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Article

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Abstract: This study investigates the use of artificial intelligence (AI) and ethnobotanical knowledge to identify natural compounds with potent antiviral activity against HPV-16. Through molecular docking simulations, we evaluated the binding affinities of various essential oil components, including Apigenin, against the key antigenic determinants of HPV-16 (E6, E7 oncoproteins, and L1 major capsid protein). Apigenin exhibited strong binding affinities, particularly to E6 Oncoprotein. To further enhance these findings, we employed an AI-driven reverse engineering approach to predict a natural compound with superior efficacy. The AI identified Luteolin, a flavonoid with additional hydroxyl groups, as a promising candidate. Comparative docking studies demonstrated that Luteolin possesses significantly stronger binding affinities to all three HPV-16 targets, surpassing the efficacy of Apigenin. This work highlights the potential of AI in accelerating the discovery and optimization of natural antiviral agents, providing a powerful tool for pharmacological and ethnopharmacological screenings. Our findings suggest that AI-guided identification of natural compounds can lead to the development of more effective antiviral therapies, warranting further experimental validation and potential synthesis in laboratory settings.

Keywords: HPV-16; Molecular Docking; Artificial Intelligence; Ethnobotany; Natural Compounds; Antiviral Agents; Luteolin; Apigenin; Pharmacological Screening; Ethnopharmacology; Computer Simulations; Laboratory Synthesis

1. Introduction

Human papillomavirus (HPV) is a diverse group of DNA viruses, with over 200 known genotypes [1], classified into low-risk and high-risk types based on their association with benign or malignant lesions, respectively. HPV-16 is one of the high-risk strains, responsible for a significant proportion of cervical cancers and other anogenital and oropharyngeal malignancies [2].

Understanding the structural and genetic makeup of HPV-16 is crucial for developing effective therapeutic strategies.

HPV-16 is a non-enveloped virus with a double-stranded circular DNA genome approximately 7.9 kilobases in length [3]. The viral particle is icosahedral in shape, about 55 nm in diameter, and consists of 72 pentameric capsomeres [4]. The capsid is composed of the L1 major capsid protein [5], which forms the bulk of the virion, and the L2 minor capsid protein [6]. The viral genome is divided into early (E) and late (L) regions [7], with the early region encoding proteins involved in viral replication and oncogenesis (E1, E2, E4, E5, E6, and E7) [8] and the late region encoding the structural proteins L1 and L2 [9].

In terms of viral classification, HPV-16 belongs to the family Papillomaviridae, ge-nus Alphapapillomavirus [10, 11]. According to the Baltimore classification system, it is a Group I virus, meaning it possesses a double-stranded DNA genome. The oncogenic potential of HPV-16 is primarily attributed to the E6 and E7 oncoproteins, which interact with and degrade tumor suppressor proteins p53 and retinoblastoma (Rb), respectively, leading to uncontrolled cell proliferation and malignant trans-formation [12].

HPV-16 is associated with both acute and chronic diseases. Acute infections often manifest as benign warts, while persistent infections can lead to chronic conditions and progression to cancers [13]. The pathogenesis of HPV-related cancers is a mul-tistep process involving persistent infection, viral integration into the host genome, and subsequent genetic alterations. Current treatments for HPV infections include surgical removal of warts and lesions [17], cytotoxic agents [19], and antiviral medications [14]. Vaccination remains the most effective preventive measure, with vaccines like Gardasil and Cervarix [15] providing protection against HPV-16 and other high-risk types [20-23].

Despite the availability of vaccines, there is a significant need for effective thera-peutic agents to treat existing infections and HPV-related cancers [16]. Natural compounds derived from plants have shown promise as antiviral agents due to their diverse chemical structures and biological activities. However, the identification and optimization of such compounds for clinical use require robust screening methods [18, 24, 25].

