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***Research Article** (Open Access)*

EXPLORING NATURAL COMPOUNDS AS POTENTIAL INHIBITORS OF DENGUE VIRUS NS5 RDRP THROUGH MOLECULAR DOCKING

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ABSTRACT
Dengue, a mosquito-borne virus affecting millions globally, lacks a widely accepted and effective cure, highlighting the urgent need for novel antiviral drugs targeting viral replication. This study aims to identify potential inhibitors of dengue virus replication using natural phytochemicals from our in-house ligand library. Focusing on the RNA-dependent RNA polymerase (RdRp), a critical enzyme for viral replication and survival, we screened various phytochemicals and identified five candidates with binding affinities ranging from -8.4 to -9 kcal/mol. These compounds demonstrated superior affinity for the RdRp compared to co-crystallized ligands. Subsequent ADME analysis confirmed favorable pharmacokinetic characteristics, underscoring their potential as promising antiviral inhibitors.

***Keywords*:** *Dengue Virus, Natural Compounds, Phytochemicals, RNA-dependent RNA Polymerase, Molecular Docking, Drug-Likeness*

INTRODUCTION
Dengue fever is a contagious viral illness that affects millions globally, with a vast portion of the population susceptible to infection. The risk of infection continues to rise, primarily due to the presence of the dengue carrying mosquito in tropical and subtropical regions. Moreover, the increasing temperatures associated with global warming create more favorable environments for these mosquitoes, exacerbating the spread of dengue (San Martín *et al.*, 2012). Dengue virus (DENV) has four types: DENV-1, DENV-2, DENV-3, and DENV-4. It spreads through the bite of infected female Aedes mosquitoes, mainly Aedes aegypti, and sometimes Aedes albopictus. This can cause severe health conditions such as dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) (Brady *et al.*, 2012).
The dengue virus contains a single-stranded RNA which is approximately 11 kilobases long. This RNA carries instructions for producing three structural proteins (Capsid, pre-Membrane, and Membrane), which are vital for constructing the virus, and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5), which are essential for the virus to replicate. These proteins play crucial roles in both the formation of the virus and its ability to multiply within the body (Tanbin *et al.*, 2021). Recent antiviral research has focused on finding ways to stop funcunting key viral enzymes involved in the infection process (Cabarcas-Montalvo *et al.*, 2016). **S**everal research studies have reported that the NS5 RdRp is among the most commonly targeted proteins in antiviral research. This particular enzyme, NS5 DENV RdRp, is crucial for the dengue virus to replicate and spread within the body (Elshahawi *et al.*, 2019). Furthermore, the amino acid sequences of NS5 exhibit an approximate similarity of 70% among the four DENV serotypes (Shimizu *et al.*, 2019) Thus, NS5 RdRp might be considered a primary focus for therapeutic intervention.
Nowadays researchers have been using molecular docking and virtual screening techniques to find potential medications for dengue. In this study we aimed to identify an effective drug for treating dengue, a disease that has been the focus of numerous studies with many therapeutic candidates being explored. To find a promising drug candidate with high inhibition activity and low binding energy, we targeted the RNA-dependent RNA polymerase (RdRp) protein, which plays a key role in the virus's replication

process. By using computer-aided drug discovery methods, we aimed to find out compounds that can effectively inhibit this protein, thereby stopping the virus from replicating and offering a potential new treatment for dengue.

In our study we focused on phytocompounds as ligands because Phytocompounds, derived from various plants, have emerged as promising drug candidates in the field of Computer-Aided Drug Design (CADD). These natural compounds possess a rich chemical diversity and have been honed through evolutionary processes to interact effectively with biological targets. Their inherent "drug-like properties"—such as bioactivity, stability, and favorable pharmacokinetic profiles—make them excellent candidates for drug development (Newman & Cragg, 2012; Harvey *et al.,* 2015**)**. By leveraging computational tools to predict interactions and optimize these natural compounds, CADD accelerates the discovery and refinement of new, effective drugs derived from phytocompounds.

MATERIALS AND METHODS

Protein Preparation

The crystallography structure of the target protein, RNA dependent RNA polymerase (RdRp) of the Dengue virus solved at 2.01 Å resolution determined using X-ray crystallography was retrieved from the RCSB Protein Data Bank with PDB ID: 5K5M (Lim *et al.,* 2016). In our study, we targeted dengue virus RdRp because dengue virus RdRp is promising due to its essential role in viral replication (Selisko *et al.,* 2014) and the absence of a mammalian counterpart (Malet *et al.,* 2008; Lim *et al.,* 2015) which minimizes off-target effects. This makes RdRp a safe and effective drug target. Developing RdRp inhibitors could provide a crucial antiviral therapy for dengue and related flaviviruses.

