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Anti-viral Activity of Neem Over Zika Virus Using Autodocking

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ABSTRACT

Zika Virus (5IRE) is a single stranded RNA genome. Neem components like nimbin, nimbolide, catechin, gallic acid, gedunin, azadirachtin, mahmoodin, epicatechin, margolone, margolonone, ismoargolononе have better antiviral activity over zika virus. Neem has various other properties like anti-inflammatory, spermicidal, anti-arthritic, antipyretic, hypoglycemic, antiulcer, immunomodulatory, antifungal, antibacterial, diuretic, antimalarial. The library of ligands was taken from NCBI (National Centre for Biotechnology Information), and protein from RCSB Protein Data Bank. The top docked result for both agonist and antagonist was taken and their analogues were created and thus autodocking plays a vital role in drug discovery.

Keywords: Zika(5IRE), autodock, neem, ncbi.

1. INTRODUCTION

Zika virus (ZIKV) is a member of the virus family *Flaviviridae* and the genus *Flavivirus*. It is transmitted by *Aedes* mosquitoes[1], such as *A. aegypti* and *A. albopictus*. *Aedes aegypti* is also responsible for causing dengue and chikungunya fevers. Common symptoms of the zika virus include acute onset of fever, maculopapular rash, arthralgia, or non-purulent conjunctivitis. The virus was first isolated in 1947 in the Zika Forest of Uganda. There is a close link between Zika virus with dengue, yellow fever, Japanese encephalitis, and West Nile viruses.

In north brazil, Zika strain was detected in the amniotic fluid of pregnant women. In prenatal ultrasounds, microcephaly [2], was developed in the fetus. Microcephaly is a disease with decreased head circumference, that result in abnormal brain development and leads to mental and motor defects such as cerebral palsy. This strongly indicates the correlation to this virus and the development of fetal malformation. The zika infected mother having a febrile rash during pregnancy. Zika infections in adults may cause Guillain–Barré syndrome.

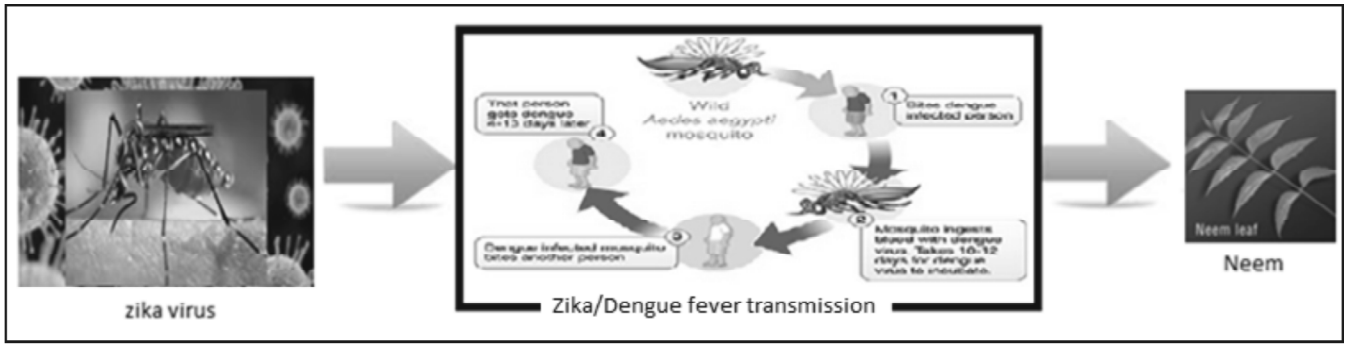

Figure 1: Neem control the breeding of aedes aegypti mosquities

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Zika virus prevention: Reducing exposure to mosquito bites reduces the risk caused by zika virus. EPA registered insect repellents are advisable for pregnant women. Neem control the breeding of aedes aegypti mosquities [3], and it has good larvicidal efficacy.

2. STRUCTURE OF ZIKA VIRUS

Zikavirus is enveloped and icosahedral and has a nonsegmented, single-stranded, 10 kilobase positive-sense RNA genome. It is most closely related to the Spondweni virus.

