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# Bioactive Phytoconstituents of *Leucas zeylanica* and its Molecular Docking Study

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Bioactive phytoconstituents **1-3** were isolated from the chloroform extract of aerial parts of *Leucas zeylanica.* Structure of all the three compounds were elucidated by the spectroscopic analysis data. Three compounds showed good activities, but compound **2** exhibited significant *in vitro* antioxidant activity. In molecular docking study of compound **2** also showed good docking score within binding pocket of the selected proteins.

**Keywords: *Leucas zeylanica*, Phytoconstituents, Antioxidant activity, Molecular docking.**

## INTRODUCTION

*Leucas zeylanica* is popularly known as shetodrone in Bangladesh. It belongs to the plant family Lamiaceae and produces in dry and sunny areas such as sea beaches, roadside and paddy fields. It has a wide variety of medicinal applications against severe ailments [1]. It is familiar in the treatment of several fatal diseases such as abdominal pain, gout, malaria, burning urination, flatulence and abdominal skin tightening after delivery [2]. Plant juice is also used in the treatment of fever, scabies, headache and colds [2]. Earlier phytochemical screening on the genus *Leucas* revealed the presence of a variety of natural bioactive compounds [3-8]. Nidhal *et al*. [9] isolated several components including triterpenoids, flavonoids, glycosides, steroids, fatty acids and their derivatives in *Leucas zeylanica*. As a part of our ongoing search for bioactive natural products, the current phytochemical investigation of the aerial parts of *Leucas zeylanica* has led to the isolation of three new natural products **1-3**. This work also present the elucidation of structures, the antioxidant activity of compounds and the molecular docking of all the three new natural products.

## EXPERIMENTAL

The IR spectra (KBr pellets) were recorded on a Shimadzu FT-IR 20 spectrophotometer, Japan. The NMR experiments ($^1$H, $^{13}$C, COSY, HSQC and HMBC) were carried out on a Bruker AVANCE 400 spectrometer (Bruker AG, Germany). Mass spectra were measured on a MAT 95XL Finnigan instrument (Thermo Quest Finnigan, Germany) for electrospray ionization (ESI).

**Plant material:** Matured and flowering plants of *Leucas zeylanica* were collected from the hilly region of the district of Chattogram, Bangladesh during the month of August 2020. The plant was identified by Prof. A. Rahman, Department of Botany, and a voucher specimen was deposited at Department of Botany, University of Chittagong, Bangladesh.

**Extraction and isolation:** The plant materials (3.5 kg) were dried powdered and extracted with chloroform (3 × 72 h). The total chloroform extract was evaporated under reduced pressure to afford chloroform extract of 35 g. The chloroform extracts (4 g) were chromatographed on a silica gel column eluted with *n*-hexane-EtOAc (4:1) to give compound **1** (177 mg), compound **2** (210 mg) and a mixture of 520 g. Solid mass 520 mg was rechromatographed on a silica gel column and eluted with *n*-hexane-EtOAc (2:1) to give compound **3** (270 mg). Three compounds were purified by repeated crystallization from solvent system *n*-hexane-EtOAc (3:1).

**Compound 1:** White amorphous solid, m.p.: 170-172 ºC, soluble in $CHCl_3$. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3380 (O-H). $^1$H and $^{13}$C NMR (400 and 100 MHz, $CDCl_3$) (Table-1). EI-MS *m/z* 481.5632 [M+1], 410, 340, 284, 218, 182, 162, 123.

