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IN SILICO STUDIES ON DENGUE AND NIPAH VIRAL PROTEINS WITH SELECTED *AZADIRACHTA INDICA* LEAVES CONSTITUENTS

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ABSTRACT

Dengue vrirus contains seven proteins and Nipah virus contains five proteins, which are considered to be most potent for drug designing. Recent studies have shown that these proteins can effectively cause the deactivation of dengue and nipah disease in humans. *Azadirachta indica* phytochemicals are found to have anti-bacterial and anticancer properties. In this study, the binding efficiency of five compounds that are present in the *Azadirachta indica* with all the twelve proteins through Insilico methods was carried out. By our virtual screening and molecular docking result, we found that the 3,7,11,15- tetramethyl-2-hexadecen-1-ol and 8,11,14- Eicosatrienoic acid have highest binding affinities with the proteins.

KEYWORDS: Neem, molecular docking, hydrogen bonding, chemsketch.

1. INTRODUCTION

Azadirachta indica, is one of two species in the genus *Azadirachta* commonly known as nimtree, neem, or Indian lilac has attracted worldwide prominence in recent years. It is a tree in the family *Meliaceae*. Owing to its wide range of medicinal properties it has been extensively used in Homoeopathic medicine, Unani and Ayurveda. Neem is a tree that grows fast in India and it can reach a height of up to 15–20 m (about 50–65 feet) tall, and sometimes even to 35–40 m (115–131 feet). More than 140 compounds have been isolated from different parts of *Azadirachta indica*. There leaves, seeds, roots, fruits, flowers and bark have been used traditionally for the treatment of different disease. The medicinal utilities of *Azadirachta indica* leaf and its constituents have been described especially to exhibit anti-inflammatory, antimalarial, immunomodulatory, antiviral, antihyperglycaemic, antifungal, antiulcer, antibacterial, antioxidant, anticarcinogenic and antimutagenic properties.[1] Neem is used in non-pesticidal management (NPM), providing a natural alternative to synthetic pesticides. *Azadirachta indica* also suppresses the hatching of pest insects from their eggs. Neem-based fertilizeres have been effective against the pest. Neem cake is often sold as a fertilizer.[2] Neem-coated urea is being used an alternate to plain urea fertilizer in India. It reduces pollution, improves fertilizer's efficacy and soil health.[3]

GC-MS chromatogram of the methanolic extract of *Azadirachta indica* showed five major peaks indicating the presence of five phytocomponents. From the results, it was observed that presence of 9, 12, 15-Octadecatrienoic acid (synonym: Linolenic acid; α-Linolenic acid), 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol (synonym: Phytol), 8,11, 14-Eicosatrienoic acid (Synonym: Homo-γ-linolenic acid), Tridecanoic acid (synonym:Tridecylic acid) and N-Hexadecanoicacid (synonym:Palmitic acid) were the major components in the extract. Linolenic acid shows antibacterial and antifungal properties, Phytol posses anti-diuretic, antioxidant and anticancer property. Homo-γ-linolenic acid has anti-inflammatory and anticoagulant property. Palmitic acid shows Hypercholesterolemic, Pesticide, antialopecic, antiandrogenic and antifibrinolytic properties.[4]

Dengue fever is caused by Dengue virus. It is a mosquito-borne single positive-stranded RNA virus of the family *Flaviviridae*; genus *Flavivirus*.[5,6] Four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4)[7] of the virus have been found, all of which can cause the full spectrum of disease.[8] Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are the severe forms.[9] Dengue is found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas.[10] There are ten proteins in this virus, out of which three are structural proteins and seven are nonstructural proteins.[11] The seven non structural proteins are NS1

protein, NS2B/NS3 protease, trans – membrane domain of NS2A, NS3 helicase, envelope protein, capsid protein, and NS5 protein. NS2B-NS3 protease is a crucial enzyme for the viral replication.[12] The N-terminal of the NS3 protein will associate with the NS2B cofactor that is important for the viral replication. This protein is hetero dimeric protein which consists of NS2B and NS3protein. The other non structural protein used for this study was the trans-membrane domain of theNS2A of dengue virus type 2. NS2A is a functionally active component of viral replication complex which is involved in the assembly of the virion and also it acts as an antagonist to the host immuneresponse.[13] NS3 helicase belongs to the non-structural and a multi-domain dengue virus replication protein.[14] The structural protein of dengue virus is the Envelope protein which is involved in the viral assembly. The envelope protein domain III of the dengue type 4 viruses is utilized for the study. It is classified under structural protein immune system.[15] The capsid protein used for this study was from dengue virus type 2 (strain Puerto Rico/PR159-S1/1969).[16] It is one of the structural proteins, which is involved in the encapsidation of the viral genome. The protein used for this study is the non-structural 5 (NS5) protein from the dengue virus type 3 (strain Sri Lanka / 1266 / 2000). This protein is classified under the transferases. The RNA – dependent – RNA - polymerase (RdRp) domain of the NS5 protein is a main protein which is involved in the viral genome replication. RNA is synthesized via "de novo" by NS5 protein.[17]

