GMR
The Original

MOLECULAR DOCKING STUDY OF SIDDHA FORMULATION SEENTHIL CHOORANAM TARGETING TRANSFORMING GROWTH FACTOR-B1 (TGF-B1) AND HUMAN PAPILLOMAVIRUS ONCOPROTEINS OF CERVICAL CARCINOMA

Shiyamala Janakiraman*[1], Parvatha Rajakumaran V. S[2], Hemalatha Kanagarajan[3], P. Sukumaran[4], M. K. Malathi[5], M. K. Seeni[6]

[1]Associate Professor, National Institute of Siddha, Tambaram Sanatorium, Chennai, Tamil Nadu, India.
[2]Research Scholar, Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai, Tamil Nadu, India.
[3]Head, Department of Pharmaceutical Analysis, Saveetha College of Pharmacy (SCOP), Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai, Tamil Nadu, India.
[4]Professor & Head, UG Department of Varmam Puramaruthuvam Sirappu Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai – 600106, Tamil Nadu, India.
[5]Associate Professor, UG Department of Varmam Puramaruthuvam Sirappu Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai – 600106, Tamil Nadu, India.
[6]Deputy Director (Technical) I/C, Government Research & Development Wing for ISM, Arumbakkam, Chennai – 600106, Tamil Nadu, India.
*Corresponding Author: Shiyamala Janakiraman, Email: shyamalaragav123@gmail.com

ABSTRACT

Background: Cervical carcinoma remains one of the leading causes of cancer-related mortality among women worldwide, with persistent infection by high-risk Human Papillomavirus (HPV), particularly HPV-16, being the primary etiological factor. The HPV E6 oncoprotein and Transforming Growth Factor-β1 (TGF-β1) are key molecular targets involved in Cervical carcinogenesis, tumor progression, and metastasis. Seenthil Chooranam, a classical Siddha polyherbal formulation containing Tinospora cordifolia and Eclipta alba, is rich in bioactive phytoconstituents with reported anticancer properties.

Objective: To investigate the molecular interactions and therapeutic potential of phytoconstituents of Seenthil Chooranam against HPV E6 oncoproteins and TGF-β1 using an integrated in silico approach.

Methods: Twelve phytoconstituents were screened for drug-likeness using SwissADME, and seven compounds satisfying Lipinski's Rule of Five were further evaluated for ADMET properties using pkCSM. Molecular docking was performed using AutoDock Vina implemented in PyRx against HPV E6 proteins (PDB IDs: 4XR8 and 4GIX) and TGF-β1 (PDB ID: 1VJY). Protein–ligand interactions were analyzed using BIOVIA Discovery Studio Visualizer.

Results: Seven phytoconstituents demonstrated favorable physicochemical properties and acceptable predicted pharmacokinetic profiles. Molecular docking revealed that Tinocordiside exhibited the highest binding affinity against HPV E6 proteins, with docking scores of −9.6 kcal/mol for 4XR8 and −8.5 kcal/mol for 4GIX, while Luteolin (−10.4 kcal/mol) and Apigenin (−10.3 kcal/mol) displayed binding affinities comparable to the standard ligand (−10.5 kcal/mol) against TGF-β1. Protein–ligand interaction analysis showed that the lead compounds established multiple conventional hydrogen bonds, carbon–hydrogen bonds, π-mediated interactions, hydrophobic contacts, and van der Waals interactions with key active-site residues, supporting stable protein–ligand complex formation.

Conclusion: The findings suggest that the phytoconstituents of Seenthil Chooranam possess promising multitarget inhibitory potential against HPV E6 oncoproteins and TGF-β1, indicating their potential to simultaneously modulate viral oncogenic activity and host signaling pathways involved in Cervical cancer progression. Among the evaluated compounds, Tinocordiside emerged as the most promising inhibitor of HPV E6, whereas Luteolin and Apigenin demonstrated excellent binding affinity toward TGF-β1. Further experimental validation is warranted to confirm their therapeutic efficacy and safety.

