



## Isolation, Identification, Antibacterial Activity and Docking of Fatty acid and Fatty Alcohol from *Rumex dentatus* Leaf Extract

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### ABSTRACT

*Rumex dentatus* is a traditional medicinal plant that has been used in the treatment of anti-dermatitis, anti-inflammatory, anti-tumour, diarrhoea, eczema, constipation, and locally known Toothed dock. This article describes the isolation of compounds from CH<sub>3</sub>OH extract of *Rumex dentatus* leaf. The structures of the isolated compounds were identified by spectroscopic analysis, such as <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, DEPT-135, COSY, HMBC, and HSQC. The extract and compounds were evaluated for antibacterial activity. The isolated compounds and antibacterial relationships were performed by molecular docking. The antibacterial activity was determined on *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* by agar well diffusion assay. Molecular docking studies were performed with the help of software such as Auto Dock Tools-1.5.6, Auto dock vina, chem3D pro 12.0.2.1076 and Discovery Studio Visualizer. The extract was showed maximum inhibition zone with *Staphylococcus aureus*, which indicates good antibacterial activity. The molecular docking was exhibited best results with DNA gyrase. The antibacterial and docking results were revealed that extract and compounds might be beneficially for antibacterial activities.

**Keywords:** *Rumex dentatus*, Hexacosanol, Hexacosanoic acid, COSY, DNA gyrase, antibacterial.

### INTRODUCTION

*Rumex dentatus* is commonly known as Toothed dock, Aegean dock and Golden dock belong to the family Polygonaceae. It is a widely distributed many temperate and tropical countries such as India, Afghanistan, Pakistan, East Asia and is used in the Mediterranean diet<sup>1</sup>. *Rumex dentatus* shows allelopathic activity against wheat and mustard<sup>2</sup>. Mostly, allelochemicals of plants are phenolic compounds which increase the generation of reactive oxygen species (ROS) and biological stress<sup>3</sup>. The highest concentration of all ROS is dangerous for organisms, and oxidative stress state in the cell occurs. The high production of ROS can damage to the cell by causing peroxidation of lipid, enzyme inhibition, oxidation of protein, damage to nucleic acids, activation of programmed cell death and ultimately leading to the death of the cells. The plants have the antioxidant functioning power to detoxify the effect of ROS. *Rumex* plant has been used in traditional medicines for anti-inflammatory, bactericidal, anti- dermatitis, astringent, anti-tumor.<sup>4-6</sup> Traditionally, the root of the plant is used for treating acariasis, diarrhoea, eczema, constipation.<sup>7</sup> Various pharmacological active compounds such as flavonoids have been isolated from CH<sub>3</sub>OH crude extract of this plant, but hexacosanol and hexacosanoic acid first time isolated from this plant. Interestingly, long-chain alcohols were developing rat sciatic nerves and brain biosynthesis<sup>8-9</sup>. Hexacosanol have been isolated from *Protasparagus falcatus*<sup>10</sup>, *Acacia Nilotica*<sup>11</sup>, *Sonchus wightianus*<sup>12</sup>, *Euphorbia retusa*<sup>13</sup>, *Euphorbia peplus*<sup>14</sup>, *Baliospermum Axillare*<sup>15</sup>, *Symplocos racemosa*<sup>16</sup>, *Mallotus metcalifianus*<sup>17</sup>,

*Sapium baccatum*<sup>18</sup> and showed biological activity such as Insulin secretion<sup>19</sup>, anti-HIV<sup>20</sup>, acetylcholinesterase inhibitory activity<sup>21</sup>. Hexacosanoic acid has been isolated from *Caesalpinia digyna*<sup>22</sup>, *Millettia speciosa*<sup>23</sup>, *Egyptian Propolis*<sup>24</sup>, *Citrus reticulata*<sup>25</sup>.

CH<sub>3</sub>OH crude extract and isolated compounds were screened for antibacterial activities on *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The bacterial infection is a major cause for human communities worldwide.<sup>26</sup> Therefore, there is urgently needed to kill bacteria (bactericidal) or stop bacterial growth (bacteriostatic) and develop new bacterial antibiotic resistance. DNA gyrase is present in all bacteria, play a key role in bacterial growth, which is the best target of the drug.<sup>27</sup> Isolated compounds were docked with DNA gyrase *in silico*.

