MEDDOCS
Open Access Publisher

ISSN: 2578-8760

Journal of Nanomedicine

Open Access | Research Article

Computational Study for Identification of Antiviral Peptides Targeting Oncogenic Human Papillomavirus (HPV) Infections

Deepak Saravanan; Monisha Mohan*

Department of Science and Humanities, Indian Institute of Information Technology, Design and Manufacturing, Kancheepuram, Chennai, Tamil Nadu, India.

***Corresponding Author(s):** Monisha Mohan
Department of Science and Humanities, Indian Institute of Information Technology, Design and Manufacturing, Kancheepuram, Chennai, Tamil Nadu, India.
Email: monisha@iiidm.ac.in

Received: Dec 02, 2022

Accepted: Jan 02, 2023

Published Online: Jan 04, 2023

Journal: Journal of Nanomedicine

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: HPV oncoproteins; Antiviral peptide; Cervical cancer; Protein-peptide complex

Abstract

HPV infection is one of the leading causes of cancer mortality in women worldwide. Early treatment of the precancerous lesions would prevent the progression of cervical cancer. The oncogenic viral proteins namely E2, E6, and E7 play a critical role in the regulation of the cell cycle, immortalization, transformation of cervical cells, and maintaining the chromosomal integrity. The viral E2 protein is a key player involved in the replication of the genome, transcription, and partitioning of epigenomes during viral DNA replication. The binidng E6 and E7 interfere with the tumor suppressor functions of p53 and Rb respectively thereby targeting them to the ubiquitin proteolytic pathway. The present study identifies novel plant-derived antiviral peptides that target the oncoproteins against the life-threatening HPV infection. Docking calculations revealed that the antiviral peptides namely AP01049, AP00355, and AP01062 bind HPV E6, E7, and E2 respectively with the lowest binding energy. The docking score of AP01049 with E6 was found to be -1076.4 kcal/mol, while the docking score of AP01062 and AP00355 was determined to be -1005.1 kcal/mol and -1066.8 kcal/mol respectively.

Introduction

Human Papillomavirus (HPV), a dsDNA virus, is one of the root causes of cervical cancer in women. Currently, more than 200 different types of HPV have been identified [1,2]. Among them, 30 types of HPV are reported which induce genital warts and cancer risk. Depending on the ability of the virus to trigger cervical cancer, HPV is broadly classified into 2 types namely; high-risk and low-risk HPV. The High-risk HPV includes HPV-16, -18, -31, -45, and -56 while the low-risk types include HPV-4, -11, -44, and -65 [3]. Every year, around 450,000 cases of cervical cancer are reported and it is the second leading cause of death in women with cancer worldwide [4]. Even though chemotherapy and surgical treatment have been commonly employed for the prevention of cervical cancer, the lack of suitable targets for early detection and failure to respond to the currently available chemotherapeutic drugs are major challenges for the treatment. The oncogenicity of HPV is highly contributed by E2, E6, and E7 viral proteins [5]. E2 protein has an N-terminal transactivation domain which is involved in transcriptional regulation and a C-terminal domain DNA-binding domain that is important for DNA replication. E6 viral protein activates

Cite this article: Saravanan D, Monisha M. Computational Study for Identification of Antiviral Peptides Targeting Oncogenic Human Papillomavirus (HPV) Infections. J Nanomed. 2023; 6(1): 1056.

various host cellular proteins including p53, E6AP, and MAML1 resulting in uncontrolled cellular proliferation and inhibition of apoptosis [6,7]. For instance, HPV E6 oncoprotein induces cervical cancer by inhibiting the critical tumor suppressor function of p53 by targeting it for proteasomal degradation. The binding of E6 to proteins such as FADD/procaspase inhibits apoptosis [8,9]. Therefore, inhibitors targeting HPV E6-FADD/procaspase 8 interaction could resensitize the HPV-infected cells to apoptosis offering a promising therapy to treat cervical cancer. The high-risk E7 interacts with the Rb protein to bypass the G1/S checkpoint which results in the accumulation of genetic errors facilitating the development of the tumor [10,11]. Therefore, HPV E2, E6, and E7 viral oncoproteins activate various molecular pathways driving the cells toward malignancy [12]. Extensive research across the globe has demonstrated the potential application of peptides in the therapy and diagnosis of multiple cancers. Peptides that are plant-derived exhibit excellent anticancer properties. The present study aims to identify a novel plant derived peptide that inhibits HPV oncoproteins for the targeted therapy of cervical cancer.

