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Antiviral screening of seaweed native fucoidan and its derivatives against dengue virus NS2B/NS3 Protease (3L6P) and Methyltranferase (MTase -1L9K); An In-silico docking, ADMET and molecular dynamic study

Saravanan Muniyappan[1], Kothai Ramalingam[1], D. Vinod Kumar[2] and Arul Balasubramanian[1]*

[1]Department of Pharmacy Practice, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation (Deemed to be University), Salem-636008 (Tamilnadu), India.

[2]Department of Biomedical Engineering, Vinayaka Mission's Kirupananda Variyar Engineering College, Vinayaka Mission's Research Foundation (Deemed to be University), Salem-636308 (Tamilnadu), India.

(Corresponding author: Arul Balasubramanian)*

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ABSTRACT: Dengue virus (DENV) infections are the major life threat around the globe with millions of people life is at risk. The cure for DENV infection lays an hectic challemge due to the lack of Antidengue drugs or vaccines which had lead to the enormous demand for a potent lead molecules for the cure, prophylaxis and treatment of Dengue infections. Here an in-silico docking work on native seaweed fucoidan and its derivatives in structure-function investigation of DENV protease and Methyltransferase, the largest non-structural protein of DENV, which is responsible for replication of the viral genome, RNA capping and suppression of host interferon responses was presented. DENV-1 NS2B/NS3 Protease (3L6P) and Methyltranferase (MTase -1L9K) was designated as a prime targets because of its major role in the viral replication cycle. Theses enzymes were selected for the research on antiviral drugs. The sulfated polysaccharide Fucoidan was derived from brown seaweed *Stoechospermum marginatum*. It was subjected to acid hydrolysis to yield low molecular weight fucoidan monomer and it was characterised by FTIR, NMR, and Mass spectroscopic techniques. The SMFUC molecule was designed to yield 40 derivatives by the structural modification of the two methyl (-CH_3) and two hydroxyl (-OH) of the native fucoidan. The methyl moieties were converted to carboxyl group and it was further converted to its ester, amide, aldehyde, acid chloride and ketone, the hydroxyl groups were acetylated, benzoylated, aminated, sulphated and phosphorylated. Further Desulphated SMFUC was designed for the Insilco docking, ADMET, MMSD and Molecular dynamic study against DENV-1 NS2B/NS3 Protease 3L6P and DENV-2 1L9K Methyltranferase. 3,4-diacetyl fucoidan showed highest ligand binding affinity GLIDE score of -9.849 kcal/mol against DENV-1 NS2B/NS3 Protease (3L6P) and 3,4-dibenzoyl fucoidan showed more binding affinity against DENV-2 1L9K Methyltranferase with a GLIDE score of -9.2kcal/mol amongst the native fucoidan and its derivatives. *In-silico* pharmacokinetic study of the these molecules were screened using SwissADME and sarADMET studies proved that these compounds were well absorbed by GI tract, and do not cross the blood brain barrier, Simulated toxicity simulation studies proved except phosphate fucoidans they are non-toxic and without a metabolic enzyme inhibition activity. Simulated molecular dynamics study against the optimized fucoidans showed a stable RMSD score of 3.8-4.2 for the Acetyl fucoidan-3L6P complex and 1.5-1.8 for Benzoyl fucoidan-1L9K complex. Hence it may conclude that seaweed fucoidan and its derivatives have high antiviral potential against DENV. These molecules might be lead for the emergence of antiviral drugs against DENV infections.

Keywords: Dengue Virus; Fucoidan; *in-silico*; NS2B/NS3 Protease; Methyl Transferase

INTRODUCTION

Dengue fever is considered to be a very crucial disease with the diagnostic rate of infections of about 2 million people per year across the world. This fever is caused by the dengue virus, belonging to the *Flaviviridae* family. According to the World Health Organization, one-third of with a variety of the strains of virus polypeptide (Guzman *et al.*, 2016; Bhatt *et al.*, 2013).

Earlier, the disease was mainly restricted to urban and semi-urban areas of the country due to the availability of breeding sites of the mosquito vector species, Aedes aegypti, and then the rural areas. This situation has led to the proliferation of *Aedes aegypti* mosquito. This had resulted in frequent outbreaks of dengue/DHF in rural areas of the country (Jagtap *etal.*, 2009).

Five serotypes of dengue virus have been identified which are DENV-1, DENV-2, DENV-3 and DENV-4 and DENV-5 (Añez *et al.*, 2016). These first four serotypes are closely related in structure; however, they interact differently with the antibodies, present in the human blood serum. Here viruses share a similarity of almost 65% in the genome while there are genetic variations within a single serotype. However, these four

serotypes cause the same diseases despite having genomic variations. Genome of DENV-5, as compared to the other four serotypes, is less characterized and not much information is present regarding this serotype. The polypeptide of the dengue virus is encoded by a long single-stranded RNA, referred as positive sense RNA, and is further cleaved into ten different proteins (seven nonstructural proteins and three structural proteins) (Guha-Sapir and Schimmer 2005).

Dengue viral infection causes three major diseases in the human host namely dengue fever, dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF) (Chen *et al.,* 2018*).* DSS is the most deadly disease amongst the DENV infections. There is a lacuna in the cure for DENV infections because of the non-availability of drugs or vaccines. (Malavige *et al.,* 2004). Seaweeds are thallus which grows under the sea water and its extracts were proven to use for various ailments. Antiviral potential of seaweeds are studied in depth in the last two decades. It was utilized and proved to be a potent antiviral drug against HSV, HPV, DENV and Hepatitis infections. Fucoidans are sulfated polysaccharides present abundant in the intracellular spaces of the seaweed thallus. It is built by repeated sulfated fucopyranose units. Fucoidans was studied extensively for its biological and antiviral activity (Mustafa *et al.* 2015; Messer *et al.,* 2003; Wang *et al.,* 2020; Mendes Marques *et al.,* 2018; Han *et al.,* 2009*).*

Several plant biomolecules were identified and in-silico studies were carried out in recent years. But almost all the natural entities are repurposed synthetic derivatives which were used for various other ailments (Sharoen Yu Ming Lim *et al.,* 2021). It is notable that all the compounds so far tested in dengue clinical trials are repurposed from molecules developed for other indications (Simanjuntak *et al.,* 2015).

The current research work is based on structural modification of native fucoidans derived from brown seaweed *S. marginatum* and subjected to structural modification to get the possible and chemically acceptable fucoidan derivatives and followed by for the *in-silico* docking, ADMET and molecular dynamic studies to retrieve potent fucoidan derivatives for the cure of DENV infections.

MATERIALS AND METHODS

Seaweed *Stoechospermum marginatum*. The brown seaweed *S.marginatum* was collected from the coastal region of Tuticorin Tamilnadu, in the month of July 2021(Fig. 1). The seaweeds collected were cleaned with seawater initially, followed by tap water to remove the adhering epiphytes. The wet seaweeds were then dried in sunlight and pulverized in to fine powder and stored securely.

Extraction of fucoidans. The sulfated polysaccharide fucoidan was isolated by chitosan mediated eco-friendly extraction using continuous hot extraction using distilled water and the crude fucoidan was washed, purified, crystallized, dried and stored for further characterization studies (Muniappan *et al.,* 2020).

