Science & Technology Development Journal, 23(4):800-807

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Research article

Two new compounds from leaves of *Bruguiera cylindrica* (L.) Blume with the *in vitro* α-glucosidase inhibitory activity

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ABSTRACT

Introduction: *Bruguiera cylindrica* is one of the mangrove plants belonging to *Bruguiera* genus. This genus is characterized by the presence of a large number of compounds, but the research on bioactivities has not been investigated so far. In the present research, the α-glucosidase inhibitory activity, as well as chemical constituents of the ethyl acetate extract of this plant, were studied. **Methods**: The chemical structures of two new compounds were elucidated by spectroscopic and computational methods. **Results**: Two new compounds, benzobrugierol (**1**) and bruguierine (**2**), were isolated from leaves of *Bruguiera cylindrica* (L.) Blume, together with nine known ones, including lupeol (**3**), betulin (**4**), chrysoeriol (**5**), glut-5-ene-3-ol (**6**), cholesta-4-ene-3-one (**7**), 3α-(*Z*)-coumaroyllupeol (**8**), 3α-(*E*)-coumaroyllupeol (**9**), 3β-hydroxycholesta-5-ene-7-one (**10**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**11**). Extracts and some isolated compounds were evaluated for α-*glucosidase* inhibitory activities. **Conclusion**: The results showed that most of the extracts and tested compounds exhibited activities better than the positive control acarbose, especially two new compounds **1** and **2** with their IC_{50} values of 17.9 $\pm$ 0.4 and 34.6 $\pm$ 0.7 (mg/mL), respectively.

Key words: Bruguiera cylindrica, mangrove, new compound, α-glucosidase inhibition

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History

- Received: 2020-09-04
- Accepted: 2020-12-19
- Published: 2020-12-31

DOI : 10.32508/stdj.v23i4.2457

INTRODUCTION

Bruguiera cylindrica (L.) Blume grows widely at Can Gio mangrove forest, Vietnam. Three others of this genus are also found in Vietnam as *Bruguiera gymnorrhiza, Bruguiera parviflora,* and *Bruguiera sexangula*. The genus *Bruguiera* is characterized by the presence of a large number of compounds, many of which show a broad range of biological activities. These include insect antifeedant, antioxidant, antifungal, cytotoxic, antimalarial, and antibacterial activities [1,2]. Two sulfur-containing compounds, gymnorrhizol, and bruguiesulfurol, from *Bruguiera gymnorrhiza*, showed antidiabetic activities with IC_{50} values of 14.9 and 17.5 μM, respectively [3].

Bruguiera cylindrica has traditionally been used for treating diarrheoa, hepatitis, blood pressure, ulcers, infections, anti-inflammatory agent, and diabetes [4]. Following up with our interest in mangrove plants, the chemical constituent of *Bruguiera cylindrica* was also carried out.

MATERIALS - METHOD

Plant materials

Leaves of *Bruguiera cylindrica* (L.) Blume (Rhizophoraceae) were collected at Can Gio mangrove forest, Ho Chi Minh City, Viet Nam in August of 2014. A voucher specimen (N^o US-B013) was deposited in the laboratory of Faculty of Biotechnology, Ho Chi Minh City Open University. The scientific name of species was authenticated by Dr. Pham Van Ngot, Faculty of Biology, Ho Chi Minh City University of Pedagogy.

General experimental procedures

The NMR spectra were recorded on a Bruker Avance III, Institute of Chemistry (Vietnam Academy of Science and Technology, Hanoi, Vietnam). HR-ESI-MS spectra were obtained on a Shimadzu +IDA TOF MS. TLC was performed on precoated silica gel 60 F_{254} (Merck, Darmstadt, Germany). Gravity column chromatography was performed on silica gel 60 (0.040–0.063 mm, Merck) and Sephadex LH-20 (GE Healthcare Bio-Science AB, Uppsala, Sweden). α-Glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae* (750 UN) and *p*-nitrophenyl-α-D-glucopyranoside were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acarbose and dimethyl sulfoxide were obtained from Merck.

Cite this article : Thuy N T L, Tung B T, Chi T M, Tu P D C, Trung N T, Phung N K P. **Two new compounds from leaves of *Bruguiera cylindrica* (L.) Blume with the *in vitro* α -glucosidase inhibitory activity**. *Sci. Tech. Dev. J.;* 23(4):800-807.

