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IN SILICO STUDIES ON DENGUE AND CHIKUNGUNYA VIRAL PROTEINS WITH SELECTED *EMBLICA OFFICINALIS (AMLA)* LEAVES CONSTITUENTS

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ABSTRACT

Dengue and chikungunya virus contains seven proteins, which are considered to be the most effective for drug designing. Recent studies have shown that these proteins can effectively cause the inactivation of dengue and chikungunya disease in humans. Phytochemicals present in *Emblica offiinalis* are found to have anti-bacterial and anti-cancer properties. In our study, the binding efficiency of 4 compounds that is present in the *Emblica officinalis* with all the fourteen proteins was performed through Insilico methods. By our virtual screening and molecular docking result, we found that the Benzoic acid, 3,4,5-trihydroxy- have highest binding affinity with the proteins and we also predicted the binding site amino acid residues and the type of hydrogen bonding.

KEYWORDS: Amla, docking, hydrogen bonding, dengue virus, chikungunya virus.

INTRODUCTION

Medicinal plants are natural gift to human lives to promote disease from healthy life.[1] Abundant medicinal plants are presented in the Indian traditional systems of medicine like Ayurveda, Unani, Siddha etc.[2] These plants plays an important role in medicinal systems which provides a rich resource for research and development of natural drug.[1] Among all the plants *Phyllanthus emblica Linn or Emblica officinalis* commonly known as Amla.[4] It is widely distributed in tropical and sub-tropical areas and has therapeutic potential against deleterious disease. Thus, the word Amla is derived from the Sanskrit word Amalaki which means the "The Sustainer or Prosperity".[1] These fruit has been used in traditional medicine for generations to treat symptoms ranging from constipation to the treatment of tumors. Amla is widely used in the Indian system of medicine as diuretic, laxative, liver tonic, stomachic, restorative and anti-pyretic.[2] Phytochemicals studies on amla discovered major chemical constituents including tannis, alkaloids, polyphenols, vitamins and minerals, gallic acid, ellagic acid, embilcanin A and B. Apart from that amla shows some anti-aging, expectorant, puragative, antibacterial, anti-oxidant, anti-cancer and hypoglycemic properties.[3]

GC-MS chromatogram of the methanolic extract of Emblica officinalis showed four major peaks, 1,2,3 – benzenetriol (synonym: Pyrogallol), 2-Furancarboxyaldehyde, 5-(hydroxymethyl) (synonym: 5-hydroxymethylfurfural) and 2-Acetyl-5-methylfuran (Synonym: 5-methyl-2-furylmethylketone) and Benzoic acid, 3,4,5-trihydroxy-(synonym: Gallic-acid) were the major components in the extract. Pyrogallol is a polyphenol is known for its fungicidl and fungi static properties. In addition it has also shown antitumor, antiviral, antibaerial, cardioprotetive, pro oxidant and anti-mutagenic activities. The gallic acid has a wide spectrum of biological activities like antimicrobial, anticancer, antiviral, anti-inflammatory, analgesic and anti-HIV activities.[5]

Dengue is an arboviral disease. These are transmitted by the bite of the Ades aegypti mosquito infected with one of the four dengue virus (DENV-1, DENV-2, DENV-3 and DENV-4) serotypes.[6] Its spectrum ranges from asymptomatic infection to dengue hemorrhagic fever and dengue shock syndrome.[7] Its genome comprises a single-strand of positive-sense RNA encoding three structural and seven non-structural proteins.[8] The seven non-structural proteins are capsid protein, envelope protein, NS1 protein, transmembrane domain of NS2A, NS2B/NS3 protease, NS3 helicase and NS5 protein. NS2B-NS3 protease is a crucial enzyme for the viral replication.[9] NS2B/ NS3 protease has an important role in the viral life cycle.[10] Envelope protein is a structural protein which is involved in the viral assembly. The capsid protein is one of the structural proteins, which is involved in the encapsidation of the viral genome. The capsid protein used for this study was from dengue virus type 2 (strain Puerto Rico/PR159-S1/1969).[11] NS2A is a non-structural protein and it is a component of viral

replication complex which is functionally active in the assembly of the virion and also it acts as an antagonist to the host immune response.[12] NS3 helicase belongs to the non-structural and a multi-domain dengue virus replication protein.[13] 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 involved in the replication of the viral genome. RNA is synthesized via "de novo" by NS5 protein.[14]