KEYWORDS: Seenthil Chooranam, Siddha Medicine, Cervical Carcinoma, Molecular Docking, HPV E6, E7, TGF-β1, Tinospora cordifolia, AutoDock Vina

INTRODUCTION

Cervical carcinoma is the fourth most prevalent malignancy among women globally. Cervical cancer ranks as fourth most common cancer amongst all other cancers with an incidence rate of 604,127 new cases and 341,831 deaths globally. Approximately 99% of Cervical cancers are associated with persistent infection by high-risk HPV strains,

predominantly HPV-16.[1] Human Papilloma Virus type 16 (HPV-16) is highly oncogenic with the E6 and E7 oncogenes playing crucial roles in the pathogenesis of HPV-related Cervical carcinogenesis. Targeting these oncoproteins with specific Natural inhibitors offers a novel encouraging approach for therapeutic intervention.

Although prophylactic HPV vaccines have significantly reduced the incidence of new HPV infections and precancerous Cervical lesions, they are preventive rather than therapeutic and therefore cannot eliminate established HPV infections or treat existing Cervical malignancies. Furthermore, vaccine coverage remains suboptimal in many regions, and the long latency between HPV infection and cancer development means that HPV-associated cancers continue to pose a major global health burden. Consequently, the development of therapeutic agents targeting HPV oncoproteins, particularly E6 and E7, remains an important strategy for the treatment of Cervical carcinoma.[2]

The HPV E6 protein induces degradation of p53 through ubiquitin-mediated pathways, while HPV E7 promotes degradation of retinoblastoma protein (pRb), leading to uncontrolled cell proliferation. Among the various molecular pathways involved in Cervical carcinogenesis, TGF-β1 has emerged as an important prognostic biomarker and therapeutic target.[3] TGF-β1 has emerged as a promising prognostic biomarker to investigate Cervical cancer. Advanced tumours exploit this signaling pathway and promote epithelial–mesenchymal transition (EMT), angiogenesis, immune suppression, extracellular matrix remodeling tumour invasion and finally metastasis. However, TGF-β1 exhibits tumour suppressive effects during early carcinogenesis.Therefore, inhibition of TGF-β1 signalling together with HPV oncoproteins represents an attractive therapeutic strategy.[4] Molecular docking provides a rapid computational approach to predict ligand–protein interactions and identify promising therapeutic candidates before laboratory validation.

Exploration of alternative treatments derived from medicinal plants offers a promising avenue for the development of safer and more effective cancer therapies. Medicinal plants, with their diverse phytochemical profiles, have historically been used in various traditional medicine systems across the globe to treat a myriad of ailments, including cancers. These natural compounds often exhibit potent pharmacological activities, such as anti-inflammatory, antioxidant, and direct cytotoxic effects, making them promising candidates for targeting cancer cells while potentially minimizing the side effects associated with conventional chemotherapy.[5]

Natural products contain structurally diverse bioactive compounds with anticancer properties, including the ability to induce apoptosis, inhibit cell proliferation, suppress epithelial–mesenchymal transition (EMT), and interfere with multiple molecular pathways involved in cancer progression, making them valuable sources for the discovery of novel anticancer agents.[6]

Seenthil Chooranam is a classical Siddha polyherbal formulation traditionally indicated for inflammatory disorders, chronic diseases, immune dysfunction, and neoplastic conditions. Its phytochemicals possess antioxidant, immunomodulatory, anti-inflammatory, antiproliferative, and apoptotic properties. Tinospora cardifolia (Seenthil)and Eclipta alba (Vellai Karisalai) are the ingredients mentioned in classical Siddha text.[7]

MATERIALS AND METHODOLOGY

Collection of Phytoconstituents

Twelve phytoconstituents were selected from Tinospora cordifolia and Eclipta alba based on published phytochemical literature. Chemical structures were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) in Structure Data File (SDF) format. The compounds were screened for drug-likeness using Lipinski's Rule of Five through the SwissADME web server. Seven phytoconstituents that fulfilled the drug-likeness criteria were selected for further computational analyses. These compounds included Palmatine (CID: 19009), Berberine (CID:2353), Wedelolactone (CID:5281813), Apigenin (CID:5280443), Demethylwedelolactone (CID:5489605), Luteolin (CID:5280445), and Tinocordiside(CID:177384).

Physicochemical and Drug-Likeness Analysis

The selected phytoconstituents were evaluated for their physicochemical properties using the SwissADME web server. Molecular weight, octanol-water partition coefficient (LogP), hydrogen bond donors, hydrogen bond acceptors, rotatable bonds, and topological polar surface area (TPSA) were determined. Lipinski's Rule of Five was applied to identify compounds possessing favorable oral drug-like characteristics.

