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Research Article

Anti-Inflammatory and α-Glucosidase Inhibitory Activities of Chemical Constituents from *Bruguiera parviflora* Leaves

Tung Thanh Bui,[1] Khanh Phuong Thi Nguyen,[1] Phung Phi Kim Nguyen,[2] Dung Tien Le,[1,3] and Thuy Le Thi Nguyen[1]

[1]*Department of Biotechnology, Ho Chi Minh City Open University, Vietnam*
[2]*Department of Organic Chemistry, University of Science, VNU-HCM, Vietnam*
[3]*Department of Pharmaceutical Biochemistry, Institute of Applied Materials Science, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam*

Correspondence should be addressed to Dung Tien Le; inpcdung@yahoo.com and Thuy Le Thi Nguyen; thuy.ntl@ou.edu.vn

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Bruguiera parviflora (Rhizophoraceae) is one of the *Bruguiera* genus-based mangrove plants which has not been investigated for the chemical compositions as well as biological activities so far. The present study was aimed at investigating the phytochemicals as well as anti-inflammatory and α-glucosidase inhibitory activities of *B. parviflora* leaves. The results showed that the crude extract and its fractions significantly increased the percentage inhibitory activity against α-glucosidase and decreased NO production in LPS-stimulated RAW 264.7 cells in a dose-dependent manner. The most effective fraction BP5 was further chromatographed and purified. As a result, eight compounds were isolated and elucidated, including five flavonoids (1–5) and three triterpenoids (6–8). All isolated compounds were evaluated for the anti-inflammatory and α-glucosidase inhibitory effects. The results indicated that flavonoids namely taxifolin (1), quercetin (2), myricetin (3), rutin (4), and kaempferol (5) exhibited potent anti-inflammatory as well as α-glucosidase inhibitory activities. Among them, compound 2 showed the most potent inhibitory effect against an α-glucosidase activity with an IC_{50} value of $3.4 \pm 0.5\,\mu$g/mL and the LPS-induced NO production of $11.8\,\mu$M at the concentration of $100\,\mu$g/mL. These findings suggest that flavonoids (1–5) from *B. parviflora* leaves may be useful as the potential α-glucosidase inhibitor as well as anti-inflammatory agent.

1. Introduction

Medicinal plants have long been recognized as important sources containing bioactive compounds such as phenolics, flavonoids, alkaloids, and triterpenoids. The recent research keeps on contributing important achievements in finding natural compounds that could be of potential medical and pharmacological effects [1]. *Bruguiera*, the largest genus in the family Rhizophoraceae, includes approximately 120 species distributed in mangrove forests from Africa to Asia. Numerous studies reported that *Bruguiera* possesses several biological activities such as antioxidant [2], antifungal, cytotoxic [3], antimalarial [4], antidiabetic [5], and antibacterial activities [6, 7]. Traditionally, fruits of *B. gymnorrhiza* were widely used as drugs to treat eye diseases and herpes [7], and its bark was used as an astringent treatment of diarrhea and malaria [8]. Chemical investigations of *Bruguiera* genus resulted in the isolation of alkaloids, diterpenoids, triterpenoids, and flavonoids [9, 10], while few compounds have been isolated from *B. parviflora*.

Inflammation is the response of the body's immune cells to strange antigens in order to destroy them, but when the inflammatory response is exacerbated and continual, it can damage tissues. Therefore, inflammation is commonly associated with the development and progression of many diseases such as infections, obesity, diabetes, and cancer. Recent studies showed that chronic inflammation is implicated in the pathophysiology of type 2 diabetes, and therefore, targeting inflammation may ameliorate diabetes, preventing its progression and vascular complications [11, 12]. Following up with our interest in research in biological activities as well as phytochemicals of

mangrove plants [13, 14], the objective of this work was to determine the anti-inflammatory and antidiabetic activities of extracts and isolated compounds from *B. parviflora* leaves collected in the Can Gio mangrove forest, Vietnam, in order to confirm their pharmaceutical support.

2. Materials and Methods

2.1. General Experimental Procedure. Column chromatography was performed on silica gel 60 (70–230 mesh, Merck, Darmstadt, German) or Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), monitored by analytical thin-layer chromatography (TLC) on a precoated silica gel 60 F-254 (Merck, Darmstadt, German). Spots were visualized under UV light (254 and 365 nm) or sprayed with 10% H_2SO_4 solution and then heated at 105°C for 5 min. Melting points were determined on an IA9100 Digital Melting Point Apparatus (Cole-Parmer, United States). A spectrometer Bruker Avance III (125 and 500 MHz) was used for NMR spectra. $CDCl_3$ or DMSO-d_6 was used as solvents and TMS as the internal standard. APCI-MS were recorded on an $X500_R$ QTOF-Q mass spectrometer. ESI-MS was recorded by MSQ-Plus-DAD. A mouse macrophage cell line (RAW 264.7) was obtained from the American Type Culture Collection (Manassas, Virginia, USA). Lipopolysaccharide (LPS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), *N*-(1-naphthyl) ethylenediamine dihydrochloride (NED), Griess Reagent, Dulbecco's modified Eagle's medium (DMEM), and fetal bovine serum (FBS) were purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). α-Glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae* (750 UN), *p*-nitrophenyl α-D-glucopyranoside, acarbose, and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). Solvents were of analytical grade, and water was distilled.

2.2. Extraction, Isolation, and Purification. Leaves of *B. parviflora* were collected at the Can Gio mangrove forest, Ho Chi Minh City, Vietnam, in August 2019. A voucher specimen (No. US-B014) was deposited in the laboratory of the Faculty of Biotechnology, Ho Chi Minh City Open University, Vietnam.

The crude ethanol residue (300 g) of dried leave powder of *B. parviflora* (3 kg) was obtained by the maceration method with ethanol (5 × 40 L) at room temperature. This crude was fractionated by flash column chromatography with solvent systems of *n*-hexane and ethyl acetate (step-wise, 10 : 0, 9 : 1, 8 : 2, 5 : 5, and 0 : 10, *v*/*v*) then ethyl acetate and methanol (step-wise, 9 : 1, 8 : 2, 5 : 5, and 0 : 10, *v*/*v*) to afford nine fractions (BP1–9). A sample was taken from each fraction for preliminary screening of anti-inflammatory and α-glucosidase inhibitory activities. The bioassay result showed that the fraction BP5 was potent; therefore, it was chosen to be chemically studied.

The fraction BP5 (200 mg) was fractionated by a silica gel column chromatography using a mixture of *n*-hexane and ethyl acetate (95 : 5 to 0 : 100, *v*/*v*) then ethyl acetate and methanol (0 : 100 to 100 : 0, *v*/*v*) to yield ten subfractions (BP5.1–10). The subfraction BP5.1 (37.8 g) was chromatographed on a silica gel column with *n*-hexane-ethyl acetate (0 : 100 to 100 : 0, *v*/*v*) as eluent to obtain four subfractions (BP5.1.1–4). The subfraction BP5.1.2 (80 mg) was rechromatographed on a Sephadex LH–20 eluting with chloroform–methanol (1 : 1, *v*/*v*) to obtain compounds 1 (10.2 mg) and 4 (7.2 mg). The subfraction BP5.1.3 (128 mg) was further subjected to a silica gel column and eluted with *n*-hexane-chloroform (95 : 5 to 0 : 100, *v*/*v*) to get compounds 3 (5.1 mg) and 6 (14.2 mg). The fraction BP5.3 (33.3 g) was chromatographed on Sephadex LH–20 using chloroform–methanol (1 : 1, *v*/*v*) as eluent to obtain four compounds, 5 (6.7 mg), 2 (9.8 mg), 7 (8.8 mg), and 8 (9.5 mg).