The resolution of the protein structure provides an understanding of the stable conformations and accuracy of protein interactions characterizing the stability of the structures. The retrieved RdRp protein structure shows some missing residues during the structure analysis. To fix this problem we used modeler MODELLER plugin of CHIMERA tool (Yang *et al.,* 2012) to remodel missing residues Furthermore, the obtained remodeled structure of the RdRp protein was prepared by removing the native ligand, 68 T along with Water molecules and other heteroatoms and then the amino acids residues of protein were renumbered using the UCSF Chimera software (Pettersen *et al.,* 2004).

Screening of Ligands

We screened our in-house library of 138 phytocompounds to identify potential inhibitors for Dengue virus RdRp. Using drug-likeness parameters, including molecular weight (<500), Log P (<5), topological polar surface area (TPSA <120), hydrogen bond acceptors (HBA <10), hydrogen bond donors (HBD <5), and Lipinski's Rule of Five, we filtered and identified 32 suitable compounds. These parameters ensure that the compounds have appropriate chemical and physical properties for drug development (Walters & Namchuk, 2003).

The 32 selected phytocompounds were then docked using CB-Dock-2 software (Yang Liu, *et al.,* 2022; Xiaocong Yang, *et al.,* 2022) an enhanced version of the protein-ligand blind docking tool. CB-Dock-2 integrates curvature-based cavity detection with AutoDock Vina-based molecular docking, facilitating accurate identification of potential binding sites and interactions. This approach enabled us to evaluate the suitability of the phytocompounds as inhibitors for the Dengue virus RdRp.

RESULTS AND DISCUSSION

DOCKING USING CB-DOCK-2

The CB-Dock-2 protein-ligand docking approach was employed to independently dock 32 phytocompounds. CB-Dock-2, an advanced online docking software based on the AutoDock Vina program, offers a robust platform for molecular docking studies. This software streamlines the docking process by automatically identifying potential binding sites on the target protein, calculating the precise centers and sizes of these sites, and adjusting the docking box dimensions to accommodate the specific

query ligands. Once these parameters are set, CB-Dock-2 executes the molecular docking process using the AutoDock Vina algorithm (Mishra & Nandi, 2021; Padhi *et al.*, 2021).

Prior to docking, the PDB file of the receptor and the ligands were uploaded. The software automatically selected several top cavities for further analysis (cavity sorting), and molecular docking was conducted at each of these sites. The first conformation obtained was considered the optimal binding posture, with the corresponding location deemed the best binding site for the query ligand. The binding interactions were analyzed, and the docked position with the highest AutoDock Vina score and cavity size (first pose) was chosen for subsequent testing.

