EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 9
520-524
Year: 2018

IN SILICO DRUG DESIGNING STUDIES ON DENGUE VIRUS NS3 HELICASE

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Article Received on 11/07/2018 **Article Revised on 31/07/2018** **Article Accepted on 20/08/2018**

ABSTRACT
The key protein involved in causing dengue is NS3 helicase, which is considered as major therapeutic target for dengue drug development. Recent studies have reported positively for NS3 helicase in dysregulation of causing dengue process in humans. Dragon fruit seed phytochemicals are reported to have antioxidant and antiviral properties. In the present study we studied binding efficiency of 11 compounds that are present in the dragon fruit seeds with NS3 helicase through Insilico methods. By our virtual screening and docking result, we found that the Compound A, Compound C and Compound B have the highest binding affinity with the NS3 helicase and also we predicted the binding site amino acid residues and the nature of hydrogen bonding. However more invivo experimental validation of our results with animal models will enlighten the development of more potent drugs from these compounds for treatment of dengue.

KEYWORDS: NS3 helicase, Binding interaction, molecular docking, dengue.

INTRODUCTION
Pitaya is most commonly called as "Dragon Fruit" in English and it belongs to the Cactacea family. It has the generic name *Hylocereus.*[1] Dragon fruit has three varities; *Hylocereus undatus, Hylocereus polyrhizus* and *Hylocereus megalanthus. Hylocereus undatus* and *Hylocereus polyrhizus* have red peel but differ in the colour of the pulp. *Hylocereus megalanthus* has yellow peel and white pulp and it is a hybrid of *Hylocereus costaricensis* and *Selenicereus inermis.* Small, edible, black seeds are present interspersed in the pulp of the fruit.[2] The dragon fruit stems bear 4-7 fruits which range in length from 4-10 centimeter. The colour of the fruit ranges from blue-green to grayish-brown. The thickness of the fruit ranges from 3-8 centimeters. Dragon fruit is believed to be a native of Mexico and then transplanted by Europeans to Central America.[3] It is now widely cultivated in the Southeast Asia, Australia, The United States, Israel and Cyprus.[4] The fruit extracts were found to be effective in the treatment of various diseases[5] and they are also found to have anti-microbial and antioxidant properties.[6] The red colour of the fruit was reported to be because of the nitrogen-containing compound called betacyanin which plays a major role in the antioxidant property of the fruit.[7] Recent studies on the seeds were reported to contain large amounts of polyunsaturated fatty acids (PUFA) like linolenic acid and linoleic acid.[1] [8] Previous studies on the dragon fruit seeds reported the presence of the most probable compounds like tetradecanoic acid, octadecanoic acid, phytol, 9,12,15-octadecatrienoic acid, 9,12-octadecanoic acid, 12-chloroethyl linoleate, 8-hexadecyne present in them.[4]

Dengue, a haemorrhagic fever[9], is caused due to all the four serotypes of dengue virus (DENV-1, DENV-2, DENV-3 and DENV-4).[10] Dengue viruses contain 10 proteins out of which seven are non-structural proteins and three are structural proteins. NS3 helicase belongs to the non-structural and a multi-domain dengue virus replication protein.[11]

The protein used for this study was from the Dengue Virus type-4. It is classified under hydrolases. NS3 helicase is also called as NS3 ATPase.[12]

Bioinformatics is an interdisciplinary branch of science which utilizes statistics, computer and mathematics to analyse biological data.[13] 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.[14] 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.[15]

MATERIALS AND METHODOLOGIES

Preparation of macromolecule NS3 helicase

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. Envelop protein of dengue virus was used for this study. The 3D structure of this protein was downloaded from PDB and saved in pdb format. The downloaded protein was viewed in Py-Mol viewer.

Preparation of ligands

Ligands selected were from the previous studies on this fruit seeds. 11 ligands were used for the study. Ligands were constructed using ChemSketch.[16] The constructed ligands were optimized to add the hydrogen bonds and the obtained structures were saved in mol for docking analysis.

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.[16] The protein and the ligands were loaded and the out path was set. Standard docking parameters were used for docking (population size=200, generations=70 and no.of solutions=2). The docking process was initiated. After the docking process, the best docking pose for the individual ligands can be obtained. 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.

