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Research Article

IN SILICO INVESTIGATION OF *ECHINACEA PURPUREA* PHYTO LIGANDS TARGETING HUMAN PAPILLOMAVIRUS TYPE 18'S L1 PROTEIN: IMPLICATIONS FOR CERVICAL CANCER MANAGEMENT

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ABSTRACT

Objectives: Human papillomavirus (HPV) is a highly oncogenic virus responsible for the majority of intraepithelial lesions and cervical cancer. Among various HPV types, 16 and 18 contribute to approximately 70% of cervical cancer cases globally, making them the most prevalent high-risk oncogenic variants associated with this disease. Numerous vaccines (Gardasil 9, Gardasil, and Cervarix) have been approved by FDA to combat HPV infections; however, their widespread implementation faces challenges due to their limited cost-effectiveness.

Methods: *Echinacea purpurea's* components have already been studied for *in silico* analysis against HPV Type 16's L1 protein. In the present analysis, we aimed to explore the potential interaction between *E. purpurea* phytoligands (curcumin, echinacoside, and chicoric acid) and the major capsid protein L1 of HPV type 18 (2R5I) through molecular docking analysis.

Results: Molecular docking analysis revealed that the echinacoside, one of the components of *E. purpurea*, has the best binding affinity (−7.9 kcaL/moL) against the L1 protein of the HPV type 18.

Conclusion: The molecular docking analysis indicates that *E. purpurea* could act as an inhibitor against HPV infection. Further research and *in vivo* studies are necessary to confirm its efficacy as a cost-effective alternative to present HPV vaccines.

Keywords: Bioinformatics, Human papillomavirus infection, Human papillomavirus type 18, Human papillomavirus type 16, 2R5I, 2R5H, Cervical Cancer, Molecular Docking, Computational analysis.

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INTRODUCTION

Human papillomavirus (HPV) is a widespread group of viruses that commonly infect the skin and mucous membranes in humans. One particular variety, HPV type 18 is recognized as a high-risk oncogenic variant. HPV type 18 is mainly transmitted through sexual contact and primarily affects the genital and anal regions. Cervical cancer, as well as other vaginal and anogenital malignancies, are all highly associated with HPV type 18 infection that persists over time. HPV type 18 is widely accepted as the second most carcinogenic HPV type after HPV16 [1]. Cancer of the cervix became the fourth most prevalent cancer in women globally in 2020 [2]. HPV comprises various types, and among them, HPV type 16 and HPV type 18 are classified as high-risk oncogenic strains. These two types are particularly significant due to their association with certain cancers, especially cervical cancer, as well as other genital and anogenital cancers. Both HPV type 18 and HPV type 16 are transmitted through sexual contact and can lead to persistent infections, raising the risk of cancer development over time [3]. The development of keratinocytes, the cells that make up the outer layer of the skin, is intricately linked to the life cycle of HPVs. HPV expresses its early genes after infecting undifferentiated basal cells, promoting the early phases of the viral life cycle. Late genes are expressed as a result of this process, which makes it easier to produce the structural proteins needed to build viral capsids [4].

In its genome, the HPV features a distinctive circular, double-stranded DNA molecule. The viral genome consists of three main segments: The early region (E Region), the late region (L Region), and the long control region (LCR). These segments play essential roles in the virus's life cycle and replication process. Conversely, the L Region contains viral late genes, notably L1 and L2. The viral capsid (Fig. 1), which encloses the viral DNA to produce new contagious viral particles, emerges by the gene expression toward the end of the viral life cycle. The HPV type 18 L1 (Late 1) gene is an essential part of the virus that codes for the important structural protein L1.

Previous research by Lukman *et al.* in 2022 [5] demonstrated the significance of positive interaction and its potential implications in the viral life cycle and good binding mode of the "major capsid protein L1 of HPV type 16" (PDB ID: 2R5H) with *Echinacea purpurea* components. By exploring this novel association, we aim to study the interaction of "major capsid protein L1 of HPV type 18" (PDB ID: 2R5H) with *E. purpurea* phytoligands (curcumin, echinacoside, and chicoric acid) [5]. While different HPV types may have distinct genetic variations, the L1 protein is relatively conserved among HPV type 16 and 18 strains. Moreover, it is essential for the effective infection that L1 interacts with the heparan sulfate (HS) carbohydrates present on host cells during the first stage of papillomavirus infectious entrance [6]. Blocking this interaction with soluble heparin or enzymatic removal of HS inhibits virion binding and infectious entry. A structural alteration brought on by the viral protein L1's binding to the cell surface makes protein L2 accessible. This promotes the virus's capacity to bind to a cell receptor and allows cellular furin protease to cleave L2. Lacking L1 and L2, virus particles are unable to release their DNA, essentially halting the progress of infection [6].