Chikungunya virus (CHIKV) is an acute febrile illness caused by an arthropod-borne alpha virus that belongs to the Togaviridae family.[15] It is transmitted by the bites of infected female Aedes mosquitoes, mainly Ae. Aegypti and Ae. Alboictus. These are the main vectors of CHIKV, and both are highly invasive species and closely associated with the human peridomestic environment.[16] Its genome structure includes two open reading frames (ORFs) that encodes for two polyproteins (non-structural polyprotein and structural polyprotein),which can be cleaved respectively into four non-structural proteins (nsP1,nsP2,nsP3,nsP4) which are required for virus replication and five structural proteins (C, E3,E2,6K,E1) by viral and cellular proteases.[17] The structural proteins are synthesized as a long polyprotein, which is the post-translationally cleaved into C, E1, 6K and P62. A total of 240 copies of the C protein associate with a newly synthesized genomic RNA to form a nucleocapsid in the host cell's cytoplasm.[18] Where these Nucleocapsid forms a fusion loop on glycoprotein E1 to produce an infectious virus.

Bioinformatics is an interdisciplinary branch of science which utilizes statistics, computer and mathematics to analyse biological data.[19] Recent technological development of large-scale gene expression analysis using DNA microarrays and proteomics experiments has further boosed the importance of bioinformatics methods. Bioinfomatics is the use of information technology in biotechnology for the data storage, data warehousing and analysing the DNA sequences and became an indispensable part of the biological and clinical research of this century.[20] Thus, this is due to its role in the development of computers able to determine the peptide sequence, programs to recognize and display structures for use in X-ray crystallography and computational methods for protein sequence comparison, allowing us to infer the evolutionary connections among kingdom.[21] Docking analysis can be conducted for the protein and the ligand to analyse the fitness and the interaction with each other in the form of energy. This interaction could be used as the pharmaceutical approach for drug production.[22]

The aim of our study is to compare the best docking fit for the selected *Phyllanthus emblica Linn or Emblica officinlis* (Amla) leaves constituents with the Dengue and Chikungunya viral proteins.

1. MATERIALS AND METHODOLOGIES

1.1. Preparation of Dengue and Chikungunya viral proteins

The protein data bank (PDB) was used to obtain the three-dimensional structure of the macromolecule. PDB contains large number of proteins which are experimentally determined and stored in this site. The structures are downloaded and saved either in mm CIF or PDB format. Proteins of dengue and chikungunya virus were used for this study. The 3D structure of all the fourteen proteins were downloaded from PDB and saved in PDB format. The downloaded proteins were viewed in Py-Mol viewer.[23]

1.2. Preparation of ligands

Ligands selected were from the previous studies on GCMS analysis on *Emblica officinalis* leaves extract.[5] 4 ligands were used for the study. Ligands were constructed using ChemSketch. 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 and C respectively.

1.3. 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.[24] The proteins and the ligands were loaded and the out path was set. Standard docking parameters were used for docking (population size=200, generations =70 and Number of solutions =2). The docking process was initiated. After the docking process, the best docking pose for the individual ligands can be obtained for all the seven dengue viral proteins. The best binding pose, the binding affinity and the total binding energy values were saved in the output folder. The saved files were visualized in Py-Mol viewer.[25]

2. RESULTS

2.1. Total Binding Energy (kcal/mol) profile for Dengue and Chikungunya viruses proteins with 4 ligands

Table 1: The Total Binding Energy (kcal/mol) profile for Dengue and Chikungunya viruses non-structural proteins with 4 ligands.

Ligand	Compound name	Dengue Virus					Chikungunya Virus	
		NS1 protein	Trans membrane domain of NS2A	NS2B / NS3 protease	NS3 helicase	NS5 protein	NSP2	NSP3
A	1,2,3 –benzenetriol	-72.59	-372.21	-59.33	-68.75	-65.2	-63.56	-69.39
B	2-Furancarboxaldehyde,5-(hydroxymethyl)	-69.48	-466.73	-61.01	-66.7	-68.97	-73.03	-71.65
C	2-Acetyl-5-methylfuran	-63.14	-379.29	-55.32	-62.42	-59.72	-64.93	-76.48
D	Benzoic acid,3,4,5-trihydroxy-	-84.1	-476.18	-64.94	-87.89	-80.2	-86.11	-84.21

Table 2: The Total Binding Energy (kcal/mol) profile for Dengue and Chikungunya viruses structural proteins with 4 ligands.