Extraction and isolation

The dried powder (8 kg) of leaves was macerated with ethanol (20 L) at room temperature for two days. After filtration, the ethanol solution was evaporated to dryness under reduced pressure to yield a crude ethanol residue (900 g). This crude ethanol residue *was fractionated* according to the solid phase extraction method *and* eluted consecutively with -hexane, ethyl acetate, and finally with ethanol to yield -hexane (100 g), ethyl acetate (300 g), and ethanol (380 g).

The ethyl acetate fraction (300 g) was fractionated by silica gel column chromatography using a mixture of ethyl acetate–methanol (stepwise, 98:2 to 0:100, *v/v*) to yield six fractions (E1–E6). These were then continuously separated using silica gel and ephadex LH-20 and eluted with appropriate solvent systems of $CHCl_3$-MeOH to give 11 compounds. As a result, fraction EA1 afforded **3** (15.1 mg), **4** (10.3 mg) and **11** (10.3 mg), fraction EA2 gave **7** (10 mg), **8** (5 mg), **9** (8 mg) and **10** (7 mg) and fraction EA3 obtained **1** (5 mg), **2** (5 mg), **5** (5 mg) and **6** (8.5 mg).

Benzobrugierol (1)

White amorphous powder. **HR-ESI-MS**, *m/z*: 167.0190 $[M+H]^+$ (calcd for $C_8H_6O_2$ +H, 167.0167). **^{1}H-NMR** (500 MHz, Acetone-d_6): 7.19 (2H, *m* , H-5,6), 7.51 (1H, *dd*, 7.0, 2.0 Hz, H-7), 8.03 (1H, *s*, H-3), 8.15 (1H, *dd*, 7.0, 2.5 Hz, H-4). **^{13}C - NMR** (125 MHz, Acetone-d_6): 112.8 (C-7), 122.0 (C-4), 123.3 (C-5&C-6), 127.5 (C-3a), 132.7 (C-3), 137.8 (C-7a), 166.3 (C-2).

Bruguierine (2)

White amorphous powder. **HR-ESI-MS**, *m/z*: 425.1143 $[M+Na]^+$, (calcd. for $C_{23}H_{18}O_5N_2$+Na, 425.1113). **^{1}H-NMR** (500 MHz, CD_3OD): 5.30 (1H, *s*, H-2), 6.77 (2H, *d*, 8.5 Hz, H-3',5'), 6.92 (4H, *d*, 8.5 Hz, H-2",6",2"',6"'), 7.23 (2H, *d*, 8.5 Hz, H-2',6'), 7.77 (4H, *d*, 8.5 Hz, H-3",5",3"',5"'), 9.76 (2H, *s*, C**H**O). **^{13}C - NMR** (125 MHz, CD_3OD): 104.9 (C-2), 115.8 (C-3',5'), 116.9 (C-4,5,2",6",2"',6"'), 129.0 (C-2',6'), 130.3 (C-4",4"'), 130.5 (C-1'), 133.4 (C-3",5",3¢",5"'), 158.8 (C-4'), 165.2 (C-1",1"').

α -glucosidase inhibitory assay

The α-glucosidase inhibitory activity was evaluated on some isolated compounds according to the method of Apostolidis *et al.*[5]. Acarbose was used as a positive control. All experiments were carried out in triplicate.

Computational Details

All DFT calculations were performed with Gaussian 09 package[6]. The geometric optimization of predicted structures was done at B3LYP/6-311++G(2d,p) in both the gas phase and in the methanol solvent. The frequency calculations were also taken at the same level to ensure these structures are minimum on the potential energy surface. The relative energies with ZPE correction for optimized structures were evaluated based on the difference between the total energy of each configuration. Theoretical ^{1}H and ^{13}C NMR chemical shifts were deduced from the isotropic magnetic shielding tensors by using Gauge-Independent Atomic Orbital (GIAO) methodology at B3LYP/6-311+G(d,p)[7–9]. The modified DP4+ probability was performed to assign the exact conformer using online implementation available from http://www-jmg.ch.cam.ac.uk/tools/nmr/DP4/[10].

