RESEARCH PAPER

Structure-Based Evaluation of Theophylline as a Potential Inhibitor of HPV E6 and E7 Oncoproteins in Cervical Cancer

Poornima D[1], Keerthi B R[2], Punith R[3], Monika M K[4], Yadunandan Chikkaboregowda[5], Haleshappa R[6], Anil Kumar K M[7], Ishwar Prasad Sahu[8], Sumitha Elayaperumal[9], Raghunath M[10]*, Avinash KO[11]*

[1]Associate Professor of Biotechnology, Tumkur University, Tumakuru – 572103, Karnataka, India. ORCID ID- 0000-0001-7665-3890
[2]Department of Computer Science, Acharya Bangalore B School(AUTONOMOUS), Bengaluru-560091, Karnataka, India.
[3]Assistant professor, Radiation oncology, AJ insititute of medical sciences, Mangalore.
[4]Assistant Professor, Department of Biochemistry- MLT BGS Global institutions of Allied Health Sciences Bangalore
[5]Department of Studies in Biotechnology, Davangere University, Davangere-577 007, Karnataka, India.
[6]Department of Chemistry and Biochemistry, Government Science College, Nrupatunga University, Bengaluru-560001, Karnataka India
*[7]*Department of Environmental Science, School of Life Sciences, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India.*
[8]Department of Mechanical Engineering, Rungta College of Engineering & Technology, Rungta International Skills University, Bhilai - 490024, Chhattisgarh, India
*[9]*Department of Biotechnology and Bioinformatics, School of Life Sciences, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India.*
*[10]*Division of Medical Statistics, School of Life Sciences, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India, ORCID ID- 0000-0001-8957-0138*
*[11]*Department of Biochemistry, Shri Maruthi College of Allied Health Sciences. Bengaluru 560089;*

Corresponding author(s).
Raghunath M, Avinash KO**
E-mail(s)- raghunath.m@jssuni.edu.in, avinashavi2291@gmail.com

ABSTRACT
Cervical cancer remains one of the leading causes of cancer-related morbidity and mortality among women worldwide, with persistent infection by high-risk human papillomavirus (HPV), particularly HPV-16 and HPV-18, serving as the primary etiological factor. The viral oncoproteins E6 and E7 promote cervical carcinogenesis through degradation of the tumour suppressors p53 and retinoblastoma protein (pRb), respectively, making them attractive therapeutic targets. In the present study, an integrated computational and experimental approach was employed to identify potential natural inhibitors targeting HPV E6 and E7 oncoproteins. A structure-based virtual screening of 120,823 phytobioactive compounds was performed using the Schrödinger Suite against homology-modelled E6 and E7 proteins. Lead compounds were further evaluated through molecular docking, density functional theory (DFT) analysis, and 100 ns molecular dynamics (MD) simulations. Among the screened compounds, theophylline demonstrated the most favourable binding profile, exhibiting strong interactions with critical functional residues, including Cys58 in E6 and Arg65/Arg94 in E7, along with stable protein–ligand interactions throughout MD simulations. DFT analysis revealed favourable electronic properties, suggesting enhanced molecular reactivity and binding potential. In vitro investigations demonstrated significant antioxidant activity in the DPPH radical scavenging assay, with an IC_{50} value of 57.22 µg/mL, alongside notable anti-hemolytic and antiangiogenic properties. Collectively, these findings suggest that theophylline may interfere with HPV-mediated oncogenic pathways by destabilising E6/E7 functional interactions, restoring redox balance, and suppressing tumour-promoting mechanisms. The study highlights theophylline as a promising candidate for repurposing as a targeted therapeutic agent against HPV-associated cervical cancer, warranting further validation through cellular and in vivo studies.
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Author for Correspondence: Raghunath M, Avinash KO

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INTRODUCTION

Cervical cancer is a malignant neoplasm arising from the epithelial cells of the cervix and remains a significant global public health burden (1). It is currently the fourth most frequently diagnosed cancer and the fourth leading cause of cancer-related mortality among women worldwide. According to GLOBOCAN 2022 estimates, approximately 660,000 new cases and 350,000 deaths were reported globally in 2022, with a disproportionate burden observed in low- and middle-income countries. (2) In India alone, 127,356 new cases and 79,906 deaths were recorded, corresponding to age-standardised incidence and mortality rates of 17.7 and 11.2 per 100,000 women, respectively. Projections indicate that, if current trends persist, the annual incidence of cervical cancer may exceed 191,000 cases by 2040, underscoring the urgent need for improved preventive and therapeutic strategies. (3)

