International Journal of Basic and Applied Biology
Print ISSN: 2394-5820, Online ISSN: 2349-2539, Volume 2, Number 3; October-December, 2014 pp. 124-129

http://www.krishisanskriti.org/ijbab.html

Finding Natural Inhibitor for NS3 Protease Enzyme from Dengue Virus via Molecular Modeling

Chandramohan Jana[1], Indu Cahturvedi[2], Ophelie Robine[3], Anshul Nigam[4], Subrata Sinha[5] and Ashwani Sharma[6,#]

[1]New Era Proteomics Drug Discovery Research C-1/31, Yamuna Vihar New Delhi-110053, India
[2]Department of Animal Nutrition Central Institute for Research on Goats Makhdoom, Mathura-281 122, UP, India
[3]Former, University of Rouen, Normainde France
[3]Current: New Era Proteomics Drug Discovery Research 56 Route de Darnetal, 76000, Rouen, France
[4]IPLS project, School of Life Science Pondicherry University,Puducherry- 605014, India
[5]Centre of Bioinformatics Studies Dibrugarh University, Assam-786004, India
[6]New Era Proteomics Drug Discovery Research C-1/31, Yamuna Vihar New Delhi-110053, India
E-mail: [1]chandramohanjana@gmail.com, [2]indu_0012@yahoo.co.in, [3]ophrob@hotmail.fr, [4]anshulnigam2006@gmail.com, [5]subratasinha@dibru.ac.in, [6]ashwansharma@gmail.com

Abstract: *The NS3 protease enzyme of the dengue virus is derived from the translation of dengue virus RNA genome guided by host cell ribosome. Further, the activated NS3 protein helps in viral replication in the host cell therefore always a major drug target for anti-viral drug therapy against dengue disease. Chemical anti-viral drugs are not specific and corporate higher cost of treatment. Therefore, our study was focused on finding natural inhibitor against NS3 protease enzyme of dengue virus. Molecular docking and dynamics approaches were explored for fast screening of natural compounds databases. Azadirachtin (A known anti-microbial compound) from seeds of the neem tree shows best affinity for NS3 protease with lowest interaction energy of -111.95 as compare to other compounds. The corresponding Z score was found to be 0.015 which raised confidence interval of 98.50% on the docking affinity of Azadirachtin for NS3 protease. Therefore, our computational study is able to find natural inhibitor against NS3 protease which may act as potential candidate for designing natural anti-viral therapy against dengue virus.*

1. INTRODUCTION

Dengue virus is classified in the family of Flaviviridae associated with genus Flavi virus. The transmission of dengue virus is taken place to humans by the help of Aedes mosquitoes. Dengue virus causes major diseases such as mild flu-like syndrome (known as dengue fever), coagulopathy, increased vascular fragility, and permeability (dengue hemorrhagic fever) in the tropical and subtropical areas [1, 2]. In south asia and Indian subcontinent, the dengue virus infects the children (< 15 years old) greater than the adult [3-6]. The aedes mosquitoes injects the dengue virus into the bloodstream of the epidermis and dermis which results in the infection of immature Langerhans cells [7, 8] and keratinocytes [7]. Then these infected cells migrate from site of infection to lymph nodes, where monocytes and macrophages are recruited, which become targets of infection. The dengue virus utilizes the ribosomes machinery of the host cells and translates its genomic RNA to the precursor poly-protein. Subsequently, the viral poly-protein is cleaved by the host cell furin and dengue NS2B-NS3 serine protease (NS2B-NS3pro) at various regions to produce structural and nonstructural viral proteins [9-11]. The NS3 protein is a viral non-structural protein that possesses enzymatic activity. The N-terminal of this protein contains 180 amino acid residues that represent NS3 protease [12, 13]. On interaction with its cofactor i.e. NS2B, the NS3 protease forms a NS2B-NS3pro complex and activated [14]. Works have been reported about disruption of NS2B-NS3pro functions in order to inhibit viral replication [15]. Therefore, NS2B-NS3pro is considered as a potential target for the design of antiviral drugs against dengue virus [16, 17]. In recent years, many investigators focus on plants and their derivatives to develop new antiviral drugs [18-20]. Recently, Ehsan et al 2014 [21] shows that baicalein (5, 6, 7-trihydroxyflavone, a flavonoid isolated from *Scutellaria baicalensis*) is able to inhibit the *in vitro* replication of dengue virus in Vero cells, functioning at different stages of virus replication[9]. Therefore, in this paper, we are proposing the application of molecular docking and molecular dynamics for finding natural inhibitor against NS3 protease enzyme. We predict that the binding of natural inhibitor at the active site of NS3 protease enzyme may inhibit its action to replicate viral genome which indirectly inhibits the further infection of dengue virus in the host cell. Our computational approach detected best affinity of natural compound Azadirachtin for the NS3 protease enzyme from dengue virus and may be used to design anti-viral drug therapy.

