.06 (1H, d, *J* = 8.4 Hz, H-5), 7.03 (1H, d, *J* = 1.8 Hz, H-2), 6.90 (1H, dd, *J* = 8.4, 1.8 Hz, H-6), 6.54 (1H, d, *J* = 15.6 Hz, H-7), 6.20 (1H, dd, *J* = 15.6, 6.0 Hz, H-8), 4.83 (1H, overlap, H-1‴), 4.12 (2H, dd, *J* = 6.0, 1.2 Hz, H-9), 3.83 (3H, s, OCH₃), 3.84 (1H, m, H-6‴α), 3.65 (1H, m, H-6‴β), 3.58 (2H, m, H-1′), 3.56 (2H, m, H-2′), 3.46 (1H, m, H-4‴), 3.45 (1H, m, H-2‴), 3.44 (2H, m, H-1″), 3.36 (1H, m, H-3‴), 3.35 (1H, m, H-5‴), 1.52 (2H, m, H-2″), 1.34 (2H, m, H-3″), 0.88 (3H, t, *J* = 7.2 Hz, H-4″). ¹³C-NMR (CD₃OD, 150MHz): δ 133.4 (C-1), 111.4 (C-2), 150.9 (C-3), 147.8 (C-4), 117.9 (C-5), 120.9 (C-6), 133.3 (C-7), 125.9 (C-8), 72.7 (C-9), 70.5 (C-1′), 71.2 (C-2′), 72.1 (C-1″), 32.8 (C-2″), 20.3 (C-3″), 14.2 (C-4″), 102.7 (C-1‴), 74.9 (C-2‴), 78.2 (C-3‴), 71.3 (C-4″), 77.8 (C-5‴), 62.5 (C-6‴), 56.7 (OCH₃). HR-ESIMS *m/z* 477.1917 [M + Cl]⁻ (calcd. for $C_{22}H_{34}O_9Cl$, 477.1891).

8,3′,4′-Trihydroxyflavone-7-O-(6″-O-p-coumaroyl)-β-D-glucopyranoside (**3**): brownish-yellow amorphous powder; $[\alpha]_D^{25}$ +7.22 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 414, 316 nm; IR (KBr) ν_{max} 3405, 2923, 2853, 2026, 1688, 1649, 1603, 1513, 1443, 1383, 1278, 1168, 1129, 1076 cm⁻¹; ¹H-NMR (DMSO-d_6, 600 MHz): δ 7.54 (1H, d, *J* = 16.2 Hz, H-7‴), 7.53 (2H, d, *J* = 9.0 Hz, H-2‴, 5‴), 7.44 (1H, d, *J* = 1.8 Hz, H-2′), 7.33 (1H, dd, *J* = 7.8, 1.8 Hz, H-6′), 7.11 (1H, d, *J* = 8.4 Hz, H-5), 7.04 (1H, d, *J* = 8.4 Hz, H-6), 6.85 (1H, d, *J* = 7.8 Hz, H-5′), 6.79 (2H, d, *J* = 9.0 Hz, H-3‴, 6″), 6.67 (1H, s, H-3), 6.39 (1H, d, *J* = 16.2 Hz, H-8‴), 5.02 (1H, d, *J* = 7.8 Hz, H-1″), 4.44 (1H, d, *J* = 9.6 Hz, H-6″α), 4.21 (1H, dd, *J* = 12.0, 7.2 Hz, H-6″β), 3.75 (1H, m, H-5″), 3.43 (1H, m, H-2″), 3.37 (1H, m, H-3″), 3.27 (1H, m, H-4″). ¹³C-NMR (DMSO-d_6, 150 MHz) : δ 145.8 (C-2), 113.2 (C-3), 182.6 (C-4), 114.4 (C-5), 112.0 (C-6), 152.3 (C-7), 132.8 (C-8), 117.4 (C-9), 154.4 (C-10), 123.6 (C-1′), 118.6 (C-2′), 145.7 (C-3′), 148.7 (C-4′), 116.3 (C-5′), 125.2 (C-6′)), 101.4 (C-1″), 73.4 (C-2″), 75.8 (C-3″), 70.2 (C-4″), 74.4 (C-5″), 63.5 (C-6″), 125.3 (C-1‴), 130.6 (C-2‴), 116.1 (C-3‴), 160.2 (C-4‴), 116.1 (C-5‴), 130.6 (C-6‴), 145.2 (C-7‴), 114.2 (C-8‴), 166.7 (C-9‴). HR-ESIMS: *m/z* 593.1316 [M − H]⁻ (calcd for $C_{30}H_{25}O_{13}$, 593.1295).