Persistent infection with high-risk human papillomavirus (HPV (4), particularly genotypes HPV-16 and HPV-18 HPV-16 and HPV-18, is the principal etiological factor in cervical carcinogenesis. HPV DNA is detected in nearly all cervical cancer cases (5), establishing one of the strongest causal associations in cancer epidemiology. Progression from infection to malignancy is influenced by several cofactors, including tobacco use, high parity, prolonged oral contraceptive use, and co-infections with pathogens such as *Chlamydia trachomatis* or herpes simplex virus type 2. (6) Additional behavioural and immunological risk factors, including early sexual debut, multiple sexual partners, and immunosuppression (e.g., HIV infection), further increase susceptibility to malignant transformation. (7)

The molecular pathogenesis of HPV-driven cervical cancer is primarily mediated by the viral oncoproteins E6 and E7 (8), which disrupt host cell cycle regulation and genomic stability. HPV is a small, non-enveloped DNA virus encoding 8–10 proteins, among which E6 and E7 are constitutively expressed in malignant cells. The E6 oncoprotein promotes carcinogenesis by recruiting the E6-associated protein (E6AP (9) an E3 ubiquitin ligase, leading to ubiquitin-mediated degradation of the tumour suppressor p53 (10). This interaction inhibits apoptosis and permits the survival and proliferation of genetically damaged cells. The structural integrity and oncogenic function of E6 are critically dependent on cysteine residue Cys58, a key component of its zinc-binding domain, as well as Tyr39, which facilitates E6-E6AP-p53 complex formation and contributes to immune evasion through modulation of interferon regulatory pathways. (11)

Similarly, the E7 oncoprotein drives uncontrolled cellular proliferation by binding to and promoting the degradation of the retinoblastoma protein (pRb) (12), resulting in the release of E2F transcription factors and inappropriate progression from the G1 to S phase of the cell cycle. The zinc-binding domain of E7, particularly residues Arg65 and Arg94, is essential for interactions with multiple host proteins, including PTPN14, a regulator of epithelial differentiation (13). Degradation of PTPN14 by E7 suppresses cellular differentiation and promotes malignant transformation. These observations indicate that continuous expression of both E6 and E7 is required for the initiation and maintenance of cervical cancer, and that therapeutic inhibition of oncoproteins may effectively suppress tumour growth. (14)

In recent years, dietary supplements and naturally derived compounds have attracted increasing interest as potential modulators of cancer progression due to their antioxidant, anti-inflammatory, and chemo preventive properties (15). Compounds such as vitamin D, curcumin, and green tea polyphenols have demonstrated potential anticancer effects through the attenuation of oxidative stress and inflammatory signalling. Given the absence of clinically approved drugs that specifically target HPV E6 and E7 oncoproteins, the development of targeted therapeutic agents remains a critical unmet need. Structure-based drug design offers a rational approach to exploit the known structural and functional vulnerabilities of E6 and E7, particularly by targeting key amino acid residues such as Cys58 and Tyr39 in E6, and Arg65 and Arg94 in E7, to disrupt oncogenic protein-protein interactions and restore tumor suppressor function. (16)

2. Materials and Methods

2.1. Structure-based virtual screening

In virtual screening, compounds with a high probability of binding to the target are identified by docking possible drug candidates onto the protein structure using computational tools.

The Schrödinger Suite 2023 was used for the studies *in silico analysis*. It is a piece of software for chemical and biological applications that offers a variety of instruments that facilitate the study of chemical system structures, reactivities, and properties. These tools also enable the creation of high-quality molecular visualizations for the presentation of structural findings. Water molecules and non-essential atoms were removed before docking (17).

2.2. Protein Selection and Preparation

The SWISS-MODEL server, which generated homology models using FASTA sequences retrieved from NCBI, was used to generate three-dimensional protein models of the E6 and E7 oncoproteins. H-bonds were optimised, missing loops and side chains were filled with Prime, bond orders were assigned, and het atoms and water molecules were eliminated (18).

2.3. Ligand Library Preparation

A library containing 120,823 natural compounds, selected based on their neuroprotective, anticancer, anti-inflammatory, and antioxidant properties, was retrieved from PubChem. Neuroprotective, anticancer, anti-inflammatory & antioxidant properties were retrieved from PubChem and imported into the workspace of Maestro. To prepare the library for HTVS, the LigPrep tool was used.

The criteria for ligand preparation included OPLS3 force field, generation of one conformer for each ligand and pH 7.0 ± 2.0 to generate possible ionisation states (19, 20).

2.4. Binding Site Prediction and Grid Receptor Generation

The Schrödinger Suite's Sitemap program was used to retrieve the binding sites for both proteins. To create a grid around the binding site amino acid residues for both proteins, the suite's receptor grid generating tool selected the top-ranked possible binding site (site 1) and produced grid zip files as an output (21).

2.5. High-Throughput Virtual Screening

Next, a structure-based virtual screening process was applied to the ligand output file using the Glide module of the "Schrödinger Suite. The HTVS precision was chosen with one pose per ligand as the output after the receptor grid file was imported into the ligand docking panel.

