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Small Molecules Screening Against *Type 2 dengue virus envelope protein (DenV e)* from Phyto Antiviral Ligands–An Insilico Analysis

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Abstract

Dengue fever is one of the mosquito-borne diseases caused by all the four serotypes of Dengue viruses (DenV1, V2, V3, and V4). Dengue viruses are single positive-stranded RNA virus, its genome codes for three structural proteins and seven nonstructural proteins, among these the glycosylated envelope protein is major surface protein and mediate both cell attachment and fusion with host cellular membrane. 158 Ligands from medicinal plants were screened against DenV adhesion. Structures of DenV E protein and ligands were downloaded and used to further modifications and analysis like Lipinski drug filter analysis, removal of hetero atoms, energy minimization, docking, binding site analysis and toxicity analysis by using Koba^{MIN}, Openbabel, PYRX, PYMOL, chimera, ADME Tox . Naringenin, Quercetin and Fisetin molecules show excellent docking results with very minimal toxicity.

Keywords: DenV E protein, Koba^{MIN} server, Openbael, ADME Tox and Chimera-1.8rc, Naringenin, Quercetin and Fisetin.

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1. Introduction

Dengue virus is a single positive stranded RNA virus, belongs to *Flaviviridae* family. Most commonly the dengue fever is transmitted through *Aedes aegypti* mosquito and *Aedes albopictus* also act as a vector for DenV in India. In recent years, dengue fever has emerged as one of the world's most rapidly spreading and important infectious diseases. Viral RNA have 11000 bp and codes for three structural proteins such as Capsid protein C, Membrane protein M, Envelope protein E along with seven non-structural proteins, that as NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5. The glycosylated envelope protein is a major surface protein which mediates both cell attachment and fusion of the viral cell with host cell [1]. Dengue envelope protein is composed of three distinct domain referred to domain I (DI), II (DII) and III (DIII), are identified as antigenic domains [3]. In general electrostatic interactions between the negatively charged sulphate groups in the Glycosaminoglycans (GAGs) of host and exposed basic residues such as lysine and arginine in the DenV protein are thought to mediate the molecular recognition process

[5]. Site directed mutagenesis analysis on Den V II prove that the lysine of 291 and 295 in Domain III is responsible for interaction with host cell surface Glycosamino Glycans [1]. In this research we carried out docking studies of antiviral ligands from medicinal plants to inhibit initial attachment towards the host cell surface GAGs.

2. Materials and Method

Molecules pretreatment:

Envelope protein of type 2 virus structure was retrieved from PDB. Hetero atoms were removed by using BIOSIUTE 4.0, KoBA^{MIN} server is used to minimize energy to get an optimal stability and activity protein structure. Common antiviral ligands from medicinal plants were retrieved from PUBCHEM and filtered by Lipinski filter tool. OPENBABEL software was used to energy minimization for ligands.

Sources of Ligands:

We select the following 28 medicinal plants from literature studies which has been exhibiting common antiviral properties for our analysis. Kenyan Carissa edulis, Phytophthora infestans, Phytolacca Americana, Phytolacca farmosus, Nicotiana glutinosum, Lyconis radiate, Lyconis radiate, Chelidonium manis, Psychatria impacachuanha, Cabuncala sp, Striolata sp, Bochmeria cylindrical, Clivia miniata, Olives sp, Sorbus ancuporia, Quercus sp, Prunus domestica, Citrus sp, Chlorophora tinctoria, Acacia sp, Rhus sp, Citrus sp, Acacia sp, Citrus sp, Citrus sinensis, Balduina angustifolia, *Malus domestica* , Leucothac keikei and Zizia aptera [6,8,9].

Docking:

158 antiviral ligands from 28 plats were downloaded in sdf format and Energy minimized using OPENBABEL [2]. All ligands were docked against type 2 DenV E protein by using PYRX 8.0. From the docking result top five ligands were selected for further analysis

Binding site analysis:

After docking the structures of protein and ligands are saved separately, PyMOL is used to get a docked structure and then chimera is used to get efficient analysis about atom interactions, types of bonds between ligand and protein with its localization.

Risk Analysis (ADME Tox): Pharmaco kinetics and Toxicity analysis were carried out for the ligand molecules using -ADME Tox tool.

