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Molecular interactions of plant-derived inhibitors from *Euphorbia hirta* against binding sites of HPV E6 and E7 oncoproteins - An *in-silico* analysis

Research Article

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Abstract

Although no cure for Human Papilloma virus (HPV) exists at the moment, effective vaccines are present that can prevent the most harmful strains of the virus from infecting people. While potential cures like therapeutic vaccines, immunotherapy, and gene therapy are still in the early stages of development and may take years to become widely available, we focussed on using traditional *Siddha*-based medicines (phytochemicals of *Euphorbia hirta L.*) as a prospect through *in-silico* docking studies. Twelve phytochemicals from *Euphorbia hirta L.* were selected to investigate the antiviral properties against HPV. These ligand structures were obtained from PubChem database, prepared for docking with two major HPV targets i.e., E6 and E7 oncoproteins obtained from PDB, using AutoDock tools. The analysis revealed the potential of lobeline and euphol ligands effectively binding to the active sites of E6 and E7 proteins respectively. Using the ADMET lab 2.0 server, the ligands were evaluated for their commercial drug potential by considering their absorption, distribution, metabolism, excretion, and toxicity properties.

Keywords: Molecular docking, E6 and E7 oncoproteins, *Euphorbia hirta L.,* Natural inhibitors**,** Anti-tumor, Anti-viral.

Introduction

Cervical cancer is a form of cancer that originates in the cervix, the section of the uterus that links to the vagina. Based on the 2021 WHO report, cervical cancer is among the top four types of cancer in women globally and is associated with around 311,000 deaths annually. The primary contributor to cervical cancer is the long-standing infection by specific high-risk types of human papillomavirus (HPV), mainly HPV16 and HPV18 (1). Currently, the available treatment options for cervical cancer are surgery, chemotherapy, and/or radiotherapy, which can be used alone or in combination (2). These treatment options have undergone significant advances; however, their clinical efficacy is restricted by complications associated with surgery, the possibility of disease recurrence, and side effects associated with therapy (3). Various research studies have proposed new therapeutic approaches such as immunotherapies, targeted therapies, genetic treatments, and combination therapies as potential alternatives to traditional chemotherapy (2). As a result, it has become crucial to identify additional complementing and substituting strategies that can help decrease the side effects of chemotherapy. Multitarget therapies, such as polypharmacology, have been proposed as a solution to drug resistance in cancer treatment. These therapies have been shown to offer greater benefits than using combinations of drugs, as they can reduce side effects and better target cancer cells (4). *Euphorbia hirta* is a very common weed found throughout India and South east Asia. The succulent herb has different regional names such as *Dudhi*, *Dugdhika*, and *kshira* in Sanskrit, due to the milky sap it possesses. It is also referred to as *Amman Pacharisi* in the *Siddha* medicine system. The various medicinal uses of this common weed have been indicated in Siddha materia medica *Gunapadam Mooligai vaguppu.*

The phytopharmacological investigations revealed that *Euphorbia hirta* Linn, possesses various pharmacological properties due to its bioactive components. These bioactive components include terpenoids, alkaloids, steroids, tannins, fats, oils, mucilages, glycosides, saponins, coumarin, anthroquinones, chlorophyll, carotenoids, flavonoids, and their derivatives, such as rhamnose, quercetin rhamnoside, chlorophenolic acid, rutin, leucocyanidin, myricitrin, cyanidin 3,5-diglucoside, camphol, flavonol, inositol, tetraxerol, β-sitosterol, and kaempferol, Afzelin, euphorbin-A, euphorbin-B, euphorbin-C, euphorbin-D, gallic acid, and protocatechuic acid. Additional compounds include amyrin acetate, diterpenes such as 12-deoxyphorbol-13-phenylacetate-20-acetate, ingenol triacetate, and phytosterols such as campestrol, cholesterol, and stigmasterol. These bioactive components exhibit a myriad of pharmacological effects including anti-inflammatory, antimicrobial, antidiarrheal, sedative,

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analgesic, anti-pyretic, anti-oxidant, antiasthmatic, anti-tumor, larvicidal, and diuretic effects. (5)