ADMET Prediction

The pharmacokinetic and toxicity profiles of the selected phytoconstituents were predicted using the pkCSM web server. Parameters evaluated included water solubility, Caco-2 permeability, intestinal absorption, skin permeability, P-glycoprotein substrate/inhibitor status, volume of distribution (VDss), blood-brain barrier permeability, central nervous system permeability, cytochrome P450 interactions, total clearance, renal OCT2 substrate specificity, AMES mutagenicity, hERG inhibition, hepatotoxicity, skin sensitization, and acute and chronic toxicity. These parameters were used to assess the pharmacokinetic suitability of the selected compounds prior to molecular docking.

Protein Preparation

The three-dimensional crystal structures of Human Papillomavirus E6 oncoproteins (PDB IDs: 4XR8 and 4GIX) and structure of Transforming Growth Factor-β1 (TGF-β1) (PDB ID: IVJY) were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB). Protein preparation was carried out using Molegro Molecular Viewer. Water molecules, unwanted heteroatoms, and non-essential molecules were removed, and the proteins were optimised for docking. The active binding pocket was identified based on the position of the native ligand present in the crystal structure.[8]

(A) (b) (c)

Figure-1. Three-dimensional crystal structures of the target proteins used for molecular docking (a) HPV16 E6 protein (4GIX), (b) HPV16 E6 protein (4XR8), and (c) Transforming Growth Factor-β1 (TGF-β1; PDB ID: 1VJY)

Ligand Preparation

The selected phytoconstituents were downloaded from PubChem in SDF format and imported into PyRx Virtual Screening Tool (Version 0.8). Ligand structures were subjected to energy minimization using the Open Babel module integrated within PyRx to obtain stable conformations suitable for docking studies.

Molecular Docking

Molecular docking was performed using PyRx Virtual Screening Tool (Version 0.8) employing the AutoDock Vina docking algorithm. Initially, the co-crystallized ligand was re-docked into the active binding pocket of both HPV E6 proteins (4XR8 and 4GIX) and structure of Transforming Growth Factor-β1 (TGF-β1) (PDB: IVJY) to validate the docking protocol. Subsequently, the seven selected phytoconstituents were docked into the same binding site using identical docking parameters along with their active ligand. Binding affinity (kcal/mol) was used as the primary scoring function to rank ligand-protein interactions, with more negative values indicating stronger binding. The best docking pose for each ligand was selected based on the lowest binding energy and RMSD values.

Protein–Ligand Interaction Analysis

The best docking conformations were analyzed using BIOVIA Discovery Studio Visualizer. The two-dimensional interaction diagrams were generated to identify interactions, π-π stacking, π-alkyl interactions, π-cation interactions, van der Waals interactions, and the amino acid residues involved in ligand binding.

RESULTS

Drug-Likeness Screening

Table - 1: Physicochemical properties of selected phytoconstituents

Compound	CID	MW (Da)	LogP	HBA	HBD	RB	TPSA (Å²)	Lipinski
Palmatine	19009	352.41	3.38	4	0	4	152.24	Pass
Berberine	2353	336.37	3.1	4	0	2	144.87	Pass
Wedelolactone	5281813	314.25	2.82	7	3	1	127.1	Pass
Apigenin	5280443	270.24	2.58	5	3	1	112.52	Pass
Demethylwedelolactone	5489605	300.22	2.51	7	4	0	120.41	Pass
Luteolin	5280445	286.24	2.28	6	4	1	117.31	Pass
Tinocordiside	177384	396.48	0.39	7	4	4	164.89	Pass

Among the twelve phytoconstituents screened using Lipinski's Rule of Five, seven compounds satisfied the drug-likeness criteria and were selected for further analyses. These included Palmatine, Berberine, Wedelolactone, Apigenin, Demethylwedelolactone, Luteolin, and Tinocordiside. The molecular weights of the selected compounds ranged from 270.24 to 396.48 Da, while LogP values ranged from 0.39 to 3.38. The compounds exhibited acceptable hydrogen bond donor and acceptor profiles, rotatable bond counts, and topological polar surface area (TPSA), indicating favorable physicochemical properties for oral drug candidates (Table 2).