Nipah virus belongs to the family *Paramyxoviridae*, genus Henipavirus. Its name originated from Sungai Nipah. Malaysia (NiVM) and Bangladesh (NiVB) are the two distinct strains of NiV, Differences in transmission patterns and mortality rates suggest that NiVB may be more pathogenic than NiVM. Nipah virus genome is non-segmented, single-stranded negative-senseRNA.The genome is 18.2 kb in length. The genome contains six transcriptional units that encode the viral structural proteins nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), attachment (G), and the RNA-dependent RNA polymerase (L) protein as well as nonstructural proteins (V, W and C), in the order 3′-N-P/V/W/C-M-F-G-L-5′. The viral structural proteins all have essential roles throughout the viral life cycle. Of these, the viral core or nucleocapsid that consists of N, P, and L proteins contains all factors necessary for viral transcription and genome replication. The V and C proteins play key roles in NiV pathogenicity and. P, V and W proteins are involved in IFN-responses, with the W protein being the most efficient and the P protein the least. NiV V and W proteins can also inhibit virus-induced activation of the IRF3-responsive promoter and IFN-β promoter. And only W protein will show strong inhibition to the promoter activation in response to the Toll-like receptor 3 stimulation. NiV C protein was also suggested to have weak IFN antagonist activity, but the target is unclear.[18] NiV envelope proteins is of functional importance for viral pathogenesis either by regulating cytopathogenicity or by modulating recognition of infected cells by the immune system, nucleoprotein and polymerase L protein has a distinct function in replication. Glycoprotein has a critical role in attachment and fusion.[19,20]

Bioinformatics is an interdisciplinary branch of science which consists and uses statistics, computer and mathematics to analyze the biological data.[21] Bioinformatics is now utilized for many research aspects such as evolution. Protein Data Bank (PDB) is a protein storage bioinformatics tool. It contains the structures of large numbers of proteins, ligands and other macromolecules.[22] Docking analysis could be conducted for the protein and the ligand to analyze the fitness and the interaction with each other in the form of binding energy. This interaction could be used as the pharmaceutical approach for drug production.[23]

The aim of our study is to compare the best docking fit for the selected *Azadirachta indica* leaves constituents with the Dengue and Nipah viral proteins.

2. MATERIALS AND METHODOLOGIES

2.1. Preparation of viral proteins

The protein data bank (PDB) that contains large number of proteins which are experimentally determined and stored was used to obtain the three-dimensional structure of the macromolecule. PDB The structures are downloaded and saved either in mm CIF or PDB format. Proteins of dengue and nipah virus were used for this study. The 3D structure of all the twelve proteins were downloaded from PDB and saved in PDB format. Only those proteins are downloaded and docked which have PDB ID. The downloaded proteins were viewed in Py-Mol viewer.[24]

2.2. Preparation of ligands

Ligands selected were from the previous studies on GCMS analysis on *Azadirachta indica* leaves extract.[25] 5 ligands were used for the study. Ligands were constructed using ChemSketch.[26] The constructed ligands were optimized to add the hydrogen bonds and the obtained structures were saved in mol for docking analysis and named as A, B, C, D and E respectively.

2.3. Docking study

Docking studies were conducting using iGEMDOCK software. IGEMDOCK (Generic Evolutionary Method for molecular Docking) is a graphical-automatic drug design system for docking, screening and post-analysis. The proteins and the ligands were loaded and the out path was set. Standard docking parameters like (population size=200, Number of solutions =2 and generations =70) were used for docking. The docking process was initiated. After the docking process, the best docking pose can be obtained for all the seven individual ligands of dengue viral proteins. The binding affinities of the compounds the binding pose and the total binding energy values that are best were saved in the output folder. The saved files were visualized in Py-Mol viewer.[27]

3. RESULTS

3.1. Total Binding Energy (kcal/mol) profile for Dengue and Nipah viruses non structural proteins with 5 ligands.

Table 1: The Total Binding Energy (kcal/mol) profile for Dengue and Nipah viruses non structural proteins with 5 ligands.

Ligand	Compound name	Dengue Virus					Nipah virus
		NS1 protein	Transmembrane domain of NS2A	NS2B / NS3 protease	NS3 helicase	NS5 protein	NiV- W
A	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-	-90.76	-581.88	-88.35	-89.54	-102.159	-71.54
B	3,7,11,15- tetramethyl-2-hexadecen-1-ol	-83.75	-639.09	-80.85	-97.79	-88.2922	-75.67
C	8,11,14- Eicosatrienoic acid	-110.14	-595.87	-78.91	-107.66	-93.7538	-91.3
D	Tridecanoic acid	-81.17	-598.14	-64.37	-84.41	-78.5582	-69.48
E	N-Hexadeconoic acid	-92.03	-622.96	-82.85	-86.51	-101.62	-73.66

3.2. Total Binding Energy (kcal/mol) profile for Dengue and Nipah viruses structural proteins with 5 ligands

Table 2: The Total Binding Energy (kcal/mol) profile for Dengue and Nipah viruses structural proteins with 5 ligands.

Ligands	Compound name	Dengue Virus		Nipah virus			
		Capsid protein	Envelope Protein	Phosphoprotein	Nucleoprotein	Glycoprotein	Fusion protein
A	9,12,15- Octadecatrienoic acid,(Z,Z,Z)-	-84.19	-72.24	-90.76	-84.14	-77.93	-68.33
B	3,7,11,15- tetramethyl-2-hexadecen-1-ol	-83.23	-85.55	-84.8	-81.68	-77.55	-71.41
C	8,11,14- Eicosatrienoic acid	-101.08	-78.55	-96.08	-95.48	-89.04	-79.33
D	Tridecanoic acid	-74.89	-80.21	-69.93	-77.65	-87.34	-65.75
E	N-Hexadeconoic acid	-81.74	-75.62	-73.25	-83.15	-83.69	-72.26

3.3. H – Bond profile for Dengue and Nipah viruses non structural proteins with 5 ligands

Table 3: H – Bond profile for Dengue and Nipah viruses non structural proteins with 5 ligands.