2. MATERIALS AND METHODS

2.1 Receptor file

The NS3 protease enzyme from Dengue virus was selected for our drug discovery study. The 3D structure of ND3 protease enzyme has been submitted in RCSB protein data bank with PDB ID Code 2M9P. We selected its chain A for our Molecular docking and Molecular dynamics studies.

2.2 Compounds database

Compounds database was generated from the anti-viral natural compounds obtained from plants. Their action mechanism, efficacy for treating the viral disease and sources information were collected from the literatures. The SMILES strings of the compounds were obtained from the PUBCHEM database and converted into 3D structure via CORINA server (http://www.molecular-networks.com/online_demos/corina_demo). In addition, the 3D structures of the natural compounds were also downloaded from CHEMDB database and all structures were stored in one database in PDB file format.

2.3 Ligand Binding site

The PDB structure (2M9P) of NS3 protease enzyme from dengue virus is available with bounded serine protease inhibitor PRD_001171 at the active site of enzyme. We extracted the putative ligand binding site residues at 8Å from the ligand where PRD_001171 was taken as center. These residues were chosen as potential docking target for our docking study.

2.4 Molecular Docking

The binding site residues selected by the earlier step was chosen for next step of docking process. The compounds database was screened against the selected binding site residues from 2M9P. All the docking process was performed by iGemdockv2.1 software. Genetic algorithm was chosen for performing the docking process with the the *Drug Screening* platform with parameters such as Population size: 200, Number of generations: 70 and Number of solutions: 3. Later, after the docking process, the compounds were ranked based on their interaction energies and fitness values with the ligand binding site residues. The natural compound that produced lowest interaction energy was further selected for Protease-Ligand complex molecular dynamics study for refinement of natural compound interaction with the protease enzyme.

2.5 Standard score analysis

Standard score known as the Z score was calculated for the interaction energies produced by iGemdock after the docking of compounds with the protease enzyme. The Z score is able to measure uniqueness and confidence in prediction of interaction energies after the docking process and further able to find unique compound showing best affinity for the protease enzyme which is unlikely to produce by other compounds in random population. In extension of the standard score, the Z score is associated with the probability score known as P value. The P value is able to obtain confidence on interaction energy and affinity of the best compound for protease enzyme. The standard P value is 0.05 which is equivalent to 95% confidence interval. The best compound was selected with based on more negative Z score of interaction energy and P value less than < 0.05.