ADMET Prediction

Table - 2: Summary of ADMET prediction

Compound	Intestinal Absorption (%)	BBB	AMES	hERG I	Hepatotoxicity
Palmatine	97.08	No	Yes	No	Yes
Berberine	97.15	No	Yes	No	Yes

Wedelolactone	93.75	No	Yes	No	No
Apigenin	93.25	No	No	No	No
Demethylwedelolactone	76.07	No	No	No	No
Luteolin	81.13	No	No	No	No
Tinocordiside	71.47	No	Yes	No	Yes

The predicted ADMET profiles of the selected phytoconstituents are presented in Table 3. Human intestinal absorption ranged from 71.47% to 97.15%. None of the compounds were predicted to inhibit hERG class I channels or cross the blood-brain barrier. Apigenin, Demethylwedelolactone, Luteolin, and Wedelolactone were predicted to be non-hepatotoxic, whereas Palmatine, Berberine, and Tinocordiside showed predicted hepatotoxicity. AMES mutagenicity was predicted for Palmatine, Berberine, Wedelolactone, and Tinocordiside, while Apigenin, Demethylwedelolactone, and Luteolin were predicted to be non-mutagenic.

Molecular Docking Analysis

Table 3: Molecular docking scores of selected phytoconstituents against HPV E6 proteins (4GIX and 4XR8) and TGF-β1 (1VJY)

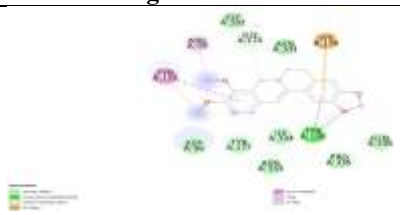
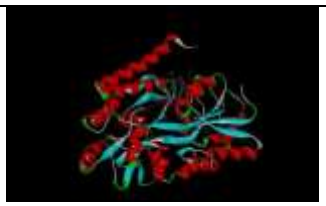
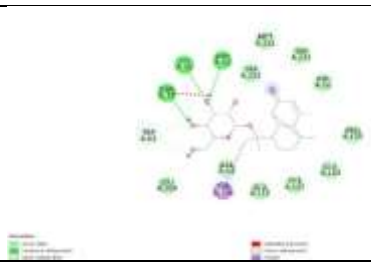
Binding Affinity (kcal/mol)					
Compound Name	**4XR8**	**Compound Name**	**4GIX**	**Compound Name**	**1VJY**
Tinocordiside	-9.6	Active Ligand	-9.2	Std Ligand	-10.5
Demethylwedelolactone	-9.4	Tinocordiside	-8.5	Luteolin	-10.4
Wedelolactone	-9.3	Berberine	-8.4	Apigenin	-10.3
Berberine	-9.2	Demethylwedelolactone	-8.3	Wedelolactone	-9.4
Luteolin	-9.2	Luteolin	-8.2	Demethylwedelolactone	-9.4
Apigenin	-8.8	Apigenin	-8.2	Palmatine	-9.2
Palmatine	-8.3	Palmatine	-7.9	Berberine	-9.1
Active Ligand	-8.3	Wedelolactone	-7.9	Tinocordiside	-8.8

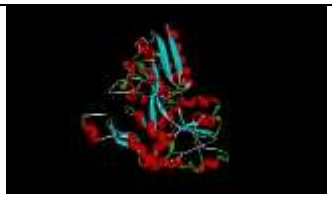
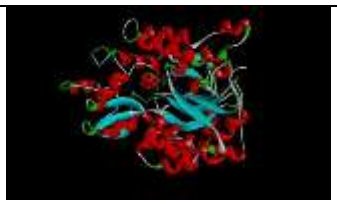
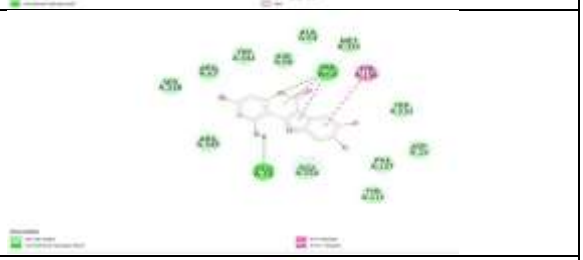
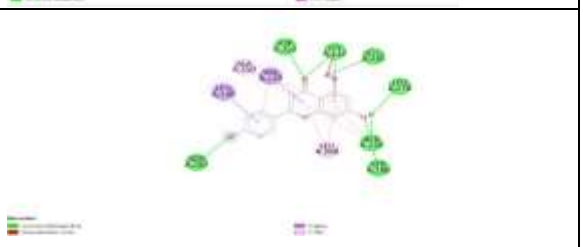
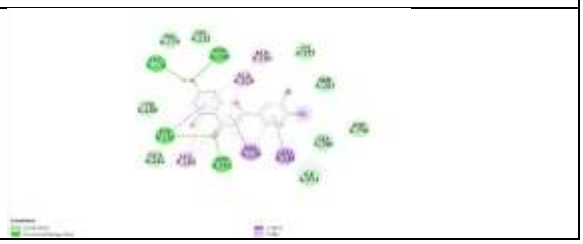
The molecular docking results against HPV E6 proteins (4XR8 and 4GIX) and TGF-β1 (1VJY) are summarized in Table 4.For the 4XR8 protein, Tinocordiside exhibited the highest binding affinity among the phytoconstituents with a docking score of −9.6 kcal/mol, followed by Demethylwedelolactone (−9.4 kcal/mol), Wedelolactone (−9.3 kcal/mol), Berberine (−9.2 kcal/mol), Luteolin (−9.2 kcal/mol), Apigenin (−8.8 kcal/mol), and Palmatine (−8.3 kcal/mol). The native ligand exhibited a binding affinity of −8.3 kcal/mol.

