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In silico molecular simulation studies on fucoidan derived from the brown seaweed *Stoechospermum marginatum* (C Agardh) kutzing and its derivatives against dengue virus NS2B/NS3 Protease 3L6P

Saravanan Muniappan[2], Arul Balasubramanian[1*], Kothai Ramalingam[3]

[1] Department of Pharmacy Practice, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation, Deemed to be University, Salem, Tamil Nadu, India

[2] Department of Pharmacy Practice, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation, Deemed to be University, Salem, Tamil Nadu, India

[3] Department of Pharmacology, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation, Deemed to be University, Salem, Tamil Nadu, India

Abstract

Dengue viral infection was emerging as a most significant lethal disease, found it's widespread pathogenesis against the mankind. The treatment and eradication of dengue viral infections becomes challenging since there is no vaccines and drugs to combat the disease. The proposed study is based on the *In silico* screening of fucoidan (sulfated polysaccharide) derived from brown seaweed *Stoechospermum marginatum* against Dengue Virus (DENV) 3L6P Non Structural proteins NS2B/NS3 Protease (NS2B/NS3 pro) of DENV-2 serotype. The conventional wet-lab on optimized fucoidan derivatives will serve as the ideal source of lead molecule in the treatment of dengue viral infections. Since there were no approved antiviral drugs or vaccines against DENV infection demands an extensive study. The current *In silico* molecular docking study stands useful for the design and development of novel drug fucoidan compound having better inhibitory activity against the dengue viral proteins 3L6P NS2B/NS3 Protease of DENV-2 strain. Aminated, sulfated, phosphorylated, and acetylated fucoidans were docked with the dengue viral protein (NS2B/NS3 Protease). The docking scores were highest for when amino fucoidans were docked against NS2B/NS3 Protease giving a docking score of -10.8 kcal/mol followed by Over sulfated fucoidan (-9.9 kcal/mol), and phosphorylated fucoidans (-8.913 kcal/mol). Among the docked proteins against fucoidan derivatives, acetyl fucoidans gave a minimum docking score of -6.2 kcal/mol), and unsubstituted fucoidan showed less binding affinity when compared with the fucoidan derivatives, which concludes that fucoidan controls NS2B/NS3 Protease expression. The fucoidan derivatives will be a potential drug candidate if further be validated by wet-lab studies for their proper function.

Keywords: fucoidan; dengue viral proteins; ns2b/ns3 protease; ligands; binding affinity

Introduction

Drug discovery has taken a long stride in the last few decades with the aid of novel computational techniques in identifying efficient lead molecules. Molecular dynamic approach has stamped their importance in evaluating drug targets binding affinity on the target sites of the biological entities. This study will gives us the pharmacokinetic and dynamic properties of the derived core drug molecule. The efficacy and pharmacological potential of the lead biomolecules can be segregated according to its effect on the proposed targets. The identified molecules with promosing activity can be further modified in to a dfferent derivatives of its base or core structure. Further structural alterations were indulged to promoteand improve the binding affinity characteristics of the optimized drug targets with the binding receptors of the biological system. Novel computing softwares and the web world guide us in deriving high quality data and transferring complex structural entities in to a data retrievable for the identification of newer and potent drug leads

The emergence of the In-silico screening method has opened a new arena in all fields of novel drug discovery. The structure-based design of lead molecules will help medical researchers to build an efficacious drug molecule. Novel computing software and the web world guide us in deriving high-quality data and transferring complex biological data into retrievable knowledge in recent trends to discover novel drug molecules [2].

Stoechospermum marginatum

Brown seaweeds found to possess rich biologically active polysaccharides with a broad spectrum of biological activities [3]. Fucoidans, laminarians, carrageenan, and alginic acids are the polysaccharides derived from seaweeds [4]. *S.marginatum* is one of the important species belonging to the genus Stoechospermum and a wide range of bioactive properties have been reported from this species [5]. It is widely distributed on the southern coasts of Tamil Nadu, India, and many parts of Asia and it is reported to be used as animal feed, food ingredients, and fertilizer. Since *S. marginatum* is available in large quantities, it appears to be the most suitable raw material for commercial exploitation. Aquesous extracts of *S.marginatum* were screened and documented for its Anti-Proliferative and Angio-Suppressive Effect [6]. *S. marginatum* also shows a good amount of flavonoids in support and its antioxidant activity

indicating that this species is an ideal target for investigating the activity of the biomolecules present in S. marginatum for various medical and industrial applications [7].

