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Research article

MANGROVE PHYTOCHEMICALS INHIBIT AGAINST WEST NILE VIRUS REPLICATION *IN-SILICO* ANALYSIS

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ABSTRACT: West Nile Virus is an arthropod borne virus of genus Flavivirus. Mosquitoes are predominate arthropod vector. WNV is a single stranded, positive sense RNA of about 11 kb containing a single long open reading frame flanked by Non coding region at both ends. The RdRp polymerase activity of Nonstructural protein NS5 has essential role in viral replication. The RdRp duplicates the single stranded RNA genome during a single, continuous polymerization event. In this study we performed docking study on NS5 Methyltransferase protein as target and ligand compounds are selected from the natural products of mangrove derived compounds and performed the docking to inhibit the viral replication process**.** The screened mangrove derived compounds were binding site of WNV RdRp which inhibits the viral replication.
Keywords: West Nile Virus; NS5 Methyltransferase protein; Flavivirus; Mosquito; Mangrove

INTRODUCTION

West Nile virus (WNV) is an arthropod-borne virus of the genus Flavivirus belongs to the family Flaviviridae. It is a member of the Japanese Encephalitis Virus (JEV), Dengue virus, Yellow fever virus, Tick-borne encephalitis virus, and St.Louis encephalitis virus. WNV has now spread globally, with the first case in the Western Hemisphere being identified 1999. [1] WNV has widely distributed throughout the Northern and Central America. [2, 3] Mosquitoes are well-known to transmit dreadful human diseases such as malaria, dengue, Japanese encephalitis and yellow fever at an increasing magnitude with global warming. Mosquito-borne diseases play a major role in loss of human welfare and economy with an annual incidence of 350-500 million clinically manifested cases and death of 1.1 to 2.1 million people. In general, mosquito control programs are largely a failure because of ever increasing resistance of mosquitoes to insecticidal chemicals. [4]. WNV was originally isolated in 1937 from the blood of a febrile patient in the west Nile district of Northern Uganda. [5] Mosquitos are predominate arthropod vector. Mosquito are the competent vector for the WNV, in which the appropriate receptors are present on the endothelial cell lining of the mid gut that allows WNV to invade and replicate in the cell. The virus must be able to escape from the mid gut, penetrate and replicate in the salivary glands. [6] The potential for mosquito saliva to impact the course of WNV disease was demonstrated. [7, 8, 9] The West Nile virus (WNV) is transmitted through female mosquitoes, the infected mosquito species *Culex pipiens*, *Culex tarsalis* and *Culex quinquefasciatus* are the main sources. [10]. The genome of WNV is a single stranded, positive sense RNA of about 11 kb containing a single long open reading frame flanked by Non coding region at both ends. The 5' region of the genome encodes structural protein, whereas the 3' region encodes non-structural proteins. The Nucleocapsid of about 30 nm in diameter consists of capsid and genomic RNA and it is surrounded by a lipid bilayer in which the viral envelope and membrane protein are embedded, approximately 11 kb in length. [11] RNA contains a 5' untranslated region with single open reading frame (ORF) and 3' untranslated region. The ORF encodes 10 viral protein with three structural capsid (C), pre-membrane (PrM) or membrane (M) and envelopes with seven Non-structural (NS1, NS2, NS2B, NS3, NS4A, NS4B and NS5) proteins. [12] The plus genomic RNA is transcribed into a complementary minus sense RNA which in turn serves as the template for the synthesis of more plus sense genomic RNA. [13, 14] The synthesis of plus and minus sense RNA is asymmetric; plus sense RNA is produced in 10 to 100 fold excess over minus sense RNA. [15] The RNA dependent RNA polymerase (RdRp) activity of the Non-structural protein (NS5) is a key activity for viral replication WNV RdRp domains were determined at 3.0 and 2.35 Å resolution. [16] NS5 methyltransferase is also one of the important targets for antiflaviviral drug discovery. [17, 18, 19].

The mosquito species of *Aedes* and *Haemogogus* are the vectors which transmit YFV and its related viruses. It serves as a reservoir for the virus; humans and monkeys are the primary host for viral infection. [20] Marine plants compounds screened for mosquito larvicidal activity and found pyrethrum as a bioactive compound derived from coastal mangrove plants. [21, 22, 23, 24, 25] Computational selections of chemical inhibitors for the sterol carrier protein-2 (AeSCP-2) are highly reliable and novel methods for discovery of potent compounds to control mosquitoes. [26] *In-Silico* docking analysis on Yellow Fever Virus using mangrove derived compounds. [27] The natural drugs from marine sources have received as considerable with fewer side effects based on literature we performed the docking analysis against NS5 methyltransferase protein to inhibit the West Nile Virus viral replication.