For the 4GIX protein, the native ligand showed the highest binding affinity (−9.2 kcal/mol). Among the phytoconstituents, Tinocordiside demonstrated the strongest binding affinity (−8.5 kcal/mol), followed by Berberine (−8.4 kcal/mol), Demethylwedelolactone (−8.3 kcal/mol), Wedelolactone (−8.2 kcal/mol), Luteolin (−8.2 kcal/mol), Apigenin (−8.2 kcal/mol), and Palmatine (−7.9 kcal/mol).

Docking against TGF-β1 (1VJY) showed that the standard ligand exhibited the highest binding affinity (−10.5 kcal/mol). Among the phytoconstituents, Luteolin demonstrated the strongest binding affinity (−10.4 kcal/mol), followed by Apigenin (−10.3 kcal/mol), Wedelolactone (−9.4 kcal/mol), Demethylwedelolactone (−9.4 kcal/mol), Palmatine (−9.2 kcal/mol), Berberine (−9.1 kcal/mol), and Tinocordiside (−8.8 kcal/mol).

Figure - 2: Protein–Ligand Interaction Analysis

S.No	3D dimensional 4GIX Protein structure	Molecular ligand interactions
1.	 **4GIX - Berberine**	
2.	 **4GIX - Tinocordiside**	

3.	 **4XR8 - Tinocordiside**	
4.	 **4XR8 - Demethylwedelolactone**	
5.	 **1VJY - Luteolin**	
6.	 **1VJY - Apigenin**	

NOTE: Green represents the hydrogen bond acceptor region, and purple represents the hydrogen bond donor region with active binding site residues

Table - 4: Major interactions of the best docked compounds

Protein	Ligand	Binding Energy (kcal/mol)	Major Hydrogen Bonds	Other Important Interactions
4GIX	Tinocordiside	−8.5	Asn13, Lys16, Asp15	Trp63 (carbon H-bond), Tyr156 (π–sigma),
4GIX	Berberine	−8.4	Tyr100	Gly175 (carbon H-bond), Tyr172 (π–π T-shaped), Arg99 & Tyr100 (π–alkyl), Lys176 (π–cation),
4XR8	Tinocordiside	−9.6	Asn13, Lys43, Glu45, Ala64	Asn13 (carbon H-bond), Met331 (alkyl)
4XR8	Demethylwedelolactone	−9.4	Glu45, Asn13, Lys43, Ala64	Met331 (alkyl)
1VJY (TGF-β1)	Apigenin	−10.3	Leu278, Ser280, Lys232, Asp351	1VJY (TGF-β1) Apigenin −10.3 Leu278, Ser280, Lys232, Asp351 Val219 (π–sigma), Leu260, Leu340, Ala230, Ala350 (π–alkyl)
1VJY (TGF-β1)	Luteolin	−10.4	Asp351	Leu278, Leu260, Leu340, Tyr249, Lys232, Ala230, Val219 (π–alkyl)

Two-dimensional protein–ligand interaction analysis was performed for the best-docked complexes using BIOVIA Discovery Studio Visualizer (Figures 2–7). The interaction maps demonstrated that the selected phytoconstituents established multiple conventional hydrogen bonds, carbon–hydrogen bonds, π–π interactions, π–sigma interactions, π–cation interactions, π–alkyl interactions, alkyl interactions, and van der Waals contacts with residues located within the binding pockets of the target proteins.

