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Molecular Docking studies on phytocompounds from the methanol leaf extract of *Carica papaya* against Envelope protein of dengue virus (type-2)

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ABSTRACT

Dengue viruses, a member of Flaviviridae family are mosquito born and are the causative agents for dengue fever. Dengue infection becomes a serious health concern globally because of the high mortality rate and the unviability of any proper treatment. Virus attachment to the host cell and subsequent fusion process are mediated by the envelope glycoprotein (E protein). The fusion process is driven by low pH induced conformational change of envelope protein in the endosomal compartment of the host cell. Due to the high prevalence of dengue viral infections and having no specific treatment, the development of novel antiviral agents is essential. Antiviral substances obtained from natural products, including medicinal plants, are potentially good targets to study. Extracts from the Carica papaya leaves, are commonly prescribed for the dengue patients but there are no scientific evidences for its activity against dengue. Hence we tried to investigate the anti-viral activity of compounds present in the leaves of Carica papaya against envelope protein of dengue 2 virus (DENV-2). Molecular docking approach using Autodock 4.2 was used in this study and results reveled that six compounds showed high inhibitory activity against the E protein. Six compounds (Stigmast-5-en-3-ol, (3á,24S) (M-30); D:A-Friedooleanan-7-one, 3-hydroxy (M-28); 5-Heptadecene, 1-bromo (M-26), 2-(4'-Chlorophenyl)naphtho[2,3-b]furan-4,9-dione (M-27); Neurosporaxanthin methyl ester (M-25); 3,6-bis(t-Butyl)fluorenone (M-20); 5,11,17,23-Tetrakis(1,1-dimethylethyl) pentacyclo [19.3.1.1(3,7).1 (9,132).1(15,19)] octacosa-1(25),3,5,7(28),9,11,1 3(27), 15,17,19(26),20,22-dodecaene-4,12,16-triol-24-one (M-22)) showed high inhibitory activity against the β-OG pocket (hydrophobic pocket between the domain I and II) of envelope protein. These findings concludes that this selected compounds could serve as antiviral drugs for dengue infections. Further in-vitro and in-vivo studies are necessary to confirm their efficacy and to evaluate their drug potency.

Keywords: Dengue virus, Envelope protein, Docking, *Carica papaya*, *in-silico* study.

INTRODUCTION

Dengue viruses (DENV) are members of *Flaviviridae* family, a group of 70 virus including West Nile virus (WNV), yellow fever virus, Japanese encephalitis virus (JEV), tick-borne encephalitis (TBEV) [1,2,3]. Dengue viruses are mosquito-borne and are the causative agent of dengue fever. In the recent years dengue infection has become a major public health problem and emerging viral disease of the humans [4]. There exists 4 distinct serotypes of dengue virus (DENV-1, DENV-2, DENV-3 and DENV-4). Each type shares 65% of the genome, and, cause nearly identical syndromes in humans, ranging from self-limited febrile illness called dengue fever (DF) to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [5,6]. All the serotypes of dengue virus, are transmitted from one host to the other by mosquitoes, primarily *Aedes aegypti* and *Aedes albopictus* [3,7].

Dengue virus is a positive–stranded RNA virus. The 11 kb RNA genome encodes for a single polyprotein. This polyprotein is then cleaved in the cytoplasm into three structural and seven non-structural proteins [1,8]. The structural proteins includes Capsid (C) protein, Membrane (M) protein and Envelope (E) protein. These protein plays an important role in the viral particle formations. The non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) are involved in the replication, assembly and immune response escape [3,8,9].

The entry of the dengue virus into the host cell is mediated by E protein [8,10]. Envelope protein present at the surface of the virus enhances the fusion of viral cell membrane and host cell membrane [9]. Initially, E protein is arranged as a heterodimer in the immature state of dengue virus. The presence of premembrane protein which covers the protein peptide, will generate a spiky surface on E protein. Later, E protein transforms into homodimer at Golgi apparatus, during the virion maturation [6]. During maturation, a polygonal confirmation is adapted by the E protein, where the dimers are arranged in polygons covering the surface of the viral particles [8].