2.3. Anti-Inflammatory Assay

2.3.1. Cell Culture. The RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% L-glutamine. The cells were incubated at 37°C in humidified atmosphere containing 5% CO_2.

2.3.2. Cell Viability. Cells (3×10^5) were cultured in 96 well plates containing DMEM supplemented with 10% FBS and incubated at 37°C, 5% carbon dioxide for 24 hours. After, the cells were treated with 100 μL of samples (25, 50, 75, and 100 μg/mL) in the presence of 100 μg/mL LPS and incubated for 24 hours with 5% CO_2 at 37°C. Then, 20 μL of MTT solution (5 mg/mL) was added to each well and further incubated for 2 hours. MTT is reduced by mitochondrial dehydrogenases to the water-insoluble pink compound formazan, depending on the viability of cells. After incubation, the medium was removed, and the remaining formazan crystals were dissolved with 100 μL of dimethyl sulfoxide (DMSO). After 30 minutes, the absorbance was measured at 570 nm by using a microplate reader. The percentages of cell viability were calculated using the following equation:

$$\text{Cell viability } (\%) = \frac{[A]_{sample}}{[A]_{control}} \times 100. \quad (1)$$

Dexamethasone (Dexan), commonly used to treat inflammation, is a positive control at a concentration of 15 μM [15].

2.3.3. Determination of NO Production. The NO level was determined by the Griess reaction. RAW 264.7 cells (3×10^5 cells/well) were pretreated with the samples at the various concentrations of 25, 50, 75, and 100 μg/mL for 30 minutes and then stimulated with 2 μL LPS (100 μg/mL) for 24 hours at 37°C in an incubator with 5% CO_2. A reaction mixture containing 20 μL of the Griess reagent, 150 μL of each supernatant, and 130 μL of distilled water was incubated at room temperature for 30 minutes. The absorbance of the mixture was recorded at 540 nm by a microplate reader. A standard curve was prepared using $NaNO_2$ as a standard solution in the same manner and was used to calculate the concentration of NO [16].

2.4. α-Glucosidase Inhibitory Assay. The α-glucosidase inhibitory assay was based on the hydrolysis of the substrate 1-nitro-4-hydroxybenzene α-D-glucopyranoside (pNPG) of α-glucosidase. The reaction mixture containing 60 μL of sodium

phosphate buffer (100 mM, pH 6.8), 20 μL of the sample at different concentrations, and 20 μL of α-glucosidase (0.3 IU/mL) in the phosphate buffer was incubated in 96-well plates at 37°C for 10 minutes. After 10 minutes, the reaction was started with the addition of 100 μL of p-nitrophenyl-α-D-glucopyranoside solution (200 μM) in phosphate buffer (substrate), and the mixture was incubated at 37°C for 10 minutes. Then, the reaction was stopped by adding 100 μL of a NaOH solution (50 mM). The α-glucosidase inhibitory activity was measured by determining the colour of the released *p*-nitrophenol (pNP) from pNPG and expressed as inhibition percentage by the following equation:

$$\text{Inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100. \quad (2)$$

The inhibitory concentration (IC_{50}) for each sample was calculated using a regression analysis from the graph plotting the percentage of inhibition and the different sample concentrations [13].

2.5. Statistical Analysis. All results were expressed in the mean values ± standard deviation (SD) of triplicate determinations. Differences between test samples were assessed for significance using one-way analysis of variance (ANOVA) and Duncan's test, where the probability ($p < 0.05$) was considered significant.

3. Results and Discussion

3.1. Effect of BPFs on Cell Viability. To determine the cytotoxic effect of *B. parviflora* fractions (BPFs), the viability of RAW 264.7 cells was measured using the MTT assay. Cells were treated with various BPFs at different concentrations from 25 to 100 μg/mL and incubated for 24 hours. The cell viability was greater than 90% for the BPFs. These results indicated that BPFs at the tested concentrations were not cytotoxic to RAW 264.7 cells, compared to that of nontreated control (Figure 1). Based on this, concentrations ranging from 25 to 100 μg/mL were used for determining the anti-inflammatory effect in LPS-stimulated RAW 264.7 cells of BPFs.

3.2. Effect of BPFs on NO Production in LPS-Induced RAW 264.7 Cells. NO plays as a proinflammatory mediator and is synthesized by inducible nitric oxide synthase (iNOS). Finding medicinal resources that can reduce NO production has been characterized as a new pharmacological strategy for the treatment of inflammation-related diseases. The inhibitory influence of all fractions was assessed the anti-inflammatory effect in LPS-stimulated RAW 264.7 cells as an *in vitro* model. Cells were simultaneously treated with LPS and different concentrations of BPFs. The concentration of NO in the cell culture supernatants was determined by measuring the accumulation of nitrite using the Griess reaction. As displayed in Figure 2, all the fractions appreciably inhibited the LPS-induced NO production in a concentration-dependent manner. Among them, the fraction BP5 exhibited a significant reduction of NO production to 14.12 μM at a concentration of 100 μg/mL as compared with the LPS-treated cells.

3.3. Effect of BPFs on α-Glucosidase Inhibition. α-Glucosidase is an intestinal enzyme that breaks the polysaccharides-linked α-1,4 down to α-glucose which leads to a high blood sugar level. The development of an α-glucosidase inhibitor derived from natural products is an important contribution to the treatment of diabetes. The crude extract and its fractions from *B. parviflora* leaves were evaluated for their α-glucosidase inhibitory activity. It was observed that four fractions BP1, BP2, BP8, and BP9 exhibited 31.5, 66.8, 57.8, and 26.8% inhibitory activities at 200 μg/mL, respectively (Table 1). Meanwhile, fractions BP4, BP5, and BP6 exhibited strong inhibitory activities with 88.1, 98.8, and 83.8%, respectively, better than the one of the positive control (acarbose) with 79.1% inhibition at 200 μg/mL. Among them, the fraction BP5 (IC_{50} value of 44.3 ± 0.2 μg/mL) showed the best. As a result, the increase in the α-glucosidase inhibitory activity shown by different BPFs would be due to their chemical components such as flavonoids, terpenoids, steroids, alkaloids, and glycosidic compounds. These suggested that the fraction BP5 was a remarkable inhibition potential against the α-glucosidase.