2.6 Protease-Ligand complex Molecular Dynamics

The Protease-Ligand complex was generated from the best natural compound showing lowest interaction energy with the protease enzyme. The complex was seeded to classical molecular dynamics simulation by GROMACS.v.4.5.5 software. The MD process was divided into following steps: (1): The topology file was prepared for protease enzyme by using GROMOS9643A1 atoms force field with the help of *pdb2gmx* command. (2): The 3D coordinates of the ligand was submitted to PRODRG server for GROMACS topology file generation. We maintained the chirality on and full charges were provided to the compound structure during PRODRG processing. Later, partial charges were adjusted for the compound in the topology file according to the force field. (3): Subsequently, both the topology files (for protease enzyme and ligand) were merged together and saved in *.gro* format. (4): The molecular dynamics was performed in the condensed phase by adding 15472 water molecules in the protease-ligand complex system within a cubic periodic boundary condition with box size of 1.0 nm using *editconf* and *genbox* commands. (5): Furthermore, total charge of the system was neutralized by adding 10 number of Na^+ ions using *genion* command. (6): The system was pre-minimized for 1000 steps using steepest descent method by *grompp* tool. (7): Furthermore, the minimized system was slowly heated to constant temperature of 300 K using NVT (constant number of particles, volume and temperature (300 K) ensemble dynamics for 100 ps (time steps, dt=0.002 and nsteps =50000) with leap-frog integrator. The temperature of the system was kept constant by using Andersen thermostat. (8): Next, NPT (constant number of particles, pressure and temperature) ensemble dynamics was run for 100 ps where the pressure coupling of 1 bar was provided with Berendsen algorithm. (9): Subsequently, production phase of the dynamics was performed for 4 ns with time steps of 2fs and number of steps of 2000000.

Post dynamics analysis were performed by using GROMACS utility commands such as Total Energy, Potential Energy, Temperature (by using *g_energy)*, Radius of gyration for measuring degree of compactness (using *g_gyrate command)* and root mean square deviation (RMSD) of the complex structure from the original complex structure(using *g_rms*), analysis of number of hydrogen bonds between ligand and

protease enzyme (using *g_hbond*), Radial Distribution Function for analysis of distance of ligand from the protease enzyme during the simulation (using *g_rdf* command) and conformational clustering by *g_cluster* module of GROMACS package.

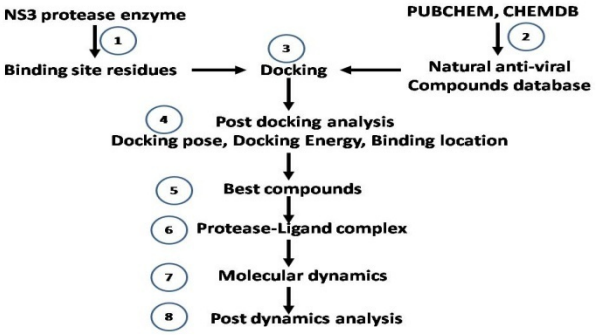


Fig. 1: Schematic for the overall methodology.

(1): Downloaded structure of NS3 protease from RCSB data bank (PDB Code: 2M9P, chain A) and extracted binding site residues at 8Å taking bounded inhibitor as center. (2): Obtained natural anti-viral compounds from PUBCHEM and CHEMDB and made anti-viral compounds database. (3): Screened compounds database against binding site of protease enzyme by docking process. (4): Post docking analysis for ligand binding pose, interaction energies and binding location on protease enzyme. (5): Selected best compound based on lowest interaction energy. (6): Made protease-ligand Complex for molecular dynamics. (7): Complex refinement by molecular dynamics. (8): Post dynamics analysis.

3. RESULTS

Total 159 natural compounds were screened with the binding site of NS3 protease enzyme from dengue virus via iGemdock software. The compound Azadirachtin produced lowest interaction energy with the protease enzyme. It produced very stable binding with the enzyme binding site and generated interaction energy (fitness value) of -111.95 kcal/mole as compared to other natural compounds.