Methods

Preparation of crystal structure of HPV drug targets

The protein data bank (https://www.rcsb.org/) was used for retrieving the PDB file of drug targets of HPV infection such as E2 (PDB ID: 1DTO), E6 (PDB ID: 4GIZ) and E7 proteins (Accession no. AAD33253.1). Since there is no availability of PDB structure of E7 from the protein data bank, the 3D structure was modeled by the SWISS-MODEL server. SWISS-MODEL performs the homology modeling which consists of four steps: (i) identification of template from the evolutionarily related proteins solved structures; (ii) sequence alignment for mapping residues of retrieved sequence and template structure; (iii) construction of the tertiary structure model on the basis of the alignment; and (iv) analysis the quality of the model by novel version of QMEAN Score [13]. The quality of the model proteins were assessed using Ramachandran Plot. The structures of the modeled peptides were ionized using EPIK at pH±2 and energy minimized using the OPLS 2005 force field of the Schrodinger suite.

Modelling of antiviral peptides from plant source

Totally 30 antiviral peptides of plant source have been retrieved from the Antimicrobial Peptide Database (https://aps.unmc.edu/). The retrieved sequence was submitted in the following server to obtain the 3D structures, SWISS-MODEL, PEPstrMOD, and PEP-FOLD3. The PEP-FOLD (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/) constructs the 3D model in three consecutive steps: (i) the prediction of SA letters, (ii) the assembly of the SA selected amino acids based on the sOPEP coarse grained force field and their refinement was done by the Monte-Carlo procedure, and (iii) all atoms conformation and the clustering procedure by 100 simulations [14].

Molecular docking analysis

The obtained drug targets of HPV were docked with the retrieved antiviral peptides. The docking of peptide and HPV sequence was done by ClusPro online server (https://cluspro.bu.edu/login.php) which have two important steps: (i) it runs on the systematic grid conformational program named PIPER using the Fast Fourier Transform. The function of docking score includes an electrostatic energy term, desolvation contributions calculated by a structure based pairwise potential and the van der waals interaction energy; (ii) PIPER uses the pairwise RMSF for the clustering of the 1000 top structures. Cα interface RMSF is the radius used in the clustering. The hierarchical approach was used after the clustering was completed in order to minimize the energy results in the removal of the side chain possible for collision by the CHARMM potential [15].

Visualization and interaction of drug target - antiviral peptide complexes

The docked protein-protein complex was visualized using Pymol software (https://pymol.org/2/). This is used for the creation of high-quality images for publication which is easy to use for the molecular docking analysis [16]. The interaction of the protein-protein complex was analyzed by the PIMA server (http://caps.ncbs.res.in/pima/). It identifies the inter-interaction of complexes based on the hydrogen bonds, van der waals interaction, electrostatic energy, salt bridges, and total stabilizing energy [17].

Molecular Dynamic (MD) simulation for the protein flexibility

The top hit protein-peptide complex obtained from the ClusPro was evaluated for flexibility by CABS-flex 2.0 server (http://biocomp.chem.uw.edu.pl/CABSflex2) and result was evaluated by the RMSF (root mean square fluctuation) plot. Based on the MD trajectory and NMR ensemble (default), the RMSF plot was obtained. The protein model developed by the CABS has an unique characteristics spatial resolution. They use the K-medoids method, a clustering trajectory for generating the output. The protein flexibility simulation takes place at a high rate with high resolution (10ns). The parameter for the simulation is default with 50 cycles [18].