TABLE-1
$^1$H AND $^{13}$C NMR DATA OF COMPOUND **1** ($CDCl_3$, δ, ppm, *J*, Hz)*

| C atom | $^{13}$C, δ | $^1$H, δ | C atom | $^{13}$C, δ | $^1$H, δ |
|---|---|---|---|---|---|
| 1 | 129.2 | | 10′ | 33.5 | 1.64 (m) |
| 2 | 107.8 | 7.22 (d, *J* = 7.2) | 11′ | 29.5 | 1.27 |
| 3 | 128.8 | 6.42 (t, *J* = 8.4) | 12′ | 29.3 | 1.27 |
| 4 | 127.1 | 7.69 (m) | 13′ | 29.2 | 1.27 |
| 5 | 128.7 | 7.42 (d, *J* =7.6) | 14′ | 29.2 | 1.27 |
| 6 | 128.6 | 6.40 (t, *J* =8.4) | 15′ | 29.1 | 1.27 |
| 7 | 102.8 | 5.36 (s) | 16′ | 29.0 | 1.27 |
| 1′ | 129.1 | | 17′ | 29.2 | 1.27 |
| 2′ | 128.4 | 6.75 (d, *J* = 2.1) | 18′ | 28.9 | 1.27 |
| 3′ | 139.2 | | 19′ | 14.0 | 0.88 (s) |
| 4′ | 156.9 | | 20′ | 114.0 | 5.84 (d, *J* = 6.8) |
| 5′ | 130.3 | 6.37 (d, *J* = 8.0) | 21′ | 37.2 | 2.37 (t, *J* =7.6) |
| 6′ | 127.0 | 7.09 (d, *J* = 8.0) | 22′ | 24.7 | 1.27 (m) |
| 7′ | 53.8 | 4.93 (s) | 23′ | 31.9 | 1.43 (m) |
| 8′ | 127.3 | | 24′ | 11.9 | 0.90 (s) |
| 9′ | 77.2 | 4.96 (t, *J* =10.8) | 25′ | 11.5 | 0.90 (s) |

*The assignment was based on COSY, HMBC, HSQC and DEPT (135°) experiments.

**Compound 2:** White amorphous solid, m.p.: 190-192 ºC, soluble in $CHCl_3$. IR (KBr, $\nu_{max,}$ $cm^{-1}$): 3432, 1737, 1697. $^1$H & $^{13}$C NMR data (400 and 100 MHz, $CDCl_3$) (Table-2). EI-MS *m/z* 969.4236 [M+1], 951, 894, 807,750, 733, 608, 521, 491, 361, 284.

**Compound 3:** White amorphous solid, m.p.: 180-182 ºC, soluble in $CHCl_3$. IR (KBr, $\nu_{max,}$ $cm^{-1}$): 3417, 1710, 1740. $^1$H & $^{13}$C NMR data (400 and 100 MHz, $CDCl_3$) (Table-3). EI-MS *m/z* 961.5236 [M+1], 506, 413, 266, 248, 240, 206, 204.

**Antioxidant activity assay:** The antioxidant efficacy of the isolated compounds was assessed by using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method [10]. An ethanolic DPPH solution was prepared by 24 h constant stirring and isolated compounds were dissolved in this solution to prepare five different dilutions such as 500, 250, 125, 62.5 and 31.25 µg/mL. 4.0 mL DPPH solution and 100 µL of sample solution were placed in a test tube and stored in dark conditions. Similarly, the standard sample solution was prepared to use as a positive control. Each tube was then vortexed and incubated at 26 ºC for 15 min. The maximum absorbance was read by UV-visible spectrometer at 517 nm for each solution against blank. The percent of inhibition was determined by using the following equation:

TABLE-2
$^{13}$C NMR AND $^1$H NMR SPECTRAL DATA OF COMPOUND **2** ($CDCl_3$, δ, ppm, *J*, Hz)*

| C atom | $^{13}$C, δ | $^1$H, δ | C atom | $^{13}$C, δ | $^1$H, δ |
|---|---|---|---|---|---|
| 1 | 29.6 | 1.72 (m); 1.02 (m) | 33 | 29.3 | 1.28-1.32 (m) |
| 2 | 51.6 | 2.08 (m) | 34 | 29.2 | 1.28-1.32 (m) |
| 3 | 77.2 | 3.60 (d, *J* = 6.4) | 35 | 24.0 | 1.28-1.32 (m) |
| 4 | 33.1 | | 36 | 12.1 | 0.91 (t, *J* = 6.8) |
| 5 | 32.9 | 1.65 (t, *J* = 7.2) | 37 | 31.9 | 1.28-1.32 (m) |
| 6 | 29.5 | 1.41 (m); 0.98 (m) | 38 | 31.0 | 1.28-1.32 (m) |
| 7 | 29.4 | 1.61 (m); 1.00 (m) | 39 | 24.3 | 1.28-1.32 (m) |
| 8 | 49.3 | 1.68 (t, *J* = 7.2) | 40 | 12.2 | 0.88 (t, *J* = 6.8) |
| 9 | 48.4 | 1.68 (t, *J* = 7.2) | 1′ | 177.4 | |
| 10 | 34.0 | | 2′ | 127.5 | 6.29 (d, *J* = 7.8) |
| 11 | 43.2 | 2.38 (t, *J* = 7.2) | 3′ | 126.4 | 7.99 (t, *J* = 8.6) |
| 12 | 118.2 | 5.46 (t, *J* = 2.4) | 4′ | 125.3 | 6.23 (d, *J* = 7.8) |
| 13 | 120.3 | | 5′ | 124.4 | |
| 14 | 38.7 | | 6′ | 178.1 | |
| 15 | 28.2 | 1.65 (m); 1.22 (m) | 6′(OH) | | 8.64 (s) |
| 16 | 32.7 | 1.83 (m); 1.54 (m) | 7′ | 97.6 | 3.90 (d, *J* = 6.4) |
| 17 | 42.4 | | 8′ | 82.5 | 3.43 (t, *J* = 7.2) |
| 18 | 51.3 | 2.08 (d, *J* = 4.4) | 9′ | 82.5 | 3.27 (t, *J* = 7.2) |
| 19 | 52.8 | 1.67 (m) | 10′ | 91.6 | 3.72 (d, *J* = 6.4) |
| 20 | 64.7 | | 11′ | 138.2 | |
| 21 | 36.0 | 1.55 (m); 1.28 (m) | 12′ | 129.3 | 7.72 (s) |
| 22 | 32.9 | 1.73 (m); 1.43 (m) | 13′ | 170.2 | |
| 23 | 204.1 | | 14′ | 127.1 | |
| 24 | 69.5 | 4.25 (m) | 15′ | 171.1 | |
| 25 | 23.5 | 1.02 (d, *J* = 6.8) | 13′(OH) | | 9.61 (s) |
| 26 | 23.1 | 1.02 (d, *J* = 6.8) | 15′(OH) | | 9.47 (s) |
| 27 | 14.0 | 0.91 (s) | 16′ | 130.1 | 7.56 (s) |
| 28 | 15.1 | 0.91 (s) | 17′ | 33.7 | 2.10 |
| 29 | 17.2 | 0.93 (s) | 18′ | 29.1 | 1.28-1.32 (m) |
| 30 | 18.1 | 0.93 (s) | 19′ | 24.7 | 1.28-1.32 (m) |
| 31 | 129.2 | 5.85 (s) | 20′ | 11.2 | 0.88 (t, *J* = 6.8) |
| 32 | 123.2 | 5.11 (m) | | | |

*The assignment was based on COSY, HMBC, HSQC and DEPT (135°) experiments.

