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RESEARCH ARTICLE

In silico screening of plant peptides against the envelope protein of dengue virus

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ABSTRACT

Peptide therapeutics are found to be an emerging and attractive class of treatment due to their highly specific and safe nature. Hence twenty plant peptides were subjected to screening by molecular docking against the envelope protein of the dengue virus using Clus Pro, Patch Dock, and HADDOCK servers. Physicochemical parameters, allergenicity, and toxicity profile of the plant peptides were estimated by Protparam analysis, AllergenFP, and ToxinPred web servers. Six potential compounds namely Ginkbilobin, Cycloviolin-D, Circulin-B, Circulin-A, Cycloviolacin-013, and Circulin-C showed the highest binding energy with both nonallergenic and nontoxic properties. They also exhibited desirable half-lives extending to 30 hrs except for Ginkbilobin, which showed the least half-life of 4.4 hours and non-polar activity. The residues of Ala-4 of Ginkbilobin; Arg-30 of Cycloviolin D; Arg-29 of Circulin A and C interacted with the Try 101 of the domain II of Envelope protein, implying the possible inhibition of the insertion process of the trimeric E protein during fusion with the host cells. Thus, the identified plant peptides could serve as potential leads upon further subjection to *in vitro* studies.

Keywords: DENV; antiviral peptides; protein-protein docking; allergenicity; domain II.

INTRODUCTION

Dengue is one of the most emerging mosquito-borne infections with which tropical countries have been contending in the last 20 years (Guo *et al*., 2017). According to current estimates, up to 390 million dengue infections occur each year making it a public health emergency of international concern (Hasan *et al*., 2016). Many of these illnesses are asymptomatic or subclinical and are transmitted in sylvatic cycles between mosquitoes of the genus Aedes and nonhuman primates in Africa, Southeast Asia, and South Asia (Powell, 2018).

Dengue Virus (DENV) belongs to the genus Flavivirus of the Flaviviridae family and is classified into four closely related serotypes DENV-1, 2, 3, and 4 sharing the sequence homology of 65–70% (Weaver & Vasilakis, 2009).DENV genome is a positive sense single-stranded RNA (ssRNA) with 11kb encoding 3 structural proteins (Capsid, Envelope, and prM) and 7 non-structural proteins (NS1, NS2A/B, NS3, NS4A/B, NS5) involved in viral RNA replication(Gebhard *et al*., 2011). Dengue viral infection represents broader clinical presentations with clinical symptoms from dengue viral fever to dengue shock syndrome (DSS) (Hadinegoro *et al*., 2015). Currently, no specific treatment exists for the management of dengue viral infections, and are treated symptomatically (Kalayanarooj, 2011).

This accentuates an urgent need for the development of novel drugs for the prevention and treatment of diseases. Although, Dengvaxia, a tetravalent vaccine been licensed for use in a few dengue-endemic countries, the main concerns behind it are the moderate to low efficacy against the serotypes DENV-1 and 2 and the safety of children below the age of 9 years. Supportive treatment by intravenous fluid replacement therapy and symptomatic treatment remains the major therapeutic option to reduce the fatality rate.

Hence, designing peptides against DENV E (envelope) protein will be an ideal strategy as it may inhibit the entry and fusion of the virus with the host cell. The envelope protein of the dengue virus appears as a dimer on the surface of the mature viral particle, and each monomer of the E protein has three ectodomains (ED1 to ED3) as well as a transmembrane region. The dimerization interface, two glycosylation sites, and the peptide of cellular membrane fusion are all found in ED2. ED3 is a polypeptide segment that is continuous and has an immunoglobulin-like structure that protrudes from the virion surface, with a membrane-proximal stem and a transmembrane anchor (Rodenhuis-Zybert *et al*., 2010).

Peptide therapeutics are found to be emerging and attractive drugs as they are found to be naturally occurring and safer, specific and efficacious than synthetic drugs (Low *et al*., 2017).

Identification of plant peptide molecules targeting the dengue envelope protein will be an effective approach to inhibiting the virus infection as it could prevent the virus from attaching and entering the host cells (Fahimi *et al*., 2018).

Therefore, this study was designed to assess the antiviral activity of naturally occurring plant peptides and to illustrate the mechanism of inhibition through molecular docking against the

envelope protein of the dengue virus. The allergenic, Safety, and Physico-chemical properties of peptides were also evaluated by *in silico* studies. Thus, the identified peptides would help in the development of novel peptides for the designing of antiviral drugs against the dengue virus.

