Antioxidant activity of isolated compounds from ethyl acetate extract of *Bruguiera parviflora*

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ARTICLE INFO

DOI:10.46223/HCMCOUJS.tech.en.12.2.2329.2022

Received: May 27th, 2022

Revised: August 10th, 2022

Accepted: August 17th, 2022

Keywords:

antioxidant; bioactivity; *Bruguiera parviflora*; DPPH; mangrove plant; phytochemical

ABSTRACT

Bruguiera parviflora belongs to *Bruguiera* genus, which possesses several biological activities such as antidiabetic, anti-inflammatory, and antioxidant. Phytochemical investigation of the ethyl acetate extract from *Bruguiera parviflora* leaves was carried out bioassay-guided isolation and obtained three known compounds, namely kochioside A (**1**), oleanolic acid (**2**) and quercitrin (**3**). The chemical structures of isolated compounds were identified by spectroscopic method and comparison with previously published data. All isolated compounds were evaluated for antioxidant activity with the free radical scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH). Among them, quercitrin (**3**) was the most potent compound against DPPH radical with the IC_{50} value of 16.4 ± 0.2 µg/mL, compared to vitamin C, which was used as a positive control.

1. Introduction

Free radicals are molecules or atoms that have one or more unpaired electrons, therefore, they are highly reactive and unstable (Robert, Kerri, & Pablo, 2009). Consequently, they can react quickly with other substances to take the required electron to obtain stability. The attacked molecule becomes a free radical by losing its electron and a chain reaction causing damage to the living cell. As a result, free radical damage contributes to the etiology of many chronic health problems such as diabetes, inflammation, cancer, ischemia, and anemia (Anjaneya et al., 2012). Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition. Thus, the search for effective and nontoxic natural compounds with antioxidative activity has increased in recent years.

Bruguiera parviflora, a member of the Rhizophoraceae family, grows widely at the Can Gio mangrove forest, in Vietnam. Some *Bruguiera* species were widely used as drugs to treat eye diseases, herpes (Haq, Sani, Hossain, Taha, & Monneruzzaman, 2011), diarrhea, and malaria (James & Norman, 2006). A literature survey on the chemical constituents of the genus *Bruguiera* revealed the presence of alkaloids, diterpenoids, triterpenoids, and flavonoids (Han et al., 2004; Homhual, Bunyapraphatsara, et al., 2006; Nguyen et al., 2020). These compounds have been isolated from barks, stems, roots, leaves, flowers, and fruits of the genus *Bruguiera* (Cai et al., 2011; Huang et al., 2009; Karalai & Laphookhieo, 2005; Laphookhieo, Karalai, Ponglimanont, & Chantrapromma, 2004). We have previously reported the anti-inflammatory and antidiabetic activities as well as phytochemicals of the *Bruguiera* genus (Bui, Nguyen, Nguyen, Le, & Nguyen, 2022; Nguyen et al., 2020). We here report the isolation of antioxidant compounds from the ethyl acetate extract of *Bruguiera parviflora* leaves through bioassay-guided fractionation.

2. Materials and methods

2.1. Plant material

Leaves of *Bruguiera parviflora* were collected at 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.

2.2. Chemicals

Silica gel (230-400 mesh), sephadex LH-20, silica gel 60 F_{254} for TLC, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid and solvents. All chemicals and reagents were purchased from Merck (Germany) and Sigma-Aldrich (USA).

2.3. Extraction

The fresh leaves were washed and then dried and ground into powder. The powder (3kg) was macerated with ethanol (40L) at room temperature for two days. This maceration was repeated ten times. After filtration, the ethanol solution was evaporated to dryness under reduced pressure to yield a crude ethanol residue (300g). This crude ethanol residue was fractionated according to the solid phase extraction method with the silica gel as an adsorbent. It was applied to a silica gel column (ϕ = 15cm, d = 7cm), eluted consecutively with *n*-hexane, ethyl acetate, and finally with methanol. After evaporation under reduced pressure, three extracts were obtained, *n*-hexane (50g), ethyl acetate (135g), and methanol (60g). A sample was taken from each extract for preliminary screening of antioxidant activity.