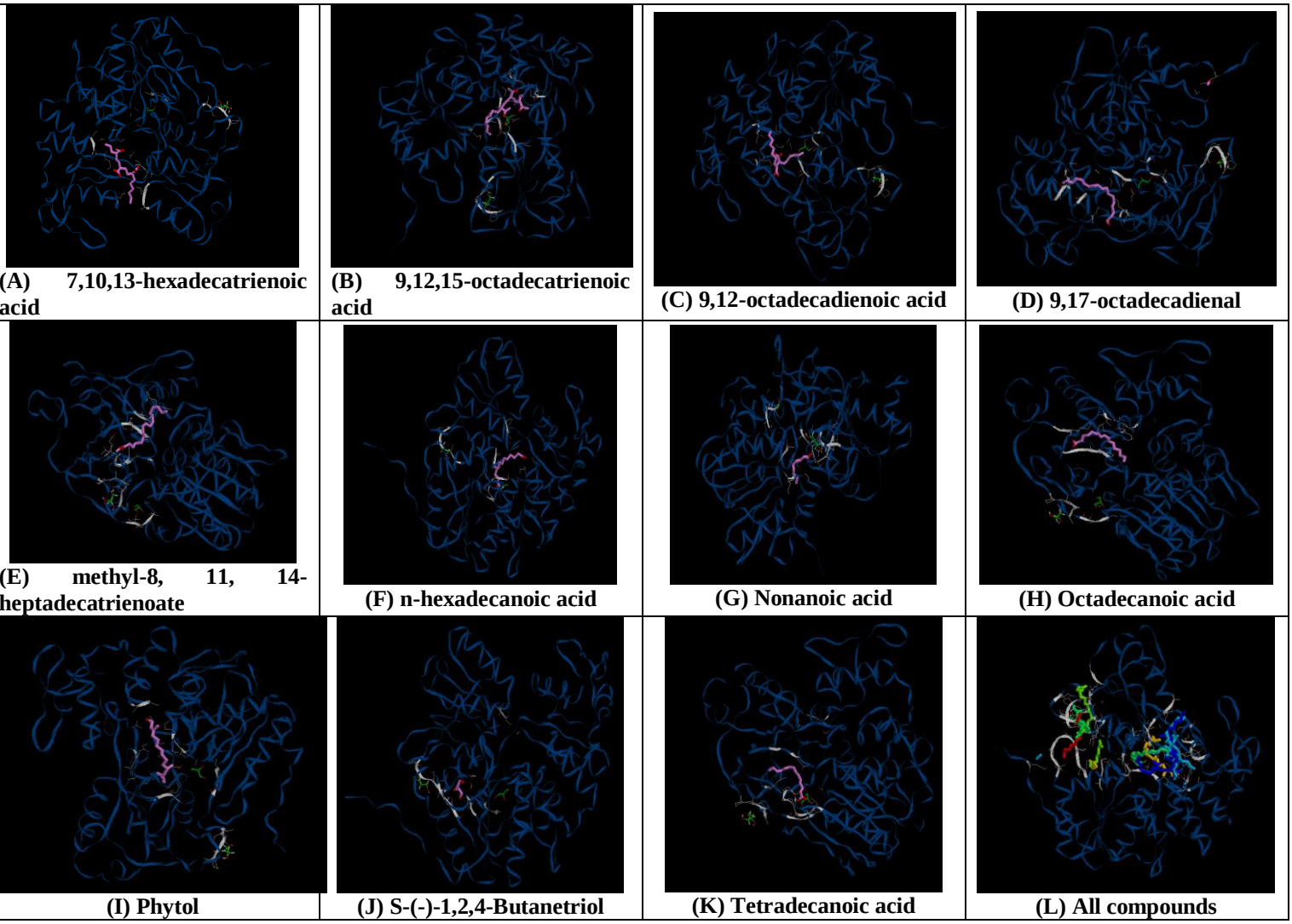
RESULTS AND DISCUSSION

Table 1: The fitness and the interaction profile for NS3 helicase protein with the ligands.

Ligands	Compounds	Total Binding Energy (kcal/mol)	Vander Waal's Force (kcal/mol)	E (pharma)	H-S Arg 387	H-S Arg 599	V-S Arg 387	H-Bond Energy	Electrostatic Force	AverCon Pair
				Z-score =>	1.65	2.66	2.88			
				W(pharma) =>	0.62	1.00	1.00			
A	7,10,13-hexadecatrienoic acid	-110.38	-85.14	-106.3	-6.4	-3.5	-11.1	-18.20	-7.04	21.92
B	9,12,15-octadecatrienoic acid	-101.77	-87.77	-101.8	-10.5	-3.5	-14	-14	0	20.89
C	9,12-octadecadienoic acid	-102.08	-82.33	-102.1	-3.5	-3.5	-10.1	-13.02	-6.73	22
D	9,17-octadecadienal	-85.35	-84.34	-113.4	0	0	-6.1	-1.01	0	26.38
E	methyl-8,11,14-heptadecatrienoate	-85.78	-72.15	-101.8	0	0	0	-13.63	0	27.15
F	n-hexadecanoic acid	-91.51	-79.73	-92.3	0	0	-3.9	-6.88	-4.90	29.06
G	Nonanoic acid	-73.12	-53.18	-58.2	0	0	0	-15.38	-4.55	33.18
H	Octadecanoic acid	-90.33	-83.75	-102.1	0	0	0	-6.59	0	26.3
I	Phytol	-78.93	-69.94	-87.3	0	-3.1	-4.5	-8.99	0	25.52
J	S-(-)-1,2,4-Butanetriol	-58.21	-35.36	-96.1	0	0	0	-22.85	0	35.43
K	Tetradecanoic acid	-96.06	-76.84	-85.3	0	0	0	-19.50	0.29	29.19