MATERIALS AND METHODS

Peptide screening and Protein Preparation

Potential Antiviral peptides were retrieved from the data repository of APD (https://aps.unmc.edu/) and the structures of 20 peptides were retrieved from Protein Data Bank (PDB) (https://www.rcsb.org) and the unavailable structures were modeled using swiss model (Waterhouse *et al*., 2018*)*. The envelope protein of the DENV (PDB ID: 4UTC) was retrieved from the protein data bank with a resolution of 3.08 A°.

Molecular docking

The Envelope protein structure of DENV as receptor molecule and peptides as ligands were subjected to the protein-protein docking analysis in Cluspro, Patchdock, Hpepdock, and HADDOCK web servers. In Patchdock, final clustering was selected based on the RMSD value. The information-driven flexible docking method HADDOCK (High Ambiguity Driven Protein-Protein docking) was used to model biomolecular complexes.

Physicochemical properties

Physicochemical properties of the peptides were estimated using the Expasy ProtParam tool (https://web.expasy.org/protparam/) (Gasteiger *et al*.,2005*)*. This tool provides relevant properties including molecular weight, theoretical pI, extinction coefficient, anticipated half-life, instability index, aliphatic index, and grand average of hydropathicity (GRAVY).

Allergenicity and Toxicity Prediction

Allergenic properties of the peptides were calculated using allergenFP by employing a five E-descriptor-based fingerprinting method. The toxicity of the peptides was predicted using the ToxinPred webserver (https://webs.iiitd.edu.in/raghava/toxinpred/).

RESULTS

About 20 proteins were subjected to the protein-protein docking approaches using different software to identify the binding affinities of various peptides against the Envelope protein. Among the subjected 20 peptides, Ginkbilobin-(-103.8 kJ/mol), Cycloviolin-d (-78.6 kJ/mol), Circulin-B (-78.1 kJ/mol), Circulin-A (-76.9 kJ/mol), Cycloviolacin-013 (-76.9 kJ/mol), Circulin-c (-73.8 kJ/mol) showed highest binding energy and the binding energy of other peptide molecules using different software is shown in Table 1.

Ginkbilobin interacted with the envelope protein through hydrogen bonds at six places and formed 3 salt bridges. The residue Arg 26 of the Ginkbilobin peptide interacted with the Glu 71 residue of the Envelope protein with a distance of 2.61. Likewise, Cycloviolin D formed seven hydrogen bond interactions and among which the Glu 7 of the peptide interacted with Ser 72 at the hydrogen bond distance of 2.61. Cycloviolacin -013 showed six hydrogen bond interactions in which Glu 6 interacted with ser 72 at a distance of 2.60. Circulin A, B, and C formed five, two, and four hydrogen bond interactions respectively. The detailed hydrogen bond interaction of the peptides with the hydrogen bond distance is given in Table 2 and Figure 1.

Through Toxin pred, the peptides were nontoxic but the variability in allergenicity between the compounds were noted. The allergenicity profiles of the peptides were evaluated using the allergenFP webserver which characterised Palicouerin, VHL-1, circulin-D, circulin-E, cycloviolacin-024, cycloviolacin-y1, cycloviolacin-y4, cycloviolacin-y5 as allergens. Circulin A,Circulin B ,Circulin C, Cycloviolacin-VY1, Cycloviolacin-013, Cycloviolacin-014, Cycloviolin-A, Cycloviolin-C , Cycloviolin-D, Ginkbilobin, Kalata-b8, Tricycloan-A ,others were found to be non-Allergen. The allergenicity and toxicity profile of different peptides is shown in Table 3.

The theoretical PI was highest for Ginkbilobin showing 11.71 and for the other compounds Cycloviolin-D, Cycloviolacin-013, Circulin A, Circulin B, Circulin C showed 8.33. A negative GRAVY value indicated that the protein is non-polar and a positive value indicated the other proteins as non-polar compounds. Hence in the subjected analysis, Ginkbilobin and Cycloviolin-D were found to be non-polar

Table 1. The binding energy of different plant peptides towards envelope protein of dengue virus