HPV and Cervical cancer viral proteins

HPV is a DNA virus that infects skin and mucous membranes, including the cervix. It has a small circular genome of approximately 8 kilobases and encodes for several viral proteins, including E6 and E7, which play a crucial role in the development of cervical cancer (6)

There are several potential drug targets for HPV, including:

Viral proteins

HPV encodes several proteins that are critical for the virus life cycle and pathogenesis, such as HPV-E6 and HPV-E7. Various cellular factors interact with these proteins, including tumour suppressors, and can promote cell proliferation and survival. Inhibiting these viral proteins could potentially prevent or treat HPV-associated cancers.

Cellular signalling pathways

The cell signalling pathways, such as PI3K/Akt and Ras/MAPK relating to cell growth and survival, can be activated at HPV infection. These pathways could be targeted with specific inhibitors to restrict HPV-infected cell proliferation and prevent cancer development.

Immune system

HPV infection can suppress the immune system, which normally helps to detect and eliminate cancerous cells. Targeting immune checkpoints or activating immune responses against HPV-infected cells could potentially enhance the immune system's ability to recognise and eliminate infected or cancerous cells.

DNA replication

HPV replicates its DNA using host cellular machinery, and targeting the enzymes involved in DNA replication could potentially disrupt the virus life cycle and prevent its replication. It has been demonstrated that the E6 protein found in high-risk HPV types plays a significant role as an oncogene in the development of cervical cancer. E6 is a compact protein that consists of around 150 amino acids and is capable of interacting with various host proteins to induce cell growth, obstruct programmed cell death, and interfere with typical cell cycle control mechanisms (7). The E6 targets the critical tumour suppressor protein p53 which plays a vital role in preventing cancer by inducing cell cycle arrest or apoptosis in response to DNA damage or other stresses. The degradation of p53 is promoted by E6 through binding to E6AP, which is a cellular ubiquitin ligase that targets p53 via the proteasome. This leads to a loss of p53 function and allows the infected cells to proliferate and accumulate mutations, eventually leading to cervical cancer. In addition to its effects on p53, E6 also interacts with other host proteins including the tumour suppressor proteins pRb, MAML1, DLG1 and the cellular protein PDZ. These interactions contribute to the oncogenic effects of E6 by promoting cell cycle progression, inhibiting apoptosis, and disrupting normal cell polarity and adhesion (6).

Materials and methods

Software tools

Bioinformatics tools such as Chimera 1.12 (1), LigPlot+ v.2.2.8 were used and molecular docking was calculated using AutoDock tools v.1.5.7 and MGL tools v.1.5.7 packages. Additionally, online resources such as Pubmed, Pubchem database, Protein Data Bank ADMET lab v.2.0 were used for obtaining the chemical structures and data collection.

