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Identification of Novel Antiviral Peptides Targeted for E Protein of Dengue Virus by *In-Silico* Approaches

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ABSTRACT

Dengue fever, caused by the Dengue virus (DENV), is a significant global public health concern. The disease spectrum ranges from asymptomatic or mild febrile illness to severe conditions like Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Antiviral peptides have shown promise in combating Dengue virus infections by targeting specific regions of viral proteins to inhibit viral replication and entry into host cells. In this study, in-silico approaches were employed to select antiviral peptides targeting the E protein of the Dengue virus. The three-dimensional structure of the E protein of DENV was predicted using AlphaFold2, and docking studies were conducted against the antimicrobial peptides (AMPs) from the CAMPR4 database using the ClusPro protein-protein docking server. The selection of AMPs was based on the length of the sequence, experimental validation, the presence of experimentally determined structures, antiviral activity, whether they were naturally or synthetically derived, and their relevance to viral taxonomy. Based on the energies resulting from the docking studies, it was observed that the E protein had the highest negative interaction energy with the MBD-4 (11-40) / P9 [H21R, K23R, K28R] (P9R) complex, indicating the strongest binding, followed by Rp 71955, Antihypertensive protein BDS-1, Alstotide S1, LL-37, and Dermaseptin-4.

KEY WORDS: Antimicrobial peptides, dengue virus, serotype, in-silico.

INTRODUCTION

Over the past 20 years, dengue fever—which is caused by the dengue virus has reemerged on a global scale. This resurgence has resulted in increased epidemic activity and the spread of several serotypes, including the emergence of dengue haemorrhagic fever in previously unaffected areas (Gubler, 1998). It affects over 2.5 billion people worldwide, with 390 million infections, 500,000 hospitalizations, and 25,000 deaths reported annually (Bhatt *et al*., 2013; Guzman and Harris, 2015). DENV is classified under the genus Flavivirus which belongs to the family Flaviviridae (Wilder-Smith *et al*., 2016).

The dengue virus is classified into four serotypes, which are dengue virus type 1-4 (DENV-1-4) (Mishra *et al*.,2014). Each serotype of the Dengue virus can be categorized into different genotypes. For DENV-1, there are genotypes I, II, III, IV, V, and VI. DENV-2 has genotypes known as Asian/American, Asian 1, Asian 2, Cosmopolitan, and American. DENV-3 has genotypes I, II, III, and IV. Lastly, DENV-4 has genotypes that vary based on different geographical regions (Wei and Li,2017, Araujo *et al*.,2009, Gallichotte *et al*.,2018). These serotypes play a crucial role in the clinical manifestations and severity of dengue fever, with studies showing that the infecting serotype can impact disease outcomes (Yung *et al*., 2015; Suppiah *et al*., 2018). Severe cases of dengue fever are characterized by the sudden onset of fever, rash, headache, muscle pain, and, in certain cases, spontaneous bleeding (Mishra *et al*., 2014; Kosasih *et al*., 2016). The presence of multiple serotypes contributes to the complexity of dengue dynamics, as different serotypes can co-circulate and lead to severe disease manifestations like DHF and DSS (Pooja *et al*., 2017). The circulation of specific serotypes in different regions can also influence the clinical presentation of the disease, with certain serotypes being associated with more severe outcomes (Yung *et al*., 2015; Suppiah *et al*., 2018).

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The DENV RNA genome is approximately 11 kb in size, and it encodes for a polyprotein which is made up of three structural proteins, namely nucleocapsid (C), precursor membrane (prM) or membrane (M), and envelope (E) proteins and seven non-structural (NS) proteins comprising NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 proteins (Chambers *et al*., 1990; Falgout and Markoff, 1995).

A major obstacle in the fight against dengue is the absence of medically endorsed antiviral medications that specifically target the virus. Present treatment approaches primarily involve providing support, with an emphasis on alleviating symptoms and addressing complications. The lack of effective antiviral treatment emphasizes the immediate requirement for innovative therapeutic strategies that can decrease viral load and avert severe disease symptoms. Current research work is primarily dedicated to the identification and development of antiviral agents that can efficiently hinder the replication and transmission of the dengue virus. Peptides have gained significant attention because of their inherent benefits, such as their high specificity for targets, ease of synthesis and modification, and generally low toxicity profiles. Peptides possess specific characteristics that make them highly suitable for the development of antiviral drugs.