For 4GIX, Tinocordiside formed conventional hydrogen bonds with Asn93, Lys16, Asp15, and Ala18, together with hydrophobic interactions involving Trp231, Trp341, Met331, Tyr156, and Phe157. Berberine established a conventional hydrogen bond with Asn101 and additional interactions involving Arg99, Tyr172, Asp96, and Tyr177.
For 4XR8, Tinocordiside formed hydrogen bonds with Lys23, Asn31, Gly40, and Asn43, together with hydrophobic interactions involving Trp63, Leu44, Met331, and Phe157. Demethylwedelolactone formed conventional hydrogen bonds with Glu45 and Asp66, along with additional interactions involving Trp341, Trp231, Tyr156, and Arg67.
For TGF-β1 (1VJY), Apigenin and Luteolin established multiple hydrogen-bonding and hydrophobic interactions within the active-site cavity. Both compounds formed stable interaction networks consisting of conventional hydrogen bonds, π-mediated interactions, and van der Waals contacts with residues lining the binding pocket, similar to the interaction profile of the standard ligand.

DISCUSSION

Persistent HPV infection, particularly HPV-16, plays a pivotal role in Cervical carcinogenesis through the oncogenic activities of E6 and E7 proteins, while aberrant TGF-β1 signaling contributes to epithelial–mesenchymal transition (EMT), tumor progression, immune evasion, and metastasis. Therefore, simultaneous targeting of viral oncoproteins and host signaling pathways represents a promising therapeutic strategy for Cervical Cancer. The present study investigated the therapeutic potential of phytoconstituents from Tinospora cordifolia and Eclipta alba, the principal ingredients of the Siddha formulation Seenthil Chooranam, against HPV E6 oncoproteins (PDB IDs: 4XR8 and 4GIX) and Transforming Growth Factor-β1 (TGF-β1; PDB ID: 1VJY) using an integrated in silico approach comprising drug-likeness evaluation, ADMET prediction, molecular docking, and protein–ligand interaction analysis.
Among the twelve phytoconstituents initially screened, seven compounds satisfied Lipinski's Rule of Five, indicating favorable drug-like characteristics. Their molecular weights ranged from 270.24 to 396.48 Da, they displayed moderate lipophilicity (LogP 0.39–3.38), and they showed acceptable hydrogen bond donor and acceptor profiles. These physicochemical parameters are well recognized as important determinants of membrane permeability, bioavailability, and pharmacokinetic behavior, supporting their suitability for oral drug development.
The ADMET analysis further supported the pharmacological potential of the selected phytoconstituents. All compounds demonstrated high predicted intestinal absorption (71.47–97.15%), indicating the possibility of efficient gastrointestinal uptake following oral administration. None of the compounds were predicted to inhibit hERG class I potassium channels, suggesting a low risk of cardiotoxicity.[9] Although Palmatine, Berberine, and Tinocordiside were predicted to exhibit hepatotoxicity, these findings are computational predictions that require confirmation through experimental toxicity studies. Overall, the ADMET profile suggests that the majority of the investigated phytoconstituents possess acceptable pharmacokinetic characteristics suitable for further drug development.[10]
Molecular docking demonstrated that several phytoconstituents exhibited strong binding affinities toward both HPV E6 proteins and TGF-β1. Among the HPV E6 targets, Tinocordiside consistently displayed the strongest binding affinity, with docking scores of −9.6 kcal/mol against 4XR8 and −8.5 kcal/mol against 4GIX. The docking score obtained against 4XR8 exceeded that of the co-crystallized ligand (−8.3 kcal/mol), indicating excellent binding compatibility with the E6 active site. Demethylwedelolactone, Wedelolactone, Berberine, and Luteolin also demonstrated docking energies comparable to or better than the native ligand against 4XR8, highlighting their potential as HPV E6 inhibitors. Although the native ligand exhibited the strongest affinity toward 4GIX (−9.2 kcal/mol), Tinocordiside remained the best-performing phytoconstituent, suggesting consistent recognition of conserved functional regions across different HPV E6 conformations.
Docking against TGF-β1 (1VJY) further demonstrated the therapeutic potential of the selected phytoconstituents. Luteolin and Apigenin exhibited docking scores of −10.4 and −10.3 kcal/mol, respectively, which were comparable to the standard ligand (−10.5 kcal/mol). Since TGF-β1 is a key mediator of epithelial-mesenchymal transition, extracellular matrix remodeling, angiogenesis, and immune suppression during Cervical cancer progression, inhibition of this signaling pathway may complement HPV E6 inhibition by suppressing tumor invasion and metastatic potential. These findings suggest that Seenthil Chooranam phytoconstituents may possess multitarget activity against both viral and host molecular pathways involved in Cervical carcinogenesis.
Protein–ligand interaction analysis provided additional insight into the molecular basis of ligand recognition and binding stability. The docked phytoconstituents established extensive networks of conventional hydrogen bonds, carbon–hydrogen bonds, π–π interactions, π–sigma interactions, π–cation interactions, π–alkyl interactions, alkyl interactions, and van der Waals contacts, all of which contribute significantly to protein–ligand stability and binding specificity.
For the 4GIX protein, Berberine exhibited a stable binding orientation through a conventional hydrogen bond with Tyr100, together with a carbon–hydrogen bond involving Gly175. Aromatic interactions including π–π T-shaped interactions with Tyr172, π–alkyl interactions involving Arg99 and Tyr100, and a π–cation interaction with Lys176 further enhanced complex stability. Numerous van der Waals contacts with residues surrounding the binding pocket indicated efficient accommodation of the ligand. Similarly, Tinocordiside formed multiple conventional hydrogen bonds with Asn13, Lys16, and Asp15, together with a carbon–hydrogen bond involving Trp63 and a π–sigma interaction with Tyr156. Although one unfavorable donor–donor interaction with Asn13 was observed, the extensive hydrogen-bonding network and multiple hydrophobic contacts likely compensated for this interaction, resulting in a stable protein–ligand complex.
Within the 4XR8 binding pocket, Tinocordiside established multiple conventional hydrogen bonds involving Asn13, Lys43, Glu45, and Ala64, together with hydrophobic interactions surrounding Met331 and neighboring aromatic residues. These interactions collectively explain its superior docking affinity and stable accommodation within the active site. Likewise, Demethylwedelolactone exhibited a favorable interaction profile characterized by conventional hydrogen bonds

