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# Drug repurposing of Daclatasvir and Famciclovir as antivirals against dengue virus infection by *in silico* and *in vitro* techniques

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Drug repurposing is a technique for reusing an existing drug to treat another ailment. It is common knowledge that nearly all medicines used in human therapy have more than one target impact in addition to their primary action. The present work is aimed to repurpose existing antiviral drugs for dengue disease. A molecular docking study is performed with the DENVE protein for the identification of the suitable drug candidate which acts in the fusion process. For all repurposed drugs at the active site of DENVE, molecular docking experiments were performed using CLC Drug Discovery Workbench Software (PDB ID: 1OKE). The relative binding modes and the affinities of all the selected drugs were predicted and compared with the co-crystallized *n-octyl-beta-D-glucopyranoside (βOG)*. The Daclatasvir (Score-53.52) makes hydrogen bonds with ALA50 and THY48. According to the docking score evaluation, the entire drug candidates had docking result ranging from −32.15 to −53.52. Among the drugs tested the two drugs namely Daclatasvir and Famciclovir have been identified as HITS for combating DENVE protein.

**Keywords**: Dengue virus, Drug repurposing, Envelope protein, Hinge region, Molecular docking, *n-Octyl-beta-D-glucopyranoside (βOG)*

Dengue viral disease, a mosquito-borne viral pathogen dengue virus (DENV), has been a significant public health issue in recent decades. Dengue is currently present in 119 countries throughout the world. Fifty-hundred (50-100) million people in tropical and subtropical countries are infected with DENV per year, resulting in approximately 5,000,000 existence diseases and 25,000 deaths. DENV belongs to the Flavivirus family of the Flaviviridae family. The genome of flavivirus consists of approximately 11,000 base pairs (bp) of RNA, which translate into three structural proteins, including membrane [M], capsid [C], & envelope [E], and 7 non-structural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The viral envelope consists of two transmembrane proteins, the envelope (E) and the premembrane (prM). E binds directly to cellular receptors and facilitates viral and cell membrane fusion through viral cell penetration and is the primary site for antibody neutralization[1]. Interruption of virus replication at the initial point of contact[2] can be an attractive technique. Membrane fusion is the main molecular event that occurs during the viral entrance into the host cell[3]. The envelope protein (E) constitutes the main component of viral surface. It is very important for fusion process through which the virus merges into host receptor[4]. Among the three domains DI, DII, and DIII present in the E protein, the migration of domains I and II in the hinge region promotes the mechanism of fusion. Rearrangement and/or conformational changes in the hinge area by small molecules can disrupt the process of fusion[5-7].

Keeping the above facts, the present work is aimed to repurpose existing antiviral drugs for dengue disease. A molecular docking study is performed with the DENVE protein for the identification of the drug candidate which acts in the fusion process. The drugs obstructing the βOG pocket have thought to interact with conformational changes within the envelope protein that are basic for configuration. The following seven medicines were chosen based on their mechanism of action and therapeutic efficacy: Acyclovir inhibits

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Suppl. Data available on respective page of NOPR

nucleic acid synthesis; Lamivudine and Zidovudine inhibit reverse transcriptase. Famciclovir and Ganciclovir are DNA polymerase inhibitors; Oseltamivir inhibits progeny virus release and Daclatasvir is a non-nucleoside polymerase inhibitor.

## Materials and Methods

### Molecular docking research

Molecular docking investigations were conducted using CLC Drug Discovery Workbench Tools to provide exact proof of optimized validation for both ligand (selected drug candidates) and their target receptor DENVE protein to form a stable compound. The chemical structures of the selected drugs have been depicted in (Suppl. Table S1). Dengue virus type 2 envelope proteins (E) PDB ID: 1OKE were retrieved from the protein data bank at a resolution of 2.1[8].

### Preparation of ligands

Ligands (selected drug candidates) were prepared using the SPARTAN'14 software kit. Their molecular structures have been defined using the baseline standard of DFT/B3LYP/6-31 G[9].