The E protein monomer is composed of β-barrels and organized into 3 structural domains: domain I, domain II and domain III [1,5,6,11]. Domain I, which is the central domain, contains aminoterminus and two disulphide bridges. Domain II, which is rich in glycine, is an extended finger like domain that bears the fusion peptide and stabilizes the dimer. It contains three disulphide bridges. Domain III, the immunoglobulin like domain, contains the C-terminal domain, the receptor-binding motif and one disulphide bridges [6,8]. Domain I, is poisoned in-between domain II and domain III [5].

Blocking of virus entry and replication is a widely accepted tactic in the design of antiviral [12]. The entry of the virus particle into the host cell requires the fusion of viral and cellular membranes via receptor mediated endocytosis [6,12]. This process is mediated by complex structural changes in the dengue E protein. At the initial stage of process, acidic environment of endosome catalyzes the protonation of selected, highly conserved histidine residues in E protein which triggers a reversible dissociation of the virion surface E protein dimers. The fusion peptide loop exposure at the tip of domain II is then facilitated by the movement of domain II around a flexible hinge domain at the domain I–domain II junction, allowing its insertion into the host cell membrane. In the transitionary stage, the envelope protein connects the cellular membrane and the viral envelope. Both membranes are finally pulled into proximity for fusion while the E protein reassociates into trimers. After merging of the membranes, E protein homotrimers are irreversibly formed [2].

A significant findings in the reported work in E protein structure, is the identification of a binding pocket in the DI–DII hinge domain (Figure 1), that accommodates a hydrophobic ligand, in this case a molecule of β-N-octylglucoside (β-OG) added during crystallization [2,13]. But whereas in the other structures, this pocket is closed by the *kl* loop [12]. The binding of the β-OG in to this binding pocket alters the confirmation of one of the loops connecting two domains [2]. The β-OG pocket may therefore a logical target for structure-based design of potential antiviral agents because ligands that bind there could alter the conformational equilibrium around this region of E protein that drives membrane fusion [2,11].

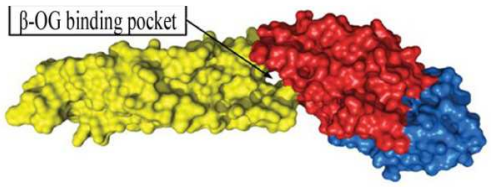

Figure 1. Structure of the DENV E ectodomain highlighting the β-OG binding site [2]

Plant derived compounds remains a significant source for the development of antiviral drug. Studies on dengue virus reveals the importance if photochemical agents against dengue [3]. *Carica papaya*, commonly known as Papaya, is a plant that belongs to the family of *Caricaceae.* Papaya, is an herbaceous succulent plants that possess self-supporting stems [14,15,16]. The leaf extracts of *Carica papaya* are prescribed for the treatment of pyrexia, gonorrhea, diabetes, syphilis, inflammation, fever, and for dressing foul wounds [3]. In a recent study, it is reported that aqueous extracts of *Carica papaya* leaves exhibited potential activity against dengue fever by increasing the PLT count from $55x10^3$/μL to 168 $x10^3$/μL, WBC from $3.7x10^3$/μL to 7.7 $x10^3$/μL and NEUT from 46% to 78.3%

[3,4]. In this study, we report the virtual screening of methanol extracted compounds of *Carica papaya* leaf against the β-OG binding site in the dengue virus envelope protein.

MATERIALS AND METHODS

Plant Materials:
The *Carica papaya* leaves were handpicked at the local area of Thrissur city. Collected leaves were washed with distilled water and allowed to shade dry. The dried leaves were then mechanically into fine powder [17].

Extraction:
The powdered plant material (15g) was sequentially extracted in a Soxhlet extractor using 200 ml of solvent. This sequential extraction was started with Petroleum ether, which helps in reducing the chlorophyll pigment in the green leaves, followed by dichloromethane, ethyl acetate, acetone and methanol [18]. The concentrated extracts were transferred to preweighed vials, dried in room temperature, and stored at -20^{o}C for further study.

