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DOCKING STUDY OF SELECTED *CALOTROPIS GIGANTEA* LEAVES CONSTITUENTS ON DENGUE VIRAL PROTEINS – AN *IN SILICO* APPROACH

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ABSTRACT

Dengu virus has seven major non structural proteins which are considered to be most effective for drug designing. Recent studies have shown that these proteins can effectively cause inactivation of dengue process in humans. *Calatropis gigantea* phytochemicals are reported to have antiinflamatory and antimicrobial properties. In the present study, the binding efficiency of 5 compounds that are present in the *Calatropis gigantea* with all the seven proteins through in silico methods was carried out. By our virtual screening and docking result, we found that the Compound D and Compound E have highest binding affinity with the proteins and also we predicted the binding site amino acid residues and the type of hydrogen bonding.

KEYWORDS: Binding Interaction, Molecular Docking, Hydrogen Bond.

1. INTRODUCTION

Herbal extracts that act as drugs are important because of their efficiency, low toxicity and no side effects. The world of flora is more of importance beacuse of its renewable non-exhaustive source of bioactive compounds. The therapeutic uses of folklore Indian herbal drugs have been evaluated successfully and many new medicinal plants have been discovered along with its therapeutic uses. *Calotropis gigantea* is a plant which is of greater herbal importance. This plant belongs to *Apocynaceae* family which includes latex bearing plants. The plant *Calotropis gigantea* is known by different names in English such as "Crown flower", "giant Indian milkweed". In Hindi *Calatropis gigantea* is also called as "Aak", "Arka", "Madar" and in Sanskrit "Ganarupa", "Mandara", "Vasuka", "Svetapushp" etc. In India *Calotropis gigantea* occupies special importance because of its large industrial uses and economic values. It has various medicinal properties. The different parts of the plant have are most potential in curing various diseases and disorders like asthma, cold, epilepsy, fever, indigestion, leprosy, piles, skin diseases etc., and exhibiting activities that are antiinflammatory, anthelmintic, anticancer and antitumor as observed in various polyherbal preparations.[1]

The GCMS chromatogram of methanolic leaves extract showed nearly 160 compounds. Of these reported, 5 compounds were found to be having high retention time. Those include n -Hexadecanoic acid, 9, 12, 15 octadecatrienoic acid (z, z, z), Methyl 8, 11, 14 – heptadecatrienoate, Di isooctyl phthalate, Bis (2 – ethylehexyl) phthalate.[2] n-Hexadecanoic acid reported to have anti-inflammatory and antiandrogenic properties.[3] 9, 12, 15 octadecatrienoic acid reported to have Antiinflammatory, Hypocholesterolemic, anticancer, Hepatoprotective, Antihistaminic, Antiarthritic, Anticoronary, Antieczemic properties.[4] Methyl 8, 11, 14 - heptadecatrienoate reported to have antimicrobial, antifungal properties.[5] Di isooctyl pthallate reported to have antimicrobial activity.[6] Bis (2-ethylehexyl) pthallate is used as plasticizer in medical devices such as intravenous tubing and bags.[2]

Dengue, a haemorrhagic fever[7], is caused due to all four serotypes of dengue virus (DENV-1, DENV-2, DENV-3 and DENV-4).[8] These viruses contain ten proteins out of which three are structural proteins and seven are non structural proteins.[9] The seven non structural proteins are capsid protein, envelope protein, NS1 protein, trans – membrane domain of NS2A, NS2B/NS3 protease, NS3 helicase and NS5 protein. NS2B-NS3 protease is a crucial enzyme for the viral replication.[10] This protein is hetero dimeric protein of NS2B and NS3protein. The N-terminal of the NS3 protein forms associates with the NS2B cofactor which is important for the viral replication. NS2B/ NS3 protease has an important role in the viral life cycle.[11] Envelope protein is a structural protein which is involved in the viral assembly. The protein utilized for the study is the envelope protein domain III of the dengue type 4 viruses (strain Dominica / 814669 / 1981). It is classified under structural protein

immune system.[12] 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).[13] The protein used for this study was the trans-membrane domain of theNS2A of dengue virus type 2. 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 immuneresponse.[14] NS3 helicase belongs to the non-structural and a multi-domain dengue virus replication protein.[15] 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 isinvolved in the replication of the viral genome. RNA is synthesized via "de novo" by NS5 protein.[16]