Seaweed fucoidans derived from *S.marginatum* were well studied and documented for their potential antiviral activity against Human immunodeficiency virus (HIV), Herpex Simplex Virus (HSV-1 & HSV-2), Human Papilloma Virus (HPV), Dengue Virus (DENV), and Influenza infections [8, 9].

Fucoidan

Fucoidan is, a chemical constituent of the cell wall of most brown seaweeds and a linear polymer of high molecular weight consisting of β-(1—>3) linked D-mannuronic acid and L-guluronic acid units in the pyranose ring form is the only extract presently obtained from the brown seaweeds [10]. From the many commercially important chemicals derived from seaweeds, fucoidans find their application in food, pharmaceuticals, cosmetics, papers, and textile industries [11]. The biological activity of fucoidans is related to a molecular structure, which includes fucose linkage, the sugar type, sulfate content; molecular weight being the most important determinant [12]. Chemically Fucoidan is composed of α-(1-3) linked sulfated L-fucose. The main component of fucan primarily composed of a polymer of α [1-3] linked fucose with sulfate groups substituted at the 4 positions on some of the fucose residues [13].

Fucoidans' structure was further confirmed with the chitosan-mediated extraction of sulfated polysaccharides from the brown seaweed *Stoechospermum marginatum* [14, 15]. Fucoidan has also been shown to have cytoprotective properties. Chemotherapeutic anticancer drugs are effective against cancer cells, but because of a lack of selectivity, they also attack normal immune cells. It has been demonstrated that fucoidan can protect dendritic cells from the effect of 5-Fluorouracil a representative cancer drug [16].

Fucoidans as Viral Inhibitors

Antiviral activity of fucoidan was extensively studies and the inhibition property of fucoidan got multiple mechanism in preventing viral infection like inhibition of adsorption, internalization, release and transcription against lethal viral pathogens [17]. Fucoidans isolated from the brown seaweed was found to be selective antiviral agents against herpes simplex virus (HSV) types 1 and 2 and human cytomegalovirus [18]. Fucoidans showed high efficiency and low toxicity against influenza outbreaks [19]. The inhibitory effect of fucoidan on the replication of the African swine fever virus (ASFV) in vitro was investigated and proved effective [20]. It was found to have antiviral activity by inhibition of hanta virus adsorption [21]. The effect on human parainfluenza virus type 2 (hPIV-2) was well documented [22]. Fucoidan is a potent and effective against influenza as viral entry inhibitor [23, 24]

Drug designing is a process of selecting novel drug candidates in which many necessary steps are taken to banish such drug molecules that have side effects and also represent interaction with other drug candidates. There are vast numbers of software that play a vital role In silico pharmacological research. The In silico drug designing software are used to investigate molecular modeling of the gene, protein sequence analysis, and 3D structure of target proteins.

Dengue Virus NS2B-NS3 protease

The NS2B-NS3 viral protease is an attractive antiviral target because it plays an important role in viral cell wall cleavage, which is required for viral replication. Thus, we aimed to identify novel inhibitors of the Dengue Virus NS2B-NS3 protease. DENV-2 viral strains are more prevalent in the global spreading of dengue viral disease. To that aim fucoidan present in brown algae *S.marginatum* compounds and 12 structurally diverse derivatives to assess its antiviral potential. Were screened and investigated by structure-based virtual In silico studies [25].

In the present study seaweed, fucoidan derivatives used in this study for docking against DENV NS2B/NS3 and their interactions are observed for the discovery of effective drugs. These compounds are further evaluated on the basis of Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties.

Materials and Methods

***In silico* docking studies**

To predict the mode of action of the ligands Fucoidan and its derivatives against NS2B/NS3 protease (3L6P) protein of DENV-2 viral strain In silico molecular docking studies were carried out. Following databases and tools are used in the present investigation such as Protein Data Bank, PyrX, MarwinSketch, Discovery Biovia, and PyMol Viewer.

Preparation of NS2B/NS3 Protease (3L6P)

The crystal structure of DENV1NS2B/NS3 protease of DENV-2 strain was downloaded from the PDBSum database and the PDB ID was: 3L6P. (Figure 1). The water molecules and active sites were removed from the protease 3l6P and hydrogen atoms were added to the dengue viral macromolecule.