MATERIALS AND METHODS

Preparation of ligand structures

The docking analysis of the chemical compounds (Pyrethrum, Triterpenoid, Stigmasterol, Tricin) were selected and derived from mangrove plants. ChemSketch (Chemically intelligent drawing interface freeware developed by Advanced Chemistry Development, Inc., (http://www.acdlabs.com) was used to construct the structure of the ligands. Using draw mode of Chemsketch, the ligands were generated and the three dimensional optimizations were done and then saved in MOL file (a file format for holding information about the atoms, bonds, connectivity and coordinates of a molecule).

Retrieval of the protein structure

The structures of the target receptor binding sites of West Nile Virus (PDB ID: 2OY0), NS5 Methyltransferase protein were obtained from the RCSB Protein Data Bank, http://www.rcsb.org/ pdb

Protein-ligand docking:

Crystal structure of the West Nile virus methyltransferase (2OY0) protein has been taken to found binding sites by DETECT CAVITIES under the PREPARATION option of Molegro Virtual docker (Free trial version), it is detects 5 cavities by default with a 10 Å resolution. Default parameters are used in the molecular docking program Molegro.

RESULTS

Docking analysis

The Mangrove derived phytochemicals (Pyrethrum, Triterpenoid, Stigmasterol, and Tricin) (Fig: 1) were docked with the binding site of the protein NS5 Methyltransferase responsible for viral replication. The ligand molecules docked on active site of West Nile Virus NS5 Methyltransferase protein [PDB ID: 2OY0] (Fig: 2) offers different binding modes for these compounds as they are strongly dependent on the attached substituent. Each phytochemicals formed more number of H-bond interactions. The protein-ligand interaction energy of inhibitors was given by Pose organizer in Molegro Virtual docker (Free trial version) for different poses of inhibitors. The poses values are negative values for interaction energy it reflects the positive docking approach. Hydrogen bond interaction, Electrostatic interactions and binding pocket amino acid residues are shown in the Fig: 3, 4, 5 and Table: 2. The best pose interaction energy for compound are Stigmasterol is -152.073, Pyrethrum is -148.358, Triterpenoid is -117.962 and Tricin is -103.503. The no. of hydrogen bond binding pose and interaction values are shown in the table: 3. The 2D structure and properties of the phytochemical compounds Molecular weight, number of hydrogen bond donors and acceptors for the active principles were noted and listed below Table: 1

Properties of Ligand Molecules

Table: 1. Properties of phytochemical compounds

Properties	Pyrethrum	Triterpenoid	Stigmasterol	Tricin
Molecular Formula	$C_{21}H_{28}O_3$	$C_{30}H_{48}O_7S$	$C_{29}H_{48}O$	$C_{17}H_{14}O_7$
Molecular Weight	328.44522 [g/mol]	552.76292 [g/mol]	412.69082 [g/mol]	330.28886 [g/mol]
H bond acceptors	3	7	1	7
H donors	0	3	1	3