2.4. DPPH radical scavenging activity assay

The free radical scavenging ability was determined using DPPH assay according to the method described previously (Gyamfi, Yonamine, & Aniya, 1999). Samples (80µL) at different concentrations were mixed with 120µL DPPH (80µM, in methanol). The reaction mixture was vortexed and incubated in the dark at room temperature for 30 minutes. The absorbance of the mixture was recorded at 517nm by a microplate reader. Ascorbic acid standards as positive controls were prepared in a similar manner, as for the test group except for the antioxidant solution's replacement. The scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \text{x100 \%} \quad (1)$$

2.5. Isolation and purification

The ethyl acetate extract was potent antioxidant activity therefore it was chosen to be chemically studied. It was fractionated by a silica gel column chromatography using a mixture of ethyl acetate-methanol (stepwise, 98:2 to 0:100, *v/v*) to yield five fractions (E1-E5). The fraction E1 (300mg) was chromatographed on a Sephadex LH-20 eluting with chloroform-methanol (1:1, *v/v*) to give four subfractions (E1.1-E1.4). The subfraction E1.3 (70mg) was rechromatographed on a silica gel column using chloroform-ethyl acetate (stepwise 98:2 to 0:100, *v/v*) to obtain compound **1** (10.0mg), compound **2** (12.7mg) and compound **3** (20.0mg).

2.6. Structural identification

The isolated compounds were dissolved either in chloroform or dimethyl sulfoxide depending on their solubility for analysis. The structures of isolated compounds were elucidated from the data obtained from ^{1}H and ^{13}C-NMR spectra.

2.7. Statistical analysis

All antioxidant assays were carried out in triplicate, and their results were expressed as the mean values ± Standard Deviation (SD). Differences between test samples were assessed for significance using a one-way analysis of variance (ANOVA) and Duncan's test, where the probability ($p < 0.05$) was considered significant.

3. Result and discussion

3.1. Results

3.1.1. DPPH radical scavenging activity

Antioxidants are important substances that possess the ability to protect the body from damage caused by free radical induced various diseases. The radical scavenging activity of different extracts of *Bruguiera parviflora* leaves was estimated by discolouring the DPPH solution from purple to yellow. The degree of colour change is proportional to the concentration and potency of the antioxidants. As displayed in Figure 1a, the DPPH radical scavenging ability of all extracts increased with increasing concentration. It was observed that three extracts crude ethanol, ethyl acetate, and methanol exhibited 58.94, 71.64, and 69.78% radical scavenging activity at 200 µg/mL, respectively. Among them, the ethyl acetate extract exhibited a significantly radical scavenging ability with IC_{50} value of 98.1 ± 0.1 µg/mL (Table 1). These suggested that it contains phytochemical constituents that are potent antioxidants. Therefore, it was chosen to be chromatography on silica gel and Sephadex LH-20 to give three known compounds **1-3** (Figure 2). These compounds were evaluated for the DPPH radical scavenging ability. As the results, in Figure 1b shows that compounds **1**, **2** and **3** displayed good antioxidant effects with DPPH radical scavenging ability to 77.17, 53.57 and 78.88%, respectively, at the concentration of 100 µg/mL. Among them, the most effective one was compound **3** with an IC_{50} value of 16.4 ± 0.2 µg/mL as compared with ascorbic acid (Table 1).