### Molecular docking simulation

CLC Drug Discovery Workbench tools were used in a molecular docking research to generate exact estimations of the optimal configuration for ligands and their target receptor protein, as well as the resultant complexity. The docking modeling was performed in according to the docking procedure, which includes the stages are: In the molecule project, create a binding site and a binding pocket; dock ligands entered into the molecule table; and evaluate the docking results. Redocking was utilized to check the docking techniques and requirements employed in order to verify the dependability of ligand configurations and locations obtained from molecular docking investigations. Docking findings and 2D amino acid interactions predict drug binding affinities, modalities of binding, and orientation on the active protein receptor site[10].

### Calculation of molecular properties

#### *Molinspiration batch property calculation toolkitmib*

Molinspiration is a Java-written toolkit for molecular processing and property estimation. The toolkit could be used in batch mode to handle a huge amount of molecules (approximately 10,000 molecules per min) or can be reached directly from your intranet via a web interface.

Molecular properties such as LogP (octanol/water partition co-efficient), molecular polar surface area, molecular volume, rule of 5, and number of rotatable bonds-nrotb have been calculated using Molinspiration software[11-15] and depicted in (Table 1).

### Molecular Dynamics Study

CABS-flex-2.0 was used to perform the molecular dynamics analysis. It is a rapid modeling technique for protein structure flexibility simulation. It is predicted on the CABS model, which is a well-known coarse-grained protein modeling method. This work provides the consensus view of protein near-native dynamics derived from ten-nanosecond MD simulations is presented here (explicit water, all-atom, for all protein metafolds using the four most popular force-fields). The CABS model utilizes stochastic dynamics (a Monte Carlo technique) and a knowledge-based force-field that is not biased toward a simulated protein's natural structure. Combining the CABS method with all-atom MD offers an efficient method for long-term multiple stage protein systems of molecular modeling with mechanistic precision because CABS-based interactions (dynamics) enable for the simulation of the whole assembling in a perfect run[16].

### Anti-viral study

#### *Cell lines and culture medium*

Vero (African Green Monkey, Kidney) cell lines used were collected from the National Cell Science

Table 1 — Selected drug candidates and their physicochemical properties

| Compounds | miLogP | TPS | natoms | MW | Nviolations | nrotb | Volume |
|---|---|---|---|---|---|---|---|
| Acyclovir | −1.61 | 119.06 | 16 | 225.21 | 0 | 4 | 187.75 |
| Lamivudine | −1.09 | 90.38 | 15 | 229.26 | 0 | 2 | 187.07 |
| Baloxavir | 2.44 | 75.01 | 34 | 483.50 | 0 | 1 | 389.09 |
| Daclatasvir | 7.77 | 174.65 | 54 | 738.89 | 1 | 13 | 683.66 |
| Famciclovir | 0.48 | 122.24 | 23 | 321.34 | 0 | 9 | 285.31 |
| Ganciclovir | −2.17 | 139.29 | 18 | 255.23 | 0 | 5 | 212.60 |
| Stavudine | −0.54 | 84.33 | 16 | 224.22 | 0 | 2 | 192.96 |
| Zidovudine | −0.10 | 134.08 | 19 | 267.25 | 0 | 3 | 224.06 |
| Oseltamivir | 0.85 | 90.66 | 22 | 312.41 | 0 | 8 | 309.60 |

Centre, Pune, India. Cells were grown in Dulbecco's middle Eagle medium (DMEM) supported by Cells mature with trypsin, EDTA (197.16 mg/L), glutamine (0.5 g/L) and PBS (1L) in Dulbecco's middle Eagle medium (DMEM) assisted by Fetal Calf Serum and the cells are kept in the CO2 incubator at 37°C. This medium was used in the subculture for adherent cell use. The supernatant was withdrawn and the mixture was added and incubated with 10 mL of trypsin/EDTA. In the flask, 10 mL of DMEM was applied and passed to the centrifuge. Cells then spun for 5-7 min at 1300-1500 rpm. The *in vitro* Cytotoxicity assay was performed according to Jubie *et al*., 2020)[17].

