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RESEARCH ARTICLE

In silico Study of Natural inhibitors for Human papillomavirus-18 E6 protein

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ABSTRACT:
Globally, the leading cause of death from cancer in women is infection with the human papillomavirus (HPV). This calls for imperative actions to explore anticancer drugs against this threatening viral infection, in which case, natural ingredients are presumed to be a promising source. Several studies show that plant-origin compounds such as allicin, apigenin, capsaicin, cyanidin, fisetin, genistein, laricitrin, naringenin, piperine, and syringetin have demonstrated therapeutic effects against several cancer types. In this study, the interaction mechanism of these compounds with HPV-18 E6 oncoprotein, that is known to downregulate tumor suppressor p53, was predicted using an *in silico* approach. Molecular docking simulations of natural ligands and E6 protein were performe, followed by chemical interaction analysis and 3D molecular visualization. Results indicated that fisetin is the best natural inhibitor as it has the lowest binding energy. It is highly recommended that the results of this study be used as a reference in designing anticancer drugs *in vitro* and *in vivo*.

KEYWORDS: HPV, E6, cervical cancer, inhibitors, virtual screening.

INTRODUCTION:

More than a hundred types of cancer attack humans, and this has lead to it being placed as the second-leading cause of death worldwide. In 2015, cancer has resulted in 8.8 million mortalities and is expected to rise to 10 million in 2020[1]. Some scientists believe that most cancers (90-95%) are caused by mutations that are triggered by environmental and lifestyle factors, and the rest (5-10%) are due to genetic inheritance. Globally, approximately 18% of cancer deaths are attributed to

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infectious agents[2]. Inhibition of the activity of the major transcription factors appears to be a very potent approach to cancer therapy[3,4].

Human papillomavirus (HPV), a sexually transmitted virus from the papillomavirus family, is a causative agent of several types of cancer including cervical, head and neck, vaginal, penile, and anal cancer[5]. Over 200 subtypes of HPV have been identified and characterized over the years. Based on its oncogenic potential, HPV is classified into high-risk and low-risk groups[6,7]. High-risk types such as HPV-18 and -16 are responsible for causing anogenital cancer which account for 15.7% and 62.6% of cervical cancers, respectively, whereas low-risk HPV-6 and -11 induce only genital warts[8,9]. Thus, HPV 16 and 18 became the main targets in the design of anticancer drugs.

HPV is a double-stranded virus that invades the epithelium[10]. The HPV genome consists of three main regions. First, the long control region (LCR) has the function of regulating DNA replication by controlling the transcription of viral genes. Th early region (E) is responsible for encoding non-structural proteins involved in viral replication (E1,E2,E4) and oncogenesis (E5,E6,E7). Late regions are known to encode structural proteins (L1 and L2) involved in viral capsid formation, transmission, and spread of virions[11-13].

E6 is one of the oncoproteins expressed in HPV-16 and -18, which contributes to malignant transformation, immortality, and carcinogenicity[2]. This oncoprotein targets p53, which plays an important role as a tumor suppressor and checkpoint regulator in the cell cycle[14]. E6 forms a heterotrimeric complex with p53 and E6-associated protein (E6AP) via the ubiquitination pathway which leads to p53 degradation[15]. Decreased p53 content causes it to be unable to repair DNA damage resulting in malignancy[2].

For more than three decades, HPV has been identified as the causative agent for cervical cancer, but finding effective therapies to fight HPV infection remains to be established[16]. Developing drugs for the prevention and treatment of cancer based on natural compounds of plant origin has emerged as a promising approach in recent years[17].

Allicin is reported to suppress the viability of cervical cancer cells depending on the time and dose of therapy[18]. Apigenin arrests the cycle of human cervical cancer cells (HeLa) in the G1 phase and induces apoptosis via DNA fragmentation[19]. Capsaicin exhibits potent anticancer properties on various types of cancer cells, and its combination with radiotherapy or conventional chemotherapy improves sensitivity, reduces side effects, and increases patient tolerance of therapy[20]. Cyanidin-3-O-β-glucopyranoside has an anti-proliferative effect by activating caspase-3 and inducing p21 expression on human prostate cancer cells DU145 and LnCap[21]. Fisetin which is found in many fruits and vegetables, induces apoptosis and interferes with the mitochondrial membrane potential of HeLa cell lines[22,23]. Genistein triggers apoptosis in HeLa cells through increased caspase-9 and caspase-3 activity[24]. Laricitrin can reverse lung cancer cells-induced dendritic cell paralysis, by inhibiting STAT3 (signal transducer and activator of transcription 3) phosphorylation[25]. Naringenin, a flavanone derivative in citrus fruits, has been shown to cause cell death and inhibit the cell cycle in the G0/G1 (24 h) and S (48 h) phases in a 3D spheroid culture of cervical cancer cells, as well as to increase the therapeutic effect of cervical cancer by cisplatin[26,27]. *Atriplex halimus* containing syringetin is also reported to have selective cytotoxicity against breast (MCF-7) and prostate (PC3) cancer cells[28].