Ligand	Compound name	Dengue Virus					Nipah virus
		NS1 protein	Trans membrane domain of NS2A	NS2B / NS3 protease	NS3 helicase	NS5 protein	NiV- C
A	9,12,15- Octadecatrienoic acid,(Z,Z,Z)-	H-S	_	H-M H-S	H-S	H-M H-S	H-S
B	3,7,11,15- tetramethyl-2-hexadecen-1-ol	H-M H-S	H-M H-S	H-M H-S	H-M	H-M	H-M
C	8,11,14- Eicosatrienoic acid	H-M H-S	H-M H-S	H-M H-S	H-M H-S	H-M	H-S
D	Tridecanoic acid	H-M H-S	H-M H-S	H-M	H-S	H-S H-M	H-M H-S
E	N-Hexadeconoic acid	H-M H-S	H-S	H-M	H-S	H-S H-M	H-M H-S

3.4. H – Bond profile for Dengue and Nipah viruses structural proteins with 5 ligands

Table 4: H – bond profile for Dengue and Nipah viruses structural proteins with 5 ligands.

Ligands	Compound name	Dengue Virus		Nipah virus			
		Capsid protein	Envelope Protein	Phosphoprotein	Nucleoprotein	Glycoprotein	Fusion protein
A	9,12,15- Octadecatrienoic acid,(Z,Z,Z)-	H-M H-S	H-M	H-S	H-M	H-M	H-M H-S
B	3,7,11,15- tetramethyl-2-hexadecen-1-ol	H-M H-S	H-M H-S	H-M	H-M	H-S	H-M H-S
C	8,11,14- Eicosatrienoic acid	H-M H-S	–	H-M H-S	H-M H-S	H-M H-S	H-M H-S
D	Tridecanoic acid	H-M H-S	H-M H-S	H-S	H-S	H-M	H-M H-S
E	N-Hexadeconoic acid	H-M H-S	H-S	H-S	H-M	H-S	_

3.5. Amino acid position profile for Dengue and Nipah viruses non structural protein with 5 ligands

Table 5: Amino acid position profile for Dengue and Nipah viruses non structural proteins with 5 ligands.

Ligand	Compound name	Dengue Virus					Nipah virus
		NS1 protein	Transmembrane domain of NS2A	NS2B / NS3 protease	NS3 helicase	NS5 protein	NiV- W
A	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-	Lys(206)	–	Gly(96) Lys(26)	Asn(369)	Thr(50)/ Thr(51) Thr(51)	Tyr(194)
B	3,7,11,15- tetramethyl-2-hexadecen-1-ol	Ile(242) Lys(206)	Gly(3) Asp(1)	THR(94) Asn(152)	Gly(198)/ Lys(199)/ Thr(200) Thr(200)	Ile(251)	Gln(241)
C	8,11,14- Eicosatrienoic acid	Ile(242) Ser(252)	Gly(5) Lys(16)	Ser(131) Asn(105)	Ile(203)/ Leu(204)/ Gly(462)/ Arg(463) Asp(470)	Ala(341)	Arg(229)
D	Tridecanoic acid	Ser(252) Asn(225)	Asp(1)	Thr(94)	Asp(409)	Arg(57) Arg(38)	Cys(122) Cys(121)
E	N-Hexadeconoic acid	Lys(206) Ile(242)	Asp(1)	Gly(96)	Arg(381)	His(800) His(512)/ Gln(802)	Lys(437)

3.6. Amino acid position profile for Dengue and Nipah viruses structural protein with 5 ligands

Table 6: Amino acid position profile for Dengue and Nipah viruses structural proteins with 5 ligands.

Ligands	Compound name	Dengue Virus		Nipah virus			
		Capsid protein	Envelope Protein	Phosphoprotein	Nucleoprotein	Glycoprotein	Fusion protein
A	9,12,15- Octadecatrienoic acid,(Z,Z,Z)-	Lys(76) Arg(55)	Gly(628)/ Arg(629)	Asp(492)	Ala(265)	Asn(187)	Tyr(473) Lys(471)
B	3,7,11,15- tetramethyl-2-hexadecen-1-ol	Thr(25) Glu(87)	Ile(618) Lys(625)	Ile(527)	Gln(171)	Asn(586)	Gln(468) Gln(469)
C	8,11,14- Eicosatrienoic acid	Asn(21) Arg(68)	-	Arg(495) Arg(532)/ His(535)	Lys(240)	Lys(386) Lys(386)	Gln(468)
D	Tridecanoic acid	Ala(77) Lys(45)	Ile(618) Lys(625)	Lys(476)	Arg(352)	Gly(227)	Gln(478) Tyr(473)
E	N-Hexadeconoic acid	Arg(22) Arg(68)	Arg(672)	Arg(532)	Ala(248) Val(249)	Lys(560)	–