A positive-sense RNA genome can be directly translated into viral proteins. The structural proteins surrounds the virus. The replicated RNA strand is held within a nucleocapsid formed from 12-kDa protein blocks; the capsid is contained within a host-derived membrane modified with two viral glycoproteins[4].There are two lineages of Zika: the African lineage and the Asian lineage. The epidemic virus spreading in the America is 89% identical to African genotypes, but in French Polynesia, it was found to be Asian strain.

A. LIGAND

B. Nimbin

Nimbin is a triterpenoid seperated from Azadirachta indica[14]. It act as anti-inflammatory, agent, antipyretic, fungicidal, medicine and antiseptic properties. Nimbin has good antiviral efficacy against the Zika/dengue fever virus.

C. Nimbolide

Nimbolide is a terpenoid lactone derived from Azadirachta indica. Nimbolide exerts its antimalarial activity by inhibiting the growth of *Plasmodium falciparum*. It also shows antibacterial activity against *S. aureus* and *S. coagulase* and also acts as an anti-tumor agent[15].

D. Azadirachtin

Azadirachtin is a highly oxygenated C-secomeliacins isolated from neem seed. It is a tetranortriterpenoids[5]. It is structurally resembles insect hormones known [16,17] as “ecdysones” that manage the process of metamorphosis. Azadirachtin act as a “ecdysones blocker” and it break the metamorphosis. Thus it exerts its insecticidal effect. It is having strong antifeedant activity as well as antimalarial property and it inhibits the growth of malarial parasites.

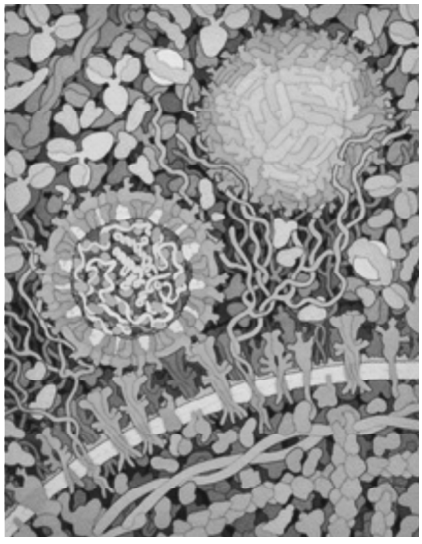

Figure 2: Cross-section of Zika virus, with capsid layer (pink), membrane layer (purple), and RNA genome (yellow)

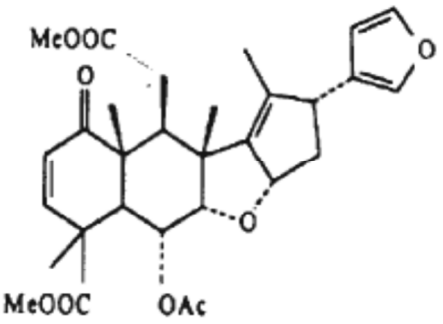

Figure 3: Structure of Nimbin (NEEM)

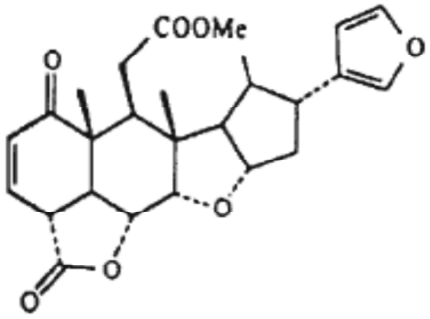

Figure 4: Structure of nimbolide

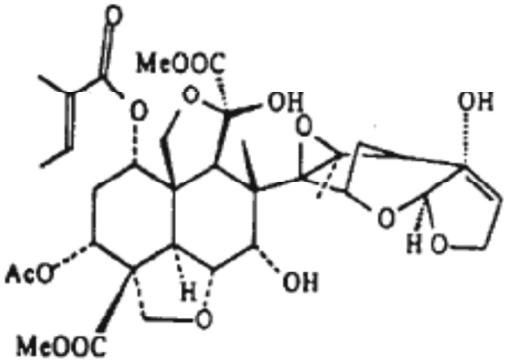

Figure 5: Structure of Azadirachtin

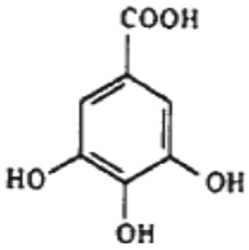

Figure 6: Structure of gallic acid

E. Gallic Acid

Gallic acid, a trihydroxybenzoic acid, is a sort of synthetic resin acid found in gallnuts, sumac, witch hazel, tea leaves, oak bark. Its molecular formula is $C_6H_2(OH)_3COOH$. Gallic acid is found freely as a part of chemical reaction tannins[16]. Gallic acid acts on the cell membrane of the virus through hydrophobicity changes, decrease of negative surface charge, and occurrence of local rupture or pore formation in the cell membranes with consequent leakage of essential intracellular constituents.