Ligand	Compound name	Dengue Virus		Chikungunya Virus			
		Capsid protein	Envelope protein	Capsid protein	Envelop protein E1	Envelop protein E2	Envelop protein E3
A	1,2,3 –benzenetriol	-60.24	-63.94	-62.17	-72.94	-63.63	-59.17
B	2-Furancarboxaldehyde,5-(hydroxymethyl)	-68.86	-70.03	-68.74	-61.49	-74.27	-64.29
C	2-Acetyl-5-methylfuran	-63.75	-60.78	-61.22	-64.08	-66.61	-60.87
D	Benzoic acid,3,4,5-trihydroxy-	-79.45	-69.3	-82.31	-90.54	-77.21	-77.8

2.2. H – Bond profile for Dengue and Chikungunya viruses protein with 4 ligands

Table 3: H – Bond profile for Dengue and Chikungunya viruses non structural proteins with 4 ligands.

Ligand	Compound name	Dengue Virus					Chikungunya Virus	
		NS1 protein	Trans membrane domain of NS2A	NS2B / NS3 protease	NS3 helicase	NS5 protein	NSP2	NSP3
A	1,2,3 –benzenetriol	H-M	H-M	H-S	H-M	H-S	H-S	H-M
B	2-Furancarboxaldehyde,5-(hydroxymethyl)	H-S	H-M	H-S	H-M	H-S	H-M	H-M
C	2-Acetyl-5-methylfuran	H-M	H-M	H-S	H-M	H-S	H-S	H-M
D	Benzoic acid,3,4,5-trihydroxy-	H-M	H-S	H-M	H-S	H-S	H-S	H-M

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

Ligand	Compound name	Dengue Virus		Chikungunya Virus			
		Capsid protein	Envelope protein	Capsid protein	Envelope protein E1	Envelope protein E2	Envelope protein E3
A	1,2,3 –benzenetriol	H-S	H-M	H-S	H-M	H-S	H-M
B	2-Furancarboxaldehyde,5-(hydroxymethyl)	H-S	HM	H-S	H-M	H-M	H-S
C	2-Acetyl-5-methylfuran	H-M	H-M	H-M	H-M	H-M	H-S
D	Benzoic acid,3,4,5-trihydroxy-	H-S	H-M	H-M	H-S	H-S	H-S

2.3. Amino acid position profile for Dengue and Chikungunya viruses protein with 3 ligands

Table 5: Amino acid position profile for Dengue and Chikungunya viruses non structural proteins with 4 ligands.

Ligand	Compound name	Dengue Virus					Chikungunya Virus	
		NS1 protein	Trans membrane domain of NS2A	NS2B / NS3 protease	NS3 helicase	NS5 protein	NSP2	NSP3
A	1,2,3 –benzenetriol	Ile(242)	Leu(11)	Arg(55) Asp(58)	Asn(329)	His(53)	His(1151) Gln(1232)	Ser(112)
B	2-Furancarboxaldehyde,5-(hydroxymethyl)	Arg(294)	Gly(5) Met(4)	Asn(152)	Phe(417)	Asp(808)	Asn(1317)	Ala(25)
C	2-Acetyl-5-methylfuran	Ser(185)	Leu(11)	Asn(152)	Lys(618)	Lys(578)	Asn(1054)	Ser(112) Gly(114) Tyr(116)
D	Benzoic acid,3,4,5-trihydroxy-	Glu(205)	Arg(18)	Leu(149)	Asn(329)	Ser(776)	Glu(1128)	Gly(114)

Table 6: Amino acid position profile for Dengue and Chikungunya viruses structural proteins with 4 ligands.

Ligand	Compound name	Dengue Virus		Chikungunya Virus			
		Capsid protein	Envelope protein	Capsid protein	Envelope protein E1	Envelope protein E2	Envelope protein E3
A	1,2,3 –benzenetriol	Arg(41)	Ile(618) Lys(625) Gly(628) Arg(629) Ile(630)	Lys(252)	Gly(227)	Asn(39)	Asp(132)
B	2-Furancarboxaldehyde,5-(hydroxymethyl)	Phe(47)	Ile(618)	Trp(245)	His(82)	Leu(326)	His(331)
C	2-Acetyl-5-methylfuran	Arg(22)	Arg(629)	Asn(111)	Tyr(85) Gly(227)	Gly(227)	Arg(223)
D	Benzoic acid,3,4,5-trihydroxy-	Arg(41)	Gly(628)	Asn(111)	His(195)	Gly(260)	Glu(308)