The dengue virus envelope (E) protein is an essential element of the viral structure, serving a crucial function in facilitating the virus's entry into host cells. The E protein is responsible for the initial binding of the virus to receptors on host cells and enables the fusion of the viral and cellular membranes. This fusion allows the viral genetic material to enter the host cell and initiate the infection process. Certain antiviral peptides have the potential to disrupt these crucial processes by specifically targeting the E protein. Such disruption can effectively prevent the virus from establishing infection and spreading within the host.

To date, there is no reliable antiviral drug available to treat dengue caused by the four DENV serotypes. Therefore, there is a significant demand for antiviral drugs for the management of dengue. In this study, we focused on identifying antiviral peptide(s) from the peptide database CAMPR4 by using computational approaches aimed at interacting with and inhibiting the function of the E protein of the dengue virus. CAMPR4 (Collection of Anti-Microbial Peptides) has been created to expand and accelerate antimicrobial peptide (AMP) based studies by providing curated information on natural and synthetic AMPs.

METHODOLOGY

Collection of Sequences

The E protein sequence of dengue virus was retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/protein/QBK46950.1).
The sequence was analysed using ProtParam to determine its phyochemical characteristics.

Prediction of secondary and tertiary structure and evaluation

To the protein sequence obtained from NCBI predicted secondary structure by using PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) and SOPMA (https://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/_NPSA/npsa_sopma.html). Further to the sequence three-dimensional structure was predicted by using AlphaFold2 (Jumper *et al*., 2021) and to minimize the tortional effects in the structure structural refinement was conducted by using the Galaxy Refine module (https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE). In order to assess the quality of predicted proteins evaluation was done by PROCHECK (http://bioinf.cs.ucl.ac.uk/psipred/&uuid=56b7f320-1e5b-11ef-80c1-00163e100d53) and ProSA (https://prosa.services.came.sbg.ac.at/prosa.php) servers. The successful structure was used for docking with antiviral peptides.

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Selection of antimicrobial peptides

The antimicrobial peptides (AMPs) sequences were obtained from the CAMPR4 database (http://www.camp.bicnirrh.res.in/seqDb.php?page=0). The criteria used to select AMPs included the length of the sequence (ranging from 1 to 100 mer), experimental validation, the presence of experimentally determined structure, antiviral activity, whether they were naturally or synthetically derived, and their relevance to viral taxonomy. The peptide sequences obtained were analyzed for their antimicrobial peptide (AMP) probability using the Antimicrobial Peptide Scanner vr.2 (https://www.dveltri.com/ascan/v2/), with a threshold cutoff value set at >0.5.

Study of physiochemical properties of AMPs

The ProtParam tool (https://web.expasy.org/protparam/) was used to analyze the sequences of potential antiviral peptides (AMPs) in order to determine their important physicochemical properties, such as molecular weight, theoretical pI, instability index, net charge, and grand average of hydropathicity (GRAVY). Peptides with a calculated isoelectric point (pI) exceeding 40 were chosen for additional investigation. Additionally, the peptides underwent assessment for their hydrophobicity and hydrophilicity using the PEPTIDE 2.0 server (https://www.peptide2.com/N_peptide_hydrophobicity_hydrophilicity.php). In addition, the solubility of the peptides was evaluated using the Protein-Sol server, which can be accessed at https://protein-sol.manchester.ac.uk/. Peptides exhibiting hydrophobicity and hydrophilicity levels exceeding 30% were selected for subsequent docking investigations. This thorough assessment guarantees the choice of peptides with ideal characteristics for their antiviral effectiveness and durability.

Molecular Docking between E protein and AMPs

Molecular docking is a crucial process for comprehending the interaction between the ligand and receptor. E protein was identified as the receptor in this study, while the potential AMPs were regarded as ligands. The ClusPro server (https://cluspro.org/login.php) was utilized to perform protein-protein docking. In the beginning, the three-dimensional structures of the chosen potential antimicrobial peptides were obtained from the PDB structure database. These structures were then improved using the GalaxyRefine server and utilized as ligands in this work. The selection of the most promising candidate was based on the binding energies observed between and E protein the antiviral peptides.