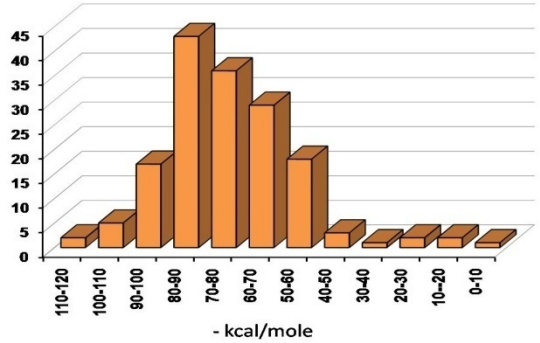


Fig. 2: Distribution of the interaction energies produced for natural anti-viral compounds.

Second rank was obtained by the compound Diosmin which also produced similar affinity for the protease enzyme with interaction energy of -111.94 kcal/mole (Fig. 2).

The standard score known as Z score was calculated for measuring uniqueness of the docking results and selection of the best compound. The Azadirachtin and Diosmin natural compounds produced Z score of -2.10 as compare to other compounds which shows that affinity produced by these compounds are unlikely to be produced by other compounds (Fig. 3). The corresponding P value for the Z score of Azadirachtin and Diosmin are 0.015 which are lesser than the standard P value cut off of 0.05 (95% confidence interval). Therefore, the confidence interval for the interaction energies and affinities of Azadirachtin and Diosmin compounds are 98.50%.

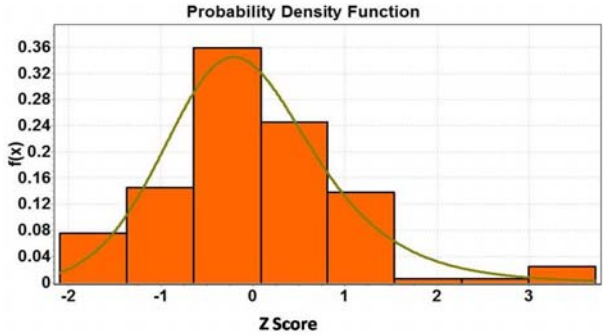


Fig. 3: Z scores of the docking interaction energies with respect to their probabilities distribution (P values) for anti-viral compounds.

Azadirachtin is a natural compound classified in the limonoid group. It is the secondary metabolite product of neem (*Azadirachta indica*) seeds. It is a highly oxidized tetranortriterpenoid which boasts a plethora of oxygen functionality, comprising anenol ether, acetal, hemiacetal and tetra-substituted oxirane as well as a variety of carboxylic esters. Diosmin is a flavone compound, belong to the flavonoid family (polyphenol compound) found in *Teucrium gnaphalodes*.

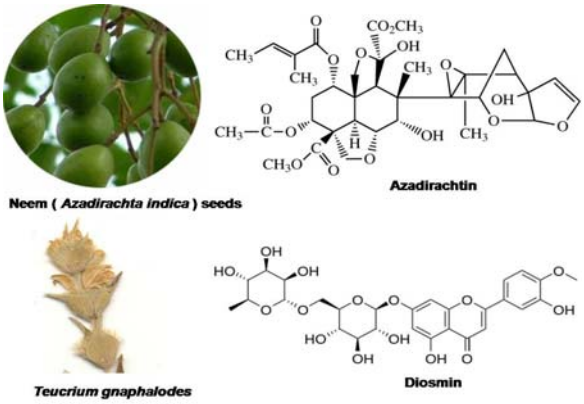


Fig. 4: 2D structure of Azadirachtin and Diosmin compounds.

3.1. Binding site Residues

The binding of Azadirachtin was analyzed at the active site of NS3 protease enzyme. After docking the compound Azadirachtin binds at the active site of protease enzyme. Furthermore, we extracted the amino acid residues making hydrogen bonds interaction with the Azadirachtin compound. We found that residues Glu 47, Ser 96 and Thr 195 make strong hydrogen bonds with the Azadirachtin.