TABLE-3
$^{13}$C NMR AND $^{1}$H NMR SPECTRAL DATA OF COMPOUND **3** ($CDCl_3$, δ, ppm, *J*, Hz)*

| C atom | $^{13}$C, δ | $^{1}$H, δ | C atom | $^{13}$C, δ | $^{1}$H, δ |
|---|---|---|---|---|---|
| 1 | 33.0 | 1.76 (m); 1.10 (m) | 2′ | 33.8 | 2.30 (d, *J* = 6.4) |
| 2 | 37.2 | 1.98 (m); 1.13 (m) | 3′ | 40.4 | 1.66 (m) |
| 3 | 71.8 | 3.55 (t, *J* = 5.2) | 4′ | 33.8 | 2.41 (t, *J* = 6.0) |
| 4 | 36.5 | | 5′ | 121.7 | 5.14 (dt, *J* = 8.8, 5.4) |
| 5 | 45.8 | 1.51 (t, *J* = 3.2) | 6′ | 129.3 | 5.07 (dd, *J* = 8.8, 5.2) |
| 6 | 31.6 | 1.42 (m); 1.10 (m) | 7′ | 50.1 | 1.98 (m) |
| 7 | 31.8 | 1.62 (m); 1.12 (m) | 8′ | 39.7 | 1.81 (t, *J* = 7.6) |
| 8 | 42.2 | | 9′ | 91.6 | 5.10 (t, *J* = 3.2) |
| 9 | 42.2 | 1.51 (t, *J* = 3.2) | 12′ | 89.6 | 5.09 (t, *J* = 3.2) |
| 10 | 39.8 | | 13′ | 204.1 | |
| 11 | 42.2 | 2.06 (t, *J* = 7.2) | 14′ | 39.6 | 1.81 (t, *J* = 7.6) |
| 12 | 138.2 | 5.37 (d, *J* = 4.4) | 15′ | 36.1 | 1.51 (t, *J* = 3.2) |
| 13 | 140.7 | | 16′ | 29.3 | 1.27-1.32 (m) |
| 14 | 42.3 | | 17′ | 29.2 | 1.27-1.32 (m) |
| 15 | 29.6 | 1.61 (m); 1.16 (m) | 18′ | 29.1 | 1.27-1.32 (m) |
| 16 | 30.2 | 1.85 (m); 1.55 (m) | 19′ | 28.7 | 1.27-1.32 (m) |
| 17 | 50.2 | | 20′ | 28.2 | 1.27-1.32 (m) |
| 18 | 51.2 | 2.01 (t, *J* = 7.2) | 21′ | 26.1 | 1.27-1.32 (m) |
| 19 | 29.5 | 1.72 (m); 1.15 (m) | 22′ | 25.3 | 1.27-1.32 (m) |
| 20 | 36.5 | | 23′ | 24.7 | 1.27-1.32 (m) |
| 21 | 29.4 | 1.46 (m); 1.18 (m) | 24′ | 24.7 | 1.27-1.32 (m) |
| 22 | 29.4 | 1.63 (m); 1.46 (m) | 25′ | 24.3 | 1.27-1.32 (m) |
| 23 | 178.0 | | 26′ | 24.3 | 1.27-1.32 (m) |
| 24 | 21.2 | 1.07 (s) | 27′ | 24.0 | 1.27-1.32 (m) |
| 25 | 19.8 | 0.95 (s) | 28′ | 23.0 | 1.27-1.32 (m) |
| 26 | 19.0 | 0.93 (s) | 29′ | 29.1 | 1.51 (m) |
| 27 | 18.9 | 0.90 (s) | 30′ | 11.8 | 0.84 (d, *J* = 7.2) |
| 28 | 18.7 | 0.90 (s) | 31′ | 11.9 | 0.84 (d, *J* = 7.2) |
| 29 | 14.0 | 0.96 (s) | 32′ | 12.0 | 0.88 (s) |
| 30 | 15.3 | 0.98 (s) | 33′ | 21.0 | 1.07 (s) |
| 1′ | 178.2 | | 34′ | 12.2 | 0.87 (s) |

*The assignment was based on COSY, HMBC, HSQC and DEPT (135°) experiments.

$$\text{Inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

where, $A_{control}$ = absorbance of DPPH radical and $A_{sample}$ = absorbance of DPPH with the sample. The concentration-inhibition curves were utilized to calculate the $IC_{50}$ values for ascorbic acid and isolated analogs.

**Molecular docking study:** To analyze the binding mode of the isolated compounds in the active site of human antioxidant enzyme receptor molecular docking was conducted by Gaussian 09, PyRx 0.8, PyMOL and Discovery Studio 4.1 software [11-14]. The three-dimensional crystal structure of human antioxidant enzyme receptor 3MNG was retrieved from the Protein data bank (www.rcsb.org) and prepared through PyMol (version 2.4) to remove all the water molecules, hetero atoms and inhibitors present in the structure. The swisspdb viewer was utilized to minimize the energy of the target protein structure. Structure optimization of compound **2** was done by using Chem Draw 16 pro software.

## RESULTS AND DISCUSSION

Compound **1** was obtained as white amorphous powder. The IR spectrum of **1** showed broad absorption for O-H group at $\nu_{max}$ 3380 $cm^{-1}$. The mass spectrum of **1** exhibited a molecular ion peak at *m/z* 481.5632 [M+1] consistent with the molecular formula $C_{32}H_{48}O_3$. The $^{13}$C DEPT (135 ºC) spectrum of compound **1** revealed the presence of 13 methylene carbons, 11 methine carbons and 3 methyl carbons in the molecule. The absorption positions of all 32 carbons in $^{13}$C NMR spectrum are given in Table-1. The $^{1}$H NMR spectrum of compound **1** showed the presence of 8 aromatic protons at δ 7.22 (1H, d, *J* = 7.2 Hz, H-2), 6.42 (1H, t, *J* = 8.4 Hz, H-3), 7.69 (1H, m, H-4), 7.42 (1H, t, *J* = 7.6 Hz, H-5), 6.40 (1H, d, *J* = 8.4 Hz, H-6), 6.75 (1H, d, *J* = 2.1 Hz, H-2′), 6.37 (1H, d, *J* = 8.0 Hz, H-5′) and 7.09 (1H, d, *J* = 8.0 Hz, H-6′). The absorption positions of two olefinic protons at δ 5.84 (2H, d, *J* = 6.8 Hz, H-20′), 3 methine protons at δ 5.36 (1H, s, H-7), 4.96 (1H, t, *J* = 10.8 Hz, H-9′) and 1.43 (1H, m, H-23′). Absorption positions of 12 sets of methylene protons and 3 sets of methyl protons are given in Table-1. Absorption patterns, number of carbons and protons of compound **1** suggest the presence of two side chains in the molecule. The connectivity of the side chain and important $^{1}$H-$^{1}$H correlations in 2D COSY spectrum and $^{1}$H-$^{13}$C correlations in HMBC spectrum are presented in Fig. 1. The molecular ion peak of 1 *m/z* 481.5632 [M+1] along with other mass peaks