3.4. Isolation and Identification of the Potentially Bioactive Compounds. In the present study, the fraction BP5 demonstrated the highest α-glucosidase inhibitory as well as anti-inflammatory activities compared to other fractions. Therefore, BP5 was selected for further isolation and elucidation of the potential bioactive compounds. The fraction BP5 was subjected to a silica gel column and Sephadex LH-20 for fractionation. Repeated column chromatography was performed for subfractions to yield eight compounds. The chemical structures of the isolated compounds were identified by 1D and 2D NMR spectroscopic analysis as well as compared with the reported data in the literature. They were five flavonoids, taxifolin (1) [17], quercetin (2) [18], myricetin (3) [19], rutin (4) [20], and kaempferol (5) [21], and three triterpenoids, lupeol (6) [22], betulinic acid (7) [23], and corosolic acid (8) [24]. Among these compounds, taxifolin (2) and corosolic acid (8) have not yet been previously reported from the *Bruguiera* genus. Especially, all compounds were isolated for the first time from *B. parviflora*. Their spectral data (Figure S1–S25 in supplementary file) were described in detail in published data, and their structures are shown in Figure 3.

Compound 1 (quercetin). Yellow amorphous powder; m.p. 313–315 °C. ESI-MS *m/z* 301.04 $[M\text{-}H]^-$, calcd. for $C_{15}H_{10}O_7$-H, 301.24

^{1}H NMR (500 MHz, DMSO-d_6): 6.18 (1H, *d*, 2.0 Hz, H-6), 6.41 (1H, *d*, 1.5 Hz, H-8), 7.67 (1H, *d*, 2.0 Hz, H-2′), 6.88 (1H, *d*, 8.5 Hz, H-5′), 7.54 (1H, *dd*, 8.5, 2.0 Hz, H-6′), 12.48 (1H, *s*, 5-OH)

^{13}C NMR (125 MHz, DMSO-d_6): 147.7 (C-2), 135.8 (C-3), 175.9 (C-4), 160.7 (C-5), 98.2 (C-6), 164.0 (C-7), 93.4 (C-8), 156.2 (C-9), 103.0 (C-10), 122.0 (C-1′), 115.1 (C-2′), 145.1 (C-3′), 146.8 (C-4′), 115.6 (C-5′), 120.0 (C-6′)

Compound 2 (taxifolin). Pale yellow powder; m.p. 230–232 °C; $[\alpha]_D^{20}$+44.0 (*c* 1.3, Acetone). ESI-MS *m/z* 303.30 $[M\text{-}H]^-$, calcd. for $C_{15}H_{12}O_7$-H, 303.25

^{1}H NMR (500 MHz, DMSO-d_6): 4.96 (1H, *d*, 11.0 Hz, H-2), 4.48 (1H, *d*, 11.0 Hz, H-3), 5.84 (1H, *d*, 2.0 Hz, H-6), 5.89 (1H, *d*,

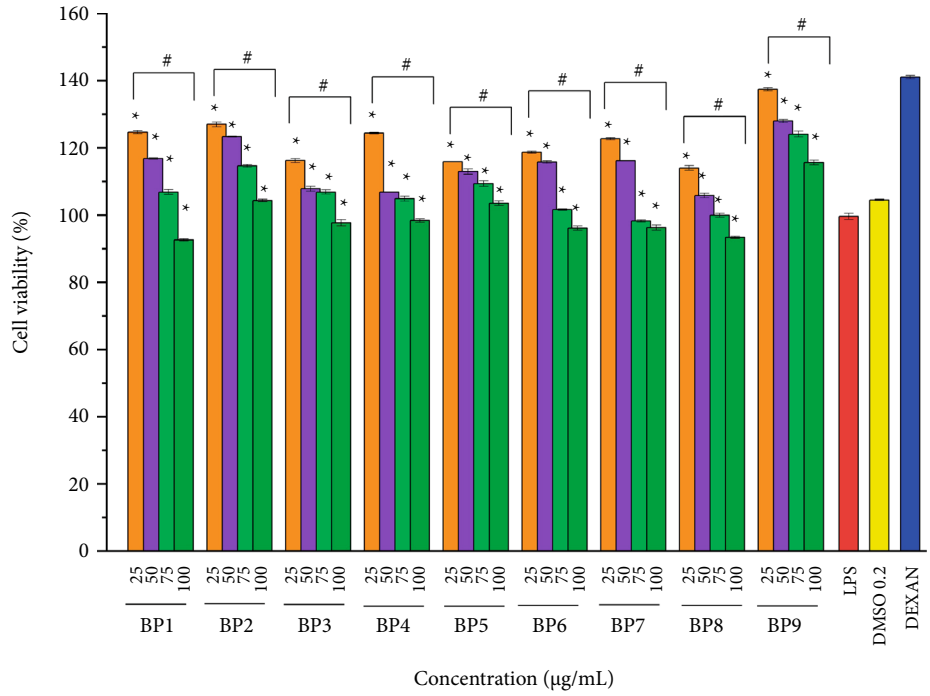


Figure 1: The effect of BPFs on the survival rate of RAW 264.7 macrophages cells. The cells were pretreated with concentrations (25, 50, 75, and 100 μg/mL) of BPFs for 24 hours. The data show the mean ± SD of three independent experiments performed in triplicates. $^{*}p < 0.05$ versus the LPS-treated group, $^{\#}p < 0.05$ compared to Dexan (positive control).

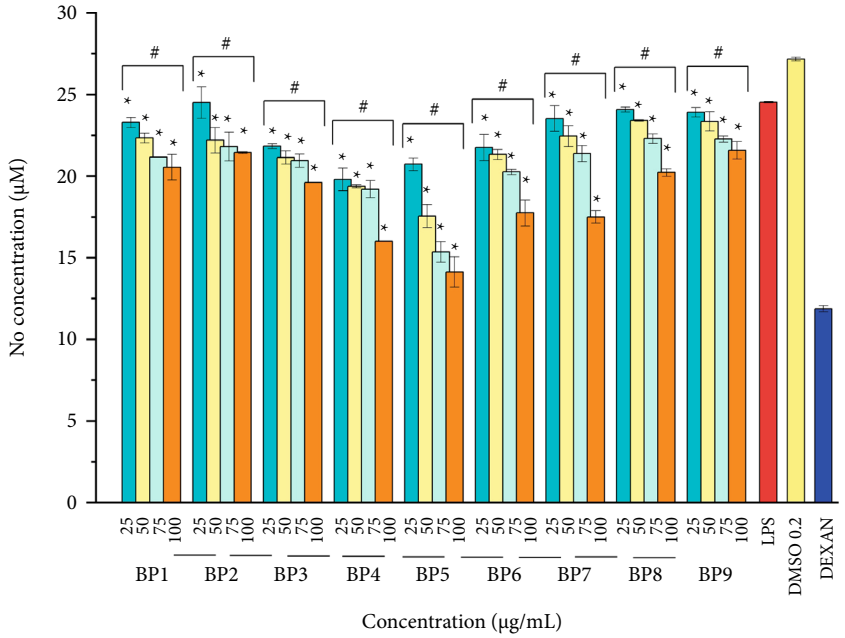


Figure 2: The effect of BPFs on the production of NO in LPS-induced RAW 264.7 macrophages. The cells were pretreated for 30 minutes with concentrations (25, 50, 75, and 100 μg/mL) of BPFs and then stimulated with LPS (100 μg/mL) for 24 hours. The data show the mean ± SD of three independent experiments performed in triplicates. $^{*}p < 0.05$ versus the LPS-treated group, $^{\#}p < 0.05$ compared to Dexan (positive control).