Research in the field of drug design is very time consuming and expensive, and the *in silico* approach can be used as a preliminary study on the potency of natural compounds as drug candidates[29]. Computational drug design simulations can predict the molecular mechanisms of candidate compounds through several analyzes based on specific research objectives, such as prediction of biological pathways, binding energies, types of interactions, and molecular dynamics[30]. This study predicted the potential molecular mechanisms of ten different natural compounds from several plant sources as antiviral candidates for HPV-18 via an *in silico* approach.

MATERIALS AND METHODS:

Hardware and Softwares:

The study was conducted on Dell Latitude E7240 with Intel® Core™ i7-4600U and 2.10 GHz processor, 16 GB RAM, and 250 GB hard disk drive. The bioinformatics softwares used were PyRx 0.8 and PyMol 2.0. Online resources such as PubChem (https://pubchem.ncbi.nlm.nih.gov/), UniProt (https://www.uniprot.org/), RCSB Protein Data Bank (PDB) (https://www.rcsb.org/), SwissADME (http://www.swissadme.ch), and Protein Plus (https://proteins.plus/) were also utilized in this study.

Compounds and Protein Preparation:

This study involved ten natural compounds namely allicin, apigenin, capsaicin, cyanidin, fisetin, genistein, laricitrin, naringenin, piperine, and syringetin. Their chemical 3D structures were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). RCSB PDB (https://www.rcsb.org/) was used to reveal the HPV-18 E6 protein target samples.

Drug-Likeness Analysis:
Drug-likeness analysis was performed to qualitatively assess the possibility of a molecule becoming an oral drug based on its bioavailability. It was done on the SwissADME (http://www.swissadme.ch) and referred to Lipinski's rule of five[31,32].

Molecular Docking Study:
The compounds that met Lipinski's rule were analyzed for their binding energy with HPV-18 E6 protein by using docking analysis. A blind docking type was performed using the Vina program in PyRx 0.8[33].

Protein-Ligand Interactions:
This analysis identified the interaction position and the type of chemical bond formed when a potential compound binds to the E6 protein target. The Protein Plus web server (https://proteins.plus/) was involved in this analysis[34].

Molecular Visualization:
PyMol 2.0 was employed for visualization of representative color and structural selection[35].

RESULTS AND DISCUSSION:

The Potency of Natural Compounds as Drug Candidates:
Ten samples of chemical compounds namely allicin, apigenin, capsaicin, cyanidin, fisetin, genistein, laricitrin, naringenin, piperine, and syringetin were obtained from the PubChem database. The sample data consist of the 3-dimensional structure of the target compound in structural data format (.sdf) which was then converted into a protein data bank (.pdb) format that can be used for subsequent analysis. The selection of the target compound structure is shown in the representative form and colored based on the chemical building blocks in the PyMol 2.0 software (Figure1).