Purple coneflower, also known as *E. purpurea*, possesses antiviral, anti-inflammatory, and immunomodulatory activities that suggest it

may be used as a chemotherapeutic [7]. According to certain research, some chemicals in *E. purpurea* may slow the growth of cancer cells and trigger apoptosis.

METHODS

To find the best affinity of ligand molecules curcumin, echinacoside, and chicoric acid against the protein 2R5I, molecular docking was performed. 2D structure analysis was performed to understand the structural configuration of 2R5I with the use of PDBsum (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/). The Ramachandran plot was created with the use of PDBsum to determine the accuracy of the predicted protein structure. All the analysis was performed using 8.00 GB RAM and 11th Gen Intel ® Core (TM) i3-1115G4 processor on Windows 11. Docking analysis was done in software AutoDock Vina and structures were visualized using DS Biovia Discovery Studio software.

Retrieval of ligands

Curcumin, echinacoside, and chicoric acid were selected to perform molecular docking against protein 2R5I. The ChEMBL database "https://www.ebi.ac.uk/chembl/" was used to retrieve molecular structure (Fig. 2), 3D SDF file, and SMILES data of ligands molecules. SMILES data were used further to perform SwissADME analysis and molecular structure prediction of ligands. PyMOL was used to visualize the ligand structure [8].

Table 1 : Grid dimensions of ligands

Ligands	Grid dimensions		
	X	Y	Z
Curcumin	−0.004	−0.187	0.078
Echinacoside	0.008	−0.112	−0.037
Chicoric acid	0.002	−0.049	0.000

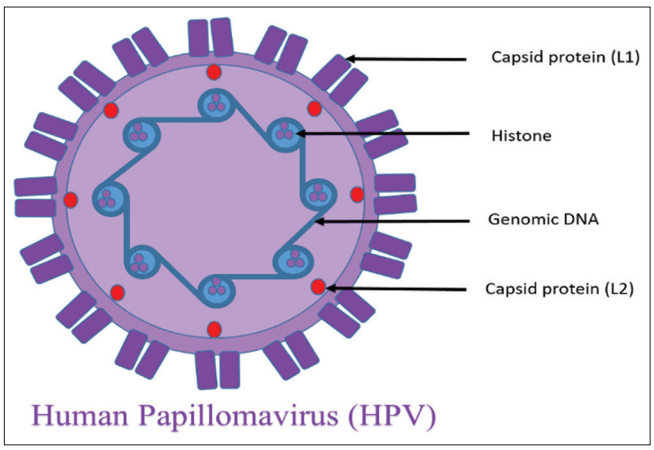

Fig. 1: HPV has a non-enveloped, icosahedral capsid composed of L1 and L2 proteins, enclosing its circular double-stranded DNA genome with a regulatory LCR region and linked to proteins that resemble histones. This structure facilitates viral entry, replication, and pathogenesis in host cells, contributing to diseases such as genital warts and cancer

Retrieval of protein

The major capsid protein L1 of HPV type 18, represented by PDB ID 2R5I, was sourced using the RCSB PDB data bank (https://www.rcsb.org/) in PDB format [9]. The protein had a 3.40 Å resolution and the X-ray diffraction method was used for protein retrieval. The visualization software Chimera 1.16 was used for visualization of protein 3D structure.

Protein and ligand preparation

The crystallographic structure of the protein is not supported by the free energy of water molecules so all water molecules of protein 2R5I were deleted with the use of software Chimera 1.16 [10]. The protein-ligand binding and docking results can be affected by the presence of water molecules. The protein structure was simplified by removing all additional chains. The X-ray diffraction method of protein retrieval does not contain charges and hydrogen atoms, so hydrogen atoms and Kollman charges were inserted in the protein structure for better optimization. All steps of protein purification were performed with the use of the software Chimera 1.16. The purified protein structure (Fig. 3) was used to analyze the Ramachandran plot and secondary structure prediction of protein with the use of PDBsum [10]. Ligand preparation was done by selecting a molecule for Autodock4 in Autodock tool 1.5.7 for three ligands, this step was performed to add charges and merge non-polar hydrogens [11].