4. Conclusions

A new polyacetylene glucoside (3*E*,5*E*,11*E*)-tridecatriene-7,9-diyne-1,2,13-triol-2-*O*-β-D-glucopyranoside (**1**), a new phenylpropanoid glucoside, 2′-butoxyethylconiferin (**2**), and a new flavonoid glycoside, 8,3′,4′-trihydroxyflavone-7-*O*-(6″-*O*-*p*-coumaroyl)-β-D-glucopyranoside (**3**), together with fifty-three known compounds **4–56** have been obtained from *B. frondosa*. Except compounds **20**, **24**, **26**, **28**, **33**, **34**, the other compounds were reported to isolate from *B. frondosa* for the first time. Compounds **1**, **2**, **3**, **9**, **15**, **21**, **24** and **51** showed significant activities in anti-inflammatory assays. And they exhibited good anti-inflammatory effects in a dose-dependent manner. The observed potential anti-inflammatory activity warrants further investigations.

Supplementary Materials

Supplementary data (Figure S1, 1D-, 2D-NMR spectra and HR-ESIMS data of compounds **1**, **2** and **3**, HPLC profiles of compounds **1**, **2**, **3**, **9**, **15**, **21**, **24**, **51** and EtOH extract of *B. Frondosa*, Table S1) associated with this article are available online.

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/20/10/18496/s1.

Acknowledgments

Author Contributions

Zhijun Wu and Wansheng Chen designed the study. Jiamei Le and Zhijun Wu performed the experiment and wrote the manuscript. Jiamei Le and Wenquan Lu analyzed the data. Xiaojuan Xiong collected and identified the *B. frondosa*. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Rahman, A.; Bajpai, V.K.; Dung, N.T.; Kang, S.C. Antibacterial and antioxidant activities of the essential oil and methanol extracts of *Bidens frondosa* Linn. *Int. J. Food Sci. Tech.* **2011**, *46*, 1238–1244.
2. Venkateswarlu, S.; Panchagnula, G.K.; Subbaraju, G.V. Synthesis and antioxidative activity of 3′,4′,6,7-tetrahydroxyaurone, a metabolite of *Bidens frondosa*. *Biosci. Biotech. Bioch.* **2004**, *68*, 2183–2185.
3. Brandao, M.G.; Krettli, A.U.; Soares, L.S.; Nery, C.G.; Marinuzzi, H.C. Antimalarial activity of extracts and fractions from *Bidens pilosa* and other *Bidens* species (Asteraceae) correlated with the presence of acetylene and flavonoid compounds. *J. Ethnopharmacol.* **1997**, *57*, 131–138.
4. Zhao, J.; Wang, C.G. Antidiarrheal activity of *Bidens frondosa*. *J. Nanjing Univ. TCM* **1999**, *15*, 299–300.
5. Pagani, F.; Romussi, G. A new polyinic D-glucoside from *Bidens frondosa* flowers. *Phytochemistry* **1971**, *10*, 2233.
6. Pagani, F.; Romussi, G.; Bohhnann, F. Notiz uber die struktur des polyin-glucosids aus *Bidens frondosa* L. *Chem. Ber.* **1972**, *10*, 3126–3127.
7. Romussi, G.; Pagani, F. Constituents of *Bidens frondosa* L. *Boll. Chim. Farm.* **1970**, *109*, 467–475.
8. Karikome, H.; Ogawa, K.; Sashida, Y. New acylated glucosides of chalcone from the leaves of *Bidens frondosa*. *Chem. Pharm. Bull.* **1992**, *40*, 689–691.

9. Le, J.M.; Wu, Z.J.; Xiong, X.J. Chemical constituents of *Bidens frondosa* L. *Chin. Pharm. J.* **2014**, *49*, 38–42.

10. Xi, F.M.; Li, C.T.; Han, J.; Yu, S.S.; Wu, Z.J.; Chen, W.S. Thiophenes, polyacetylenes and terpenes from the aerial parts of *Eclipata prostrata*. *Bioorgan. Med. Chem.* **2014**, *22*, 6515–6522.