S. No	Name	Cluspro Kj/mol	Patchdock Kj/mol	Hpep dock Kj/mol	Haddock Kj/mol
1	Circulin-A	-34.968	-286.13	-159.378	-76.9
2	Circulin-B	-34.968	-186.78	-167.523	-78.1
3	Circulin-C	-34.968	-88.03	-151.720	-73.8
4	Circulin-D	-34.968	-289.54	-191.98	-91.4
5	Circulin-E	-34.968	-104.82	-166.988	-84.9
6	Cycloviolacin-Y5	-34.968	-191.66	-161.427	-67.3
7	Cycloviolacin-024	-34.968	-272.88	-170.22	-72.6
8	Cycloviolacin-VY1	-34.968	-10.16	-173.157	-57.5
9	Cycloviolacin-Y1	-34.968	-42.02	-193.05	-83.4
10	Cycloviolacin-Y4	-34.968	-16.93	-166.175	-69.0
11	Cycloviolacin-013	-32.476	-47.12	-167.750	-76.7
12	Cycloviolacin-014	-32.476	-127.11	-184.828	-62.7
13	Cycloviolin-A	-32.476	-288.42	-164.516	-79.8
14	Cycloviolin-C	-323.476	-88.03	-151.720	-69.9
15	Cycloviolin-D	-34.968	-51.78	-170.065	-78.6
16	Ginkbilobin	-34.968	-102.12	-212.31	-103
17	Kalata-B8	-32.476	-274.62	-161.460	-60.8
18	Palicouerin	-34.968	-376.58	-180.39	-66.7
19	VHL-1	-34.968	-321.30	-170.93	-70.9
20	Tricyclon-A	-34.968	-31.77	-187.517	-68.9

Kj/mol represents the binding energy of the different plant peptides.

Table 2. Interaction analysis of the Compounds against the Envelope protein of dengue virus

S. No	Compound	Peptide Sequence	Hydrogen Bond Interaction	Distance (Å)
1.	Ginkbilobin	ANTAFVSSAHNTQKIPAGAPFNRNLRAMLADLRQNAAFAG	Glu71-Arg26	2.61
			Ser72-Arg26	2.79
			Arg73-Asn22	3.02
			Glu79-Asn11	3.29
			Ser81-Pro16	2.77
			Gly101-Ala4	2.82
2.	Cycloviolin-D	GFPCGESCVFIPCISAAIGCSCKNKVCYRN	Ser72-Glu7	2.61
			Trp101-Arg30	2.76
			Gly102-Arg30	2.71
			Asn103-Pro4	2.68
			Lys246-Val2	2.91
			Lys247-Ser8	2.71
			Lys247-Lys26	2.69
3	Circulin-B	GVIPCGESCVFIPCISTLLGCSCKNKVCYRN	Asn103-Pro3	2.83
			Lys246-Gly1	2.85
4	Cycloviolacin-013	GIPCGESCVWIPCISAAIGCSCKSKVCYRN	Ser72-Glu6	2.60
			Trp101-Arg29	2.74
			Gly102-Arg29	3.08
			Asn103-Pro3	2.71
			Lys247-Lys25	3.06
			Gln248-Ser7	3.28
5	Circulin-A	GIPCGESCVWIPCISAALGCSCKNKVCYRN	Thr70-Ser7	2.83
			Ser72-Glu6	2.62
			Trp101-Arg29	2.68
			Gly102-Arg29	2.84
			Asn103-Pro3	2.77
6	Circulin-C	GIPCGESCVFIPCITSVAGCSCKSKVCYRN	Ser72-Glu6	2.62
			Trp101-Arg29	2.69
			Gly101-Arg29	2.81
			Asn103-Pro3	2.80