Table 2: The cluster table for NS3 helicase protein and the ligands.

Ligands	Compound	H – Bond	Amino Acid Position	H – Bond Energy
A	7,10,13-hexadecatrienoic acid	H-M	Arg (387)	-2.8
		H-S	Arg (387)	-6.4
B	9,12,15-octadecatrienoic acid	H-S	Arg (387)	-10.5
C	9,12-octadecadienoic acid	H-S	His (487)	-6
D	9,17-octadecadienal	-	-	-
E	methyl-8,11,14-heptadecatrienoate	H-M	Asn (464)	-3.5
		H-S	Asp (470)	-4
F	n-hexadecanoic acid	H-S	His (487)	-3.5
G	Nonanoic acid	H-S	Arg (460)	-8.4
H	Octadecanoic acid	H-M	Lys (357)	-3.5
I	Phytol	H-S	Glu (490)	-3.7
J	S-(-)-1,2,4-Butanetriol	H-M	Leu (193)	-7
		H-S	Asp (192)	-5
K	Tetradecanoic acid	H-M	Thr (200)	-4
		H-S	Thr (200)	-5



(A) 7,10,13-hexadecatrienoic acid | (B) 9,12,15-octadecatrienoic acid | (C) 9,12-octadecadienoic acid | (D) 9,17-octadecadienal

(E) methyl-8, 11, 14-heptadecatrienoate | (F) n-hexadecanoic acid | (G) Nonanoic acid | (H) Octadecanoic acid

(I) Phytol | (J) S-(-)-1,2,4-Butanetriol | (K) Tetradecanoic acid | (L) All compounds

Fig. 1: Interaction of compounds with NS3 helicase protein.

From the Table – 1, the 3D structure coordinates of NS3 helicase is optimized and 11 compounds from dragon fruits seeds are identified. Their total binding energy was calculated using iGEMDOCK. Evaluation of binding conformation of 11 compounds with NS3 helicase protein is performed using iGEMDOCK. From docking study, we listed binding affinity of 11 compounds based on ligand binding energy (Table.1).

The binding pose for each ligand molecule into the NS3 helicase is analyzed and the one having lowest ligand binding energy with NS3 helicase among the different poses are generated. The lower energy scores represent better target protein-ligand binding affinity compared to higher energy score. Among the 11 analogs, compound A, B and C are found to have lower ligand binding energy value than other analogs. Compound "A" has least binding energy score with target protein (binding energy value = - 110.38 kcal/mol), compound "C" has ligand binding energy value of -102.08 kcal/mol and compound "B" has ligand binding energy value of - 101.77 kcal/mol. We further analyzed the docked pose for finding the binding mode of compound "A", compound "C" and compound "B" to NS3 helicase to validate the reasonable binding conformations.

Docking of compound – A into NS3 helicase protein

From Table – 2 and Figure – 1, the docking simulation of compound - A is performed for NS3 helicase. From the docking study, we observed that compound – A has best binding affinity with the target protein. Interaction analysis of binding mode of compound –A in the target protein reveals that it forms two strong hydrogen bonds, one with branched chain residue Arg 387 having - 6.4 kcal/mol as its bond energy and another hydrogen bond is observed at Arg 387 with – 2.8 kcal/mol as bond energy with the backbone. A close-up view of binding mode of compound – A with NS3 helicase protein is shown in Fig.2.

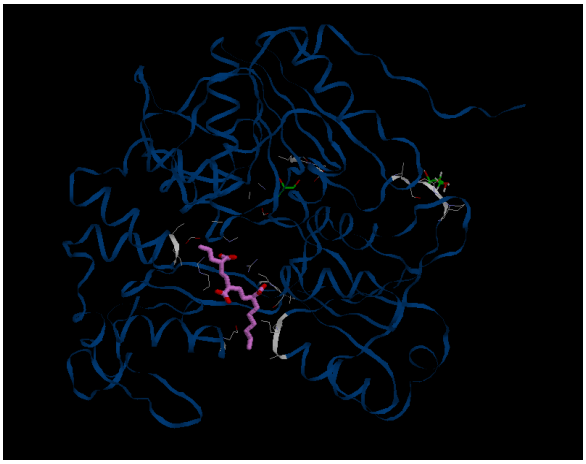


Fig. 2: A close-up view of binding mode of compound – A with NS3 helicase.