Table 1: α-Glucosidase inhibitory activity of *Bruguiera parviflora* extracts.

Fraction	Concentration (μg/mL)					IC_{50} (μg/mL)
	25	50	100	150	200	
BP1	1.0 ± 0.1	7.9 ± 0.5	13.0 ± 0.1	23.2 ± 0.2	31.5 ± 0.4	—
BP2	12.6 ± 0.1	20.8 ± 0.1	28.5 ± 0.1	55.5 ± 0.1	66.8 ± 0.1	146.2 ± 0.1[h]
BP3	26.9 ± 0.2	39.8 ± 0.1	48.4 ± 0.1	63.3 ± 0.3	69.8 ± 0.2	106.5 ± 0.3[f]
BP4	30.3 ± 0.1	44.6 ± 0.2	66.3 ± 0.1	77.7 ± 0.3	88.1 ± 0.3	69.8 ± 0.2[c]
BP5	36.6 ± 0.1	55.5 ± 0.3	74.2 ± 0.2	89.7 ± 0.1	98.8 ± 0.3	44.3 ± 0.2[a]
BP6	38.8 ± 0.2	54.6 ± 0.1	63.8 ± 0.1	76.7 ± 0.1	83.8 ± 0.1	49.4 ± 0.1[b]
BP7	20.2 ± 0.1	31.6 ± 0.2	54.0 ± 0.1	68.3 ± 0.1	80.2 ± 0.1	102.5 ± 0.1[e]
BP8	30.1 ± 0.3	37.2 ± 0.1	40.7 ± 0.2	49.8 ± 0.1	57.8 ± 0.2	150.8 ± 0.1[i]
BP9	2.7 ± 0.1	3.6 ± 0.6	8.8 ± 0.3	15.5 ± 0.1	26.8 ± 0.2	—
The crude	35.3 ± 0.3	44.0 ± 0.1	54.1 ± 0.5	63.3 ± 0.2	79.8 ± 0.1	83.0 ± 0.4[d]
Acarbose	4.7 ± 0.4	10.5 ± 0.2	39.5 ± 0.3	62.4 ± 0.1	79.1 ± 0.3	127.7 ± 0.2[g]

Values are presented as the mean ± SD values of triplicate determinations. Means with lower cases in the same column indicate significant differences at a 5% statistical level; -: inactive.

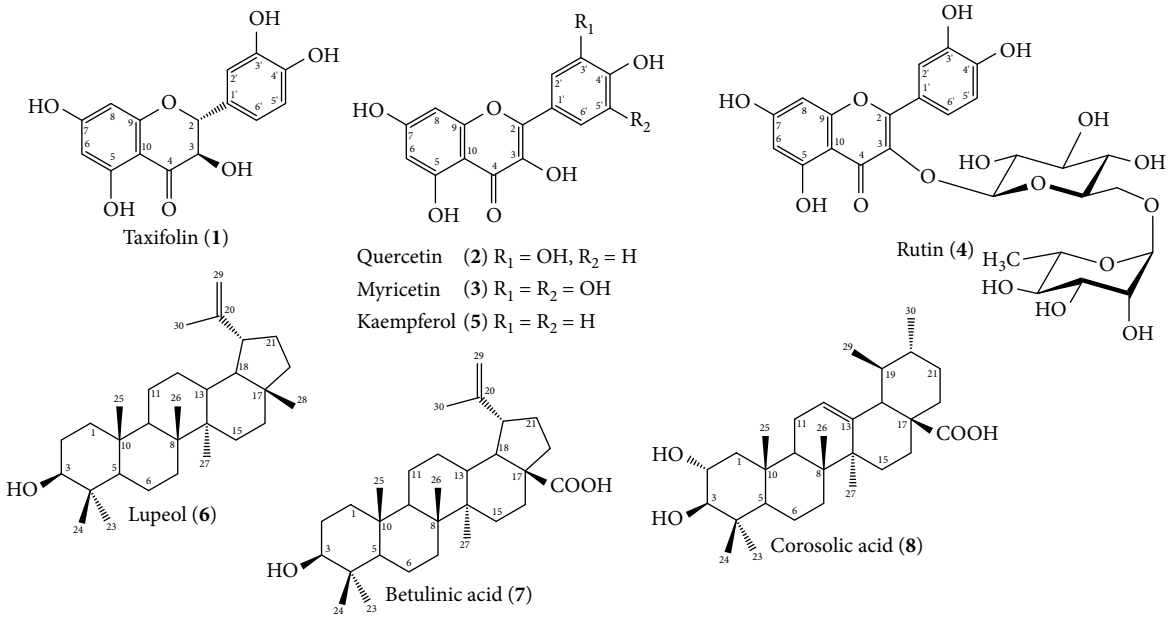


Figure 3: The chemical structures of isolated compounds from *Bruguiera parviflora*.

1.5 Hz, H-8), 6.87 (1H, *s*, H-2′), 6.73 (1H, *d*, 8.0 Hz, H-5′), 6.75 (1H, *d*, 9.5 Hz, H-6′), 5.73 (1H, *brs*, 3-OH), 11.88 (1H, *s*, 5-OH), 8.98 (2H, *s*, 3′,4′-OH).

^{13}C NMR (125 MHz, DMSO-d_6): 83.1 (C-2), 71.6 (C-3), 197.6 (C-4), 163.4 (C-5), 96.1 (C-6), 167.1 (C-7), 95.1 (C-8), 162.6 (C-9), 100.4 (C-10), 128.1 (C-1′), 115.2 (C-2′), 145.8 (C-3′), 145.0 (C-4′), 115.4 (C-5′), 119.4 (C-6′)

Compound 3 (myricetin). Yellow amorphous powder; m.p. 213–215 °C. ESI-MS *m/z* 317.22 $[M-H]^-$, calcd. for $C_{15}H_{10}O_8$-H, 317.24

^{1}H NMR (500 MHz, DMSO-d_6): 6.18 (1H, *d*, 1.5 Hz, H-6), 6.37 (1H, *d*, 1.5 Hz, H-8), 7.24 (2H, *s*, H-2′,6′), 12.49 (1H, *s*, 5-OH)

^{13}C NMR (125 MHz, DMSO-d_6): 146.9 (C-2), 135.9 (C-3), 175.8 (C-4), 160.8 (C-5), 98.2 (C-6), 163.9 (C-7), 93.2 (C-8), 156.1 (C-9), 103.0 (C-10), 120.8 (C-1′), 107.2 (C-2′), 145.8 (C-3′), 135.9 (C-4′), 145.8 (C-5′), 107.2 (C-6′)

Compound 4 (quercetin 3-O-rutinoside, rutin). Yellow amorphous powder; m.p. 190–192 °C.