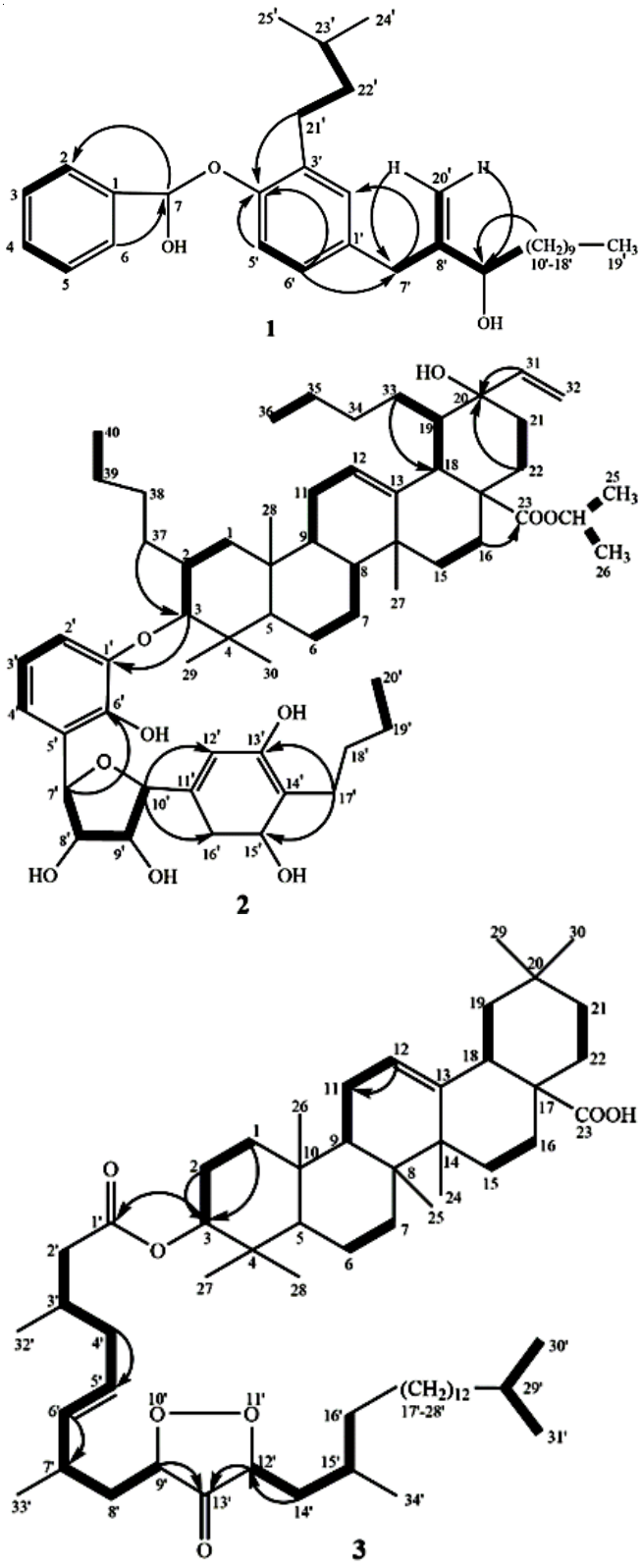


Fig. 1. Selected ▬ COSY and ⟶ HMBC correlations of **1**, **2** and **3**

*m/z* 463, 410, 340, 284, 218, 182, 162, 123 can be explained nicely with the given structure of compound **1**. Compound **1** is thus characterized as 4′-[7-[hydroxyl(phenyl)methoxy]-3′-isopentylbenzyl]tridec-8′-en-9′-ol and is reported first time from *Leucas zeylanica*.