RESULTS

The crude extract of *Bruguiera cylindrica* leaves was fractionated and eluted with *n*-hexane, ethyl acetate, and ethanol, to yield the corresponding residues: -hexane, ethyl acetate, and ethanol fractions. These fractions were evaluated on the α-glucosidase inhibitory activity. The result indicated that except for the ethanol fraction, the other fractions were potent inhibitors. There was a dose-dependent increase in the percentage inhibitory activity against the α-glucosidase enzyme. The ethyl acetate fraction was the most efficient one with the IC_{50} value of 61.8 ± 0.3 mg/mL (Table 1). Then, it was chromatographed on silica gel and Sephadex LH-20 to give two new compounds, benzobrugierol (**1**) and bruguierine (**2**), and nine known ones **3**-**11** (Figure 1).

The known compounds were identified from spectroscopic analysis and comparison with literature data, including lupeol (**3**)[11], betulin (**4**)[11], chrysoeriol (**5**)[12], glut-5-ene-3-ol (**6**)[13], cholesta-4-ene-3-one (**7**)[14], 3α-(*Z*)-coumaroyllupeol (**8**)[15], 3α-(*E*)-coumaroyllupeol (**9**)[16], 3β-hydroxycholesta-5-ene-7-one (**10**)[17] and β-sitosterol 3-*O*-β-D-glucopyranoside (**11**)[18,19]. Except for two compounds **8** and **9**, all of them were isolated from leaves of *B. cylindrica* for the first time.

The α-glucosidase inhibitory activity was evaluated on two new compounds, **1** and **2**, and some of the known ones, **3**, **4**, **5,** and **11** (the other compounds were not tested because the samples did not well dissolve in the tested media). The results showed that all of the test compounds exhibited better activities than the positive control acarbose. Among them, two new

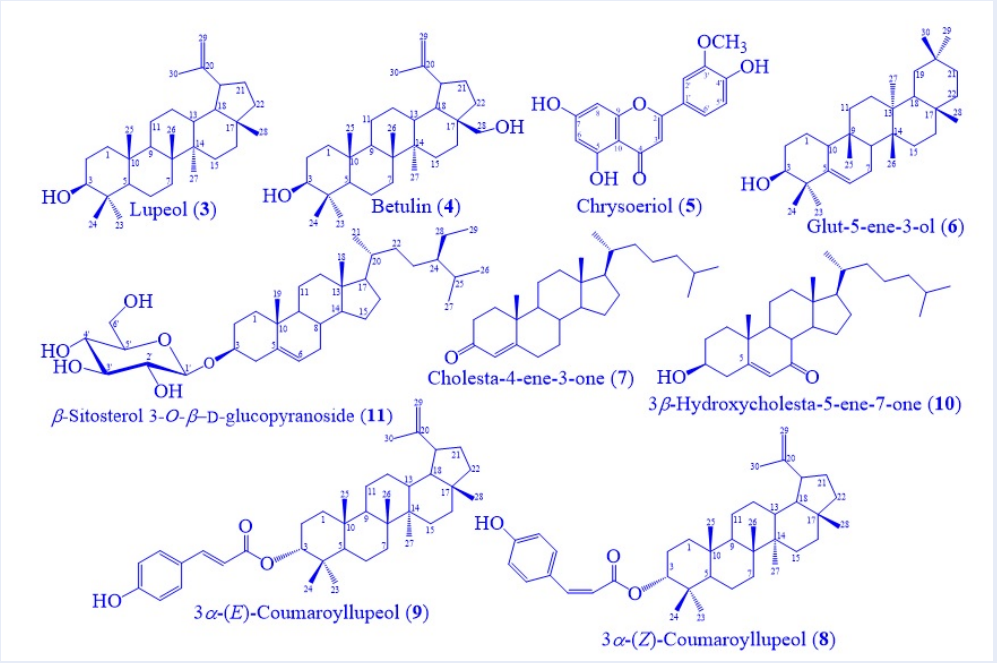


Figure 1: Structure of known compounds **3**-**11**

compounds, benzobrugierol (**1**) and bruguierine (**2**) were the most potent inhibitors with IC_{50} values of 17.9 ± 0.4 and 34.6 ± 0.7 μg/mL, respectively (Table 2).