Fig 1: DENV-2 Dengue Viral NS2B/NS3 Protease 3L6P protein structures.

Preparation of ligand structure

Fucoidan compounds used for the docking study were selected from the literature [6]. MarwinSketch, drawing interface software was used to construct the structure of the ligands. Fucoidan derivatives were drawn using the MarwinSketch structure drawing tool. The six-membered

fucopyranoses have two have hydroxyl groups in the 3 and 4 positions. Possible structures were drawn by targeting the 2 active functional group sites. The substituted derivatives are aminated, sulfated, phosphorylated, and acetylated fucoidans. It was clearly depicted in Tab.1. The structures of fucoidan derivatives are shown in Fig.3. The ligands were generated and three-dimensional (3D) optimizations were done and then saved in a. mol file. The derived ligands were saved as "pdbq" format using the PyRx tool.

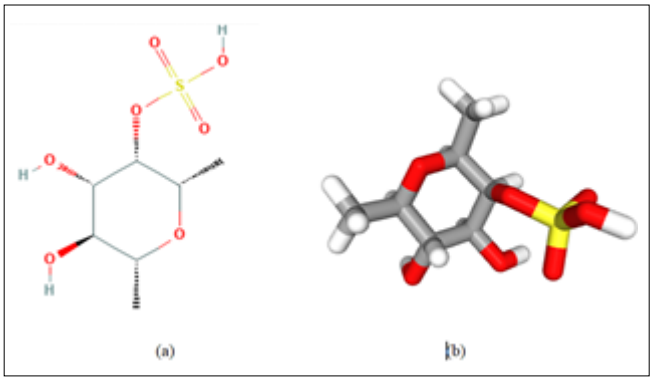

Fig 2: Pubchem structure of fucoidan (a) 2D structure (b) 3D structu

Docking Study with PyRx tool

In this present docking, the study analyzed using PyRx docking software. PyRx is a program to build graphic representations of molecular models. The program will be able to show molecular models to pupils, or even design matters by combining different elements. That will be able to include in the model several atoms, residues, groups, and calculations. The pdpq files of the protein macromolecule and the fucoidan ligands were then docked. Each ligand will be docked in eight confirmers and ligand binding affinity will be presented as a table at the end of a simulation study. A total of 13 compounds were docked with the Dengue Virus NS2B/NS3 protease 3l6P.

Table 1: Binding affinity of fucoidan derivatives against DENV-2 NS2B/NS3 Protease

Viral Enzyme	Fucoidan Ligand Code	Binding Affinity
Fucoidan	SMFU	-6.2
3-Amino Fucoidan	3AMFU	-10.4
4-Amino Fucoidan	4AMFU	-10.5
3,4-Diamino Fucoidan	34DAMFU	-10.8
3-Sulfonyl Fucoidan	3SUFU	-9.4
4-Sulfonyl Fucoidan	4SUFU	-9.7
3,4-Sulfonyl Fucoidan	34DSUFU	-.9.9
3-Phosopo Fucoidan	3POFU	-8.2
4-Phosopo Fucoidan	4POFU	-8.4
3,4-Diphosopo Fucoidan	34POFU	-8.7
3-Acetyl Fucoidan	3ACFU	-6.8
4-Acetyl Fucoidan	4ACFU	-6.7
3,4-Di acetyl Fucoidan	34DACFU	-6.9

Visualization of Protein using Discovery Biovia Viewer

The discovery bio via software interactively displays molecular models and creates publication-quality images. A `ribbon drawing' is featured here. The Discovery Biovia software depicts the simulation and binding scores in terms of ball and stick, space-filling, surface drawing, and density map contour. The docked structures were then visualized using the PyMol Viewer software and the results were predicted (Figure 5,6,7 &8).

ADMET and drug-likeness prediction

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) of all the fucoidan derivatives were calculated using PreADMET [22]. The ADME prediction helped to predict the pharmacological potency of the predicted fucoidan derivatives. The molecular structure file was utilized for the predictions and the pharmacokinetics and pharmacodynamic properties were seriously evaluated for the interaction of designing fucoidan ligands with the viral NS2B/NS3 protease.

Results

***In silico* Molecular Docking Studies**

To study the interaction between ligands (fucoidan) and dengue viral protein 3L6P (NS2B/NS3 Protease) to explore their binding mode, a docking study was performed by using Discovery Biovia docking software.