4. DISCUSSION

Considering all the tables from Table – 1, to Table - 6, the 3D structure coordinates of seven non proteins of Dengue and five proteins of Nipah viruses are optimized and 5 compounds from *Azadirachta indica* leaves extract are identified. The total binding energy of the compounds with all the twelve proteins was calculated using iGEMDOCK. Evaluations of binding conformation of these 5 compounds with seven Dengue as well as five Nipah viral proteins are performed using iGEMDOCK. From docking study, we listed binding affinities of 5 compounds based on ligand binding energy (Table- 1 and Table - 2). The binding pose for each ligand molecule into the Dengue and Nipah viral proteins are analyzed and the one having lowest ligand binding energy with these proteins among the different poses are generated. The lower energy scores represent better protein-ligand target binding affinity compared to higher energy score. Considering the structural proteins of Dengue virus, among the 5 analogs, compound "B" is found to have lower ligand binding energy (binding energy value= -85.5 kcal/mol), than other analogs for Envelope protein. Compound "C" has least binding energy score with caspid protein (binding energy value= -101.08 kcal/mol), the structural proteins of Nipah virus had following binding energies, Nucleoprotein('C' binding energy value= -95.48kcal/mol), Glycoprotein('C', binding energy value= -89.04kcal/mol), Phosphoprotein('C', binding energy value=-96.08kcal/mol) and Fusion protein('C' binding energy value=-79.33kcal/mol).The non structural proteins of Dengue virus had these binding energy values: Trans membrane domain of NS2A ('B', binding energy value= -639.09kcal/mol), NS2B / NS3 protease ('A', binding energy value= -88.35kcal/mol), NS3 helicase ('C', binding energy value= -107.66kcal/mol), NS5 protein ('A', binding energy value= -102.159 kcal/mol) and NS1 protein ('C', binding energy value = -110.4kcal/mol). And the non structural proteins of Nipah viruses have,Niv W protein ('B', binding energy value= -75.6kcal/mol). We further analyzed the docked pose for finding the binding mode of compound "B" and compound "C" in to seven dengue and five Nipah viral proteins to validate the reasonable binding conformations.

4.1. Non-Structural proteins of Dengue Virus

4.1.1. The Total Binding Energy for Dengue virus NS1 protein with 5 ligands

From Table – 1, Table – 3 and Table – 5, the docking simulation of 5 ligands were performed for Dengue virus NS1 protein. From the docking study, we observed that compound-C has best binding affinity with the target NS1 protein with the binding energy value of -110.14kcal/mol. Interaction analysis of binding mode of compound –C in dengue virus NS1 protein reveals that it forms two hydrogen bonds with low energy, with Ile(242) and Ser(252). A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS1 protein with 5 ligands: is shown in Fig.1.

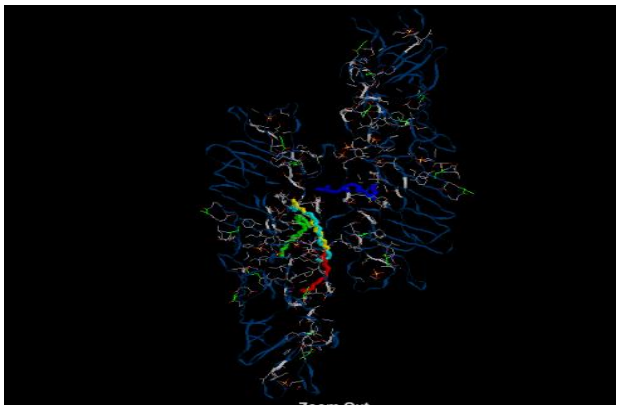

Fig. 1: The Total Binding Energy profile for Dengue virus NS1 protein with 5 ligands.

4.1.2. The Total Binding Energy for Dengue virus Trans membrane domain of NS2A with 5 ligands

From Table – 1, Table – 3 and Table – 5, the docking simulation of 5 ligands were performed for Dengue virus Trans membrane domain of NS2A.From the docking study, we observed that compound – B has best binding affinity with the target Trans membrane domain of NS2A with the binding energy value of -639.09kcal/mol. Interaction analysis of binding mode of compound –B in dengue virus NS1 protein reveals that it forms two hydrogen bonds with low energy, with Gly(3) and Asp(1). A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus Trans membrane domain of NS2A with 5 ligands: is shown in Fig.2.

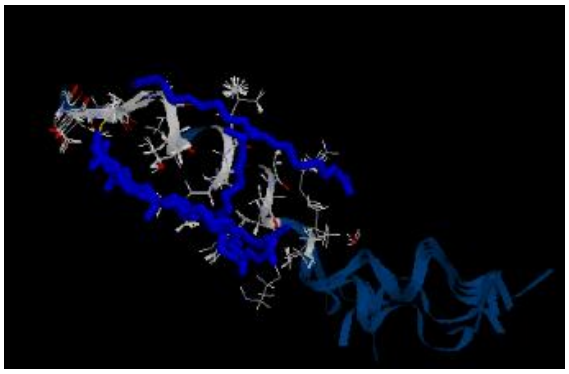

Fig. 2: The Total Binding Energy profile for Dengue virus Trans membrane domain of NS2A with 5 ligand.

4.1.3. The Total Binding Energy for Dengue virus NS2B / NS3 protease with 5 ligands

From Table – 1, Table – 3 and Table – 5, the docking simulation of 5 ligands were performed for Dengue virus NS2B / NS3 protease. From the docking study, we observed that compound – A has best binding affinity with the target NS2B / NS3 protease with the binding energy value of -88.35 kcal/mol. Interaction analysis of binding mode of compound –A in dengue virus NS2B / NS3 protease reveals that it forms two hydrogen bonds with low energy, with Gly(96) and Lys(26) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS2B / NS3 protease with 5 ligands: is shown in Fig.3.

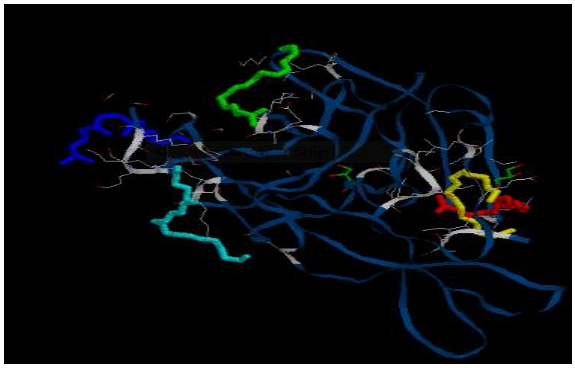

Fig. 3: The Total Binding Energy profile for Dengue virus NS2B / NS3 protease with 5 ligands.