^{1}H NMR (500 MHz, DMSO-d_6): 6.19 (1H, *d*, 2.0 Hz, H-6), 6.38 (1H, *d*, 1.5 Hz, H-8), 7.54 (2H, *dd*, 8.0, 2.0 Hz, H-2′,6′), 6.84 (1H, *d*, 7.0 Hz, H-5′), 5.34 (1H, *d*, 6.0 Hz, H-1″), 4.38 (1H, *brs*, H-1‴), 12.59 (1H, *s*, 5-OH), 0.99 (3H, *d*, 5.0 Hz, H-6‴)

^{13}C NMR (125 MHz, DMSO-d_6): 156.6 (C-2), 133.3 (C-3), 177.4 (C-4), 161.2 (C-5), 98.7 (C-6), 164.1 (C-7), 93.6 (C-8), 156.4 (C-9), 104.0 (C-10), 121.2 (C-1′), 115.2 (C-2′), 144.8

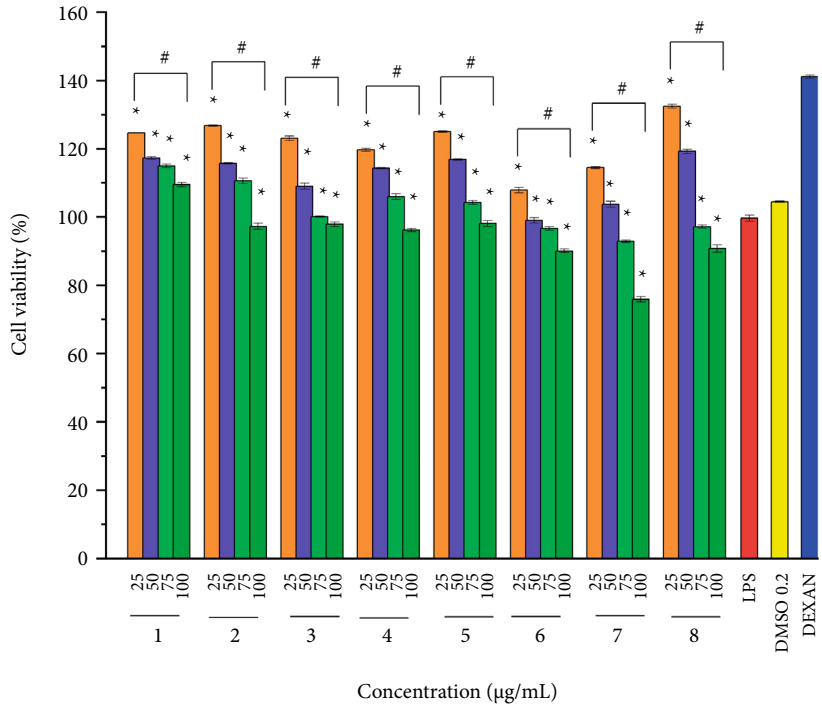


Figure 4: The effect of the isolated compounds on the production of NO in LPS-induced RAW 264.7 macrophages. The cells were pretreated for 30 minutes with concentrations (25, 50, 75, and 100 μg/mL) of compounds 1–8 and then stimulated with LPS (100 μg/mL) for 24 hours. The data show the mean ± SD of three independent experiments performed in triplicates. $^{*}p < 0.05$ versus the LPS-treated group, $^{\#}p < 0.05$ compared to Dexan (positive control).

(C-3′), 148.4 (C-4′), 116.3 (C-5′), 121.6 (C-6′), 101.2 (C-1″), 74.1 (C-2″), 76.5 (C-3″), 70.0 (C-4″), 75.9 (C-5″), 67.0 (C-6″), 100.8 (C-1‴), 70.4 (C-2‴), 70.6 (C-3‴), 71.9 (C-4‴), 68.2 (C-5‴), 17.7 (C-6‴)

Compound 5 (kaempferol). Yellow amorphous powder; m.p. 230–233 °C.

^{1}H NMR (500 MHz, DMSO-d_6): 6.21 (1H, *s*, H-6), 6.46 (1H, *s*, H-8), 8.03 (2H, *d*, 8.5 Hz, H-2′,6′), 6.93 (2H, *d*, 9.0 Hz, H-3′,5′), 9.34 (1H, *s*, 3-OH), 12.46 (1H, *s*, 5-OH), 10.85 (1H, *s*, 7-OH), 10.13 (1H, *s*, 3′-OH)

^{13}C NMR (125 MHz, DMSO-d_6): 146.8 (C-2), 135.6 (C-3), 175.9 (C-4), 156.2 (C-5), 98.2 (C-6), 164.0 (C-7), 93.5 (C-8), 160.7 (C-9), 103.0 (C-10), 121.6 (C-1′), 129.5 (C-2′,6′), 115.4 (C-3′,5′), 159.2 (C-4′)

Compound 6 (lupeol). White powder; m.p. 212–214 °C. APCI–MS *m/z* 409.49 [M-H_2O+H]$^{+}$, calcd. for $C_{30}H_{50}O$–H_2O+H, 409.72

^{1}H NMR (500 MHz, $CDCl_3$): 3.18 (1H, *dd*, 11.5, 5.0 Hz, H-3), 0.68 (1H, *d*, 9.0 Hz, H-5), 2.37 (1H, *sextet*, 6.0 Hz, H-19), 0.96 (3H, *s*, H-23), 0.76 (3H, *s*, H-24), 0.83 (3H, *s*, H-25), 1.03 (3H, *s*, H-26), 0.94 (3H, *s*, H-27), 0.79 (3H, *s*, H-28), 4.68 (1H, *brs*, H-29a), 4.56 (1H, *s*, H-29b), 1.68 (3H, *s*, H-30)

^{13}C NMR (125 MHz, $CDCl_3$): 38.9 (C-1), 27.6 (C-2), 79.1 (C-3), 39.0 (C-4), 55.5 (C-5), 18.5 (C-6), 34.4 (C-7), 41.0 (C-8), 50.6 (C-9), 37.3 (C-10), 21.1 (C-11), 25.3 (C-12), 38.2 (C-13), 43.1 (C-14), 27.6 (C-15), 35.7 (C-16), 43.0 (C-17), 48.5 (C-18), 48.1 (C-19), 151.1 (C-20), 30.0 (C-21), 40.2 (C-22), 28.1 (C-23), 15.5 (C-24), 16.3 (C-25), 16.1 (C-26), 14.7 (C-27), 18.2 (C-28), 109.5 (C-29), 19.5 (C-30)

Compound 7 (betulinic acid). White powder; m.p. 315–317 °C. APCI–MS *m/z* 455.38 [M-H]$^{-}$, calcd. for $C_{30}H_{47}O_3$–H, 455.69

^{1}H NMR (500 MHz, $CDCl_3$): 3.19 (1H, *dd*, 11.0, 4.5 Hz, H-3), 0.68 (1H, *d*, 9.5 Hz, H-5), 3.00 (1H, *td*, 11.0, 5.0 Hz, H-19), 0.93 (3H, *s*, H-23), 0.75 (3H, *s*, H-24), 0.82 (3H, *s*, H-25), 0.98 (3H, *s*, H-26), 0.96 (3H, *s*, H-27), 4.74 (1H, *brs*, H-29a), 4.61 (1H, *brs*, H-29b), 1.69 (3H, *s*, H-30)