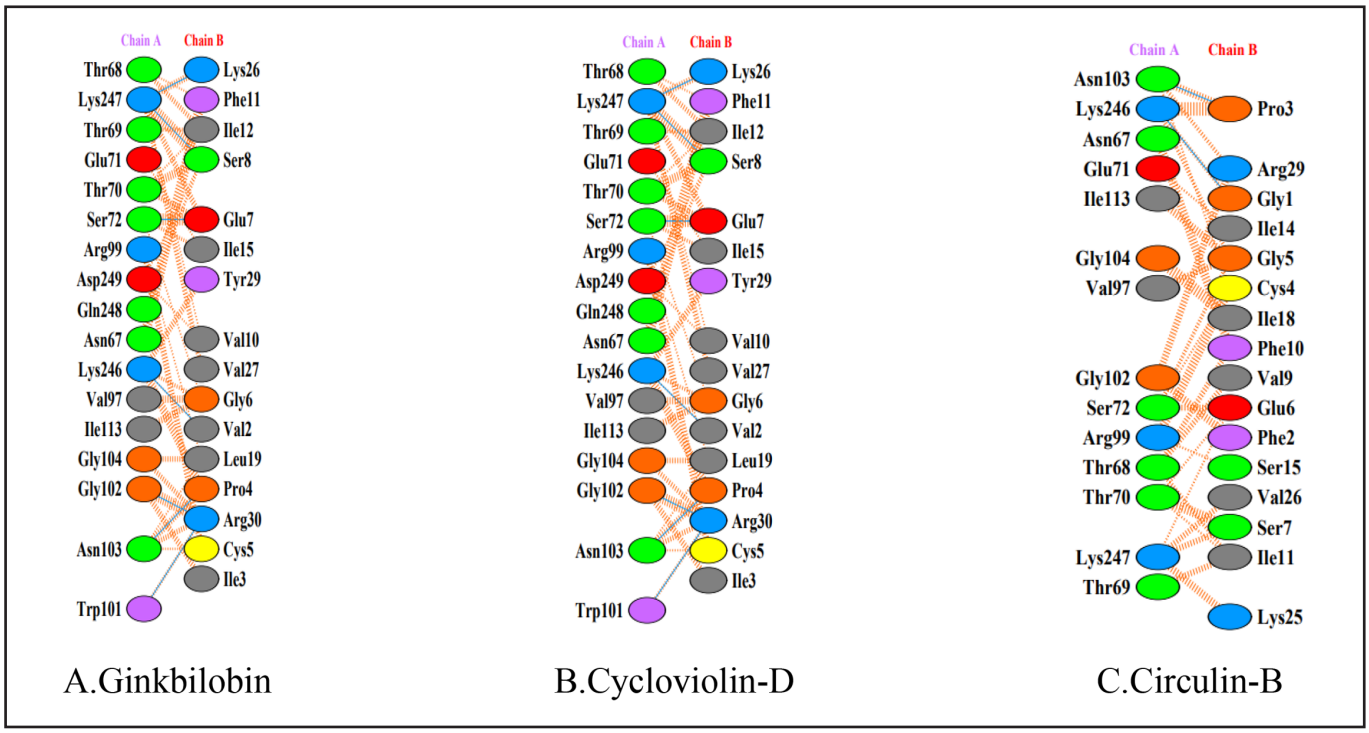

Figure 1. Protein -Protein docking and the molecular interactions of top 3 plant peptides A. Ginkbilobin, B. Cycloviolin-D, and C. Circulin-B against the Envelope Protein of dengue virus.

Table 3. Allergenicity and toxicity profile of the peptides

S. No	Compounds	Allergenicity	Toxicity prediction
1	Circulin-A	Non-allergen	Non-toxin
2	Circulin-B	Non-allergen	Non-toxin
3	Circulin-C	Non-allergen	Non-toxin
4	Circulin-D	Allergen	Non-toxin
5	Circulin-E	Allergen	Non-toxin
6	Cycloviolacin-Y5	Allergen	Non-toxin
7	Cycloviolacin-024	Allergen	Non-toxin
8	Cycloviolacin-VY1	Non-allergen	Non-toxin
9	Cycloviolacin-Y1	Allergen	Non-toxin
10	Cycloviolacin-Y4	Allergen	Non-toxin
11	Cycloviolacin-013	Non-allergen	Non-toxin
12	Cycloviolacin-014	Non-allergen	Non-toxin
13	Cycloviolin-A	Non-allergen	Non-toxin
14	Cycloviolin-C	Non-allergen	Non-toxin
15	Cycloviolin-D	Non-allergen	Non-toxin
16	Ginkbilobin	Non-allergen	Non-toxin
17	Kalata-b8	Non-allergen	Non-toxin
18	Palicouerin	Allergen	Non-toxin
19	Tricycloan-A	Non-allergen	Non-toxin
20	Vhl-1	Allergen	Non-toxin

and the other compounds to be polar. Ginkbilobin exhibited a very less half-life of 4.4 hrs while the other compounds showed an increased half-life to 30 hrs. The physicochemical characteristics of the subjected peptides are represented in Table 4.

DISCUSSION

Peptide drugs act as future ideal and effective drugs for treating many diseases and are approved for treatment by the FDA. In this study, twenty plant peptides with potential antiviral activity against other viruses were chosen and tested in-silico against the envelope protein of the dengue virus. Six potential compounds namely Ginkbilobin, Cycloviolin-D, Circulin-B, Circulin-A, Cycloviolacin-013, Circulin-C showed the highest binding energy with both non-allergenic and non-toxic properties. They also had desirable half-lives of up to 30 hours and were polar in nature, with the exception of the peptide Ginkbilobin, which had the shortest half-life of 4.4 hours and non-polar activity.

Envelope protein has 495 amino acid residues and consists of three distinct domains- a central domain (EDI), a dimerization domain (EDII), and an immunoglobulin (Ig)-like C terminal domain (EDIII) (Perera & Kuhn, 2018).