DISCUSSION

Compound **1** was obtained as a white amorphous powder and appeared purple on TLC plate under UV light at 365 nm. Its molecular formula was established as $C_8H_6O_2$ through the pseudo molecular ion peak in the HR-ESI-MS spectrum at *m/z* 167.0190 $[M+H]^+$ (calcd. for $C_8H_6O_2$ +H, 167.0167, with the error of 2.3 millimass). The ^{1}H-NMR spectra of **1** indicated five aromatic proton signals at d_H 8.15 (1H, *dd*, 7.0, 2.5 Hz, H-4), 8.03 (1H, *s*, H-3), 7.51 (1H, *dd*, 7.0, 2.0 Hz, H-7) and 7.19 (2H, *m* , H-5, and H-6), corresponding to the carbon signals at δ_C 122.0 (C-4), 132.7 (C-3), 112.8 (C-7) and 123.3 (C-5 and C-6) in the ^{13}C-NMR and HSQC spectra. Besides, the ^{13}C-NMR spectrum of **1** revealed signals of two quaternary aromatic carbons at d_C 137.8 (C-7a), 127.5 (C-3a), and one oxygenated carbon at d_C 166.3 (C-2). The ^{1}H-^{1}H COSY experiment of **1** showed the correlations of adjacent aromatic protons H-4/H-5/H-6/H-7 (Figure 2).

These spectral data resembled those of indol 3-carboxylic acid[20]. However, its molecular formula

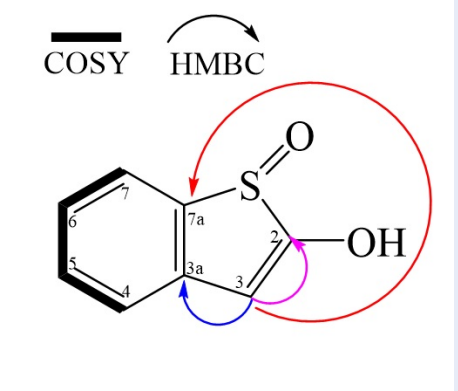


Figure 2: Key COSY and HMBC correlations of compound **1**

($C_9H_7O_2N$+H, 162.0555 amu) did not fit the experimental HR-MS spectrum of **1**. The combination of 2D-NMR and HR-ESI-MS data suggested that **1** could be composed of the 1,2-disubstituted benzene ring (counted for a structural formula of C_6H_4) fused with a certain x-membered ring whose partial structure formula of $C_2H_2O_2S$. This structure was also confirmed via the HMBC correlations of the H-4 proton at δ_H 8.15 to three carbons at δ_C 123.3 (C-5,C-6) and 137.8 (C-7a), of the H-7 proton at δ_H 7.51 to two carbons at δ_C 122 (C-4) and 127.5 (C-3a), of H-

5 and H-6 protons at δ_H 7.19 (2H, *m*) to four carbons at δ_C 112.8 (C-7), 122.0 (C-4), 127.5 (C-3a) and 137.8 (C-7a). Additionally, the HMBC correlations of a singlet at δ_H 8.03 (1H, *s*) to three carbons at δ_C 127.5 (C-3a), 137.8 (C-7a) and 166.3 (an oxygenated olefin carbon of the $C_2H_2O_2S$ moiety) demonstrated that the hydroxy group located at C-2 or C-3 of the five-membered ring bearing a S=O group. Up to this point, there were two structures **1x** and **1y,** that could satisfy all the NMR and HR-MS data (Figure 4).

In order to assign the correct structure of the isolated compound (**1x** or **1y**), their stable geometries were optimized as given in Figure 3, and the DP4 probability was performed based on their parameters of NMR chemical shift to determine the true configuration.

Following the relative energy, the results in the Table 1 showed that the isomer **1y** was estimated to be more stable than the remaining one by 1.9 kcal.mol^{-1} in gas phase and by 4.35 kcal.mol^{-1} in methanol solvent. The DP4 calculation resulted in the prediction of **1y** with 99.85% probability. Accordingly, 3-hydroxybenzo[*b*]thiophene-1-dioxide (**1y**) was assigned for **1** and was named benzobrugierol.

Five-membered ring compounds containing a thiolane oxide group, e.g. brugierol, isobrugierol, had been reported in some species of this genus such as in *Bruguiera conjugata*[21], *B. cylindrica*[22], *B. sexangula*[23] and *B. gymnorrhiza*[24]