RESULTS AND DISCUSSION

Antiviral peptides that specifically target the E protein of the Dengue virus have demonstrated encouraging outcomes in the advancement of potent antiviral treatments. These peptides have shown to hinder viral replication and entry processes, making them valuable resources in fighting against Dengue virus infections (Damen *et al*.,2022; Bai *et al*.,2007). These peptides can hinder viral fusion with host cells, a critical step for viral infectivity, by interfering with the formation of structures necessary for fusion (Porotto *et al*., 2006).

In this study, the sequence of the E protein was collected from the polyprotein of Dengue virus type 2 (DENV-2) available in the NCBI database (accession number QBK46950.1). The total length of the polyprotein is 3391 amino acids, with the E protein spanning from positions 281 to 775, making it 495 amino acids in length. The physiochemical properties of the E protein were analyzed using ProtParam, which revealed a molecular weight of 54,438.04 daltons. The theoretical isoelectric point (pI) was calculated to be 7.91. Additionally, the instability index, aliphatic index, and grand average of hydropathicity (GRAVY) were determined to be 29.31, 84.42, and -0.109, respectively. These properties provide insights into the stability, solubility, and overall behavior of the E protein, which are crucial for understanding its function and interaction with antiviral peptides.

The secondary structure of the E protein, as analyzed by SOPMA and PSIPRED, revealed the following composition: alpha helix (16.57%), extended strands (29.9%), and random coil (53.54%) (fig1). The three-dimensional structure was predicted using AlphaFold2 (fig 2A). AlphaFold is a neural network-based machine learning approach that has significantly advanced the field of protein structure prediction. It utilizes deep learning techniques to predict the 3D

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structures of proteins with remarkable accuracy, even in cases where similar structures are not known (Jumper *et al*., 2021; Yin and Pierce, 2023). AlphaFold's success has been recognized in multiple evaluations, including the Critical Assessment of Protein Structure Prediction (CASP), where it surpassed other methods in generating precise protein structures with high accuracy (Senior *et al*., 2020; Poitras, 2023).The integration of AlphaFold2 in this study has provided a detailed and accurate model of the E protein, which is essential for understanding its interactions with antiviral peptides and for developing potential therapeutic strategies against Dengue virus infections.

Fig 1. secondary structure prediction of E protein of dengue virus by SOPMA.

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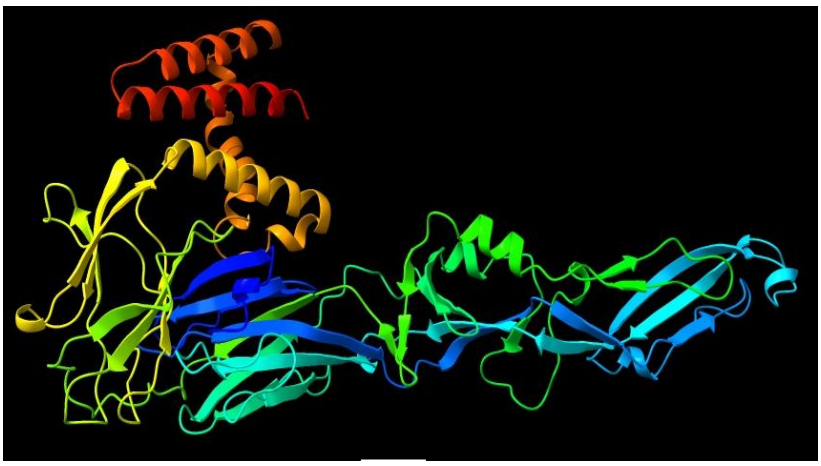