with Glu45, Asn13, Lys43, and Ala64, together with hydrophobic interactions involving Met331, Trp63, Trp231, Glu154, Leu44, Asp42, and Pro41. The combination of polar and hydrophobic interactions suggests that both compounds effectively occupy the HPV E6 binding cavity and may interfere with its biological activity.[8]
Interaction analysis of TGF-β1 (1VJY) revealed that Apigenin formed multiple conventional hydrogen bonds with Leu278, Ser280, Lys232, and Asp351, supported by π–sigma, π–alkyl, and extensive van der Waals interactions that stabilized the complex. Similarly, Luteolin established a stable interaction network through hydrogen bonding with Asp351 together with multiple hydrophobic contacts involving Leu278, Leu260, Leu340, Tyr249, Lys232, Ala230, and Val219. The interaction profiles of both compounds closely resembled those of the standard ligand, indicating their ability to occupy the functional binding pocket of TGF-β1 effectively.[11]

CONCLUSION

Molecular docking and protein–ligand interaction analyses of Seenthil Chooranam revealed strong binding affinities and stable interactions against HPV E6 oncoproteins (4XR8 and 4GIX) and Transforming Growth Factor-β1 (TGF-β1; 1VJY). The Phytoconstituent Tinocordiside demonstrated the highest binding affinity against HPV E6 proteins, while Luteolin and Apigenin showed binding affinities comparable to the standard ligand against TGF-β1. Collectively, these findings suggest that the phytoconstituents of Seenthil Chooranam may exert therapeutic effects by simultaneously targeting viral oncogenic proteins and host signaling pathways involved in Cervical cancer progression. Further in vitro and in vivo studies, and well-designed clinical investigations is essential to confirm their stability, efficacy, safety, and therapeutic potential for the management of HPV-associated Cervical carcinoma.