3. Results and Discussion

Results

List of Top five ligands:

Among the 158 Ligands top five ligands which required very minimal energy to bind are selected to risk analysis

Table 1: List of Top five ligands

S.No	Plant Sources	Ligands	Binding Energy (kcal/mol)	Active site (Ligand Binding site on the Protein)
1.	*Kenyan Carissa edulis*	CID 73145 (Beta amyrin)	-9.4	A35.B, E13.B, F337.B, N355.B, R350.B, V354.B,
2.	*Citrus paradisi*	CID 439246 (Naringenin)	-9.1	D249.B, E44.A, G28.A, H244.B, H27.A, K246.B, L247.B, L277.A, N276.A, P243.B
3.	*Malus domestica*	CID 5280343 (Quercetin)	-8.6	D249.B, E44.A, G28.A, H244.B, H27.A, K246.B, K247.B, N242.B, R2.A,
4.	*Malus domestica*	CID 5281614 (Fisetin)	-8.4	A245.B, E44.A, G28.A, H244.B, H27.A, K246.B,, K247.B, P243.B, R2.A
5.	Kenyan Carissa edulis	CID 259846 (Lupeol)	-8.1	A35.B, E13.B, K36.B, L351.B, N355.B, N37.B, R350.B, V354.B

Note: Underlined Amino acids indicate polar (Hydrogen bond) interaction between protein and ligand.
Non-Underlined Amino acids indicate non-polar interaction between protein and ligand.

Table 2: Drug Likeness Property

S.No	Ligands	Lipinski Rule				
		MW	**HBA**	**HBD**	**Log P**	**MR**
1.	CID 73145	426	0	1	7.137	130.60
2.	CID 259846	426	0	1	6.993	130.53
3.	CID 439246	272	2	3	2.510	66.32
4.	CID 5281614	286	6	4	1.970	111.13
5.	CID 5280343	302	2	5	0.974	73.91

HBA = Hydrogen Bond Acceptor; **HBD** = Hydrogen Bond Donar; **MW** = Molecular Weight;
MR = Molecular Refractivity;

Risk Analysis:

ADME Tox tool was used to find structural properties (MW, H bonds, lipophilicity, shape & reactivity), physicochemical properties (solubility, permeability & chemical stability), biochemical properties (metabolism, transportor affinity & target affinity) and pharmacokinetics (half-life & bioavailability) of the ligand were analyzed.

Table 3: Risk Analysis

S.No	Ligands	Lipinski rule of 5	Veber Rule	Egan Rule	Bayer oral phychem score	GSK 4/400 Rule	Pflzer 3/75 Rule	Phospho-lipidosis Non inducer
1.	CID 73145	MR	NR	NR	NR	HR	HR	NR
2.	CID 259846	MR	NR	NR	NR	HR	HR	NR
3.	**CID 439246**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**
4.	**CID 5281614**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**
5.	**CID 5280343**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**

NR = NO RISK**; MR** = MEDIUM RISK**; HR** = HIGH RISK

Structure of Target Protein

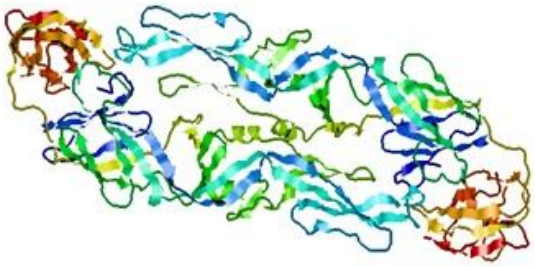


Figure1: DenV2 E (1OKE)