GC-MS analysis:
The GC-MS analysis of *Carica papaya* leaf extracts in methanol was performed using the Thermo GC - TRACE Ultra Ver: 5.0, Thermo MS DSQ II. Experimental conditions as follows: DB 5 - MS capillary standard non - polar column (dimension: 30 Mts, ID: 0.25 mm, Film: 0.25 μm) was used and helium was used as the carrier gas, flow rate was set at 1.0 ml/min. In the gas chromatography section, temperature programmed (oven temperature) was oven temp 70º C raised to 260 °C at 6 C /min. The injection volume was 1 μL [19]. Samples which dissolved in methanol were run fully at a range of 50-650 m/z. The total GC running time was 38.53 min.

Identification of Compounds:
The phytocomponents in the methanol extracts of the *Carica papaya* leaves were identified based on the retention time on DB 5 - MS capillary standard non – polar column, Mass spectrum were interpreted using the database of National Institute Standard and Technology (NIST). The name, molecular weight, and structure of the components of the methanol extract were identified.

Selection and refinement of receptor:
Initial step in the in-silico drug designing procedure is the identification and selection of the appropriate drug target or receptor [7]. Envelope protein of dengue virus is essential for the fusion of virus and host cell. The three dimensional structure of type 2 dengue virus envelope protein was retrieved from Protein Data Bank (www.rcsb.org/pdb) using PDB ID: 1OKE [2]. To optimize the structure, H_2O molecules and other heteroatoms were removed. Moreover, energy minimization of the protein was carried out using GROMACS molecular dynamic package and GROMOS96 43a1 force field [20].

Ligand Input File Preparation and Optimization:
Ligand input structures were drawn using Marvin Sketch. The drawn structures were cleaned in 3D format and optimized using Marvin Sketch. The resulting structures were then saved in pdb format for molecular docking studies [22].

Docking:
The docking of the ligand into the active site of E protein is carried out using Autodock 4.2. Autodock has reported to be an effective tool capable of quickly and accurately predicting bound conformations and binding energies of ligands with macromolecular targets [21]. Polar hydrogen atoms were added to the enzyme and its nonpolar hydrogen atoms were merged. For the ligands, Gasteiger charges were added and nonpolar hydrogen atoms were merged. All the rotatable bonds were set to be rotatable. Protein-ligand docking was done using the Lamarckian Genetic Algorithm (LGA) method. The grid box with a dimension of 70 x 62 x 62 points and 0.375 Å grid spacing was used around the β-OG pocket to cover the entire protein binding site and accommodate ligands to move freely. After docking searches were completed, the best conformation was chosen from the most populated cluster with the minimum binding energy. The interaction of docked protein-ligand complex conformations, including hydrogen bond and other interactions, were analyzed using Discovery Studio Visualizer 4.1 [23].

RESULTS AND DISCUSSION

GC-MS Results:

In the present study, GC-MS profile of the methanol extract of *Carica papaya*, has shown 30 peaks representing 30 phytocompounds in the extract (Figure 2). The identified compounds namely, Formic acid, 2-methylhex-3-yl ester (M-1), (2S)-N-Methylaspartic acid (M-2), Thiazole, 4-(1,1-dimethylethyl) (M-3), Benzene, methyl (M-4), Indene-2-D1 (M-5), d,l-Phenylalanine Amide (M-6), 2-Methoxy-4-vinylphenol (M-7), (3S,5S)-3,5-Dihydroxy-3,5-dimethyl-1-phenyloct-7-en-4-one (M-8), anti-7-Methoxybenzonorbornene (M-9), 2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one (M-10), 1-Dodecanol (M-11), 2-Allyl-5-t-butylhydroquinone (M-12), Methane, isothiocyanato (M-13), Benzyl 3-Trimethylsilylbutanoate (M-14), Tetradecanoic acid, methyl ester (M-15), 2,2'-dihydroperoxy-2,2'-dimethyl-2,2'-dipropyl peroxide (M-16), Phthalic acid, hept-4-yl isobutyl ester (M-17), Hexadecanoic acid, methyl ester (M-18), Hexadecanoic acid (M-19), 3,6-bis(t-Butyl)fluorenone (M-20), Hexadecatrienoic acid, methyl ester (M-21), 5,11,17,23-Tetrakis(1,1-dimethylethyl) pentacyclo [19.3.1.1(3,7). 1(9,132) . 1(15,19)]octacosa-1(25),3,5,7(28),9,11,1 3(27), 15,17,19(26),20,22-dodecaene-4,12,16-triol-24-one (M-22), Di-(2-ethylhexyl)phthalate (M-23), Nonacosane (M-24), Neurosporaxanthin methyl ester (M-25), 5-Heptadecene, 1-bromo (M-26), 2-(4'-Chlorophenyl)naphtho[2,3-b]furan-4,9-dione (M-27), D:A-Friedooleanan-7-one, 3-hydroxy (M-28), 4-Nitro-2,2'-bipyridine-1-oxide (M-29), and Stigmast-5-en-3-ol, (3á,24S) (M-30). The GC-MS results confirmed the presence of these 30 phytocompounds with the retention time 4.49, 5.49, 6.15, 6.58, 6.93, 8.19, 9.43, 10.26, 10.77, 11.84, 12.15, 12.81, 13.63, 16.51, 17.44, 19.48, 20.22, 21.58, 22.29, 24.23, 24.85, 29.27, 31.25, 31.74, 33.37, 36.69, 37.45, 39.45, 39.75, 40.12 (Table 1).

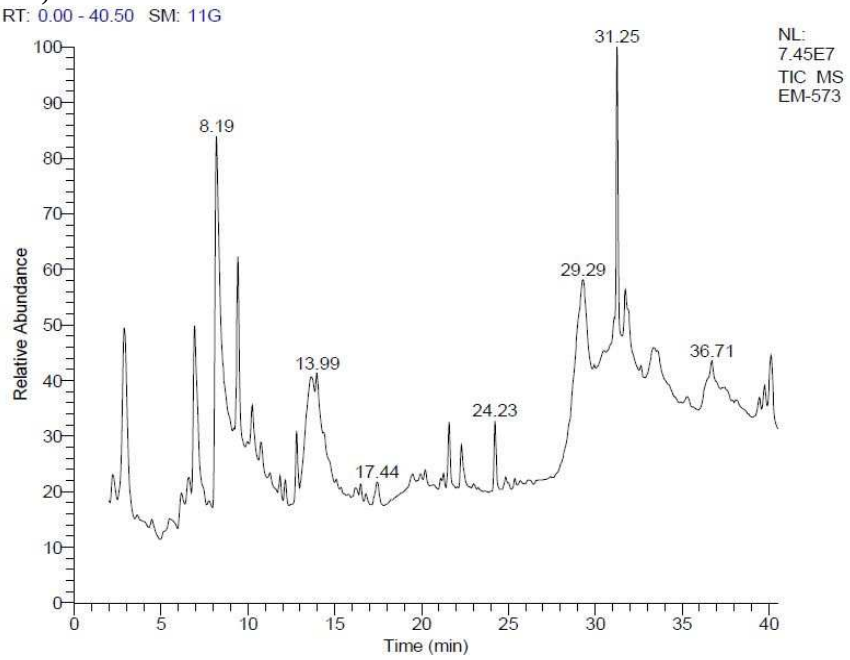

Figure 2. GC-MS profile of methanol leaf extract of *Carica papaya*

Table 1. Phytocomponents identified in the methanol leaf extracts of *Carica papaya*

S.No	RT	Name	M.F	M.W	Area (%)
1	4.49	Formic acid, 2-methylhex-3-yl ester	$C_8H_{16}O_2$	144	0.54
2	5.49	(2S)-N-Methylaspartic acid	$C_5H_9NO_4$	147	1.49
3	6.15	Thiazole, 4-(1,1-dimethylethyl)-	$C_7H_{11}NS$	141	1.31
4	6.58	Benzene, methyl-	C_7H_8	92	6.58
5	6.93	Indene-2-D1	C_9H_7D	116	8.49
6	8.19	d,l-Phenylalanine Amide	$C_9H_{12}N_2O$	164	18.04
7	9.43	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	6.40
8	10.26	(3S,5S)-3,5-Dihydroxy-3,5-dimethyl-1-phenyloct-7-en-4-one	$C_{16}H_{22}O_3$	262	2.41
9	10.77	anti-7-Methoxybenzonorbornene	$C_{12}H_{14}O$	174	0.82
10	11.84	2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	$C_{15}H_{24}O_2$	236	0.53