Compound **2** was obtained as a white amorphous powder. It gave Salkowski and Liebermann-Bürchard reaction test for the identification of steroid and terpenoid [10]. The IR spectrum of compound **2** showed a broad absorption for O-H stretching at $\nu_{max}$ 3432 $cm^{-1}$. It also showed sharp absorptions at $\nu_{max}$ 1737 and 1697 $cm^{-1}$ for >C=O function of an ester. $^1$H NMR spectrum of compound **2** showed the presence of a huge number of methyl, methylene and methine protons in the molecule. The $^{13}$C NMR of compound **2** revealed the presence of 9 methyl carbons, 18 methylene carbons and 19 methine carbons in the molecule. Earlier literature review had revealed that genus *Leucas* contains several steroids, terpenoids along with a long alkyl chain [4,8, 15]. Several alkyl chains were observed in compound **2**, which is consistent with the attempt to correlate this molecule with those that have been previously reported [8,9]. $^1$H NMR spectrum of **2** showed the presence of 5 aromatic protons at δ 6.29 (1H, d, *J* = 7.8 Hz, H-2′), 7.99 (1H, d, *J* = 8.6 Hz, H-3′), 6.23 (1H, d, *J* = 7.8 Hz, H-4′), 7.72 (1H, s, H-12′) and 7.56 (1H, s, H-16′). Absorptions of three phenolic protons attached to C6′ (OH), C13′ (OH) and C15′ (OH) are shown at δ 8.64 (s), 9.61 (s) and 9.47 (s), respectively. Presence of three olefinic protons at δ 5.85 (1H, s, H-31), 5.46 (1H, t, *J* = 2.4 Hz, H-12), 5.11 (1H, m, H-32) and six lower field methine protons at δ 3.60 (1H, d, *J* = 6.4 Hz, H-3), 4.25 (1H, m, H-24), 3.90 (1H, d, *J* = 6.4 Hz, H-7′), 3.43 (1H, t, *J* = 7.2 Hz, H-8′), 3.27 (1H, t, *J* = 7.2 Hz, H-9′) and 3.72 (1H, d, *J* = 6.4 Hz, H-10′) are confirmed in the $^1$H NMR spectrum of compound **2**. $^{13}$C NMR (DEPT 135 ºC) spectrum of compound **2** revealed the presence of 19 methylene carbons, 25 methine carbons and 9 methyl carbons in the molecule. Absorptions of 12 aromatic carbons appeared at δ 177.4 (C1′), 127.5 (C2′), 126.4 (C3′), 125.3 (C4′), 124.4 (C5′), 178.1 (C6′), 138.2 (C11′), 129.2 (C12′), 170.2 (C13′), 127.1 (C14′), 171.1 (C15′) and 130.1 (C16′). Chemical shifts of 60 carbons in $^{13}$C NMR spectrum of compound **2** are given in Table-2. The important $^1$H-$^1$H and $^1$H-$^{13}$C correlations in 2 observed from 2D COSY and HMBC spectra are shown in Fig. 1. The mass spectrum of **2** exhibited the highest molecular ion peak at *m/z* 969.4236 [M+1] corresponding to the molecular formula $C_{60}H_{88}O_{10}$. The molecular ion peak *m/z* 969.4236 [M+1] along with other mass fragments 951, 894, 807,750, 733, 608, 521, 491, 361, 284 are very much consistent with the proposed structure of compound **2**. The spectral data compound **2** supported the structure as isopropyl 2,19-dibutyl-3-[5′-[11′-[14′-butyl-13′,15′-dihydroxypentyl]-8′,9′-dihydroxytetrahydrofuran-10′-yl]-6′-hydroxyl phenoxy]-20-hydroxy-14,4,4,10-tetramethyl-20-vinyl-1,2,3,4,5,6,7,8,9,10,11,14,15, 16,17,18,19, 20,21,22-isosahydropicene-17-caboxylate.