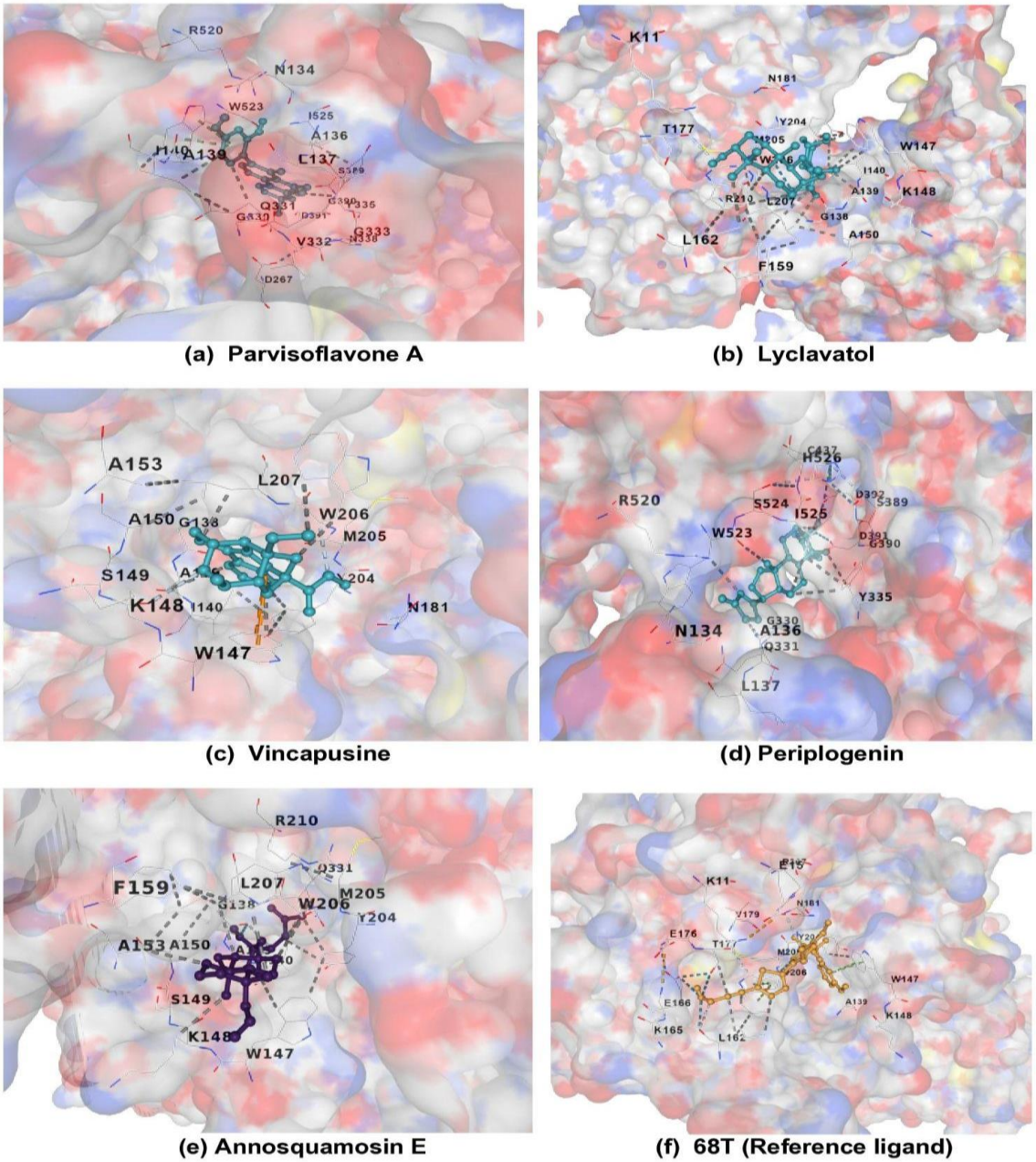

Fig. 1. Molecular docking interaction analysis of top 5 lead compounds (a) Parvisoflavone A (b) Lyclavatol (c) Vincapusine (d) Periplogenin (e) Annosquamosin E (f) 68T(Reference ligand) with DENV-2 NS5 RdRp (PDB ID: 5K5M).

Table 1: Docking results of the selected top 5 phytocompound and the reference ligand with DENV-2 NS5 RdRp (PDB ID: 5K5M)

Compound Name	Docking score	Cavity volume (Å^3)	Center			Size			Contact Amino acids residues
			x	y	z	x	y	z	
Parvisoflavone A	-9.3	4206	-21	-27	-11	30	32	35	ASN134 ALA136 LEU137 ALA139 ILE140 ASP267 GLY330 GLN331 VAL332 GLY333 TYR335 ASN338 SER389 GLY390 ASP391 ARG520 TRP523 ILE525
Lyclavatol	-9.0	4206	-21	-27	-11	30	32	35	LYS11 GLY138 ALA139 ILE140 TRP147 LYS148 ALA150 PHE159 LEU162 THR177 ASN181 TYR204 MET205 TRP206 LEU207 ARG210
Vincapusine	-8.9	4206	-21	-27	-11	30	32	35	GLY138 ALA139 ILE140 TRP147 LYS148 SER149 ALA150 ALA153 ASN181 TYR204 MET205 TRP206 LEU207
Periplogenin	-8.7	4206	-21	-27	-11	30	32	35	ASN134 ALA136 LEU137 GLY330 GLN331 TYR335 SER389 GLY390 ASP391 ASP392 CYS437 ARG520 TRP523 SER524 ILE525 HIS526
Annosquamosin E	-8.5	4206	-21	-27	-11	30	32	35	GLY138 ALA139 ILE140 TRP147 LYS148 SER149 ALA150 ALA153 PHE159 TYR204 MET205 TRP206 LEU207 ARG210 GLN331
68T (Reference ligand)	-8.1	4206	-21	-27	-11	30	32	35	LYS11 GLU15 ALA139 TRP147 LYS148 LEU162 LYS165 GLU166 GLU176 THR177 VAL179 ASN181 TYR204 MET205 TRP206 ARG307