11. He, J.; Shen, Y.; Jiang, J.S.; Yang, Y.N.; Feng, Z.M.; Zhang, P.C.; Yuan, S.P.; Hou, Q. New polyacetylene glucosides from the florets of *Carthamus tinctorius* and their weak anti-inflammatory activities. *Carbohyd. Res.* **2011**, *346*, 1903–1908.

12. Yue, Z.G.; Qin, H.; Li, Y.H.; Sun, Y.; Wang, Z.P.; Yang, T.H.; Liu, L.; Wang, M.C.; Feng, F.; Mei, Q.B. Chemical Constituents of the Root of *Jasminum giraldii*. *Molecules* **2013**, *18*, 4766–4775.

13. Yang, X.W.; Huang, M.Z.; Jin, Y.S.; Sun, L.N.; Song, Y.; Chen, H.S. Phenolics from *Bidens bipinnata* and their amylase inhibitory properties. *Fitoterapia* **2012**, *83*, 1169–1175.

14. Dai, Z.; Wang, G.L.; Lin, R.C. Chemical constituents of *Balanophora simaoensis* (III). *Chin. Tradit. Herb Drugs* **2006**, *37*, 1608–1610.

15. Shimoda, K.; Kondo, Y.; Nishida, T.; Hamada, H.; Nakajima, N.; Hamada, H. Biotransformation of thymol, carvacrol, and eugenol by cultured cells of *Eucalyptus perriniana*. *Phytochemistry* **2006**, *67*, 2256–2261.

16. Chen, Y.J.; Wang, H.T.; He, L.P.; Zhang, H.G.; Yin, J.L. Study on chemical constituents from bark of *Fraxinus mandschurica* Rupr. *Chin. Pharm. J.* **2009**, *44*, 1133–1136.

17. Ishikawa, T.; Fujimatu, E.; Kitajima, J. Water-soluble constituents of anise: New glucosides of anethole glycol and its related compounds. *Chem. Pharm. Bull.* **2002**, *50*, 1460–1466.

18. Liu, Y.B.; Qin, J.J.; Cheng, X.R.; Zhu, J.X.; Zhang, F.; Jin, H.Z.; Zhang, W.D. Three new neolignans and one new phenylpropanoid from the leaves and stems of *Toona ciliata* var. *Pubescen*. *Helv. Chim. Acta* **2011**, *94*, 1685–1691.

19. Zhao, L.; Li, L.Z.; Peng, Y.; Song, S.J. Isolation and identification of chemical constituents from seeds of *Crataegus pinnatifida* Bge. *J. Shenyang Pharm. Univ.* **2012**, *29*, 9–11.

20. Kikuzaki, H.; Hara, S.; Kawai, Y.; Nakatani, N. Antioxidative phenylpropanoids from berries of *Pimenta dioica*. *Phytochemistry* **1999**, *52*, 1307–1312.

21. Ouyang, M.A.; Chen, P.Q.; Wang, S.B. Water-soluble phenylpropanoid constituents from aerial roots of *Ficus microcarpa*. *Nat. Prod. Re*s. **2007**, *21*, 769–774.

22. Wang, W.; Ding, Y.; Xing, D.M.; Wang, J.P.; Du, L.J. Studies on phenolic constituents from leaves of pineapple(*Ananas comosus*). *Chin. J. Chin. Mater. Med.* **2006**, *31*, 1242–1244.

23. Machida, K.; Nakano, Y.; Kikuchi, M. Phenolic glycosides from *Viburnum dilatatum*. *Phytochemistry* **1991**, *30*, 2013–2014.

24. Ma, H.Y.; Sun, Y.; Zhou, Y.Z.; Hong, M.; Pei, Y.H. Two new constituents from *Artemisia capillaris* Thunb. *Molecules* **2008**, *13*, 267–271.

25. Cao, X.; Li, C.J.; Yang, J.Z.; Wei, B.X.; Yuan, S.P.; Luo, Y.M.; Hou, Q.; Zhang, D.M. Four new neolignans from the leaves of *Tripterygium wilfordii*. *Fitoterapia* **2012**, *83*, 343–347.

26. Aboul-Ela, M.A.; El-Lakany, A.M.; Abdel-Kader, M.S.; Alqasoumi, S.I.; Shams-El-Din, S.M.; Hammoda, H.M. New quinic acid derivatives from hepatoprotective *Inula crithmoides* root extract. *Helv. Chim. Acta.* **2012**, *95*, 61–66.