Results and discussion

Modelling of antiviral peptides from plant source

The sequence of all the antiviral peptides derived from plant source was retrieved from the antimicrobial peptide database (**Table 1**). The tool for modeling of the three dimensional structures of the peptide was selected according to the length of the peptide sequence. The SWISS-MODEL and PEPstrMOD were used to build peptide models with length above and below 30 amino acid residues respectively. The structures of the modeled peptides were optimized and energy minimized using the OPLS force field of the Schrodinger suite (**Figure 1**).

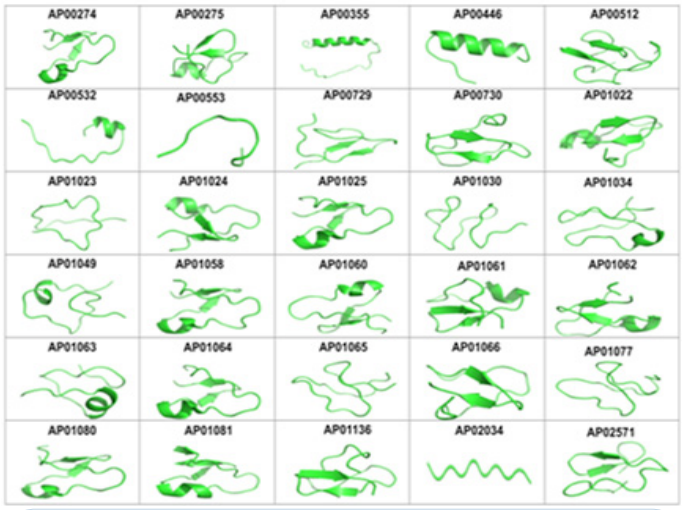


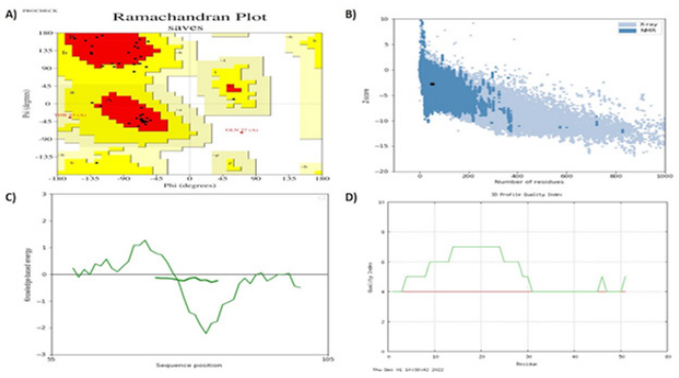
Figure 1: The modeled 3D structure of the 30 antiviral peptide derived from plant source.

Table 1: The retrieved sequence of antiviral peptides of plant sources from the antimicrobial peptide database.