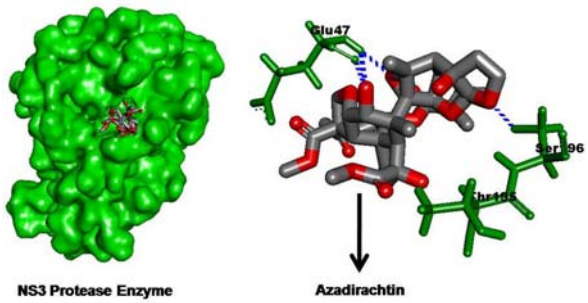


Fig. 5: Amino acid residues making Hydrogen bonds interaction with the Azadirachtin compound at the binding site of NS3 protease enzyme.

3.2. Molecular Dynamics of Protease

Azadirachtin complex The complex of Protease-Azadirachtin was stabilized after 4 ns of simulation. The stabilized system produced average potential energy of -6.795 x10^{-5} kj/mole. Radius of gyration measured degree of compactness of the system during the simulation. RDF analysis reveals that the system remains compact during the simulation time of 4 ns and produced average Rg value of 1.73 nm. This may be due to strong affinity of Azadirachtin natural compound for the Protease enzyme (Fig. 6).

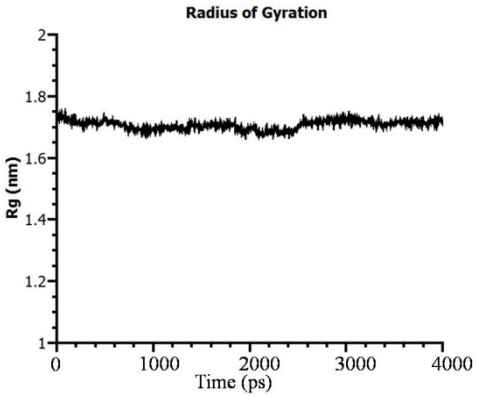


Fig. 6: Radius of gyration for Protease- Azadirachtin complex.

The atomic fluctuations were measured by calculating backbone-backbone RMSD during the simulation of 4 ns. Fig. 7 shows the backbone-backbone conformations RMSD and predicts that from 0 ps to 2200 ps of the simulation, the RMSD slowly increases to 0.35 nm and later becomes constant until end of the simulation of 4 ns. (Fig. 7). This shows that the numbers of atomic fluctuations are quite low after 2200 ps and reveals the stability of the system after 4 ns.

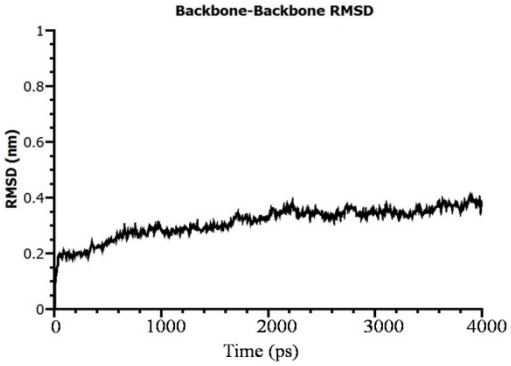


Fig. 7: Backbone-Backbone conformations RMSD for the Protease- Azadirachtin complex during the MD simulation of 4 ns.

Furthermore, the number of hydrogen bonds formed between the Protease enzyme and Azadirachtin during the MD simulation was measured. Fig. 8 shows that always minimum two hydrogen bonds are established between the protease enzyme and Azadirachtin during most of the simulation time of 4 ns. From 2000 ps to 2500 ps and from 3000 ps to 4000 ps, one addition hydrogen bond is forming between protease enzyme and Azadirachtin.