Fig. 1. Seaweed *Stoechospermum marginatum* thallus.

Preparation of fucoidans The purified fucoidan was subjected to acid hydrolysis using 0.1N HCl and it was continuously boiled for 6 hours at 80ºC. The LMW native fucoidan was collected and purified by gel chromatography and utilized for structural characterization.

Characterization of Fucoidan. Chemically fucoidans are sulfated polysaccharides composed of individual fucose connected through 1-3 and 1-4 linkage. It possess two active hydroxyl (-OH) group, two methyl (-CH_3) and a sulfate (-SO_4) moiety

ATR-FTIR Studies. Functional group analysis of native fucoidan was done using BRUKER ALPHA II ATR-FTIR Spectrophotometer by scanning in the region of 4000–450cm^{-1} region. The wavenumbers retrieved were identified and correlated for the identification fucoidans (Doménech-Carbó *et al.,* 2020).

H^1NMR Studies. Nuclear magnetic resonance (NMR) spectroscopic studies were conducted using JEOL ECS 400 MHz NMR spectrophotometer. The study reveals the presence of Hydrogen in their environments and will help to elucidate the structure of fucoidan (Rouessac Francis & Annick Rouessac 2022).

Mass Spectroscopic Studies. The molecular weight determination of fucoidan is the direct correlation of the presence of fucoidans. The fucoidan posses a molecular weight of 243, which was referred with the data, retrieved from the PUBchem resources. PerkinElmer GC-MS Mass spectrophotometer was used to carryout the studies (Lin *et al.,* 2008).

Designing Fucoidan and 3,4-disubstituted derivatives. Fucoidan is chemically called sulphated polysaccharides. It has two active hydroxyl groups at C3 and C4 position and two methyl groups at C2amd C6 position. The dihydroxyl groups were designed in to their amino, acetyl, sulpho, phosphor and its benzoylated derivatives. The derivatives are named as Fucoidan, 3,4 diamino fucoidan, 3,4-diacetyl fucoidan, 3,4-disulfonyl fucoidan, 3,4-diphospho fucoidan and 3,4-dibenzoyl fucoidan. The designing of structures were done using Marwin Sketch software drawing tool (Bera *et al.,* 2007) (Fig. 2).

Designing of Desulfated 3,4-disubstituted fucoidans. The 3.4-dihydroxyl groups were designed in to their amino, acetyl, sulpho, phosphor and its benzoylated derivatives. The same compounds were modified in to

their desulfated 2,6 disubstituted derivatives. The derivatives are named as, desulfated fucoidan , 3,4 dimethoxy desulfated fucoidan, 3,4-diamino desulfated fucoidan, 3,4-disulfonyl desulfated fucoidan , 3,4-benzoyl desulfated fucoidan, 3,4-phenoxy desulfated fucoidan , 3,4-phospho desulfated fucoidan, and 3,4-diacetyl desulfated fucoidan (Fig. 3).

Designing of 2,6-disubstituted fucoidans. The 2,6 dimethyl groups were designed in to their carboxylic acids. The carboxylic acids were designed in to their acetyl, amide, acid chloride, ester, and aldehyde and ketone derivatives. The derivatives are named as fucoidan 2,6-dicarboxylate, fucoidan 2,6-dicarbonyl aldehyde, fucoidan 2,6-dicarboxamide, fucoidan 2,6-dimethylcarboxylate, fucoidan 2,6-dicarbonyl chloride, fucoidan 2,6-dibenzoate, and fucoidan 2,6-diacetate (Fig. 4).

Designing of desulfated 2,6-disubstituted fucoidans. The dimethyl groups were designed in to their carboxylic acids. The carboxylic acids were designed in to their acetyl, amide, acid chloride, ester, and aldehyde and ketone derivatives. The same compounds were modified in to their desulfated 2,6 disubstituted derivatves. The derivatives are named as desulfated fucoidan 2,6-dicarboxylate, desulfated fucoidan 2,6-dicarbonyl aldehyde, desulfated fucoidan 2,6-dicarboxamide, desulfated fucoidan 2,6-dimethylcarboxylate, desulfated fucoidan 2,6-dicarbonyl chloride, desulfated fucoidan 2,6-dibenzoate, and desulfated fucoidan 2,6-diacetate (Fig. 5).

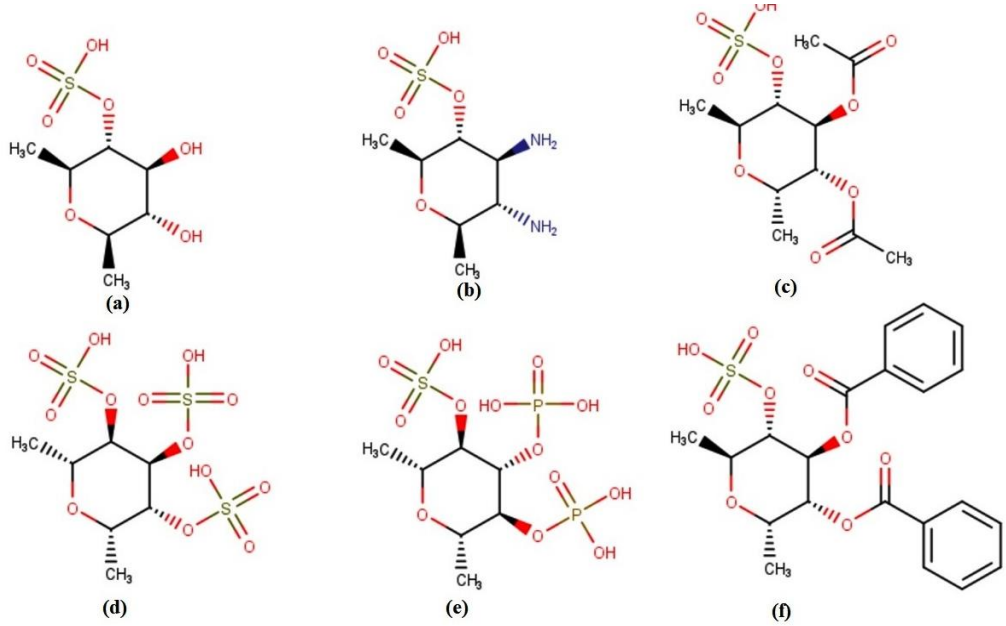


Fig. 2. Structure of fucoidan and its 3,4-disubstituted derivatives (a) Fucoidan (b) 3,4 diamino fucoidan (c) 3,4-diacetyl fucoidan (d) 3,4-disulfonyl fucoidan (e) 3,4-diphospho fucoidan and (f) 3,4-dibenzoyl fucoidan.

