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Research Article

Identification of Potential Anti-dengue Lead from Nilavembu Through *In-silico* Study

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ABSTRACT

Dengue fever is a severe mosquito-borne global health concern caused by the Dengue virus. There are no effective vaccines or anti-virals against dengue, even though several medications are under developmental stages. As we all know, the traditional medicine system mainly depends on plants to treat various types of diseases caused by bacteria, viruses, and other micro-organisms. In this scenario, the present study focussed on identifying the inhibitory potential of phytoconstituents from a well-known antipyretic medicinal herb *Andrographis paniculata* (Burm.f.) Nees against MTase domain of NS5 protein from the virus and IMPDH2 from the host through Molecular docking to identify the hit compounds and further drug-likeness, pharmacokinetics, and toxicity studies were carried out to ascertain a lead candidate. Through molecular interaction results, it was identified that in the case of NS5, about 28 compounds showed the least binding energy than native ligand SAH and were recommended as hits, out of which 12 compounds interact specifically with the active site residues and were selected as top hits. In the case of IMPDH2, 13 compounds were identified as hits since they showed less binding energy than native ligand RVP, and among that, nine compounds were selected as top hits based on their interaction with the active site residues. Furthermore, the selected hit molecules were subjected to drug-likeness, pharmacokinetics, and toxicity prediction and identified Oleanolic acid as the best lead candidate against both the targets NS5 and IMPDH-II. The study further emphasizes Oleanolic acid as the best lead candidate because naturally, triterpenoid compounds possess anti-viral activity but further *in vitro* and *in vivo* studies are essential to propose Oleanolic acid as an anti-dengue compound.

Introduction

Dengue is a mosquito-borne viral disease distributed in tropical and subtropical regions of the world. Dengue virus is mainly transmitted by the female mosquito species *Aedes aegypti* and, to a lesser extent, by *Aedes albopictus.*[1] These two species also act as vectors for transmitting other dreadful viruses such as chikungunya, yellow fever, and Zika. According to World Health Organisation (WHO), dengue is defined as one of the neglected tropical diseases, and globally large number of dengue cases was reported in the year 2019.

Dengue virus (DENV) belongs to the family Flaviviridae and the genus *Flavivirus,* which comprises other pathogenic viruses such as West Nile virus, Tick-borne Encephalitis Virus, Yellow Fever Virus, and Zika virus.[2] There are four distinct but closely related dengue virus serotypes, namely DENV1, DENV2, DENV3, and DENV4, whereas DENV2 causes more lethality. Infection with any serotypes provides lifelong immunity against that serotype but does not confer protection against secondary infection with a heterologous serotype. The symptoms of dengue viral infection range from self-limiting mild Dengue fever to severe forms such as Dengue Hemorrhagic fever (DHF) and dengue shock syndrome (DSS) which might be fatal.[3]

Dengue virus has a positive sense single-stranded RNA genome of ~11kilobases which encodes a poly-protein that is post-translationally modified into three structural proteins, namely Capsid(C), Envelope(E),

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and precursor membrane protein (prM). It forms the building blocks of mature virus and seven non-structural proteins, namely NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5, which are involved in assembly, maturation, and host immune response modulation.[4] Among the seven non-structural proteins, NS5 is the largest protein encoded in the DENV genome with 900 amino acid residues. It is a multifunctional and most conserved protein among the four DENV serotypes and possesses two catalytic domains, C-terminal RNA dependent RNA Polymerase(RdRp) domain, and N-terminal S-adenosyl methionine methyltransferase(MTase) domain. The MTase domain of NS5(1-262) is responsible for capping the viral RNA genome. The presence of a cap ensures stability and translation into viral polyproteins by host cell ribosomes. It catalyzes methylation at the N7 atom of Guanine and 2'-Oatom of the ribose of Adenosine which contributes to the escape of the virus from the host cell's innate immune response.[5] The N-terminal MTase domain is connected to the C-terminal RdRp domain through ten linker residues. The RdRp domain (273-900) stimulates the formation of both positive and negative sense double-stranded RNA intermediates. Further, the negative-sense RNA strand serve as a template for the synthesis of new positive-sense genomic RNA.[6] Hence the role of NS5 in viral replication and host immune response modulation makes it an excellent target for DENV drug discovery.