**MTT antiviral assay**

The highly sensitive *in vitro* antiviral compound evaluation approach emphasizes on the spectrophotometrical analysis of the viability of pathogen and longer independent cells by lowering in situ MTT tetrazolium pigment. Mitochondrial cells secrete MTT yellow water-soluble pigment to soluble purple, non - soluble formazan in viable cells. At 490/650 nm, the quantity of the formazan compound present from each well of the microtitre plate is spectrophotometrically identified. The toxic effects of different test compounds to host cells were evaluated in a certain microtitre plate simultaneously[18]. The medium was extracted after a 24 h incubation time. We introduced HSV-1 & II at a dosage of 100 $TCID_{50}$ for 2 h in order to guarantee that the virus attached to the cell. The medium was removed after a 24 h incubation period. In order to guarantee that the virus was attached to the cell, 100 $TCID_{50}$ of HSV-1 and HSV-II was administered for 2 h. A compound growth average solution was added to the cells after 2 h of cleaning with PBS. As cell control, merely 100 µL of medium was incorporated and 100 µL of 100 $TCID_{50}$ doses were added as virus control. The supernatant was removed after three days of incubation and 50 mL of MTT solution (2 mg/mL) was applied for 4 h at 37°C to each well. Thus, to disperse the crystals of formazan, 100 µL of dimethyl sulphoxide (DMSO) was added among each well. The colour response in an electronic micro plate reader was analyzed at 490 nm after vigorously shaking the plates for ten min to remove the crystals. The untreated control was randomly set at 100%.

**Larvicidal Bioassay**

Larvicidal activity was determined in Aedes aegypti larvae of the third instar using WHO standards with slight changes. In triplicate, the tested compounds had been combined with 50 mL of dechlorinated distilled water and fifty five larvae of Aedes aegypti breeds tested at different concentrations from 25-100 g/mL. In a 3:1 ratio, the larvae were fed dry yeast powder. Single vessel with no drug suspension was utilized as a control. The number (amount) of dead larvae was counted after 24, 48 and 72 h. Infectious drugs were produced and more than half of the larvae were killed[19]. The larvicidal efficacy of the repurposed drugs towards *Aedes aegypti* was determined by varying drug solution concentrations. The affected larvae then examined under a stereo zoom microscope after 72 h for each proportion (concentration).

## Results

In order to produce a stable compound, molecular docking studies were performed on both drug candidates (as ligands) and their target receptor protein. The protein-ligand combination is based on the protein data bank structure of *n-octyl-beta-D-glucopyranoside* with dengue virus E protein (PDB ID: 1OKE). Following the import and processing of the protein receptor file from the protein data bank, the next step was to search for the binding pockets and binding site. The reference ligand *n-Octyl-beta-D-glucopyranoside* was utilized to compare treatment candidates and docking findings. The search for the ligand-binding mode was conducted inside the volume of the binding site. Binding pockets have been used to direct molecular docking of ligand. The binding domain (site) and docking position of co-crystallized n-Octyl-β-D-glucopyranoside interfacing with amino acid residues are depicted in (Supplementary Table S2) and the binding pocket are represented. The docking result, interacting group, and hydrophobic interactions and hydrogen bonds formed by the amino acids of group interaction atoms are all depicted in (Table 2). Daclatasvir (Score-53.52) makes 2 hydrogen bonds with the ALA50 and THY48. Docking result assessments revealed that all pharmaceutical entities had docking scores ranging from-32.15 to-53.52. The drug-likeness of selected drug candidates was ascertained by evaluating the physicochemical properties using mol inspiration toolkit and the outcomes were tabulated in (Table 1). The outcomes revealed that all have drug-like properties.