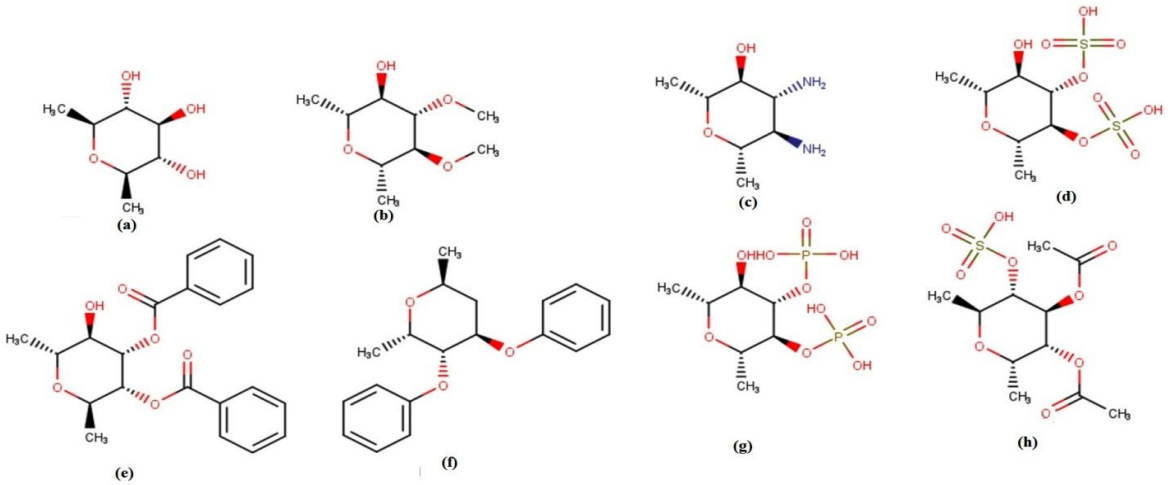


Fig. 3. Structure of fucoidan and desulfated 3,4-disubstituted fucoidans (a) desulfated fucoidan (b) 3,4 dimethoxy desulfated fucoidan (c) 3,4-diamino desulfated fucoidan (d) 3,4-disulfonyl desulfated fucoidan (e) 3,4-benzoyl desulfated fucoidan, (f) 3,4-phenoxy desulfated fucoidan , (g) 3,4-phospho desulfated fucoidan, and (h) 3,4-diacetyl desulfated fucoidan.

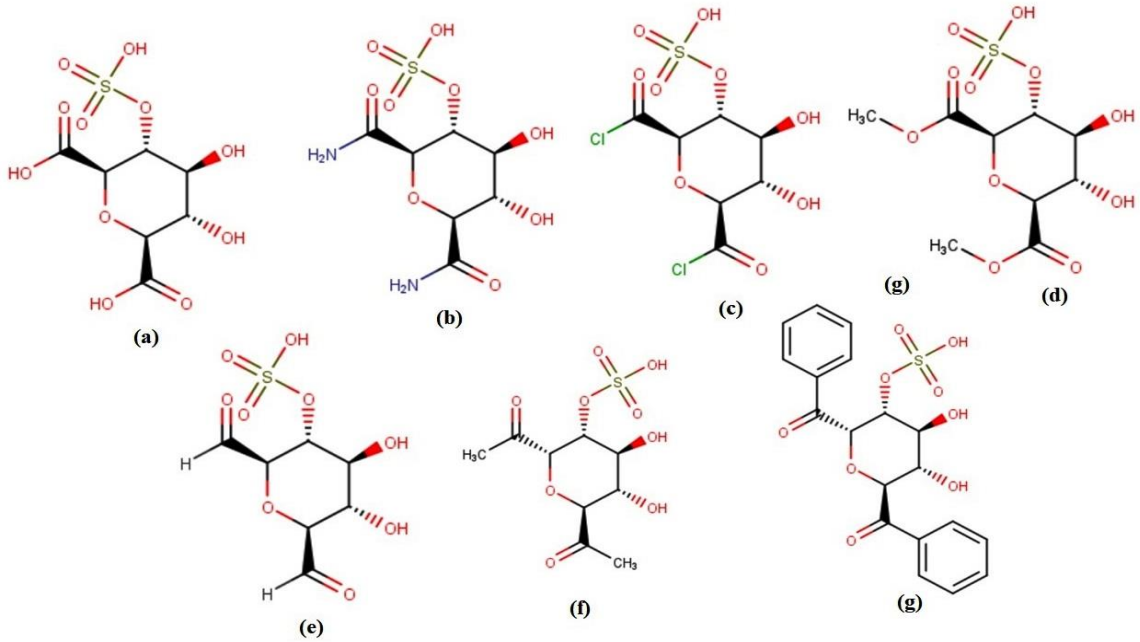


Fig. 4. Structure of 2,6-disubstituted fucoidans (a) fucoidan 2,6-dicarboxylate (b) fucoidan 2,6-dicarbonyl aldehyde (c) fucoidan 2,6-dicarboxamide (d) fucoidan 2,6-dimethylcarboxylate (e) fucoidan 2,6-dicarbonyl chloride , (f) fucoidan 2,6-dibenzoate , and (g) fucoidan 2,6-diacetate.

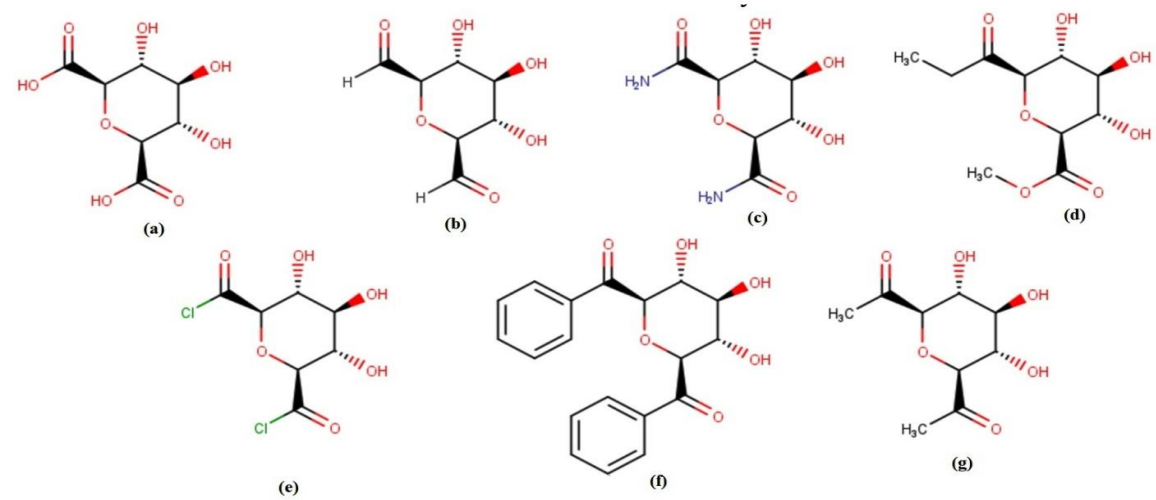


Fig. 5. Structure of desulfated 2,6-disubstituted fucoidans (a) desulfated fucoidan 2,6-dicarboxylate (b) desulfated fucoidan 2,6-dicarbonyl aldehyde (c) desulfated fucoidan 2,6-dicarboxamide (d) desulfated fucoidan 2,6-Dimethylcarboxylate (e) desulfated fucoidan 2,6-dicarbonyl chloride, (f) desulfated fucoidan 2,6-dibenzoate, and (g) desulfated fucoidan 2,6-diacetate.