IMPDH (Inosine 5'monophosphate dehydrogenase) is another important target selected from the host cell involved in purine nucleotides' biosynthesis. It catalyzes the conversion of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP), a rate-limiting step in the de novo synthesis guanine nucleotides. Human IMPDH has two isoforms, type I and type II, with 84% sequence similarity. IMPDH type I is prevalent in normal human leukocytes and lymphocytes, whereas type II is overexpressed in rapidly proliferating cells (malignant) and virus-infected cells, where significant amounts of nucleotides are required for rapid viral proliferation. Inhibition of the enzyme could be possible by occupying suitable compounds in the binding site of either natural substrate (IMP) or cofactor (NAD^+). Thus IMPDH-II has been suggested to be an important target for anti-viral drug discovery, especially against infectious RNA viruses.[7]

There are no clinically approved drugs or vaccines against dengue so far, even though a lot of studies have been conducted to attain these goals. The treatment of dengue is limited to supportive care with analgesics, fluid replacement, and bed rest.[8] Therefore it is obligatory to develop new efficient anti-virals to eradicate this global threat. More studies were carried out on natural products during the past few decades by considering their low cost and less adverse effects. Several plants have anti-dengue properties[9,10] and are being used by traditional healers. Phytochemicals present in medicinal plants form an attractive substitute for developing drugs against dengue viral infection. In this context, the present study aimed to validate the anti-dengue efficacy of the common medicinal herb *A. paniculata* (Burm.f.) nees and scrutinize the plant's active lead phytochemical through *in-silico* molecular docking, pharmacokinetics, and Toxicity analysis.

Materials and Methods

Selection and Preparation of Target

The MTase domain of NS5 with (PDB ID:4V0Q) from the dengue virus and human type II Inosine Monophosphate Dehydrogenase (IMPDH-II) from the host side(PDB ID: 1NF7) were selected as the targets. The targets were visualized using 'UCSF Chimera' and removed the co-crystallized ligands from them. Further, the structures were prepared using DockPrep option in chimera by deleting water molecules, adding hydrogens, assigning partial charges, repairing truncated side chains, and finally, energy minimization of the targets was carried out. The prepared targets were subsequently converted to Pdbqt format using Autodock 4.2.[11]

Active Site Determination

The active sites of the targets were identified by analyzing the amino-acid residues already occupied by the co-crystallized ligands. The MTase domain of NS5 is complexed with natural ligand SAH. Therefore, the residues bound to SAH form the active site residues, including Gly81, Cys82, Gly86, Ser56, Gly58, Trp87, Lys105, Glu111, His110, Asp131, Val132 and Asp146. In the case of IMPDH2, the residues bound with the native ligand RVP(Ribavirin Monophosphate) form the active site residues, including Ser68, Met70, Gly328, Ser329, Ile330, Cys331, Asp364, Gly365, Gly366, Gly387, Ser388, Tyr411, Met414, and Gln441.

Selection and Preparation of Ligand

About 151 phytochemicals identified from *A. Paniculata* (Burm.f.) Nees were chosen as ligands for docking. The 3D structures of 113 phytochemicals were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and the remaining 38 phytochemicals were drawn using Chemsketch, and their canonical smiles were generated. The canonical smiles were submitted to an online file format converter (Open Babel version 2.4.1) to generate 3D structures in sdf format. Subsequently, Gypsum DL an open source program was used to enumerate appropriate ionization, tautomeric, chiral, cis/trans isomeric, and ring-conformational forms of the 3D structures.[12] Finally, they were loaded into PyRx software to perform energy minimization using Universal Force Field (UFF)with the conjugate gradient algorithm for 200 steps,[13] followed by conversion of sdf to pdbqt format.

Validation of Docking Protocol

Before initiate the docking studies, validation of the docking protocol was carried out by removing the natural

ligands S-Adenosyl-L-Homocysteine (SAH) in the NS5 and Ribavirin Monophosphate in the IMPDH-II from their binding sites and re-docking it to the crystal structures of dengue virus NS5 (PDB ID: 4V0Q) and Human IMPDH-II (PDB ID: 1NF7) respectively. The root means square deviation (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation of SAH was 1.47. In the case of IMPDH-II, the RMSD between the predicted conformation and the observed X-ray crystallographic conformation of RVP (Ribavirin Monophosphate) was observed to be 1.49. This indicates the reliability of the docking method in reproducing the experimentally observed binding mode.