Grid preparation

The AutoDock tool 1.5.7 "https://ccsb.scripps.edu/mgltools/" was used for grid preparation. Dimensions of the grid were automatically predicted with the use of AutoDock tool 1.5.7 for docking because the binding site of the protein was not identified (Table 1).

Pharmacological studies

The pharmacological properties of ligands were studied with the use of SwissADME (http://www.swissadme.ch/) (Daina *et al*., 2017a) (Tables 1-4). LIPINSKI rule of five and physicochemical properties such as fraction Csp3, rotatable bond, TPSA, lipophilicity, MLogP, hydrogen donors, hydrogen acceptors, blood–brain barrier penetration, PGP substrate, GI absorption, solubility (LOGSw-SILICOS IT), and molar refractivity were studied in SwissADME. ADMElab 2.0 ("https://admetmesh.scbdd.com/") was used to analyze LIPINSKI rule of five or Pfizer's rule of five, all values analyzed in SwissADME were accepted by LIPINSKI rule of five or Pfizer's rule of five in ADMElab 2.0 [12].

Molecular docking

AutoDock Vina was used to perform docking of curcumin, echinacoside, and chicoric acid against protein 2R5I to determine their inhibitory properties (Eberhardt *et al*., 2021). Purified protein structure and ligands structure were converted into.Pdbqt format with the use of PyMOL before docking. The docking was performed for all three ligands against protein 2R5I with the dimensions predicted by AutoDock tool

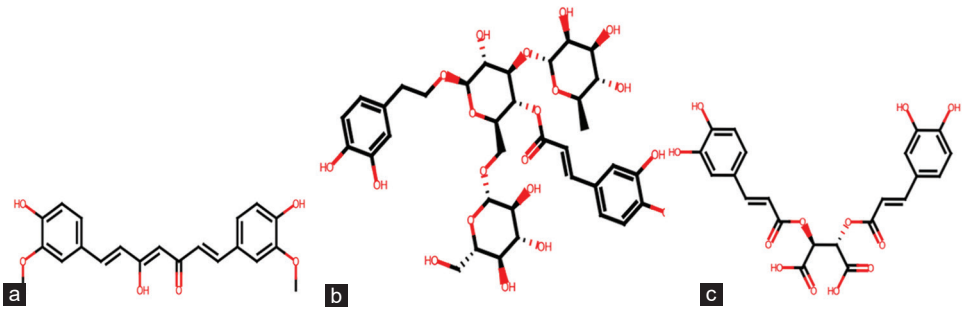

Fig. 2: Molecular structure of ligands (a) curcumin (b) echinacoside (c) chicoric acid [4]

Table 2: Ligand physicochemical properties

Ligands	Molecular weight	Fraction Csp3	Rotatable bonds	TPSA	Lipophilicity
Curcumin	368.38 g/moL	0.10	7	96.22 Å^2	3.21
Echinacoside	786.73 g/moL	0.57	14	324.44 Å^2	0.92
Chicoric acid	474.37 g/moL	0.09	11	208.12 Å^2	0.88

Table 3: Lipinski filter analysis

Ligands	Molecular weight	MLogP	Hydrogen donors	Hydrogen acceptors	Molar refractivity
Curcumin	368.38 g/moL	1.47	3	6	103.70
Echinacoside	786.73 g/moL	−4.47	12	20	180.81
Chicoric acid	474.37 g/moL	0.14	6	12	114.00

Table 4: ADME prediction

Ligands	BBB	GI absorption	PGP substrate	Solubility (LOGSw-SILICOS IT)
Curcumin	No	High	No	−3.61
Echinacoside	No	Low	No	1.63
Chicoric acid	No	Low	Yes	−0.87