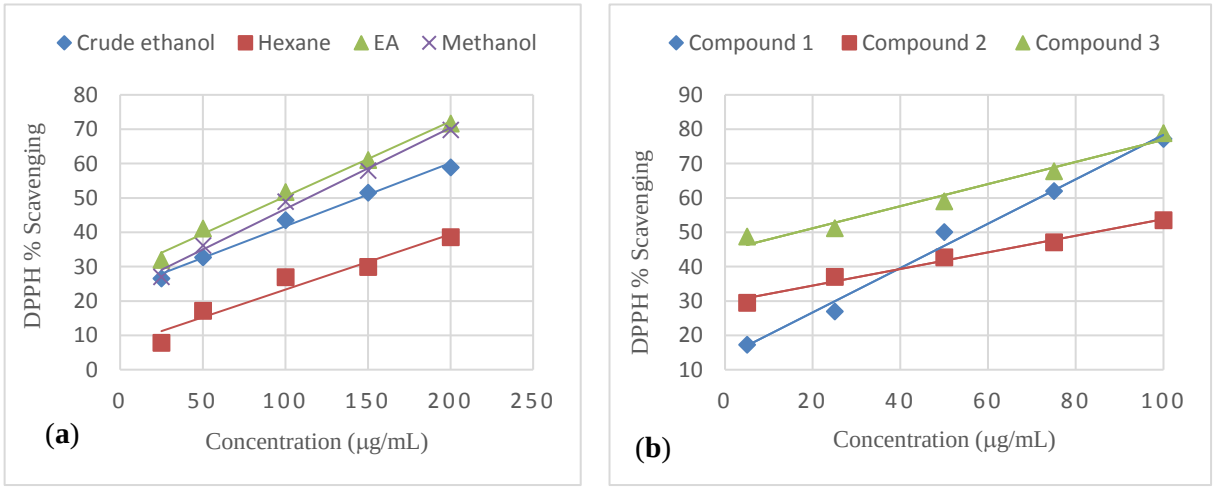

Figure 1. DPPH radical scavenging activity of (**a**) different extracts and (**b**) isolated compounds from *Bruguiera parviflora* leaves by different solvents at various concentrations

Though the antioxidant potential of extracts and isolated compounds were lower than those of ascorbic acid, this study revealed that the ethyl acetate extract and compounds from *Bruguiera parviflora* leaves have prominent antioxidant activity.

Table 1

The IC_{50} values of the DPPH radical scavenging effect of extracts and isolated compounds from *Bruguiera parviflora*

Extract	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)
Crude ethanol	144.9 g ± 0.2	**1**	56.1 c ± 0.1
Hexane	-	**2**	84.1 d ± 0.1
Ethyl acetate	98.1 e ± 0.1	**3**	16.4 b ± 0.2
Methanol	113.6 f ± 0.3	Ascorbic acid (Positive control)	10.1 a ± 0.1

Data 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 using DMRT (Duncan Multiple Range Test); - : inactive

3.1.2. Structural identification

The ethyl acetate extract was chromatographed on silica gel and Sephadex LH-20 to give three known compounds. Their structure was identified from spectroscopic analysis and comparison with literature data. They were kochioside A (**1**) (Imran, Ahmed, & Malik, 2007), oleanolic acid (**2**) (Tian, Dai, Li, & Wang, 2009) and quercitrin (**3**) (Ishikawa et al., 2014). All compounds were isolated for the first time from *Bruguiera parviflora*. Their spectral data (Figure S1-S6 in supplementary file) were described in detail in published data, and their structures are shown in Figure 3.

Compound 1 (Kochioside A). White amorphous powder.

^{1}H-NMR (500 MHz, DMSO–d_6): 5.29 (1H, *t*, 2.5 Hz, H-6), 3.50 (1H, *m*, H-3), 1.00 (3H, *d*, 6.5 Hz, H-28), 0.94 (3H, *s*, H-19), 0.85 (3H, *d*, 6.0 Hz, H-21), 0.79 (3H, *d*, 7.5 Hz, H-26), 0.77 (3H, *s*, H-29), 0.75 (3H, *d*, 8.0 Hz, H-27), 0.61 (3H, *s*, H-18); **glucose pyranoside**: 4.33 (1H, *d*, 7.5 Hz, H-1′), 3.77 (1H, *dd*, 12.0, 3.0 Hz, H-6a′), 3.68 (1H, *dd*, 12.0, 5.0 Hz, H-6′b), 3.16-3.38 (*m*, H-2′-5′)