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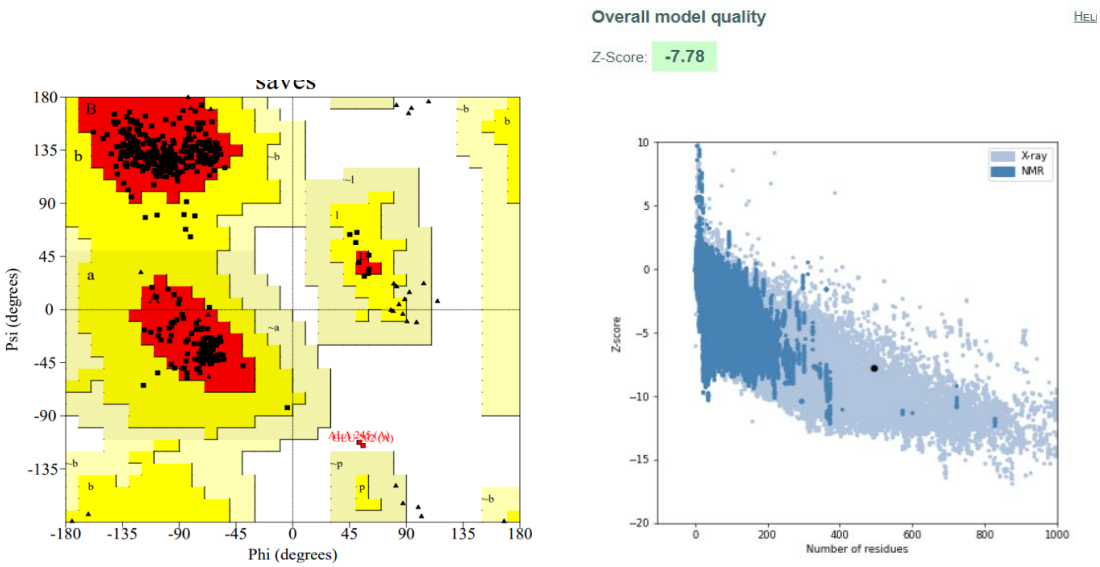

B C

Fig 2. 3D structure prediction a valuation, A) 3D structure of E protein Ramachandran plot for E protein and C) ProSA analysis o. E rotein of dengue virus.

To enhance the precision of the atomic positions in the protein structure and minimize potential errors from predicted models, we conducted refinement using the GalaxyRefine server. Prior to refinement, the Ramachandran plot analysis showed that 383 residues (90.8%) were in the most favored regions, 33 residues (7.8%) were in additionally allowed regions, and 3 residues (0.7%) were in generously allowed regions. Post-refinement, these values improved significantly, with 406 residues (96.2%) in the most favored regions, 14 residues (3.3%) in additionally allowed regions, and 2 residues (0.5%) in generously allowed regions (fig 2B). Additionally, the ERRAT overall quality factor, which assesses the statistical accuracy of non-bonded atomic interactions, increased from 85.86 to 93.33 following refinement. This improvement indicates a substantial enhancement in the overall structural quality and reliability of the refined protein model.

For further structural evaluation, ProSA was used. The predicted E protein model yielded an overall quality score of -7.78 (fig 2C), which is comparable to values typically observed in high-quality X-ray diffraction structures. The Z-score provided by ProSA is a measure of the model's quality. It is determined by comparing the model to a database of known protein structures. Models with Z-scores similar to experimentally determined structures, such as those obtained through X-ray crystallography, are considered to be of high quality. In addition, the ProSA energy plot

confirmed that the predicted structure exhibited a favourable energy distribution, thereby providing further validation of the reliability and accuracy of the refined protein model. The thorough assessment highlights the efficacy of the GalaxyRefine server in generating top-notch structural models.

In order to accomplish the goal of this research, antiviral peptides were obtained from the CAMPR4 database, following specific criteria including sequence length (1-100 amino acids), experimental verification, determined structure, antiviral effectiveness, whether they are naturally occurring or artificially synthesized, and viral classification. A total of 24 AMP sequences were obtained based on these criteria, as shown in **Table 1**. The peptides' physicochemical properties were analyzed using the ProtParam server.

By applying a prediction probability cutoff value of >0.5, a total of 19 peptides with lengths ranging from 17 to 95 amino acids were selected. These peptides were further evaluated based on their instability index, using a threshold value of 40. Peptides with an instability index below 40 were considered stable, leading to the selection of 12 peptides (Table 2). The instability index (II) is a vital measure used to evaluate the stability of proteins. An instability index value below 40 indicates protein stability, whereas a value above 40 implies protein instability (Guruprasad *et al*., 1990). The value of 40 serves as an important threshold for determining the likelihood of a protein being stable or unstable.