BBB: Blood–brain barrier

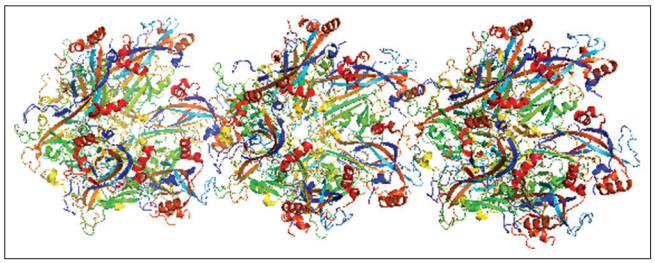

Fig. 3: Purified structure of 2R5I

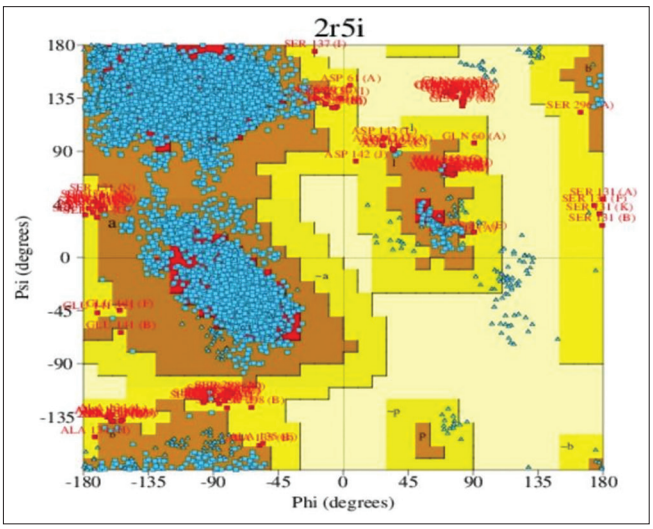

Fig. 4: Ramachandran plot of 2R5I

1.5.7. AutoDock Vina predicted nine possible configurations of protein-ligand binding according to the grid box for all three ligands against protein 2R5I. The lower binding affinity is considered the best degree of binding and best docking configuration. Echinacoside had the lowest binding affinity than curcumin and chicoric acid, and the best binding affinity of individual three ligands was visualized with the use of DS Biovia Discovery Studio [13].

Visualization

DS Biovia Discovery Studio was used to visualize 3D and 2D structure of the best binding affinity of individual three ligands against protein 2R5I [13]. 3D and 2D structures of the best binding affinity of three ligands with protein were generated and downloaded to predict amino acids and bonds involved in protein-ligand binding.

RESULTS

Protein structure analysis

The Ramachandran plot was used to analyze the torsion angle distribution of a protein structure. The Ramachandran plot represented in Fig. 4 was performed using PDBsum, and the secondary structure of the protein (Fig. 5) was also created in PDBsum [10]. Red areas on the graph represent stable peptide conformation, which is known as the sterically permissible region. The allowed region contains 80.2% or 4365 residues, whereas the disallowed region contains 0.3% or 15 residues. A total of 6345 residues are made up of 465 glycine residues, 375 proline residues, and 5445 non-glycine and non-proline residues. The secondary structure of 2R5I contains seven sheets, three helix-helix

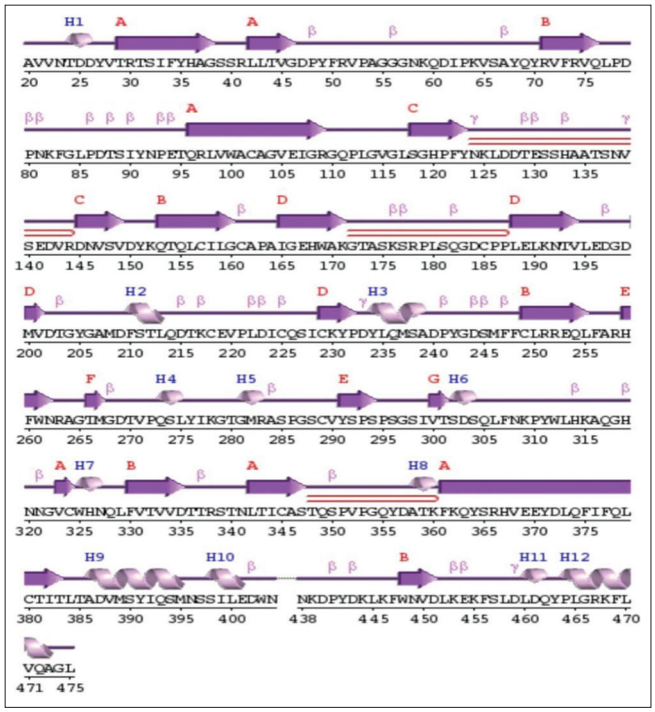

Fig. 5: Secondary structure of protein 2R5I

interacs, one beta alpha beta unit, 21 strands, three beta hairpins, five beta bulges, 12 helices, 40 beta turns, and four gamma turns.