Docking Analysis of E6 and E7 oncoproteins with *Euphorbia hirta L.* ligands

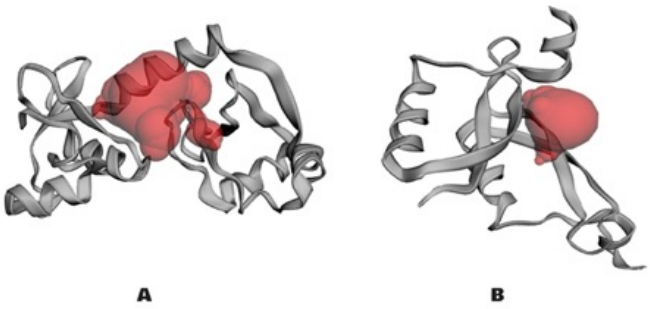
Twelve natural compounds' chemical structures were obtained from the PubChem library. Chimera 1.12 was used to obtain the structures in smiles format, convert them to PDB, and then minimise the PDB format structures so they could be saved in PDB files. PDB file was sent to AutoDock Tools (ADT) for PDBQT file preparation. The PDB database was used to obtain the three-dimensional structures of HPV's E6 (PDB ID: 4GIZ) and E7 (PDB ID: 2B9D) protein structures. The PDB file (4GIZ) that was obtained from the Protein Data Bank had extra chains (A, B, and D), water molecules, and ligands that were removed. Finally, the PDB file was assessed and sent to Autodock Tools (ver. 1.5.7) for PDBQT File preparation before the C chain (4GIZ). In the preparation of the PDB files for the docking operation, ADT was used to automatically perform several tasks. These included the removal of non-standard residues, the computation of Gasteiger charges for protein atoms, the detection of aromatic carbons and rotatable bonds, and the setting of TORSDOF. Only polar hydrogens were retained in the process. Finally, the prepared receptor and ligand PDB files were converted into PDBQT file format. The Auto Grid program was used to calculate three-dimensional grid maps of the receptor proteins for use in the docking process. The dimensions of the grid maps were determined based on the location of polar residues within the binding sites. Specifically, for the E6 receptor, the grid map had an x-dimension of 56 points, a y-dimension of 50 points, and a z-dimension of 86 points, with a grid spacing of 0.375 Å and box centre coordinates of X = 2.851, Y = 50.522, and Z = 27.126. For the E7 receptor, the grid map had an x-dimension of 54 points, a y-dimension of 42 points, and a z-dimension of 44 points, with a grid spacing of 0.375 Å and box centre coordinates of X = 16.89, Y = 35.16, and Z = 44.31. we utilised the Lamarckian genetic algorithm (LGA) to investigate the interaction pattern of selected natural metabolite inhibitors (ligands) with the receptor. We performed 100 genetic algorithm (GA) runs using a set of default parameters for the Docking process, including a population size of 150 for every 25 × 105 energy evaluations, 27,000 maximum number of generations, a mutation rate of 0.02, and a crossover rate of 0.8 for the LGA. The results demonstrated the effectiveness of the LGA in analysing the interaction pattern between the receptor and ligands. All the

inhibitors underwent molecular docking operations in distinct conformations and found to be with varying binding energies. The binding energies of ligands to E6 and E7 oncoproteins were obtained and tabulated.

Results

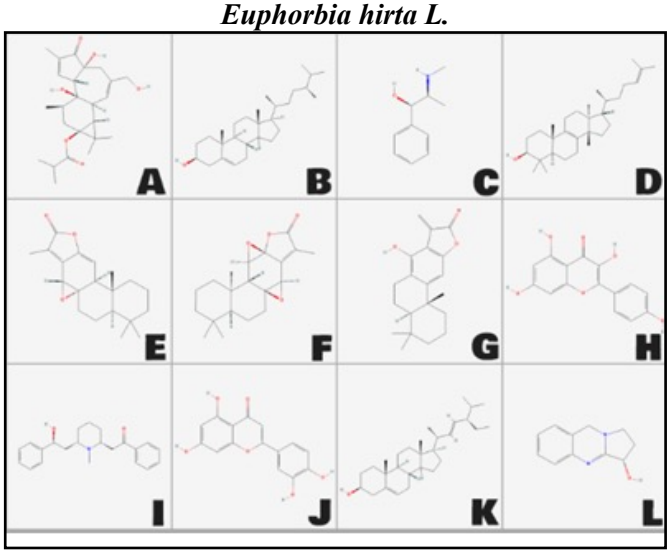
The CASTp pocket detection analysis showed a prominent pocket area = 308.251 Å^2 and volume = 276.183 Å^3 for C - chain of HPV E6. A smaller active pocket for CR3 domain of HPV E7 was detected, with an area = 119.909 Å^2 and volume = 98.538 Å^3. The prominent binding sites of E6 and E7 oncoproteins are shown in figure 1.

Fig 1: Structure of the target proteins (A) binding site of HPV E6 C - chain, (B) binding site of HPV E7 CR3 marked as red bubbles



The docking studies show that all the 12 selected ligands (Figure 2) have the ability to bind with varying binding affinities to the E6 and E7 oncoproteins of HPV which can hinder the binding capability of HPV onto tumour suppressor proteins within the human body. The binding affinities of the ligands after docking are tabulated in table 1.