The highly constructive conformation, posture, and favourable bonded complexes formed by receptor

ligand interaction were employed as inputs for the drug Daclatasvir in Molecular dynamics simulation (MD) utilizing CABS flex 2.0. The optimized protein was then subjected to a rapid simulation (dynamic) of structural versatility by using the CAB-flex 2.0 server and a random number generation seed of 4956, with all other parameters set to default. The server-analyzed protein's contact map and root-mean-square fluctuations (RMSFs) were acquired. The RMSF values of each receptor-ligand complex were plotted to see how the drugs affected structural stability and integrity. The RMSF quantifies of the average movement of atoms or groups of atoms in relation to the corresponding structure (configuration). The overlapping of the configuration and connection map of ten models of versatility computation of DENVE protein (1OKE) Daclatasvir complex was shown in (Fig. 1), respectively, to determine whether a structure is reliable over the time-scale of the computations (simulations) or whether it is differing from the early (initial) coordinates. The active site amino acid sequence was extrapolated using the RMSF. (Fig. 2) revealed that all of the catalytic residues in the DENVE protein (1OKE) Daclatasvir combination were stable.

***In vitro* cytotoxicity assay**

HSV-1 and HSV-2 viruses were tested for antiviral activity. For HSV-1 and HSV-2 viruses, a virus concentration of 100 $TCID_{50}$ was employed. When compared to Famciclovir (58.08 percent ) and a selectivity index of 5.11 against HSV-1 virus, the medication Daclatasvir demonstrated a good percentage of cell protection (74.45%) and a selectivity index ($CC_{50}/IC_{50}$) of 13.33. When compared to Famciclovir (43.45%) and a selectivity

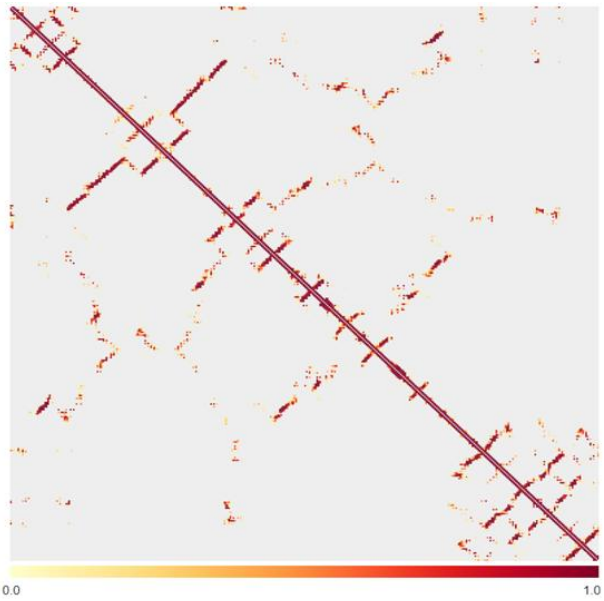


Fig. 1 — The CAB-flex 2.0 server provided a contact map of superimposition of the top ten simulated structures of 1OKE-Daclatasavir

Table 2 — Docking parameters for the selected compounds

| Compounds | Canonical SMILES | Score | HB | Metal Int.ac | Steric Int.ac | Ligand config | RMSD |
|---|---|---|---|---|---|---|---|
| Acyclovir | C1=NC2=C (N1COCCO) N=C (NC2=O) N | −36.14 | −2.00 | 0.00 | −34.95 | 0.81 | 91.84 |
| Lamivudine | C1C (OC (S1) CO) N2C=CC (=NC2=O) N | −39.31 | −5.95 | 0.00 | −34.10 | 0.74 | 93.65 |
| Baloxavir | C1COCC2N1C (=O) C3=C(C (=O) C=CN3N2 C4C5=C (CSC6=C C=CC=C46) C (=C(C=C5) F)F)O | −33.05 | 0.00 | 0.00 | −42.94 | 9.89 | 93.26 |
| Daclatasvir | CC(C) C(C (=O) N1CCCC1C2=NC=C (N2) C3=CC=C(C=C3) C4=CC=C(C=C4) C5=CN=C (N5) C6CCCN6C (=O) C(C(C) C) NC (= O) OC)NC (=O) OC | −52.77 | −7.63 | 0.00 | −58.89 | 14.74 | 85.54 |
| Famciclovir | CC (=O) OCC (CCN1C=NC2=CN=C (N=C21)N) COC (=O) C | −50.29 | 0.00 | 0.00 | −56.55 | 6.26 | 92.21 |
| Ganciclovir | C1=NC2=C (N1COC (CO) CO) N=C (NC2=O) N | −40.82 | −11.23 | 0.00 | −33.23 | 3.63 | 94.49 |
| Stavudine | CC1=CN(C (=O) NC1=O) C2C=CC (O2) CO | −37.34 | −0.00 | 0.00 | −35.95 | 0.61 | 92.51 |
| Zidovudine | CC1=CN(C (=O) NC1=O) C2CC(C (O2) CO) | −40.67 | −6.72 | 0.00 | −35.30 | 1.35 | 95.17 |
| Oseltamivir | CCC (CC) OC1C=C (CC (C1NC (=O) C) N) | −43.45 | −5.83 | 0.00 | −41.67 | 4.05 | 94.94 |
| *N-octyl-β-D-glucopyranoside* | CCCCCCCCOC1C(C(C(C (O1) CO) O) O) O | −53.58 | −18.94 | 0.00 | −39.59 | 5.18 | 91.34 |