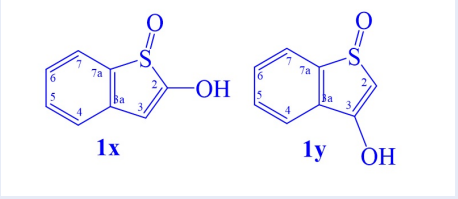


Figure 4: Structure of new compound **1**

Compound **2** was obtained as a white amorphous powder. Its molecular formula was established as $C_{23}H_{18}O_5N_2$ through the pseudo molecular ion peak in the HR-ESI-MS spectrum at *m/z* 425.1143 $[M+Na]^+$ (calcd. for $C_{23}H_{18}O_5N_2$+Na, 425.1113 with the error of 3.0 millimass). In the ^{1}H-NMR spectrum of **2** four doublet signals integrated for twelve protons in the aromatic region at δ_H 7.77 (4H, *d*, 8.5 Hz, H-3",H-5",H-3"',H-5"'), 7.23 (2H, *d*, 8.5 Hz, H-2',H-6'), 6.92 (4H, *d*, 8.5 Hz, H-2",H-6",H-2"',H-6"') and 6.77 (2H, *d*, 8.5 Hz, H-3',H-5') revealed the presence of three symmetrical 1,4-disubstituted benzene rings. Furthermore, the singlet proton signal at δ_H 9.76 (2H, *s*) as well as the carbon signal at δ_C 192.8 in the ^{13}C-NMR spectrum of **2** suggested the presence of an aldehyde group in the molecule. In addition, five oxygenated aromatic carbon signals were observed at δ_C 165.2 (C-1',C-1"'), 158.8 (C-4'), and 116.9 (C-4,C-5) along with twelve aromatic methine carbon signals at δ_C 133.4 (C-3",C-5",C-3"',C-5"'), 129.0 (C-2',C-6'), 116.9 (C-2",C-6",C-2"',C-6"') and 115.8 (C-3',C-5'), one methine carbon-bearing two heteroatom at δ_C 104.9 (C-2) and three quaternary aromatic carbon signals at δ_C 130.5 (C-1') and 130.3 (C-4",C-4"'). The combination of NMR and HR-ESI-MS data suggested that **2** could be composed of three 1,4-disubstituted benzene rings (A, B, C rings), one imidazole, one hydroxy, and two aldehyde groups. The HSQC and HMBC correlations of two aldehyde proton signals with C-2",C-3",C-4",C-5",C-6" and C-2"', C-3"',C-4"',C-5"',C-6"' indicated the attachment of the first aldehyde group at C-4" of the aromatic ring and of the second aldehyde one at C-4"' of the C ring (Figure 5). The HMBC cross-peak of H-2 with C-3',C-5',C-2',C-6' and C-4' indicated the attachment of the C-1' of the aromatic A ring with carbon C-2 of the imidazole ring.

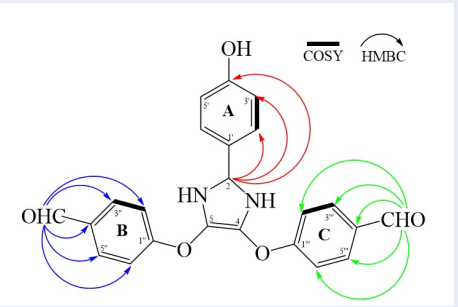
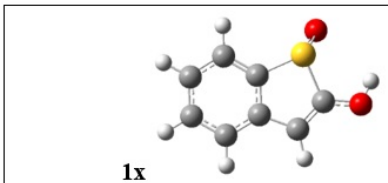


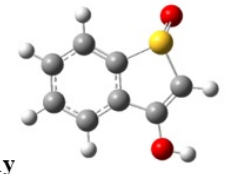
Figure 5: Key COSY and HMBC correlations of compound **2**

Based on these analyses, there were two structures **2x** and **2y** (Figure 7), which could fit all the experimental HR-MS and NMR data. It was obvious that the geometric structure **2x** possessed an imidazole ring while **2y** having a dioxole one. The calculated results in Figure 6 showed that **2x** was the more energetic-favorable structure as compared to **2y** by the relative energy of 16.01 kcal.mol^{-1} in the gas phase and 18.06 kcal.mol^{-1} in methanol. The PD4 analysis gave a great probability of 100% for **2x**. Thus, **2 x** was predicted to be the structure of compound **2**, named 4,5-di(4-formylphenoxy)-2-(4-hydroxyphenyl)-2,3-dihydro-1*H*-imidazole, or ruguierine.