4.1.4. The Total Binding Energy for Dengue virus NS3 helicase with 5 ligands

From Table – 1, Table – 3 and Table – 5, the docking simulation of 5 ligands were performed for Dengue virus NS3 helicase. From the docking study, we observed that compound – C has best binding affinity with the target NS3 helicase with the binding energy value of -107.66 kcal/mol. Interaction analysis of binding mode of compound –C in dengue virus NS3 helicase reveals that it forms two hydrogen bonds with low energy, with Ile(203), leu(204), Gly(462), Arg(463), Asp (470) residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS3 helicase with 5 ligands: is shown in Fig.4.

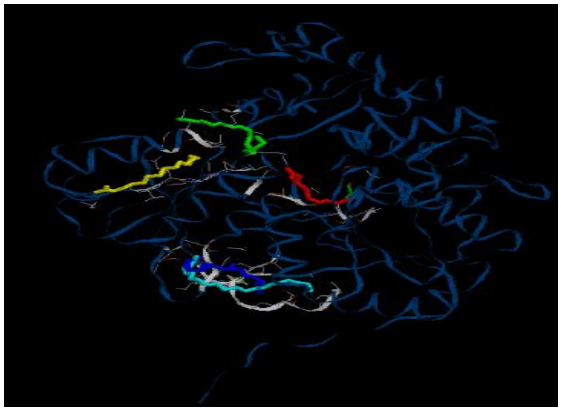

Fig. 4: The Total Binding Energy profile for Dengue virus NS3 helicase with 5 ligands.

4.1.5. The Total Binding Energy for Dengue virus NS5 protein with 5 ligands

From Table – 1, Table – 3 and Table – 5, the docking simulation of 5 ligands were performed for Dengue virus NS5 protein. From the docking study, we observed that compound – A has best binding affinity with the target NS5 protein with the binding energy value of -102.159 kcal/mol. Interaction analysis of binding mode of compound –A in dengue virus NS5 protein reveals that it forms two hydrogen bonds with low energy, with Thr(50), Thr(51), Thr(51) residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS5 protein with 5 ligands: is shown in Fig.5.

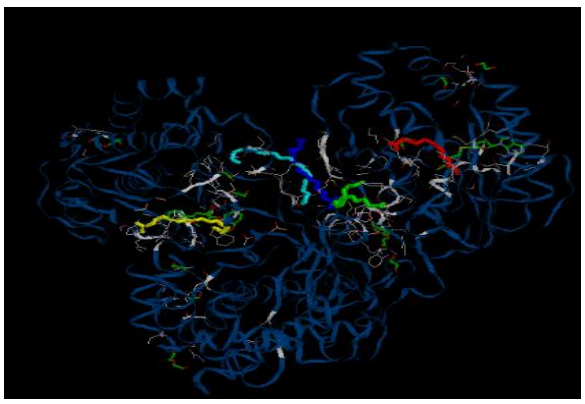

Fig. 5: The Total Binding Energy profile for Dengue virus NS5 protein with 5 ligands.

4.2. Non-Structural proteins of Nipah Virus

4.2.1. The Total Binding Energy for Nipah virus NiV-W protein with 5 ligands

From Table – 1, Table – 3 and Table – 5, the docking simulation of 5 ligands were performed for Nipah virus NiV-W protein. From the docking study, we observed that compounds – B has best binding affinity with the target protein NiV-W with the binding energy values of -75.67 kcal/mol. Interaction analysis of binding mode of compounds – B in Nipah virus protein NiV-W reveals that it forms one hydrogen bond with low energy, with Gln(241). A close-up view of the Total Binding Energy (kcal/mol) profile for Nipah virus NiV-W protein with 5 ligands: is shown in Fig.6.

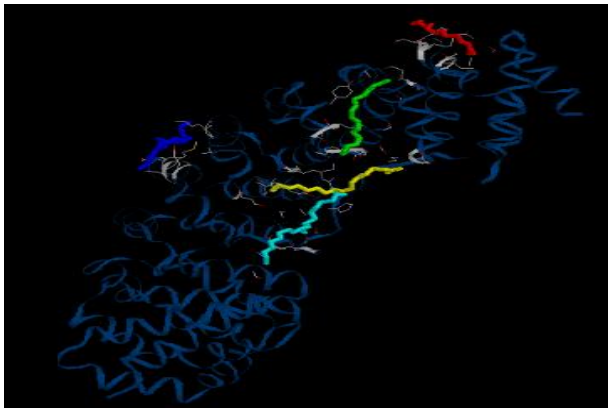

Fig. 6: The Total Binding Energy profile for Nipah virus NiV-W protein with 5 ligands.

4.3. Structural proteins of Dengue virus

4.3.1. The Total Binding Energy for Dengue virus Capsid protein with 5 ligands

From Table – 2, Table – 4 and Table – 6, the docking simulation of 5 ligands were performed for Dengue virus Capsid protein. From the docking study, we observed that compound – C has best binding affinity with the target Capsid protein with the binding energy value of -101.08 kcal/mol. Interaction analysis of binding mode of compound –C in dengue virus Capsid protein reveals that it forms two hydrogen bonds with low energy, with Asn(21) and Arg(68) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus Capsid protein with 5 ligands: is shown in Fig.7.