Figure 2: 2D structures of selected ligands from *Euphorbia hirta L.*



(A) 12-Deoxyphorbol-13-Isobutyrate, (B) Campesterol, (C) Ephedrine, (D) Euphol, (E) Jolkinolide A, (F) Jolkinolide B, (G) Jolkinolide C, (H) Kaempferol, (I) Lobeline, (J) Luteolin, (K) Stigmasterol, and (L) Vasicine

Table 1: Interactions and binding affinities of selected ligands from *Euphorbia hirta L.* towards HPV E6 and E7 oncoproteins

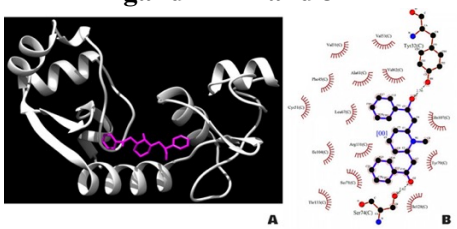
Compound name	Mol. Wt.	Mean binding energy (kcal/mol)		Number of hydrogen bonds		Residues involved in hydrogen bonding		Residues involved in other interactions	
		E6	E7	E6	E7	E6	E7	E6	E7
12-Deoxyphorbol-13-Isobutyrate	418.24	-6.6	-6.7	2	1	Tyr32, Cys51	Lys57	Leu50, Val62, Leu67, Tyr70	Tyr46, Ala47, Val49, Lys57, Val59, Arg60
Campesterol	400.37	-7.3	-6.1	1	0	Cys51	NA	Val31, Phe45, Leu50, Val62, Ile104, Ile128, Arg131, Thr133	Tyr46, Val49, Lys57, Val59, Arg60
Ephedrine	165.12	-5.0	-4.4	1	2	Tyr32	Ser51	Val31, Phe45, Leu50, Val53, Val62, Leu67	Ala50, Leu77
Euphol	426.39	-7.2	-7.7	0	0	NA	NA	Leu50, Val62, Leu67, Tyr70, Gln107	Tyr46, Ala47, Val49, Leu58, Arg60, Pro86

Jolkinolide A	314.19	-7.4	-6.5	2	2	Ser71, Gln107	Lys57, Leu58	Tyr32, Leu67, Tyr70, Ile73	Gln44, Glu56, Lys57
Jolkinolide B	330.18	-7.2	-6.3	3	3	Ser71, Ser74, Gln107	Lys57, Arg60	Tyr32, Phe45, Leu50, Val53, Val62, Leu67, Tyr70,	Val49, Arg60. Lys57
Jolkinolide C	312.17	-7.1	-6.8	0	0	NA	NA	Val31, Tyr32, Phe45, Leu50, Val53, Val62,	Gln44, Pro45, Ala47, Lys57
Kaempferol	286.05	-7.5	-6.2	2	3	Tyr32, Tyr60	Gln44, Glu56, Leu58	Val31, Phe45, Leu50, Val53, Val62, Gln107	Gln44, Tyr46, Ala47, Leu58
Lobeline	337.2	-7.9	-6.5	2	2	Tyr32, Ser74	Tyr46, Ala47	Val31, Phe45, Val53, Val62, Leu67, Ile104, Ile128, Thr133	Gln44, Tyr46, Val49, Lys57, Leu58, Arg60
Luteolin	286.05	-7.2	-6.2	5	4	Lys11, Cys51, Tyr60	Ala47, Val49, Glu74	Val31, Phe45, Leu50, Val53, Val62	Tyr46, Ala47
Stigmasterol	412.37	-7.0	-6.7	1	1	Cys51	Val49	Val31, Leu50, Val62, Leu67, Glu75, Ile104, Ile128, Arg131, Thr133	Val49, Lys57, Val59, Arg60, Pro86, Leu87
Vasicine	188.09	-6.4	-5.1	2	2	Tyr32	Glu56, Leu58	Val31, Tyr32, Phe45, Leu50, Val53, Val62, Leu67	Gln44, Tyr46, Leu58