Retrieval of Protein Structure:

The 3D structure of NS2B/NS3 Protease was derived from PDB and Pymol for using them as a target for docking simulation (Fig. 1).

Retrieval of ligands structure:

The 3D structure of the fucoidan was derived from Pubchem software. Fucoidan derivatives namely aminated, sulfated, phosphorylated, and acetylated Ligands were created and derived for the docking procedure using Marwinsketch, and the 2D structure of the ligands obtained was shown in the (Figure. 3). The optimized fucoidan derivatives with good ligand binding affinity is depicted in Figure.4.

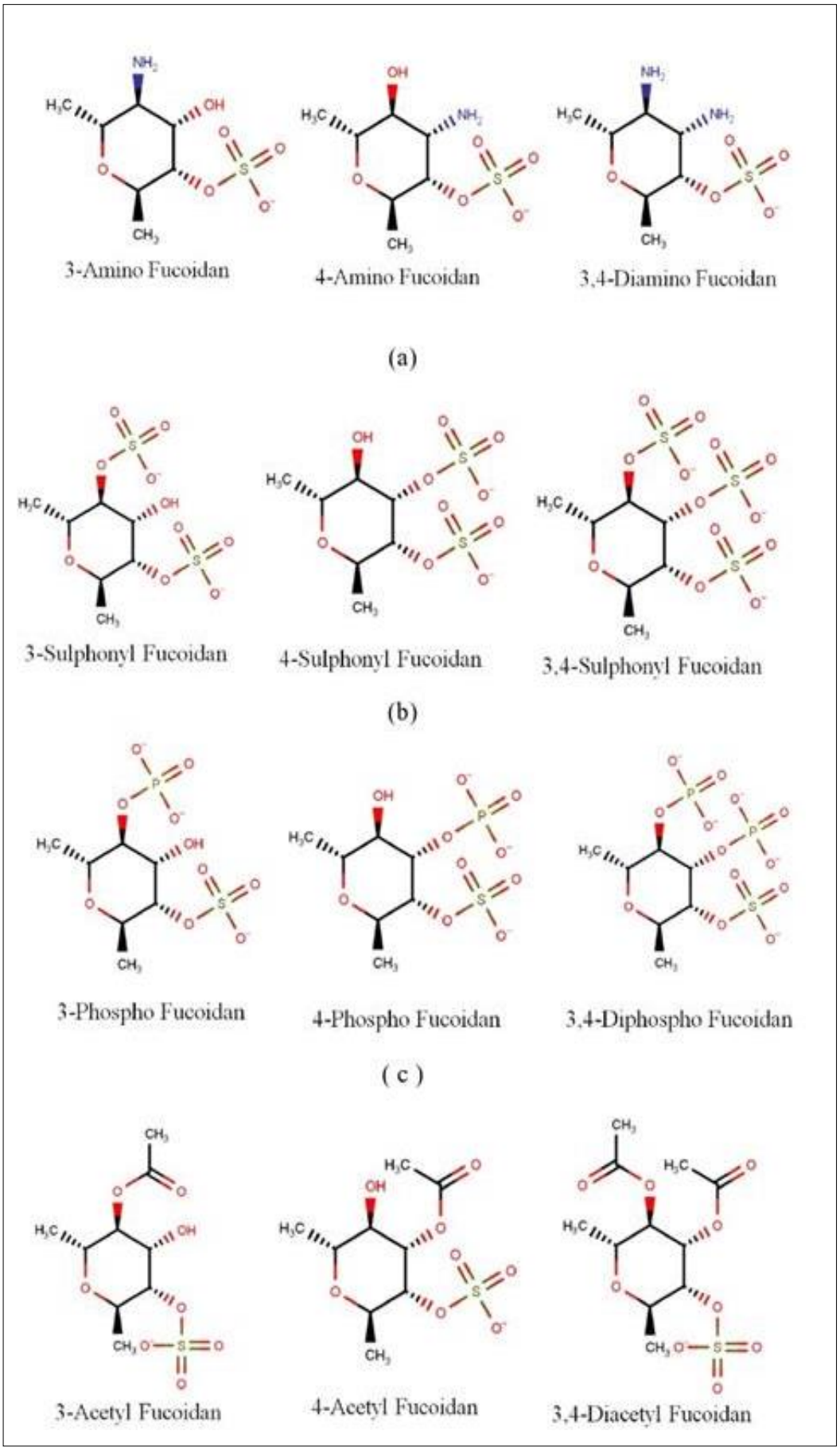

Fig 3: 2D structures of fucoidan derivatives (a) Aminated fucoidans, (2) Sulphated fucoidans, (c) Phosphorylated fucoidans and (d) Acetylated fucoidans