2.6. Conceptual DFT studies of top lead compounds

To determine the electrostatic properties of the phytobioactives, Density Functional Theory (DFT) analysis was performed using the optimisation module of the Jaguar v11.7 package. The calculations employed the DFT method with the B3LYP module, which combines Becke's exchange functional parameters with the LYP correlation functional and utilised the 6-31G (d,p) basis sets. The energy gap between the frontier molecular orbitals, specifically the highest occupied molecular orbitals (HOMOs) and the lowest unoccupied molecular orbitals (LUMOs), was examined. The HOMO and LUMO energy profiles describe the electron donor and acceptor characteristics of each chemical, providing insight into the compounds' receptor reactivity (22).

2.7. Molecular Dynamics (MD) Simulation

The compounds were then filtered based on the docking score, protein-ligand nonbonded interactions, and hydrogen bonds with specific amino acids. Based on the docking score with the E6 AND E7 proteins, one was selected for further analysis. The top two complexes were further subjected to MD simulations via the Desmond module of the Schrödinger Suite 2022-3 to analyse the intermolecular interactions and the stability of the complex at various time scales. To build the system, the TIP3P solvent model and orthorhombic water box shape were selected, and counterions were added to neutralise the system. The obtained model system was then loaded into the molecular dynamics work panel, and the simulation run time was set at 100 ns. After the completion of the simulation run, the complexes were analysed via the simulation interaction diagram panel of the Desmond module (23).

2.8. Cell lines and culture conditions

2.9. DPPH radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl)

The antioxidant assay of theophylline was conducted to assess its capacity to scavenge free radicals. The assay assesses the ability of a compound to neutralise free radicals and protect against oxidative damage. Different concentrations of theophylline were prepared to check for its antioxidant activity. The assay was conducted in triplicate, absorbance at 517 nm was taken using a UV spectrophotometer and graphs were plotted to conclude.

2.10. Anti-hemolytic Assay

The anti-hemolytic test assesses a substance's capacity to shield red blood cells from rupturing due to substances such as hydrogen peroxide. Haemoglobin release is detected at 540 nm when treated RBCs are incubated with the substance. Reduced absorbance suggests membrane stability and the tested compound's potential for cytoprotection or antioxidant effects (24).

3. Results

3.1 Molecular Docking Analysis

3.1. The library of phytobioactives

It was prepared based on the anti-inflammatory, antioxidant, and neuroprotective properties of the phytobioactives, as reported in the literature. Each compound was carefully selected for its demonstrated potential in these three critical areas, ensuring a comprehensive collection of phytobioactives with promising therapeutic benefits.

3.2. Protein Structure

The three-dimensional structures of E6 and E7 oncoproteins were retrieved through homology modelling by using (swissmodel.expasy.org)

E6 Protein (4XR8)

E7 Protein (2B9D)

Figure 3.1. 3D Structures of Proteins E6 and E7.

3.3 High-Throughput Virtual Screening (HTVS)

Using the Glide module from the Schrödinger Suite, a high-throughput virtual screening of an extensive natural compound library was performed to identify the binding sites of the HPV oncoproteins E6 and E7. For comparative analysis, both proteins were docked with currently available standard medications used to treat cervical cancer. The results of the virtual screening showed that several natural substances formed robust hydrogen bonding interactions with key amino acid residues in the active sites of E6 and E7, including Cys58, Tyr39, Arg94, and Arg65. In contrast to conventional therapeutic agents, compounds from the phytobioactive library notably showed better docking scores, indicating a possible higher binding affinity and therapeutic relevance.

With the highest docking score and two stable hydrogen bond formations with the Cys58 critical residue, a site known to be essential for E6 function, theophylline emerged as the most promising of the compounds screened. E6 interacts with a ubiquitin ligase called E6-associated protein (E6AP) to facilitate the breakdown of the tumour suppressor p53. The zinc finger domain of E6, which includes the Cys58 residue, is crucial to its structure and functionality, including its ability to bind to p53 and E6AP. Among the cysteine residues in the E6 zinc finger that coordinate zinc ions is Cys58. Theophylline may potentially inhibit E6 function by altering the structural integrity of the zinc-binding domain, thereby disrupting interactions with E6AP and p53.

In HPV-infected cells, this may contribute to the restoration of cell cycle regulation and apoptosis, a crucial mechanism of cancer suppression, by restoring p53 levels. Protein-protein interactions that are essential for E6's carcinogenic activity are disrupted when crucial residues like Cys58 are targeted. Cervical cancer progression may be inhibited, apoptosis may increase, and proliferation may decrease as a result. Theophylline's binding to Cys58 may disrupt the zinc-binding motif, which would destabilize the three-dimensional structure of E6 and result in its loss of function. On targeting cysteine 58, these findings led to theophylline being selected for further molecular dynamics (MD) simulations to assess the protein-ligand complex's stability and dynamic behaviour in physiological settings. The Glide docking process calculates docking scores, which are correlated with the free energy of binding and represent the binding affinity between ligands and their targets. Stronger predicted binding affinity is indicated by a more negative Glide score. Figure 3.2 displays the docking results, which include a graphical depiction of the Glide scores.