index of 3.85, Daclatasvir demonstrated efficacy against HSV-2 virus with a percent cell protection of (77.25%) and a selectivity index of 15.44. The findings indicate that the Daclatasvir and Famciclovir demonstrated efficacy against both the viruses and more specifically drug Daclatasvir when compared to Famciclovir. All the results were tabulated in (Tables 3-5).

The larval growth (mortality) of the selected designed ligands was investigated to understand the association among ligands growth rate and larvae mortality. The $LC_{50}$ and $LC_{90}$ for the highest larvicidal activity were identified over the 72 h exposure period. Even after 24, 48, and 72 h of testing, Daclatasvir demonstrated significant larvicidal efficacy at low dosages (Table 6).

Mortality was determined after 24, 48, and 72 h of exposure using varied concentrations. Concentration and exposure duration have an effect on the death rate. Observing Daclatasvir therapy at extremely low concentrations for 24 and 48 h, on the other hand, resulted in the greatest morbidity range. Famciclovir demonstrated modest mortality (morbidity) in both the 24, 48, and 72 h exposure periods, although it impeded larval growth at the early pupal stage. Some damage may occur at the cellular level (Fig. 3).

## Discussion

*In silico* molecular docking simulations have been conducted to position all selected drugs at the desired binding site of the DENVE protein receptor, to estimate binding affinities, binding modes, and ligands orientation, correlating with co-crystallized n-Octyl-β-D-glucopyranoside. Docking results have shown that all molecules have strong docking scores. The results discussed in this paper illustrate the significance of the molecular docking approach in designing and producing novel bioactive compounds. The prediction of the binding affinity of the novel compound (ligand) to the selected target (enzyme/protein) is a key factor in the design of the new drug. Among the drugs tested two drugs namely Daclatasvir and Famciclovir have been identified as HITS for combating DENVE protein. The *in silico* study is supported by the *in vitro* screening in which both the compounds are effective against HSV-1 & 2 and more precisely the Daclatasvir compound relative to Famciclovir. The larvicidal activity of Daclatasvir and Famciclovir was measured by $LC_{50}$ and $LC_{90}$ values. After 24, 48, and 72 h of exposure, Daclatasvir had substantial larvicidal efficacy at low concentrations.

Drug repurposing has been used by a variety of organizations to find effective therapies for dengue disease. Methods used in these studies required repositioning of medications based on scientific experience in the prevention of dengue symptoms. The

Table 3 — Cytotoxicity studies

| Sample | $CC_{50}$ µg/mL |
|---|---|
| Daclatasvir | 208.21 ± 8.22 |
| Famciclovir | 195.01 ± 7.49 |