Docking of compound – C into NS3 helicase protein

From Table – 2 and Figure – 1, the docking studies of 11 compounds are performed for the target protein. In our results on the binding conformation modes of compounds with the target protein, compound - C shows higher affinity with the NS3 helicase. In examining the binding interaction and position of the compound C with the target protein ligand binding site predicted by your docking procedure, it is found that one strong hydrogen bond is formed at His 487 having - 6.0 kcal/mol as its bond energy. A close-up view of binding mode of compound – C NS3 helicase is shown in Fig.3.

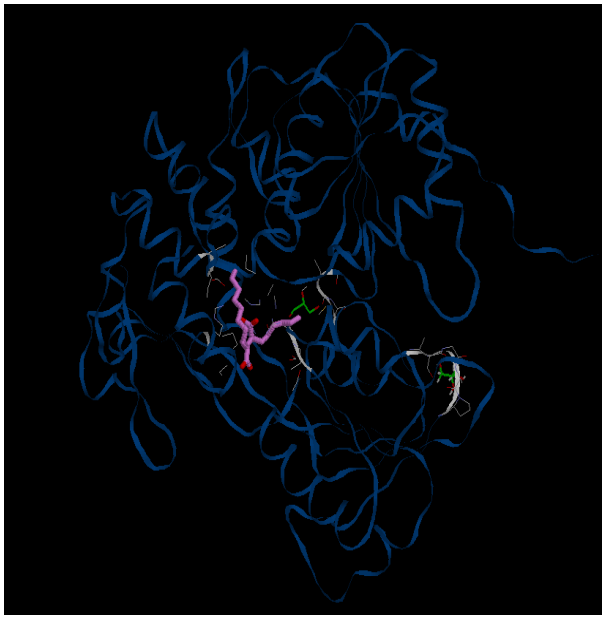


Fig. 3: A close-up view of binding mode of compound – C with NS3 helicase protein.

Docking of compound – B into NS3 helicase protein

From Table – 2 and Figure – 1, the docking simulation of compound - B is performed for NS3 helicase. From the docking study, we observed that compound – B has best binding affinity with the target protein. Interaction analysis of binding mode of compound –B in the target protein reveals that it forms one strong hydrogen bonds at Arg 387 with the bond energy of -10.5 kcal/mol. A close-up view of binding mode of compound – B with NS3 helicase protein is shown in Fig.4.

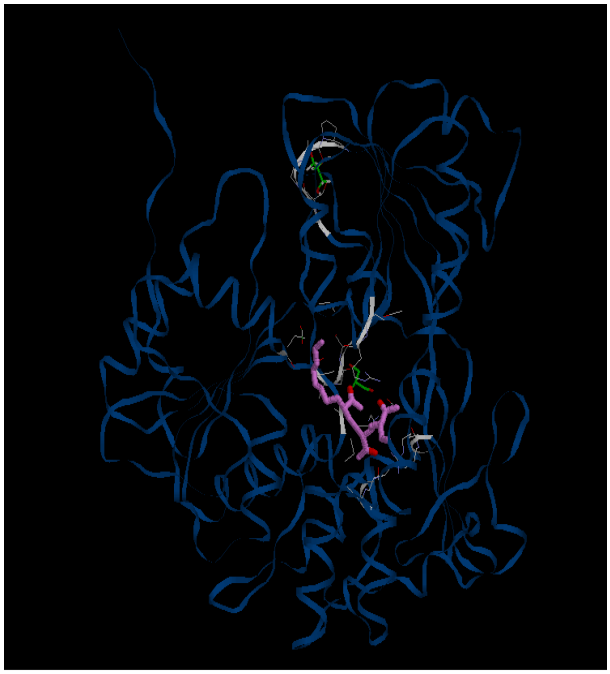


Fig. 3: A close-up view of binding mode of compound – B with NS3 helicase protein.

CONCLUSION

Our molecular docking studies explored the possible binding modes of 11 compounds that are present in dragon fruit seed with NS3 helicase protein. It revealed that all the 11 compounds show minimum affinity with the target protein. Especially the compound A (7,10,13-hexadecatrienoic acid), compound C (9,12-octadecadienoic acid) and compound B (9,12,15-octadecatrienoic acid) shows best result when compared with 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 A, Compound C and Compound B have highest binding affinity with the NS3 helicase. 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 the target protein. Our study is probably the first such attempt to predict the binding site, 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.

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