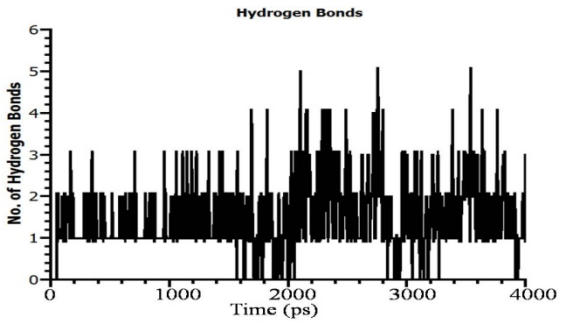
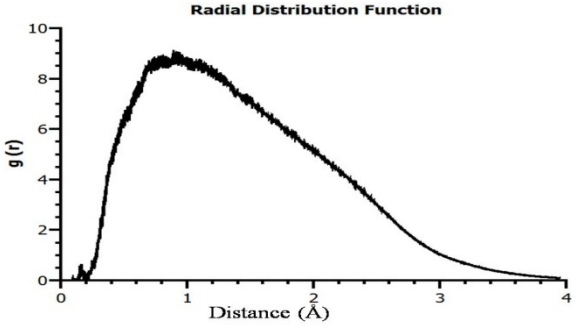


Fig. 8: Pattern of hydrogen bonds formed between Protease and Azadirachtin during the MD simulation of 4 ns.

Fig. 9 represents the distance between the protease enzyme and Azadirachtin during the MD simulation.



9: Radial Distribution Function between Protease and Azadirachtin.

The Radial distribution function analysis reveals that the density of protease enzyme and Azadirachtin vary with maximum distance of 1 Å.

The different conformations generated during the MD simulation of NS3 protease-Azadirachtin complex was clustered using single linkage (nearest neighbor) method within RMSD cut off 0.1 nm from the centre structure. All the conformations were sorted into two clusters. First cluster contains very few structures with all conformations from 0 ps to 50 ps are stored in first cluster. Later, after 50 ps until 4 ns, all conformations are stored in cluster 2. This shows that there are little changes in the conformations of the complex and system becomes stable after 50 ps.

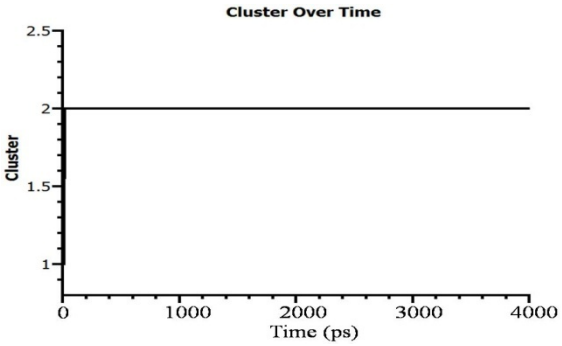


Fig. 10: Clustering of different conformations of Protease-Azadirachtin complex during the MD simulation.

Snapshot was extracted at the end of the simulation for considering stabilized complex system to find hydrogen bonds interaction between Protease and Azadirachtin. Fig. 11 reveals that Azadirachtin makes hydrogen bonds with the Ser 196, Thr 195 and Ile 97 amino acid residues of protease enzyme. Therefore, the complex molecular dynamics simulation is able to find stabilized complex and refined the interaction between protease and Azadirachtin compound.

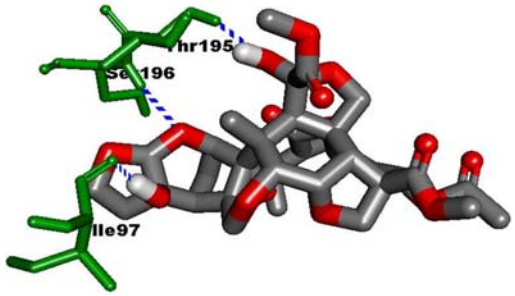


Fig. 11: Stabilized Protease-Azadirachtin complex structure at 4000 ps of the MD simulation time.

