Anti-Inflammatory Constituents from *Bidens frondosa*

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**Abstract:** A new polyacetylene glucoside (3E,5E,11E)-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 (3E,5E,11E)-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 coumaroaryl)-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), threeo-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-protopocatechuoyl)-β-glucopyranoside (25), quercetin-3-O-glucopyranoside (26), 8,3′,4′-trihydroxyflavonone-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), sulforetin-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), 7R,11R-phytol (41), 1-octacosanol (42), indole-3-carboxylic acid (43), 1H-indole-3-carboxylic acid (44), niacinamide (45), β-sitosterol (46), stigmasterol (47), hiziprafuran (48), 5-hydroxy-2-furaldehyde (49), α-tocopherol (50), 4-hydroxy-2-furaldehyde (51), α-tocospirio A (52), ethyl linolate (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 H₂O 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 C₁₉H₂₄O₈Na, 403.1369), consistent with the formula C₁₉H₂₄O₈, 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 ¹³C-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 \( \delta_C \) 81.9, 81.5, 76.2, 73.8 were ascribed to two acetylene bonds. The signals at \( \delta_C \) 149.5, 145.5, 137.9, 129.8, 109.1, 106.4 were ascribed to three double bonds. The signals at \( \delta_C \) 102.7, 77.1, 76.8, 74.0, 70.2, 61.0 were assigned to a glucopyranosyl unit. In the ¹H-NMR spectrum, the signals at \( \delta_H \) 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, \( J = 16.2 \) Hz) implied the presence of three conjugated double bonds with \( E \) geometry as deduced from the coupling constants [10]. The ¹H- and ¹³C-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-\( \beta \)-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′ (\( \delta_H \) 4.27, d, \( J = 7.8 \) Hz) to C-2 (\( \delta_C \) 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-\( \beta \)-D-glucopyranoside (Figure 1).

![Figure 1. Key ¹H-¹H COSY and HMBC (H→C) correlations of compound 1.](image)

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 C₂₂H₃₄O₉Cl, 477.1891), consistent with the formula C₂₂H₳₄O₉, indicating the presence of six degrees of unsaturation. The ¹³C-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 \( \delta_C \) 133.3, 125.9 were ascribed to a double bond and the signals at \( \delta_C \) 150.9, 147.8, 133.4, 120.9, 117.9, 111.4 were
ascribed to an aromatic ring. The DEPT spectrum showed signals at δC 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 δH 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 δH 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 1H- and 13C-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′ (δH 3.56) to the C-1′ (δC 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 (1H, 13C, 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 1H-1H 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 C30H25O13, 593.1295), consistent with the formula C30H26O13, 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 13C-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 δC 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 δC 166.7, 160.2, 145.2, 130.6, 125.3, 116.1, 114.2 were assigned to a p-coumaroyl group. The signals at δC 101.4, 75.8, 74.4, 73.4, 70.2, 63.5 were ascribed to a glucopyranosyl unit. In the 1H-NMR spectrum, the signals at δH 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 δH 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 δH 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‴ (δH 4.44, 4.21) to C-9‴ (δC 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](image)

**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′-hydroxyethyl)-phenol-1-β-D-glucopyranoside (6) [16], (1′R,2′R)-guaiacyl glycerol 3′-O-β-D-glucopyranoside (7) [17], threeo-5-hydroxy-3,7-dimethoxyphenylpropane-8,9-diol (8) [18], 3-(4-hydroxy-3-methoxyphenyl)-3-methoxypropene-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], caffeoylcherynanin (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′-hydroxyxycutellarein-7-O-(6′-O-proto-catechuoyl)-β-glucopyranoside (25) [33], quercetin-3-O-glucosylranoside (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], (−)-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], 7R,11R-phytol (41) [45], 1-octacosanol (42) [46], indole-3-carboxylic acid (43) [47], 1H-indole-3-carboxdehyde (44) [48], niacinamide (45) [49], β-sitosterol (46) [50], stigmasterol (47) [51], hiziprauran (48) [52], 5-hydroxy-2-furaldehyde (49) [53], α-tocopherol (50) [54], 4-hydroxy-2-furaldehyde (51) [55], α-tocspiro 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–H2O at 2.0 mL/min. Precoated silica gel GF254 and HF254 plates were used for TLC, and Zones were visualized under UV light (254 nm and 365 nm) or by spraying with 10% H2SO4–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 CH2Cl2–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 CH2Cl2–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 CH2Cl2–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 CH2Cl2–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 CH2Cl2–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 CH2Cl2–MeOH (1:1) to afford compounds 41 (27 mg).

The EtOAc-soluble part (214 g) was fractionated by silica gel CC eluted with CH2Cl2–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 CH2Cl2–MeOH (40:1→20:1→10:1→4:1) and further purified by Sephadex LH-20 column eluted with MeOH–H2O (40%→60%→80%) and finally purified by Sephadex LH-20 column eluted with MeOH–H2O (50%→70%→90%) and finally purified by Sephadex LH-20 column eluted with MeOH–H2O (50%→70%→90%).
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–H₂O, 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 H₂O) to afford two subfractions (Fr.18-1–Fr.18-2). Fr.18-1 (21 g) was purified by semi-preparative HPLC (30% MeOH–H₂O, 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–H₂O, 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 H₂O) to afford two subfractions (Fr.19-1–Fr.19-2). Fr.19-1 (22 g) was purified by semi-preparative HPLC (30% MeOH–H₂O, 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–H₂O, 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 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-diyn-1,2,13-triol-2-O-β-D-glucopyranoside (1): brownish amorphous powder; [α]D₂⁰ = −16.25 (c 0.04, MeOH); UV (MeOH) λmax (log ε) 339, 317, 298, 268, 253 nm; IR (KBr) νmax 3362, 3005, 2922, 2852, 2025, 1658, 1632, 1467, 1423, 1411, 1383, 1075, 812 cm⁻¹; 1H-NMR (DMSO-d₆, 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-6α), 3.45 (2H, m, H-1), 3.43 (1H, m, H-6β), 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-d6, 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 C19H24O8Na, 403.1369).

2′-Butoxyethylconiferin (2): white amorphous powder; [α]D20 +27.22 (c 0.06, MeOH); UV(MeOH) λmax (log ε) 259, 202 nm; IR (KBr) νmax 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′′). 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 C22H24O10Cl, 477.1891).

8,3′,4′-Trihydroxyflavone-7-O-(6′-O-p-coumaroyl)-β-D-glycopyranoside (3): brownish-yellow amorphous powder; [α]D20 +7.22 (c 0.03, MeOH); UV (MeOH) λmax (log ε) 414, 316 nm; IR (KBr) νmax 3405, 2923, 2853, 2026, 1688, 1649, 1603, 1513, 1443, 1383, 1278, 1168, 1129, 1076 cm⁻¹; 1H-NMR (DMSO-d6, 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-d6, 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 C30H25O13, 593.1295).

4. Conclusions

A new polyacetylene glucoside (3E,5E,11E)-tridecatriene-7,9-diyn-1,2,13-triol-2-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.

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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.

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

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