DOCKING RESULTS

We used the CB-Dock-2 service to analyze how 32 plant-derived compounds interact with the dengue virus NS5 protein's RdRp domain to find potential inhibitors. Our molecular docking analysis identified five compounds with better binding energy (-9.3 to -8.5 kcal/mol) than the reference ligand 68T (-8.1 kcal/mol) (Table 1). Parvisoflavone A showed the highest binding affinity at -9.3 kcal/mol, interacting with key amino acids (ASN134, ALA136, LEU137, ALA139, ILE140, ASP267, GLY330, GLN331, VAL332, GLY333, TYR335, ASN338, SER389, GLY390, ASP391, ARG520, TRP523, ILE525).
It also passes drug likeness and ADME therefore has the most remarkable ability to inhibit the DENV-2 NS5 RdRp. Molecular docking is essential in structure-based drug design, as it predicts the interactions between potential drug molecules and target proteins at an atomic level. By simulating binding affinity and orientation, it aids in identifying and optimizing lead compounds. This accelerates the drug discovery process and enhances the development of precise and effective therapeutics. Our study suggests that Parvisoflavone A and other phytocompounds could be effective inhibitors of the dengue virus, warranting further experimental validation (Fig 1).

EVALUATION OF DRUG-LIKENESS FOR THE SELECTED LIGAND

We initially filtered the phytocompounds based on the following parameters: molecular weight (<500 Da), Log P (<5), topological polar surface area (TPSA <120 Å²), hydrogen bond acceptors (HBA <10), hydrogen bond donors (HBD <5), and compliance with Lipinski's Rule of Five. The drug-likeness of the top five lead compounds, along with a reference ligand, was further evaluated using SwissADME software (Table 2) (Daina *et al.,* 2017).

Table 2: Drug-likeness assessment of the top 5 lead compounds

Compound Name	Molecular Weight	H-bond acceptors	H-bond donors	LogP	TPSA	Lipinski's Rule Violations	Rotatable Bonds	GI Absorption
Parvisoflavone A	**390.51**	**5**	**3**	**2.75**	**86.99**	**0**	**1**	**High**
Lyclavatol	**378.50**	**5**	**1**	**3.46**	**72.83**	**0**	**5**	**High**
Vincapusine	**450.69**	**4**	**4**	**4.31**	**80.92**	**0**	**3**	**High**
Periplogenin	**368.43**	**5**	**1**	**3.13**	**63.93**	**0**	**3**	**High**
Annosquamosin E	**352.34**	**6**	**3**	**2.67**	**100.13**	**0**	**1**	**High**

In drug design and molecular docking, parameters such as molecular weight, Log P, TPSA, HBA, and HBD are crucial for assessing drug-likeness. Lipinski's Rule of Five states that an orally active drug should have no more than one violation of these criteria: molecular weight ≤500 Da, Log P ≤5, TPSA ≤140 Å², HBA ≤10, and HBD ≤5. These parameters are vital for predicting a compound's pharmacokinetics, including its absorption, distribution, metabolism, and excretion (ADME). Ensuring potential drugs meet these criteria increases the likelihood of bioavailability and effectiveness, thereby enhancing the efficiency of drug discovery and development.

CONCLUSION

Dengue, a virus spread by mosquitoes, affects millions worldwide, and currently, there is no widely accepted and effective treatment. To find new drugs that can stop the virus from replicating, we explored natural plant compounds from our own collection. The virus needs a specific protein, called RNA-dependent RNA polymerase (RdRp), to replicate and survive, making it a good target for antiviral drugs. In our study, we found five plant compounds that bind strongly to this protein, with binding affinities between 8.4 and 9 kcal/mol. These compounds were selected after thorough screening and showed better binding to the target protein compared to existing ligands. We also tested their pharmacokinetic properties using ADME analysis, which predicts how well the compounds can be absorbed, distributed, metabolized, and excreted in the body. The goal of this study was to see if plant compounds could inhibit the dengue virus by binding to a key protein, DENV-2 RdRp. Our docking experiments showed that the top five plant compounds interact strongly with the important parts of this protein, indicating they could be effective inhibitors.

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