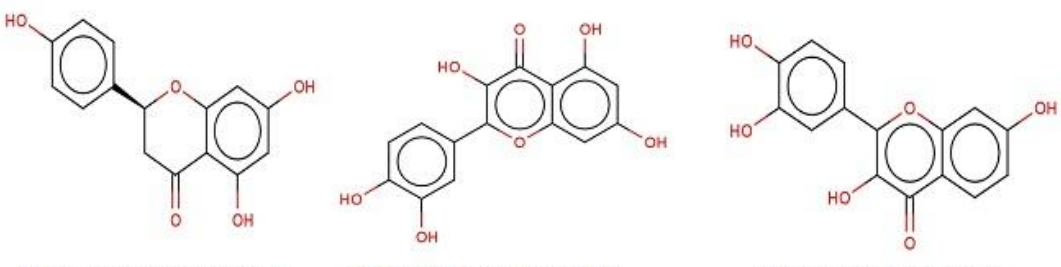
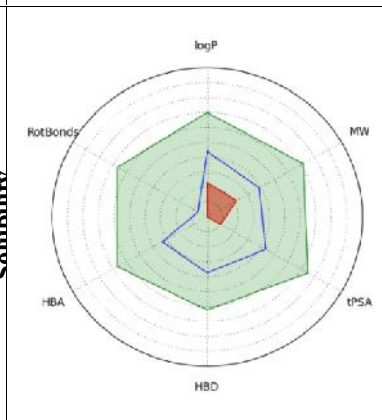
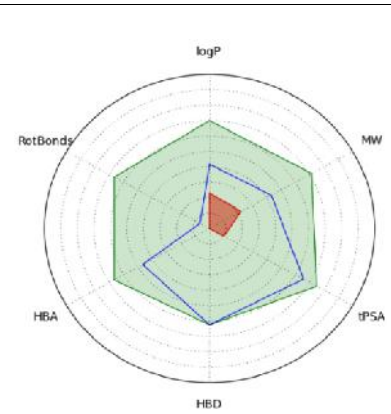
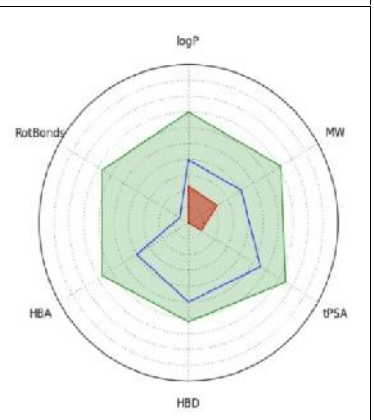
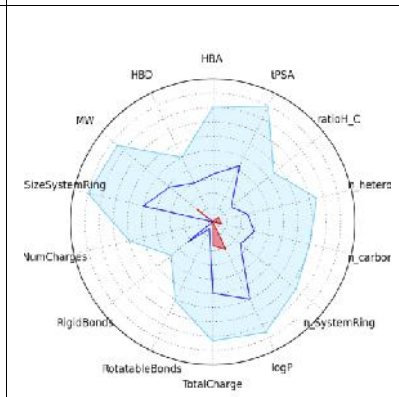
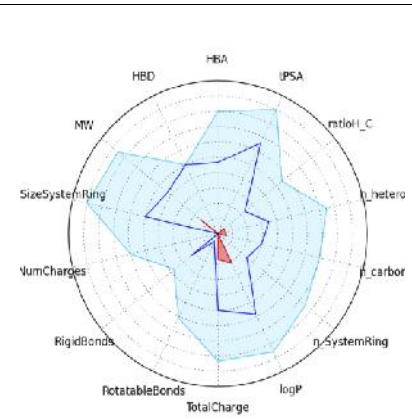
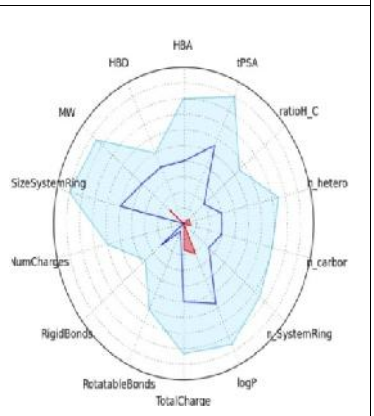
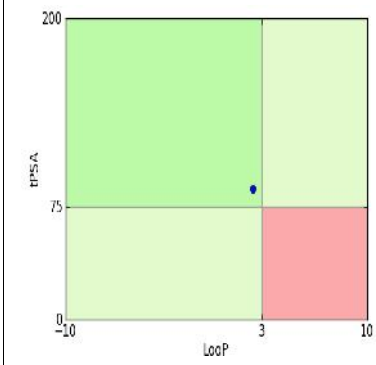
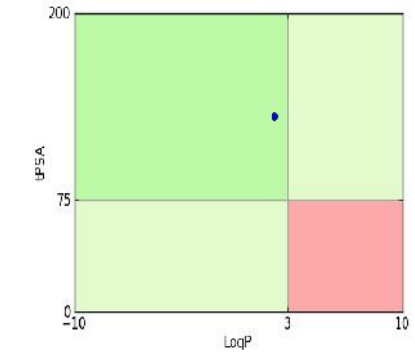
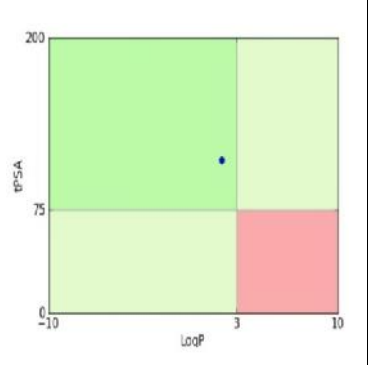