SwissADME analysis

Screening of small molecules and their medication properties are determined using the Lipinski rule of five. All three ligands were accepted by Lipinski's rule of five according to ADMElab 2.0 [12]. All minimum and maximum values of SwissADME characteristics, Lipinski rule of five, and ligand physicochemical properties can be studied from SwissADME [8].

Molecular docking and visualization

The ligand echinacoside had better binding affinity than curcumin and chicoric acid against HPV type 18. Possible nine binding affinities of individual three ligands derived from AutoDock Vina are listed in Table 5. RMSD i.b. represents intra-batch RMSD, where RMSD is calculated in a single docking batch. RMSD u.b. represents inter-batch RMSD, where RMSD is calculated in different docking batches.

The ligand curcumin, echinacoside, and chicoric acid had the best binding affinity scores −6.9, −7.9, and −6.3, respectively. All individual best binding affinity scores were analyzed with the use of DS Biovia Discovery Studio for 3D and 2D structure visualization of protein-ligand binding (Figs. 6-8). Detailed information about the amino acids engaged in the interactions and the specific type of interactions established between 2R5I and phyto-ligands is presented in Table 6.

DISCUSSION

Two types of HPV strain (18 and 16) are responsible for 70% of HPV causing cancer of the cervix (World Health Organization, 2015) [2]. Reduction of HPV L1 capsid antigen expression is observed in the progression of invasive cancer [14]. HPV type 18 and type 16 are isolates of one HPV type according to their intratypic molecular variants, only <10% variation in the nucleotide sequence of L1 is observed [15]. The L1 is a conserved region in HPV type 18 and HPV type 16 and heparin is observed in binding sites of both HPV type 18 and 16, which is involved in HPV infection [5,16].

HPV vaccination cost is beyond the limit for the average citizen, so *E. purpurea* as an affordable herbal therapy was reported in Nigeria for HPV infection. Immune system improvement and reduction in viral replication were reported with the use of *Echinacea* along with affordability and accessibility for the average citizen [5]. At present, there is no scientific report to prove the efficiency of *Echinacea*, but the potentiality of the plant for research on *E. purpurea* for HPV infection was reported through molecular docking analysis. Molecular docking analysis of *Echinacea* ligands (curcumin, echinacoside, and chicoric acid) was done against the pentamer structure of 2R5H [5]. L1 conserved region of protein 2R5H was targeted to check the binding affinity of *Echinacea* ligands and chicoric acid (−8.7 kcaL/moL) was reported as the best ligand according to docking results [5].

Depending upon the pentamer structure of HPV type 16 L1 (2R5H) docking analysis against *Echinacea*, we performed docking on the pentamer structure of HPV type 18 (2R5I) against *Echinacea*. In the present molecular docking analysis, the same ligands *Echinacea* were used against protein 2R5I because of their dissimilarity in the L1 region, the presence of heparin at the binding site of the protein, and the role of both proteins in cervical cancer. We checked the binding affinity of ligands *Echinacea* against protein 2R5I to compare their binding affinity with protein 2R5H for ideas of future research. The chicoric acid