11	12.15	1-Dodecanol	$C_{12}H_{26}O$	186	0.57
12	12.81	2-Allyl-5-t-butylhydroquinone	$C_{13}H_{18}O_2$	206	1.83
13	13.63	Methane, isothiocyanato-	C_2H_3NS	73	10.50
14	16.51	Benzyl 3-Trimethylsilylbutanoate	$C_{14}H_{22}O_2Si$	250	0.99
15	17.44	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	242	1.32
16	19.48	2,2'-dihydroperoxy-2,2'-dimethyl-2,2'-dipropyl peroxide	$C_{10}H_{22}O_6$	238	0.86
17	20.22	Phthalic acid, hept-4-yl isobutyl ester	$C_{19}H_{28}O_4$	320	1.25
18	21.58	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.96
19	22.29	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.	1.75
20	24.23	3,6-bis(t-Butyl)fluorenone	$C_{21}H_{24}O$	292	2.04
21	24.85	Hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	264	0.60
22	29.27	5,11,17,23-Tetrakis(1,1-d imethylethyl)pentacyclo [19.3.1.1 (3,7) . 1(9,132).1(15,19)]octacosa1(25),3,5,7(28),9,11,13(27),15,17,19(26),20,22-dodecaene-4,12,16-triol-24-one	$C_{44}H_{54}O_4$	646	11.21
23	31.25	Di-(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390	7.85
24	31.74	Nonacosane	$C_{29}H_{60}$	408	2.89
25	33.37	Neurosporaxanthin methyl ester	$C_{36}H_{48}O_2$	512	2.96
26	36.69	5-Heptadecene, 1-bromo-	$C_{17}H_{33}Br$	317	3.31
27	37.45	2-(4'-Chlorophenyl)naphtho[2,3-b]furan-4,9-dione	$C_{18}H_9ClO_3$	308	0.46
28	39.45	D:A-Friedooleanan-7-one, 3-hydroxy-	$C_{30}H_{50}O_2$	442	0.80
29	39.75	4-Nitro-2,2'-bipyridine-1-oxide	$C_{10}H_7N_3O_3$	217	1.46
30	40.12	Stigmast-5-en-3-ol, (3á,24S)-	$C_{29}H_{50}O$	414	3.40

Docking and Interaction analysis:

Docking studies were carried out in order to find the inhibitory activity of the compounds obtained from GC-MS analysis of methanol extract of *Carica papaya.* Among the thirty compounds, 6 compounds showed best docking result based on the binding energy. Docking studies and binding free energy calculations of these thirty compounds revealed that M-30 has maximum interaction energy (-9.99 kcal/mol), followed by M-28 (-9.79 kcal/mol), M-27 (-8.66 kcal/mol), M-25 (-8.31 kcal/mol), M-20 (-7.9 kcal/mol) and M-22 (-7.22 kcal/mol) (Table 2). Figure 3, 4 and 5 shows the ligand site interactions of compounds M-30, M-28, M-27, M-25, M-20 and M-22.