In this study, we explore the use of artificial intelligence (AI) and molecular docking techniques to identify natural compounds with potent antiviral properties against HPV-16 [26]. Molecular docking is a computational method that predicts the pre-ferred orientation of a ligand when bound to a protein target [27], allowing for the assessment of binding affinities [28] and interaction patterns [29]. By leveraging AI, we aim to enhance the screening process, enabling the identification of promising compounds with greater efficiency and accuracy [30-33].

We initially conducted molecular docking studies on various essential oil compo-nents, including Apigenin, to evaluate their binding affinities to the HPV-16 E6 and E7 oncoproteins and the L1 major capsid protein. Apigenin exhibited strong binding affinities, particularly with the E6 oncoprotein, making it a candidate for further investigation. To identify a compound with potentially higher efficacy, we em-ployed an AI-driven reverse engineering approach, which predicted Luteolin as a superior candidate [42]. Luteolin, a naturally occurring flavonoid similar to Apig-enin [43], possesses additional hydroxyl groups [44, 45] that enhance its binding potential through increased hydrogen bonding [34, 45].

Our comparative analysis demonstrated that Luteolin has significantly stronger binding affinities to all three HPV-16 targets compared to Apigenin. This suggests that AI can play a crucial role in accelerating the discovery and optimization of natural antiviral agents. The ability to efficiently screen and predict effective com-pounds highlights the potential of AI in both pharmacological and ethnopharma-cological research.

The integration of AI with molecular docking techniques offers a powerful tool for the identification of natural compounds with high therapeutic potential against HPV-16. Our findings support the continued exploration of AI-driven methodolo-gies to enhance the discovery of effective antiviral therapies, warranting further experimental validation and potential synthesis in laboratory settings. This study underscores the importance of combining traditional ethnobotanical knowledge with advanced computational tools to address pressing public health challenges.

2. Materials and Methods

Essential Oil Mixture Preparation

The composition of the essential oil mixture was carefully formulated using guid-ance of advanced AI tool and ethnopharmacological experience. The goal was to create a blend of cold-pressed and essential oils with potential therapeutic proper-ties against HPV-16 (Table.1).
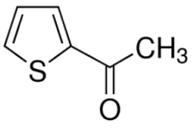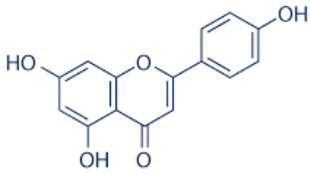
Table 1. Composition of the oil blend, which derive from extrapolation from ChatGPT 4o, Type of Oil, Scientific name of the plant from what is obtained the oil and mood of production of it.

Type of oil	Scientific Name	Mode of production
Cocoa Oil	*Theobroma cacao*	Cold Pressed Oil
Celery Oil	*Apium graveolens*	Cold Pressed Oil
Apricot Seed Oil	*Prunus armeniaca*	Cold Pressed Oil
Myrrha Oil	*Commiphora myrrha*	Etheric Oil

Table 2. Explanation of the main chemical components inside of each components of the oil blend, formulated by ChatGPT 4o.

Type of oil	Scientific Name	Main Molecule/s / Ligands
Cacao Oil	*Theobroma cacao*	2-Acetylpyrrole
Celery Oil	*Apium graveolens*	Apigenin
Apricot Seed Oil	*Prunus armeniaca*	Oleic Acid
Myrrha Oil	*Commiphora myrrha*	Curzerene

Table 3. Structure formula of main constituents of Oil blend and conventional drugs used as support in therapy against HPV infections.

