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Research Article

Potential Inhibitor of DENV-2 Virus Protease (NS2B-NS3): An *In-Silico* Studies of Anti-Viral Plants

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Abstract

Dengue virus (DENV) is a mosquito-borne pathogen that affects millions of people worldwide. The DENV-2 protease is a vital enzyme responsible for viral replication and is a promising target for antiviral therapy. The objective of the study is to identify potential inhibitors of DENV-2 protease using *In-Silico* approaches with phytocompounds from ten antiviral plants. Initially, 133 phytoconstituents were collected with anti-dengue properties from previously reported studies which were virtually screened using SWISS ADME for ADME properties. The DENV-2 protease structure (2FOM) was obtained from the Protein Data Bank and molecular docking was performed using AutoDock Vina. The best-scoring compounds were evaluated and top five potential inhibitors with high binding affinity and stability were selected. The top-scoring compounds were Ligand-91 (Terchebin, -8.1 kcal/mol), Ligand-13 (7-desacetyl-7-benzoylgedunin, -7.8 kcal/mol), Ligand-100 (Triterpenoid, -7.8 kcal/mol), Ligand-12 (7-desacetyl-7-benzoylazadiradione, -7.7 kcal/mol), Ligand-20 (Azadirolic acid, -7.7 kcal/mol), Ref.1 (Doxycycline, -6.6 kcal/mol), Ref.2(Monosdenvir, -7.5 kcal/mol), and Ref.3 (Zanamivir, -5.6 kcal/mol). The result of the study shows that 7-desacetyl-7-benzoylazadiradione and 7-desacetyl-7-benzoylgeduninas compounds with high binding affinity for the target protein. These compounds are found in *Azadirachta indica* making it a promising candidate for further experimental validation and development of antiviral agents against DENV-2.

Keywords: Molecular docking, Anti-dengue, Anti-viral, ADME analysis

1. INTRODUCTION

Medicinal plants have been used in healthcare in traditional medicine since time immemorial. It has been a cornerstone of traditional medicine for centuries, providing natural remedies for various ailments. These plants are a rich source of bioactive compounds that have evolved to protect them from environmental stresses, pathogens, and insects. Humans have tapped into this chemical arsenal to develop medicines that prevent and treat diseases. Studies have been carried out globally to focus on plant-based chemicals for drug discovery which have led to the production of plant-based medicines [1]. Medicinal plants have also played a crucial role in the development of modern medicine. Many conventional drugs are derived from plants, such as aspirin from willow bark, quinine from cinchona trees, and morphine from opium poppies. Additionally, plants are a rich source of leads for drug discovery, with many pharmaceutical companies exploring plant-based compounds for new medicines [2]. Herbal medicines have gained its popularity in the last few decades for its therapeutic treatments like Ayurveda, Unani, Homeopathy, Sidha, etc, which use herbs as a major constituent. Plants consist of multiple alkaloids/compounds that occur naturally (as against single extracts) exhibit synergistic actions such as antiviral, antibacterial, anti-protozoa, and antioxidant [3]. Medicinal plants used in traditional medicine and indigenous knowledge to treat a variety of ailments include bulbs, shrubs, ferns, and trees[4].Among the different plant parts, the leaves were most frequently used for the treatment of diseases followed by root, fruit, flower, tuber, rhizome, bark, stem[5]. Herbal medicines can be administered as a paste, decoction, infusion, juice or poultice, or taken orally with no preparation. Medicinal plants contain a wide variety of secondary metabolites or compounds such as tannins terpenoids, alkaloids, flavonoids and possess significant antibacterial, antifungal, anticancer, antidiuretic, anti-inflammatory and anti-diabetic. Pharmacognosy provides a vast understanding of potential medicinal plants applications for the prevention and treatment of numerous conditions and diseases including Dengue[6]. There is an immense ongoing interest among the researchers in developing new antiviral therapeutic agents from natural products or the extract of plants. The numbers of studies that have been undertaken to document and preserve medicinal plant knowledge in Nagaland are few. The numbers of scientific research into the efficacy and safety of phytomedicines and scientific investigations are carried out to confirm the anti- viral properties which can be used to combat

various viral diseases. The following antiviral plants are considered for the study, viz., *Azadirachta indica*, *Brassica campestris*, *Carica papaya*, *Catharanthus roseus*, *Euphorbia hirta*, *Ocimum sanctum*, *Psidium guajava*, *Allium sativum*, *Houttuynia cordata*, and *Mimosa catechu*[7].

Dengue is a virus-based infection caused by the dengue virus (DENV), a member of the genus *Flavivirus* in the family *Flaviviridae* which is transmitted to humans through the bites of infected female mosquitoes, primarily the *Aedes aegypti* mosquito. The virus in other species within the Aedes genus such as *Aedes albopictus, Aedes polynesiensis, Aedes scutellaris*, *Aedes (Stegomyia) aegypti*, *Aedes albopictus*, etc can also act as vectors, but their contribution is secondary to *Aedes aegypti*[8-9]. Along with the dengue virus, *Flavivirus* includes Japanese Encephalitis Virus (JEV), Yellow fever virus (YFV), West Nile virus (WNV) and tick-borne Encephalitis virus (TBEV). Zika virus outbreak has been reported in various countries which is a prominent human-pathogenic flaviviruses [10-11]. Dengue is endemic in more than 125 countries, a significant cause of mortality in tropical and subtropical regions. Infections range from asymptomatic self-limited, acute, febrile disease called dengue fever (DF) to life-threatening dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). There has been a drastic increase case of Dengue and over 2.5 billion people, in more than 100 countries, are at risk of dengue infection, with millions of cases occurring around the world every year. [12-13]. According to WHO, an estimation of 390 million infections is reported annually worldwide, of which 96 million are clinically apparent. The largest number of dengue cases reported was in 2023. The WHO Region of the Americas reported 4.5 million cases, with 2300 deaths. A high number of cases were reported in Asia: Bangladesh (321 000), Malaysia (111 400), Thailand (150 000), and Vietnam (369 000). Dengue is considered to be ubiquitous throughout the tropics, with local spatial variations in risk influenced strongly by rainfall, temperature and the degree of urbanization. Dengue fever can be better framed for health interventions in terms of specific environmental features and assemblages of high-risk spaces. [14-15]. Half of the world's population is at risk of dengue infection as there is no specific antiviral treatment that act against DENV is currently available despite considerable efforts to find potent inhibitors for the Dengue protease. The only dengue vaccine available on the market is CYD-TDV, which was developed by Sanofi Pasteur (marketed as Dengvaxia and is currently approved in 20 countries in Latin America, Asia, and Australia. While CYD-TDV is effective for the prevention of severe infection in previously infected people, it also increases the risk of severe dengue in individuals who have not been previously infected by dengue. The results highlighted were important in directing future vaccine development to identify immune correlates of protection [16-17].