27. Brandao, G.C.; Kroon, E.G.; Souza, D.E.; Souza Filho, J.D.; Oliveira, A.B. Chemistry and antiviral activity of *Arrabidaea pulchra* (Bignoniaceae). *Molecules* **2013**, *18*, 9919–9932.

28. Jiang, Z.H.; Hirose, Y.; Iwata, H.; Sakamoto, S.; Tanaka, T.; Kouno, I. Caffeoyl, coumaroyl, galloyl, and hexahydroxydiphenoyl glucoses from *Balanophora japonica*. *Chem. Pharm. Bull.* **2001**, *49*, 887–892.

29. Nishibe, S.; Tamayama, Y.; Sasahara, M.; Andary, C. A phenylethanoid glycoside from *Plantago asiatica*. *Phytochemistry* **1995**, *38*, 741–743.

30. Silva, D.B.; Okano, L.T.; Lopes, N.P.; de Oliveira, D.C. Flavanone glycosides from *Bidens gardneri* Bak. (Asteraceae). *Phytochemistry* **2013**, *96*, 418–422.

31. Wang, Y.J.; Yang, X.W.; Guo, Q.S. Studies on chemical constituents in Huangjuhua (flowers *Chrysanthemum morifolium*). *Chin. J. Chin. Mater. Med.* **2008**, *33*, 526–530.

32. Xu, Y.; Wu, C.L.; Liu, Y.; Zhang, Z. Studies on chemical constituents of *Saussurea tangutica* Maxim. *Chin. Tradit. Herb Drugs* **2010**, *41*, 1957–1960.

33. Saracoglu, I.; Varel, M.; Harput, U.S.; Nagatsu, A. Acylated flavonoids and phenol glycosides from *Veronica thymoides* subsp. *Pseudocinerea. Phytochemistry* **2004**, *65*, 2379–2385.

34. Zhou, Z.H.; Yang, C.R. Chemical constituents of crude green tea, the material of Pu-er tea in Yunnan. *Acta Bot. Yunnanica* **2000**, *22*, 343–350.

35. Lu, Y.R.; Foo, L.Y. Flavonoid and phenolic glycosides from *Salvia officinalis*. *Phytochemistry* **2000**, *55*, 263–267.

36. Kraut, L.; Mues, R.; Sim-Sim, M. Acylated flavone and glycerol glucosides from two *Frullania* species. *Phytochemistry* **1993**, *34*, 211–218.

37. Jiang, R.Z.; Chen, Y.H.; Luo, H.M.; Xu, D.D.; Gao, Y.; Gao, Q.P. Isolation and structure identification of water soluble constituents from *Ixeris Sonchifolia. Nat. Prod. Res. Dev.* **2010**, *22*, 430–432.

38. Zhao, A.H.; Zhao, Q.S.; Peng, L.Y.; Zhang, J.X.; Lin, Z.W.; Sun, H.D. A new chalcone glycoside from *Bidens pilosa. Acta Bot. Yunnanica* **2004**, *26*, 121–126.

39. Wang, J.; Wang, N.L.; Yao, X.S.; Kitanaka, S. Structures and anti-histamine activity of chalcones & aurones compounds from *Bidens parviflora* Willd. *Asia. J. Tradit. Med.* **2007**, *2*, 23–29.

40. Cai, L.; Liu, C.S.; Fu, X.W.; Shen, X.J.; Yin, T.P.; Yang, Y.B.; Ding, Z.T. Two new glucosides from the pellicle of the walnut (*Juglans regia*). *Nat. Prod. Bioprospect.* **2012**, *2*, 150–153.

41. Jeong, D.M.; Jung, H.A.; Kang, H.S.; Choi, J.S. Peroxynitrite scavengers from *Phellinus linteus. Nat. Prod. Sci.* **2008**, *14*, 1–11.

42. Wu, Z.J.; Ou-Yang, M.A.; Yang, C.R. Polyphenolic constituents of *Salvia sonchifolia. Acta Bot. Yunnanica* **1999**, *21*, 393–398.

43. Huang, Y.; Chang, R.J.; Jin, H.Z.; Zhang, W.D. Phenolic constituents from *Tsoongiodendron odorum* Chun. *Nat. Prod. Res. Dev.* **2012**, *24*, 176–178.

44. Duan, J.; Li, W.; Hu, X.J.; Koike, K.; Fu, H.Z. Constituents of *Silene rubicunda* Franch. *Chin. Tradit. Herb Drugs* **2009**, *40*, 528–530.