^{13}C NMR (125 MHz, $CDCl_3$): 38.9 (C-1), 27.6 (C-2), 79.2 (C-3), 39.0 (C-4), 55.5 (C-5), 18.4 (C-6), 34.5 (C-7), 41.9 (C-8), 50.7 (C-9), 37.4 (C-10), 21.0 (C-11), 25.7 (C-12), 38.6 (C-13), 42.6 (C-14), 30.7 (C-15), 32.3 (C-16), 56.5 (C-17), 47.1 (C-18), 49.4 (C-19), 150.6 (C-20), 29.9 (C-21), 37.2 (C-22), 28.1 (C-23), 15.5 (C-24), 16.3 (C-25), 16.2 (C-26), 14.9 (C-27), 180.5 (C-28), 109.8 (C-29), 19.5 (C-30)

Compound 8 (corosolic acid). White powder; m.p. 250-252 °C. APCI–MS *m/z* 471.43 [M-H]$^{-}$, calcd. for $C_{30}H_{48}O_4$-H, 471.69

^{1}H NMR (500 MHz, DMSO-d_6): 3.41 (1H, *m*, H-2), 2.74 (1H, *d*, 9.5 Hz, H-3), 5.15 (1H, *dt*, 14.5, 3.6 Hz, H-12), 2.11 (1H, *d*, 11.5 Hz, H-18), 0.92 (3H, *s*, H-23), 0.71 (3H, *s*, H-24), 0.92 (3H, *s*, H-25), 0.75 (3H, *s*, H-26), 1.04 (3H, *s*, H-27), 0.82 (1H, *d*, 6.0 Hz, H-29), 0.91 (3H, *d*, 7.0 Hz, H-30)

^{13}C NMR (125 MHz, DMSO-d_6): 46.8 (C-1), 67.2 (C-2), 82.3 (C-3), 38.5 (C-4), 54.8 (C-5), 18.0 (C-6), 32.6 (C-7), 38.9 (C-8), 47.0 (C-9), 37.6 (C-10), 22.9 (C-11), 124.5 (C-

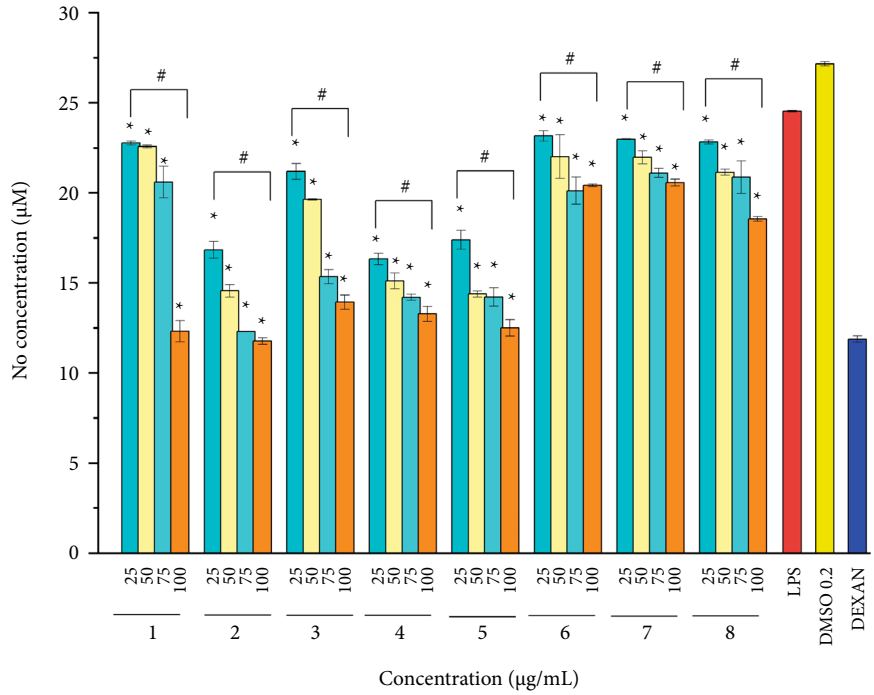


FIGURE 5: The effect of the isolated compounds on the survival rate of RAW 264.7 macrophages cells. The cells were pretreated with concentrations (25, 50, 75, and 100 μg/mL) of compounds 1–8 for 24 hours. The data show the mean ± SD of three independent experiments performed in triplicates. $^{*}p < 0.05$ versus the LPS-treated group, $^{\#}p < 0.05$ compared to Dexan (positive control).

12), 138.3 (C-13), 41.7 (C-14), 27.5 (C-15), 25.6 (C-16), 47.1 (C-17), 52.4 (C-18), 38.5 (C-19), 38.5 (C-20), 30.2 (C-21), 36.3 (C-22), 28.8 (C-23), 16.4 (C-24), 16.9 (C-25), 17.0 (C-26), 23.3 (C-27), 178.4 (C-28), 17.2 (C-29), 21.1 (C-30)

3.5. Effects of Isolated Compounds 1–8 on NO Production in LPS-Induced RAW 264.7 Cells. All isolated compounds 1–8 were evaluated for their inhibition of NO production in LPS-stimulated RAW 264.7 cells using the Griess assay with dexamethasone (Dexan) as a positive control. The anti-inflammatory activities of the tested compounds increased in accordance with the increased concentration. As shown in Figure 4, compounds 1–5 displayed a potential inhibition with NO production from 11.77 to 13.92 μM at a concentration of 100 μg/mL as compared with the LPS-treated cells, in which all of them belonged to flavonoids. Therefore, it was possible to indicate that the anti-inflammatory activity of *B. parviflora* would be derived from its flavonoic compounds. Among these flavonoids, compounds 1, 2, and 5 showed good anti-inflammatory effects with NO production to 12.32, 11.77, and 12.50 μM, respectively, at the concentration of 100 μg/mL. Besides, the effects of the tested compounds on cell viability were evaluated to determine whether their anti-inflammatory activities were due to the cytotoxicity. As the results in Figure 5 show, these compounds did not show any significant cytotoxicity with LPS treatment for 24 hours up to 100 μg/mL. Based on all the above-mentioned data, the flavonoids from *B. parviflora* leaves would play an important role in inhibiting LPS-stimulated NO release.