*Author for Correspondence: Raghunath M, Avinash KO

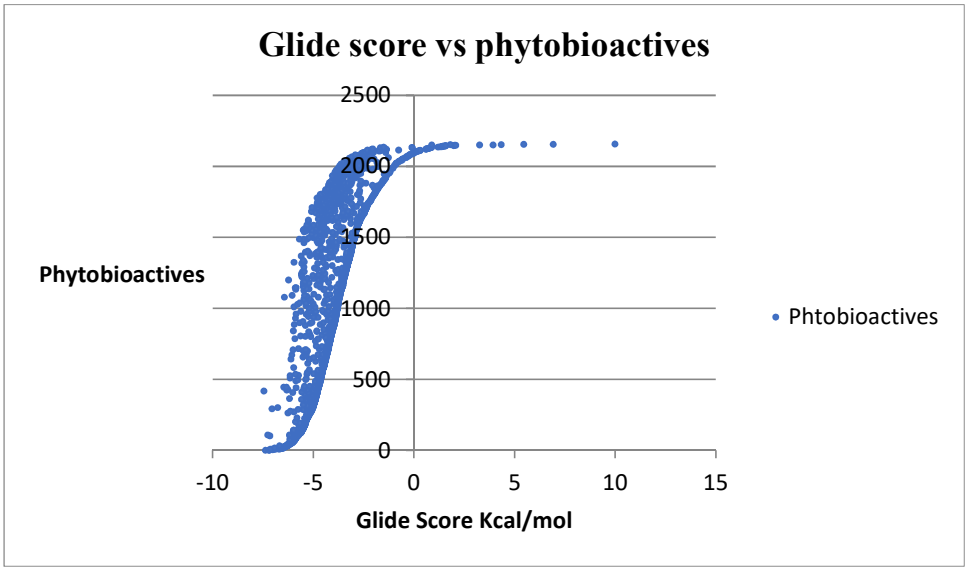

Fig 3.2 Graphical representation of the Glide score (kcal/mol) with E6 and E7 oncoprotein, where each data point indicates a ligand. After thorough filtering of compounds based on Lipinski's rule of five, Glide score, hydrogen bonds with cysteine 58 and tyrosine 39 and non-bonded interactions the top five compounds-Theophylline (P1), Chrysophanol (P2), Cytidine (P3), Casanthranol (P4), and Gossypin (P5), were considered for further analysis. Figure 3.3.1 represents two-dimensional protein ligand interactions of the E6 protein docked complexes, respectively

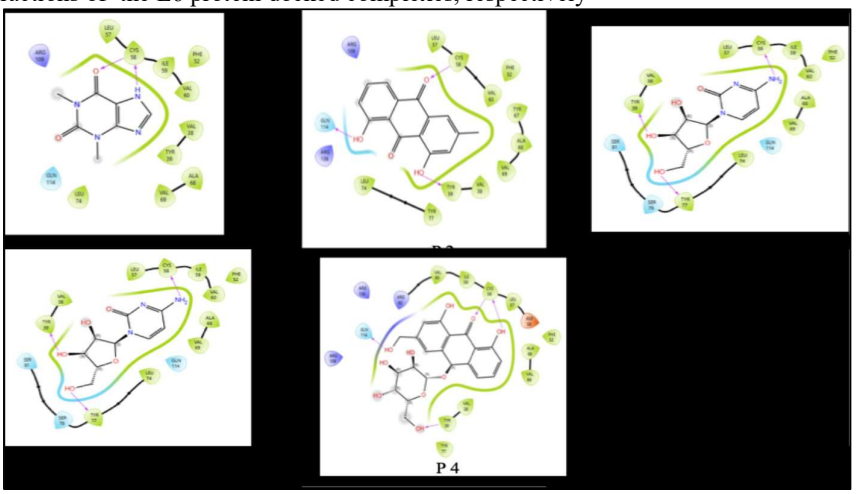

Figure 3.3.1. Two-dimensional interaction diagrams of phytobioactive compounds (P1-P5) docked with the HPV E6 protein, illustrating key binding interactions such as hydrogen bonds, hydrophobic contacts, and amino acid residues within the active site.

After thorough filtering of compounds based on Lipinski's rule of five, Glide score, hydrogen bond with arginine 94 and arginine 65 and non-bonded interactions, the top 5 compounds, 3 AMINO-beta-penine (P1), Purpurogallin-4-Carboxylic acid (P2), Hieracin (P3), Theobromine(P4), and Aphyllic acid (P5) were considered for further analysis. Figure 3.3.2 represents two-dimensional protein ligand interactions of the E7 protein docked complexes, respectively.