^{13}C-NMR (125 MHz, DMSO-d_6): 140.4 (C-5), 122.1 (C-6), 79.3 (C-3), 56.9 (C-9), 50.4 (C-17), 46.1 (C-4), 42.4 (C-13), 42.4 (C-14), 39.9 (C-22), 38.9 (C-24), 37.4 (C-1), 36.8 (C-10), 36.2 (C-8), 34.1 (C-15), 32.0 (C-16), 32.0 (C-20), 29.7 (C-7), 29.4 (C-25), 26.4 (C-2), 24.3 (C-23), 23.2 (C-11), 21.1 (C-12), 21.1 (C-28), 19.7 (C-27), 19.3 (C-19), 19.1 (C-21), 18.8 (C-26), 11.9 (C-18), 11.8 (C-29); **glucose pyranoside**: 101.2 (C-1′), 76.5 (C-2′), 75.8 (C-3′), 73.7 (C-5′), 70.5 (C-4′), 62.2 (C-6′)

Compound 2 (Oleanolic acid). White amorphous powder.

^{1}H-NMR (500 MHz, $CDCl_3$): 5.28 (1H, *t*, 3.0 Hz, H-12), 3.22 (1H, *dd*, 9.5, 3.5 Hz, H-3), 2.82 (1H, *dd*, 11.5, 3.5 Hz, H-18), 1.13 (3H, *s*, H-27), 0.99 (3H, *s*, H-29), 0.93 (3H, *s*, H-30), 0.91 (3H, *s*, H-25), 0.90 (3H, *s*, H-23), 0.77 (3H, *s*, H-24), 0.76 (3H, *s*, H-26)

^{13}C-NMR (125 MHz, $CDCl_3$): 183.3 (C-28), 143.7 (C-13), 122.8 (C-12), 79.2 (C-3), 55.4 (C-5), 47.8 (C-9), 46.7 (C-17), 46.0 (C-19), 41.8 (C-14), 41.1 (C-18), 39.4 (C-8), 38.9 (C-1), 38.6 (C-4), 37.2 (C-10), 33.9 (C-21), 33.2 (C-29), 32.8 (C-22), 32.6 (C-7), 30.8 (C-20), 29.9 (C-23), 28.3 (C-15), 27.8 (C-2), 27.3 (C-27), 26.1 (C-29), 23.7 (C-16), 23.5 (C-11), 18.5 (C-6), 17.3 (C-26), 15.7 (C-24), 15.5 (C-25)

Compound 3 (Quercitrin). Yellow amorphous powder.

^{1}H-NMR (500 MHz, DMSO-d_6): 12.65 (1H, *s*, 5-OH), 7.30 (1H, *d*, 2.0 Hz, H-2′), 7.25 (1H, *dd*, 8.5, 2.0 Hz, H-6′), 6.87 (1H, *d*, 8.5 Hz, H-5′), 6.39 (1H, *d*, 2.0 Hz, H-8), 6.21 (1H, *d*, 2.0 Hz, H-6), **rhamnose pyranoside:** 5.26 (1H, *s*, H-1″), 3.13-3.98 (*m*, H-2″-5″), 0.82 (3H, *d*, 6.0 Hz, H-6″)

^{13}C-NMR (125 MHz, DMSO-d_6): 177.8 (C-4), 164.2 (C-7), 161.3 (C-5), 157.3 (C-9), 156.5 (C-2), 148.5 (C-4′), 145.2 (C-3′), 134.3 (C-3), 121.2 (C-6′), 120.8 (C-1′), 115.7 (C-5′), 115.5 (C-2′), 104.1 (C-10), 98.7 (C-6), 93.7 (C-8), **rhamnose pyranoside:** 101.9 (C-1″), 71.2 (C-4″), 70.6 (C-3″), 70.4 (C-2″), 70.1 (C-5″), 17.5 (C-6″)