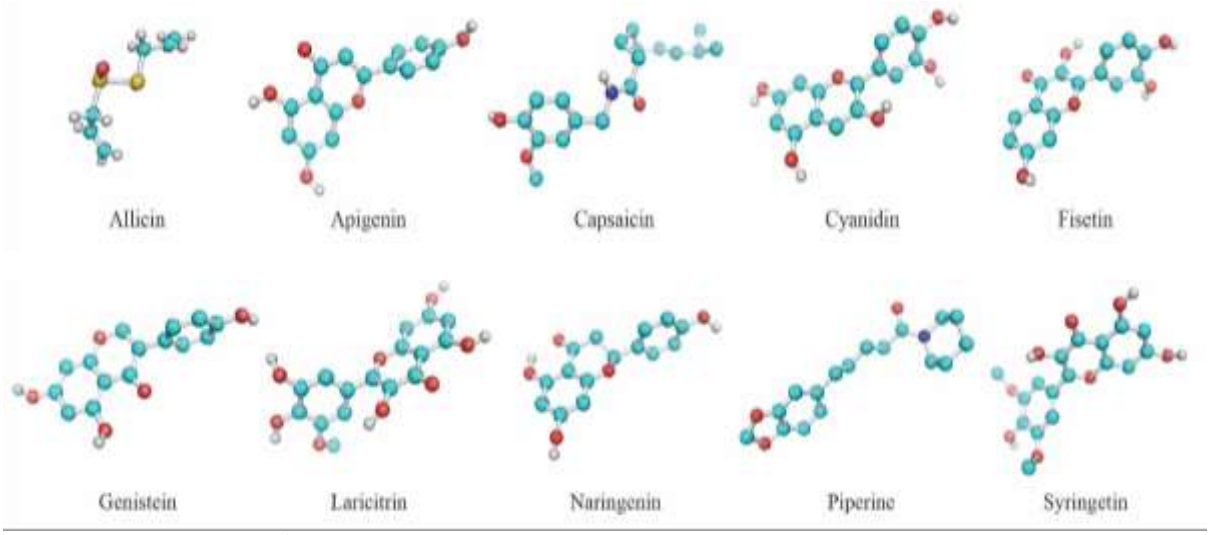

Figure 1. Chemical structure of natural compounds.

The drug-ability of these chemical compounds was then predicted on the SwissADME server (http://www.swissadme.ch) based on Lipinski's rule of five. The rule of five (Ro5) is defined as a combination of parameters capable of identifying potential drug candidates that may cause problems with absorption and permeability[36]. Lipinski's rule states that oral drugs must not have more than one violation of the five criteria that consist of molecular weight ≤ 500 Dalton, hydrogen bond donors ≤ 5, hydrogen bond acceptors ≤ 10, lipophilicity ≤ 5, and molar refractivity between 40-130[37,38]. The prediction results show that all the chemical compounds are drug-like (Table 1). These compounds can proceed to the next stage of analysis, i.e. the determination of the binding energy to the target protein.

Table 1. Prediction results of drug-like molecules based on Lipinski's rule.

Compounds	Lipinski's Rule of Five				
	MW (Da)	HBD	HBA	Log P	MR
Allicin	162.27	0	1	1.18	45.88
Apigenin	270.24	3	5	0.52	73.99
Capsaicin	305.41	2	3	2.69	90.52
Cyanidin	287.24	5	6	0.32	76.17
Fisetin	286.24	4	6	-0.03	76.01
Genistein	270.24	3	5	0.52	73.99
Laricitrin	332.26	5	8	-0.83	84.53
Naringenin	272.25	3	5	0.71	71.57
Piperine	285.340	0	3	2.39	85.47
Syringetin	346.29	4	8	-0.59	89.00

Note. MW: Molecular Weight; HBD: Hydrogen Bond Donors; HBA: Hydrogen Bond Acceptors; Log P: Lipophilicity; MR: Molar Refractivity.

Interaction Study of Natural Ligands with HPV-18 E6 via Docking Analysis:

Molecular docking is a valuable computational modeling technique for estimating the interaction of ligand-receptor macromolecules and determining the binding energies generated[39,40]. The protein was prepared using PyMol 2.0 software to eliminate the water molecules[41]. In this study, the blind docking method was employed due to the fact that the functional domain of the target receptor was unknown[42].

Chemical compounds consisting of allicin, apigenin, capsaicin, cyanidin, fisetin, genistein, laricitrin, naringenin, piperine, and syringetin were used as ligands and E6 (2I04) as the target. Docking simulations for them were carried out in PyRx 0.8 software with a grid docking center x: -6.8275; y: -10.748; z:11.2834 and dimensions (Å) x: 47.2043; y: 54.2914; z: 44.6647. Molecular visualization of docking results was conducted by structural selection and coloring using PyMol 2.0 software (Figure 2).