Bioinformatics is an interdisciplinary branch of science which utilizes statistics, computer and mathematics to analyse biological data.[17] Bioinformatics is now utilized for many researches to identify many aspects such as evolution. Protein Data Bank (PDB) is a protein storage bioinformatic tool. It contains the structures of large numbers of proteins, ligands and other macromolecules.[18] 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.[19]

The aim of our study is to find the best docking fit from the selected 5 compounds of *Calatropis gigantea* extract with seven different non structural Dengue viral proteins.

2. MATERIALS AND METHODOLOGIES

2.1. Preparation of dengue 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. Seven proteins of dengue virus were used for this study. The 3D structure of all the seven proteins were downloaded from PDB and saved in PDB format. The downloaded proteins were viewed in Py-Mol viewer.[20]

2.2. Preparation of ligands: Ligands selected were from the previous studies on GCMS analysis on *Calotropis gigantea* leaves extract. 5 ligands were selected based on the retention area exhibited on GCMS and those were used for this docking study. Selected 5 ligands were constructed using ChemSketch.[21] 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.[22] 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 each individual 5 ligands were 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.[23]

3. RESULTS

3.1. Total Binding Energy (kcal/mol) profile for Dengue viruses protein with 5 ligands.

Table. 1: The Total Binding Energy (kcal/mol) profile for Dengue viral protein with 5 ligands.

Ligand	Compound name	Envelope protein	NS1 protein	Trans membrane domain of NS2A	NS2B / NS3 protease	NS3 helicase	NS5 protein	Capsid protein
A	n-Hexadecanoic acid	-68.4	-88	-492.7	-76.3	-87.6	-88.4	-75.5
B	9,12,15 octadecatrienoic acid	-86.6	-98.5	-655.7	-84.7	-96.9	-90.9	-92.6
C	Methyl 8,11,14-heptadecatrienoate	-76.6	-91.6	-545.6	-84.4	-84.8	-87.5	-75.0
D	Di isooctyl phthallate	-94.7	-101.8	-761.1	-111.1	-109.3	-107.0	-108.7
E	Bis (2-ethylehexyl) phthallate	-107.4	-90.7	-701	-95.4	-108.1	-110.4	-107.1

3.2. H – Bond profile for Dengue viruses protein with 5 ligands.

Table. 2: H – Bond profile for Dengue viral protein with 5 ligands.

Ligand	Compound name	Envelope protein	NS1 protein	Trans membrane domain of NS2A	NS2B/NS3 protease	NS3 helicase	NS5 protein	Capsid protein
A	n-Hexadecanoic acid	H-S	H-S	H-S	H-S	H-S H-M	H-S H-M	-
B	9,12,15 octadecatrienoic acid	H-M	H-S	H-S	H-S	H-S H-M	H-S	H-S H-M
C	Methyl 8,11,14-heptadecatrienoate	H-S	H-M	-	H-S	H-S H-M	H-S	H-S
D	Di isooctyl phthallate	-	H-S	H-S	H-M	-	H-S	H-S H-M
E	Bis (2-ethylehexyl) phthallate	H-S H-M	H-S	-	H-S H-M	-	H-S H-M	H-S H-M

3.3. Amino acid position profile for Dengue viruses protein with 5 ligands

Table. 3: Amino acid position profile for Dengue viral protein with 5 ligands.