45. Brown, G.D.; Liang, G.Y.; Sy, L.K. Terpenoids from the seeds of *Artemisia annua*. *Phytochemistry* **2003**, *64*, 303–323.

46. Zhang, X.; Lan, Z.; Dong, X.P.; Deng, Y.; Hu, X.M.; Peng, T.; Guo, P. Study on the active components of *Nardostachys chinensis*. *Chin. Med. Mat.* **2007**, *30*, 38–41.

47. Guo, S.H.; Zhong, H.M.; Lin, W.H. Studies on the chemical constituents of the Marine Sponge (*Xetospongia testudinaria*) from the South China Sea. *J. Qingdao Univ. Sci. Technol.* **2007**, *28*, 502–504.

48. Li, X.; Song, S.J.; Piao, S.J.; Dong, T.; Lin, H.W. Studies on chemical constituents of marine sponge *Aplysinopsis* sp. *Chin. J. Mar. Drugs* **2009**, *28*, 21–25.

49. Shi, B.J.; Chou, G.X.; Wang, Z.T. Chemical constituents from *Senecio nemorensis*. *J. Chin. Pharm. Univ.* **2010**, *41*, 26–28.

50. Cheng, J.; Bai, Y.J.; Zhao, Y.Y.; Wang, B.; Cheng, T.M. Studies on the phenylpropanoids from *Eucommia ulmoides. Chin. J. Chin. Mater. Med.* **2002**, *27*, 38–40.

51. Ainiwaer, A.; Rena, K. Studies on the chemical constituents of seed from *Nigella glandulifera*. *Chin. Med. Mat.* **2007**, *30*, 1259–1261.

52. Boukamcha, H.; Jannet, H.B.; Bouazizi, Y.; Mighri, Z. Isolation and structure determination of a novel furanic ester from the aerial part of *Prasium Majus. Nat. Prod. Res.* **2003**, *17*, 63–66.

53. Ai, F.W.; Zhang, S.; Li, Y.F.; Ma, Y.L. Studies on the chemical constituents of *Typhonium giganteum. Chin. Tradit. Herb Drugs* **2010**, *41*, 201–203.

54. Kitajima, J.; Kimizuka, K.; Arai, M.; Tanaka, Y. Constituents of *Ficus pumila* leaves. *Chem. Pharm. Bull.* **1998**, *46*, 1647–1649.

55. Yu, Z.X.; Wang, G.L.; Bian-Ba, C.R.; Lin, R.C. Studies on chemical constituents in root of *Phlomis medicinalis* I. *Chin. J. Chin. Mater. Med.* **2006**, *31*, 656–658.

56. Chiang, Y.M.; Kuo, Y.H. Two novel α-tocopheroids from the aerial roots of *Ficus microcarpa*. *Tetrahedron. Lett.* **2003**, *44*, 5125–5128.

57. Chang, M.H.; Wang, G.J.; Kuo, Y.H.; Lee, C.K. The low polar constituents from *Bidens Pilosa* L. *var. Minor* (Blume) Sherff. *J. Chin. Chem. Soc.* **2000**, *47*, 1131–1136.

58. Retief, L.; Mckenzie, J.M.; Koch, K.R. A novel approach to the rapid assignment of ^{13}C-NMR spectra of major components of vegetable oils such as avocado, mango kernel and macadamia nut oils. *Magn. Reson. Chem.* **2009**, *47*, 771–781.

59. Mannina, L.; Luchinat, C.; Patumi, M.; Emanuele, M.C.; Rossi, E.; Segre, A. Concentration dependence of ^{13}C-NMR spectra of triglycerides: Implications for the NMR analysis of olive oils. *Magn. Reson. Chem.* **2000**, *38*, 886–890.

60. Zeng, N.; Shen, Y.; Li, L.Z.; Jiao, W.H.; Gao, P.Y.; Song, S.J.; Chen, W.S.; Lin, H.W. Anti-inflammatory triterpenes from the leaves of *Rosa laevigata. J. Nat. Prod.* **2011**, *74*, 732–738.

61. Gao, H.; Zhao, F.; Chen, G.D.; Chen, S.D.; Yu, Y.; Yao, Z.H.; Wang, Z.; Li, J.; Yao, X.S. Bidesmoside triterpenoid glycosides from *Stauntonia chinensis* and relationship to anti-inflammation. *Phytochemistry* **2009**, *70*, 795–806.

Sample Availability: Samples of the compounds **1**–**56** are available from the authors.