Values are the mean ± standard deviation of three different experiments

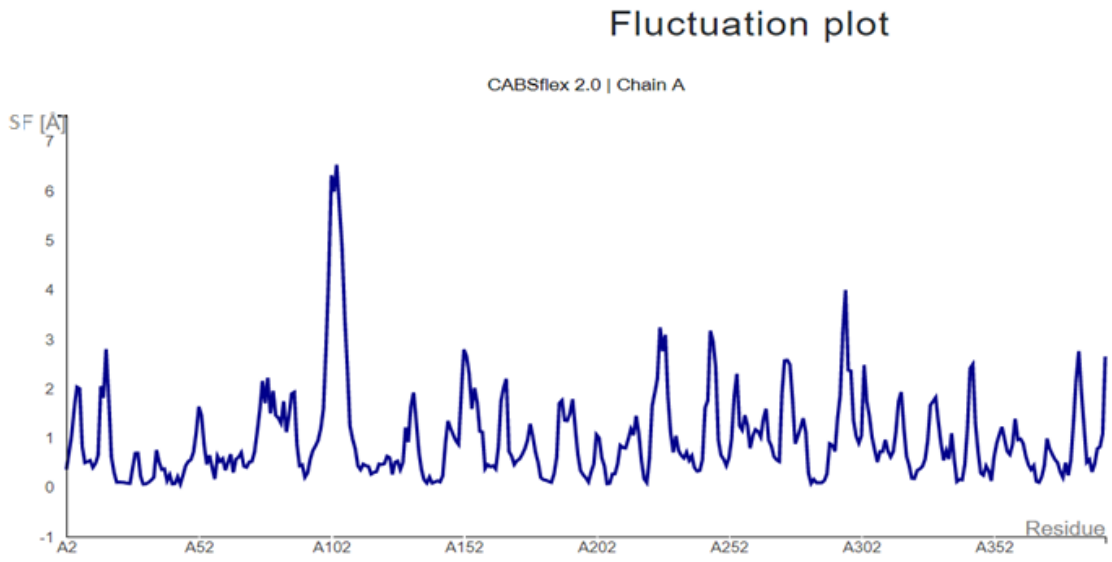


Fig. 2- The CAB-flex 2.0 server provided the root-mean-square fluctuation (RMSF) of atoms in the 1OKE-Daclatasvir complex

Table 4 — Antiviral activity of extracts against HSV-1 and HSV-2 virus at 100 $TCID_{50}$ (50% Tissue culture infectivity dose)

| | Antiviral activity of HSV-1 | | | Antiviral activity of HSV-2 | | |
|---|---|---|---|---|---|---|
| Sample | Conc. (μg/mL) | 100 $TCID_{50}$/% Virus growth | % Cell proliferation | Conc. (μg/mL) | 100 $TCID_{50}$/% Virus growth | % Cell proliferation |
| Daclatasvir | 50 | 25.55 | 74.45 | 50 | 22.75 | 77.25 |
| | 25 | 31.25 | 68.75 | 25 | 29.45 | 70.55 |
| | 12.5 | 53.85 | 46.15 | 12.5 | 41.45 | 58.55 |
| | 6.25 | 85.35 | 14.65 | 6.25 | 75.95 | 24.05 |
| Famciclovir | 50 | 41.92 | 58.08 | 50 | 56.55 | 43.45 |
| | 25 | 53.24 | 46.76 | 25 | 62.25 | 37.75 |
| | 12.5 | 78.75 | 21.25 | 12.5 | 75.45 | 24.55 |
| | 6.25 | 89.65 | 10.35 | 6.25 | 86.85 | 13.15 |

Table 5 — Anti-HSV-1 and HSV-2 activity in Vero cells

| | HSV-1 | | HSV-2 | |
|---|---|---|---|---|
| Treatment | $IC_{50}$ μg/mL | $CC_{50}$ μg/mL | $IC_{50}$ μg/mL | $CC_{50}$ μg/mL |
| Daclatasvir | 15.60 ± 3.25 | 208.31 ± 8.22 | 3.49 ± 2.79 | 208.31 ± 8.22 |
| Famciclovir | 38.09 ± 5.63 | 195.01 ± 7.49 | 50.56 ± 3.55 | 195.01 ± 7.49 |