### MATERIALS AND METHODS

#### Plant Materials

The plant leaves of *Rumex dentatus* were collected from district Aligarh, UP, India and identified by environmental botanist, Plant Taxonomist, and Ethnobotanist professor M. Badruzzaman Siddiqui, Department of Botany, Aligarh Muslim University, Aligarh, India (Accession no. 31066).

#### General Experimental Procedures

TLC was carried out by on silica gel GF<sub>254</sub> using a pre-coated glass plate. IR spectra in potassium bromide pellets were acquired on the Perkin Elmer 10.4 instruments. Measurement of the melting point was determined at



Stuart digital apparatus (SMP10), which is uncorrected.  $^1\text{H-NMR}$  (400 & 500 MHz) and  $^{13}\text{C-NMR}$  (100 & 125MHz) in  $\text{CDCl}_3$  were recorded on Bruker Avance II (400 MHz) and Bruker Avance Neo (500 MHz) with TMS as the internal standard. Mass spectra were acquired on the XEVO G2-XS QTOF instrument.

### Extraction and Isolation

Shade air-dried leaves (1.5kg) were extracted with 80% methanol at room temperature for 21 days.  $\text{CH}_3\text{OH}$  extract was evaporated using a rotary evaporator at 70-80°C and obtain 80g crude extract. Crude of  $\text{CH}_3\text{OH}$  extract was fractionated with benzene, ethyl acetate, methanol.  $\text{C}_6\text{H}_6$  soluble fraction was eluted by column chromatography using silica gel (60-120 mesh) to give seven main fractions M1-M7. Fraction M4 was further eluted using gradient petroleum ether/ benzene (3/7 7/3 v/v) by glass column chromatography to yield four subfractions M4<sub>1</sub>-M4<sub>4</sub>. Subfraction M4<sub>2</sub> was purified by CC using silica gel using gradient petroleum ether/ $\text{C}_6\text{H}_6$  (5/5 v/v) to yield a white colour powder of compound (1), and M4<sub>3</sub> was purified using gradient hexane/EtOAc (95/5 v/v) to give white powder of compound (2). The compound (1) and (2) was further characterized by various spectroscopic methods such as  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , DEPT-15, HSQC, COSY, HMBC. Spectral data are presented in Tables 1 and 2.

### Compound 1

White color solid, Melting point: 86°C, **ESI-MS** of  $\text{C}_{26}\text{H}_{54}\text{O}$   $m/z$ : 382. **IR**  $\bar{\nu}_{\text{max}}$  (KBr disc)  $\text{cm}^{-1}$ : 3303, 2919, 2850, 1467, 1062.  **$^1\text{H-NMR}$**  ( $\text{CDCl}_3$  400MHz):  $\delta_{\text{H}}$  7.30 (1H, s; OH), 3.60 (2H, t,  $J=6.65$ , H-1), 1.56 (2H, q,  $J=6.88$ , H-2), 1.37-1.17 (46H, m,  $2\text{H}\times 23$ ), 0.88 (3H, t,  $J=6.86$ , H-26).  **$^{13}\text{C-NMR}$**  (100 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  62.95 (C-1), 32.76 (C-2), 25.76 (C-3), 29.80-29.23 (C-4-23), 31.94 (C-24), 22.71 (C-25), 14.15 (C-26). **HSQC**: C-1 (62.8, 3.6;  $\text{CH}_2$ ), C-2 (32.7, 1.5;  $\text{CH}_2$ ), C-3 (25.7, 1.30;  $\text{CH}_2$ ), C-4-23 (29.8-29.2, 1.37-1.17;  $\text{CH}_2\times 20$ ), C-24 (31.9, 1.23;  $\text{CH}_2$ ), C25 (22.7, 1.27;  $\text{CH}_2$ ), C-26 (14.1, 0.88;  $\text{CH}_3$ ).