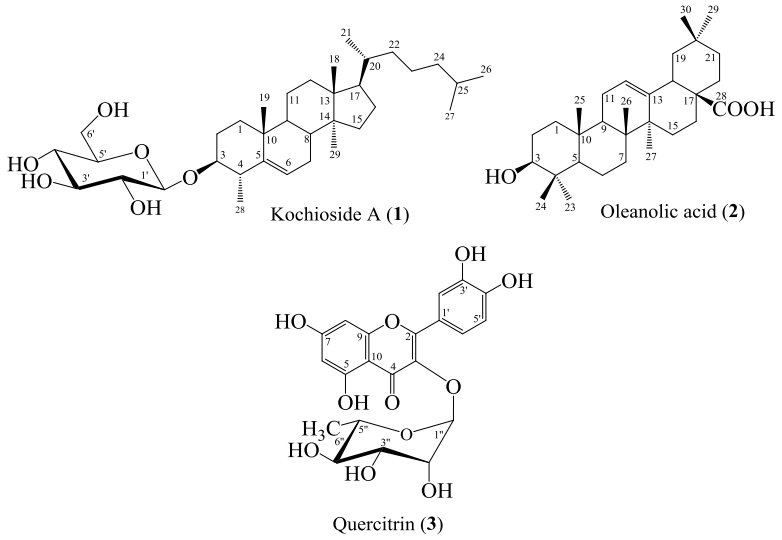

Figure 2. The chemical structures of isolated compounds from ethyl acetate extract of *Bruguiera parviflora*

3.2. Discussion

Compound **1** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum of compound **1** revealed the presence of seven methyl proton signals, including three singlet methyls at δ_H 0.94 (*s*, H-19), 0.77 (*s*, H-29) and 0.61 (*s*, H-18); four doublet methyls at δ_H 1.00 (*d*, 6.5 Hz, H-28), 0.85 (*d*, 6.0 Hz, H-21), 0.79 (*d*, 7.5 Hz, H-26) and 0.75 (3H, *d*, 8.0 Hz, H-27). The olefinic proton signal at δ_H 5.29 (*t*, 2.5 Hz, H-6) appeared to be characteristic of the cholestane skeleton. Besides, the proton signal connecting to the carbon carbinol C-3 appeared as a multiplet at δ_H 3.50 (*m*, H-3). In addition, the ^{1}H-NMR spectrum of **1** confirmed the presence of one *β*-glucose unit through a doublet signal at δ_H 4.33ppm with the large coupling constant *J* = 7.5 Hz assigning to the anomeric proton H-1′ and multiplet signals from 3.16 to 3.38ppm assigning for carbinol protons of the sugar moiety. The ^{13}C-NMR spectrum of compound **1** revealed thirty-five carbon signals of the steroid glycoside, including an anomeric carbon signal at δ_C 101.2 (C-1′), sugar carbon signals from 62.0 to 77.0ppm of C-2′- C-6′ positions, one oxygenated carbon at δ_C 79.3 (C-3), two olefinic carbon signals at δ_C 140.4 (C-5) and 122.1 (C-6) and the other carbon signals from 12.0 to 57.0ppm. In addition to the NMR data analysis, the comparison of the NMR data of compound **1** with those reported in the literature (Imran et al., 2007) was done to determine the chemical structure of **1** to be kochioside A which is isolated for the first time from *Bruguiera parviflora*.

Compound **2** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum of compound **2** showed seven tertiary methyl groups at δ_H 0.77-1.14 of an oleanane skeleton

triterpenoid. A doublet of doublet signal of one proton at δ_H 2.83 (*dd*, 13.5, 4.0 Hz, H-18) together with one olefinic proton signal at δ_H 5.29 (*t*, 3.5 Hz, H-12) suggested the oleanane-12-ene skeleton. Besides, one methine proton signal at δ_H 3.22 (*dd*, 11.5, 4.0 Hz, H-3) supported that **2** had one hydroxy group. The ^{13}C-NMR spectrum of **2** exhibited signals at δ_C 122.9 and 143.7, corresponding to carbons C-12 and C-13, respectively. The signal at δ_C 177.8 was assigned to the carboxyl group at C-28. Those data allowed us to identify compound **2** as oleanolic acid (Tian et al., 2009).