The causative agent for dengue fever (DF) is dengue virus (DENV), an RNA virus from the Flavivirus genus belonging to the *Flaviviridae* family. It is roughly spherical in shape, an enveloped single-stranded positive-sense RNA virus with an icosahedral nucleocapsid covered by the lipid bilayer. It has a positive-strand RNA genome inside a protein capsid also known as nucleocapsid and 11 kb long DENV genome can function as mRNA, and, similar to that in eukaryotes, there are untranslated regions (UTRs) at both the 5′ and 3′ end flanking the open reading frame (ORF). DENV has the ability to infect a wide range of cell types including cells of the human immune system ranging from dendritic cells, monocytes, B- and T-cells, hepatocytes, endothelial cells. Dengue infections are caused by 4 different serotypes, which are: DENV 1, DENV2, DENV3, and DENV4. These serotypes share approximately 65% of their genome similarity, but there is some genetic variation in each serotype. Despite these variations, infection with each of the dengue serotypes results in the same disease and range of clinical symptoms[13, 18].

The pathogenic female *Aedes aegypti* mosquito releases the Dengue virus via saliva into the skin of the mammalian host. Salivary components of *Aedes aegypti* mosquitoes have been identified as significant contributors to increased viral replication. A salivary protein named "34 kDa protein" increases DENV viral titer in human keratinocytes, reducing the expression of the antimicrobial peptides LL-37 and S100A7 and type I interferons. Viral replication starts in the salivary glands of the vector which leads to virion release in the saliva that initiates binding of the virus to host cell receptors for entry via receptor-mediated endocytosis [8,19,20]. DENV after receptor-mediated endocytosis, the positive-strand viral RNAis released into the cytoplasm and translated into a polyprotein. The translated polyprotein are further cleaved by viral and host proteases into three structural (capsid: C, membrane: M, and envelope: E) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins which helps in replication. The RNA genome and the capsid protein interact to form a complex, while other structural proteins form part of the virion envelope. Although the NS proteins are absent within the virion, they assist in virus replication and evasion of the immune system within an infected cell. [21,22,23,26]. C protein localized in the cytoplasm and nuclei is a foremost structural component of DENV which is thought to be crucial for its well-organized replication. The lipid bilayer of virions is formed by lipid between the nucleocapsid core and E/M outer shell.

During the replication of DENV, a membrane-bound replication complex formation helps to incorporate host factors, viral proteins, and genomic RNA. The positive-strand (+) DENV genomic RNA acts as a template to synthesize complementary negative-strand (-) RNA, which acts as a template for the synthesis of new positive-strand viral RNAs[21,24,25]. Viral replication occurs on the endoplasmic reticulum by NS proteins followed by viral assembly and trafficking of immature viral particles to the trans-Golgi network (TGN) where it undergoes pH-dependent maturation. The acidic environment of the TGN facilitates viral maturation where the pr domain of precursor membrane protein (prM) is cleaved by the host protease furin. prM shields

the envelope proteins from premature fusion and pH-induced reorganization during viral secretion. Subsequently, the fully matured infectious virus particle are then released from the hostcell.[8,25,26].

Among the four serotypes of Dengue -DENV 1, DENV2, DENV3, and DENV4, DENV2 is most prevalent globally which contains a single-stranded RNA of positive polarity. The Dengue virus (DENV) is roughly spherical in shape with a diameter of approximately 50 nm, which enveloped single-stranded positive-sense RNA virus of 11 kb long which is translated into a large polyprotein during the infectious life cycle. This polyprotein is processed by cellular and viral proteases into three mature structural proteins: the capsid (C), envelope (E), and membrane (M) proteins along with other seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). These nonstructural proteins play roles in viral replication, virion assembly and attenuation of the host antiviral response [8,27,28].