S.No	Peptide ID	Source	Sequence	Docking score		
				E2	E6	E7
1	AP00274	*Chassalia parviflora*	GIPCGESCVWIPCISAALGCSCKNKVCYRN	-890.4	-791.0	-753.3
2	AP00275	*Chassalia parviflora*	GVIPCGESCVFIPCISTLLGCSCKNKVCYRN	-926.6	-849.9	-800.2
3	AP00355	*Ginkbilobin*	ANTAFVSSAHNTQKIPAGAPFNRNLRAMLADLRQNAAFAG	-902.3	-912.8	**-1066.8**
4	AP00446	*Malabar spinach*	GADFQECMKEHSQKQHQHQG	-652.9	-793.4	-804.1
5	AP00512	*Capsella bursa-pastoris*	GYHGGHGGHGGGYNGGGGHGGHGGGYNGGGHHGGGGHG	-679.4	-890.0	-725.3
6	AP00532	*Phaseolus lunatus L.*	KTCENLADTFRGPCFATSNC	-848.2	-919.5	-782.0
7	AP00553	*Vigna sesquipedalis*	KTCENLADTY	-630.4	-695.5	-798.8
8	AP00729	*Oldenlandia affinis*	GLPVCGETCVGGTCNTPGCTCSWPVCTRN	-747.9	-905.3	-778.3
9	AP00730	*Oldenlandia affinis*	GSVLNCGETCLLGTCYTTGCTCNKYRVCTKD	-735.8	-864.0	-764.7
10	AP01022	*Leonia cymosa*	GVIPCGESCVFIPCISAAIGCSCKNKVCYRN	-915.0	-797.4	-807.1
11	AP01023	*Leonia cymosa*	GTACGESCYVLPCFTVGCTCTSSQCFKN	-696.2	-750.6	-853.0
12	AP01024	*Leonia cymosa*	GIPCGESCVFIPCLTTVAGCSCKNKVCYRN	-835.5	-781.4	-789.8
13	AP01025	*Leonia cymosa*	GFPCGESCVFIPCISAAIGCSCKNKVCYRN	-792.0	-785.5	-805.0
14	AP01030	*Viola arvensis*	GLPICGETCVGGTCNTPGCSCSWPVCTRN	-873.8	-1040.7	-806.4
15	AP01034	*Palicourea condensata*	GDPTFCGETCRVIPVCTYSAALGCTCDDRSDGLCKRN	-660.6	-815.9	-704.4
16	AP01049	*Viola betonicifolia*	GLPVCGETCFGGTCNTPGCSCTWPICTRD	-829.7	**-1076.4**	-836.4
17	AP01058	*Viola hederaceae*	SISCGESCAMISFCFTEVIGCSCKNKVCYLN	-833.6	-977.9	-784.1
18	AP01060	*Chassalia parvifolia*	GIPCGESCVFIPCITSVAGCSCKSKVCYRN	-819.1	-776.1	-742.4
19	AP01061	*Chassalia parvifolia*	KIPCGESCVWIPCVTSIFNCKCKENKVCYHD	-924.5	-1002.1	-877.9
20	AP01062	*Chassalia parvifolia*	KIPCGESCVWIPCLTSVFNCKCENKVCYHD	**-1005.1**	-937.3	-909.2
21	AP01063	*Chassalia parvifolia*	KVCYRAIPCGESCVWIPCISAAIGCSCKN	-728.7	-775.9	-715.6
22	AP01064	*Viola odorata*	GIPCGESCVWIPCISAAIGCSCKSKVCYRN	-904.5	-817.6	-731.4
23	AP01065	*Viola odorata*	GSIPACGESCFKGKCYTPGCSCSKYPLCAKN	-759.3	-708.5	-716.2
24	AP01066	*Viola odorata*	GLPTCGETCFGGTCNTPGCTCDPWPVCTHN	-736.8	-938.6	-773.8
25	AP01077	*Viola yedoensis*	GGTIFDCGETCFLGTCYTPGCSCGNYGFCYGTN	-832.1	-1050.9	-865.6
26	AP01080	*Viola yedoensis*	GVPCGESCVFIPCITGVIGCSCSSNVCYLN	-804.5	-864.4	-740.5
27	AP01081	*Viola yedoensis*	GIPCAESCVWIPCTVTALVGCSCSDKVCYN	-770.3	-905.5	-755.4
28	AP01136	*Viola tricolor*	GGTIFDCGESCFLGTCYTKGCSCGEWKLCYGTN	-733.0	-1002.5	-812.6
29	AP02034	*Cocos nucifera*	EQCREEEDDR	-587.7	-871.5	-789.5
30	AP02571	*Viola yedoensis*	CGESCVFIPCITTVLGCSCSIKVCYKNGSIP	-750.2	-721.4	-665.4