Molecular Docking Studies

In the current study, docking was performed using AutodockVina to predict each ligand's best binding mode and binding affinity against the selected targets. AutoDockVina combines both the conformational preferences of the receptor-ligand complex and experimental affinity measurements to compute its binding energy.[14] All calculations for protein-ligand docking were carried out using Lamarckian Genetic Algorithm (LGA) method. A grid box was generated around the active site of targets before docking. After the docking search was completed, the best conformation was chosen based on the least binding energy. The interaction between the targets and ligand molecules, including hydrogen bonds and hydrophobic interactions, were visualized using Discovery Studio Visualizer.

Druglikeness, Pharmacokinetics, and Toxicity Prediction

The best hit molecules identified through molecular docking studies were further filtered to select an appropriate lead candidate by evaluating Druglikeness properties using Molinspiration server (https://www.molinspiration.com/) Pharmacokinetic profiling, as well as Toxicity Prediction parameters such as Carcinogenicity, Hepatotoxicity, Acute oral toxicity, HERG (Human Ether-a-go-go-Related Gene) inhibition and Ames mutagenicity, were predicted through admetSAR2.0.[15] Additionally, another webserver ADMETlab[16] was used to revalidate HERG and Ames mutagenicity. Finally, Toxicity Checker at Mcule, an online drug discovery platform (https://mcule.com/apps/toxicity-checker/) has been used to detect potential toxic substructure in the hits.

Results and Discussion

Molecular Docking Analysis

In the present study, *in-silico* investigations of the phytoconstituents against NS5 and IMPDH-II were carried out using AutoDockVina. Results against NS5 revealed that among 151 compounds screened, 28 compounds showing free energy of binding less than that of native ligand SAH(S-Adenosyl-L-Homocysteine) were selected as hit molecules. Out of which, it was observed that four compounds showed unfavorable bonds. The formation of any unfavorable bond between/in protein-ligand complex reduces the stability of the complex as these types of bonds indicate a force of repulsion occurring between two molecules and an atom. Twelve compounds did not exhibit any hydrogen bond or hydrophobic interaction with the active site residues. The rest of the 12 compounds showed hydrogen bond and hydrophobic interaction with the active site residues, and hence they were selected as the top hits (Table 1).

In IMPDH-II, 13 compounds were selected as the hit molecules since they showed better binding affinity than native ligand RVP (Ribavirin monophosphate). The hits were further filtered based on interaction analysis, and it was observed that four compounds showed unfavorable bonds whereas the rest of the nine compounds formed either hydrogen bonds or hydrophobic interaction with the active site residues; hence, they were recommended as top hits (Table 2).

Comparing the docking results of NS5 and IMPDH-II, it was observed that Diosgenin, Bisandrographolide, Oleanolic acid, Andrographoside, and Gitoxigenin were found to be common hits.

Drug-likeness, Pharmacokinetics and Toxicity Prediction

In silico assessment of Drug-likeness, Pharmacokinetics, and Toxicity of the top hits were carried out to explore their Lead-like potential. Physiochemical parameters such as logP, Molecular weight, Topological polar surface area (TPSA), number of Hydrogen bond donors (HBD), number of Hydrogen bond Acceptors (HBA), and number of rotatable bonds were evaluated to determine "Drug-like" compounds based on Lipinski's rule of five (RO5) and Veber's rule. According to the rule of five proposed by Christopher A. Lipinski, a compound exhibits good oral bioavailability and high membrane permeability when it satisfies logP ≤ 5; MW ≤ 500 Da; HBAs ≤10 and HBDs ≤5.[17] Veber's rule proposes that a compound possesses good absorption when its TPSA ≤140 Å and several rotatable bonds ≤ 10.[18] In the current study, out of 16 hit molecules, the physiochemical properties of the 11 compounds were found to be in perfect accordance with Veber's rule and Lipinski's filter with Oleanolic acid showing slight variation in logp value (6.72). In contrast, the rest of the five compounds Andrographidine E, Andrographidine D, Bisandrographolide, 3,4-Dicaffeoylquinic acid, and Andrographoside showed violation in either of the rules mentioned above. The details of the predicted results are depicted in Table 3.