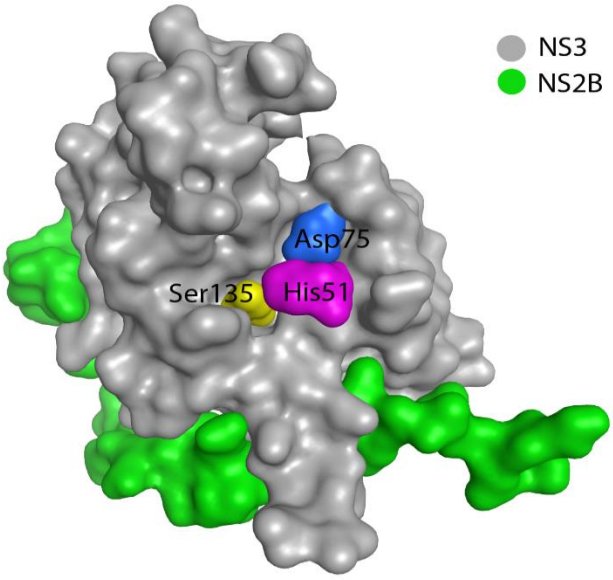


Figure 1: Dengue protease (NS2B-NS3) with catalytic site

NS3, a large multifunctional protein of 618 amino acids belongs to the superfamily 2 of RNA helicases/NTPases having Walker A, GK(S/T) and Walker B, DEx(D/H) motifs, along with other superfamily-characteristic conserved sequence motifs present in subdomains I and II (which possess the fold of the RecA protein) endowed with protease, helicase, nucleoside 5′-triphosphatase (NTPase), as well as 5′-terminal RNA triphosphatase activities, plays an important role in viral polyprotein processing and genome replication. The N-terminal of NS3 comprises a serine protease domain, with the protein NS2B acting as a membrane-anchoring cofactor, necessary for proteolytic activity (figure 1). Helicase activity of NS3 catalyzes the unwinding of the duplex RNA in the 3′–5′ direction releasing the single stranded RNA available to NS5 as a template for replication in the presence of a divalent cation and the energy derived from the hydrolysis of ATP [29,30, 31]. NS2B is a viral serine protease and, along with other NS proteins NS1, NS2A, NS4, and NS4B, plays diverse roles in viral replication, assembly, and release. The C-terminal end of the NS3 protein has three enzymatic properties: a 5′ RNA-triphosphatase (RTP), a nucleoside triphosphatase (NTPase), and a helicase. NS3 forms a complex with NS5 and assists in replication through the unwinding of viral RNA and dephosphorylation prior to 5′-end capping [8, 29, 32]. The dengue virus NS3 protease, a member of the flavivirin enzyme family (EC 3.4.21.91), is located in the N-terminal 184 residues of the multifunctional 69 kDa NS3 protein and contains a functional catalytic triad consisting of His51, Asp75 and Ser135 (figure 1). In addition to the serine protease, the NS3 protein contains enzymatic activities of a nucleoside triphosphatase, a 5' - RNA triphosphatase (RTPase) and a RNA - stimulated RNA helicase. The NS3 protease catalyses the post-translational cleavage of the viral polyprotein precursor in the non-structural region at the NS2A/NS2B, NS2B/NS3, NS3/NS4A and NS4B/NS5 sites and at additional sites within the viral capsid protein, NS2A, NS4A and within a C-terminal region of NS3 itself. The overall conformation of the dengue virus NS3 protease displays the β-barrel conformation typical for serine proteases, although the viral enzyme appears to possess higher compactness with short or absent loop structures and a relatively shallow substrate binding site [33,34]. Based on a number of studies, the methyltransferase (MTase) domain of the DV non-structural protein NS5 (NS5 MTase) is thought to be a promising antiviral target[35, 36] However, the closed conformation is the major form even in the unbound state, which thus represents the best model for structure-guided drug designs[27, 37, 38].

2. METHODS

2.1 Selection of Phytocompounds

After comprehensive search on databases, 10 plants having antiviral properties were selected to confirm their efficiency against DENV-2. A total of 133 phytoconstituents from the selected plants were identified.

Table 1: List of selected phytocompounds.

Sl. No	Phytocompounds	Name of Plants
1	Nimbin	*Azadirachta indica*[39]
2	desacetylnimbin	
3	desacetylsalannin	
4	kaempferol 3-O-rutinoside	
5	Epicatechin	
6	nimbinene,	
7	6-deacetylnimbinene	
8	Nimbandiol	
9	Nimbolide	
10	ascorbic acid	
11	n-hexacosanol	
12	7-desacetyl-7-benzoylazadiradione	
13	7-desacetyl-7-benzoylgedunin	
14	7-hydroxyazadiradione	
15	Nimbiol	
16	Quercetin	
17	ß-sitosterol	
18	Rutin	
19	Limbonin	
20	Azadirolic acid	
21	Nimbochalcin	
22	Azadirachtin	
23	Limbocidin	
24	3-p-coumaroylquinic	*Brassica campestris*[40]
25	Caffeic	
26	Ferulic	
27	sinapic acids	
28	kaempferol 3-sophoroside-7-glucosides	
29	Citric	
30	Aconitic	
31	2-ketoglutaric	
32	Maleic	
33	Shikimic	
34	fumaric acids	
35	Zeaxanthin	
36	Lutein	
37	p-coumaric (4-hydroxycinnamic	
38	Chymopapain,	*Carica papaya*[41.42]

39	Carpaine	
40	Dehydrocarpaine	
41	Oleic acid	
42	Tocopherol	
43	Squalene	
44	Neophytadiene	
45	butyl 9,12,15-octadecatrienoate	
46	n-hexadecanoic acid	
47	phytol (tetramethyl-2-hexadecen)	
48	dasycarpidan-1-methanol acetate (ester)	
49	octadecenoic acid	
50	D-limonene	
51	bis (2-(2-chloroethoxy)ethyl) ether	
52	Dimethoxydimethylsilane	
53	dibenzyl ether	
54	benzhydrazide,	
55	o-butylisourea	
56	2-chloro-5,5-dimethyl-1-phenyl-3-hexen-1-ol	
57	2-methoxybenzeneacetaldehyde	
58	Myricetin	
59	1-methyl-2-pyrrolidinone	
60	Benzonitrile	
61	Nonanal	
62	octanoic acid	
63	1-decene	
64	nonanoic acid	
65	Benzene	
66	Benzene, 1,3-bis(1,1-dimethylethyl)-5-methoxy	
67	1-iodooctadecane	
68	2-methylnaphthalene	
69	2-tetradecene	
70	10-undecenoic acid	
71	Dodecanal	
72	1,4-dimethylnaphthalene	
73	9-oxononanoic acid	
74	1-hentriacontane	
75	2,4-di-tert-butylphenol	
76	nonanedioic acid	
77	dimethyl ester	
78	1-octadecene	