1x

C	-0.69075300	0.91814800	-0.00077900
C	-0.51322900	-0.47898000	-0.08684100
C	-1.57423500	-1.37124200	-0.08516700
C	-2.87232100	-0.85403100	0.03359200
C	-3.07306300	0.52824900	0.10605000
C	-1.99341700	1.41953600	0.08187100
C	0.56908700	1.66326200	0.03891100
C	1.64472600	0.86140800	-0.04304300
H	-1.40527900	-2.44156300	-0.15771700
H	-3.72121300	-1.52971500	0.06394100
H	-4.08337000	0.91883200	0.18478400
H	-2.16487900	2.49078300	0.13619200
H	0.62439300	2.73708500	0.17981700
O	2.94767800	1.18632200	0.01551400
H	3.45697300	0.42475100	0.34354100
S	1.22336000	-0.88570300	-0.39981700
O	1.89467800	-1.75470000	0.65685400



1y

C	-0.54392100	0.68282400	-0.01385900
C	-0.21130700	-0.67273000	-0.13167900
C	-1.17549500	-1.66834800	-0.12414600
C	-2.51737500	-1.28144200	0.00701000
C	-2.86349600	0.07149000	0.10126200
C	-1.88116800	1.06888600	0.08726700
C	0.65684600	1.52739200	-0.00097400
C	1.82087400	0.85060800	-0.10239900
H	-0.90014800	-2.71554000	-0.20452300
H	-3.29382000	-2.03991300	0.03390100
H	-3.90860800	0.35196100	0.19089200
H	-2.14589200	2.11849900	0.16125200
S	1.58047100	-0.91663300	-0.36931600
O	2.17116200	-1.75269700	0.75158200
H	2.82817800	1.24568300	-0.12472900
O	0.46707500	2.86595900	0.09839100
H	1.31712000	3.32724700	0.11759700

Figure 3: Cartesian coordinates of predicted structures of compound **1**

Table 1: α-glucosidase inhibitory activity of different extracts from *Bruguiera cylindrica*

Extract	Concentration (mg/mL)					IC_{50} (mg/mL)	p-value
	10	50	100	150	200		
Crude ethanol	26.1 ± 0.3	36.5 ± 0.1	56.9 ± 0.4	72.5 ± 0.2	80.1 ± 0.4	87.3 ± 0.4	0.01
n-Hexane	29.6 ± 0.3	42.6 ± 0.2	57.0 ± 0.4	70.0 ± 0.3	86.1 ± 0.3	78.2 ± 0.2	0.00
Ethyl acetate	31.8 ± 0.4	46.0 ± 0.1	65.9 ± 0.2	79.6 ± 0.4	86.7 ± 0.1	61.8 ± 0.3	0.04
Methanol	4.7 ± 0.2	17.1 ± 0.3	37.9 ± 0.2	52.6 ± 0.1	75.5 ± 0.4	135.8 ± 0.4	0.01
Acarbose (positive control)	4.7 ± 0.4	10.5 ± 0.2	39.5 ± 0.3	62.4 ± 0.1	79.1 ± 0.3	127.7 ± 0.2	0.00

Data are presented as mean ± SD values of triplicate determinatios. A one-way analysis of variance (ANOVA) and ositive analysis was done using Duncan multiple test. Significance was accepted at P<0.05.

CONCLUSION

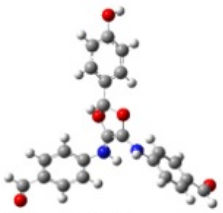
From the ethyl acetate fraction of leaves of *Bruguiera cylindrica* (L.) Blume, two new compounds namely benzobrugierol (**1**) and bruguierine (**2**), together with nine known ones, were isolated and the chemical structure elucidated. Extracts and some isolated compounds were evaluated for α-glucosidase inhibitory activities. Among them, benzobrugierol (**1**) and bruguierine (**2**) were the potent inhibitors with IC_{50} values of 17.9 ± 0.4 and 34.6 ± 0.7 (mg/mL), respectively.