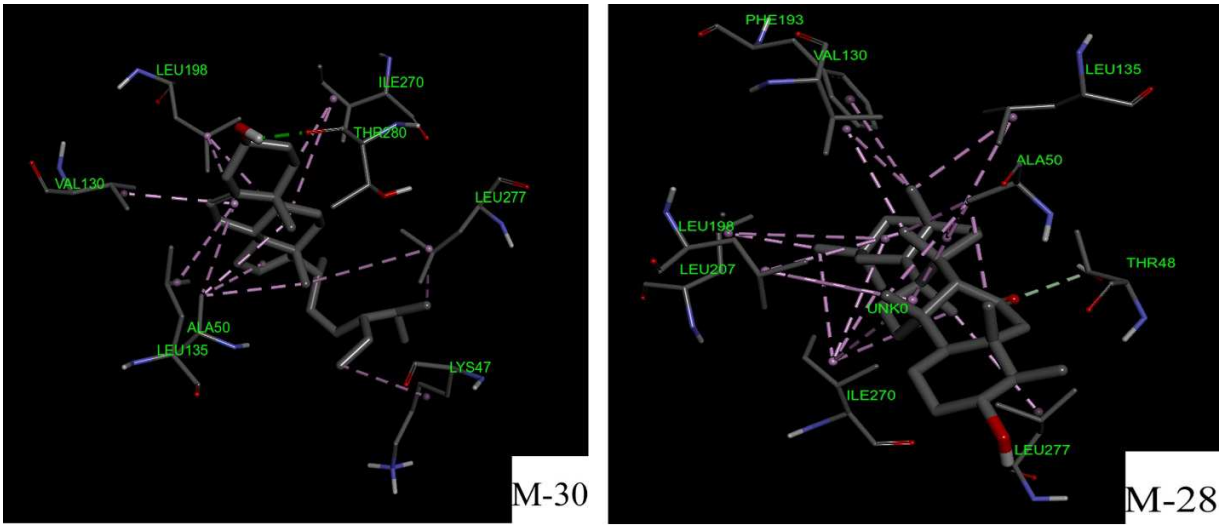

Figure 3. Interactions of the ligand M-30 and M-28 and the binding site residues of Envelope protein (PDB ID: 1oke)

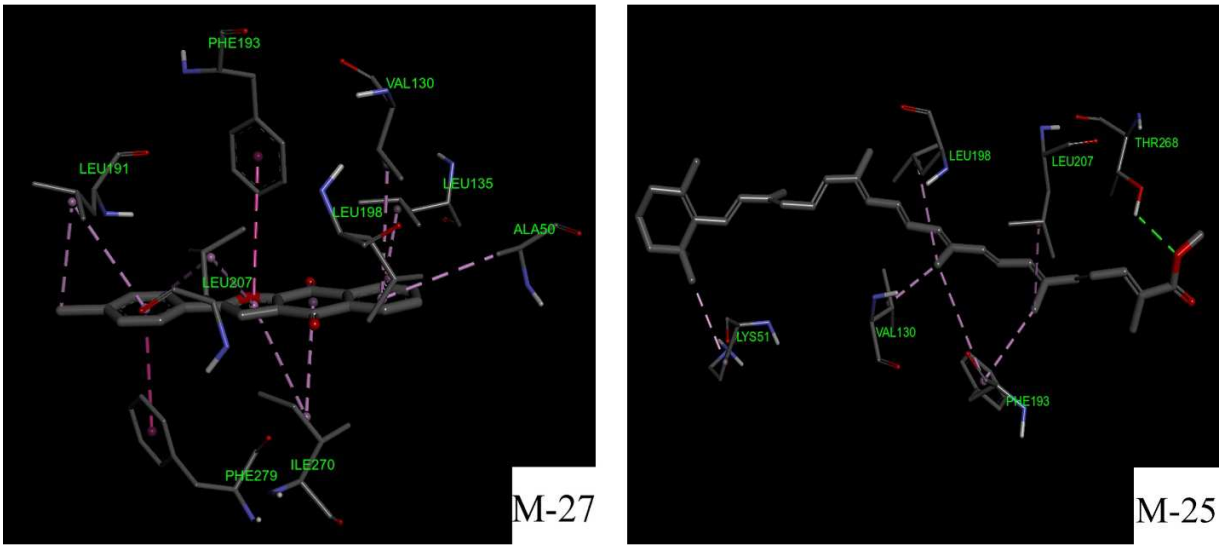

Figure 4. Interactions of the ligand M-27 and M-25 and the binding site residues of Envelope protein (PDB ID: 1oke)

Table 2. The interaction energy analysis of six ligands with that of dengue Envelope protein (PDB ID: 1OKE) using Discovery Studio Visualizer 4.1