Ligand	Compound name	Envelope protein	NS1 protein	Trans membrane domain of NS2A	NS2B/NS3 protease	NS3 helicase	NS5 protein	Capsid protein
A	n-Hexadecanoic acid	Arg (619)	Trp (232)	Arg (18)	Trp (83)	Asp (470) Arg (463)	Thr (50)	-
B	9,12,15 octadecatrienoic acid	Arg (629)	His (181)	Lys (16)	Lys (87) / Arg (24)	Gln (467) Arg (463)	His (712)	Arg (41) Leu (35)
C	Methyl 8,11,14-heptadecatrienoate	Arg (619)	Gly (235)	-	Thr (120)	Thr (200) Gly (198)	Lys(689)	Arg (68)
D	Di isooctyl phthallate	-	Arg (314)	Thr(7)	Leu (149)	-	Lys (253) / Lys (357)	Thr (25) Arg (22) / Val (23) / Ser (24)
E	Bis (2-ethylehexyl) phthallate	Lys (625) Gly (628) /Arg (629) / Ile (630)	Arg (336)	-	His (60) Arg (55)	-	Thr (51) Gln (693)	Arg (41) Leu (44)

4. DISCUSSION

From the Table – 1, Table – 2 and Table – 3, the 3D structure coordinates of seven non structural proteins of dengue virus is optimized and 5 compounds from *Calotropis gigantea* leaves extract are identified. Their total binding energy was calculated using iGEMDOCK. Evaluations of binding conformation of 5 compounds with seven non structural dengue viral proteins are performed using iGEMDOCK. From docking study, we listed binding affinity of 5 compounds based on ligand

binding energy (Table.1). The binding pose for each ligand molecule into the non structural dengue viral protein is 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. Among the 5 analogs, compound E is found to have lower ligand binding energy (binding energy value= -107.4 kcal/mol), than other analogs for Envelope protein. Compound "D" has least binding energy score with NS1 protein (binding energy value= --101.8 kcal/mol), Trans membrane domain of NS2A (binding energy value= -761.1kcal/mol), NS2B / NS3 protease (binding energy value= -111.1kcal/mol), NS3 helicase (binding energy value= -109.3kcal/mol), NS5 protein (binding energy value= -107.0 kcal/mol) and Capsid protein (binding energy value = -108.7kcal/mol). We further analyzed the docked pose for finding the binding mode of compound "E" and compound "D" in to seven non structural dengue proteins to validate the reasonable binding conformations.

4.1. The Total Binding Energy for Dengue virus envelope protein with 5 ligands: From Table – 1, Table – 2, Table – 3 and Figure – 1, the docking simulation of 5 ligands were performed for Dengue virus envelope protein. From the docking study, we observed that compound – E has best binding affinity with the target envelope protein with the binding energy value of -107.4 kcal/mol. Interaction analysis of binding mode of compound –E in dengue virus envelope protein reveals that it forms two hydrogen bonds with low energy, one with Lys (625) residue and another with Gly (628) , Arg (629) and Ile (630) residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus envelope protein with 5 ligands: is shown in Fig.1.

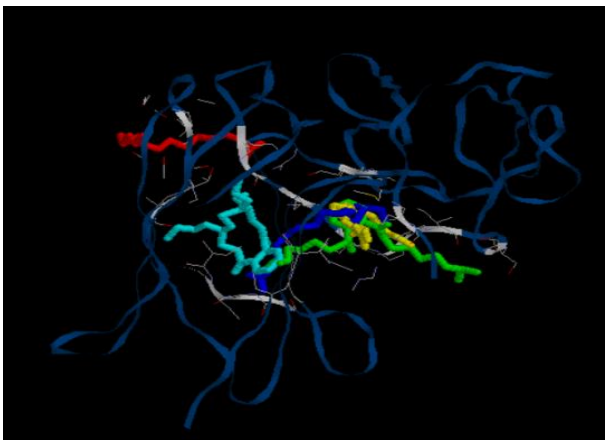

Fig. 1: The Total Binding Energy for Dengue virus envelope protein with 5 ligands.