3. DISCUSSION

Considering all the tables from Table – 1, to Table - 6, the 3D structure coordinates of seven non proteins of dengue and six proteins of chikungunya viruses are optimized and 4 compounds from *Emblica offiinalis* leaves extract are identified. The total binding energy of the compounds with all the thirteen proteins was calculated using iGEMDOCK. Evaluations of binding conformation of these 4 compounds with seven dengue as well as chikungunya viral proteins are performed using iGEMDOCK. From docking study, we listed binding affinities of 4 compounds based on ligand binding energy (Table- 1 and Table - 2). The binding pose for each ligand molecule into the dengue and chikungunya 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 4 analogs, compound "A" is found to have lower ligand binding energy (binding energy value= -60.24 kcal/mol), than other analogs for Envelope protein. Compound "A" has least binding energy score with capsid protein (binding energy value= -60.78 kcal/mol), the structural proteins of chikungunya virus had following binding energies, Capsid protein ('D' binding energy value= -82.31), Envelope protein E1 ('D' binding energy value= -90.54), Envelope protein E2 ('D', binding energy value= -77.21), Envelope protein E3 ('D', binding energy value= -77.8) The non structural proteins of Dengue virus had these binding energy values: Trans membrane domain of NS2A ('D', binding energy value= -476.18kcal/mol), NS2B / NS3 protease ('D', binding energy value= -64.94kcal/mol), NS3 helicase ('D', binding energy value= -87.89kcal/mol), NS5 protein ('D', binding energy value= -80.2 kcal/mol) and NS1 protein ('D', binding energy value = -84.1kcal/mol). And the non-structural proteins of chikungunya viruses have, NSP2 ('D', binding energy value= -86.11), NSP3 ('D', binding energy value= -84.21). We found that the compound "D" was found to have the best binding affinity with five dengue with two non-structural chikungunya viral proteins and two dengue with four structural chikungunya viral proteins.

3.1. Non-Structural proteins of Dengue Virus

3.1.1. The Total Binding Energy for Dengue virus NS1 protein with 3 ligands

From Table – 1, Table – 3 and Table – 5, the docking simulation of 4 ligands were performed for Dengue virus NS1 protein. From the docking study, we observed that compound – D has best binding affinity with the target NS1 protein with the binding energy value of -84.1kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus NS1 protein reveals that it forms one hydrogen bond with low energy, with Ile (242) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS1 protein with 4 ligands: is shown in Fig.1.

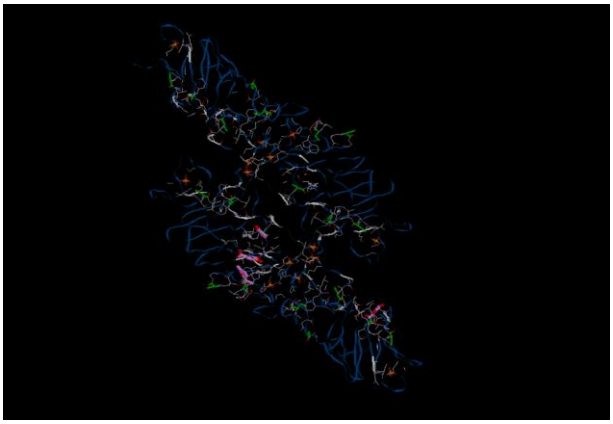

Fig. 1: The Total Binding Energy profile for Dengue virus NS1 protein with 4 ligands.

3.1.2. The Total Binding Energy for Dengue virus Trans membrane domain of NS2A with 4 ligands

From Table – 1, Table – 3 and Table – 5, the docking simulation of 4 ligands were performed for Dengue virus Trans membrane domain of NS2A. From the docking study, we observed that compound – D has best binding affinity with the target Trans membrane domain of NS2A with the binding energy value of -476.18 kcal/mol. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus Trans membrane domain of NS2A with 4 ligands: is shown in Fig.2.

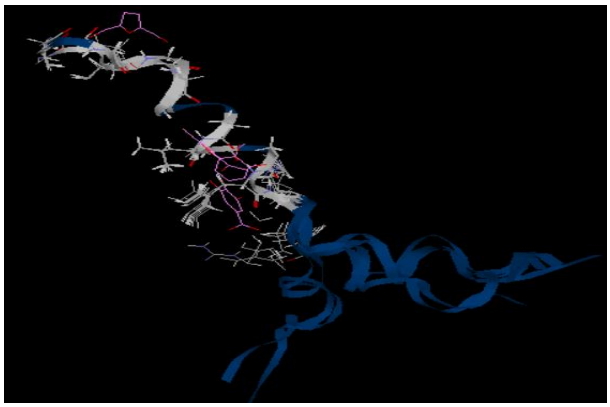

Fig. 2: The Total Binding Energy profile for Dengue virus Trans membrane domain of NS2A with 4 ligands.