NA- Not Applicable

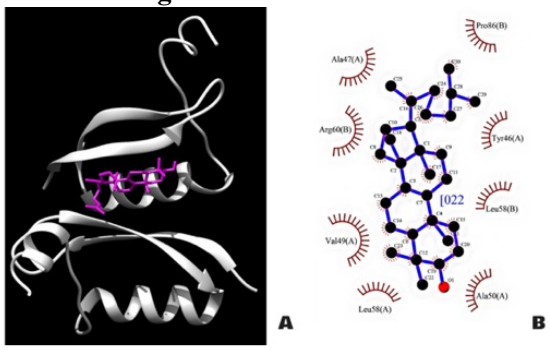
For docking with E6 as receptor, lobeline was found with the lowest binding affinity of -7.9 kcal/mol interacting with Tyr32 and Ser74 residues forming hydrogen bonds with respective distances of 2.76 Å and 2.62 Å (**Figure 3**). For E7 receptor, euphol was noted with the lowest binding affinity of -7.7 kcal/mol. Hydrogen bond interactions were not observed between the euphol and E7 oncoprotein (**Figure 4**) but hydrophobic interactions were observed between them and the target proteins.

Figure 3: Interaction between the E6 target and ligand in 2D and 3D



(A) lobeline bound to the active site of HPV-E6 protein. (B) interaction diagram of lobeline to tyr32 and ser74 residues through hydrogen bonding.

Figure 4: Interaction between the E7 target and ligand in 2D and 3D



(A) euphol bound to the active site of HPV-E7 protein. (B) hydrophobic interaction diagram of euphol.

After ADMETlab 2 analysis of the 12 ligands, the selected pharmacokinetic parameters are tabulated in table 2. Of all the ligands, jolkinolide B shows higher probability of being P-glycoprotein inhibitor and higher oral toxicity. 12-Deoxyphorbol-13-Isobutyrate shows higher probability of blood-brain barrier permeability and oral toxicity. The highest probability of blood-brain barrier permeability and carcinogenicity is seen in vasicine.

Table 2: Selected pharmacokinetic parameters of 12 ligands from *Euphorbia hirta L.* calculated using ADMETlab 2

Ligand	P-glycoprotein inhibition	Human intestinal absorption	BBB	hERG	Rat Oral Acute Toxicity	Skin Sensitivity	Carcinogenicity
12Deoxyphorbol13Isobutyrate	0.004	0.1	0.901	0.001	0.977	0.025	0.394
Campesterol	0.377	0.004	0.854	0.04	0.023	0.176	0.067
Ephedrine	0	0.015	0.275	0.057	0.596	0.079	0.027
Euphol	0.37	0.009	0.118	0.018	0.038	0.202	0.019
Jolkinolide A	0.003	0.01	0.176	0.006	0.716	0.144	0.433
Jolkinolide B	0.973	0.005	0.419	0.005	0.966	0.523	0.74
Jolkinolide C	0.898	0.006	0.04	0.008	0.654	0.874	0.107
Kaempferol	0.004	0.008	0.009	0.07	0.156	0.856	0.097
Lobeline	0.831	0.043	0.777	0.87	0.313	0.672	0.109
Luteolin	0.004	0.047	0.009	0.064	0.046	0.946	0.095
Stigmasterol	0.126	0.026	0.873	0.014	0.7	0.035	0.041
Vasicine	0	0.017	0.992	0.028	0.846	0.268	0.756

All values are depicted in the notation of "probability of being active" in which values closer to 0 are less probable and closer to 1 are highly probable.