F. Gedunin

Gedunin acts as antineoplastic agent. It modulates the cell survival and apoptosis pathways. In vitro treatment of ovarian cancer cell lines namely SKOV3, OVCAR4, and OVCAR8 with gedunin alone produced up to an 80% decrease in cell proliferation. It also exerts its antifungal and antimalarial activities[15].

G. Epicatechin

Pure epicatechin is a scentless white powder. Epicatechin may be a flavonol belonging to the cluster of flavonoids[6]. Picatechin is known to be a sturdy inhibitor that has insulin like actions.

H. Catechin

Catechins, the most important element of tea extract, have numerous physiological effects. There are few studies, however, on the results of catechins on body fat reduction in humans. It has been believed that the body mass index (BMI) correlates with the quantity of malondialdehyde and thiobarbituric acid-reactive substances within the blood. Catechins are dietary polyphenolic compounds[7,15]. The anti-inflammatory effects are activated through modulation of nitric oxide synthase isoforms and its neuroprotective capabilities helps in reducing the cerebral ischemia and it also plays role in attenuating oxidative stress.

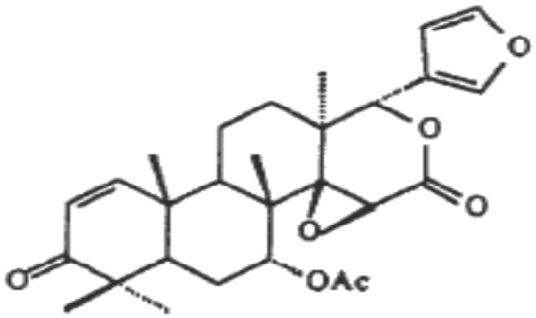

Figure 7. Structure of gedunin

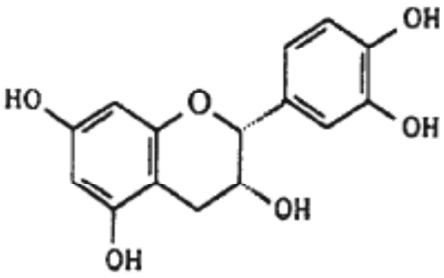

Figure 8. Structure of Epicatechin

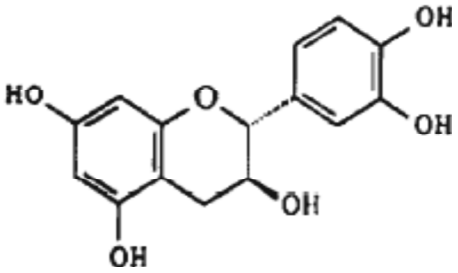

Figure 9: Structure of Catechin

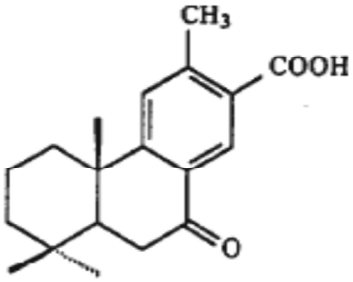

Figure 10. Structure of Catechin

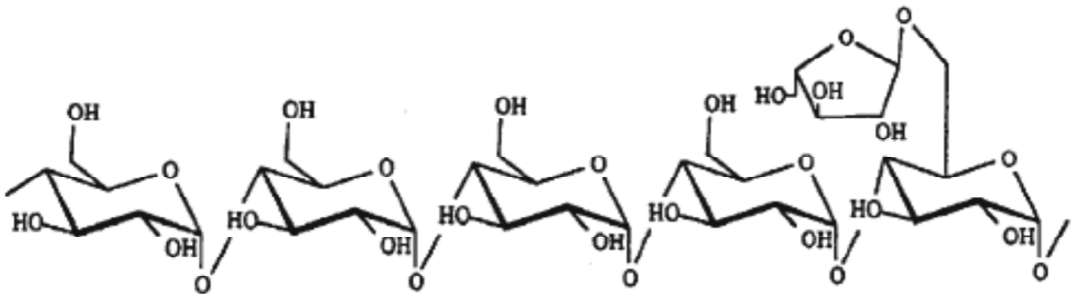

Figure 11. Structure of Gla

I. Margolone

It is an antidepressant diterpenoid isolated from Azadirachta indica stem bark area. It is known to be active against *Klebsiella, Staphylococcus* and *Serratia* species.The formula is $C_{19}H_{24}O_3$ and therefore the relative molecular mass is 396.The artificial name of margolone is 2-Phenanthrenecarboxylic acid, 4b,5,6,7,8,8a,9,10-octahydro-3,4b,8,8-tetramethyl-10-oxo-, (4bS-trans)[8,16].

J. Gla

Gamma linolenic acid (GLA) is a member of the *n*-6 family of polyunsaturated fatty acids and can be produced from linoleic acid (LA) [16,17] by the enzyme delta-6-desaturase. It act as a dietary supplement. It shows its anti-inflammatory action by regulating NF-kappa *B* and AP-1 activation in lipopolysaccharide-induced RAW 264.7 macrophages[9].

K. Mahmoodin

Mahmoodin is a deoxygedunin isolated from seed oil and it possess moderate antibacterial action against some strains of human pathogenic bacteria.