Selection of DENV proteins. DENV comprises of Non-structural proteins in the identified strains of DENV1-DENV5. There are three major non-structural proteins named as protease, RNA dependent RNA polymerase and methyltransferase. Protease is responsible for replication and methyltransferase plays a major role in transcription phase of DENV development in the host cell (Lin *et al.,* 2008).

DENV-1 NS2B/NS3 Protease-3L6P. Dengue virus strain 1 (DENV-1) non-structural protein NS2B/NS3 Protease-3L6P crystal structure was retrieved from PDB (Bera *et al.,* 2007). It was utilized for virtual screening for its antiDENV activity of the native and the designed fucoidans (Fig. 6(a)). The secondary structure showing binding spheres of (a) DENV-1 NS2B/NS3 Protease is depicted in Fig.7 (a)

DENV-2 Methyltransferase-1L9K. Dengue virus strain 2 (DENV-2) non-structural protein methyl transferase (MTase) (1L9K) crystal structure was retrieved from PDB (Morris *et al.,* 2009). It was utilized for virtual screening for its antiDENV activity of the native and the designed fucoidans (Fig. 7(a)). The secondary structure showing binding spheres of DENV-2 Methytransferase (1L9K) is shown in Fig. 7(b).

***In-silico* docking studies.** The docking study was performed to analyse the inhibition role of fucoidan and its derivatives against NS2B/NS3 proteases and methyltransferase. The ligand and protein preparation was performed in AutoDock Tools while docking was performed using Auto-Dock Vina simulation software (Trott *et al.,* 2010; Visualizer 2010). Water molecules were removed using the Discovery Studio 2.5 software, and CHARMM27 force field was applied onto these protein structures. Simultaneously, the structures were processed 1000 steps and the steric overlaps were removed using the smart-minimize algorithm. Energy minimization and three-dimensional (3D) structure optimization was carried out for fucoidans structures using MarvinSketch (Ghersi *et al.,* 2009). The polar hydrogens were added the two receptors and the ligands using AutoDock Tools. The process of interactions was enhanced by addition of polar charges. The binding pocket comprised of catalytic triad residues His, Asp and Ser. The interactions of the fucoidans were analyzed along with the estimation of binding energies using Autodock Vina (Messer *et al.,* 2003). The total number of dockings were 28 molecules against 3L6p and 28 molecules against 1L9K, performed by using a JAVA based script to automate the docking process. The focused docking approach is reported to have higher accuracy as compared to the blind docking approach (Erbel *et al.,* 2006).

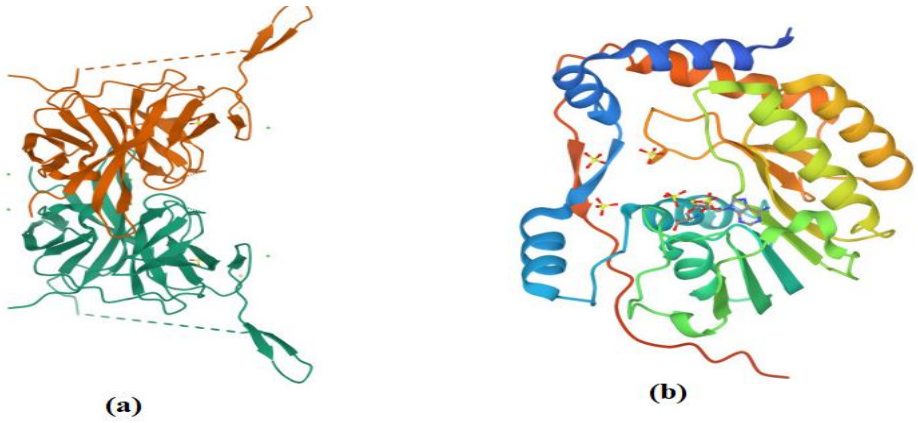


Fig. 6. Crystal structures of (a) DENV-1 Non-structural protein NS2B/NS3 protease (3L6P) and DENV-2 Methyltransferase (1L9K).

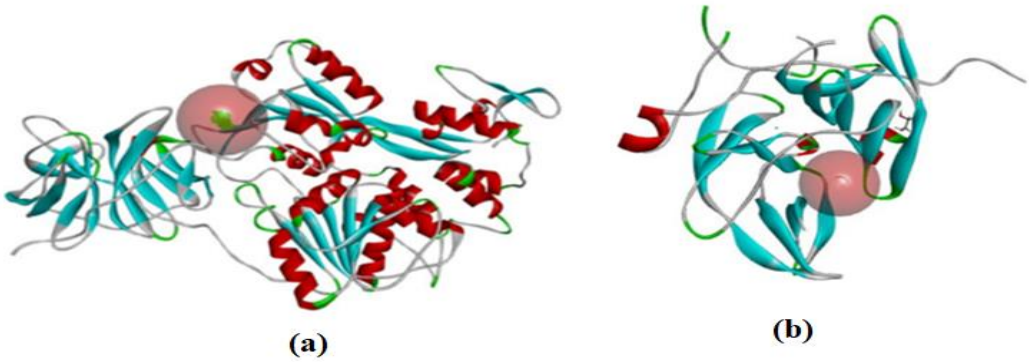


Fig. 7. Secondary structure showing binding spheres of (a) DENV-1 NS2B/NS3 Protease (b) DENV-2 Methytransferase (1L9K).

ADME Studies of fucoidans. Absorption, distribution. metabolism and excretion (ADME) of native fucoidans and derivatives were predicted by in-silico screening using SwissADME software. To support its absorption pattern, bioavailability, lipophilicity, pharmacokinetics, synthetic suitability, drug and lead likeliness will be predicted. Based on the results, the optimized molecule selection will be carried out. The predictions were made using the molecular structure file. Pharmacological properties and pharmacokinetics of the phytochemicals were analyzed using the physically significant descriptors and pharmaceutically relevant properties associated with the ligand molecules (Lee *et al.,* 2003).

Boiled Egg Plot pharmacokinetic analysis. As a part of ADME studies boiled egg plot predicts the Gastro-intestinal (GI) absorption and its permeability through the blood brain barrier. The plot delivers the drug distribution pattern and their plasma protein binding characteristics of the native Fucoidans and their modified derivatives (Malik *et al.,* 2016).

Molecular Dynamic Studies. Molecular dynamic study was done to predict the fucoidan-ligand complex

binding stability. The root mean square deviation (RMSD) of the binding stability was predicted to correlate the binding ability and interaction of ligand with the DENV protein receptors (Yunta *et al.,* 2016)

Molecular Dynamic Studies of optimized fucoidan against 3L6P. MD studies on the optimized fucoidan derivative will predict the stability of the drug-DENV-1 NS2B/NS3 protease MTase protein complex. The route mean square deviation (RMSD) plot predicts the drug protein complex on the DENV receptor target. It will help in strategizing the design modification to improve the stability of the lead molecule. The hydrophilic, hydrophobic and vanderwaals forces are the target bonding components

Molecular Dynamic Studies of optimized fucoidan against 1L9K. MD studies on the optimized fucoidan derivative will predict the stability of the drug-DENV-2 MTase 1L9K protein complex. The route mean square deviation (RMSD) plot predicts the drug protein complex on the DENV receptor target. It will help in stratagising the design modification to improve the stability of the lead molecule. The hydrophilic, hydrophobic and vanderwaals forces are the target bonding components.