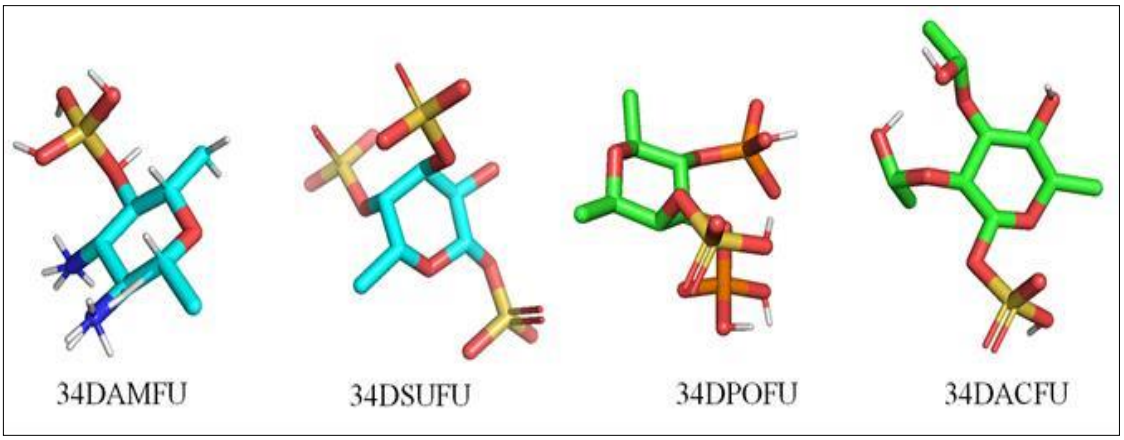

Fig 4: Optimised 3D structures of fucoidan derivatives 34DAMFU (3, 4-Diamino fucoidans), 34DSUFU (3, 4-Disulfonyl fucoidans), 34DPOFU (3, 4-Diphospo fucoidans), and 34DACFU (3, 4-Diacetyl fucoidans).

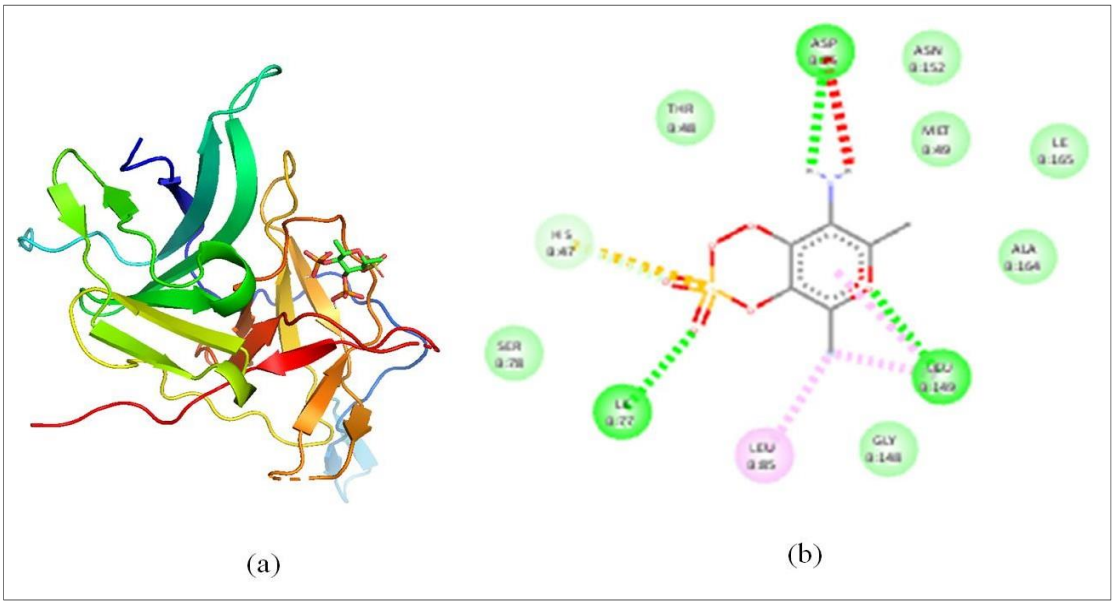

Fig 5: 3, 4-Diamino fucoidan (34DAMFU) binding sites with 3L69 NS2B/NS3 protease. (a) 3, 4-diacetyl fucoidan binding sites (b) 34DAMFU binding sites