Values are the mean ± standard deviation of three different experiments

Table 6 — Larvicidal activity of the compounds

| Sample | Time (Hrs) | $LC_{50}$ (ppm) | LCL-UCL (ppm) | $LC_{90}$ (ppm) | Intercept | Slope | $X^2$ value | *P*-value |
|---|---|---|---|---|---|---|---|---|
| Daclatasvir | 24 | 80.040 | 058.601-120.148 | 105.111 | −2.410 | 0.014 | 0.411 | 0.918 |
| | 48 | 61.123 | 081.131-178.121 | 92.240 | −1.214 | 0.010 | 0.341 | 0.968 |
| | 72 | 40.241 | 021.710-111.120 | 75.216 | −1.001 | 0.012 | 0.518 | 0.895 |
| Acyclovir | 24 | 135.625 | 062.123-165.213 | 242.124 | −1.131 | 0.010 | 1.112 | 0.810 |
| | 48 | 100.124 | 050.719-111.912 | 158.186 | −1.522 | 0.014 | 0.934 | 0.791 |
| | 72 | 60.731 | 010.114-089.127 | 134.013 | −1.551 | 0.020 | 0.821 | 0.758 |
| Lamivudine | 24 | 142.182 | 092.212-168.362 | 275.182 | −2.612 | 0.016 | 1.793 | 0.651 |
| | 48 | 129.112 | 080.118-139.260 | 231.320 | −1.827 | 0.013 | 0.342 | 0.827 |
| | 72 | 70.212 | 031.261-110.501 | 150.168 | −1.316 | 0.011 | 0.425 | 0.816 |
| Baloxavir | 24 | 198.218 | 142.084-218.424 | 292.123 | −2.810 | 0.015 | 1.812 | 0.589 |
| | 48 | 168.524 | 118.261-205.605 | 269.912 | −1.912 | 0.012 | 0.418 | 0.916 |
| | 72 | 82.418 | 069.521-154.512 | 171.225 | −1.461 | 0.010 | 0.492 | 0.892 |
| Famciclovir | 24 | 100.180 | 80.152-252.190 | 335.218 | −2.425 | 0.015 | 1.325 | 0.783 |
| | 48 | 89.252 | 68.634-181.125 | 310.112 | −2.004 | 0.011 | 1.632 | 0.758 |
| | 72 | 49.112 | 052.362-98.218 | 218.427 | −1.628 | 0.013 | 1.682 | 0.732 |
| Ganciclovir | 24 | 205.127 | 158.218-242.128 | 348.810 | −2.618 | 0.014 | 1.795 | 0.621 |
| | 48 | 152.412 | 131.810-201.425 | 321.412 | −1.571 | 0.009 | 0.301 | 0.894 |
| | 72 | 89.235 | 060.512-128.120 | 200.201 | −1.308 | 0.010 | 0.412 | 0.858 |
| Stavudine | 24 | 220.120 | 182.812-232.416 | 323.127 | −2.810 | 0.013 | 0.420 | 0.924 |
| | 48 | 132.321 | 094.231-178.123 | 258.410 | −1.832 | 0.011 | 0.389 | 0.954 |
| | 72 | 78.524 | 054.712-101.221 | 912.613 | −1.243 | 0.010 | 0.510 | 0.892 |
| Zidovudine | 24 | 234.201 | 205.712-525.812 | 372.113 | −2.149 | 0.010 | 0.628 | 0.852 |
| | 48 | 192.431 | 183.914-298.482 | 276.041 | −2.523 | 0.012 | 0.424 | 0.912 |
| | 72 | 101.898 | 085.143-184.630 | 225.510 | −1.245 | 0.011 | 0.401 | 0.928 |
| Oseltavir | 24 | 248.641 | 223.810-432.120 | 350.123 | −2.589 | 0.014 | 1.324 | 0.782 |
| | 48 | 221.425 | 171.123-284.182 | 312.241 | −2.034 | 0.011 | 1123 | 0.842 |
| | 72 | 138.520 | 175.178-175.281 | 228.417 | −1.768 | 0.013 | 0.641 | 0.923 |