Molecular docking analysis of peptides with HPV oncoproteins

The structure of oncogenic protein E2 (PDB ID: 1DTO) and E6 (PDB ID: 4GIZ) was retrieved from the protein data bank. The crystal structure of E7 protein is unavailable and therefore it was modeled using SWISS-MODEL. The structure of modeled E7 protein was validated with the Ramachandran plot using PROCHECK [19]. The stereo-chemical evaluation of backbone psi and Phi dihedral angles of the E7 protein was found to be 76.6%, 21.2% and 2.1% of amino acid residues in the preferred, allowed, and outside amino acid residue areas respectively (**Supplementary figure 1**). The structures of 30 antiviral peptides were ionized using EPIK at pH±2 and energy minimized using OPLS 2005 force field. Molecular docking of 30 antiviral peptides was performed to determine the binding potential and affinity of the peptides with HPV oncoproteins. The binding free energy was used as the parameter to estimate the strength of bonding between the peptide and HPV proteins. Lower binding free energies implies the increased strength of



Sup Figure 1: Structure validation of modeled E7 **(a)** Ramachandran plot obtained from PROCHECK. **(b)** Overall quality model obtained from the PROSA. **(c)** Local model quality obtained from the PROSA server. **(d)** 3D profile plot from the VADAR server.

bonding. Interestingly, molecular docking analysis revealed that peptides AP01049, AP00355, AP01062 bind HPV E6, E7, and E2 with higher binding energy respectively. The docking score of AP01049 with E6 was found to be -1076.4 kcal/mol, while the docking score of AP01062 and AP00355 was determined to be -1005.1 kcal/mol and -1066.8 kcal/mol respectively (**Table 2**).

Sup Table 1: Interaction of antiviral peptide with HPV oncoproteins.

Peptide	Peptide residues	Protein	Protein residues	Distance (Å)	Nature of interaction
AP01049	Thr13	HPV E6	Pro5	2.76	Hydrogen Bond
	Thr13		Arg8	3.06	Hydrogen Bond
	Gly11		Arg10	2.66	Hydrogen Bond
	Phe10		Lys11	2.79	Hydrogen Bond
	Arg28		Tyr32	3.32	Hydrogen Bond
	Cys14		Tyr54	3.01	Hydrogen Bond
	Ile25		Arg55	2.62	Hydrogen Bond
	Thr22		Ser74	2.79	Hydrogen Bond
	Asp29		Arg77	2.73	Hydrogen Bond
	Glu7		Arg102	2.88	Hydrogen Bond
	Val4		Arg129	3.11	Hydrogen Bond
	Cys9		Arg131	3.03	Hydrogen Bond
	Asp29		Arg77	2.70	Salt bridge
	Glu7		Arg102	2.87	Salt bridge
	Glu7		Arg131	2.72	Salt bridge
AP00355	Ala39	HPV E7	Thr60	2.83	Hydrogen Bond
	Gln13		Cys66	3.30	Hydrogen Bond
	Gln13		Lys67	2.68	Hydrogen Bond
	Ser7		Asp82	2.85	Hydrogen Bond
	Thr3		Arg84	2.72	Hydrogen Bond
	Arg26		Leu91	2.79	Hydrogen Bond
	Arg26		Asn92	2.67	Hydrogen Bond
	Ile15		Thr93	3.16	Hydrogen Bond
	Asn11		Ser95	2.82	Hydrogen Bond
AP01062	Gly5	HPV E2	Gln12	2.77	Hydrogen bond
	Ser7		Tyr19	2.79	Hydrogen bond
	Ile11		Tyr19	2.89	Hydrogen bond
	Trp10		Asp22	2.98	Hydrogen bond
	Gly5		Gln57	2.75	Hydrogen bond
	Pro3		Val58	3.15	Hydrogen bond
	Lys25		Glu20	2.56	Salt bridge
	Glu6		Lys68	2.73	Salt bridge