**Table 1:** 1D and 2D-NMR data for compound 1.<sup>a</sup>

Atom	Type	$\delta_{\text{C}}$	$\delta_{\text{H}}$	HMBC	COSY
1	$\text{CH}_2$	62.95	3.60 (t)	2,3	2
2	$\text{CH}_2$	32.76	[ $J=6.65$ Hz]	-----	1
3	$\text{CH}_2$	25.7	1.56 (q)	-----	-----
4-23	$-\text{[CH}_2\text{]}_4-$	29.8-	1.56 (q)	-----	-----
24	$^{23}$	29.2	[ $J=6.88$ Hz]	-----	-----
25	$\text{CH}_2$	31.94	1.3 (m)	-----	-----
26	$\text{CH}_2$	22.71	1.37-1.17	24, 25	-----
---	$\text{CH}_3$	14.15	0.88 (t)	-----	1
	OH	-----	7.30 (s)	-----	-----

<sup>a</sup>Spectra run at 400 MHz ( $^1\text{H-NMR}$ ) and 100 MHz ( $^{13}\text{C-NMR}$ ) in  $\text{CDCl}_3$ .

### Compound 2

White amorphous solid, Melting point: 88°C, **ESI-MS** for  $\text{C}_{26}\text{H}_{52}\text{O}_2$   $m/z$ : 396 [ $\text{M}^+$ ], **IR**  $\bar{\nu}_{\text{max}}$  (KBr disc)  $\text{cm}^{-1}$ : 3295, 2919, 2850, 1707, 1466, 1298, 724.  **$^1\text{H-NMR}$**  ( $\text{CDCl}_3$  500 MHz):  $\delta_{\text{H}}$  4.4 (1H, bs, OH), 2.34 (2H, t,  $J=5.8$ ; H-2), 1.63 (2H, m, H-3), 0.88 (3H, t,  $J=5.52$ ; H-26), 1.20-1.33 (42H, m,  $2\text{H}\times 21$ ), 1.34 (2H, m, H-4), 1.24 (2H, m, H-25).  **$^{13}\text{C-NMR}$**  (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  179.54 (C=O), 33.97 (C-2), 24.70 (C-3), 29.71 ( $10\times\text{CH}_2$ ), 29.67 ( $5\times\text{CH}_2$ ), 29.61 ( $\text{CH}_2$ ), 29.45 ( $\text{CH}_2$ ), 29.37 ( $\text{CH}_2$ ), 29.25 ( $\text{CH}_2$ ), 29.08 ( $\text{CH}_2$ ), 22.7 ( $\text{CH}_2$ ), 31.88 (C-25), 14.12 (C-26). **HSQC**: C-2 (33.97, 2.34;  $\text{CH}_2$ ), C-3 (24.70, 1.63;  $\text{CH}_2$ ), C-4-24 (29.0-29.71, 1.20-1.33;  $\text{CH}_2\times 21$ ), C-25 (31.88, 1.24;  $\text{CH}_2$ ), C-26 (14.2, 0.84;  $\text{CH}_3$ ).

**Table 2:**  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  and  $^2\text{D-NMR}$  spectral data of compound 2.<sup>a</sup>

Atom	Type	$\delta_{\text{C}}$	$\delta_{\text{H}}$	HMBC	COSY
1	C	179.54	-----	2,3	-----
2	$\text{CH}_2$	33.97	2.34 (t, $J=5.8$ )	3	3
3	$\text{CH}_2$	24.70	1.63	2	2,4
4-24	$-\text{[CH}_2\text{]}_4-$	29.0-	1.63	-----	-----
25	$^{24}$	29.71	(2H, m)	26	26
26	$\text{CH}_2$	31.88	1.20-	-----	25
---	$\text{CH}_3$	14.2	1.33 (m)	-----	-----
	OH	-----	4.4 (bs)	-----	-----

<sup>a</sup>Spectra run in  $\text{CDCl}_3$  solvent.

### Antibacterial assays

The antibacterial activity test of  $\text{CH}_3\text{OH}$  leaf extract and compound (1 and 2) was determined by agar well diffusion assay against two Gram-negative bacterial strains such as *Escherichia coli* (ATCC- 25922) and *Pseudomonas aeruginosa*-PAO1 as well as two gram-positive bacterial strains like *Bacillus subtilis* (NRRL-14596), and *Staphylococcus aureus* (MTCC 3160). Descriptively, the test extract and compounds were dissolved in DMSO and used in the concentration of 1 mg/ml. The overnight freshly grown cultures of bacterial test strains were adjusted to  $1.5 \times 10^8$  U/ml. An aliquot of 100  $\mu\text{l}$  of bacterial strain was spread on nutrient agar (Hi-media) plates. Wells was created in agar plates using a sterile cork borer of 8 mm diameter and wells were sealed with soft agar. Followed the created wells, 100  $\mu\text{l}$  of each test extract and compound were added in each well. The plates were left for incubation at 37 °C for 24 h. The zone of growth inhibition around each well was recorded and measured in mm with zone measuring scale.