Abbreviation

ADMET- Absorption, Distribution, Metabolism, Excretion and Toxicity
pkCSM- Pharmacokinetic properties based on graph-based signatures
LogP- Logarithm of Partition Coefficient
HBA- Hydrogen Bond Acceptor
HBD- Hydrogen Bond Donor
RB- Rotatable Bond
TPSA- Topological Polar Surface Area
BBB-Blood–Brain Barrier
AMES-Ames Mutagenicity Test
hERG I- Human Ether-à-go-go-Related Gene Inhibition

REFERENCES

1. Vaghasia H, Patel R, Prajapati J, Shah K, Saraf M, Rawal RM. Cytotoxic and immunomodulatory properties of Tinospora cordifolia, Boerhaaviadiffusa, Berberis aristata, and Ocimum basilicum extracts against HPV-positive Cervical cancer cell line. BMC Complement Med Ther. 2025 Feb 17;25(1):59. doi: 10.1186/s12906-025-04817-x. PMID: 39962423; PMCID: PMC11834557.
2. Islam MR, Rauf A, Aktar MN, Fakir MNH, Trisha SI, Asif AI, et al. Recent advances in human papillomavirus vaccines and therapeutic strategies: Combating Cervical and non-Cervical cancers. Genes Dis. 2026;13(3):101880. doi:10.1016/j.gendis.2025.101880.
3. Younas S, Malik ZI, Khan MU, Manzoor S, Rehman HM, Hammad HM, Akter S. Identification of novel therapeutic inhibitors against E6 and E7 oncogenes of HPV-16 associated with Cervical cancer. PLoS One. 2025 Oct 27;20(10):e0323595. doi: 10.1371/journal.pone.0323595. PMID: 41144400; PMCID: PMC12558539.
4. Guo, W., Liu, H., Yan, Y., Wu, D., Yao, H., Lin, K., & Li, X. (2024). Targeting the TGF-β signaling pathway: an updated patent review (2021–present). Expert Opinion on Therapeutic Patents, 34(3), 99–126. https://doi.org/10.1080/13543776.2024.2346325
5. Aljabali AAA, Obeid MA, Bashatwah RM, Qnais E, Gammoh O, Alqudah A, Mishra V, Mishra Y, Khan MA, Parvez S, El-Tanani M, Hatahet T. Phytochemicals in Cancer Therapy: A Structured Review of Mechanisms, Challenges, and Progress in Personalized Treatment. Chem Biodivers. 2025 Aug;22(8):e202402479. doi: 10.1002/cbdv.202402479. Epub 2025 Jun 11. PMID: 40192260; PMCID: PMC12351433.
6. Murugesan B, Rathinavel T, Kumar RS, Gomathi M, Maheswari SU, Ameer K, et al. Atranorin from lichens act as potential inhibitor for Cervical tumor proteins TGFbeta and kinase enzyme CDK4/CyclinD3: An anti-cancer intervention therapy. Int J Biol Macromol. 2025;320(Pt 3):145896. doi:10.1016/j.ijbiomac.2025.145896.
7. Seenthil chooranam,Agathiar Vaidya Kaviyam 1500
8. Sinthiya KGG, Keerthana A, Jacob JSP, Sivaraj C, Anantharaman R, Yesudass S, et al. In silico molecular docking analysis of phytochemicals from Phaseolus vulgaris against cancer-associated target proteins. In Silico Res Biomed. 2026;2:100397. doi:10.1016/j.insi.2026.100397.
9. Mandar BK, Khanal P, Patil BM, Dey YN, Pasha I. In silico analysis of phytoconstituents from Tinospora cordifolia with targets related to diabetes and obesity. In Silico Pharmacol. 2021 Jan 2;9(1):3. doi: 10.1007/s40203-020-00063-w. PMID: 33442530; PMCID: PMC7778662.
10. Moharana M, Pattanayak SK, Khan F. Identification of phytochemicals from Eclipta alba and assess their potentiality against Hepatitis C virus envelope glycoprotein: virtual screening, docking, and molecular dynamics simulation study. J Biomol Struct Dyn. 2023 Jul;41(11):5328-5344. doi: 10.1080/07391102.2022.2085804. Epub 2022 Jun 11. PMID: 35694813.

11. Sharma RK, Bibi S, Chopra H, Khan MS, Aggarwal N, Singh I, Ahmad SU, Hasan MM, Moustafa M, Al-Shehri M, Alshehri A, Kabra A. In Silico and In Vitro Screening Constituents of Eclipta alba Leaf Extract to Reveal Antimicrobial Potential. Evid Based Complement Alternat Med. 2022 Aug 17;2022:3290790. doi: 10.1155/2022/3290790. PMID: 36034950; PMCID: PMC9402321.