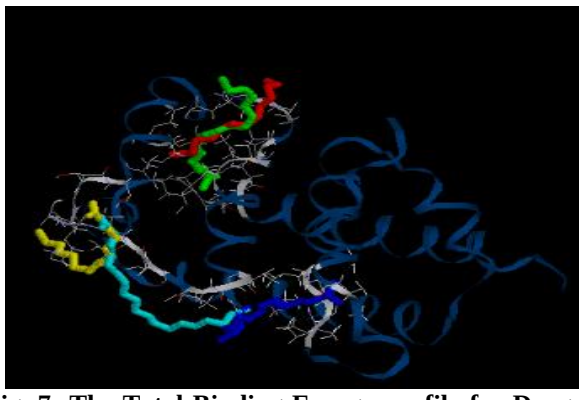

Fig. 7: The Total Binding Energy profile for Dengue virus Capsid protein with 5 ligands.

4.3.2. The Total Binding Energy for Dengue virus envelope protein with 5 ligands

From Table – 2, Table – 4 and Table – 6, the docking simulation of 5 ligands were performed for Dengue virus envelope protein. From the docking study, we observed that compound – B has best binding affinity with the target envelope protein with the binding energy value of -85.55 kcal/mol. Interaction analysis of binding mode of compound – B in dengue virus envelope protein reveals that it forms two hydrogen bond with low energy, with Ile(618) and Lys(625) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus envelope protein with 5 ligands: is shown in Fig.8.

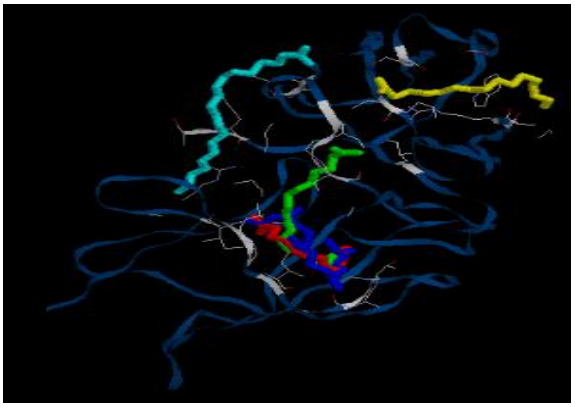

Fig. 8: The Total Binding Energy profile for Dengue virus envelope protein with 5 ligands.

4.4. Structural proteins of Nipah virus

4.4.1. The Total Binding Energy for Nipah virus Phosphoprotein with 5 ligands

From Table – 2, Table – 4 and Table – 6, the docking simulation of 5 ligands were performed for Nipah virus Phosphoprotein. From the docking study, we observed that compound – C has best binding affinity with the target Phosphoprotein with the binding energy value of -96.08 kcal/mol. Interaction analysis of binding mode of compound –C in dengue virus Phosphoprotein reveals that it forms two hydrogen bonds with low energy, withArg(495), Arg(532), His(535) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Nipah virus Phosphoprotein with 5 ligands: is shown in Fig.9.

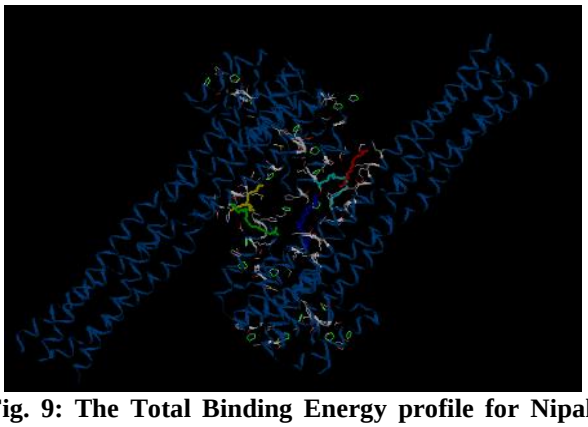

Fig. 9: The Total Binding Energy profile for Nipah virus Phosphoprotein with 5 ligands.

4.4.2. The Total Binding Energy for Nipah virus Nucleoprotein with 5 ligands

From Table – 2, Table – 4 and Table – 6, the docking simulation of 5 ligands were performed for Nipah virus Nucleoprotein. From the docking study, we observed that compound – C has best binding affinity with the target Nucleoprotein with the binding energy value of -95.48 kcal/mol. Interaction analysis of binding mode of compound –C in dengue virus Nucleoprotein reveals that it forms two hydrogen bonds with low energy, with Lys(240) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Nipah virus Nucleoprotein with 5 ligands: is shown in Fig.10.

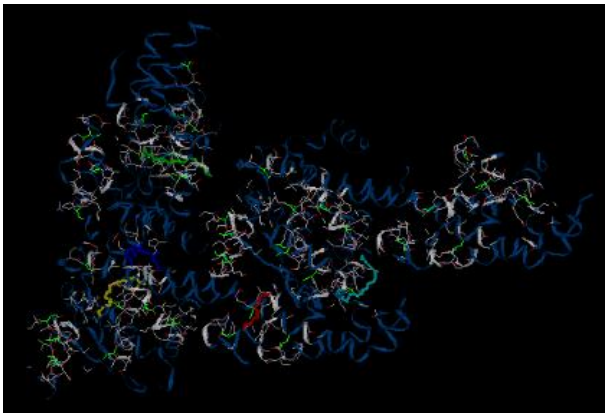

Fig. 10: The Total Binding Energy profile for Nipah virus with 5 ligands.

4.4.3. The Total Binding Energy for Nipah virus Glycoprotein with 5 ligands

From Table – 2, Table – 4 and Table – 6, the docking simulation of 5 ligands were performed for Nipah virus Glycoprotein. From the docking study, we observed that compound – C has best binding affinity with the target Glycoprotein with the binding energy value of -89.04 kcal/mol. Interaction analysis of binding mode of compound – C in dengue virus Glycoprotein protein reveals that it forms two hydrogen bond with low energy, with Lys(386) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Nipah virus Glycoprotein with 5 ligands: is shown in Fig.11.