RESULTS AND DISCUSSION

Molecular docking and binding site prediction. DENV-1 3L6P and DENV-2 Methyltransferase were docked with the fucoidan and fucoidan derivatives. These fucoidan interacted differently at the binding pocket, showing various binding affinities ranging from−4.0 to −9.8 kcal/mol. Among all the fucoidans, acetyl fucoidan showed highest binding score of -9.8 kcal/mol against DENV1-NS2B/NS3 (3L6P) and benzoylated fucoidans showed more ligand binding affinity against DENV-2 methyltransferase (1L9K). These molecules were selected on basis of effective drug-like properties and ADMET profiles (Table 1). The docking and binding energies of these selected 18 phytochefucoidans micals are discussed in this section.

Docking with DENV1-NS2B/NS3 protease-3L6P. Acetylated fucoidan, docked strongly at the binding pocket of DENV1-NS2B/NS3 protease The CDOCKER energy of the 3,4-diacetyl fucoidan is -9.8 kcal/mol. It was shown good binding interaction with NS2B/NS3 Protease receptors. Mainly, Asp 808 and Met 809 forms two hydrogen bond interactions with OH group of the 3,4-diacetyl fucoidan molecule. Moreover, the Typ 780 residue formed one Pi-lone pair interacting with the acetyl group of the 3,4-diacetyl fucoidan. The other alkyl group of this molecule forms Pi-Alkyl interaction with the active site of the 3L6P receptor (Fig. 8) (Table 2).

Docking with DENV2- methyltransferas-1L9K. The molecule 3,4-dibenzoyl fucoidan molecules have good binding integration with the CDOCKER energy of -9.2056 kcal/mol. The aromatic benzene group in this molecule forms Pi-Cation, Pi- Anion, Pi- Sulfur, and salt bridge with Arg 84, Glu 111, Lys 61, and Lys 181 residues in the active site of the Dengue Methyltransferase receptor. Despite that, the two oxygen atoms show a strong hydrogen bond with the side chain of the Lys 181 residue. Additionally, van der Waals interactions are involved to form a complex with the receptor (Fig. 9) (Table 2).

The ligand binding affinity of 3,4-diacetyl fucoidan was -9.8 kcal/mol which is comparatively higher with the ligand binding affinity of optimized phytocompounds in the range of -8.5 to -8.1 kcal/mol against DENV-1 NS2B/NS3 Protease (3L6P0.The RMSD value range between 1.5 and RMSF value with ligand and DENV enzyme distance < 2Å is comparatively more efficacious than the ligand binding affinity of existing phytoconstituents (Priyanka Purohit *et al.,* 2022).

The molecular docking study of 3,4-dibenzoyl fucoidan illustrates the DENVprotein–ligands interactions with the ligand binding affinity of -9.276kcal/mol. It was found to be the equipotent and nicely bounded into the active site of NS5 methyltransferase when compared to amino acid residues based research on natural optimized compound with the binding score of -9.24kcal/mol. (Kausar *et al.,* 2019)

Table 1: *In-silico* Ligand binding affinity of Fucoidans.

Dengue Virus Proteins	PDB Code	Fucoidans	CDOCKER energy (kcal/mol)
NS2B/ NS3 Protease	3L6P	3,4-diacetylfucoidan	-9.8963
		3,4-diphosphofucoidan	-8.0261
		3,4-dibenzoylfucoidan	-7.3855
		3,4-diaminofucoidan	-5.6660
		Fucoidan	-5.2819
		3,4-disulfonylfucoidan	-6.0259
Methyl Transferase (MTase)	1L9K	3,4-dibenzoylfucoidan	-9.2716
		3,4-diphosphofucoidan	-8.6520
		3,4-diacetylfucoidan	-6.7596
		3,4-diaminofucoidan	-4.7408
		Fucoidan	-3.6078
		3,4-disulfonylfucoidan	-3.4955

ADMET and drug likeness prediction. All the successfully docked fucoidan and its derivatives having the binding affinity −9.0 kcal/mol and above were evaluated for their drug-likeness and ADMET profiles. One of the most effective and important factor to be considered in ADME profiles is the Lipinski's rules non-violations. Physical descriptors such as molecular weight, the hydrogen donor-acceptor bonds and the lipophilicity (log*P*) of the chemicals are evaluated in these rules. A total of 40 designed fucoidan derivatives obeyed Lipinski's rules and showed drug like behavior (Table 3).

Boiled Egg Plot. Boiled egg plot of all the 28 fucoidans were predicted using SwissADME software. 3,4-diacetyl fucoidan and 3,4-dibenzoyl fucoidan to predict the pharmacokinetic properties. Both the molecules are well absorbed from GI tract and they lack BBB permeation (Fig. 10(a-b)).

Toxicity studies: The Rodent and Ames tests for toxicity prediction helped in the evaluation of fucoidans for its potential toxicity. The results showed that out of these 28 fucoidans, 24 derivatives were non-carcinogenic, non-mutagenic and non-toxic. These compounds were further evaluated on the basis of blood brain barrier (BBB) penetration behavior. From the 28 compounds, 23 non-penetrating compounds were observed to be passing BBB evaluation of which six were from 3,4-disubstituted fucoidan derivatives. (Table 3).

These compounds can be used as potential drugs, having strong inhibitory properties for DENV-2 NS2B/NS3 protein and DENV-2. Gastrointestinal Absorption of all the selected fucoidans was also high which reflected the effectiveness of these compounds to be used as drugs. Toxicity radar plot of 3,4-diacetyl fucoidan (Fig. 11) and 3,4-dibenzoyl fucoidan (Fig. 12) showed no hepatotoxicity, mutagenicity and carcinogenicity. Hence fucoidan and the acetylated, benzoylated, phosphated derivatives are considered and selected as optimized lead molecules against DENV.

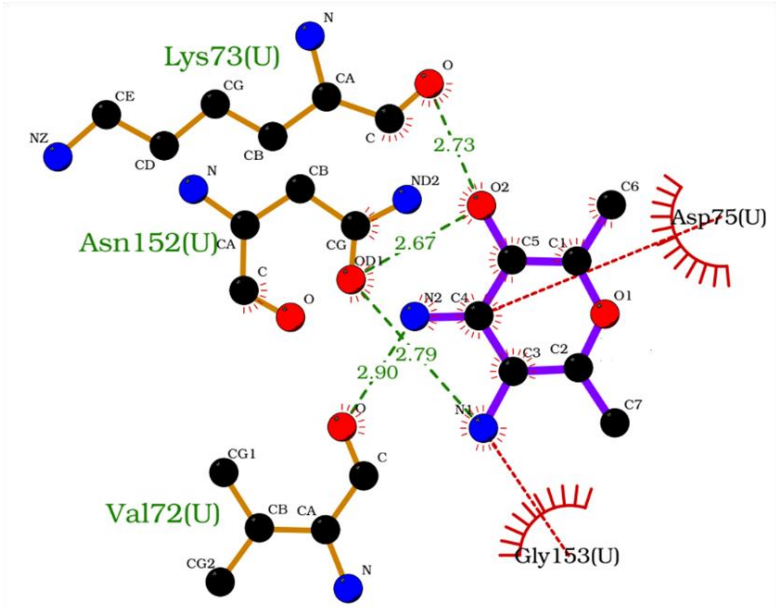


Fig. 8. *In-silico* docking 2D ligand binding complexes of acetyl fucoidan-3L6P complex complex.