4.2. The Total Binding Energy for Dengue virus NS1 protein with 5 ligands: From Table – 1, Table – 2, Table – 3 and Figure – 2, the docking simulation of 5 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 -101.8 kcal/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 Arg(314) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS1 protein with 5 ligands: is shown in Fig.2.

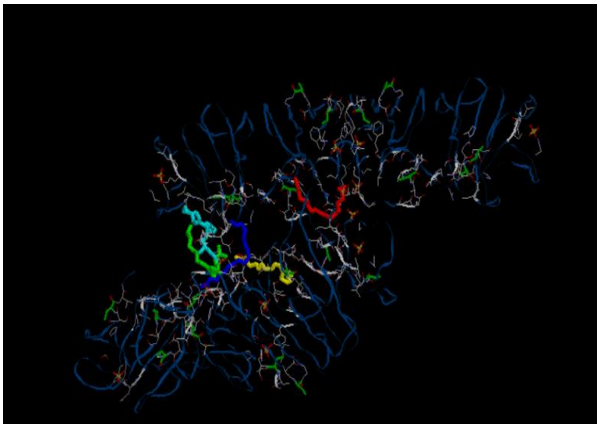

Fig. 2: The Total Binding Energy for Dengue virus NS1 protein with 5 ligands.

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

From Table – 1, Table – 2, Table – 3 and Figure – 3, the docking simulation of 5 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 -761.1 kcal/mol- . Interaction analysis of binding mode of compound –D in dengue virus Trans membrane domain of NS2A reveals that it forms one hydrogen bond with low energy, with Thr(7) residue. 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.3.

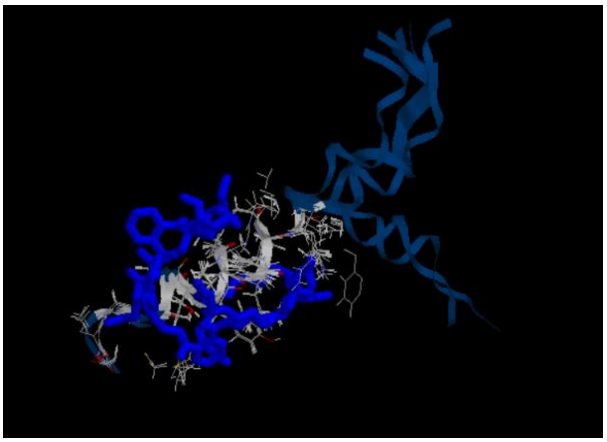

Fig.3: The Total Binding Energy for Dengue virus Trans membrane domain of NS2A with 5 ligands.

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

From Table – 1, Table – 2, Table – 3 and Figure – 4, the docking simulation of 5 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 -111.1 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 5 ligands: is shown in Fig.4.

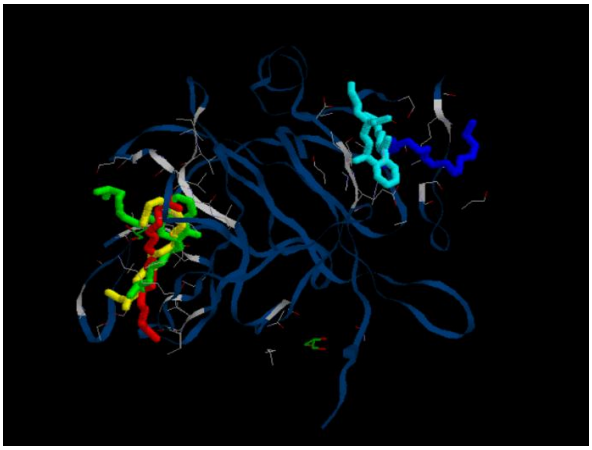

Fig. 4: The Total Binding Energy for Dengue virus NS2B / NS3 protease with 5 ligands.

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

From Table – 1, Table – 2, Table – 3 and Figure – 5, the docking simulation of 5 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 -109.3 kcal/mol . A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS3 helicase with 5 ligands: is shown in Fig 5.