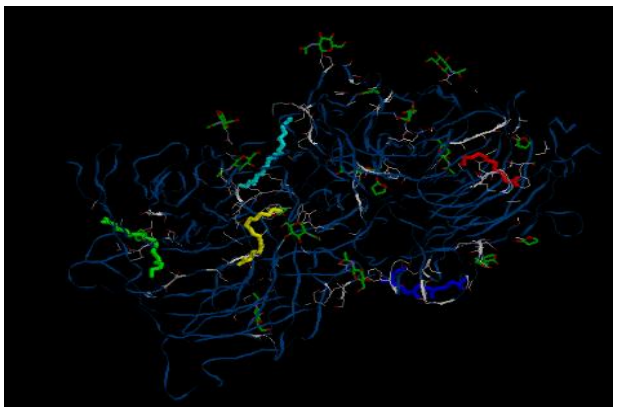

Fig. 11: The Total Binding Energy profile for Nipah virus Glycoprotein with 5 ligands.

4.4.4. The Total Binding Energy for Nipah virus Fusion protein with 5 ligands

From Table – 2, Table – 4 and Table – 6, the docking simulation of 5 ligands were performed for Nipah virus Fusion protein. From the docking study, we observed that compound –C has best binding affinity with the target Fusion protein with the binding energy value of -79.33 kcal/mol. Interaction analysis of binding mode of compound –C in Nipha virus Fusion protein reveals that it forms two hydrogen bonds with low energy, with Gln(468) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Nipah virus glycoprotein with 5 ligands: is shown in Fig.12.

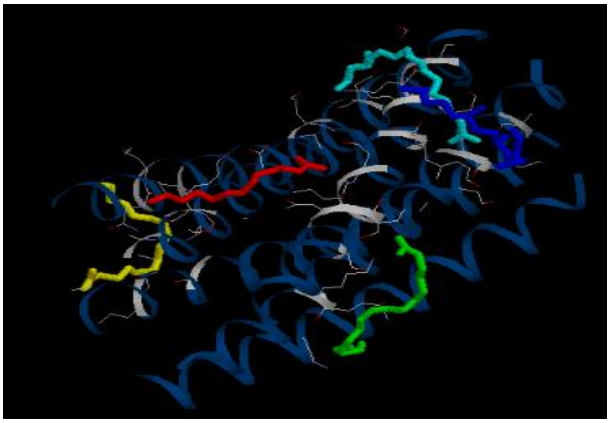

Fig. 12: The Total Binding Energy profile for Nipah virus Fusion protein with 5 ligands.

5. CONCLUSION

Our molecular docking studies explored the possible binding modes of 5 compounds that are present in *Azadirachta indica* leaf with seven proteins of Dengue virus and five proteins of Nipah virus. Dengue virus consists of envelope protein, NS1 protein, Transmembrane doamin of NS2A, NS2B/NS3 protease, NS3 helicase, NS5 protein and capsid protein; Nipah virus consists of Glycoprotein, Nucleoprotein, Phosphoprotein, Fusion protein and NiV-W. It revealed that all the 5 compounds show minimum affinity with all the proteins. The compound 'C' (8,11,14- Eicosatrienoic acid) and compound 'B' (3,7,11,15- tetramethyl-2-hexadecen-1-ol) shows best results compared to other compounds. On comparing the binding energy and the binding site residues, we found that all the compounds will differ in either of them for hydrogen bond formation. The conclusion which is drawn from our virtual screening and docking result are that the Compound C and B has highest binding affinity with the structural proteins of Dengue virus and compound C has the highest binding affinity with majority of the structural proteins of Nipah virus. Whereas the compound C is shown to have highest binding affinity with most of the non structural proteins of Dengue virus and the non structural proteins of Nipah virus has highest binding affinities with compound B and therefore it can be used as an effective drug target for Dengue virus as well as Nipah virus . Hence, the Compound C may be

considered as the effective drug target for both dengue and Nipah virus because it can effectively bind to most of the proteins of both the viruses. Though, there are many reports on the *in vitro* analysis of these compounds and its medicinal and toxic properties, there are no in silico studies that predict the binding and active regions especially with these proteins. Our study is an attempt to predict the binding site and the binding residues. However, validation of our results through *invivo* and *invitro* experiments and also with animal models will enlighten hope for the future development of more potent drugs for the treating Dengue and Nipah.