Subsequently, the hydrophobicity and solubility of these peptides were assessed using the PEPTIDE 2.0 and SolPro servers. For the Peptide Hydrophobicity/Hydrophilicity Analysis, only peptides with a hydrophobicity level above 30% were selected for further docking with E protein of dengue virus. The following 6 AMPs were ultimately chosen: Dermaseptin-4, Rp 71955, LL-37, MBD-4 (11-40) / P9 [H21R, K23R, K28R] (P9R), Alstotide S1, and Antihypertensive protein BDS-1. The three-dimensional structures of these peptides were obtained from the Protein Data Bank (Dermaseptin-4 (2DD6), Rp 71955 (1RPB), LL-37 (2FBS), MBD-4 (11-40) / P9 [H21R, K23R, K28R] P9R (6M56), Alstotide S1 (2MM6), and Antihypertensive protein BDS-1 (1BDS)) and utilized for docking studies. This comprehensive selection process ensured that the chosen peptides not only demonstrated high stability but also exhibited suitable hydrophobicity and solubility characteristics, thereby enhancing the reliability and potential effectiveness of subsequent docking and functional analyses.

Protein-protein docking studies were conducted between the predicted 3D structure of the E protein and the validated 3D structures of AMPs as mentioned above. The receptor function was performed by the E protein structure, whereas the ligand role was fulfilled by the AMP structures. The binding energies for the complexes between E protein and the AMPs were determined after a successful docking. The identified values are as follows: E-2FBS (-870.7), E-2DD6 (-774.1), E-1RPB (-1040.3), E-2MM6 (-993.8), E-1BDS (-1042.2) and E-6M56 (-1373).

Binding energy refers to the change in free energy (ΔG) that occurs when the receptor and ligand bind to form a complex. This energy change is composed of several components, including the solvation/desolvation energy, the conformational changes in the receptor and ligand, and the specific interactions between them (e.g., hydrogen bonds, van der Waals forces) (Pantsar *et al*., 2018). The negative binding energy indicates the stability of resulting complexes with receptor molecules, which is a fundamental characteristic of effective drugs (Muthu and Durairaj,2016). A more negative binding affinity corresponds to a stronger ligand-receptor interaction and leads to better molecular docking predictions (Daggupati *et al*.,2017).These results suggest that the E-6M56 (MBD-4 (11-40) / P9 [H21R, K23R, K28R] P9R) complex (fig 3) had the highest negative interaction energy, indicating the strongest binding, followed by E-1RPB (Rp 71955), E-1BDS (Antihypertensive protein BDS-1), E-2MM6 (Alstotide S1), E-2FBS (LL-37), and E-2DD6 (Dermaseptin-4). This ranking highlights the potential effectiveness of these peptides, with E-6M56 being the most promising candidate for further investigation and development as an antiviral agent against dengue virus.