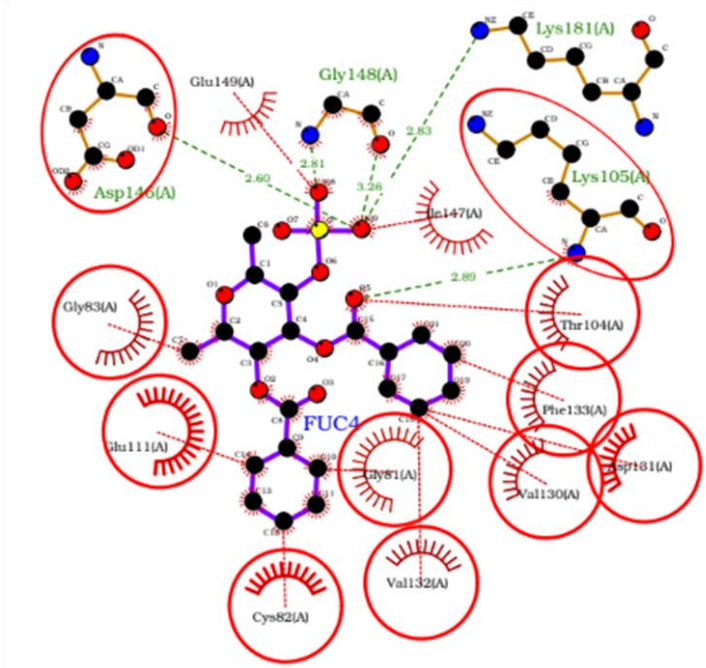
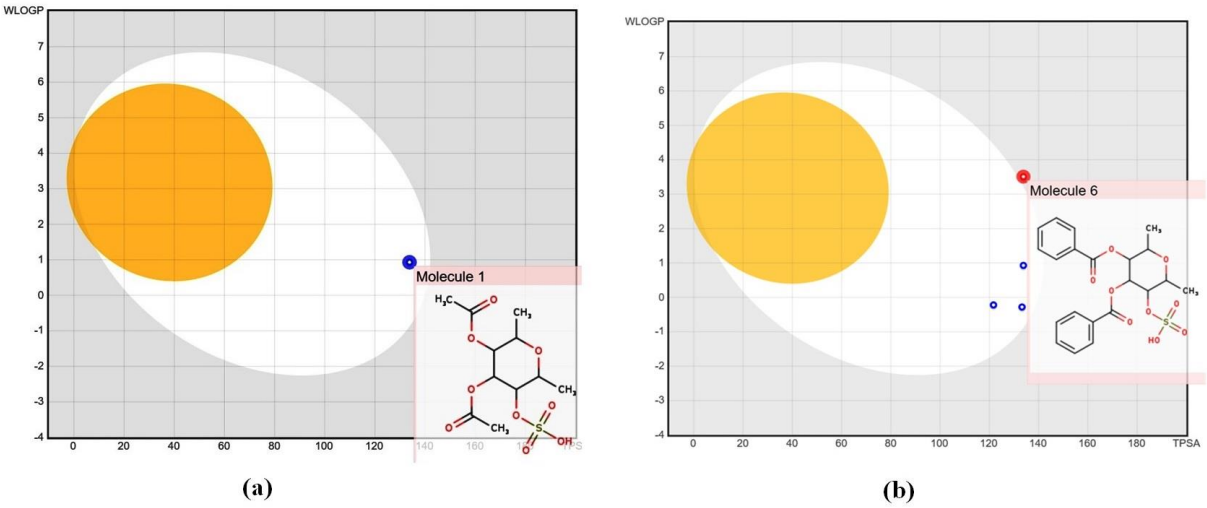


Fig. 9. *In-silico* docking 2D ligand binding complexes of acetyl fucoidan-3L6P complex complex.

Table 2: *In-silico* Pharmacokinetic study of optimized fucoidan and its derivatives.

PK parameters	Fucoidan	Amino Fucoidan	Acetyl Fucoidan	Sulfonyl Fucoidan	Phospho fucoidan	Benzoyl Fucoidan
GI absorption	High	High	High	Low	Low	Low
BBB permeant	No	No	No	No	No	No
P-gp substrate	Yes	Yes	Yes	Yes	Yes	No
CYP1A2 inhibitor	No	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No
Log K_p (skin permeation)	-8.86 cm/s	-10.83 cm/s	-8.55 cm/s	-10.38 cm/s	-11.39 cm/s	-6.95 cm/s



(a) (b)

Fig. 10. Boiled egg plot of (a) acetyl fucoidan (b) Benzoyl fucoida.

Table 3: *In-Silico* toxicity study of optimized fucoidan and its derivatives.

In-Silico toxicity	Fucoidan	Amino Fucoidan	Acetyl Fucoidan	Sulpho Fucoidan	Phospho Fucoidan	Benzoyl Fucoidan
Carcinogenicity (binary)	-	-	-	-	-	-
Carcinogenicity (trinary)	-	-	-	-	-	-
Eye corrosion	-	-	-	-	-	-
Eye irritation	-	-	-	-	-	-
Hepatotoxicity	-	+	-	+	+	-
Mitochondrial toxicity	-	+	-	-	+	-
Nephrotoxicity	-	+	-	-	-	-
Reproductive toxicity	-	-	-	-	-	-
Respiratory toxicity	-	+	-	-	-	-
Skin sensitisation	-	+	-	-	-	-

Molecular dynamic study. Acetyl fucoidan was optimized and selected for the molecular dynamic study to predict the stability of the fucoidan-DENV protease and methyltransferase non-structural proteins complexes. Fig. 13 shows the RMSD plot of Acetylfucoidan-NS2B/NS3 protease (3L6P) complex. The binding was stable throughout the run time of 100ns with a fluctuation in the equilibrium stage of 15ns. When benzoyl fucoidan was selected on the basis of its high binding affinity towards DENV Mtase. The molecular dynamic study predicted the stability of the fucoidan-methyltransferase non-structural proteins complexes. Fig. 14 shows the RMSD plot of benzoylfucoidan-methyltransferase (1L9K) complex. The binding was stable throughout the run time of 100ns with a fluctuation in the equilibrium stage of 0-5ns. Both the fucoidan lead derivatives exhibited acceptable drug binding stability towards the target proteins.