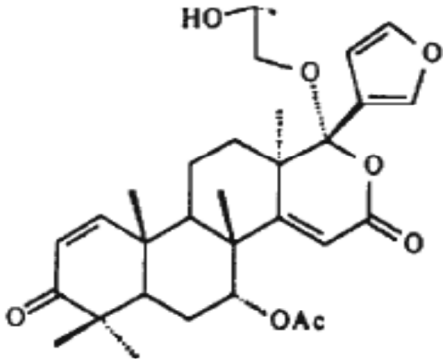

Figure 12. Structure of Mahmoodin

3. MATERIAL AND METHODS

A. Data Set Definition and Preparation

The crystalline structure of 5IRE Zika virus is obtained from the RCSB Protein Data Bank. Out of the following ligands namely nimbin, nimbolide, gedunin, azadirachtin, mahmoodin, gallic acid, epicatechin, catechin, margolone, margolonone, ismoargolonone, cyclic trisulphide, cyclic tetrasulphide, gla, glla, gllla obtained from NCBI website, 10 ligands were chosen as best ligands in terms of ligand interaction. So we have 1 protein and 10 ligands[10].

B. Virtual Screening

Virtual screening (VS) is a technique employed in drug discovery to inspect libraries of tiny molecules and also to view structures that there are a single unit comprising of the molecule that is bound to a drug target, typically a super molecule receptor or protein [11]. Virtual screening techniques play a crucial role within the lead discovery method.

C. Software Required

Autodock and AutoGrid Suite, MGL Tools 1.5.6, Open Babel, Python molecular viewer, Discovery Visual Studio. Operating system: Microsoft windows 8.1/Ubuntu 12.4.

D. Preparation

Download the ligands from the NCBI website and save them in *sdf* format. Now they are converted to *pdbqt* format in openBabael and saved in the working directory.

The receptor is downloaded from the *RCBS* website by its *PDB ID* and saved in *pdb* format in the working directory [9,10]. Unrequired chains are deleted from it by virtual screening techniques in Discovery Visual Studio software and saved into pdp format.

E. Runtime Setup

Open MGL tools and create a working directory. Then read the *pdb* file of the receptor. Delete selected atoms of chains that are not required and delete water, add hydrogens and merge non polar. Add kollman Charge. Write the *PDB* and save it. Load the pdbqt file of the receptor, detect its root and we are ready to proceed to auto grid. Polar Hydrogens are also added to the receptor[12].

F. Autogrid

Choose the receptor as the macromolecule and choose the ligand and save it in pdbqt. Set a covalent map if required. Set a grid box of 60 × 60 × 60 and spacing, center if required. Save it in gpf format and go to run and launch auto grid[11]. Wait for the process manager to close and the *gpf* file is created in our working directory. We are ready for auto dock. Any sort of error would be displayed in the terminal window.