### Molecular Docking

Docking studies of isolated compounds were determined by MGL tools and Autodock Vina. Chem3D Pro 12.0.2.1076 was used to draw the structures of compounds in 3D form. Structures of the protein or enzyme was obtained by RCSB and kollman charge, polar hydrogen added and set the grid for compound 1 with dimension  $88 \times 126 \times 112$ , center X =



$Y = Z = 0.028$  and for compound 2 with dimension  $96 \times 122 \times 106$ , center  $X = Y = Z = 0.028$  by Auto Dock Tools-1.5.6. Finally, molecular docking was completed by Autodock Vina.

## RESULTS AND DISCUSSION

### Characterization of isolated compound 1 and 2

White colour solid of compound 1 was obtained, and MASS spectra showed a molecular ion peak at  $m/z$  382, which reveals molecular formula  $C_{26}H_{54}O$ . The FT-IR (in KBr disc) was showing an absorption peak at  $3303\text{ cm}^{-1}$  (O-H stretching vibration, absorption),  $2919$  and  $2850\text{ cm}^{-1}$  (C-H stretching),  $1467\text{ cm}^{-1}$  (C-H bending vibration) and  $1062\text{ cm}^{-1}$  (C-O bending vibration). According to  $^1\text{H-NMR}$  in  $\text{CDCl}_3$ , the proton at  $\delta_H$  3.60 (t,  $J=6.6$  Hz, 2H) was integrated for two protons which indicates methylene proton and proton at  $\delta_H$  0.88 (t,  $J=6.8$  Hz, 3H) was integrated for three protons which show one methyl group and OH proton was observed at 7.30 ppm.  $^{13}\text{C-NMR}$  and DEPT-135 NMR spectra were indicated one methyl group ( $\delta_C$  14.15) and twenty-three methylene groups ( $\delta_C$  22.7, 25.7, 29.23-29.8 {20-carbon}, 31.9, 32.7, 62.9). Methyl proton at  $\delta_H$  0.86 in the HSQC spectrum was linked from methyl carbon ( $\delta_C$  14.1). The methyl carbon ( $\delta_C$  14.1) in HMBC (Figure 2) was connected from two methylene carbon ( $\delta_C$  22.7 and 31.9). It indicates that methyl carbon  $\delta_C$  14.1 was correlated from two methylene carbon  $\delta_C$  22.7 and  $\delta_C$  31.9. In HSQC spectrum, methylene proton ( $\delta_H$  3.6) was correlated from methylene carbon  $\delta_C$  62.8, in HMBC the methylene carbon ( $\delta_C$  62.8) was correlated to methylene carbon ( $\delta_C$  22.7 and 31.9) which indicates that methylene carbon ( $\delta_C$  62.8) was connected from two methylene carbon ( $\delta_C$  22.7 and  $\delta_C$  31.9). The COSY correlation (in Figure 1) was exhibited between  $\delta_H$  3.6 and  $\delta_H$  1.5. So, It was confirmed that isolated compound 1 is hexacosanol.

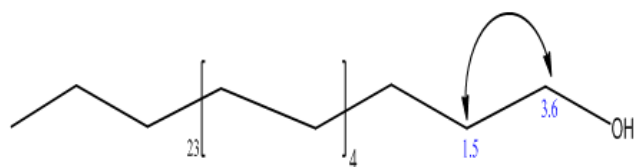


Figure 1: COSY correlation of compound (1).

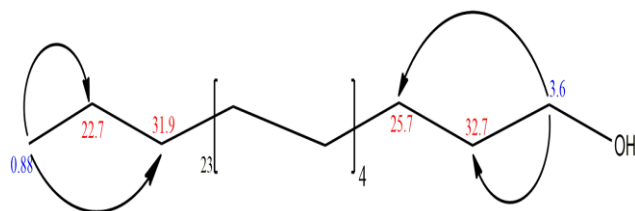


Figure 2: HMBC correlation of compound (1)