Figure 2: structures of non-risk ligands

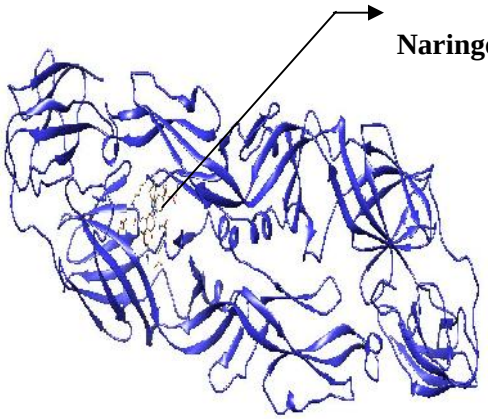
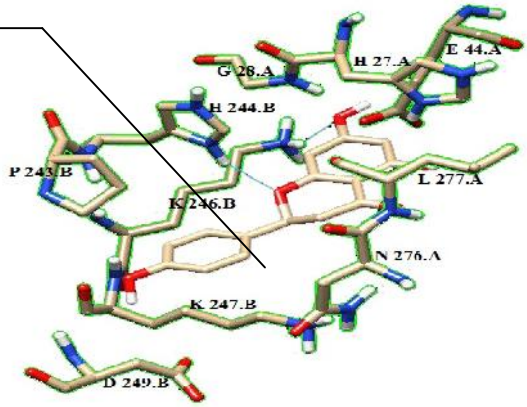
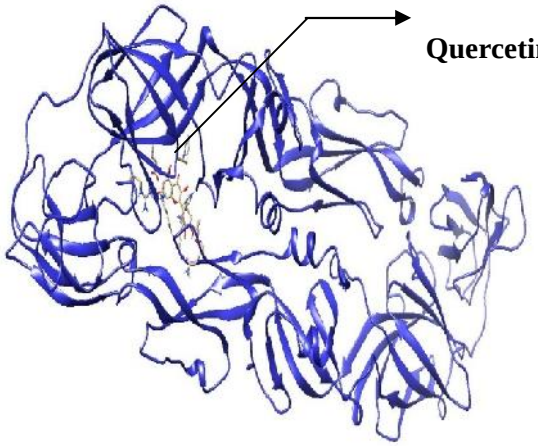
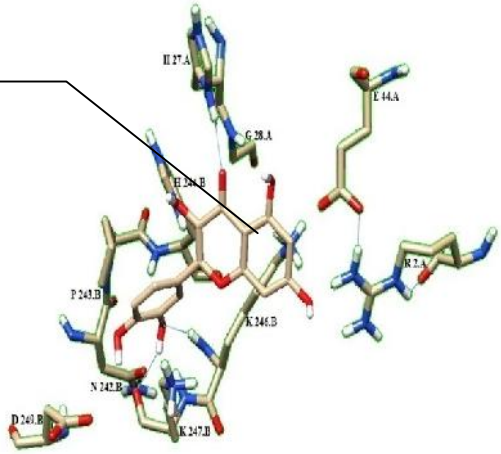
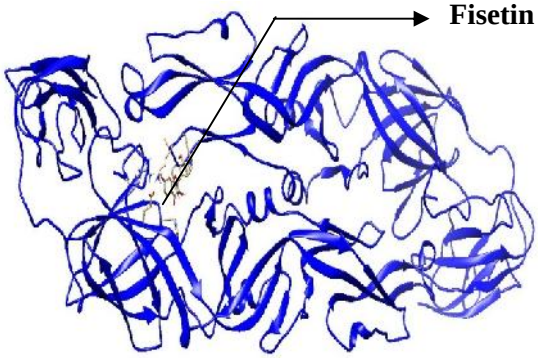
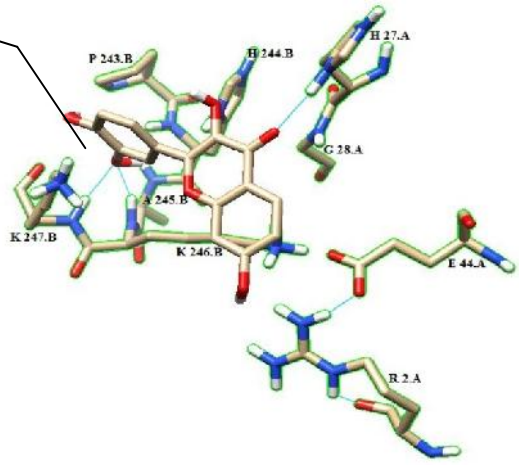
ADME Tox Analysis:

	Naringenin (CID 43924)	Quercetin (CID 5280343)	Fisetin (CID 5281614)
Oral Absorption Estimation Solubility			
Physicochemical Filter Positioning			
Toxicity Positioning			

From the above risk analysis all the three ligands have good score in oral absorption, physico- chemical properties because the position lies between minimal and maximal range and both the ligands fell in freely soluble region.

Binding site analysis:

Binding sites of ligands on two different types of proteins with respective of chains, amino acids and positions of docking details are given in the table.

	Docking	Binding site analysis
1OKE_Naringenin complex		
1OKE_Quercetin complex		
1OKE_Fisetin complex		

Discussion

Ligands bind to the pocket of domain III of type 2 dengue virus surface Glyco protein and the binding energies are identified as - 9.4, -9.1, -8.6, -8.4, -8.1 kcal/mol by β amyrin, Naringenin, Quercetin, Fisetin, Lupeol, respectively. Among the five Naringenin, Quercetin and Fisetin are identified as no risk compounds to the host and it binding site analysis shows two polar interaction (H bond), eight non polar interaction and three polar interaction (H bond), six non polar interaction and three polar interaction (H bond), five non polar interaction with their protein respectively. The no risk ligands show optimal result in drug likeness property analysis. In the ADME Tox figure the oral absorption estimation and solubility of three ligands (blue line) falls away from the negative area (pink) and also exhibits very good physico-chemical, biochemical and pharmacokinetics scores because it touches the maximum positive area (blue colored) properties. Toxicity results of ligands also in the dark green square (safe region) area; chemical structure of ligands indicates that these phytochemicals were made up of Flavonone backbone. Naringenin, Quercetin and fisetin binds with the lysine and arginine in the domain III of surface protein.

4. Conclusion

Among the 158 anti-viral ligands only three (Naringenin, Quercetin and Fisetin) possess very good activity against type 2 dengue virus initial attachment with host cell surface protein. Binding capability and drug likeness properties of Naringenin, Quercetin and Fisetin are analysed and proved by contemporary bio informatics tools. By this in-silico analysis, we conclude these three ligands are better drug molecule against dengue fever and this research will be useful for in-vivo analysis to prove as potent drugs in future.

5. References

1. Daniel Watterson, Bostjan Kobe1, and Paul R. Young, "Residues in domain III of the dengue virus envelope glycoprotein involved in cell-surface glycosaminoglycan binding'' *Journal of General Virology)*, **2012**, 93: 72–82.
2. Shao-Jun Chen and Ji-Long Ren, Identification of a Potential Anticancer Target of Danshensu by Inverse Docking, *Asian Pacific Journal of Cancer Prevention,* **2014**, 15: 111-116.
3. Guirakhoo F, Heinz F. X, Kunz C**,** Epitope model of tick-borne encephalitis virus envelope glycoprotein E: analysis of structural properties, role of carbohydrate side chain, and conformational changes occurring at acidic pH. *Virology*, **1989**, 169: 90–99**.**
4. H. William, D. Andrew, S. Klaus. VMD - Visual Molecular Dynamics, *Journal of Molecular Graphics*, **1996**, 14: 33-38.
5. Hileman R. E, Fromm J. R, Weiler J. M, Linhardt R. J, Glycosaminoglycan–protein interactions: definition of consensus sites in glycosaminoglycan binding proteins. *Bioessays*, **1998**, 20: 156–167.
6. Keivan Zandi, et al., Antiviral activity of four types of bioflavonoid against dengue virus type-2, *Virology Journal*, **2011**, 8: 560.
7. O. Trott , A. J. Olson. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, *Journal of Computational Chemistry*, **2010**, 31, 455-461.
8. Shi-you Li a,b, Cong Chena,e, Hai-qing Zhang, Identification of natural compounds with antiviral activities against SARS-associated coronavirus, *Antiviral Research,* **2005,** 67: 18–23
9. Tolo M. Festus ,Rukunga M, The antiviral activity of compounds isolated from Kenyan Carissa edulis (Forssk.) Vahl, *The Internet Journal of Alternative Medicine ISSN:* 1540-2584.