LIST OF ABBREVIATIONS

^{13} C NMR: Carbon-13 nuclear magnetic resonance;
^{1} H NMR: Proton nuclear magnetic resonance;
HR-ESI-MS: High resolution electrospray ionization mass spectrometry,
DMSO: Dimethyl sulfoxide (CD_3SOCD_3);
HSQC: Heteronuclear single quantum coherence ,
HMBC: Heteronuclear multiple bond correlation;
TLC: Thin layer chromatography;
$CDCl_3$: chloroform-*d*,
s: singlet,

2x

C	0.86811200	3.22918200	-0.36327800
C	0.78145300	4.01457000	0.78065300
C	-0.46303600	4.29155000	1.34135200
C	-1.61588500	3.77883300	0.75149600
C	-1.51627000	2.99719900	-0.39042700
H	1.83870900	3.00812600	-0.78836700
H	1.68357500	4.40951700	1.23766500
H	-2.57783500	3.99702700	1.19809600
H	-2.41426700	2.59290200	-0.83970400
O	-0.61441700	5.05486200	2.46556600
H	0.24817700	5.34992800	2.77887500
C	-0.18389800	1.92006700	-2.26552800
H	-0.25137700	2.61162500	-3.10724700
N	-1.27973200	0.91663700	-2.36971900
H	-1.72978900	0.91005800	-3.28398200
N	1.09793900	1.16793200	-2.35246300
H	1.55140900	1.25570800	-3.26085200
C	-0.27606200	2.70618600	-0.96221100
C	-0.62071400	-0.31418900	-2.10818700
C	0.70646000	-0.17403900	-2.09774500
O	-1.32353500	-1.47290400	-2.00525100
O	1.63740800	-1.15751100	-1.98012100
C	-2.21622900	-1.66139400	-0.96945700
C	-3.05566800	-2.76504600	-1.09791100
C	-2.27236100	-0.83997800	0.15674600
C	-3.95975800	-3.04634200	-0.08792900
H	-2.98123000	-3.38342300	-1.98314500
C	-3.18319700	-1.13244800	1.15694900
H	-1.61731200	0.01550100	0.24179100
C	-4.03691200	-2.23476200	1.04753600
H	-4.61616200	-3.90537000	-0.17781900
H	-3.24744300	-0.51089100	2.04156700
C	2.49063800	-1.18391100	-0.89584200
C	2.30462700	-0.41413000	0.24707300
C	3.55856200	-2.07874300	-0.99885000
C	3.20631400	-0.54936200	1.29403000
H	1.47593500	0.27637600	0.31546900
C	4.44677400	-2.20229000	0.05024200
H	3.66524300	-2.66335000	-1.90383400
C	4.28073500	-1.43579500	1.21149500
H	3.07173800	0.04370300	2.19258300
H	5.28170900	-2.88957200	-0.00595700
C	5.22079700	-1.55311900	2.33918900
H	4.98350000	-0.90275900	3.20760300
C	-5.00932900	-2.55046000	2.10824800
H	-5.62810700	-3.45194600	1.91346900
O	-5.16160800	-1.91266500	3.12679200
O	6.18779800	-2.28236000	2.36716500



2y

C	3.70756800	-1.09862300	-0.78828200
C	4.94583900	-1.35179600	-1.35619400
C	6.09304900	-0.77006500	-0.81292900
C	5.99365000	0.06227400	0.29833100
C	4.74781100	0.30740800	0.85715000
H	2.82117700	-1.55271100	-1.21255400
H	5.02572900	-2.00109300	-2.22213400
H	6.89191100	0.50511100	0.70878400
H	4.67359700	0.95585900	1.72313900
O	7.33757000	-0.98327200	-1.32969700
H	7.28090500	-1.57109900	-2.09185500
C	2.26667100	0.01791300	0.94057100
H	2.35980600	0.66787700	1.82306900
C	3.59487100	-0.26561600	0.32431100
C	0.11056900	0.25931600	0.44544000
C	0.22807400	-0.82079800	1.22910000
C	-1.31284700	2.20206200	-0.11679200
C	-0.44100500	3.17403500	0.38855900
C	-2.54596700	2.60029900	-0.66473200
C	-0.80790900	4.50894200	0.34463700
H	0.51676700	2.88365800	0.79527900
C	-2.89873800	3.93157300	-0.69964900
H	-3.21980300	1.84792000	-1.06088800
C	-2.03194200	4.91043300	-0.19441800
H	-0.12900500	5.25921200	0.73691700
H	-3.84839300	4.24236300	-1.11735800
C	-1.66259300	-2.39456600	1.08580600
C	-2.67413200	-3.05806400	1.79818800
C	-1.63843100	-2.49464100	-0.31770800
C	-3.62975300	-3.79439400	1.12454500
H	-2.70316100	-2.98390700	2.87971900
C	-2.60409500	-3.22952400	-0.97767700
H	-0.83919600	-2.02192300	-0.87482800
C	-3.61737000	-3.88782400	-0.27097800
H	-4.40657200	-4.30302700	1.68606800
H	-2.58509000	-3.31788000	-2.05722400
C	-4.64670700	-4.66977600	-0.96911200
H	-5.38843500	-5.15732600	-0.30144200
C	-2.39129600	6.33435100	-0.22394600
H	-1.62176000	7.01258400	0.20248200
O	-3.42735000	6.78605400	-0.66415800
O	-4.72507700	-4.80436300	-2.17184900
O	1.36329600	0.66910400	0.01621100
O	1.57536800	-1.17524700	1.33900500
N	-1.00708400	0.84293800	-0.10359000
H	-1.75579400	0.21369700	-0.35570300
N	-0.71943200	-1.65285200	1.78518300
H	-0.68943000	-1.78563000	2.78595000