3.1.3. The Total Binding Energy for Dengue virus NS2B / NS3 protease with 4 ligands

From Table – 1, Table – 3 and Table – 5, the docking simulation of 4 ligands were performed for Dengue virus NS2B / NS3 protease. From the docking study, we observed that compound – D has best binding affinity with the target NS2B / NS3 protease with the binding energy value of -64.94 kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus NS2B / NS3 protease reveals that it forms one hydrogen bond with low energy, with Leu (149) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS2B / NS3 protease with 4 ligands: is shown in Fig.3.

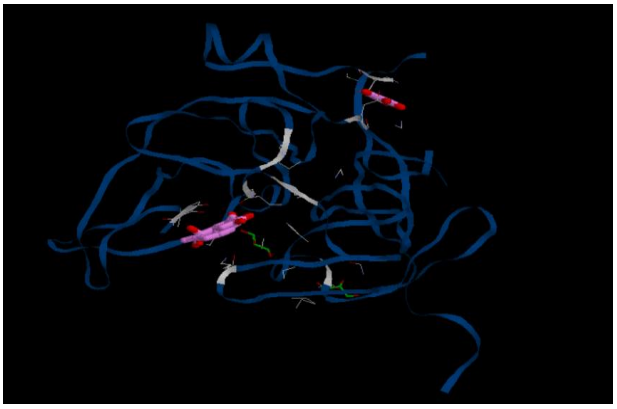

Fig. 3: The Total Binding Energy profile for Dengue virus NS2B / NS3 protease with 4 ligands.

3.1.4. The Total Binding Energy for Dengue virus NS3 helicase with 4 ligands:

From Table – 1, Table – 3 and Table – 5, the docking simulation of 4 ligands were performed for Dengue virus NS3 helicase. From the docking study, we observed that compound – D has best binding affinity with the target NS3 helicase with the binding energy value of -87.89 kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus NS3 helicase reveals that it forms one hydrogen bonds with low energy, with Asn (329) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS3 helicase with 4 ligands: is shown in Fig.4.

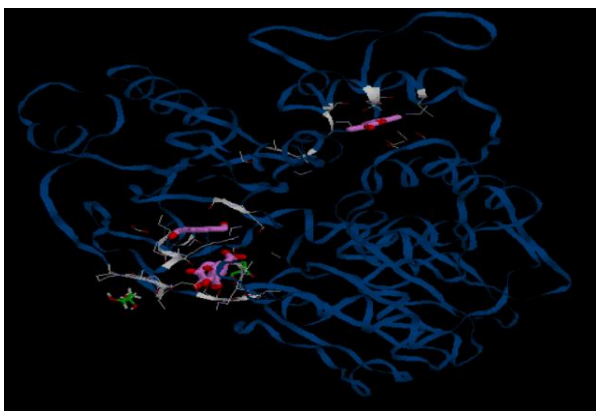

Fig. 4: The Total Binding Energy profile for Dengue virus NS3 helicase with 4 ligands.

3.1.5. The Total Binding Energy for Dengue virus NS5 protein with 4 ligands

From Table – 1, Table – 3 and Table – 5, the docking simulation of 4 ligands were performed for Dengue virus NS5 protein From the docking study, we observed that compound – D has best binding affinity with the target NS5 protein with the binding energy value of -80.2kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus NS5 protein reveals that it forms one hydrogen bonds with low energy, with serine(776). A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS5 protein with 4 ligands: is shown in Fig.

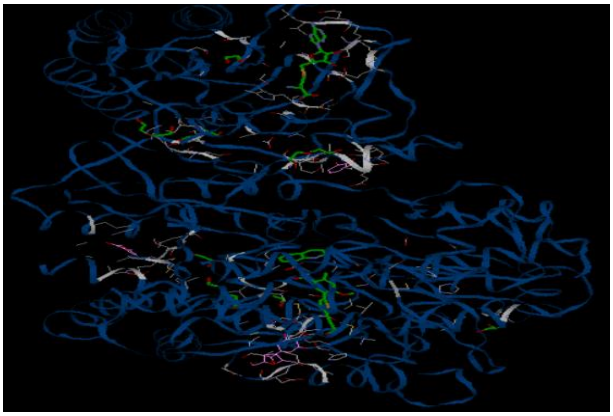

Fig. 5: The Total Binding Energy profile for Dengue virus NS5 protein with 4 ligands.