Analysis of peptide-interacting residues of E2, E6 and E7 oncoproteins

Interaction analysis of E2 with antiviral peptide AP01062 revealed that the residues namely Gly5, Ser7, Ile11, Trp10, Gly5, and Pro3 of AP01062 forms hydrogen bond with the residue of E2 namely, Gln12, Tyr19, Tyr19, Asp22, Gln57, and Val58 whereas Lys25 and GLU6 of the peptide forms the salt bridges with Glu20 and Lys68, respectively (**Figure 2 A,B and C**). Interestingly, it was found that AP01062 binds to Tyr19 and Val58 of E2 protein that are important for interaction with HPV E2 protein [20]. This implies that the peptide would interfere with the formation of the E1-E2 complex which is critical for initiation of viral DNA replication. Interaction analysis of E6 with the antiviral peptide AP01049 revealed that the residues namely Thr13, Gly11, Phe10, Arg28, Cys14, Ile25, Thr22, Asp29, Glu7, Val4, and Cys9 of AP01049 forms the hydrogen bonds with the residue of E6 namely, Arg8, Arg10, Lys11, Tyr32, Tyr54, Arg55, Ser74, Arg77, Arg102, Arg129, and Arg131 whereas ASP29 and GLU7 of the peptide forms salt bridge with the residue ARG77 and ARG131, respectively (**Figure 2 E, F and G**). Furthermore, it was previously reported that E6 residues namely; Arg8, Arg10 and Tyr54 of E6 are crucial for interaction with p53 to target it for rapid proteasomal degradation [21,22]. Lack of p53 abrogates apoptosis thereby inducing tumorigenesis. Therefore, binding of AP01049 could block the association of E6 with p53 thereby targeting HPV induced cervical cancer. In addition, interaction analysis of E7 with AP00355 shows that the residue namely Ala39, Gln13, Ser7, Thr3, Arg26, Ile15, and Asn11 of AP00355 forms the hydrogen bonds with the residue of E7 namely Thr60, Cys66, Asp82, Arg84, Leu91, Thr93, and Ser95 respectively (**Figure 2 G, H and I**) (**Table 2**). E7 residues namely; Thr60, Asp82, Arg84, Leu91 and Thr93 are crucial for interaction with Rb to target it for ubiquitin-proteasome pathway [23]. Taken together, it indicates that AP00355 would interfere with the interaction of E7 with Rb thereby inhibiting virus-induced tumorigenesis. The Kd value of the docked hit complexes was validated by the PRODIGY server. It was observed that the Kd value of docked complexes such as E6AP01049, E2AP01062 and E7AP00355 is 0.13nM, 0.25nM, and 60nM, respectively indicating a strong association of the protein-peptide complexes **(Supplementary table 1)**.

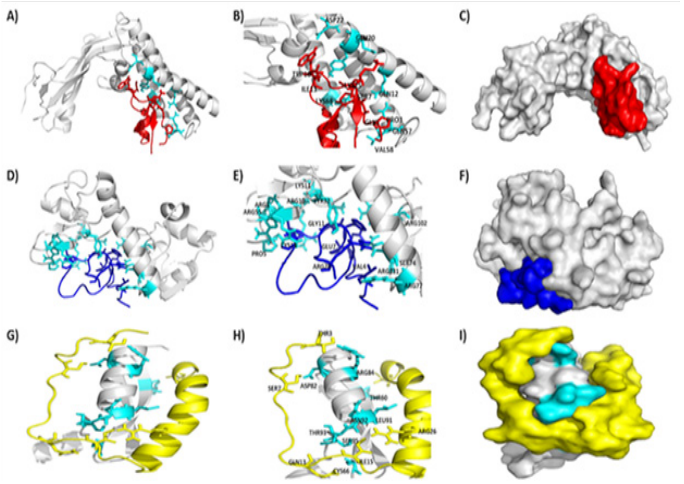


Figure 2: Interaction analysis of HPV oncoproteins with the peptides. The E2 (A, B and C), E6 (D, E and F) and E7 (G, H and I) proteins are represented in grey. The structures of AP01062, AP01049, and AP00355 are illustrated in red, blue, and yellow respectively. The residues of HPV proteins interacting with the peptide are shown in cyan sticks.

Sup Table 2: The Kd and ΔG value of the docked hit complexes of E2, E6 and E7.