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

4.6. The Total Binding Energy for Dengue virus NS5 protein with 5 ligands: From Table – 1, Table – 2, Table – 3 and Figure – 6, the docking simulation of 5 ligands were performed for Dengue virus NS5 protein From the docking study, we observed that compound – E has best binding affinity with the target NS5 protein with the binding energy value of -110.4 kcal/mol. Interaction analysis of binding mode of compound –E in dengue virus NS5 protein reveals that it forms one hydrogen bond with low energy, with Lys (253) and Lys (357) 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.6.

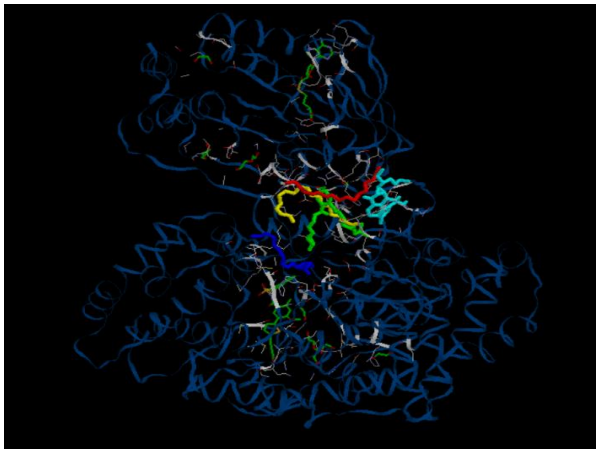

Fig. 6: The Total Binding Energy for Dengue virus NS5 protein with 5 ligands

4.7. The Total Binding Energy for Dengue virus Capsid protein with 5 ligands: From Table – 1, Table – 2, Table – 3 and Figure – 7, the docking simulation of 5 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 -108.7 kcal/mol . Interaction analysis of binding mode of compound –D in dengue virus Capsid protein. reveals that it forms two hydrogen bonds with low energy, one with Thr(25) residue and another with Arg (22), Val (23) and Ser (24) residues. 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.

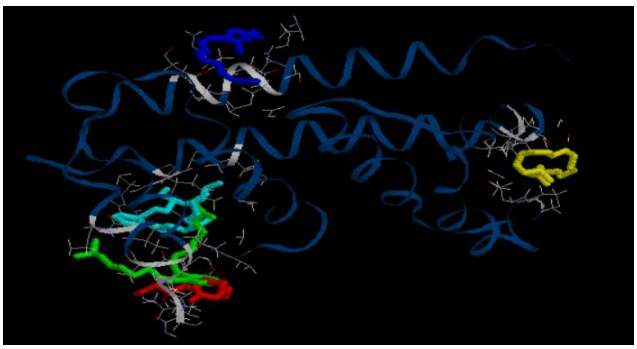

Fig. 7: The Total Binding Energy for Dengue virus Capsid protein with 5 ligands.

5. CONCLUSION

Our molecular docking studies explored the possible binding modes of 5 compounds that are present in *Calatropis gigantea* leaves extract with seven non structural proteins which are envelope protein, NS1 protein, Transmembrane doamin of NS2A, NS2B/NS3 protease, NS3 helicase, NS5 protein and capsid protein. . It revealed that all the 5 compounds show minimum affinity with all the proteins. Especially the compound D (Di isooctyl pthallate) and compound E (Bis(2-ethylehexyl) pthallate) shows best results compared to other compounds. On comparing the binding energy and the binding site residues, we found that all compounds differ either in their binding modes or with the binding site residues for hydrogen bond formation. The conclusion drawn from our virtual screening and docking result was that the Compound D has highest binding affinity with most of the proteins and it can be used as an effective drug target for Dengue virus . Though, there are many reports on the *in vitro* analysis of these compounds and its antioxidant properties, but there are no in silico studies that predict the binding and active regions especially with these proteins. Our study is probably the first such attempt to predict the binding site. However, validation of our results through *invivo* and *invitro* experiments along with animal models will enlighten hope for the future development of more potent drugs for the treating Dengue.

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