3.2. Non-Structural proteins of Chikungunya Virus

3.2.1. The Total Binding Energy for Chikungunya virus NSP2 with 4 ligands

From Table – 1, Table – 3 and Table – 5, the docking simulation of 4 ligands were performed for chikungunya virus NSP2 protein. From the docking study, we observed that compounds – D as best binding affinity with the target NSP2 protein with the binding energy values of -86.11 kcal/mol. Interaction analysis of binding mode of compounds –D in dengue virus NSP2 protein reveals that it forms one hydrogen bond with low energy, with Glu(1128). A close-up view of the Total Binding Energy (kcal/mol) profile for chikungunya virus NSP2 protein with 4 ligands: is shown in Fig.6.

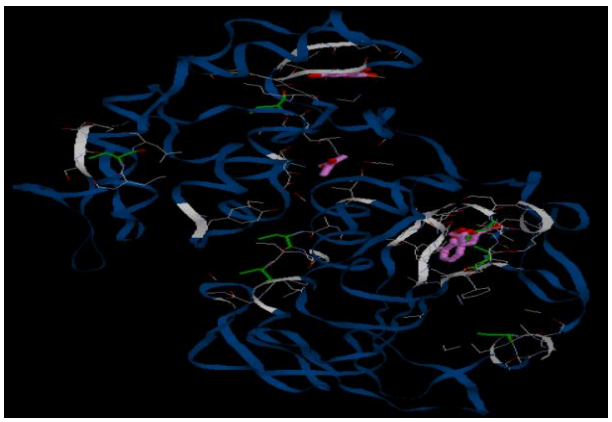

Fig. 6: The Total Binding Energy profile for Chikungunya virus NSP2 protein with 4 ligands.

3.2.2. The Total Binding Energy for Chikungunya virus NSP3 protein with 4 ligands

From Table – 1, Table – 3 and Table – 5, the docking simulation of 4 ligands were performed for chikungunya virus NSP3 protein. From the docking study, we observed that compound – D has best binding affinity with the target NSP3 protein with the binding energy value of -84.21kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus NSP3 protein reveals that it forms one hydrogen bond with low energy, with Gly(114) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for chikungunya virus NSP3 protein with 4 ligands: is shown in Fig.7.

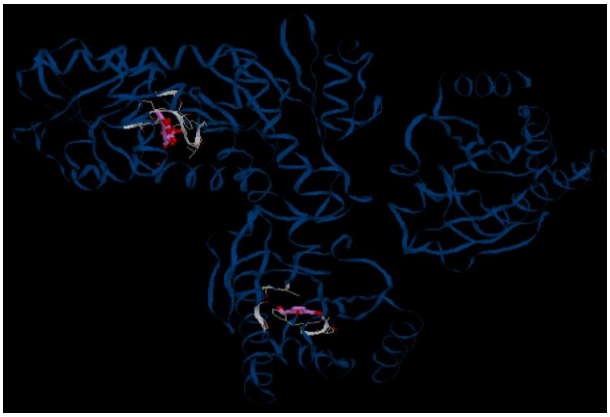

Fig. 7: The Total Binding Energy profile for Chikungunya virus NSP3 protein with 4 ligands.

3.3. Structural proteins of Dengue virus

3.3.1. The Total Binding Energy for Dengue virus Capsid protein with 4 ligands

From Table – 2, Table – 4 and Table – 6, the docking simulation of 4 ligands were performed for Dengue virus Capsid protein. From the docking study, we observed that compound – D has best binding affinity with the target Capsid protein with the binding energy value of -79.45 kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus Capsid protein reveals that it forms one hydrogen bond with low energy, with Arg(41) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus Capsid protein with 4 ligands: is shown in Fig.9.

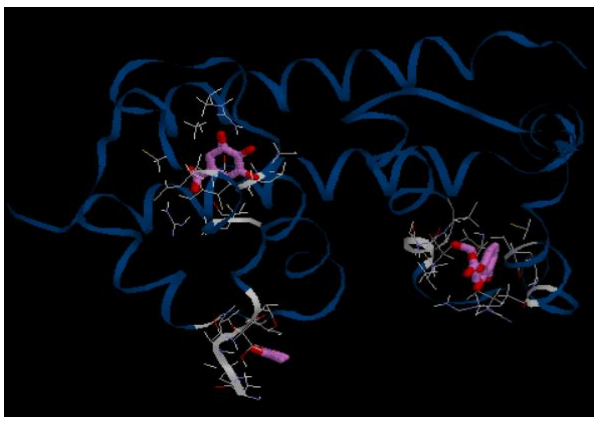

Fig. 9: The Total Binding Energy profile for Dengue virus Capsid protein with 4 ligands.