3.6. Effects of Isolated Compounds 1–8 on α-Glucosidase Inhibition. The chemical constituents 1–8 isolated from *B. parviflora* leaves were tested for α-glucosidase inhibitory activity and acarbose was used as a standard with an IC_{50} value of 127.7 ± 0.2 μg/mL. As presented in Table 2, all tested compounds 1–8 possessed significant inhibitory activities with I C_{50} values from 3.4 to 98.0 μg/mL, and these inhibitions were better than acarbose. Among the flavonoids, the most effective one was compound 2 with an IC_{50} value of 3.4 ± 0.5 μg/mL, while compound 8 showed the strongest inhibition among the tested triterpenoids with an IC_{50} value of 17.9 ± 0.4 μg/mL. These results indicated that the diversity of the structural skeleton, the substituent groups, and their positions on the skeleton could have a significant impact on α-glucosidase inhibition. From the structures of flavonoids 1–5, it would be said that the α-glucosidase inhibitory activity is strengthened by the presence of hydroxy groups directly attached to the flavonoid skeleton, because it could have the ability to make flavonoids binding into the pocket of α-glucosidase by constructing the hydrogen bond [25]. The comparison of activities of compound 2 (IC_{50} = 3.4 ± 0.5 μg/mL) and its glycosidic derivative, compound 4 (IC_{50} = 74.1 ± 0.4 μg/mL), showed that the *O*-glycosylation of the -OH group at the C-3 position weakened the *a*-glucosidase inhibitory activities. These results showed good compatibility with the structure–activity relationship studies on flavonoids as inhibitors of α-glucosidase enzyme done by Şöhretoğlu and Sari [26] and Carina et al. [27]. Furthermore, compound 7 (IC_{50} = 28.1 ± 0.4 μg/mL) exhibited stronger inhibition than compound 6 (IC_{50} = 98.0 ± 0.6 μg/mL)

Table 2: α-Glucosidase inhibitory activity of potential compounds isolated from *Bruguiera parviflora*.

Compound	Concentration (μg/mL)					IC_{50} (μg/mL)
	5	25	50	75	100	
2 (quercetin)	52.0 ± 0.5	>100	>100	>100	>100	3.4 ± 0.5[a]
1 (taxifolin)	7.3 ± 0.4	16.8 ± 0.5	44.8 ± 0.2	65.1 ± 0.2	83.6 ± 0.5	60.5 ± 0.2[d]
3 (myricetin)	1.8 ± 0.5	13.5 ± 0.5	25.1 ± 0.5	37.8 ± 0.5	51.6 ± 0.5	97.8 ± 0.5[g]
4 (rutin)	19.8 ± 0.1	26.3 ± 0.3	33.0 ± 0.5	49.8 ± 0.1	65.6 ± 0.4	74.1 ± 0.4[f]
5 (kaempferol)	26.5 ± 0.4	33.2 ± 0.6	41.4 ± 0.2	56.8 ± 0.4	66.9 ± 0.1	62.5 ± 0.4[e]
6 (lupeol)	30.2 ± 0.5	36.3 ± 0.4	39.4 ± 0.3	41.5 ± 0.8	53.0 ± 0.6	98.0 ± 0.6[g]
7 (betulinic acid)	24.0 ± 0.3	53.0 ± 0.4	72.7 ± 0.3	89.0 ± 0.2	>100	28.1 ± 0.4[c]
8 (corosolic acid)	43.2 ± 0.2	50.8 ± 0.4	85.0 ± 0.4	>100	>100	17.9 ± 0.4[b]
Acarbose	4.7 ± 0.4	10.5 ± 0.2	39.5 ± 0.3	62.4 ± 0.1	79.1 ± 0.3	127.7 ± 0.2[h]

Values are presented as the mean ± SD values of triplicate determinations. Means with lower cases in the same column indicate significant differences at a 5% statistical level.

suggesting that the replacement of the methyl group by the carboxyl group at C-28 could enhance the inhibition of *a*-glucosidase. Besides, the weaker inhibition of compound 7 compared to compound 8 (IC_{50} = 17.9 ± 0.4 μg/mL) indicated that the six-membered *E* ring would be important for the inhibition of triterpenoids. These results demonstrated that flavonoids and triterpenoids isolated from *B. parviflora* leaves were potential inhibitors against *a*-glucosidase.

4. Conclusions

The bioactive guided investigation of *B. parviflora* led to isolation and elucidation of the structures of eight compounds, including five flavonoids and three triterpenoids. Among them, flavonoids such as taxifolin (1), quercetin (2), myricetin (3), rutin (4), and kaempferol (5) exhibited the potent anti-inflammatory as well as α-glucosidase inhibitory activities. Quercetin (2) showed the most potent inhibitory effect against α-glucosidase activity with an IC_{50} value of 3.4 ± 0.5 μg/mL and the LPS-induced NO production of 11.8 μM at the concentration 100 μg/mL. The obtained result suggested that *B. parviflora* leaves are a potential natural source for the prevention of inflammation and diabetes which would be due to its bioactive compounds.

Abbreviations

DMEM: Dulbecco's modified Eagle's medium
DMSO: Dimethyl sulfoxide
DPPH: 2,2-Diphenyl-1-picryl-hydrazyl-hydrate
IC_{50}: The half-maximal inhibitory concentration
LPS: Lipopolysaccharide
BPF: *Bruguiera parviflora* fraction
TLC: Thin layer chromatography
pNPG: *para*-Nitro-4-hydroxybenzene α-D-glucopyranoside.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Supplementary Materials

Figure S1: the ESI–MS spectrum of quercetin. Figure S2: the ^{1}H–NMR spectrum of quercetin (500 MHz, DMSO–d_6). Figure S3: the ^{13}C–NMR spectrum of quercetin (125 MHz, DMSO–d_6). Figure S4: the ESI-MS spectrum of taxifolin. Figure S5: the ^{1}H–NMR spectrum of taxifolin (500 MHz, DMSO–d_6). Figure S6: the ^{13}C–NMR spectrum of taxifolin (125 MHz, DMSO–d_6). Figure S7: the APCI-MS spectrum of myricetin. Figure S8: the ^{1}H–NMR spectrum of myricetin (500 MHz, DMSO–d_6). Figure S9: the ^{13}C–NMR spectrum of myricetin (125 MHz, DMSO–d_6). Figure S10: the ^{1}H–NMR spectrum of rutin (500 MHz, DMSO–d_6). Figure S11; the ^{13}C–NMR spectrum of rutin (125 MHz, DMSO–d_6). Figure S12: the HMBC spectrum of rutin (500 MHz, DMSO–d_6). Figure S13: the ^{1}H–NMR spectrum of kaempferol (500 MHz, DMSO–d_6). Figure S14: the ^{13}C–NMR spectrum of kaempferol (125 MHz, DMSO–d_6). Figure S15: the HSQC spectrum of kaempferol (500 MHz, DMSO–d_6). Figure S16: the HMBC spectrum of kaempferol (500 MHz, DMSO–d_6). Figure S17: the APCI-MS spectrum of lupeol. Figure S18: the ^{1}H–NMR spectrum of lupeol (500 MHz, $CDCl_3$). Figure S19: the ^{13}C–NMR spectrum of lupeol (125 MHz, $CDCl_3$). Figure S20: the APCI-MS spectrum of betulinic acid. Figure S21: the ^{1}H–NMR spectrum of betulinic acid (500 MHz, $CDCl_3$). Figure S22: the ^{13}C–NMR spectrum of betulinic acid (125 MHz, $CDCl_3$). Figure S23: the APCI-MS spectrum of corosolic acid. Figure S24: the ^{1}H–NMR spectrum of corosolic acid (500 MHz, DMSO–d_6). Figure S25: the ^{13}C–NMR spectrum of corosolic acid (125 MHz, DMSO–d_6). *(Supplementary Materials)*

References

[1] A. Kumar, M. Memo, and A. Mastinu, "Plant behaviour: an evolutionary response to the environment?," *Plant Biology*, vol. 22, no. 6, pp. 961–970, 2020.