79	Ajmalicine	*Catharanthus roseus*[43,44]
80	Catharanthine	
81	Tabersonine	
82	Serpentine	
83	Vindoline	
84	Quercitol	*Euphorbia hirta*[45]
85	Alpha (α)-amyrin	
86	beta (β)-amyrin	
87	Friedelin	
88	Taraxerol	
89	Euphorbins B	
90	Euphorbin E	
91	Gallic	
92	Geraniin	
93	ellagic acid	
94	Terchebin	
95	Tannic	
96	tartaric acids	
97	lupeol fatty acid ester	
98	fatty acid phytyl esters	
99	linoleic acid	
100	Triterpenoid	
101	Eugenol	*Ocimum sanctum*[46,47]
102	β-sitosterol	
103	Stigmasterol	
104	Campesterol	
105	Reynoutrin	*Psidium guajava*[48,49]
106	Guajaverin	
107	Avicularin	
108	Isoquercitrin	
109	Hyperoside	
110	2,6-dihydroxy-3,5-dimethyl- 4-O- (6 ″ - O-galloyl-β-D-glucopyranosyl) – benzophenone	
111	flavonoids (naringin)	
112	flavonoids (hesperetin)	
113	flavonoids (daidzein)	
114	γ-glutamyl-l-cysteine peptides	*Allium sativum*[50,51]
115	Alliin (S-allyl-l-cysteine sulfoxide)	
116	diallyl thiosulfinate	
117	diallyl disulfide	
118	Garlicin	

119	diallyl trisulfide (allitridin or DATS),	
120	Ajoene	
121	vinyl-dithiins (3-Vinyl-4H-1,2-dithiin)	
122	Chlorogenic acid,	*Houttuynia cordata*[52,53]
123	Glabranin	
124	7-O-methyl-glabranine	
125	4-methyl-heptane	*Mimosa catechu*[54]
126	carboxylic acid	
127	methyl laurate	
128	2-ethyl-3-methylbut-1-ene	
129	tetra decanoic	
130	4-hydroxybenzoic acid	
131	Afzelechin	
132	Aromadendrin	
133	Baicalein	

2.2 ADME Screening

The three-dimensional structure of the phytoconstituents identified from the selected plants was obtained from the PUBCHEM[55] and the structures that are not available were drawn using ChemDRAW. The Mol2 structure of the ligands were used as the input file for screening of the drug-like properties was performed using SWISS ADME and the parameters of Lipinski Rule of 5 i.e, Molecular Weight (MW), Partition Coefficient (LogP), Hydrogen bond donors, Hydrogen Bond acceptors, Molar Refractivity (MR) were recorded.

2.3 Molecular Docking

The structures of the compounds were further evaluated via Molecular docking using Pyrx[56,57]. Structures of Ligands were minimized using Pyrx and the parameters for Energy minimization are uff for force-field and Conjugate Gradient for Optimization Algorithm. The file is then converted to pdbqt file. The structure of protease, 2FOM[58] was obtained from the Protein Data Bank. The structure of Protein was prepared using Discovery Studio where the water molecules and hetero atoms were removed. Pyrx software was employed for energy minimization and conversion to pdbqt file. Molecular Docking Analysis was performed using Autodock Vina[59,60] which is incorporated in Pyrx Graphical User Interface. The Active site selected on the protease for docking are HIS51, VAL75, LYS73, LYS74, ASP75, THR120, LEU128, PRO132, SER135, GLY151, ASN152, GLY153, VAL154, and ALA164. The active sites are centered inside the grid box where the docking is performed.

3. RESULTS AND DISCUSSION

A total of 133 phytoconstituents from 10 different plants and three reference drugs were screened using SWISS ADME. The software used for ADME Screening is SWISSADME[61] where a total number of 133 ligands from the selected plants in mol2 files were fed into SWISSADME. Based on Lipinski rule of five, all the ligands were selected for docking as there were no serious violation to the rule.

3.1 Protein Preparation

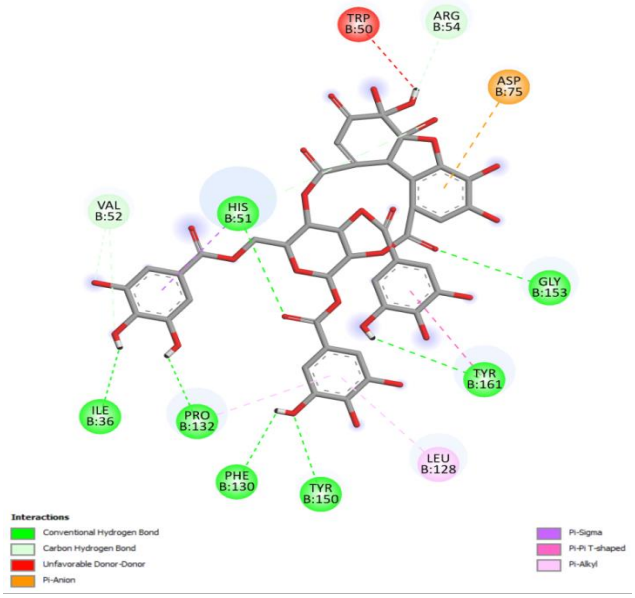
The viral Protease used for this study is Dengue Virus NS2B/NS3 Protease 2FOM. Viral Protein 2FOM structure was downloaded from PUBCHEM [62] in PDB format and prepared using MOE. The catalytic triad His51, Asp75 and Ser 135 are present