Compound **3** was obtained as a colourless amorphous powder. It gave a positive colour test for steroid and terpenoid [10]. The IR spectrum of compound **3** displayed a broad absorption at 3417 $cm^{-1}$ for OH function and sharp absorptions at $\nu_{max}$ 1710 $cm^{-1}$ carbonyl function and 1740 $cm^{-1}$ for ester and carboxylic acid carbonyl function. The $^{13}$C NMR spectrum of compound **3** showed the presence of as many as 62 carbons in the molecule Table-3. The mass spectrum of **3** exhibited a molecular ion peak at *m/z* 961.5236 [M+1] corresponding to the molecular formula $C_{62}H_{104}O_7$. Compound **3** also contains a long

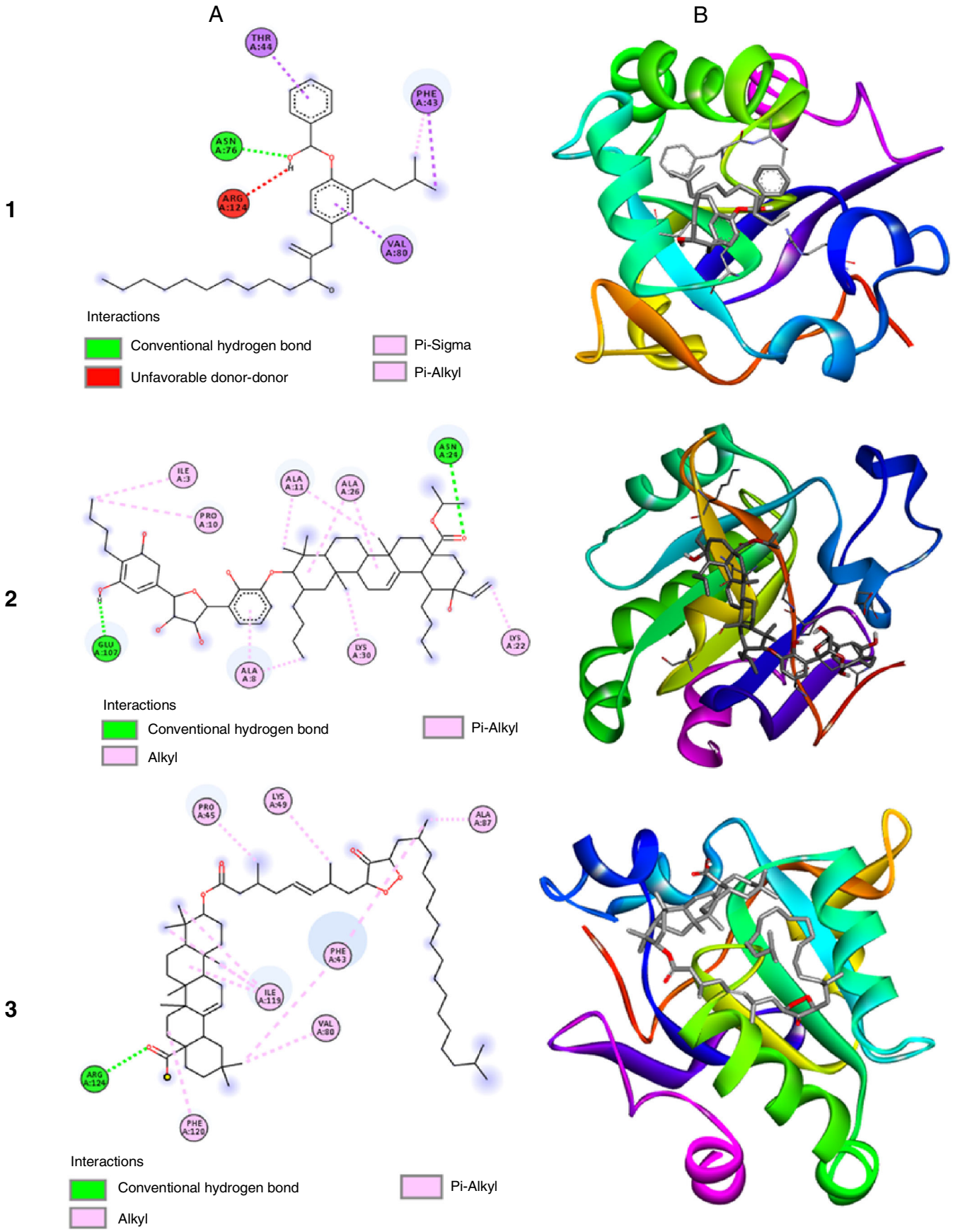


Fig. 2. Molecular docking studies of compound **1**, **2** and **3** against 3MNG protein receptors; (A) 2D interaction sketches (B) 3D docking predictions