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Title	Camp_ID	PDBID	Source Organism	Sequence	Length	Prediction Probability for AMPs
Dermaseptin-4	CAMPSQ467	2DCX,2DD6	Phyllomedusa sauvagii	ALWMTLLKKVLKAAAKALNAVLVGANA	27	1
Mytilin-B	CAMPSQ564	2EEM	Mytilus edulis	SCASRCKGHCRARRCGYYVSVLYRGRCY CKCLRC	34	1
Human Defensin-5	CAMPSQ730	1ZMP,3I5W, 4E82,4E83,4E86	Homo sapiens	ATCYCRTGRCATRESLSGVCEISGRLYRLC CR	32	0.99
Rp 71955	CAMPSQ754	1RPB	Actinomycete Sp9440	CLGIGSCNDFAGCGYAVVCFW	21	1
Palicourein	CAMPSQ762	1R1F	Palicourea condensata	GDPTFCGETCRVIPVCTYSAALGCTCDDRS DGLCKRN	37	0.99
Vhl-1	CAMPSQ763	1ZA8	Viola hederacea	CGESCAMISFCFTEVIGCSCKNKVCYLNSIS	31	0.9996
Kalata-B8	CAMPSQ956	2B38	Oldenlandia affinis	GSVLNCGETCLLGTCYTTGCTCNKYRVCT KD	31	1
Reptilian Defensin	CAMPSQ1028	2B5B	Caretta caretta	EKKCPGRCTLKCGKHERPTLPYNCGKYIC CVPVKVK	36	1
Melittin	CAMPSQ1142	1BH1,2MLT	Apis mellifera	GIGAVLKVLTTGLPALISWIKRKRQQ	26	0.99
Antihypertensive protein BDS-1	CAMPSQ1178	1BDS,2BDS	Anemonia sulcata	AAPCFCSGKPGRGDLWILRGTCPGGYGYT SNCYKWP NICCYPH	43	1
Vhl-1	CAMPSQ3000	1ZA8	Viola hederacea	SISCGESCAMISFCFTEVIGCSCKNKVCYLN	31	0.99
Retrocyclin-2	CAMPSQ3108	2LZI	Synthetic construct	GICRCICGRRICRCICGR	18	1
Antiviral lectin scytovirin	CAMPSQ3781	2QT4	Scytonema varium	GSGPTYCWNEANNPGGPNRCSNNKQCDG AR TCSSSGFCQGTSRKPDPGPKGPTYCWDEA KNPG GPNRCSNSKQCDGARTCSSSGFCQGTAGH AAA	95	1
LL-37	CAMPSQ11864	2FBS,2FBU, 2FCG,2K6O,2LMF	Homo sapiens	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNL VPRTES	37	0.99
Human Defensin-5/HD5 [E21R]	CAMPSQ19763	4RBX	Synthetic construct	ATCYCRTGRCATRESLSGVCRISGRLYRLC CR	32	1
Rev (34-50)	CAMPSQ21860	1ULL	Synthetic construct	TRQARRNRRRRWRERQR	17	0.63
Antifungal protein PAFB	CAMPSQ23410	2NC2	Penicillium chrysogenum	KFGGECSLKHNTCTYLKGGKNHVVNCGS AANK	56	0.9989

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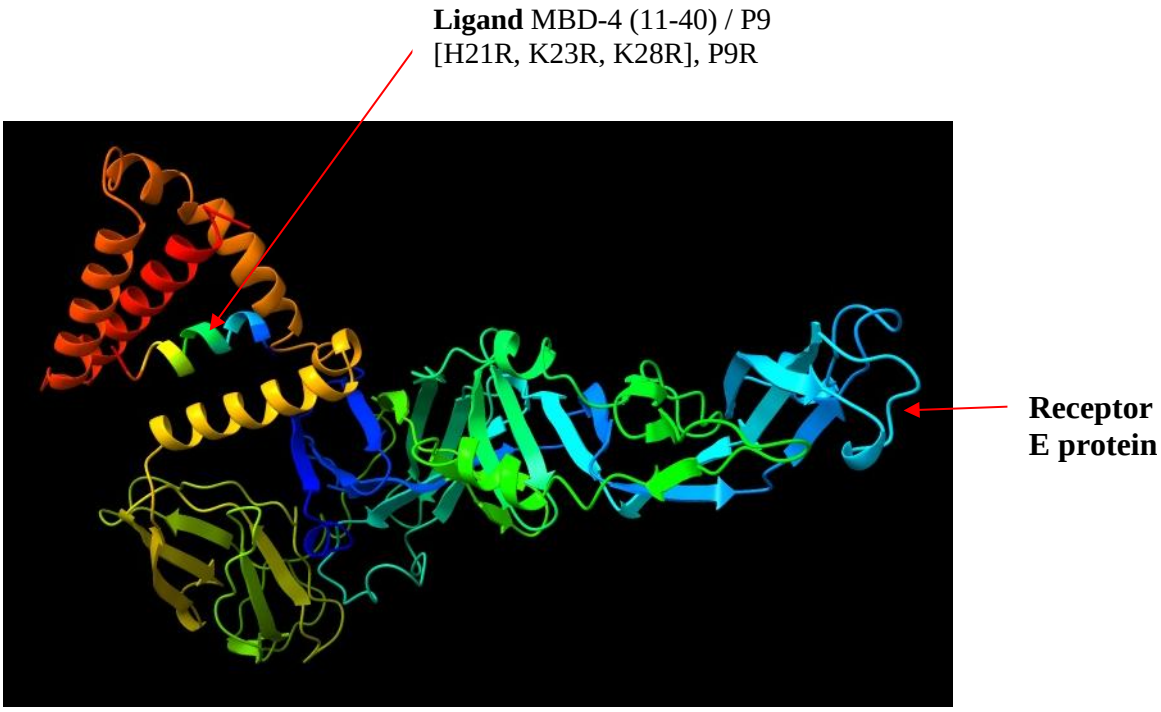
				KCKSDRHHCEYDEHHKRVDCQTPV		
MBD-4 (11-40) / P9 [H21R,K23R,K28R], P9R	CAMPSQ23847	6M56	Synthetic construct	NGAICWGPCPTAFRQIGNCGRFRVRCCRIR	30	1
Alstotide S1	CAMPSQ24145	2MM6	Alstonia scholaris	CRPYGYRCDGVINQCCDPYHCTPPLIGICL	30	0.99