Table 5: Ligands binding affinity result of docking against 2R5I

Binding score of curcumin				Binding score of echinacoside				Binding score of chicoric acid			
Mole	Affinity (kcaL/moL)	RMSD l.b.	RMSD u.b.	Mole	Affinity (kcaL/moL)	RMSD l.b.	RMSD u.b.	Mole	Affinity (kcaL/moL)	RMSD l.b.	RMSD u.b.
1	−6.9	0	0	1	−7.9	0	0	1	−6.3	0	0
2	−6.7	7.659	9.9	2	−7.7	2.922	8.185	2	−6.3	6.349	7.374
3	−6.7	4.375	8.505	3	−7.7	2.663	5.384	3	−6.2	6.191	7.714
4	−6.6	3.999	5.779	4	−7.7	2.786	5.913	4	−6.2	5.766	7.984
5	−6.6	7.226	9.206	5	−7.7	2.118	3.514	5	−6.2	5.241	7.886
6	−6.5	0.797	5.528	6	−7.6	2.922	8.776	6	−6.1	6.611	7.502
7	−6.2	4.217	6.664	7	−7.6	2.184	3.438	7	−6	3.366	5.488
8	−6.2	7.638	9.884	8	−7.5	2.614	4.992	8	−6	6.389	8.258
9	−6.2	6.301	8.509	9	−7.5	2.464	7.835	9	−6	3.567	5.864

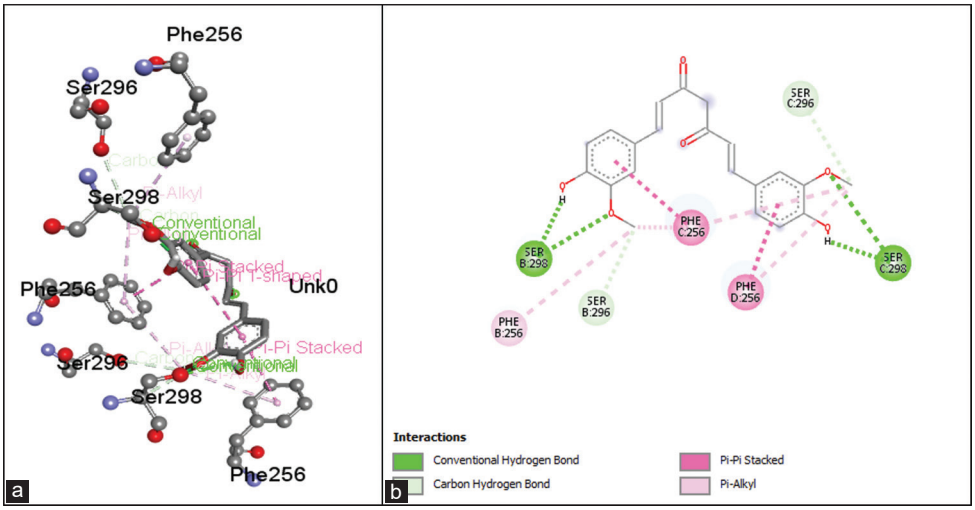

Fig. 6: Molecular structure of protein-ligand binding of curcumin against protein 2R5I (a) 3D structure (b) 2D structure

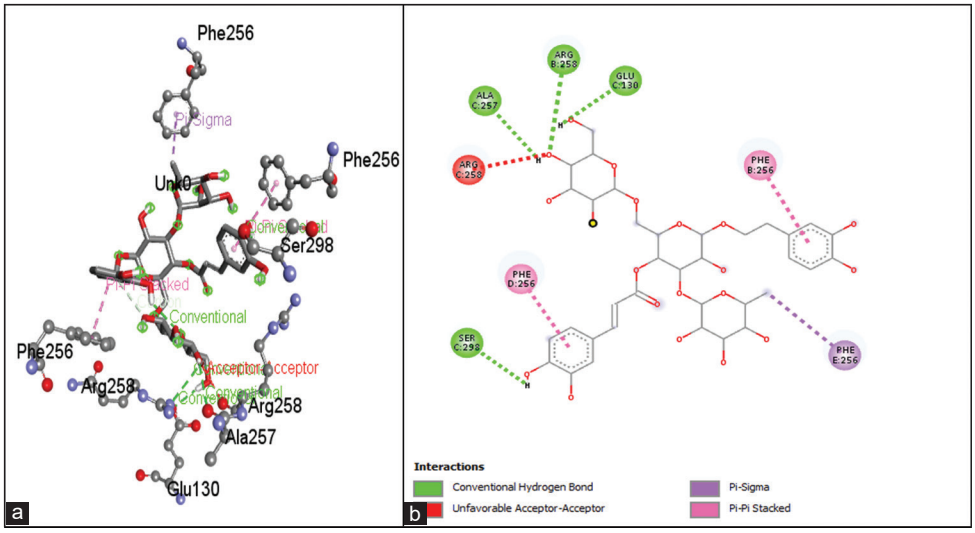

Fig. 7: Molecular structure of protein-ligand binding of echinacoside against protein 2R5I (a) 3D structure (b) 2D structure