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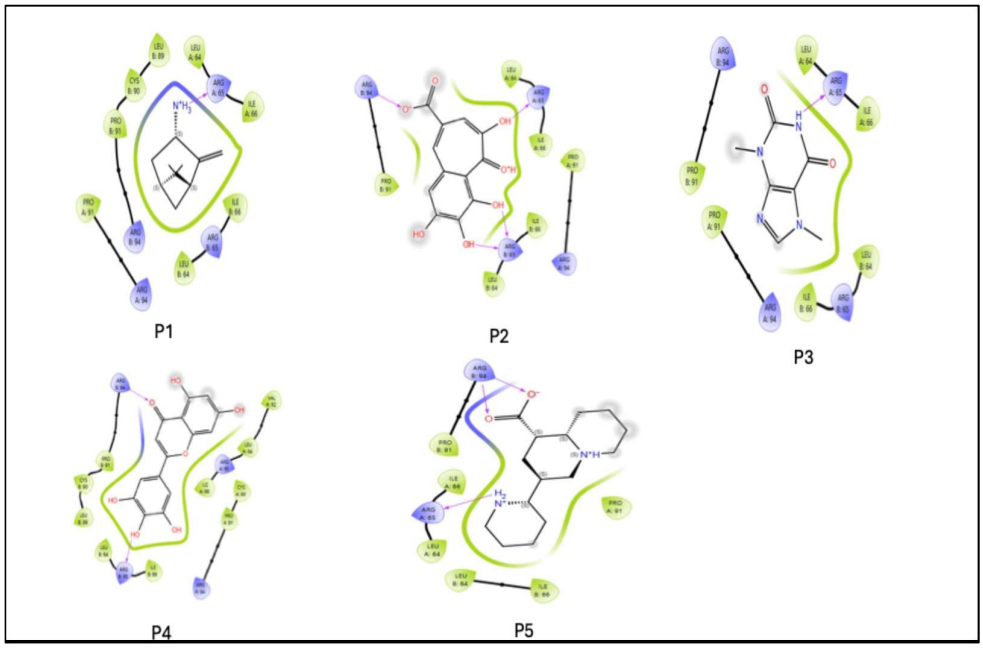


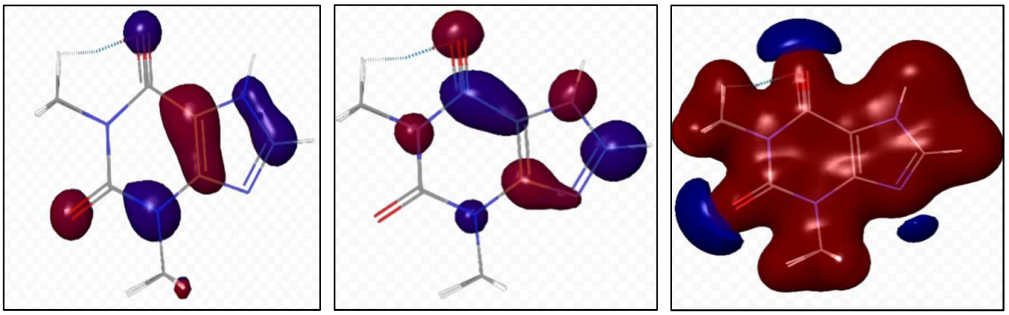
Figure 3.3.2. Two-dimensional interaction diagrams of phytobioactive compounds (P1-P5) docked with the HPV E7 protein, illustrating key binding interactions including hydrogen bonds, hydrophobic contacts, and surrounding active-site residues.

3.4 Conceptual DFT Studies of top lead Phytobioactive

In the current DFT analysis, quantum chemical calculations were conducted to optimize the structure of the phytobioactive P1 using the DFT technique. The frontier molecular orbitals (HOMO and LUMO), electrostatic potential (ESP), and interaction strength are depicted in

Figure 3.4. Analysing the ESP aids in understanding the charge-dependent characteristics of the complex's molecular structure. Figure 3.4 illustrates the ESP distribution of the molecules, highlighting the active sites and relative reactivity of the atoms

Equation 1: $\Delta E_{(gap)} = E_{LUMO} - E_{HOMO}$



A.HOMO= -0.22365 **B. LUMO= -0.03534** **C.ESP= 0.1883**

Figure 3.4.1 Representation of A. HOMO, B. LUMO and C. Electrostatic Potential (ESP) properties of the phytobioactive P1.

In the current DFT analysis, quantum chemical calculations were conducted to optimize the structure of the phytobioactive P1 using the DFT technique. The Density Functional Theory (DFT) analysis shown in the figure provides critical insights into the electronic properties of the phytobioactive compound P1. The Highest Occupied Molecular Orbital (HOMO) value is -0.22365 eV, indicating the electron-donating ability of the molecule. The Lowest Unoccupied Molecular Orbital (LUMO) is -0.03534 eV, representing the molecule's potential to accept electrons. The relatively small energy gap between the HOMO and LUMO suggests favourable chemical reactivity

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and good electronic transitions, which are important for biological interactions and stability. The Electrostatic Potential (ESP) map with a value of 0.18831 visually illustrates the charge distribution across the molecular surface, where red regions indicate electron-rich (nucleophilic) areas and blue regions represent electron-deficient (electrophilic) zones. This polarisation is significant for predicting the molecular sites involved in binding interactions with target proteins or receptors.