Table 1. Collection of AMPs and sequences from CAMPR4 database.

Title	Molecular weight	Theoretical pI	Net charge	Instability index	Peptide stability	GRAVY	Hydrophobic	Acidic	Basic	Neutral	solubility
Dermaseptin-4	2779.47	10.48	4	10.38	stable	1.004	70.37%	0%	14.81%	14.81%	76.70%
Rp 71955	2185.53	3.8	-1	33.82	stable	1.157	42.86%	4.76%	0%	52.38%	69.20%
LL-37	4493.32	10.61	6	23.34	stable	-0.724	37.84%	13.51%	29.73%	18.92%	77.50%
MBD-4 (11-40) / P9 [H21R, K23R,K28R], P9R	3412.05	10.46	6	12.8	stable	-0.15	36.67%	0%	20%	43.33%	62.70%
Alstotide S1	3373.96	6.7	0	37.59	stable	0.063	33.33%	6.67%	10%	50%	37.70%
Antihypertensive protein BDS-1	4714.42	8.64	3	29.18	stable	-0.309	32.56%	2.33%	11.63%	53.49%	70.70%
Reptilian Defensin	4080.98	9.39	7	31.6	stable	-0.608	27.78%	5.56%	27.78%	38.89%	0.863
Retrocyclin-2	2041.58	9.3	5	36.55	stable	0.517	22.22%	0%	27.78%	50%	
Antiviral lectin scytovirin	9722.48	8.59	4	30.61	stable	-1.1	22.11%	6.32%	11.58%	60%	0.795
Human Defensin-5	3588.19	8.96	4	13.79	stable	-0.113	21.88%	6.25%	18.75%	53.13%	72.10%
Human Defensin-5/HD5 [E21R]	3615.26	9.49	6	7.77	stable	-0.144	21.88%	3.13%	21.88%	53.13%	72.10%
Kalata-B8	3307.81	7.76	1	27.53	stable	-0.023	16.13%	6.45%	9.68%	67.74%	71.50%

Table 2. Predicted physiochemical properties of the AMPs



CONCLUSION

Antiviral therapy for dengue infection holds immense significance due to the global burden of the disease. In this study, antimicrobial peptides (AMPs) were meticulously collected from the CAMPR4 database and rigorously screened for potential antiviral properties. The screening process was based on specific criteria, including sequence length (1-100 amino acids), experimental verification, determined structure, antiviral effectiveness, origin (naturally occurring or artificially synthesized), viral classification, and physicochemical properties. Detailed docking studies were conducted to investigate the interactions between the three-dimensional structures of the selected AMPs and the 3D structure of the E protein of the dengue virus. These studies revealed that the peptide MBD-4 (11-40) / P9 [H21R, K23R, K28R] P9R demonstrated the highest potential, exhibiting the most significant negative binding energy. While these findings are promising, further validation through *in vitro* and *in vivo* studies is essential to confirm the efficacy and safety of this peptide as a novel therapeutic option for dengue. Continued research in this area could pave the way for new antiviral treatments that significantly impact global health.

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Conflict of Interest

The author has no conflict of interest.

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