3.3.2. The Total Binding Energy for Dengue virus envelope protein with 4 ligands

From Table – 2, Table – 4 and Table – 6, the docking simulation of 3 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 -70.03 kcal/mol. Interaction analysis of binding mode of compound –B in dengue virus envelope protein reveals that it forms one hydrogen bond with low energy, with Ile(618) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus envelope protein with 4 ligands: is shown in Fig.10.

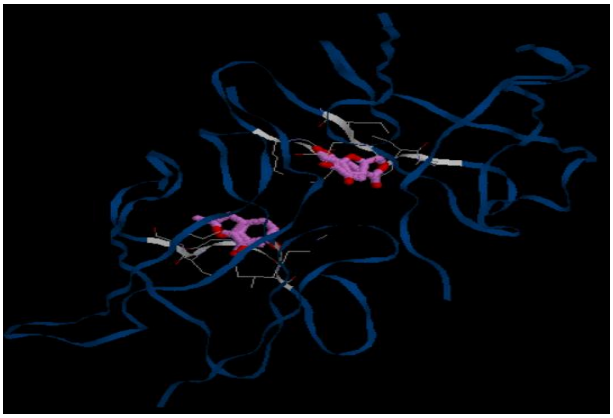

Fig. 10: The Total Binding Energy profile for Dengue virus envelope protein with 4 ligands.

3.4. Structural proteins of Chikungunya virus

3.4.1. The Total Binding Energy for Chikungunya virus Capsid protein with 4 ligands

From Table – 2, Table – 4 and Table – 6, the docking simulation of 4 ligands were performed for chikungunya virus Capsid protein. From the docking study, we observed that compound – D has best binding affinity with the target Capsid protein with the binding energy value of -82.31 kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus Capsid protein reveals that it forms one hydrogen bond with low energy, with Asn(111) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for chikungunya virus Capsid protein with 4 ligands: is shown in Fig.11.

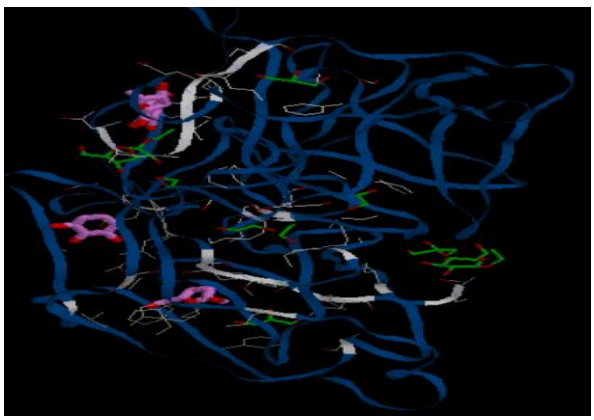

Fig. 11: The Total Binding Energy profile for Chikungunya virus capsid protein with 4 ligands.

3.4.2. The Total Binding Energy for Chikungunya virus Envelope protein E1 with 4 ligands

From Table – 2, Table – 4 and Table – 6, the docking simulation of 4 ligands were performed for chikungunya virus Envelope protein E1. From the docking study, we observed that compound – D has best binding affinity with the target Envelope protein E1 with the binding energy value of -90.54 kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus Envelope protein E1 reveals that it forms one hydrogen bonds with low energy, with His(195) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for chikungunya virus Envelope protein E1 with 4 ligands: is shown in Fig.12.

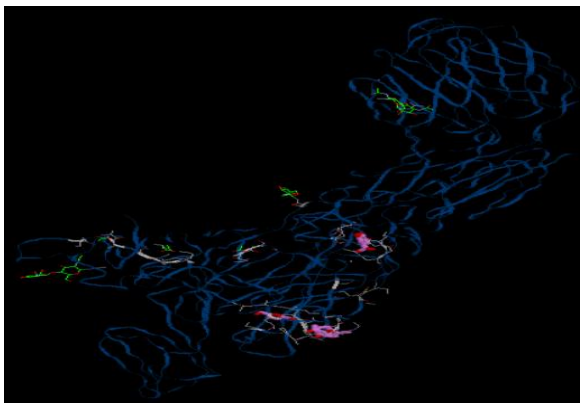

Fig. 12: The Total Binding Energy profile for Chikungunya virus Envelope protein E1 with 4 ligands.