The majority of the drug-like compounds fail due to adverse effects at a later stage of drug development. So it is important to incorporate pharmacokinetics prediction in the lead compound selection criteria by considering certain properties such as Caco-2 permeability,

Table 1: Interaction details of hits against NS5

Ligand	*Binding affinity (kcal/mol)*	*H Bond*	*Bond length (A^0)*	*Hydrophobic interaction*
SAH*	-8.0	Gly85:N-H---O:Lig Ser56:O-H---O:Lig Gly86:N-H---O:Lig Cys82:N-H---O:Lig Asp131:O---H-N:Lig Asp131:O---H-N:Lig	2.54 2.34 2.06 2.34 2.32 2.40	Nil
Andrographidine E	-10.1	Gly86:N-H---O:Lig Cys82:O---H-O:Lig Thr104:O-H---O:Lig Gly148:N-H---O:Lig	2.71 2.05 2.35 1.88	His110, Ile147.
Andrographoside	-9.8	Val132:N-H---O:Lig Lys105:N-H---O:Lig Lys130:O---H-O:Lig Asp79:O---H-O:Lig Cys82:O---H-O:Lig Trp87:N-H---O:Lig Gly86:N-H---O:Lig	2.22 2.73 2.43 1.77 2.19 1.91 2.34	His110, Ile147
Oleanolic acid	-9.2	Asp146:O-H---O:Lig	2.30	Lys105, Ile147, His110, Lys61 and Arg57.
Andrographidine D	-9.2	Trp87:N-H---O:Lig Gly86:N-H---O:Lig Arg84:N-H---O:Lig Gly148:N-H---O:Lig	2.24 2.41 1.93 1.87	Lys105, Ile147, Val132, Phe133.
Dibenz[a,c]acridine	-9.2	Nil		His110, Ile147, Val132, Lys105
Diosgenin	-9.1	Gly81:O---H-O:Lig	2.16	Ile147
Andrographidine C	-8.9	Ser56:O---H-O:Lig Gly148:N-H---O:Lig	1.88 2.18	Ile147, Val132, Lys105
Andrographidine A	-8.8	Arg84:N-H---O:Lig Asp146:O---H-O:Lig	2.04 2.06	Ile147, Val132, Lys105, Glu111.
Bisandrographolide	-8.6	Arg211:N-H---O:Lig Gly86:N-H---O:Lig Ser56:N-H---O:Lig	2.48 2.64 2.65	LYS61, Arg57, Trp87, His110.
Gitoxigenin	-8.6	Nil		Val132, Lys105 Ile147.
Wogonin	-8.2	GLY81:O---H-O:Lig GLY148:O---H-O:Lig GLY148:N-H---O:Lig	2.22 2.19 1.88	Ile147, Lys105, Val132.
Oroxylin A	-8.2	Glu111:O---H-O:Lig GLY148:N-H---O:Lig	2.20 2.58	Val132, Lys105 Ile147.

*native ligand

Human intestinal absorption, Human Oral Bioavailability, BBB (Blood Brain Barrier) permeation, P-gp substrate/ inhibitor, and Cytochrome inhibitory promiscuity (Table 4). Considering Human Intestinal Absorption, all the concerned hits were found to be absorbed through the small intestine. Prediction of membrane permeability through Caco-2(human colorectal adenocarcinoma) cellline indicates that Oleanolic acid, Diosgenin, Dibenz[a,c]acridine, Oroxylin A and Wogonin were found to be highly permeable. Regarding Human Oral Bioavailability - Oleanolic acid, Wogonin, Dibenz[a,c]acridine, Gitoxigenin, and Oroxylin A were found to possess good Oral Bioavailability. Chaturvedi *et al*., in their experimental study, observed that Blood-Brain Barrier could be damaged during dengue viral infection indicating viral invasions.[19] Some of the neurological complications associated with dengue virus infection include Encephalopathy, Encephalitis, Meningitis, Stroke, Cerebellar syndrome etc.[20] Compounds such as Oleanolic acid, Bisandrographolide, Diosgenin, Dibenz[a,c]acridine, and 14-Acetyl 3,19- isopropylidene andrographolide cross the BBB. Another important observation regarding P-glycoprotein revealed that