4. DISCUSSION

The NS3 protease enzyme from dengue virus was targeted for our study. It is a non structural protein present in the dengue virus and activated by interaction with the NS2B and forms activated NS2B-NS3 pro complex. Therefore, NS3 protease is always a major drug target for designing anti-viral drugs against dengue. Attempts have been made to find chemical drugs that hinder the function of NS3 protease enzyme. However, no specific cures have been available to treat dengue disease. Therefore, the treatment is becoming very expensive and the chemical drugs creating several side effects. In our work, we proposed the application of computational molecular docking and dynamics to find natural inhibitor for NS3 protease. Azadirachtin from seeds of the neem tree shows best affinity for NS3 protease. Azadirachtin is well known for anti-microbial activities. Its complex structure interacts with different enzymes of microbes and reduces their activity. This stops the growth of microbes in the host cell. Similarly, we propose that Azadirachtin strongly inhibit the activity of NS3 protease and hinder the dengue viral replication which prevents infection of the dengue virus. Therefore, our computational study is able to find natural inhibitor against NS3 protease which may act as potential candidate for designing natural anti-viral therapy against dengue virus.

5. CONCLUSION

Our research work concludes that natural compound Azadirachtin finds best affinity for NS3 protease enzyme. Azadirachtin is known to have anti-bacterial and anti-fungal properties. We are proposing that Azadirachtin may be used for anti-viral therapy as well. In addition, our computational approach may help in designing novel drugs for treating dengue disease. Our application of molecular docking and molecular dynamics may help the experimental biologist for fast screening of compounds which will reduce the load of testing large number of compounds by experiment.

6. ACKNOWLEDGEMENTS

Indu Chaturvedi acknowledges Indian Science and Technology Organization (ISTO) and CIRG, Mathura for providing all financial supports. IC, CJ and OR are very thankful to New Era Proteomics, Delhi and France for providing computer facilities to run computational calculations.

REFERENCES

[1] Guha-Sapir, D., and Schimmer, B., "Dengue fever: new paradigms for a changing epidemiology", *Emerg. Themes Epidemiol.*, 2**,** 2005**,** pp. 1.

[2] Halstead, S. B., **"**Observations related to pathogensis of dengue hemorrhagic Fever VI. Hypotheses and discussion", *Yale J. Biol. Med.*, 42, 1970, pp. 350-362.

[3] Carlos, C. C., Oishi, K., Cinco, M. T. C., Mapua, A., Inoue, S., Cruz, D. J.,Pancho, M. A., Tanig, C. Z., Matias, R. R., Morita, K., Natividad, F. F., Igarashi, A.and Nagatake, T., "Comparison of clinical features and hematologic abnormalities between dengue fever and dengue hemorrhagic fever among children in the Philippines", *Am. J. Trop. Med. Hyg.,* 73, 2005, pp. 435-440.

[4] Guzman, M. G., Kouri, G., Bravo, J., Valdes, L., Vazquez, S. and Halstead, S.B., "Effect of age on outcome of secondary dengue 2 infections", *Int. J. Infect. Dis.*, 6, 2002, pp. 118-0124.

[5] Kittigul, L., Pitakarnjanakul, P., Sujirarat, D. and Siripanichgon, K., "The ifferences of clinical manifestations and laboratory findings in children and adults with dengue virus infection", *J. Clin. Virol.*, 39, 2007, pp.76-81.

[6] Nakao, S., Lai, C. J. and Young, N. S., "Dengue virus, a flavivirus, propagates in human bone marrow progenitors and hematopoietic cell lines", *Blood*, 74, 1998, pp. 1235-1240.

[7] Limon-Flores, A. Y., Perez-Tapia, M., Estrada-Garcia, I., Vaughan, G., Escobar-Gutierrez, A., Calderon-Amador, J., Herrera-Rodriguez, S.E., Brizuela- Garcia, A., Heras-Chavarria, M., Flores-Langarica, A., Cedillo-Barron, L. and Flores-Romo, L., "Dengue virus inoculation to human skin explants: an effective approach to assess in situ the early infection and the effects on cutaneous dendritic cells", *Int. J. Exp. Pathol.*, 86, 2005, pp. 323-334.