3.5 DPPH Results

The antioxidant capacity of theophylline was assessed using the DPPH radical scavenging assay. Theophylline showed a dose-dependent increase in radical scavenging activity, with percentage inhibition ranging from 22.30449 at 20 µg/mL to 82.5081 at 100 µg/mL. The IC_{50} value was 57.22 µg/mL, comparable to that of ascorbic acid. These results suggest that theophylline exhibits strong antioxidant activity.

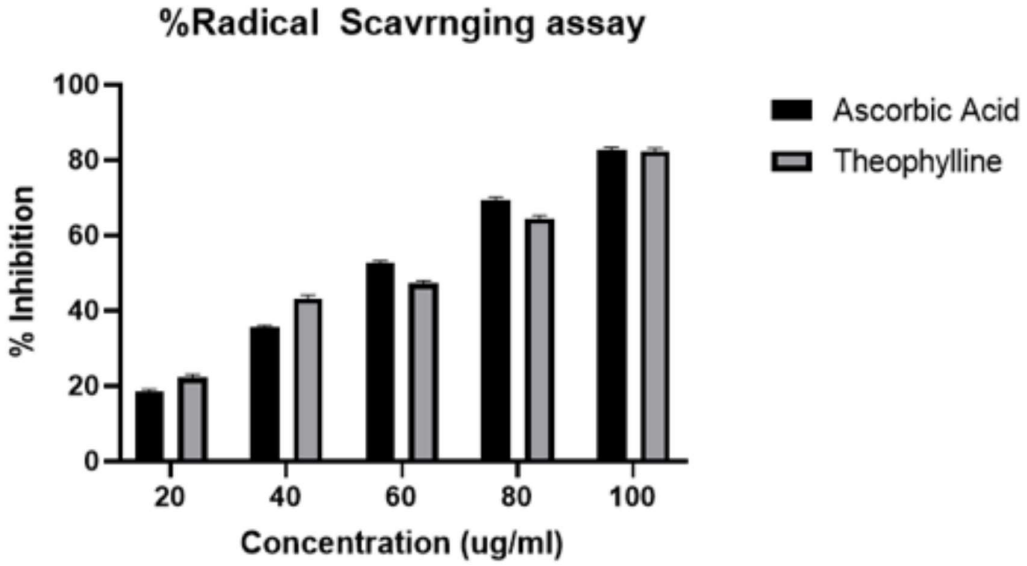

Figure 3.6.1. Graphical representation of % Inhibition of theophylline at different concentrations with the standard

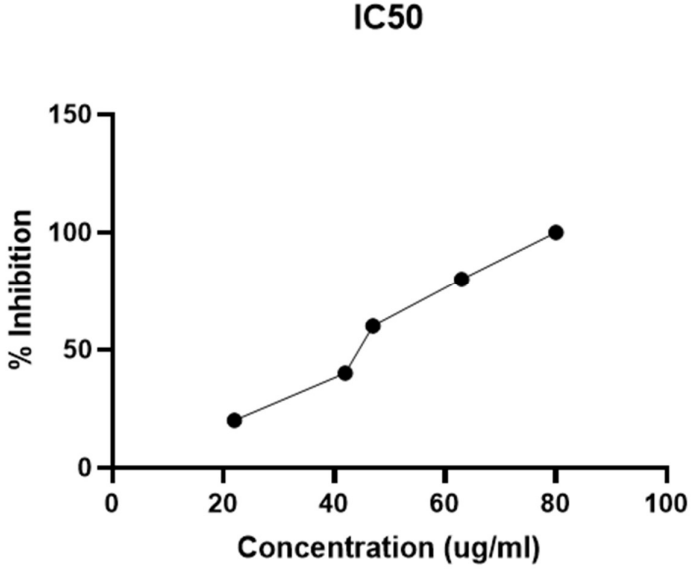

Figure 3.6.2. Graphical representation of the IC 50 value of theophylline

Theophylline has potent antioxidant qualities that help prevent cervical cancer by preventing the oxidative stress brought on by HPV infection. HPV oncoproteins, especially E6 and E7, increase reactive oxygen species (ROS) levels, which can cause DNA damage, mutations, and the activation of pro-cancer signalling pathways like PI3K/Akt

and NF-κB. Theophylline reduces oxidative DNA damage and mutagenesis by scavenging these free radicals. In addition to shielding healthy cells, this redox balance restoration blocks ROS-mediated pathways that promote angiogenesis, inflammation, and tumor formation. Furthermore, theophylline's antioxidant properties enhance its capacity to block the HPV E6 protein, especially by binding at Cys58, which can reinstate the function of the p53 tumor suppressor and encourage apoptosis. Together, these mechanisms contribute to theophylline's potential as a therapeutic agent in cervical cancer suppression.