Table 2: Interaction details of hits against IMPDH2

Ligand	*Binding affinity (kcal/mol)*	*H Bond*	*Bond length (A^0)*	*Hydrophobic interaction*
RVP *	-8.7	Asp364:N-H---O:Lig Gly387:N-H---O:Lig Gly366:N-H---O:Lig Ser329:N-H---O:Lig Tyr411:N-H---O:H	2.74 2.12 2.66 2.36 2.22	Gly328, Met70, Ile330
Diosgenin	-9.9	Gly365:N-H---O:Lig	2.38	Met70, Met385, Leu337
Bisandrographolide	-9.8	Gln441:N-H---O:Lig Gly328:N-H---O:Lig Gly324:O---H-O:Lig Asp256:O---H-O:Lig	2.56 2.16 2.78 2.32	Met414, Met420,His93
Oleanolic acid	-9.7	Gly328:N-H---O:Lig Ser327:N-H---O:Lig Val439: O---H-O:Lig	2.07 2.07 2.32	Met70, Leu337.
Andrographoside	-9.5	Gly365:N-H---O:Lig Ser276:N-H---O:Lig Asp274:O---H-O:Lig Asp274:O---H-O:Lig Ser329:O-H---O:Lig	2.48 2.14 2.59 2.54 2.08	Met70, Ile330
3,4-Dicaffeoylquinic acid	-9.3	Gln441:O---H-O:Lig Thr333:N-H---O:Lig Ser327:N-H---O:Lig Gly326:N-H---O:Lig Gly328:N-H---O:Lig Gly415:N-H---O:Lig Met414:N-H---O:Lig	2.43 1.91 2.17 2.42 2.12 2.27 2.71	Met70, Met385, Asp274
Gitoxigenin	-9.2	Gly365:N-H---O:Lig Met385:O---H-O:Lig	2.76 2.76	Met70
Paniculoside I	-9.2	Gly365:N-H---O:Lig ASN303:N-H----O:Lig	2.02 2.76	Met70, Met385, Met414, Ile330
14-Acetyl-3,19-isopropylideneandrographolide	-9.0	ASN303:N-H----O:Lig Gly326:N-H----O:Lig Gly328:N-H---O:Lig Ser327:N-H---O:Lig	1.96 2.71 2.04 1.99	Met70, Met414, Leu337.
Andropanoside	-8.9	Gln441:O---H-O:Lig Gly415:O-H---O:Lig Thr333:N-H---O:Lig Gly365:N-H---O:Lig	2.34 2.13 1.91 2.27	Met70, Ile330.

*native ligand

Oleanolic acid, Andrographoside, Andrographidine A, Andrographidine C, Dibenz[a,c]acridine, Andropanoside, Paniculoside I, and Oroxylin A neither act as substrate nor act as an inhibitor of P-gp. It is one of the most important cell surface proteins involved in xenobiotic efflux. Metabolism of xenobiotics through Cytochrome P450 isoenzymes (CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) plays a significant role in drug elimination and clearance in the liver. Thus inhibition of these isoforms might leads to drug-drug interaction due to the accumulation of drugs.[21] Among the 16 hits, Wogonin and Oroxylin A exhibited Cytochrome inhibitory promiscuity.

Early assessment of a therapeutic molecule's toxicity is crucial in drug development because the failure of drug candidates at the clinical trial stage occurs mainly due to toxicity. Currently, *in-silico* toxicity prediction is highly evolving as an alternative platform for checking toxicity to complement the existing *in-vitro* toxicity methods, thereby reducing the time, need for animal testing, and cost.[22] In the present study, a thorough appraisal of the toxicity of the concerned phytochemicals involving carcinogenicity, mutagenicity, Hepatotoxicity, HERG inhibition, and Acute Oral Toxicity was executed. Carcinogenicity of the selected phytochemicals was detected through admetSAR, and it was observed that none of the selected