Figure 6: Cartesian coordinates of predicted structures of compound **2**

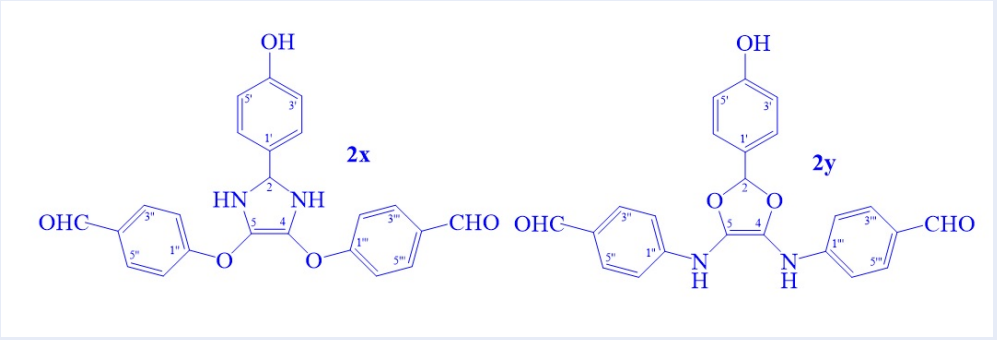


Figure 7: Structure of new compound **2**

Table 2: α-glucosidase inhibitory activity of isolated compounds from *Bruguiera cylindrica*

Compound	Concentration (mg/mL)					IC_{50} (mg/mL)	p-value
	5	**25**	**50**	**75**	**100**		
Benzobrugierol (1)	48.5 ± 0.7	51.1 ± 0.3	52.9 ± 0.8	56.6 ± 0.2	58.8 ± 0.6	17.9 ± 0.4	0.01
Bruguierine (2)	46.6 ± 0.5	49.0 ± 0.5	52.1± 0.8	54.5 ± 0.6	56.9 ± 0.5	34.6 ± 0.7	0.02
Lupeol (3)	30.2 ± 0.5	36.3 ± 0.4	39.4 ± 0.3	41.5 ± 0.8	53.0 ± 0.6	98.0 ± 0.6	0.02
Betulin (4)	17.8 ± 0.5	46.8 ± 0.7	60.3 ± 0.7	70.8 ± 0.4	> 100	38.7 ± 0.6	0.00
Chrysoeriol (5)	14.2 ± 0.2	25.9 ± 0.5	48.7 ± 0.3	65.9 ± 0.1	94.8 ± 0.4	51.1 ± 0.3	0.03
Compound	**Concentration (mg/mL)**					**IC_{50} (mg/mL)**	**p-value**
	10	**50**	**100**	**150**	**200**		
β-Sitosterol 3-O-β-D-glucopyranoside (11)	9.9 ± 0.4	32.5 ± 0.3	49.3 ± 0.2	63.2 ± 0.6	74.8 ± 0.7	114.2 ± 0.6	0.00
Acarbose (positive control)	4.7 ± 0.4	10.5 ± 0.2	39.5 ± 0.3	62.4 ± 0.1	79.1 ± 0.3	127.7 ± 0.2	0.00

Data are presented as mean ± SD values of triplicate determinations. A one-way analysis of variance (ANOVA) and ositive analysis was done using Duncan multiple tests. Significance was accepted at P<0.05.

d: doublet,
m: multiplet.

CONFLICTS OF INTEREST

The authors declare no competing financial interest

ACKNOWLEDGMENT

This research is funded by Ho Chi Minh City Open University under grant number T2020.01.1.

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