3.6 Anti-Hemolytic Assay

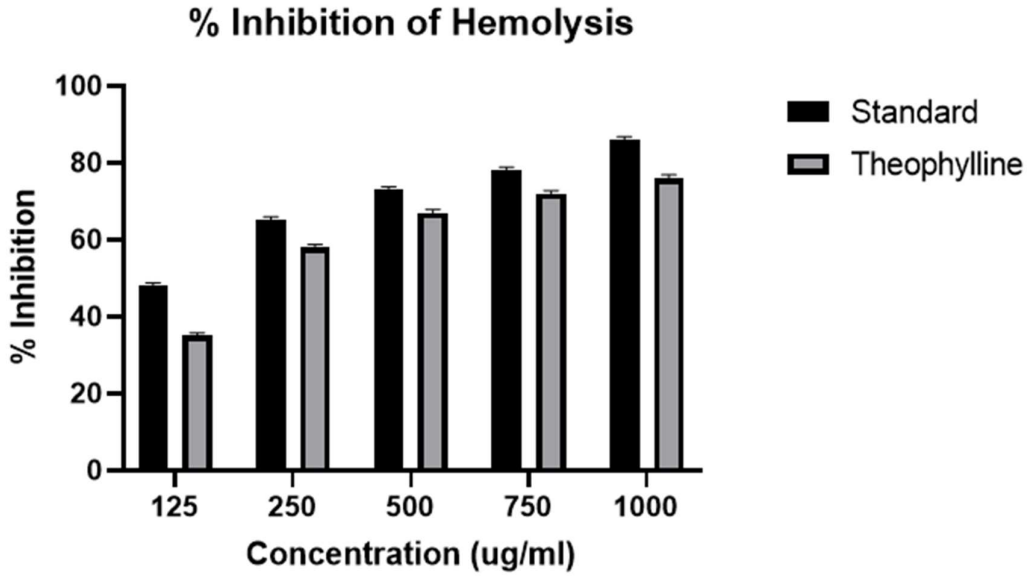

Figure 3.7.1. Graphical representation of % Inhibition of hemolysis of theophylline at different concentrations with the standard

The anti-hemolytic assay graph demonstrates that theophylline exhibits a dose-dependent protective effect against red blood cell (RBC) hemolysis, with % inhibition increasing from 125 µg/mL to 1000 µg/mL. Although the standard antioxidant shows higher inhibition at lower concentrations, theophylline's activity becomes comparable at higher doses, reaching around 75% inhibition at 1000 µg/mL. At lower concentrations (125–500 µg/mL), the standard exhibits higher inhibition than theophylline, showing superior antioxidant protection. However, at higher concentrations (750-1000 µg/mL), theophylline's performance improves significantly, nearing the inhibition levels of the standard compound.

Thus, the graph indicates that theophylline possesses effective anti-hemolytic activity that improves with increasing concentration. Although slightly less potent than the standard antioxidant, theophylline shows comparable performance at higher doses, supporting its potential as a natural antioxidant and membrane stabiliser.

4. Discussion

Persistent infection with high-risk human papillomavirus (HPV), particularly HPV-16 and HPV-18, remains the principal cause of cervical cancer development through the constitutive expression of the viral oncoproteins E6 and E7. These oncoproteins disrupt tumour suppressor pathways involving p53 and retinoblastoma protein (pRb), thereby promoting genomic instability, uncontrolled proliferation, and malignant transformation. Consequently, direct inhibition of E6 and E7 has emerged as an important therapeutic strategy for HPV-associated malignancies. In the present study, an integrated computational and experimental approach identified theophylline as a promising phytobioactive candidate capable of interacting with critical functional residues of both E6 and E7 proteins. The molecular docking analysis demonstrated that theophylline exhibited stable and high-affinity interactions with key residues, particularly Cys58 in E6 and Arg65/Arg94 in E7. These residues are functionally important for maintaining the structural integrity and oncogenic activity of the viral proteins. Previous studies have highlighted that the zinc-binding domain of E6, especially cysteine-rich residues such as Cys58, is essential for E6-mediated recruitment of E6-associated protein (E6AP) and subsequent ubiquitination and degradation of p53. Similarly, residues Arg65 and Arg94 in E7 are involved in protein–protein interactions regulating epithelial differentiation and cell-cycle progression. Therefore, targeting these conserved residues may interfere with HPV-driven carcinogenic signalling pathways.

The present findings are consistent with previous structure-based studies investigating phytobioactives against HPV oncoproteins. Harishkumar et al. reported that piceid exhibited multitarget inhibitory potential against inflammation-associated cervical cancer proteins through favourable docking interactions and molecular dynamics stability. Likewise, Jain et al. demonstrated that repurposed compounds with antioxidant and antiangiogenic properties

could effectively bind oncogenic targets involved in tumour progression. Compared with these studies, theophylline displayed comparable or improved binding stability with functionally relevant residues, suggesting its potential as a dual inhibitor of HPV E6 and E7 proteins.