Discussion

Almost 50% of cervical pre-cancers with high levels of severity are caused by various types of human papillomavirus (HPV) (10). Significantly improved survival rates can be achieved with early-stage detection of cervical cancer, which has long been regarded as a disease that is largely preventable. However there is a need to explore alternative therapies to complement and reduce the side effects of existing conventional management such as chemotherapy, surgeries, radiation therapy etc. The Siddha system of medicine in its classical text indicates *Euphorbia hirta* a common weed herb for the management of warts (*Maru*) and cervical cancer (*Uratha van megam*). Therefore, an in-silico analysis has been performed to explore plant-derived inhibitors from *Euphorbia hirta* against binding sites of HPV E6 and E7 oncoproteins. The classification of HPV strains is based on their level of risk, with two main categories being low-risk HPV (e.g., types 6 and 11) and high-risk HPV (e.g., types 16 and 18) (11). Natural products like berberine, resveratrol and curcumin have demonstrated potential as effective agents for the prevention or treatment of HPV infections. These natural compounds have been the subject of preclinical and clinical trials to investigate their efficacy (12). *Euphorbia hirta*, commonly known as asthma weed or snake weed, is a medicinal plant that has been used for centuries in traditional medicine (13). It is commonly used to treat a range of ailments, including respiratory infections, fever, and gastrointestinal disorders (14). Studies have shown that *Euphorbia hirta L.* possesses antifungal and antibacterial properties, and may be effective against a range of viral infections (15). Its antiviral properties have been studied extensively, particularly in its efficacy against Human Papillomavirus (HPV), a virus that can cause warts and cervical cancer in women (12) (16). In Siddha medicine, *Euphorbia hirta L.* has been used for various ailments, including viral infections, and recent scientific studies have confirmed its therapeutic potential (17). There are several docking studies involving screening the natural compounds from databases and consecutive in-silico docking studies (18) very little is known about the activity of *Euphorbia hirta L.* based natural compounds against HPV target proteins. In the present study, the potential anticancer properties of lobeline and euphol from *Euphorbia hirta L.* against cervical cancer is shown. Lobeline is a natural alkaloid first found in plants of the *Lobelia* genus, and has been studied for its potential as a smoking cessation aid due to its similarity to nicotine (19). It is also known to have potential therapeutic effects for a range of conditions, including schizophrenia, attention deficit hyperactivity disorder (ADHD), and Parkinson's disease (20) A pilot study on

lobeline for smoking cessation in smokers with schizophrenia found that while lobeline reduced cigarette smoking and craving, but it did not have a significant effect on other measures of nicotine withdrawal (21) The anti-viral effect of lobeline against RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 through in-silico studies has revealed high potency in inhibition of its activity (22) Euphol is a terpenoid alcohol found in plants such as *Euphorbia resinifera* and has been studied for its potential anti-inflammatory, anti-tumour and anti-viral properties. Euphol is known to have anti-inflammatory effects (23), potential anti-tumour effects through various mechanisms, including inhibition of angiogenesis and induction of apoptosis (24) and analogues of euphol having cytotoxic effects on human breast cancer cells (25). An in-silico study found that euphol could potentially bind to the active site of Mpro within SARS-CoV-2 and inhibit its activity Overall, while both lobeline and euphol have shown some potential through in-silico and preclinical studies, further clinical research is needed to determine their safety and efficacy for various health conditions.

Conclusion

The present study on molecular docking of plant-derived inhibitors from *Euphorbia hirta* against binding sites of HPV E6 and E7 oncoproteins has been performed based on the literature evidence on this traditional plant in Siddha classical literature to cure warts which has the same causative factor HPV. The study results show that among the twelve tested phytocompounds, both lobeline and euphol have shown to be effective antiviral components against binding sites of HPV E6 and E7 oncoproteins. In future preclinical studies and clinical research is needed to determine their safety and efficacy of this herb for the treatment of HPV induced health issues including cervical cancer.

Conflict of Interest: The authors declare no conflict of interest.

Supplementary Table 1: Screening of compounds based on Lipinski's rule of 5

Compound name	LogP	nHA	nHD	MW	Lipinski
12-Deoxyphorbol-13-	2.516	6	3	418.24	Accepted
Campesterol	7.308	1	1	400.37	Accepted
Ephedrine	1.032	2	2	165.12	Accepted
Euphol	7.72	1	1	426.39	Accepted
Jolkinolide A	4.833	3	1	314.19	Accepted
Jolkinolide B	4.771	4	0	330.18	Accepted
Jolkinolide C	5.367	3	1	312.17	Accepted
Kaempferol	2.656	6	4	286.05	Accepted
Lobeline	3.186	3	1	337.2	Accepted
Luteolin	2.902	6	4	286.05	Accepted
Stigmasterol	6.986	1	1	412.37	Accepted
Vasicine	1.162	3	1	188.09	Accepted

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