3.2 Molecular Docking

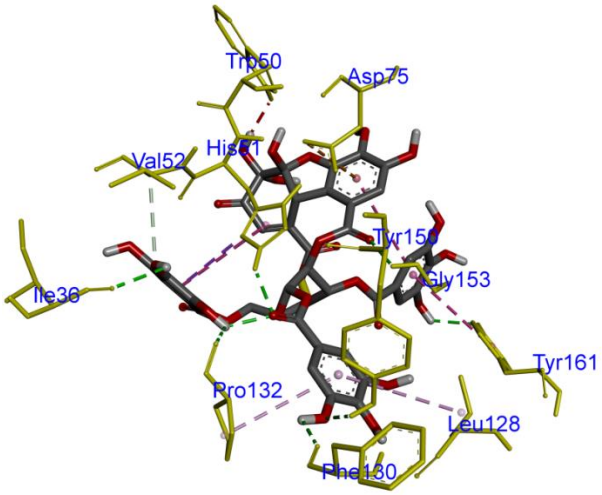
Among the selected phytoconstituents, the top 5 phytoconstituents docking scores of the interactions with 2FOM and interacting active site amino acids are ranked based on their docking scores and shown in table 2, where Ligand91 (Terchebin) being the highest with -8.1 kcal/mol and Ligand12 (7-desacetyl-7-benzoylazadiradione) and Ligand20 (Azadirolic acid) being the lowest with a score of -7.7 kcal/mol each. Docking results shows that all the selected Phytoconstituents have higher docking scores as compared to the reference drugs used in this study.

Table 2: Top docking scores with important interactions

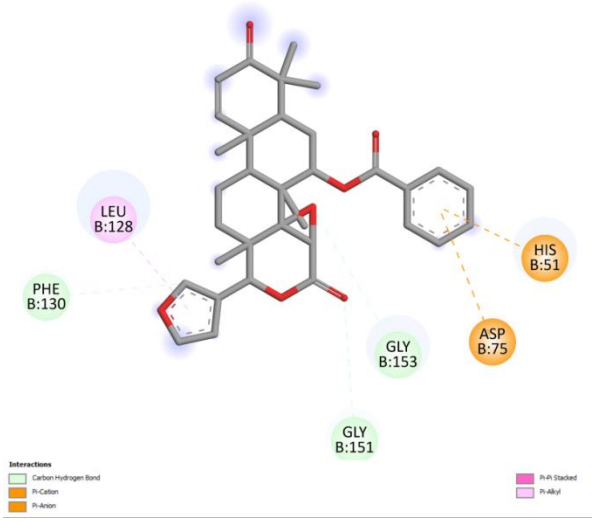
Sl. No	Ligand no	Ligands	Dock Score (kcal/mol)	Important interactions
1	91	Terchebin	-8.1	Trp50, Arg54, Asp75, Gly153, Tyr161, Leu128, Tyr150, Phe130, Pro132, Ile36, Val52, His51
2	13	7-desacetyl-7-benzoylgedunin	-7.8	Leu128, Phe130, Gly151, Gly153, Asp75, His51
3	100	Triterpenoid	- 7.8	Tyr150, Gly151
4	12	7-desacetyl-7-benzoylazadiradione	-7.7	Pro132, His51, Tyr161, Tyr150, Leu128
5	20	Azadirolic acid	-7.7	Gly151, His51, Lys73, Val72, Lys74
6	Ref.1	Doxycycline	-6.6	Val72, Trp50, Asp75, Gly151, Gly153, Val154
7	Ref.2	Monosdenvir	-7.5	Leu128, Pro132, Ser131, His51, Asp75, Asn152, Val154
8	Ref.3	Zanamivir	-5.6	Gly151, Pro132, Ser135, His51, Try150, Phe130, Leu128, Gly153



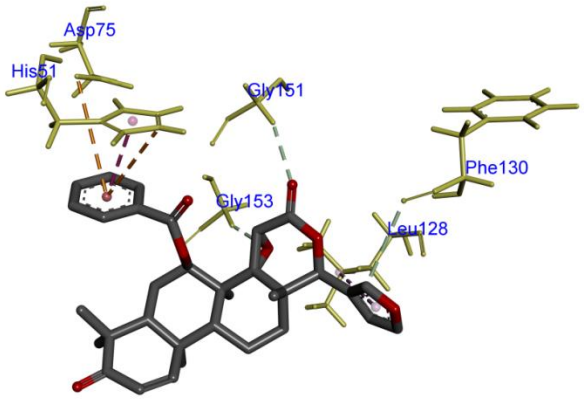
(A)



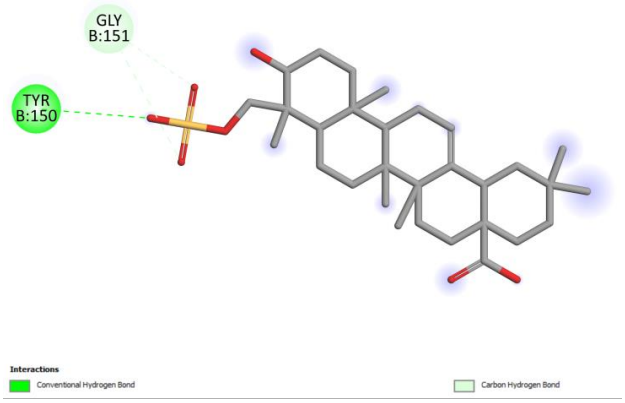
(B)