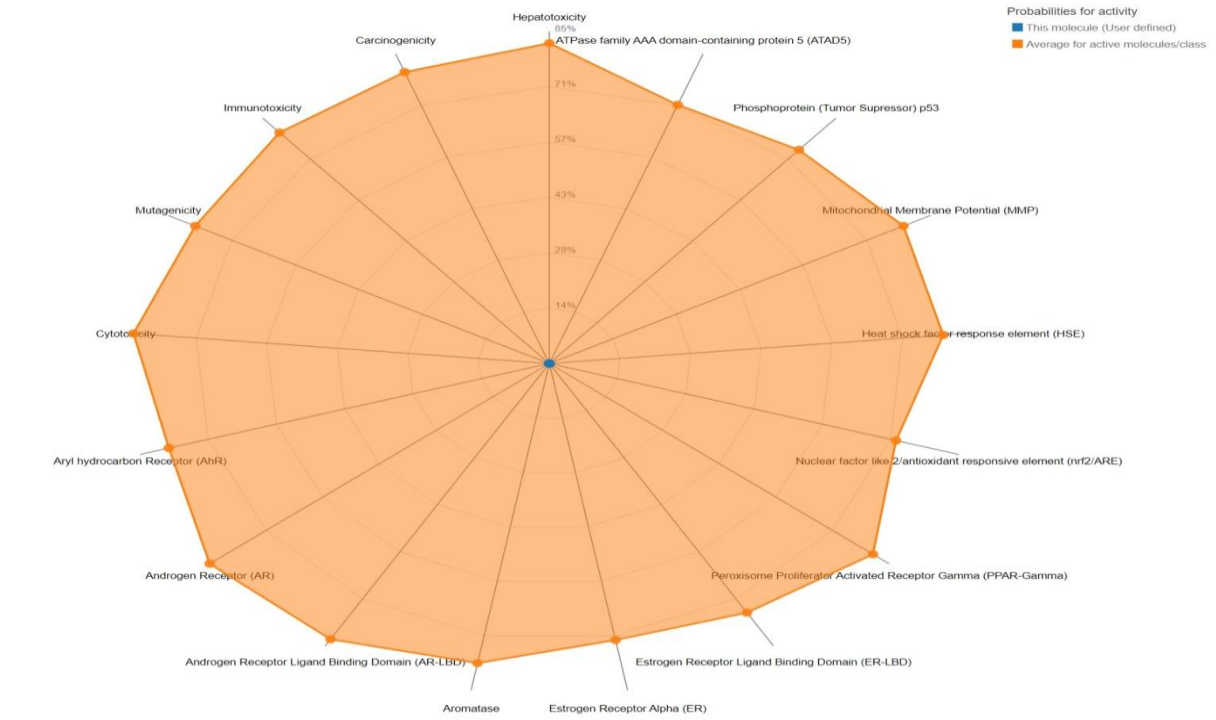


Fig. 11. Toxicity radar plot of acetyl fucoidan.

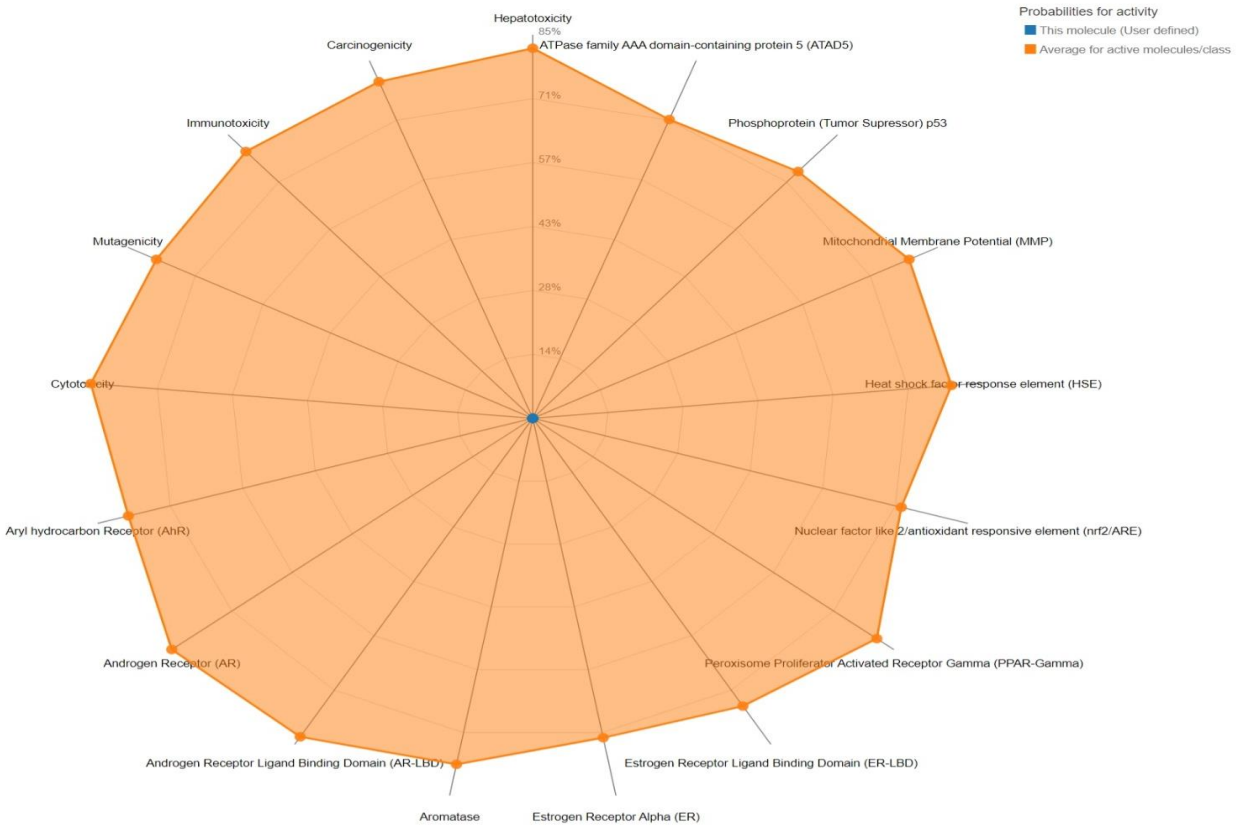


Fig. 12. Toxicity radar plot of benzoyl fucoidan.