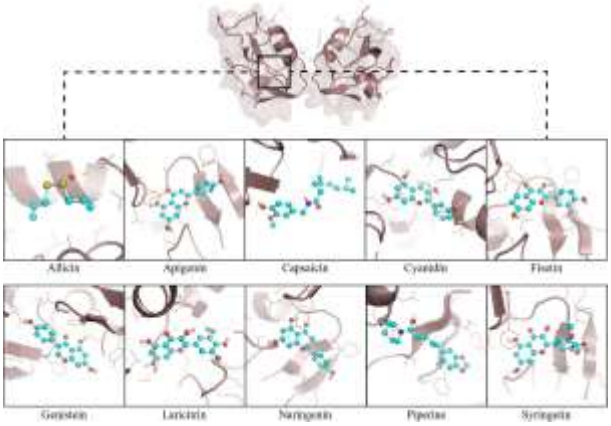

Figure 2. Molecular visualization of natural compound ligands that bind to the HPV-18 E6 receptor. The E6 protein is shown with a transparent surface and a violet cartoon structure

Since all observed natural compounds interact with E6 protein in different conformations and binding energies, the molecular complex with the lowest binding energy was selected for analysis. Molecular docking results showed that of all the compounds tested, fisetin had the lowest binding energy of -8.1 kcal/mol to HPV-18 E6. Nine other natural ligands i.e. apigenin, naringenin, syringetin, laricitrin, piperine, cyanidin, genistein, capsaicin, and allicin were observed to bind to the E6 receptor with a binding energy range of -7.4 to -3.4 kcal/mol (Table 2). In line with the objectives of this study, the tested compounds were predicted to inhibit E6 activity. The ligand with the lowest binding energy value affected the biological activity of a protein target. The lowest binding energy allowed the formation of molecular complexes under constant temperature and pressure[43]. The binding energy value is also influenced by the presence of amino acid residues in the binding region of the target protein and the chemical interaction type[33] so that fisetin was further evaluated for its binding location and the type of chemical interactions formed.

Table 2. The result of molecular docking simulation

Compound	ID	Protein Target	Binding Energy (kcal/mol)
Allicin	65036	E6	-3.4
Apigenin	5280443	E6	-7.4
Capsaicin	1548943	E6	-5.9
Cyanidin	128861	E6	-6.6
Fisetin	5281614	E6	-8.1
Genistein	5280961	E6	-6.5
Laricitrin	5282154	E6	-6.8
Naringenin	932	E6	-7.3
Piperine	638024	E6	-6.8
Syringetin	5281953	E6	-6.9

Evaluation of bond type and molecular interaction position of the natural compound with the lowest binding energy was carried out on the Protein Plus webserver. Fisetin interacts with the HPV-18 E6 domain through hydrophobic bonds at Tyr398B, Phe396B, Phe396A, Asn374B, and Ser394A, as well as hydrogen bonds at the Val397B, Phe396B, Ser394A, Ser375B, Phe396A, and Tyr398B positions (Figure 3).

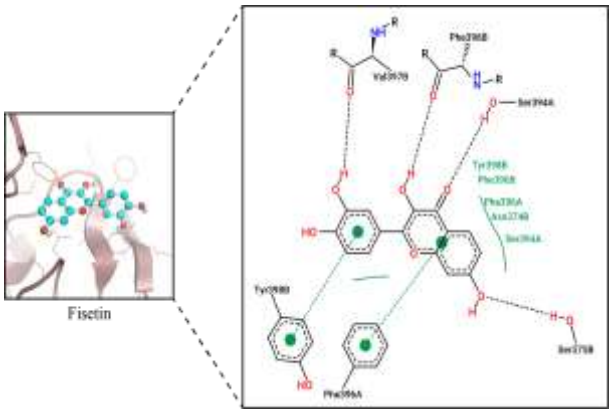

Figure 3. Chemical interaction between Fisetin and HPV-18 E6 binding domain

Chemical interactions that occur between a ligand and a protein domain have an important role in influencing the binding energy. The types of chemical interactions that can be formed are hydrophobic and hydrogen bonds[33]. This interaction is classified as a weak bond that can encourage the performance of the biological activity of certain proteins[44]. Chemical bond interactions and the position of binding amino acid residues are very important factors in determining the probability of drug molecules through computer-based studies[30]. Thus, fisetin has a good potential as a candidate for an antiviral and anticancer drug because it can bind to the HPV-18 E6 protein with the lowest binding energy and interact with hydrogen and hydrophobic bonds.

CONCLUSION:

HPV-18 antiviral molecular activities of several natural compounds were examined regarding their inhibitory mechanisms against E6 protein, and fisetin was found to be the best candidate. We suggest that the *in silico* simulation results from this study be used as a reference for drug development through *in vitro* and *in vivo* studies.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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