[2] N. Nurjanah, A. M. Jacoeb, T. Hidayat, S. Hazar, and R. Nugraha, "Antioxidant activity, total phenol content, and bioactive components of lindur leave (Bruguiera gymnorrhiza)," *American Journal of Food Science and Health*, vol. 2, no. 4, pp. 65–70, 2016.

[3] L. Li, C. G. Huang, C. Y. Wang, and Y. W. Guo, "Sexangulic acid, a new cytotoxic triterpenoid from the Chinese mangrove Bruguiera sexangula," *Natural Product Research*, vol. 24, no. 11, pp. 1044–1049, 2010.

[4] P. Chumkaew, S. Kato, and K. Chantrapromma, "A new triterpenoid ester from the fruits of Bruguiera parviflora," *Chemical and Pharmaceutical Bulletin*, vol. 53, no. 1, pp. 95-96, 2005.

[5] X. Y. Huang, Q. Wang, H. L. Liu et al., "Diastereoisomeric macrocyclic polydisulfides from the mangrove _Bruguiera gymnorrhiza_," *Phytochemistry*, vol. 70, no. 17-18, pp. 2096–2100, 2009.

[6] P. D. Abeysinghe, R. P. Wanigatunge, and R. N. Pathirana, "Evaluation of antibacterial activity of different mangrove plant extracts," *Ruhuna Journal of Science*, vol. 1, pp. 104–112, 2019.

[7] M. Haq, W. Sani, A. B. Hossain, R. M. Taha, and K. M. Monneruzzaman, "Total phenolic contents, antioxidant and antimicrobial activities of Bruguiera gymnorrhiza," *Journal of Medicinal Plants Research*, vol. 5, no. 17, pp. 4112–4118, 2011.

[8] A. A. James and C. D. Norman, "Bruguiera gymnorrhiza (large-leafed mangrove)," *Species Profles for Pacifc Island Agroforestry*, pp. 1–15, 2006.

[9] T. T. L. Nguyen, T. T. Bui, and P. K. P. Nguyen, "Two new compounds from leaves of Bruguiera cylindrica (L.) Blume with the in vitro α-glucosidase inhibitory activity," *Science & Technology Development Journal*, vol. 23, no. 4, pp. 1795–1802, 2020.

[10] L. Han, X. Huang, I. Sattler et al., "New diterpenoids from the marine mangrove Bruguiera gymnorrhiza," *Journal of Natural Products*, vol. 67, no. 9, pp. 1620–1623, 2004.

[11] G. Hua, Q. Zhang, and H. Yuan, "Nitric oxide mediates inflammation in type II diabetes mellitus through the PPARγ/eNOS signaling pathway," *PPAR Research*, vol. 2020, 7 pages, 2020.

[12] A. Mastinu, S. Bonini, and W. D. Uberti, "Gamma-oryzanol prevents LPS-induced brain inflammation and cognitive impairment in adult mice," *Nutrients*, vol. 11, no. 4, p. 728, 2019.

[13] T. T. L. Nguyen, P. E. Hansen, and P. K. P. Nguyen, "A new flavone glycoside FromLumnitzera littoreawith in Vitroα-Glucosidase inhibitory activity," *Natural Product Communications*, vol. 14, no. 6, 2019.

[14] T. T. L. Nguyen, T. T. Pham, P. E. Hansen, and P. K. P. Nguyen, "In vitro α-glucosidase inhibitory activity of compounds isolated from mangrove Lumnitzera littorea leaves," *Science & Technology Development Journal*, vol. 22, no. 1, pp. 106–113, 2019.

[15] W. Xiang, G. Zhang, F. Li et al., "Chemical constituents from the roots of Polygala arillata and their anti- inflammatory activities," *Journal of Chemistry*, vol. 2019, 8 pages, 2019.

[16] G. Zhang, H. Geng, C. Zhao et al., "Chemical constituents with inhibitory activity of NO production from a wild edible Mushroom, Russula vinosa Lindbl, May Be Its Nutritional Ingredients," *Russula vinosa Lindbl, may be its nutritional ingredients, Molecules*, vol. 24, no. 7, p. 1305, 2019.

[17] S. Zhou, Y. Shao, J. Fu, L. Xiang, Y. Zheng, and W. Li, "Characterization and quantification of taxifolin related flavonoids in Larix olgensis Henry Var. koreana Nakai extract analysis and its antioxidant activity assay," *International Journal of Pharmacology*, vol. 14, no. 4, pp. 534–545, 2018.

[18] H. Liu, Y. Mou, J. Zhao et al., "Flavonoids from Halostachys caspica and their antimicrobial and antioxidant activities," *Molecules*, vol. 15, no. 11, pp. 7933–7945, 2010.

[19] D. He, D. Gu, Y. Huang et al., "Separation and purification of phenolic acids and myricetin from black currant by high speed countercurrent chromatography," *Journal of Liquid Chromatography & Related Technologies*, vol. 32, no. 20, pp. 3077–3088, 2009.

[20] O. Monika, "Flavonoids from Prunus serotina Ehrh," *Acta Poloniae Pharmaceutica*, vol. 62, no. 2, pp. 127–133, 2005.

[21] L. Scotti, M. B. Fernandes, and M. T. Scotti, "13C NMR spectral data and molecular descriptors to predict the antioxidant activity of flavonoids," *Brazilian Journal of Pharmaceutical Sciences*, vol. 47, no. 2, pp. 241–249, 2011.

[22] T. Minqing, D. Haofu, L. Xiaoming, and W. Bingui, "Chemical constituents of marine medicinal mangrove plant Sonneratia caseolaris," *Chinese Journal of Oceanology and Limnology*, vol. 27, no. 2, pp. 288–296, 2009.

[23] T. Adrielli, M. B. Ariela, C. B. C. Catarina, B. C. Alexandre, M. M. C. Caio, and C. F. Valdir, "Chemical composition, antibacterial and antimycoplasma activities of four Eugenia species growing in Brazil," *Journal of Medicinal Plants Research*, vol. 11, no. 39, pp. 596–602, 2017.

[24] A. M. El-Moghazy, A. A. Khalifa, S. A. L. Bayoumi, and H. M. B. Sayed, "Chemical constituents of ornamental pomegranate and its antioxidant and anti-inflammatory activities in comparison with edible pomegranate," *Journal of Pharmacognosy and Phytochemistry*, vol. 5, no. 4, pp. 88–94, 2016.

[25] H. Xu, "Inhibition kinetics of flavonoids on yeast α-glucosidase merged with docking simulations," *Protein & Peptide Letters*, vol. 17, no. 10, pp. 1270–1279, 2010.

[26] D. Şöhretoğlu and S. Sari, "Flavonoids as alpha-glucosidase inhibitors: mechanistic approaches merged with enzyme kinetics and molecular modelling," *Phytochemistry Reviews*, vol. 19, no. 5, pp. 1081–1092, 2020.

[27] P. Carina, F. Marisa, and R. Daniela, "α-Glucosidase inhibition by flavonoids: an in vitro and in silico structure–activity relationship study," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 32, no. 1, pp. 1216–1228, 2017.