Linear structure of ligand molecules

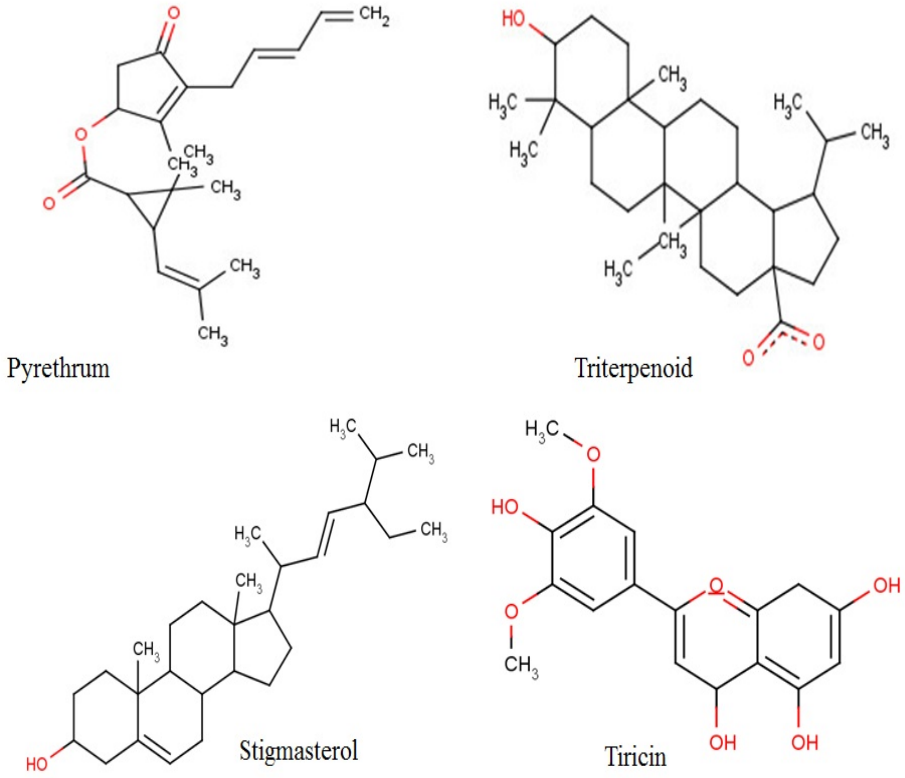

Fig: 1. 2D representation of mangrove derived compounds

Structure of protein

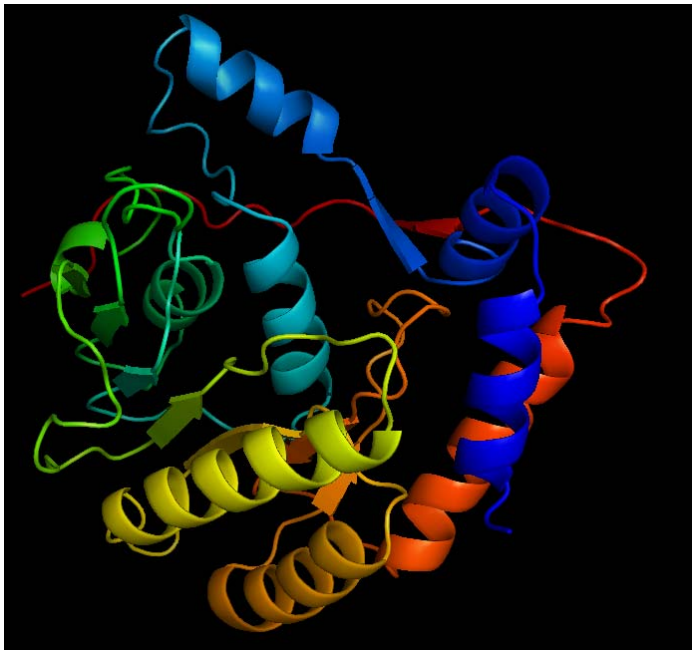

Fig:2. 3D Structure of West Nile Virus NS5 Methyltransferase protein (PDB ID: 2OY0)

Table: 2. Binding cavities Amino Acid residues and atom position

Binding sites amino acid residues

Pyrethrum		Triterpenoid		Stigmasterol		Tricin	
Residues	Molecular atom I.D	Residues	Molecular atom I.D	Residues	Molecular atom I.D	Residues	Molecular atom I.D
Val	168	Phe	133	Lys	105	Phe	133
Glu	169	Tyr	134	His	110	Tyr	134
Leu	172	Ile	147	Asp	131	Ile	147
His	173	Glu	149	Phe	133	Glu	149
Arg	174	His	159	Tyr	134	His	159
Gly	175	Arg	160	Ile	142	Arg	160
Pro	176	Thr	161	Ile	147	Thr	161
Arg	177	Ile	162	Glu	149	Ile	162
Glu	178	Arg	163	His	159	Arg	163
Phe	179	Val	164	Arg	160	Val	164
Leu	198	Met	167	Thr	161	Met	167
Arg	201	-	-	Arg	163	-	-
Tyr	202	-	-	Val	164	-	-
Gly	203	-	-	Leu	165	-	-
Trp	221	-	-	Glu	166	-	-
-	-	-	-	Met	167	-	-