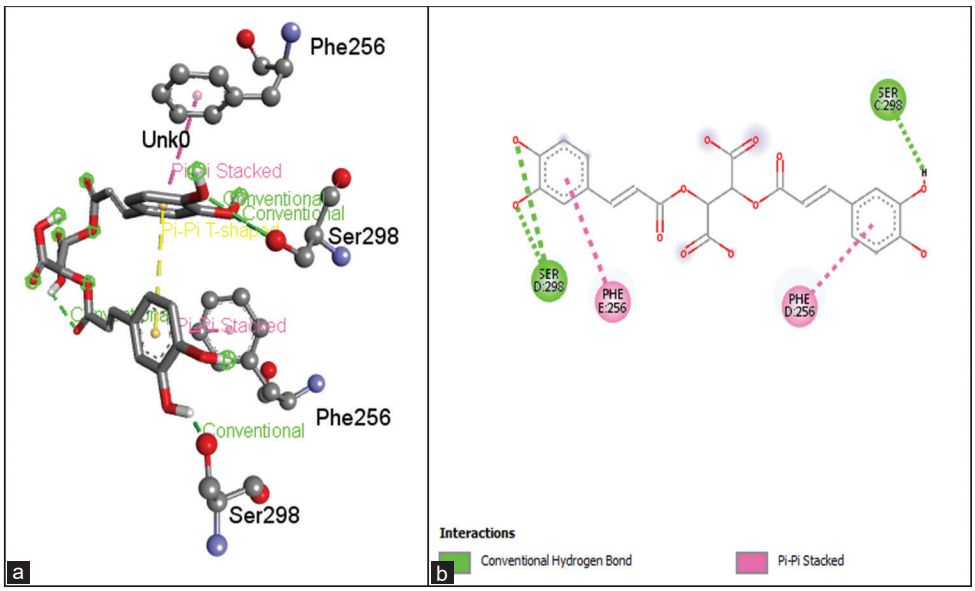

Fig. 8: Molecular structure of protein-ligand binding of chicoric acid against protein 2R5I (a) 3D structure (b) 2D structure

Table 6: Type of interaction and amino acid residues involved in protein-ligand binding

Ligand	Type of interaction	Amino acid residues
Curcumin	Conventional H-bond Pi-Pi stacked Carbon hydrogen bond Pi-alkyl	B: PHE256, B: SER296, B: SER298, C: PHE256, C: SER296, C: SER298, D: PHE256
Echinacoside	Conventional H-bond Pi-Pi stacked Unfavorable acceptor-acceptor PI-SIGMA	B: ARG258, B: PHE256, C: GLU130, C: ALA257, C: ARG258, C: SER298, D: PHE256, E: PHE256
Chicoric acid	Conventional H-bond Pi-Pi stacked	C: SER298, D: PHE256, D: SER298, E: PHE256

had the best binding affinity (−8.7 kcaL/moL) against protein 2R5H but chicoric acid was observed to have an overall low binding affinity against protein 2R5I. The echinacoside was observed to have the best docking affinity (−7.9 kcaL/moL) in protein 2R5I.

The best docking affinity score was −6.9 kcaL/moL, −7.9 kcaL/moL, and −6.3 kcaL/moL for curcumin, echinacoside, and chicoric acid, respectively. The echinacoside was observed to have interactions with B: ARG258, B: PHE256, C: GLU130, C: ALA257, C: ARG258, C: SER298, D: PHE256, and E: PHE256 amino acid residues in protein-ligand interaction along with conventional hydrogen bond, Pi-Pi stacked, unfavorable acceptor-acceptor and pi-sigma types of interactions. Therefore, echinacoside components of *E. purpurea* can have anti-bioactivities against the pentamer structure of major capsid protein L1 of HPV type 18 (2R5I).