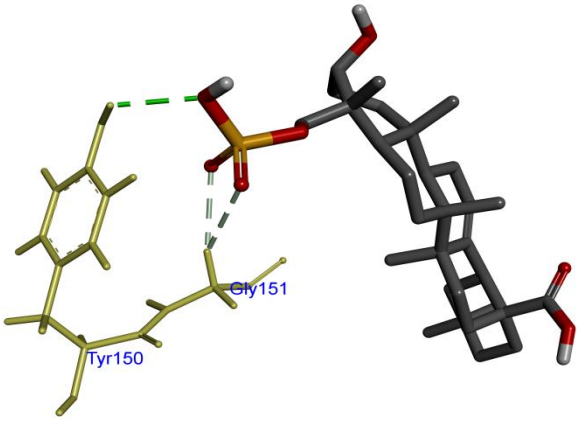
(C)



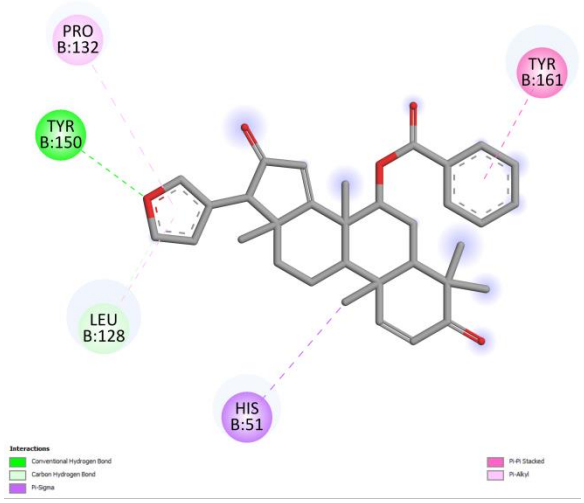
(D)


GLY
B:151
TYR
B:150
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond

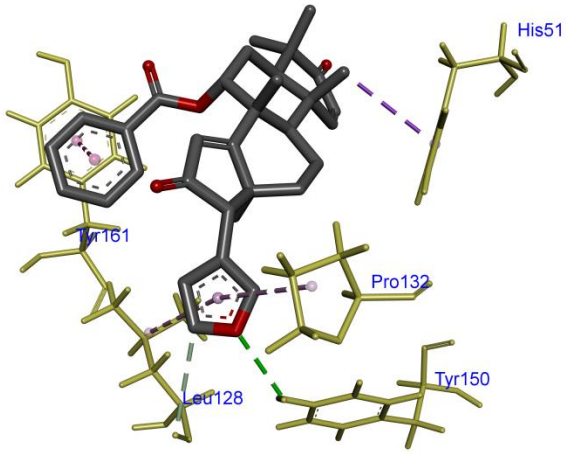
(E)


Gly151
Tyr150

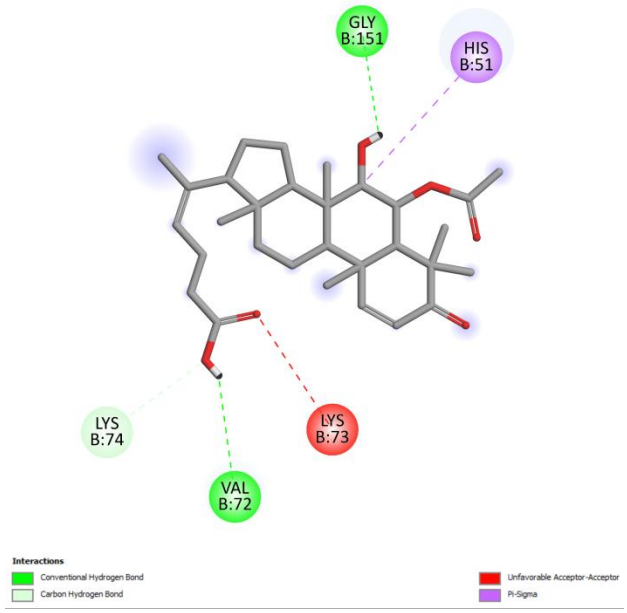
(F)


PRO
B:132
TYR
B:161
TYR
B:150
LEU
B:128
HIS
B:51
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Pi-Pi Stacked
Pi-Alkyl

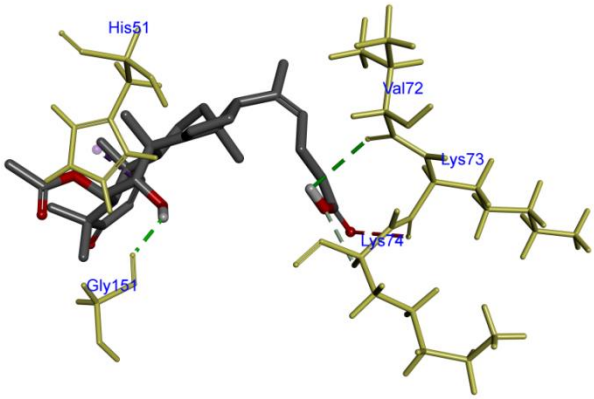
(G)


His51
Tyr161
Pro132
Tyr150
Leu128

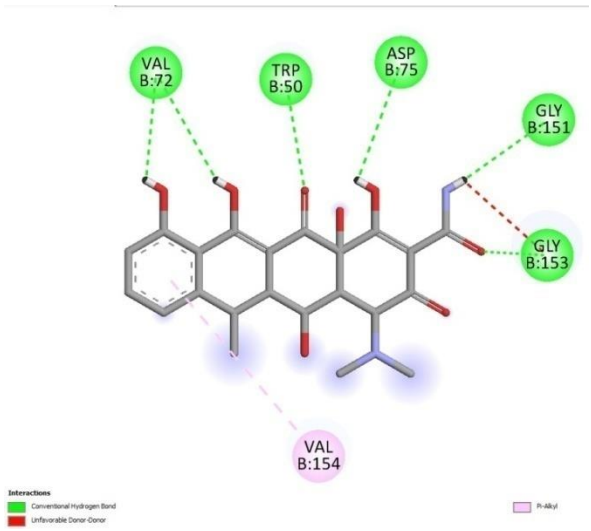
(H)