Hydrogen bond interaction

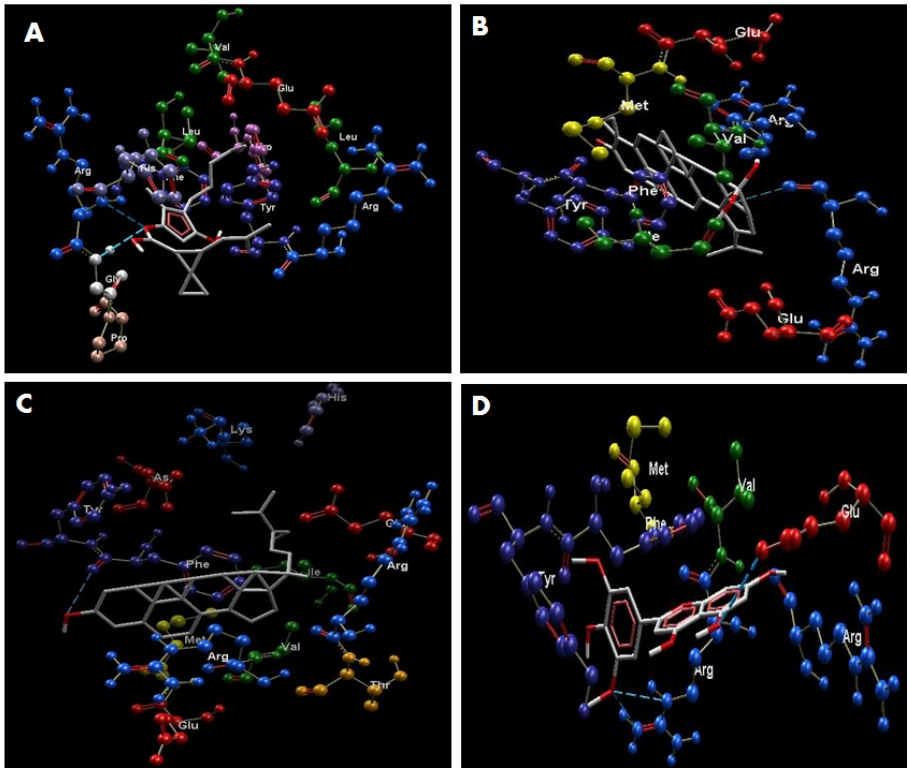

Fig: 3. Hydrogen bond interaction of the target protein active sites with phytochemicals; A-Pyrethrum interaction with target, B- Triterpenoid interaction with target, C- Stigmasterol interaction with target, D- Tricin interaction with target.

Hydrogen bond interaction values

Table: 3. Protein-ligand binding pose and hydrogen interaction value

Ligand	No.of hydrogen bond binding pose	Hydrogen bond interaction value
Pyrethrum	2	-1.85198 -2.38222
Triterpenoid	1	-1.28372
Stigmasterol	1	-1.13044
Tricin	3	-1.70441 -1.22459 -2.5

Electrostatic interaction

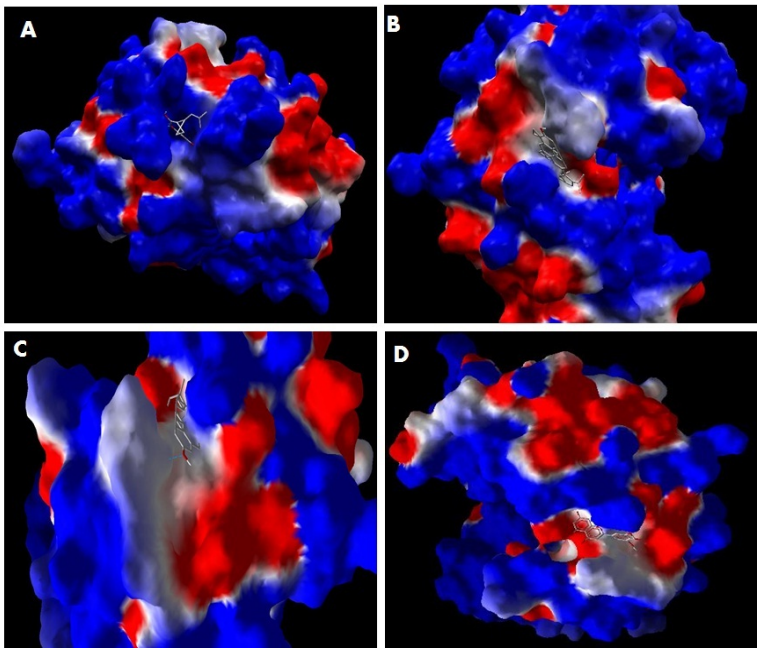

Fig: 4. Electrostatic interaction of the target protein with phytochemicals; A-Pyrethrum, B- Triterpenoid, C- Stigmasterol, D- Tricin