Molecular dynamics simulations further supported the stability of the protein–ligand complexes under physiological conditions. Stable RMSD fluctuations and sustained intermolecular interactions observed during the 100 ns simulation indicate that theophylline may maintain prolonged occupancy within the active-site region of E6 and E7. Similar observations have been reported in studies evaluating natural compounds against viral and oncogenic proteins, where sustained hydrogen bonding and hydrophobic interactions correlated with improved inhibitory potential and structural stability of ligand-bound complexes. The stability observed in the current study therefore strengthens the possibility that theophylline may effectively disrupt oncogenic protein function in biological systems.

The DFT analysis provided additional insight into the electronic characteristics and chemical reactivity of theophylline. The relatively small HOMO–LUMO energy gap observed in the present study suggests enhanced electron transfer capability and favourable molecular reactivity, properties often associated with improved ligand–receptor interactions. Previous computational investigations of phytobioactives with anticancer activity have similarly reported that lower HOMO–LUMO gaps correlate with stronger binding affinity and increased biological activity. The electrostatic potential distribution further indicated the presence of reactive electron-rich and electron-deficient regions that may facilitate stable interactions with amino acid residues within the E6 and E7 active sites.

Oxidative stress is increasingly recognised as a critical contributor to HPV-mediated carcinogenesis. HPV oncoproteins elevate intracellular reactive oxygen species (ROS), resulting in DNA damage, inflammatory signalling, angiogenesis, and genomic instability. In the present study, theophylline demonstrated substantial antioxidant activity in the DPPH radical scavenging assay, with an IC_{50} value comparable to that of ascorbic acid. These findings align with previous reports demonstrating the antioxidant potential of naturally derived methylxanthines and related compounds. The antioxidant activity of theophylline may therefore contribute synergistically to its anticancer effects by reducing ROS-mediated activation of oncogenic pathways such as NF-κB and PI3K/Akt signalling.

In addition to antioxidant activity, theophylline exhibited dose-dependent anti-hemolytic activity, indicating membrane stabilisation and cytoprotective potential. Protection against erythrocyte membrane damage is often associated with free radical scavenging capacity and inhibition of lipid peroxidation. Similar membrane-protective effects have been reported for several phytobioactives with anticancer and anti-inflammatory properties. The observed anti-hemolytic activity in the present study further supports the multifunctional therapeutic potential of theophylline in limiting oxidative and inflammatory damage associated with cervical carcinogenesis.

An important strength of the current study is the integration of large-scale virtual screening, molecular docking, DFT analysis, molecular dynamics simulations, and experimental antioxidant assays to evaluate the therapeutic potential of theophylline comprehensively. The identification of a clinically known compound with favourable pharmacological properties may also facilitate future drug repurposing approaches, potentially reducing the time and cost associated with novel drug development.

However, several limitations should be acknowledged. The protein structures used in the study were generated through homology modelling, which may not completely represent native conformational dynamics. Furthermore, although the computational and preliminary in vitro findings are promising, the current study lacks validation in HPV-positive cervical cancer cell lines and in vivo tumour models. Additional investigations are therefore necessary to confirm whether theophylline can restore p53 and pRb activity, inhibit HPV-mediated signalling pathways, and suppress tumour growth under physiological conditions. Pharmacokinetic profiling, toxicity evaluation, and dose optimisation studies will also be essential before clinical translation can be considered.

Overall, the present study provides mechanistic evidence supporting the therapeutic potential of theophylline against HPV-associated cervical cancer. By targeting functionally critical residues of HPV E6 and E7 oncoproteins while simultaneously exerting antioxidant and cytoprotective effects, theophylline may represent a promising candidate for further development as a multitarget therapeutic agent for cervical cancer management.

5. Conclusion

The present study demonstrates that theophylline possesses significant potential as a therapeutic candidate against HPV-associated cervical cancer through its dual inhibitory interactions with the HPV E6 and E7 oncoproteins. Computational analyses revealed stable binding of theophylline with critical functional residues, including Cys58 in E6 and Arg65/Arg94 in E7, supported by favourable molecular dynamics and DFT analyses. In addition, theophylline exhibited considerable antioxidant and anti-hemolytic activities, suggesting complementary cytoprotective and anti-tumour mechanisms. Collectively, these findings indicate that theophylline may disrupt HPV-mediated oncogenic pathways while reducing oxidative stress associated with cervical carcinogenesis. Although further validation using HPV-positive cervical cancer cell lines and in vivo models is necessary, the current study provides a strong foundation for the potential repurposing of theophylline as a targeted therapeutic agent for cervical cancer.

Author Contributions

Ethics, consent to participate, and consent to publish declarations

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request

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