Table 3: Physiochemical properties of the hits

Compound name	*Mol. wt. (Da)*	*Log P*	*TPSA*	*H-bond donors*	*H-bond acceptors*	*Rotatable bonds*
Diosgenin	414.63	5.93	38.70	1	3	0
Andrographidine E	490.96	1.23	157.29	4	11	7
Andrographidine D	520.49	1.04	166.53	4	12	8
Bisandrographolide	664.88	4.11	133.52	4	8	8
Oleanolic acid	456.71	6.72	57.53	2	3	1
Andrographoside	512.60	-0.66	166.14	6	10	6
Wogonin	284.27	2.96	79.90	2	5	2
Andrographidine A	462.45	0.66	144.15	4	10	6
Andrographidine C	460.44	1.22	148.06	4	10	6
Dibenz[a,c] acridine	279.34	5.74	12.89	0	1	0
Gitoxigenin	390.52	1.56	86.99	3	5	1
3,4-Dicaffeoylquinic acid	516.46	1.21	211.28	7	9	12
14-Acetyl-3,19-isopropylideneandrographolide	432.56	3.65	71.08	0	6	4
Andropanoside	496.60	0.02	145.91	5	9	7
Paniculoside I	480.60	2.04	136.68	5	8	4
Oroxylin A	284.27	2.96	79.90	2	5	2

(TPSA = Topological polar surface area, Logp = Logarithm of partial coefficient, Mol.wt = Molecular weight)

Table 4: Pharmacokinetic properties of the selected hits

	Pharmacokinetic analysis						
Hits	*Caco-2 permeability*	*HOB*	*HIA*	*BBBP*	*P-gp substrate*	*P-gp Inhibitor*	*Cytochrome inhibitory promiscuity.*
Bisandrographolide	NP	No	Yes	P	S	I	No
Oleanolic acid	P	Yes	Yes	P	NS	NI	No
Andrographoside	NP	No	Yes	NP	NS	NI	No
Wogonin	P	Yes	Yes	NP	S	I	Yes
Andrographidine A	NP	No	Yes	NP	NS	NI	No
Andrographidine C	NP	No	No	NP	NS	NI	No
Diosgenin	P	No	Yes	P	S	I	No
Dibenz[a,c] acridine	P	Yes	Yes	P	NS	NI	No
Gitoxigenin	NP	Yes	Yes	NP	S	I	No
3,4-Dicaffeoylquinic acid	NP	No	Yes	NP	S	I	No
Andrographidine E	NP	No	No	NP	NS	I	No
Andrographidine D	NP	No	No	NP	NS	I	No
Andropanoside	NP	No	Yes	NP	NS	NI	No
Paniculoside I	NP	No	Yes	NP	NS	NI	No
14-Acetyl 3,19-isopropylideneandrographolide	NP	No	Yes	P	NS	I	No
Oroxylin A	P	Yes	Yes	NP	NS	NI	Yes

(HOB = Human Oral Bioavailability, HIA= Human Intestinal Absorption, BBBP= Blood Brain Barrier Permeation, P-gp = P-glycoprotein, NP = NonPermeable, P = Permeable, NS = Non-substrate, S = Substrate)

Table 5: Details of toxicity prediction of the hit compounds

Hits	*Carcinogenicity*	*Hepato-toxicity*	*Ames muta genicity*	*HERG inhibition*	*Acute Oral Toxicity*	*Toxic substructure*
Bisandrographolide	no	no	no	yes	I	Present
Oleanolic acid	no	no	no	no	III	Absent
Andrographoside	no	no	no	no	I	Present
Wogonin	no	yes	no	no	III	Present
Andrographidine A	no	yes	no	no	III	Present
Andrographidine C	no	yes	yes	yes	III	Present
Diosgenin	no	no	no	yes	IV	Absent
Dibenz[a,c] acridine	no	yes	yes	yes	III	Present
Gitoxigenin	no	no	no	no	I	Present
3,4-Dicaffeoylquinic acid	no	yes	no	no	III	Present
Andrographidine E	no	yes	yes	yes	III	Present
Andrographidine D	no	yes	yes	yes	III	Present
Andropanoside	no	no	no	yes	I	Present
Paniculoside I	no	no	no	yes	III	Present
14-Acetyl 3,19-isopropylideneandrographolide	yes	yes	yes	no	III	Present
Oroxylin A	no	yes	no	no	III	Present