6. REFERENCES

1. Balasubramanian S, Ganesh D, Surya Narayana VVS. "GC-MS Analysis of Phytocomponents in the Methanolic Extract of *Azadirachta Indica* (Neem)". *Int J Pharm Bio Sci.*, 2014; 5(4): 258–262.
2. Sarangi KM. and Padhi S; "Dengue and its Phytotherapy A Review". *International Journal of Pharmaceutical and Phytopharmacological Research*, 2017; 4(1): 37 – 46.
3. B. D. Lindenbach, H. J. Thiel, C. M. Rice., *Flaviviridae*; the viruses and their replication, Fields Virology 2007; D. M. Knipe and P. M. Howley, Eds., 1101 – 1152.
4. Colpitts TM, Barthel S, Wang P, Fikrig E., Dengue Virus Capsid Protein Binds Core Histones and Inhibits Nucleosome Formation in Human Liver Cells, *PLoS ONE*, 2011; 6(9): e24365.
5. Rodenhuis Zybert, Izabela A, Wilschut, Jan, Smit, Jolanda M. "Dengue virus life cycle: viral and host factors modulating infectivity". *Cellular and Molecular Life Sciences*, 2010; 67(16): 2773–2786.
6. Powers CN, Setzer WN. "An In-Silico Investigation of Phytochemicals as Antiviral Agents against Dengue Fever". *Comb Chem High Throughput Screen*, 2016; 19(7): 516 – 536.
7. Mishra B, Sharma M, Pujhari SK, Ratho RK, Gopal DS, Kumar CN, Sarangi G, Chayani N, Varma SC; "Utility of Multiplex Reverse transcriptase - Polymerase Chain Reaction for Diagnosis and Serotypic Characterization of Dengue and Chikungunya Viruses in Clinical Samples". *Diagnostic microbiology and infectious disease*, 2011; 71(2): 118 – 125.
8. Saleh Hosseinzadeh, Azizollah Jafarikukhdan, Ahmadreza Hosseini, Raham Armand; "The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of Thymus vulgaris" *International Journal of Clinical Medicine*, 2015; 6: 635–642.
9. Prakash P, Gupta N. "Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review". *Indian J Physiol Pharmacol*, 2005; 49(2): 125–131.
10. Bhartiya US, Raut YS, Joseph LJ. "Protective effect of Ocimum sanctum L after high-dose 131iodine exposure in mice: an in vivo study". *Indian J Exp Biol*., 2006; 44(8): 647–652.
11. Parekh J, Chanda S; "Antibacterial and Phytochemical Studies on Twelve Species of Indian Medicinal Plants". *African Journal of Biomedical Research*, 2007; 10(2): 175–181.
12. Xie X, Gayen S, Kang C, Yuan Z, Shi PY; "Membrane Topology and Function of Dengue VirusNS2A Protein". *J. Virol*., 2013; 87: 4609 – 4622.
13. Perera R, Kuhn RJ; "Structural Proteomics of Dengue Virus". *Curr Opin Microbiol*, 2008; 11(4): 369 – 377.
14. Elahi M, Islam MM, Noguchi K, Yohda M, Toh H, Kuroda Y; "Computational Prediction and Experimental Characterization of a Size Switch Type Repacking during the Evolution of Dengue Envelope Protein Domain III (ED3)". *Biochem Biophys Acta*, 2014; 1844(3): 585–592.
15. Ma L, Jones CT, Groesch TD, Kuhn RJ Post CB; "Solution Structure of Dengue Virus Capsid Protein Reveals another Fold". *Proc. Natl. Acad. Sci*. USA, 2004; 101: 3414 – 3419.
16. Lim SP, Noble CG, Seh CC, Soh TS, El Sahili A, Chan GK, Lescar J, Arora R, BensonT, Nilar S, Manjunatha U, Wan KF, Dong H, Xie X, Shi PY, Yokokawa F. " Potent Allosteric Dengue Virus NS5 Polymerase Inhibitors: Mechanism of Action and Resistance Profiling". *PLoS Pathog*, 2016; 12(8): e1005737.
17. Park MS, Shaw ML, Munoz-Jordan J, Cros JF, Nakaya T. "Newcastle disease virus (NDV)-based assay demonstrates interferon-antagonist activity for the NDV V protein and the Nipah virus V, W, and C proteins". *J Virol*, 2003; 77: 1501–1511.
18. Shimojima M, Takada A, Ebihara H, Neumann G, Fujioka K, Irimurs T. "Tyro3 family-mediated cell entry of ebola and Marburg viruses" *J Virol*, 2006; 80(20): 10109 – 16.
19. Moller-Tank S, Maury W, Herbert A, Kuehne A, Wirchniaski A, Soh T. " Ebola viruses entry: A curious and complex series of events". *PLoS Pathalog*, 2015; 11(4): e1004731.
20. Mehmood MA, Sehar U, Ahmad N. "Use of Bioinformatic Tools in Different Spheres of Lifesciences". *Journal of Data Mining in Genomics &Proteomics*, 2014; 5(2): 1000158.
21. Berman HM, Westbrook J, Feng Z, Gilliland G,Bhat TN, Weissig H, Shindyalov IN, Bourne PE. "The Protein Data Bank". *Nucleic Acids Research*, 2000; 28(1): 235 – 242.
22. Ferreira LG, Ricardo N, Oliva G, Andricopulo AD. "Molecular Docking and Structure-Based Drug Design Strategies". *Molecules*, 2015; 20: 13384 – 13421.
23. Sushmitha H. S, Balasubramanian Sathyamurthy. "In Silico drug designing studies on Dengue Capsid Protein". *World Journal of Pharmaceutical and Life Sciences*, 2018; 4(9): 157–161.
24. Sushmitha H. S, Balasubramanian Sathyamurthy, "In Silico drug designing studies on Dengue Virus NS1 Protein". PharmaTutor, 2018; 6(10): 31–36.

25. Sushmitha H. S, Balasubramanian Sathyamurthy. "In Silico drug designing studies on Dengue Virus Envelope Protein". *World Journal of Pharmaceutical sciences*, 2018; 6(9): 138–143.
26. Anushree S, Archana S, Ashwini B M, Mahesh K, Murugan Rajadurai, Balasubramanian Sathyamurthy. "Docking Study of Selected *Calotropis Gigantea* Leaves Constituents on Dengue Viral Proteins – An *In Silico* Approach". *European Journal of Pharmaceutical and Medical Research*, 2018; 5(11): 641 – 647.
27. Smriti Chawla, Pavithra K., Rituparna Chatterjee, Balasubramanian Sathyamurthy. "Docking Study of Selected Red *Vitis Vinifera* Peel Constituents on Dengue Viral Proteins – An *In Silico* Approach". *Indo American Journal of Pharmaceutical Sciences*, 2018; 5(11): 11818 – 11826.