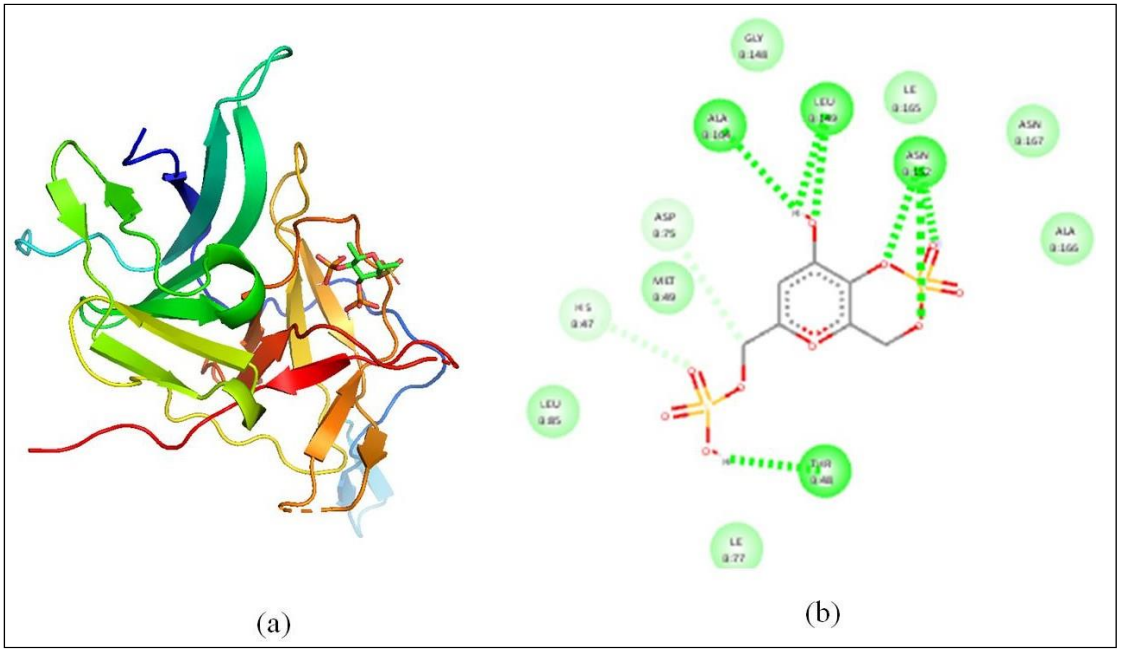

Fig 6: 3, 4-Disulphonyl fucoidan (34DSUFU) binding sites with 3L69 NS2B/NS3 protease. (a) 3,4-diacetyl fucoidan binding with viral protease (b) 34DSUFU binding sites