The Compounds with the highest docking score Ginkbilobin, Cycloviolin D, Cycloviolacin -013, Circulin A, B, and C interacted with the Domain II of Envelope virus which in particular required for the head-to-tail dimerization and for the fusion during the pH-dependent virus fusion. Cycloviolin D also interacted with Domain II at the aminoacid residues Ser 72, Trp 101, Gly 102, Asn 103, Lys 246, and Lys 247 with significant hydrogen bond formation. EDII is an elongated domain that contains a conserved fusion loop among all flaviviruses and in which the three hydrophobic amino acids (W101, L107, and F108) are found to be crucial during the fusion process for the insertion of trimeric E protein into endosomal membrane. These residues are exposed at the tip of the trimeric E protein at the early stages and among those, Ala-4 of the Compound Ginkbilobin; Arg-30 of Cycloviolin D; Arg-29 of Circulin A and C interacted with the Try 101 of the Envelope protein and hence inferring the possible inhibition of the insertion process of the trimeric E protein. Thus, this implies the potential role of the compounds in fusion inhibition. It is noteworthy that none of the compounds extended its interaction either with domain I or III. Ginkbilobin forms an $\alpha + \beta$-fold compact single-domain architecture by possessing two α-helices and a five-stranded β-sheet. Ginkbilobin has been reported to exhibit antifungal activity against Mycosphaerella *arachidic ola*, Botrytis *cinerea*, *Rhizoctonia solani*, *Coprinus comatus, Fusarium oxysporum*, and antibacterial activity against *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa*. In addition, it has also been shown to inhibit HIV-1 reverse transcriptase (Wang, & Ng, 2000).

Cycloviolacin 013 is a cyclotide reported to be isolated from *Viola odorata* (Chen *et al*., 2006). It has a Knotted structural arrangement with a circular backbone and three disulfide bridges, and the presence of the cyclic cystine knot motif is attributed to the

Table 4. Physicochemical properties of the peptides

Name	Molecular weight	Theatrical pI	EstimatedHalf-life	Instability index	Grand average of hydropathicity
Circulin-A	3175.78	8.33	30 hours	26.56 stable	0.417
Circulin-B	3307.98	8.33	30 hours	28.76 stable	0.642
Ginkbilobin	4213.75	11.71	4.4 hours	37.12 stable	-0.180
Kalata -B8	3307.81	7.76	30 hours	27.53 stable	-0.023
Cycloviolin-A	3235.88	8.33	30 hours	28.76 stable	0.681
Cycloviolin-C	3166.77	8.33	30 hours	32.98 stable	0.450
Cycloviloin-D	3170.76	8.33	30 hours	36.77 stable	-0.189
Palicourein	3928.43	4.78	30 hours	60.26 unstable	0.690
VHL-1	3340.94	5.85	1.9 hours	56.12 unstable	0.690
Circulin-C	3125.72	8.33	3o hours	18.66 stable	0.560
Circulin -D	3548.20	7.77	1.3 hours	14.86 stable	-0.016
Circulin-E	3420.03	6.71	1.3 hours	26.79 stable	0.090
Cycloviolacin-013	3148.75	8.33	30 hours	18.66 stable	0.530
Cycloviolacin-014	3203.74	8.64	30 hours	44.86 unstable	-0.187
Cycloviolacin-024	3072.44	4.35	30 hours	53.53 unstable	-0.163
Cycloviolacin-Y1	3412.77	3.67	30 hours	2.2 stable	0.142
Cycloviolacin-Y4	3025.55	4.00	30 hours	36.82 stable	1.047
Cycloviolacin-Y5	3122.67	4.37	30 hours	25.08 stable	0.793
Tricycloan-A	3504.96	4.68	30 hours	11.08 stable	0.006
Cycloviolacin-VY1	3225.88	7.77	1.2 hours	17.44 stable	0.874

enzymatic, chemical, and thermal stability (Pränting *et al*., 2010). The hydrophobic residues present in the cyclopeptides form a patch on the surface, influencing the activity of the cyclopeptides (Ireland *et al*., 2006).

Circulin A and B are macrocyclic peptides with 30 aminoacid residues stabilized by three disulfide bonds with a refined secondary structure that has been reported to play a role in biological activities such as in the inhibition of the replication of Human Immunodeficiency Virus (Balaraman & Ramalingam, 2018).

Thus, the identified compounds have already proven antimicrobial effects against HIV and other bacterial genera, but they have not been tested for anti-viral activity against dengue virus. Hence, further subjection of these selected compounds against the dengue virus through *in vitro* studies may show promising activity.

Conflicts of interest

The author declares that they have no conflict of interests.

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