GLY
B:151
HIS
B:51
LYS
B:74
LYS
B:73
VAL
B:72
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Unfavorable Acceptor-Acceptor
Pi-Sigma

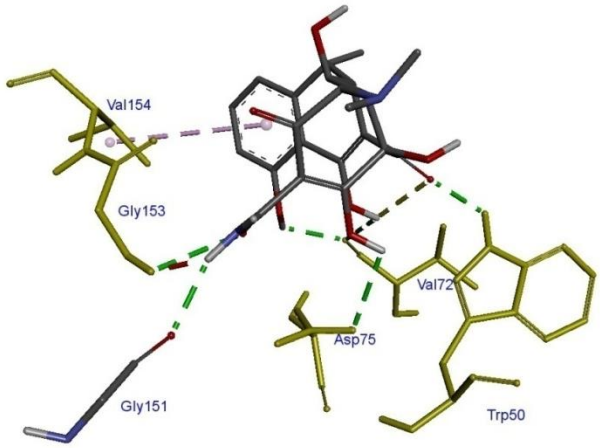
(I)


His51
Val72
Lys73
Lys74
Gly151

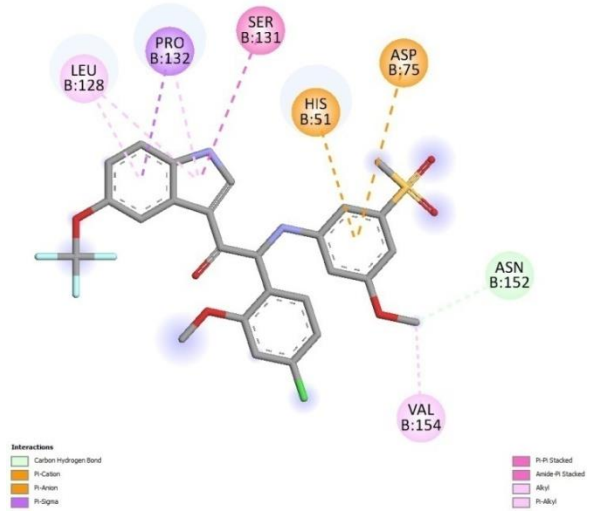
(J)



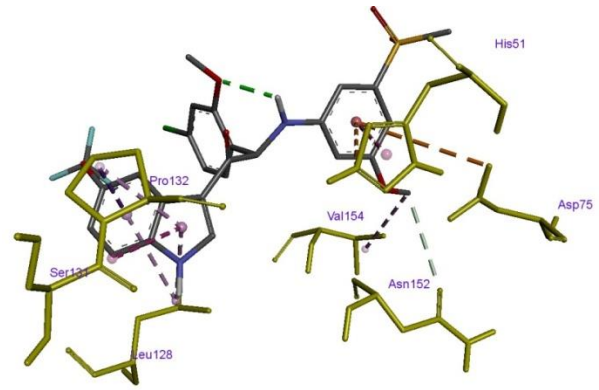
(K)



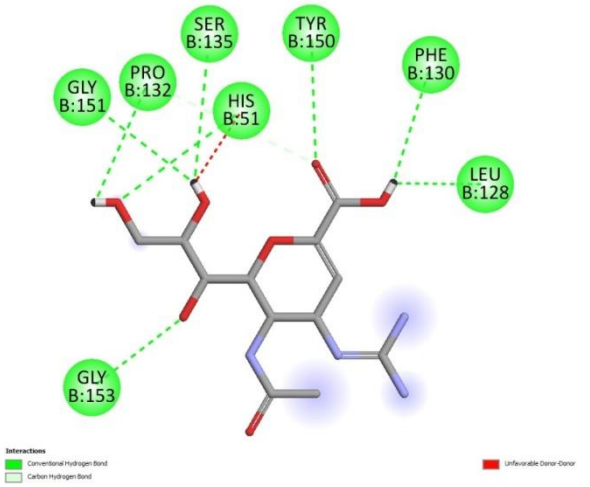
(L)



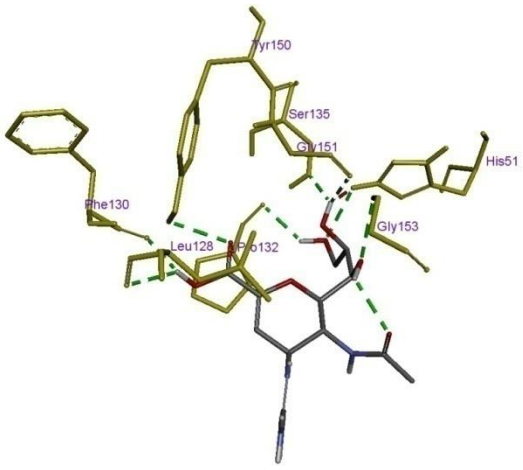
(M)



(N)



(O)



(P)

Figure 2: 2D and 3D images of Protein-Ligand interactions: (A) 2D image of protein-ligand91 interactions (B) 3D image of protein-ligand91 interactions (C) 2D image of protein-ligand13 interactions (D) 3D image of protein-ligand13 interactions (E) 2D image of protein-ligand100 interactions (F) 3D image of protein-ligand100 interactions (G) 2D image of protein-ligand12 interactions (H) 3D image of protein-ligand12 interactions (I) 2D image of protein-ligand20 interactions (J) 3D image of protein-ligand20 interactions (K) 2D image of protein-ligandRef.1 interactions (L) 3D image of protein-ligandRef.1 interactions (M) 2D image of protein-ligandRef.2 interactions (N) 3D image of protein-ligandRef.2 interactions (O) 2D image of protein-ligandRef.3 interactions (N) 3D image of protein-ligandRef.3 interactions.