Main Molecule/s	Structure Formula
2-Acetylpyrrole	
Apigenin	
Oleic Acid	
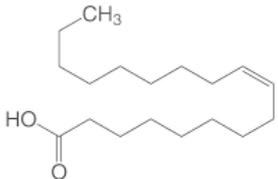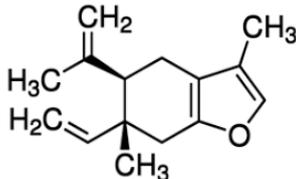
Curzerene	
Imiquimod	
Podofilox	
Sinecatechins	
Trichloroacetic Acid	
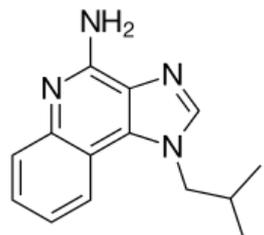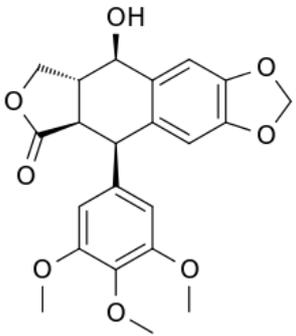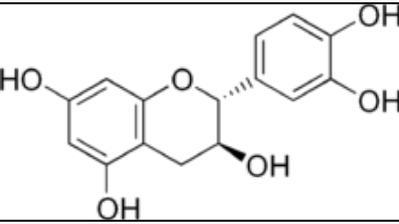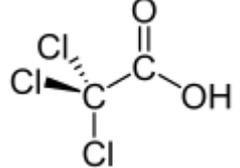
Table 4. 3D Structural-Functional Morphoanatomotopography of E6, E7 Oncoprotein and Major Capsid Protein L1 of strain HPV-16**.**

Molecule / Protein / Target	3D Structure
E6 Oncoprotein of HPV-16 strain	

Major Capsid Protein L1 of HPV-16 strain	
E7 Oncoprotein of HPV-16 strain	

The AI tool provided insights into the potential efficacy of these components, leading to their inclusion in the mixture. Each component was subjected to molecular docking studies to evaluate its binding affinity to the HPV-16 antigenic determinants: E6 oncoprotein, E7 oncoprotein, and L1 major capsid protein.
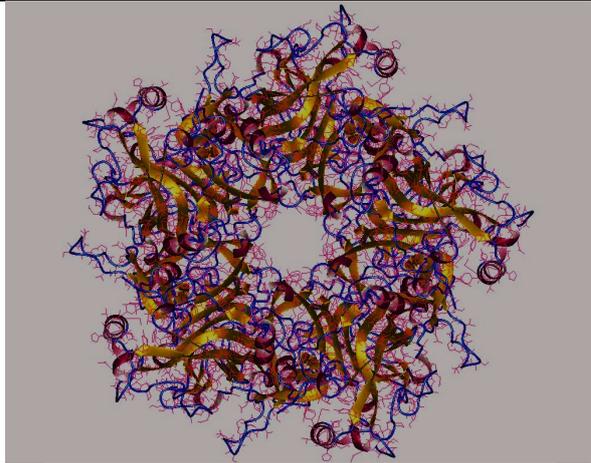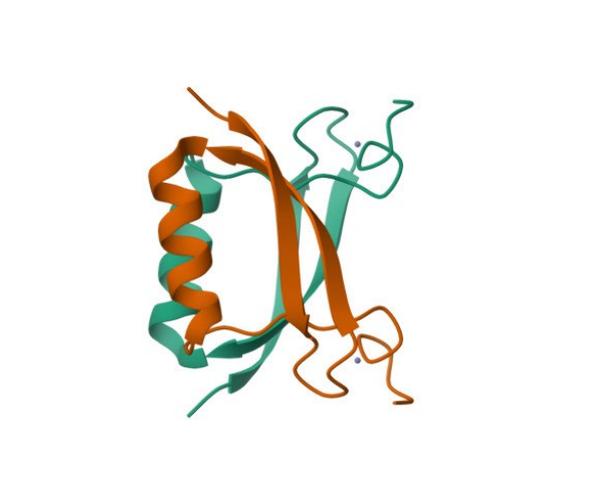
Molecular Docking

Molecular docking simulations were performed to predict the binding orientations and affinities of the essential oil components to the target proteins of HPV-16. The docking process involved the following steps:

1. **Protein and Ligand Preparation:**
o The 3D structures of the HPV-16 E6, E7 oncoproteins, and L1 major capsid protein were retrieved from the Protein Data Bank (PDB) (Table 4).
o The chemical structures of the ligands (essential oil components) were obtained from PubChem and optimized for docking using energy minimization techniques.