3.4.3. The Total Binding Energy for Chikungunya virus Envelope protein E2 with 4 ligands

From Table – 2, Table – 4 and Table – 6, the docking simulation of 4 ligands were performed for chikungunya virus Envelope protein E2. From the docking study, we observed that compound – D has best binding affinity with the target Envelope protein E2 with the binding energy value of -77.21 kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus Envelope protein E2 reveals that it forms one hydrogen bond with low energy, with Gly(260) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for chikungunya virus Envelope protein E2 with 4 ligands: is shown in Fig.13.

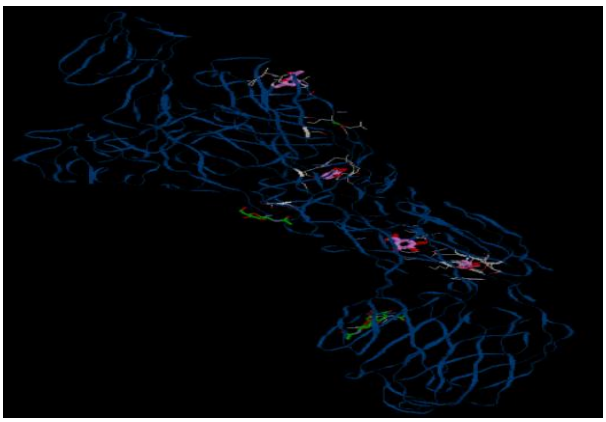

Fig. 13: The Total Binding Energy profile for Chikungunya virus Envelope protein E2 with 4 ligands.

3.4.4. The Total Binding Energy for Chikungunya virus Envelope protein E3 with 4 ligands

From Table – 2, Table – 4 and Table – 6, the docking simulation of 4 ligands were performed for chikungunya virus Envelope protein E3. From the docking study, we observed that compound –D has best binding affinity with the target Envelope protein E3 with the binding energy value of -77.8kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus Envelope protein E3 reveals that it forms one hydrogen bond with low energy, with Glu(308) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for chikungunya virus Envelope protein E3 with 4 ligands: is shown in Fig.14.

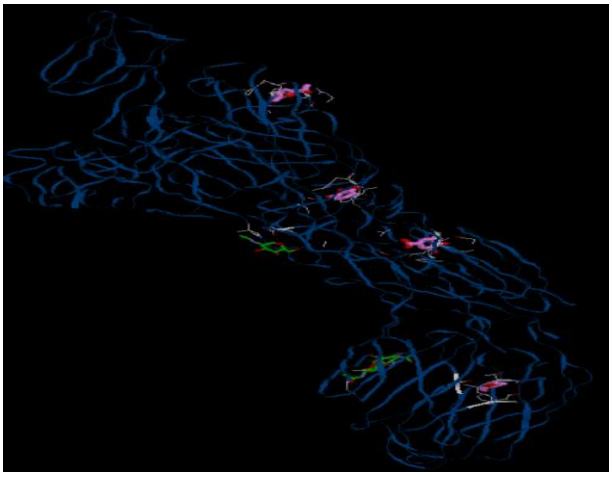

Fig. 14: The Total Binding Energy profile for Chikungunya virus Envelope protein E3 with 4 ligands.

4. CONCLUSION

Our molecular docking studies explored the possible binding modes of 4 compounds that are present in *Emblica officinalis* leaf with seven proteins of Dengue virus and six proteins of Chikungunya virus. Dengue virus consists of envelope protein, NS1 protein, Transmembrane domain of NS2A, NS2B/NS3 protease, NS3 helicase, NS5 protein and capsid protein; Chikungunya virus consists of Capsid protein, Envelope protein E1, Envelope protein E2, Envelope protein E3, NSP2 and NSP3. It revealed that all the 4 compounds show minimum affinity with all the proteins. The compound 'D' (Benzoic acid, 3, 4, 5-trihydroxy-(synonym: Gallic-acid) 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 D has highest binding affinity with most of the structural proteins of Dengue virus and compound D has the highest binding affinity with majority of the structural proteins of Chiungunya virus. Whereas the compound D is shown to have highest binding affinity with most of the non structural proteins of Dengue virus and the non structural proteins of Chikungunya virus has highest binding affinities with D compound and therefore it can be used as an effective drug target for Dengue virus as well as Chikungunya virus . Hence, the Compound D may be considered as the effective drug target for both dengue and Chikungunya 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 Chikungunya.

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