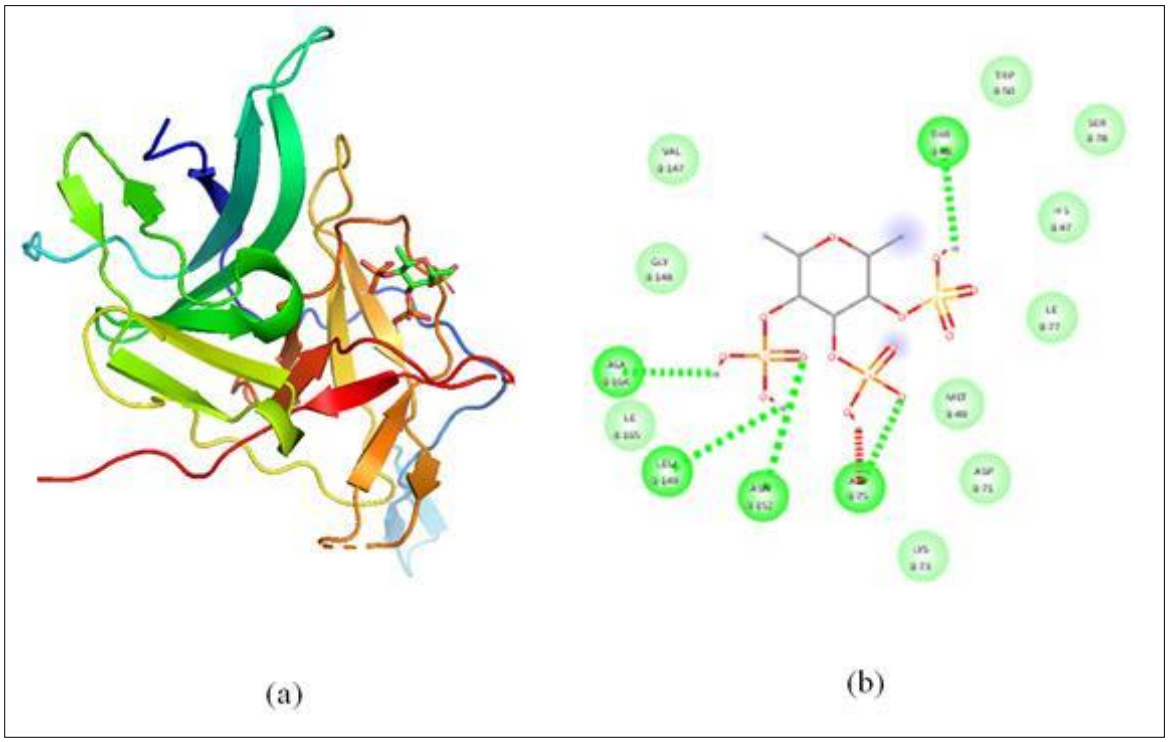

Fig 7: 3, 4-Diphospo fucoidan (34DPOMFU) binding sites with 3L69 NS2B/NS3 protease. (a) 3, 4-diacetyl fucoidan binding with viral protease (b) 34DPOMFU binding sites

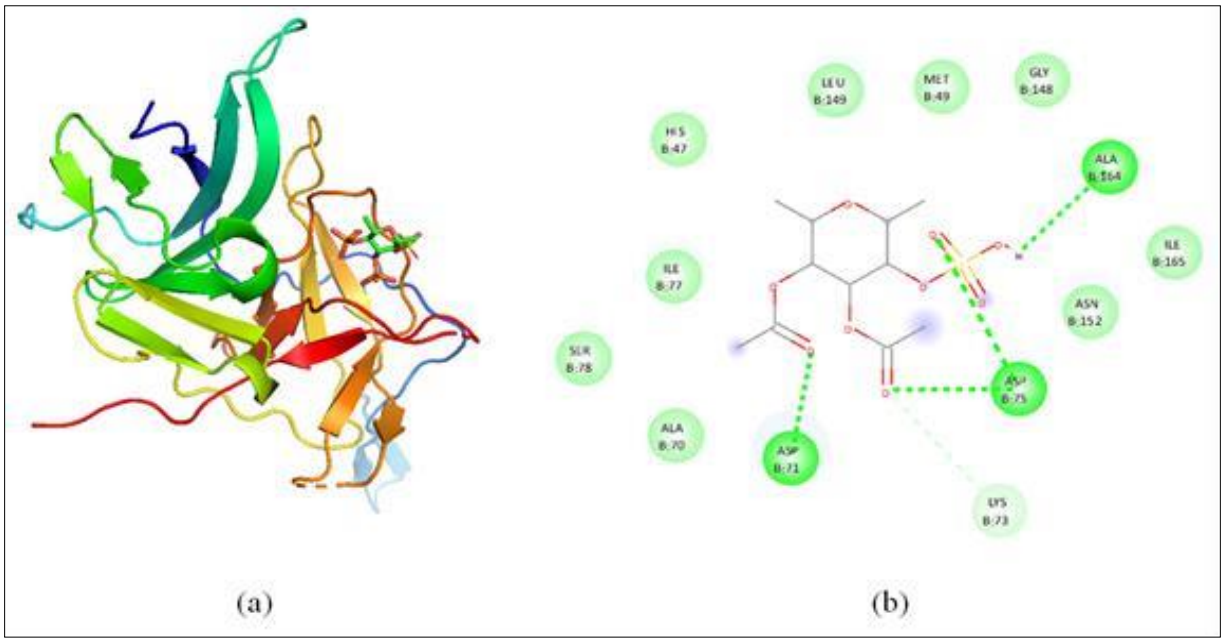

Fig 8: 3, 4-Diacetyl fucoidan (34DACFU) binding sites with 3L69 NS2B/NS3 protease. (a) 3, 4-diacetyl fucoidan binding with viral protease (b) 34DACFU binding sites