Compound 2 was obtained as a white, amorphous powder. The MASS spectra were showed a molecular ion  $[M^+]$  ion at  $m/z$  396 which indicates molecular formula  $C_{26}H_{52}O_2$ . The FT-IR (in KBr disc) was showing an absorption peak at  $3295\text{ cm}^{-1}$  (O-H),  $2919$  and  $2850\text{ cm}^{-1}$  (C-H),  $1707\text{ cm}^{-1}$  (C=O) and  $1466\text{ cm}^{-1}$  (C-H).  $^1\text{H-NMR}$  in  $\text{CDCl}_3$  was showing one triplet at  $\delta_H$  0.88 (3H, t,  $J=5.52$ ; H-26) which indicates

one methyl group and one broad signal of COOH proton was observed at 4.4 ppm.  $^{13}\text{C-NMR}$ , DEPT-135, and HSQC resolve 24 methylene, one methyl and one quaternary carbon group. HMBC experiment in Figure 4 was exhibited a correlation between  $\delta_C$  31.8 to  $\delta_H$  0.88, which indicating one methyl group at position C-26 and between  $\delta_C$  179.5 and  $\delta_H$  2.3, 1.6 reveals a carbonyl group (C=O) at position C-1.  $^1\text{H-}^1\text{H}$  correlation of  $\delta_H$  2.3 to 1.6 and 1.6 to 1.3 in COSY spectra were showed in Figure 3, which indicates methylene group connectivity. It was confirmed from the spectral technique that isolated compound 2 is hexacosanoic acid.

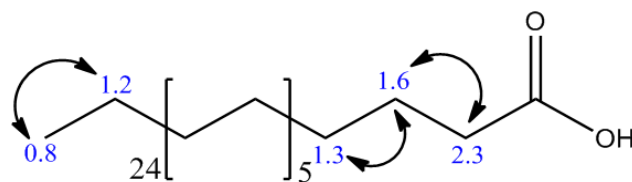


Figure 3: COSY correlation of compound (2).

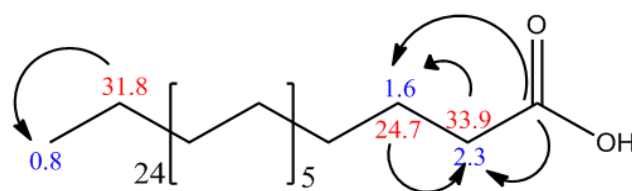


Figure 4: HMBC correlation of compound (2).

### Antibacterial Activity

Antibacterial activity of leaf extract and isolated compounds were investigated against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* using the agar well diffusion assay. The zone of inhibition of extract, hexacosanol and hexacosanoic acid were summarized in Table 3 and Figure 5.  $\text{CH}_3\text{OH}$  crude extract exhibits minimum inhibition zone 13 mm with *Bacillus subtilis* and 23 mm with *Staphylococcus aureus*. Extract with *Staphylococcus aureus* provides a large surface area for contact and show more activity. Hexacosanoic acid shows minimum inhibition zone 18 mm with *Staphylococcus aureus* for contact. Hexacosanol shows minimum inhibition zone 10 mm with *Staphylococcus aureus*, 17 mm with *Bacillus subtilis* and 20 mm with *Escherichia coli*. Hexacosanoic acid with *Escherichia coli* gives more surface area for contact and show high activity. When comparing, extract with compounds then extract showed maximum activity against gram-positive bacteria (*staphylococcus aureus*) due to its antibacterial action against cell wall synthesis. The variation between Gram-positive and Gram-negative bacterial sensitivity is expected due to differences in the composition of the cell wall of these two groups of bacteria. The gram-positive bacterial cell wall is composed of a thick layer of peptidoglycan, and short peptides are capable in the cross-linked formation of linear polysaccharide chains, thus forming more rigid structure leading to difficult penetration of the test compound



compared to the gram-negative bacteria in which the cell wall is comprised of a thin layer of peptidoglycan.<sup>28</sup>