[8] Wu, S. J., Grouard-Vogel, G., Sun, W., Mascola, J. R., Brachtel, E., Putvatana, R., Louder, M. K., Filgueira, L. M., Marovich, A., Wong, H. K., Blauvelt, A., Murphy, G. S., Robb, M. L., Innes, B. L., Birx, D. L., Hayes, C. G. and Frankel, S. S., "Human skin Langerhans cells are targets of dengue virus infection", *Nat. Med.*, 6, 2000, pp. 816-820.

[9] Lin, C., Amberg, S. M. , Chambers, T. J., Rice, C. M., "Cleavage at a novel site in the NS4A region by the yellow fever virus NS2B-3 proteinase is a prerequisite for processing at the downstream 4A/4B signalase site", *Journal of Virology*, 67, 4, 1993, pp. 2327-2335.

[10] Arias, C. F., Preugschat, F., Strass, J. H., "Dengue 2 virus NS2B and NS3 form a stable complex that can cleave NS3 within the helicase domain", *Virology*, 193, 2, 1993, pp. 888-899.

[11] Lobigs, M., "Flavivirus premembrane protein cleavage and spike heterodimer secretion require the function of the viral proteinase NS3", "*Proceedings of the National Academy of Sciences of the United States of America*, 90, 13, 1993, pp. 6218-6222.

[12] Chambers, T. J., Hahn, C. S., Galler, R., Rice, C. M., "Flavivirus genome organization, expression, and replication", *Annual Review of Microbiology*, 44, 1990, pp. 649-688.

[13] Falgout, B., Pethel, M., Zhang, Y. M., Lai, C. J., "Both nonstructural proteins NS2B and NS3 are required for the proteolytic processing of dengue virus nonstructural proteins", *Journal of Virology*, 65, 5, 1991, pp. 2467-2475.

[14] Yusof, R., Clum, S., Wetzel, M., Murthy, H. M. K. and Padmanabhan, R., "Purified NS2B/NS3 serine protease of dengue virus type 2 exhibits cofactor NS2B dependence for cleavage of substrates with dibasic amino acids *in vitro"*, *The Journal of Biological Chemistry*, 275, 14, 2000, pp. 9963-9969.

[15] Geiss, B. J., Stahla, H., Hannah, A. M., Gari, H. H., Keenan, S. M., "Focus on flaviviruses: current and future drug targets", *FutureMedicinal Chemistry*, 1, 2, 2009, pp. 327-344.

[16] Tomlinson, S. M., Malmstrom, R. D., Watowich, S. J., "New approaches to structure-based discovery of Dengue protease inhibitors", *Infectious Disorders-Drug Targets*, 9, 3, 2009, pp. 327-343.

[17] Martina, B. E. E., Koraka, P., Osterhaus, A. D. M. E., "Dengue virus pathogenesis: an integrated view", *Clinical Microbiology Reviews*, 22, 4, 2009, pp. 564-581.

[18] Zandi, K., et al. "Flavone enhances dengue virus type-2 (NGC strain) infectivity and replication in Vero cells", *Molecules*, 17, 2012a, 2437-2445.

[19] Huh, S. U., & Paek, K. H., "Plant RNA binding proteins for control of RNA virus infection", *Front Physiol*, 4, 2013, pp. 397.

[20] Kumar, S. & Pandey, A. K., "Chemistry and Biological Activities of Flavonoids: An Overview", *Scientific World Journal*, 29, 2013, pp. 165.

[21] Moghaddam, Ehsan., Teoh, Boon-Teong., Sam, Sing-Sin., Lani, Rafidah., Hassandarvish, Pouya., Chik, Zamri., Yueh, Andrew., Abubakar, Sazaly., Zandi, Keivan., "Baicalin, a metabolite of baicalein with antiviral activity against dengue virus", *Scientific Reports*, 4, 2014, pp. 5452.