Ligand	Binding Energy (kcal/mol)	Interacting residues (Hydrogen bondings)	Other interacting residues
M-30	-9.99	THR 280	LYS 47,ALA 50,VAL 130,LEU 135, LEU 198, ILE 270, LEU 277
M-28	-9.79	THR 48	ALA 50, VAL 130,LEU 135,PHE 193, LEU 198, LEU 207, ILE 270, LEU 277
M-27	-8.66	--	ALA 50, VAL 130,LEU 135,PHE 193, LEU 198, LEU 207, ILE 270, PHE 279
M-25	-8.31	THR 268	LYS 51, VAL 130,PHE 193, LEU 198, LEU, 207
M-20	-7.9	THR 48	ALA 50, LEU 198, LEU 207, ILE 270, LEU 277
M-22	-7.22	ALA 50	LYS 47, LEU 198, LEU 277

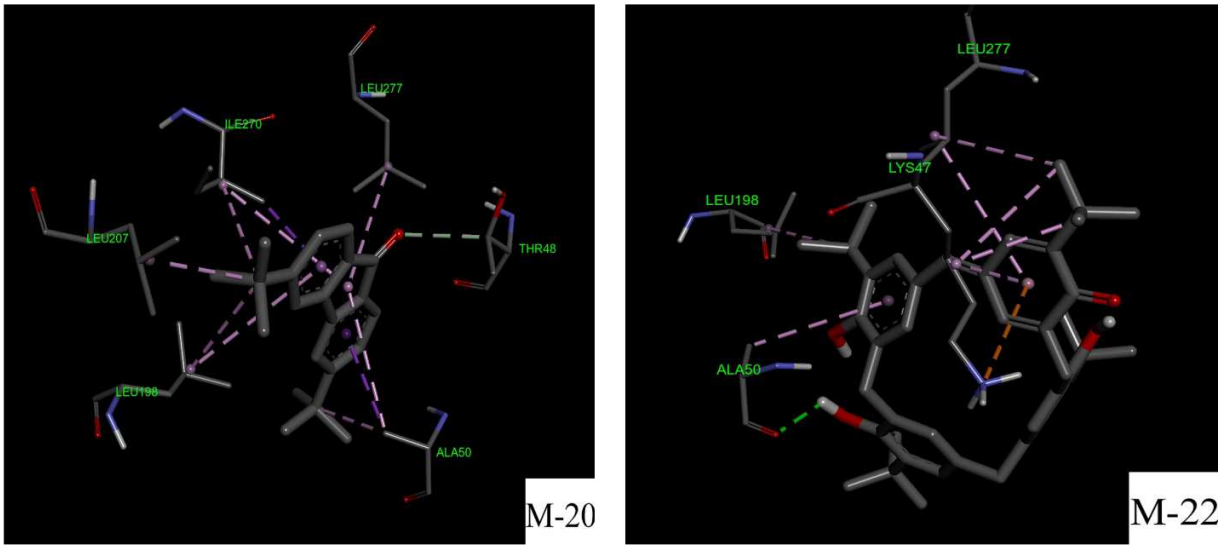

Figure 5. Interactions of the ligand M-20 and M-22 and the binding site residues of Envelope protein (PDB ID: 1oke)

CONCLUSION

Dengue has been a major health concern world widely and has proven to claim the lives of a million people yearly. Till now, no vaccine has been successfully developed for this outrageous disease. Current study was focused on assessment of the inhibitory activity of methanol extract compounds of Carica papaya leaves against dengue virus protein. Among the identified thirty compounds, six compounds (M-30, M-28, M-27, M-25, M-20 and M-22) showed better binding affinity towards the β-OG pocket of E protein. Different modes of interaction such as hydrogen bonding and other hydrophobic interactions were observed between the ligands and the E protein of dengue virus. The information acquired through this study on the binding mode of phytocompounds from C*arica papaya* and E protein will highly facilitate the synthesis and testing of these compounds as drugs against dengue virus. The study suggested that compounds from the *Carica papaya* will be strong future drug candidates against dengue virus.

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