Docking with DENV1-NS2B/NS3 protease (3L6P)

The 3D structure of NS2B/NS3 Protease was docked with fucoidan and the synthetic derivatives using The docking results were analyzed using PyMol visualization tool when Amino fucoidan was docked with NS2B/NS3 Protease it showed a docking score of -10.8 kcal/mol with 3 hydrogen bond formation. Likewise, Oversulfated fucoidans showed a docking score of -9.91 kcal/mol with 4 hydrogen bond formation and the least score was found in the acetyl fucoidans with the scores of -6.2 kcal/mol with 2 hydrogen bond formations. Among the four fucoidan derivatives when docked with NS2B/NS3 Protease compound acetyl fucoidans gave a minimum docking score with the control expression. In total, amino fucoidans docked with NS2B/NS3 Protease alone confirming the ability of the ligand for binding at the active site of the receptor was determined by this In silico docking method. The results show that there is a presence of a binding site between the viral proteins and fucoidan ligands.

Fucoidan ligand binding affinity with NS2B/NS3 Protease

Amino fucoidan docked with the binding affinity −10.8 kcal/mol against DENV-2 serine protease at His47, Asp75, and Leu 140.showing the highest binding affinity among fucoidan derivatives. Oversulfated fucoidans were docked

with the protease, −9.9 kcal/mol at Ala104, Asp148, His 77, and Tyr 40. The residues involved in the interaction with the phosphorylated fucoidans were Ala 164, Leu 140, Arg 152, Asp25, and Tyr 40 with the binding affinity -8.8 kcal/mol. Acetylated fucoidan docked with the protease at Ala164, Asp 71, and Asp 75, having a binding affinity of −7.0 kcal/mol (Table.1). The docking of the hydrogen bonding between the viral protein and fucoidan derivatives proves the strong interaction between them. The In silico docking studies proves the application of these two compounds present in S.marginatum as a potential and natural therapeutic agent to treat dengue viral infection.

ADMET and drug-likeness prediction

All the successfully docked fucoidan derivatives having the binding affinity −10.0 kcal/mol and above were evaluated for their drug-likeness and ADMET profiles. One of the most effective and important factors to be considered in ADME profiles is Lipinski's rules of 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. These compounds can be used as potential drugs, having strong inhibitory properties for NS2B/NS3 protein from the dengue virus. Estimated solubility (ESOL) of the fucoidan derivatives aminated and over sulfated fucoidans was observed to be high as compared to that of the fucoidans. Gastrointestinal Absorption of all the selected 12 fucoidan derivatives was also high which reflected the effectiveness of these chemicals to be used as drugs.

Discussion

Recently there are a few reports regarding the ligand-protein interactions of bioactive substances. In silico docking is reported protein interaction that docking of fucoidan compound against the DENV-2 proteins of dengue viral strain. In-silico molecular docking studies on the phytocompounds fucoidan present in S. marginatum against dengue viral protein NS2B/NS3 protease may throw more light on the activity of these phytocompounds in combating dengue viral infection. It is interesting to know that docking studies of compounds present in S. marginatum against dengue viral proteins have not carried out and this is the first report that is recorded. Monosubstituted and disubstituted fucoidans were derived with amino, sulphonyl, phosphate and acetyl groups. Both the derivatives have depicted equipotent ligand binding efficiency but Disubstituted fucoidan derivatives comparatively exhibited more binding affinity towards viral protease. In silico docking, the study revealed that acetyl fucoidans showed a minimum docking score with viral protein. This result shows that there is a presence of a binding site between these fucoidan derivatives. The validity of the bonding between the viral proteins was very well supported by hydrogen bonding. The result suggests the analyzed compound as the best therapeutic drug to combat dengue fever.

Conclusion

In the present study, In silico molecular docking study of fucoidan and its derivatives against dengue viral protein NS2B/NS3 protease proves the application of compounds as a potential and natural therapeutic agent to treat dengue viral diseases. The study was targeted towards the identification of potential inhibitors against NS2B/NS3 protease from the dengue virus to control the viral replication. After the evaluation of the results, Aminated and Over sulfated fucoidans are identified as potential inhibitors against dengue fever. The derived fucoidans were found to exhibit strong inhibition against NS2B/NS3 proteases from DENV-1 stereotype 3L6P.

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