Peptide	Protein	Kd (M) at 25.0 °C	ΔG (kcal mol-1)
AP01049	E6	1.3E-10	-13.4
AP01062	E2	2.5E-10	-13.1
AP00355	E7	6.0E-08	-9.8

Molecular Dynamic (MD) simulation for the protein flexibility

Molecular dynamics simulation for protein flexibility was performed to determine the stability of the HPV protein-peptide complexes [24]. The CABS- flex server generates the RMSF value based on the protein chain. The plot gives the report that the highest amplitude for the E6-AP01049 complex is residue 5 of around 3.7510Å. The E2-AP01062 complex has the highest amplitude at residue 124 of around 4.6510Å and the E7-AP00355 complex has the highest amplitude at residue 40 of around 6.3270Å. It was observed that the RMSF values of the protein-peptides complexes after 10ns simulation is less than 10Å which indicates a stable association of the peptides with

HPV oncoproteins (**Figure 3**).

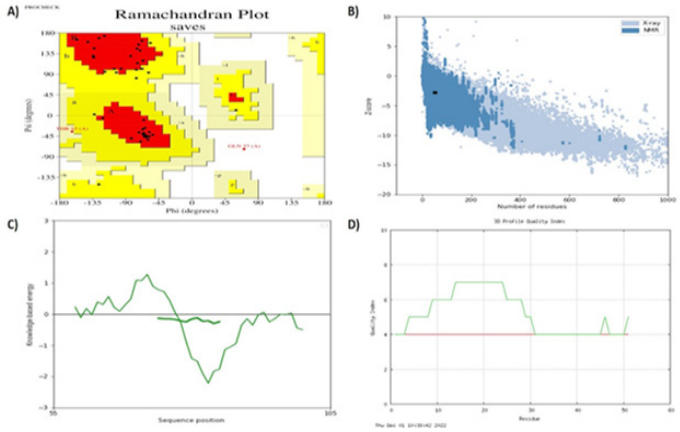


Figure 3: The RMSF profiles of **(A)** E2-AP01062 complex, **(B)** E6-AP01049 complex, and **(C)** E7-AP00355 complex obtained by CABS-fex 2.0.

Conclusion

The prediction of peptides and the identification of their binding affinities using *in silico* tools would facilitate the discovery and development of novel and potential anticancer agents, thereby saving important resources including cost, time, cost and manpower. In this study, 30 antiviral peptides derived from plant sources were screened against HPV oncoproteins. Using *in silico* approach three antiviral peptides namely AP01062, AP01049, and AP00355 that binds E2, E6, and E7 proteins with highest affinity were identified. Based on the results obtained from this study, further *in vitro* and *in vivo* evaluations of the peptides would determine their potential use as anticancer drugs for the therapy of HPV induced cervical cancer.

Conflicts of Interest

The authors declare no conflict of interest

Acknowledgement

We sincerely thank the Indian Institute of Information Technology, Design and Manufacturing – Kancheepuram for providing the necessary infrastructure to carry out this work successfully.