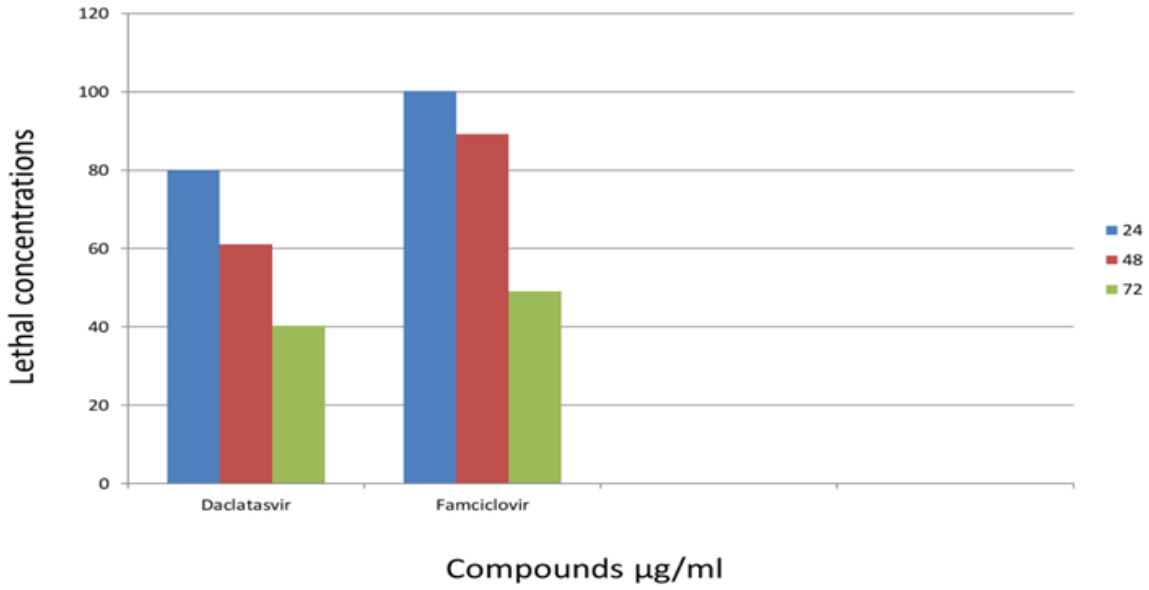


Fig. 3- Larvicidal activity of Daclatasvir & Famciclovir against *Aedes aegypti*

Previous findings showed the recurrence of Prochlorperazine[20], nordihydroguaiaretic acid[21], minocycline[22], doxycycline[23], and amodiaquine[24] in dengue infections. In addition, Chen et al.[25] examined the repurposing of a pharmacologically active compound database (LOPAC1280) using a Huh-7 cell screening technique and comprised three compounds, fluoxetine hydrochloride, salmeterol xinafoate, and N-desmethylclozapine as a dengue virus. The presence of imidazole and pyrrolidines moieties in Daclatasvir and the presence of purine moiety in Famciclovir may play a significant role in improved binding affinity 2D interactions towards the DENVE and HSV-1&2. In Daclatasvir, the increased binding affinity may be due to the presence of C2-symmetrical configuration with two fractions of imidazole rings connected by an aromatic bond and the increased *in vitro* antiviral activity may be due to the presence of non-symmetry as well as blender aromaticity[26]. The pyrrolidine rings with methyl substituent may be the reason for the increased hydrophobicity[27]. Likewise, in Famciclovir the alkyl side chains at the 2-position of the purine frame may be the reason for the increased binding affinity[28]. The study was further validated by molecular dynamics study. Daclatasvir, shown good binding affinity towards DENVE protein (1OKE) may be considered as potential hits. Overall, this study concludes that Daclatasvir as potent drug for biologically controlling *Aedes aegypti*. However, further preclinical studies can be done for establishing the proof.

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## Conflict of interest

All authors declare no conflict of interest.

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