Ligand interaction in binding pocket

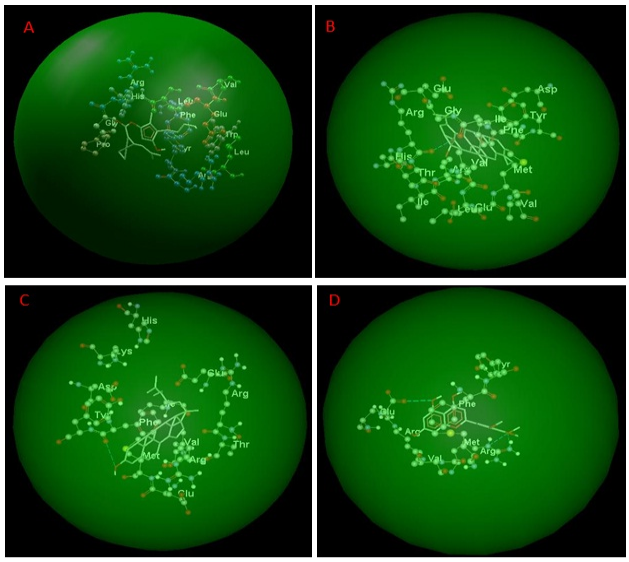

Fig: 5. Ligand binding cavity of the target protein with phytochemicals; A-Pyrethrum, B- Triterpenoid, C- Stigmasterol, D- Tricin

DISCUSSION

Mangroves are rich source of ecofriendly, marine plant are being probed an alternate source to get therapeutic compounds based on their medicinal properties. The main mode of WNV transmission is via various species of mosquitoes, currently, no vaccine against WNV infection is available. The best method to reduce the rates of WNV infection is mosquito control these chemicals are N,N-diethylmetatoluamide (DEET) and picaridin strongly disliked by biting insects such as mosquitos.[28] Insect repellents represent a pragmatic approach to the prevention of allergic reactions or vector-borne diseases such as typhus, malaria, Lyme disease, dengue fever, yellow fever, and West Nile virus that could potentially accompany an insect bite. [29] Virtual screening methods are extensively used in drug discovery process to reduce the time spent on the research as well as expenditure. In this study, natural inhibitors from mangrove plants were screened for against the West Nile Virus infection. Design .Our previous docking studies have already proved the efficacy of mangrove derived compounds dihydrofolate reductase protein, sterol containing protein (AeSCP-2). [26, 30] The mangrove derived compound pyrethrum biochemical study proved their potential in mosquito larvicide activity. [24] The ligand molecules were subjected for molecular docking program Molegro was utilized in this study resulted in identifying compound with high binding affinity towards target. The docked pose of compound revealed more number of H-bond interactions. The mangrove derived compounds (Pyrethrum, Triterpenoid, Stigmasterol, Tricin) were docked at the active site of NS5 methyl transferase protein (PDB ID: 2OY0). The molecular docking program Molegro was carried out to find out the ligand which bound in the active sites of targeted protein. Successfully the ligand molecules bind to the NS5 protein complex with best binding energy and provided excellent results as were seen by the least values of the binding energy.

CONCLUSION

Molecular docking analysis was carried out the phenolic compound (Pyrethrum, Triterpenoid, Stigmasterol, and Tricin) were screened against the WNV replication. The chemical interaction between selected ligand and the target protein NS5 Methyltransferase is arrested by the ligand and inhibits WNV replication pathway. The ligand molecules have good binding interaction with NS5 Methyltransferase protein. From this study we conclude the mangrove derived compounds were binds to the NS5 Methyltransferase it inhibits the viral replication of West Nile Virus. Therefore, this study states the importance of small molecules from plant sources as docking agents and we suggest that mangrove derived compounds should be potent drug for West Nile Virus. It is like that target prediction method. Further the *In-vitro* experiment methods for the foreseeable future.

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