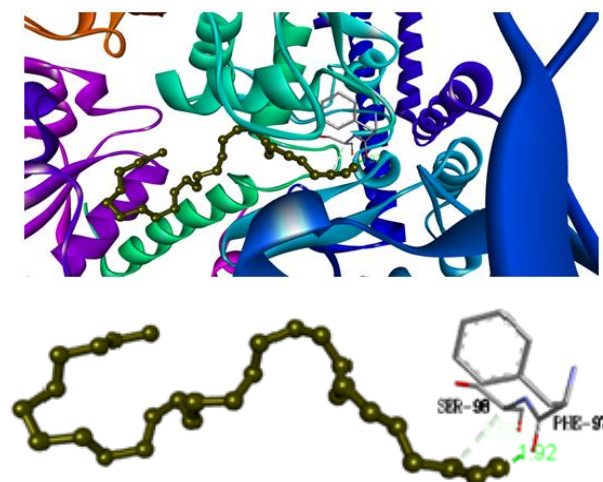


**Figure 5:** Zone of inhibition of test compounds against Gram positive and Gram negative bacteria

### Molecular Docking with Hexacosanoic acid

Molecular docking of hexacosanoic acid with DNA gyrase of *Staphylococcus aureus* is presented in Figure 6. shows -

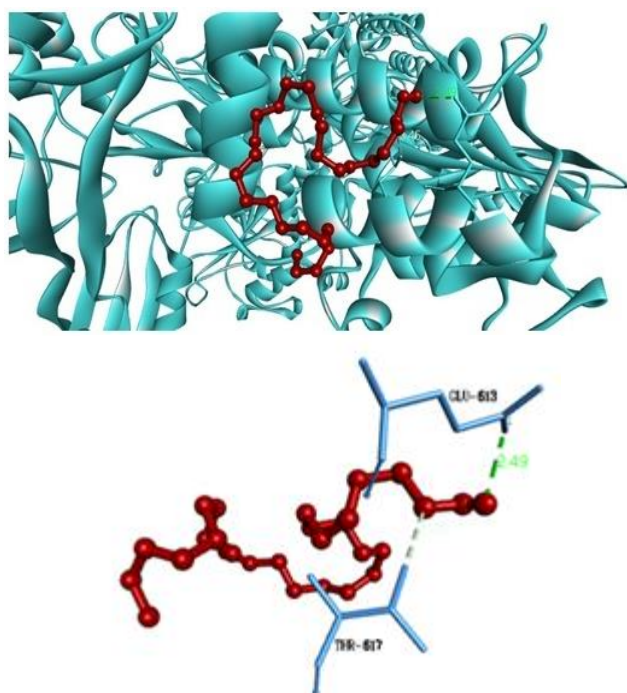
4.8 Kcal/mol binding affinity. Classical and non-classical Hydrogen binding interaction with amino acid PHE and SER appeared. Hydrogen bonding interactions of PHE-97 at 1.92 distance with a hydroxyl group, SER-98 at 3.58 distance with a carbonyl group.



**Figure 6:** Interaction of hexacosanoic acid with residues SER and PHE.

**Table 3:** Antibacterial activity of test compounds against Gram positive and Gram negative bacteria

Bacterial strains	<i>Rumex dentatus</i> CH <sub>3</sub> OH extract	Hexacosanoic acid	Hexacosanol
<i>Bacillus subtilis</i> 14596	+ve (13 mm)	-ve	+ve (17mm)
<i>Staphylococcus aureus</i> 3160	+ve (23mm)	+ve (18mm)	+ve (10mm)
<i>Escherichia coli</i> 25922	-ve	-ve	+ve (20mm)
<i>Pseudomonas aeruginosa</i> (PAO1)	-ve	-ve	-ve



**Figure 7:** Interaction of hexacosanol with amino acid GLU and THR.

### Docking Studies with Hexacosanol

Docking of hexacosanol with DNA gyrase of *Staphylococcus aureus* (in Figure 7) shows -3.8 Kcal/mol affinity. Classical and non-classical hydrogen bonding interaction with residues GLU and THR was displayed. Hydrogen bonding interaction of GLU-613 at 2.49 distance with a hydroxyl group and THR-617 at 3.45 distance with C-1.

### CONCLUSIONS

In summary, we have isolated hexacosanoic acid, and hexacosanol from the leaf extract of the *Rumex dentatus* plant and the structure of the isolated compounds were confirmed by several spectroscopic techniques. The antibacterial activities were screened on two gram-negative and two gram-positive bacterial strains. Best results were exhibited by extract and hexacosanol compound on *Staphylococcus aureus* (23 mm) and *Escherichia coli* (20 mm). The best binding affinity of isolated compounds was exhibited with DNA gyrase. The extract and compounds may be useful in bacterial disease.

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