G. Autodock

Choose the macromolecule and set the rigid filename and select the pdbqt macromolecule created during the setup of auto grid [12]. In docking, ligand choose the ligand and click defaults. For search parameters, use Genetic Algorithm and set number of GA runs as 10 (higher is suggested for better results but consumer more time) and use remaining defaults. Set docking output as Lamarckian GA (4.2) and save the output as *dpf*. Now run AutoDock and click launch and wait for the program manager to close (may take some time). Any sort of error would be displayed in the terminal window[13]

H. Analyze

Once Auto Dock completes the calculations, you can then go to: Analyze > Confirmations > Play, ranked by energy. This will show all binding confirmations in order of energy. At this point, you may analyze the results, interactions, and confirmations as needed[13]. Choose the most appropriate conformation based of the correct binding energy and inhibition constant and stabilize the results

4. RESULT

Appropriate results from the analysis phase are selected based on the appropriate conformation with the least binding energy values and the inhibition constant which is equal to the experimental value, *i.e.* Ki = Km are shown below after interacting every ligand with the 5IRE Zika Virus.

S.no	Receptor	Ligand	Binding Energy	Inhib constant
1.	5IRE (ZIKA)	Nimbin	-3.51	2.66 mM
2.		Nimbolide	-4.89	260.67 mM
3.		Gedunin	-5.22	149 mM
4.		Azadirachtin	-3.66	2.09 mM
5.		Mahmoodin	-3.79	1.67 mM
6.		Gallic Acid	-3.03	6.05 mM
7.		Epicatechin	-4.36	633.49 mM
8.		Catechin	-4.42	576.34 mM
9.		Margolone	-5.9	729.98 mM
10.		Gla	-3.16	4.82 mM

Figure 13: Protein ligand interaction value

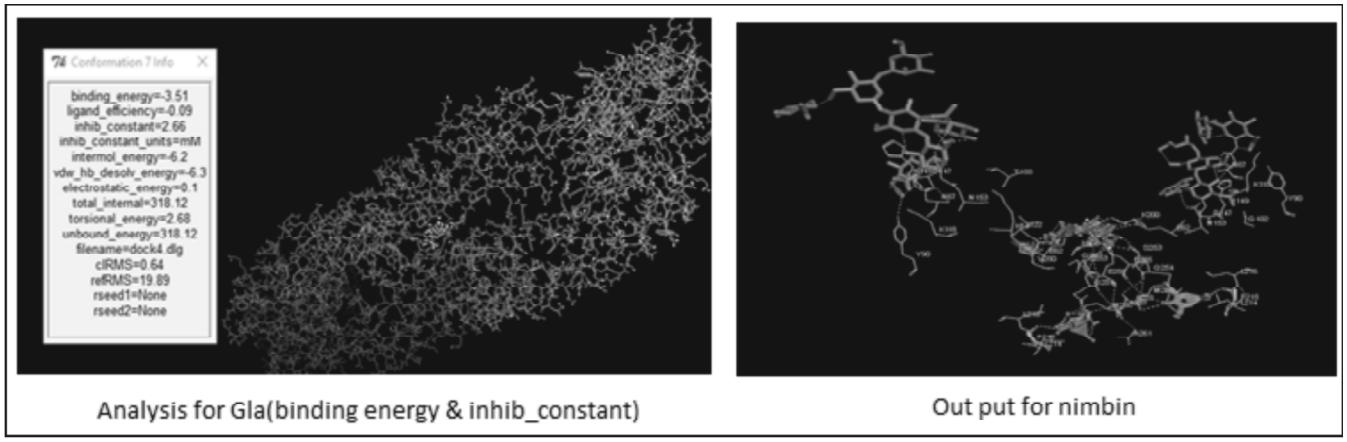

Figure 14: Protein ligand interaction value

CONCLUSION

Auto Dock performs the role in drug discovery which is less time consuming, economical. Neem auto docks with Zika virus creates novel potent drug which eradicate the virus. The drug targeting is possible in the respective receptors. So, the combination of the neem which is the natural constituent with no adverse effects with Zika, the terrific virus gives efficient natural drug to the society.

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