TABLE-5
PROTEIN-LIGAND INTERACTIONS WITH AMINO ACID RESIDUES OF 3MNG PROTEIN RECEPTOR AND THEIR BOND DISTANCES

| Compd. | Binding energy (Kcal/mol) | Hydrogen bond (Distance: Å) | Hydrophobic (Distance: Å) |
|---|---|---|---|
| **1** | -4.2 | ASN76 (3.0) | THR44 (3.6), VAL80 (3.9), PHE43 (3.6), PHE43 (4.1) |
| **2** | -6.6 | ASN24 (2.9); GLU107 (2.2) | ALA8 (4.1), ALA11 (4.0), ALA11 (4.0), ALA26 (5.1), ALA26 (4.6), ILE3 (4.1), PRO10 (4.3), LYS22 (4.6), LYS30 (5.3), ALA8 (4.2) |
| **3** | -5.9 | ARG124 (2.9) | ALA87 (3.9), ILE119 (5.4), PRO45 (4.9), LYS49 (4.6), VAL80 (4.0), ILE119 (4.3), ILE119 (4.9), ILE119 (4.2), PHE43 (5.0), PHE43 (4.5), PHE120 (5.1) |

alkyl chain in the molecule like compound **2**. The $^{1}$H NMR of compound **3** showed the presence of three olefinic protons appeared at δ 5.37 (1H, d, *J* = 4.4 Hz, H-12), 5.14 (1H, dt, *J* = 8.8, 5.4 Hz, H-5′) and 5.07 (1H, dd, *J* = 8.8, 5.2 Hz, H-6′) and three lower field methine protons appeared at δ 3.55 (1H, t, *J* = 5.2 Hz, H-3), 5.10 (1H, t, *J* = 3.2 Hz, H-9′) and 5.09 (1H, t, *J* = 3.2 Hz, H-12′) in the molecule. $^{13}$C NMR (DEPT 135 ºC) spectrum of **3** showed the presence of 27 methylene carbons, 13 methine carbons and 12 methyl carbons in the molecule. Chemical shifts of three carbonyl carbons at δ 204.1 (C13′), 178.2 (C1′) and 178.0 (C23) were confirmed from $^{13}$C NMR spectrum of **3**. Absorptions of 62 carbons in $^{13}$C NMR spectrum of **3** are shown in Table-3. Important connectivity of the side chain and the location of functional groups in the molecule were confirmed by the correlations observed in the 2D COSY and HMBC spectra Fig. 1. Based on the above spectral data, compound **3** is characterized as (*E*)-3-[[8′-[9′-[15′,29′-dimethylheptadecyl]-13′-oxo-10′,11′-dioxolan-12′-yl]-3′,7′-dimethyloct-5′-enoyl]-oxy]-4,4,8,14,10,20,20-heptamethyl-1,2,3,4,5,6,7, 8,9,10,11,14,15, 16,17,18,19,20,21,22-icosahydropicene-17-carboxylic acid.

**Antioxidant activity:** The DPPH radical scavenging method was used to determine the antioxidant activity of isolated analogues. The ascorbic acid was used as a standard reference. The standard (ascorbic acid) showed antioxidant activity with $IC_{50}$ value of 27.34 ± 1.86 µg/mL. Compound **2** revealed significant activity with $IC_{50}$ value of 63.97 ± 3.41 µg/mL whereas compounds **1** and **3** showed moderate activity as compared to standard (Table-4).

TABLE-4
ANTIOXIDANT ACTIVITIES OF COMPOUNDS **1-3**

| Compound | $IC_{50}$ (µg/mL) |
|---|---|
| **1** | 83.2 ± 3.45 |
| **2** | 63.97 ± 3.41 |
| **3** | 180.43 ± 2.39 |
| Ascorbic acid | 27.34 ± 1.86 |

**Molecular docking study:** Compounds **1**, **2** and **3** showed a binding energy score -4.2, -6.6 and -5.9 Kcal/mol when docked against the 3MNG protein receptor affords the best interaction against the selected receptor (Table-5). The number of hydrogen bonds plays a crucial role to show greater bioactivity of molecules. Fig. 2 shows a two- and three-dimensional visual representation of the docked compounds.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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