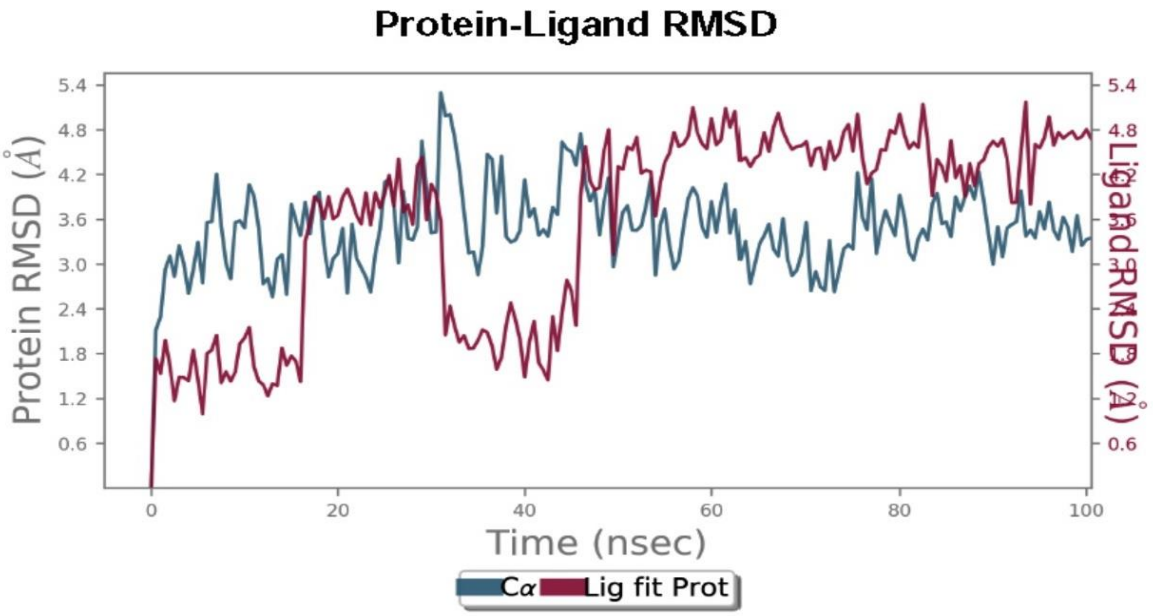


Fig. 13. RMSD plot of acetylfucoidan-DENV-1 NS2B/NS3 Protease (3L6P) complex.

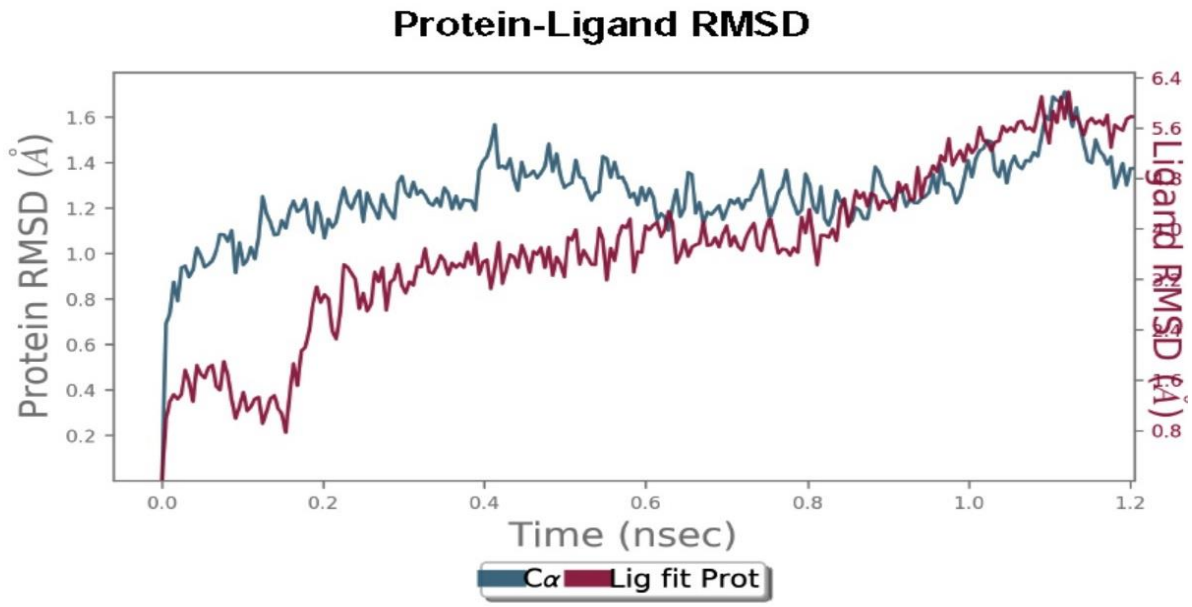


Fig. 14. RMSD plot of acetylfucoidan-DENV-2 Methyl Trans (1L9K) complex.

CONCLUSION

The dengue fever is a pathogenic disease caused by the dengue virus communicated through an mosquito vector *Aedes aegyptii*. Virus replication in host cells is carried out by the DENV non-structural proteins. These non-structural proteins are responsible for viral transcription and replication in the human host cell. In dengue virus, NS2B/NS3 protease is a trypsin-like serine protease and methyltransferase plays an major role in the cleavage of polypeptide, transcribed from the viral genome. The multi-functional property of the viral protease and methytransferase has been utilized as a prime target for drug discovery to cure the dengue fever. The present study focuses on screening and development of inhibitors of the targeted protease and methytranferase. A total of 28 fucoidans were docked against the protease and MTase. In-silico analysis are preferred before conducting any wet lab experimental work, because as it is economical and a less time consuming process.

Native fucoidan derived from *S.marginatum* and its derivatives were docked against DENV-1 NS2B/NS3 Protease and DENV-2 Methyltransferase and the results of molecular docking showed that Acetyl fucoidan inhibit the DEV-1 NS2B/NS3 protease with the binding affinity–9.8kcal/mol, and benzoylated fucoidan exhibited the inhibition against DENV-2

Methyltranferase with the binding affinity of -9.2 kcal/mol.

ADMET of the 28 selected phytochemicals revealed that these compounds have very effective drug like properties along with the non-toxic, non-carcinogenic and non-mutagenic nature. The ADMET was considered as end analysis in the screening a large number of phytochemicals, following various reported techniques. All the fucoidans were non-toxic, non-carcinogenic except aminated and phosphate fucoidans and its corresponding desulfated derivatives.

The optimized 3,4-diacetyl and 3,4-dibenzoyl fucoidan exhibited a good GI absorption and non permeable to BBB. Hence these compounds are devoid of CNS related side effects. Further they obeyed the Lipinski's rule of five without any violations and showed good drug likeliness property.MD studies prove the structural ability of the selected fucoidans to bind to the target DENV proteins for an acceptable period of analysis.

In the current research we have made an attempt to identify ideal DENV inhibitors that are specifically designed for the cure of DENV infections. The current work aimed to derive a novel molecule from the natural entity called fucoidan isolated from seaweed S.marginatum. These fucoidan derivatives are equally potent when compared with the reported natural biomolecules designed to target DENV strains. Overall, the findings of this study provide important insights into the relationship between fucoidans and the antidengie potentials of its derivatives 3,4-diacetyl fucoidan and 3,4-dibenzoyl fucoidan against DENV1 NS2B/NS3 Protease (3L6P) and against DENV2 Methyltranferase (MTase -1L9K) respectively. The goal of finding a cure for dengue in the next decade is highly feasible, judging from the encouraging Antidengue activity of fucoidan derivative against the DENV2 strain.

It was assured and hope that an efficacious anti-dengue fucoidan derivative in the foreseeable future. The future scope Indeed is to conduct in-vitro screening against live DENV strains to test the antiviral potential of the fucoidans.

Conflict of Interest. The authors declare no conflict of interest.

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