The results of molecular docking analysis of the top binding ligands with the reference are shown in figure2. Result of the docking analysis of **Protein-ligand91 Interactions** shows 7 Conventional Hydrogen Bond with Amino acids – HIS 51, ILE36, PRO 132, PHE 130, TYR 150, TYR 161, GLY 15, Hydrogen Bond interaction with catalytic Traid –HIS 51 and One unfavourable donor interaction with - TRP 50 is observed. In **Protein-ligand13 interaction,** docking analysis shows 3 Cabon Hydrogen bond with PHE 130, GLY 151, GLY 153, Pi-Cation bond with the catalytic triad – HIS 51 and Pi-Anion bond with the catalytic triad –ASP 75. Additionally, one unfavourable interaction is observed. Docking analysis of **Protein-ligand100 interactions** shows 1 Conventional Hydrogen Bond with TYR 50, 1 Cabon Hydrogen bond with GLY 151. There is no interaction with the catalytic triad and no unfavourable interaction is observed. In case of **Protein-ligand12 interactions,** it shows 1Conventional Hydrogen Bond with TYR 150, 1 Cabon Hydrogen bond with LEU 128, Pi-Sigma interaction with catalytic triad- HIS 51 and No unfavourable interaction observed. **A protein-ligand20 interaction shows** 2 Conventional Hydrogen Bond with GLY 151 and VAL 72, 1 Cabon Hydrogen bond with LYS 74. There is no interaction with the catalytic triad, however, one unfavourable Acceptor-Acceptor interaction with LYS 73 is observed. Docking analysis of **Protein-ligand Ref.1 interactions shows** 5 Conventional Hydrogen Bond with VAL 72, TRP 50, ASP 75, GLY 151, GLY 153, Interaction with catalytic triad – ASP 75 and one unfavourable interaction is observed. In **Protein-ligandRef.2 interactions,** there is1 Cabon Hydrogen bond interaction with ASN 152, Pi- Cation bond with the catalytic triad – HIS 51 and Pi-Anion bond with the catalytic triad –ASP 75. The result shows no unfavourable interaction between the ligands and the drugs. In **Protein-ligandRef.3 interactions,** 8 Cabon Hydrogen bond interactions with GLY 151, PRO 132, SER 135, HIS 51, TYR 150, PHE 130, LEU 128, GLY 153, Interaction with Catalytic traid – HIS 51and SER 135 is seen. Also, one unfavourable interaction is observed.

4. CONCLUSION

The incidence of Dengue- mosquito-borne viral fever is increasing dramatically in recent years with repeated outbreaks from many States and newer areas around the world in recent decades. While many dengue infections are asymptomatic or produce only mild illness, the virus can occasionally cause more severe cases, and even death. To date, there is no universal vaccine available to treat or prevent dengue. However, current research efforts in the development of effective vaccines and therapeutics against dengue are emerging to develop antivirals against dengue.

The present work is based on the use of the phytocompounds extracted from 10 medicinal plants found in Nagaland to inhibit Dengue virus. A total of 133 Phytocompounds were extracted from the selected plants as an anti-dengue agent targeting 2FOM (NS2B/NS3 Protease) of DENV. Virtual screening of the phytocompound was carried out using SWISS ADME to estimate the drug likeness and medicinal potential to inhibit the target protein of DENV. Reference drugs (Doxycycline, Monosdenvir, Zanamivir) which were previously studied were considered to compare its binding affinity to a target. Molecular docking of the selected phytocompounds and reference drugs was performed using Pyrx. Based on scores, 5 top ligands were selected to investigate the most effective phytocompounds owing to their binding affinity and analyze their efficacy to inhibit the target. Compounds with stronger binding affinities and their mode of interactions between the compounds with highest affinities and their binding site indicate the potential inhibitors.

The study reveals that Ligand 12 (7-desacetyl-7-benzoylazadiradione) and Ligand 13 (7-desacetyl-7-benzoylgedunin) can be potential inhibitors for DENV (NS2B/NS3 Protease). These phytocompounds are found in *Azadirachta indica*[63,64,65] commonly known as Neem which can be considered as the most potential antiviral plant selected for these studies based on its physiochemical properties.

The future of in silico studies of phytoconstituents holds great potential for advancing our understanding of these complex molecules, bioactivity, toxicity, prediction of phytoconstituent interactions and mechanisms to increase efficiency in research and development thus, enhancing drug discovery. More anti-viral plants can be considered for comprehensive study of phytocompunds applying increased computational power to simulate complex molecular interactions and analyze large datasets, expansion of databases containing phytoconstituent information to facilitate data sharing and research and visualization of molecular interactions and simulations to enhance understanding. Combination of in silico studies with experimental techniques can also help to validate results and improve accuracy to design new drugs using phytoconstituents with optimized bioactivity and reduced toxicity.

The combined methods of *In-Silico* studies with other computational methods such as Molecular Dynamic (MD) simulation, can be considered for further research to optimized and give extensive and profound results to provide deeper insights of the anti-viral activity of these natural molecules which can serves as a lead to aid in designing the therapeutic drugs against Virus infections. Besides Molecular Dynamic (MD) Simulation, other computational methods such as Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) and Molecular Mechanics General Born Surface Area (MM/GBSA) can be employed to understand the thermodynamics of protein-ligand interactions to predict the binding affinity of small molecules, estimate the binding free energy and calculate the free energy of binding between ligands and proteins which are essential in drug design and discovery.

Conflict of Interest

The author declares no conflict of interest.

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