Compound **3** was obtained as a yellow amorphous powder and appeared on TLC under UV light at 365nm. The ^{1}H-NMR spectrum of **3** showed typical signals of a 3,5,7,3′,4′-pentasubstituted flavone, including one singlet proton signal at δ_H 12.65 assigned to a chelated hydroxy group, aromatic proton doublets at δ_H 6.21 (*d*, 2.0 Hz) and 6.39 (*d*, 2.0 Hz) consistent to the *meta* protons H-6 and H-8 on the A ring and an ABX system at δ_H 7.30 (*d*, 2.0 Hz, H-2′), 6.87 (*d*, 8.5 Hz, H-5′) and 7.25 (*dd*, 8.5, 2.0 Hz, H-6′) on the B ring. Additionally, the presence of a sugar unit was demonstrated by the occurrence of an anomeric proton at δ_H 5.26 (*s*, H-1″) and a series of signals at δ_H 3.13-3.98 (*m*, H-2″-5″). Especially, the doublet proton signal of a methyl group at δ_H 0.82 (*d*, 6.0 Hz, H-6″) indicated that the sugar was rhamnopyranose. The ^{13}C-NMR spectrum of **3** displayed twenty-one carbon signals typical of a monoglycoside flavonoid, including fifteen carbon signals of the flavonol skeleton and the other ones of the rhamnopyranose sugar. The low field signal at δ_C 177.8ppm was due to the carbonyl group at C-4. The carbon signals of the rhamnopyranose were at δ_C 101.9 for the anomeric carbon, at δ_C 17.5 for the methyl group, and at δ_C 70.1 - 70.6 of the sugar carbons. The spectral data of **3** were well compatible with those reported in the literature (Ishikawa et al., 2014). On the basis of that **3** was identified as quercitrin.

4. Conclusions

The antioxidant guided investigation of *Bruguiera parviflora* led to the isolation and elucidation of the structures of three compounds, namely kochioside A (**1**), oleanolic acid, (**2**) and quercitrin (**3**). Quercitrin (**3**) exhibited the most antioxidant activity an with IC_{50} value of 16.4μg/mL. These findings contributed to the knowledge of the phytochemical properties of *Bruguiera parviflora* leaves and suggested that this plant is a potential natural source for the antioxidant effect.

Abbreviations

$CDCl_3$: Chloroform-*d*

DMSO: Dimethyl sulfoxide

^{13}C-NMR: Carbon-13 nuclear magnetic resonance

^{1}H-NMR: Proton nuclear magnetic resonance

IC_{50}: The half-maximal inhibitory concentration

TLC: Thin layer chromatography

s: singlet

d: doublet

m: multiplet

dd: doublet of doublet

t: triplet

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.

Supplementary materials

Figure S1. ^{1}H–NMR spectrum of kochioside A (500 MHz, DMSO–d_6)

Figure S2. ^{13}C–NMR spectrum of kochioside A (125 MHz, DMSO–d_6)

Figure S3. ^{1}H–NMR spectrum of oleanolic acid (500 MHz, $CDCl_3$)

Figure S4. ^{13}C–NMR spectrum of oleanolic acid (500 MHz, $CDCl_3$)

Figure S5. ^{1}H–NMR spectrum of quercitrin (500 MHz, DMSO–d_6)

Figure S6. ^{13}C–NMR spectrum of quercitrin (125 MHz, DMSO–d_6)

ACKNOWLEDGEMENTS

This research received funding sponsored by the Ministry of Education and Training, Vietnam under grant number B2020-MBS-562-04.

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