hits were predicted to be carcinogenic except 14-Acetyl 3,19-isopropylideneandrographolide. Cardiac toxicity was detected through HERG (Human Ether-a-go-go-Related Gene) inhibition. HERG encodes a potassium ion (K+) channel responsible for the electrical activity of the heart that coordinates the heart's beating.[23] Thus HERG K^+channel blockers are potentially toxic. Both admetSAR and ADMETlab were used to predict HERG inhibition and it was observed that Bisandrographolide, Andrographidine C, D & E, Diosgenin, Dibenz[a,c]acridine, Andropanoside and Paniculoside I were found to be HERG blockers. Mutagenicity of the selected hits (in correlation with Ames mutagenicity through admetSAR and ADMETlab) revealed that none of the hits were predicted to be mutagenic except Dibenz[a,c]acridine, Andrographidine C, E & D and 14-Acetyl 3,19- isopropylidene andrographolide. Comprehensive estimation of hepatotoxicity utilizing admetSAR disclosed that Oleanolic acid, Bisandrographolide, Andrographoside, Diosgenin, Gitoxigenin, and Andropanoside were found to be non-hepatotoxic. According to admetSAR data, Bisandrographolide, Gitoxigenin, Andropanoside, and Andrographoside showed category I acute oral toxicity, indicating high toxicity, and Diosgenin showed category IV, and the remaining compounds exhibited category III acute oral toxicity, which suggests less toxicity. Finally, they have also been administered to Mcule-Toxicity checker and found that among the hits, both Oleanolic acid and Diosgenin do not possess any potential toxic substructure. Toxicity prediction results are shown in Table 5.

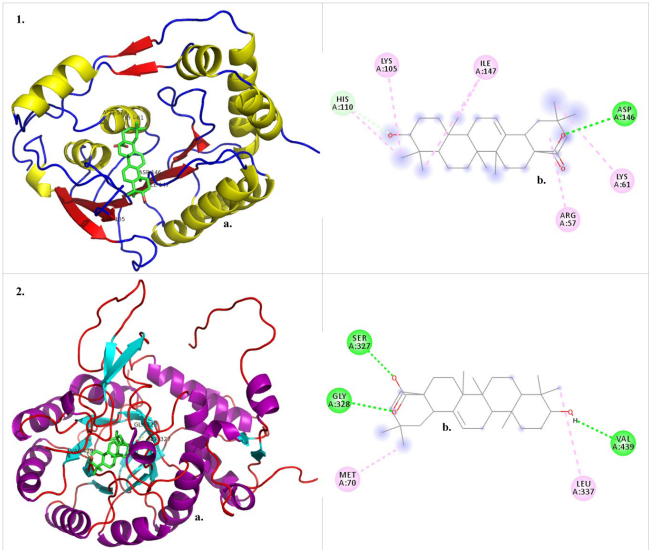

Fig 1: (1) Docking interaction between Oleanolic acid and MTase domain of NS5. (2) Docking pose of Oleanolic acid with IMPDH-II. a. 3D image and b. 2D image

Based on the above results**,** Oleanolic acid was selected as the lead molecule against both the targets as it interacts with the active site residues (Asp146 &Lys105) of NS5, and in the case of IMPDH-II it is found to be Gly328 & Met70. It also satisfied Druglikeness properties with an insignificant violation of logp value but fulfilled Pharmacokinetic properties. As far as the toxicity prediction, Oleanolic acid does not possess toxicity and potential toxic substructure. The 2D and 3D Docked images of lead molecule with the NS5 and IMPDH-II were depicted in Fig 1.

Conclusion

In the current study, the anti-dengue viral property of Nilavembu has been analyzed based on the traditional knowledge through *in silico* molecular docking combined with Druglikeness, Pharmacokinetics, and Toxicity analysis. The docking results revealed that the plants have several molecules with an inhibitory effect on NS5 and IMPDH-II. However, except Oleanolic acid, the rest of the hit compounds showed more than one violation in either Druglikeness or ADMET properties. Hence, Oleanolic acid, a pentacyclic triterpenoid present in the plant's root, was selected as the lead molecule. It has several pharmacological properties, including anti-cancer, anti-diabetic, antimicrobial, anti-viral, anti-hypertensive, antioxidant, anti-inflammatory, and anti-parasitic.[24] But further *in vitro* and *in vivo* studies are essential to confirm the *in silico* results and propose Oleanolic acid as a potential drug candidate against dengue.

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