References

1. D'Abramo CM. Small Molecule Inhibitors of Human Papillomavirus Protein - Protein Interactions. The Open Virology Journal. 2011; 80-95.
2. Iryani I, Amelia F, Iswendi I. Active sites prediction and binding analysis E1-E2 protein human papillomavirus with biphenylsulfonacetic acid. IOP Conf Ser Mater Sci Eng. 2018; 335: 012031.
3. Woodman CBJ, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer. 2007; 7: 11-22.
4. Kanodia S, Fahey LM, Kast WM. Mechanisms used by human papillomaviruses to escape the host immune response. Curr Cancer Drug Targets. 2007; 7: 79-89.
5. Kalvatchev Z, Rösl F. Human papilloma viruses: Realities and perspectives. Biotechnol Biotechnol Equip. 2007; 21: 137-144.
6. Tungteakkhun SS, Duerksen-Hughes PJ. Cellular binding partners of the human papillomavirus E6 protein. Archives of Virology. 2008; 397-408.
7. Yim E-K, Park J-S. The Role of HPV E6 and E7 Oncoproteins in HPV-associated Cervical Carcinogenesis. Cancer Research and Treatment. 2005; 319.
8. Tungteakkhun SS, Filippova M, Neidigh JW, Fodor N, Duerksen-Hughes PJ. The interaction between human papillomavirus type 16 and FADD is mediated by a novel E6 binding domain. J Virol. 2008; 82: 9600-9614.
9. Garnett TO, Filippova M, Duerksen-Hughes PJ. Accelerated degradation of FADD and procaspase 8 in cells expressing human papilloma virus 16 E6 impairs TRAIL-mediated apoptosis. Cell Death Differ. 2006; 13: 1915-1926.
10. Liu S, Tian Y, Greenaway FT, Sun M-Z. A C-terminal hydrophobic, solvent-protected core and a flexible N-terminus are potentially required for human papillomavirus 18 E7 protein functionality. Biochimie. 2010; 92: 901-908.
11. Liu X, Clements A, Zhao K, Marmorstein R. Structure of the human Papillomavirus E7 oncoprotein and its mechanism for inactivation of the retinoblastoma tumor suppressor. J Biol Chem. 2006; 281: 578-586.
12. Pappa KI, Kontostathi G, Lygirou V, Zoidakis J, Anagnou NP. Novel structural approaches concerning HPV proteins: Insight into targeted therapies for cervical cancer (Review). Oncol Rep. 2018; 39: 1547-1554.
13. Bordoli L, Kiefer F, Arnold K, Benkert P, Battey J, Schwede T. Protein structure homology modeling using SWISS-MODEL workspace. Nat Protoc. 2009; 4: 1-13.
14. Thévenet P, Shen Y, Maupetit J, Guyon F, Derreumaux P, Tufféry P. PEP-FOLD: an updated de novo structure prediction server for both linear and disulfide bonded cyclic peptides. Nucleic Acids Res. 2012; 40: W288-93.
15. Ghani U, Desta I, Jindal A, Khan O, Jones G, Hashemi N, et al. Improved Docking of Protein Models by a Combination of Alphafold2 and ClusPro.
16. Seeliger D, de Groot BL. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. J Comput Aided Mol Des. 2010; 24: 417-422.
17. Kaleeckal Mathew O, Sowdhamini R. PIMA: Protein-Protein interactions in Macromolecular Assembly - a web server for its Analysis and Visualization. Bioinformation. 2016; 12: 9-11.
18. Nag A, Verma P, Paul S, Kundu R. In Silico Analysis of the Apoptotic and HPV Inhibitory Roles of Some Selected Phytochemicals Detected from the Rhizomes of Greater Cardamom. Appl Biochem Biotechnol. 2022; 194: 4867-4891.
19. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. Journal of Applied Crystallography. 1993; 283-291.
20. Leticia Saucedo-Mendiola M, Luis Ríos-Bañuelos J, Vázquez-Vázquez A, Marcela Coria-Quiñones E, Estela Frías-Zepeda M, Alonso Gándara-Mireles J, et al. E1 and E2 viral proteins as therapeutic targets for development of antiviral agents. Viruses and Viral Infections in Developing Countries. IntechOpen; 2020.
21. Soumia M, Hajji H, El Mzibri M, Younes FZ, Mohammed B, et al. In-Silico Molecular Modeling Studies to Identify Novel Potential Inhibitors of HPV E6 Protein. Vaccines (Basel). 2022; 10.
22. Nabati F, Moradi M, Mohabatkar H. In silico analyzing the molecular interactions of plant-derived inhibitors against E6AP, p53, and c-Myc binding sites of HPV type 16 E6 oncoprotein. Mol Biol Res Commun. 2020; 9: 71-82.

23. Shaikh F, Sanehi P, Rawal R. Molecular screening of compounds to the predicted Protein-Protein Interaction site of Rb1-E7 with p53- E6 in HPV. Bioinformation. 2012; 8: 607-612.

24. Karplus M, McCammon JA. Molecular dynamics simulations of biomolecules. Nat Struct Biol. 2002; 9: 646-652.