CONCLUSION

Many research works are ongoing for HPV-related diseases to provide promising results of therapeutic agents for the management of HPV infection. *In silico* analysis in bioinformatics and molecular modeling, advancement is a boon for validating such therapeutic agents. Although, research work has been done to check the inhibitory action of natural compounds against L1 protein (2R5I). L1 protein (2R5I) is a conserved region and contains heparin in the binding site, which is a risk factor for HPV infection. This docking analysis on protein 2R5I provides promising information on how HPV-related infections can be managed with the use of plant *E. purpurea* as an inhibitor when confirmed by *in vivo* techniques.

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AUTHOR CONTRIBUTIONS

VVS conceived the whole work, performed the analysis, analyzed the results, and wrote the manuscript. SC contributed to writing the theory part of the manuscript and editing the manuscript. PK contributed to the idea of research work and review of the manuscript. SB contributed by referencing of article in the manuscript.

CONFLICT OF INTEREST

There are no competing interests that the authors categorically state might potentially influence the findings, results, or conclusions given in the study.

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ETHICAL APPROVAL

Not applicable.

REFERENCES

1. Chen AA, Gheit T, Franceschi S, Tommasino M, Clifford GM. Human papillomavirus 18 genetic variation and cervical cancer risk worldwide. J Virol 2015;89:10680-7.
2. Ahmed HG, Bensumaidea SH, Alshammari FD, Alenazi FS, ALmutlaq BA, Alturkstani MZ, *et al*. Prevalence of human papillomavirus subtypes 16 and 18 among Yemeni patients with cervical cancer. Asian Pac J Cancer Prev 2017;18:1543-8.
3. Temesgen MM, Alemu T, Shiferaw B, Legesse S, Zeru T, Haile M, *et al*. Prevalence of oncogenic human papillomavirus (HPV 16/18) infection, cervical lesions and its associated factors among women aged 21-49 years in Amhara region, Northern Ethiopia. PLoS One 2021;16:e0248949.
4. Wang X, Liu H, Ge H, Ajiro M, Sharma NR, Meyers C, et al. Viral DNA replication orientation and hnRNPs regulate transcription of the human papillomavirus 18 late promoter. mBio 2017;8:e00713-17.
5. Lukman Y, Bala DA, Malik KI, Saidu A, Saleh KA, Abubakar BJ, *et al*. Identification of HPV16's E6 gene in suspected cases of cervical lesions and docking study of its L1 protein with active components of *Echinacea purpurea*. Afr Health Sci 2022;22:98-105.
6. Ozbun MA. Extracellular events impacting human papillomavirus infections: Epithelial wounding to cell signaling involved in virus entry. Papillomavirus Res 2019;7:188-92.
7. Barrett B. Medicinal properties of *Echinacea*: A critical review. Phytomedicine 2003;10:66-86.
8. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017;7:42717.
9. Burley SK, Berman HM, Kleywegt GJ, Markley JL, Nakamura H, Velankar S. Protein Data Bank (PDB): The single global macromolecular structure archive. Methods Mol Biol 2017;1607:627-41.
10. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, *et al*. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem 2004;25:1605-12.
11. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, *et al*. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 2009;30:2785-91.
12. Yang H, Lou C, Sun L, Li J, Cai Y, Wang Z, *et al*. admetSAR 2.0: Web-service for prediction and optimization of chemical ADMET properties. Bioinformatics 2019;35:1067-9.
13. Jabir NR, Rehman MT, Alsolami K, Shakil S, Zughaibi TA, Alserihi RF, *et al*. Concatenation of molecular docking and molecular simulation of BACE-1, γ-secretase targeted ligands: In pursuit of Alzheimer's treatment. Ann Med 2021;53:2332-44.
14. Hernandez J, Elahi A, Siegel E, Coppola D, Riggs B, Shibata D. HPV L1 capsid protein detection and progression of anal squamous neoplasia. Am J Clin Pathol 2011;135:436-41.
15. King AJ, Sonsma JA, Vriend HJ, van der Sande MA, Feltkamp MC, Boot HJ, *et al*. genetic diversity in the major capsid L1 protein of HPV-16 and HPV-18 in the Netherlands. PLoS One 2016;11:e0152782.
16. Hofmann KJ, Neeper MP, Markus HZ, Brown DR, Müller M, Jansen KU. Sequence conservation within the major capsid protein of human papillomavirus (HPV) type 18 and formation of HPV-18 virus-like particles in *Saccharomyces cerevisiae*. J Gen Virol 1996;77:465-8.