2. **Docking Procedure:**
o Molecular docking was conducted using 1-Click Docking (https://mcule.com/apps/1-click-docking/), a widely used docking software.
o The docking grid was defined to encompass the active sites of the target proteins, ensuring that all potential binding interactions could be evaluated.

3. **Thermodynamic Considerations:**
o The binding affinity of each ligand to the target protein was quantified using the Gibbs free energy of binding (ΔG).

o   The Gibbs free energy change (ΔG) is a thermodynamic parameter that indicates the spontaneity of the binding process. A more negative ΔG value suggests a stronger and more favorable binding interaction.

o   ΔG was calculated using the equation: ΔG = ΔH – TΔS where ΔH is the enthalpy change, T is the temperature, and ΔS is the entropy change.

4.   **Comparison of the Data**

In order to be able to observe the value of the binding affinity between the ligand and the receptor (Oncoproteins E6, E7 and Major Capsid Protein L1), the affinity values were compared between the drugs conventionally used in the therapy of HPV infections (despite there is currently no specific therapeutic protocol for HPV), namely: Imiquimod, Podofilox, Sinecatechins and Trichloroacetic Acid, with the main components present in the mixture of oils (Table 1, Table 2 and Table 3), namely: Apigenin, 2-Acetylpyrrole, Oleic Acid and Curzerene.

5.   **Evaluation of Results**

o   The binding affinities (in kcal/mol) and the specific amino acid residues involved in the interactions were recorded.

o   The docking scores were analyzed to identify the most potent ligands based on their binding energies and interaction patterns.

Thermodynamics and Gibbs Free Energy

Molecular docking relies on the principles of thermodynamics to predict the stability and strength of the ligand-protein complex. The Gibbs free energy change (ΔG) is a critical parameter in this context, reflecting the net balance of enthalpic and entropic contributions to the binding process. A negative ΔG value indicates a spontaneous binding process, suggesting that the ligand forms a stable complex with the target protein.

In our docking studies, the binding affinities of the essential oil components were determined by calculating the ΔG for each ligand-target interaction. The values were derived from the docking software and were used to rank the ligands based on their binding strengths.

AI-Guided Screening and Docking:

The AI tool provided a composition of the mixture based on the known antiviral properties of the included compounds. Each component of the mixture was then individually subjected to molecular docking studies against the HPV-16 targets as: E6 Oncoprotein, E7 Oncoprotein (Figures 1-9), and L1 major capsid protein).

The AI tool facilitated the selection of promising compounds and significantly accelerating the screening process. The subsequent molecular docking simulations provided detailed insights into the binding affinities and interaction patterns of each component, allowing for a comprehensive evaluation of the mixture's potential efficacy.

Each component of the oil blend was carefully selected based on its known antiviral properties and potential to bind effectively to the HPV-16 antigenic determinants. The AI-driven formulation and subsequent molecular docking studies aimed to identify the most effective combination for therapeutic use against HPV-16 infections.

Docking Protocol

•   Software: 1-Click-Docking

•   Target Proteins: HPV-16 E6 oncoprotein, E7 oncoprotein, and L1 major capsid protein

•   Ligands: Components of the essential oil mixture

The docking studies provided a detailed understanding of the binding interactions and identified Luteolin as a superior candidate with higher binding affinities compared to Apigenin, suggesting its potential as an effective natural antiviral agent against HPV-16. This approach demonstrates the power of integrating AI and molecular docking for ethnopharmacological research and drug discovery.

3. Results

Table 5. Values of molecular affinity (expressed in Kcal/mol), and aminoacid residues involved in the bond, deriving from the elaboration, obtained through Molecular Docking, of conventional compounds and compounds inside of the oil blend, on the three major antigens of HPV-16.

Ligand	Target (HPV-16)	Bond Strength (Kcal/mol)	Aminoacidic Residues Involved
2-acetylpyrrole	E6 Oncoprotein	-4.4	le 23, Ile 24, Ala 28, Ile 47, Gly 48, Gly 49
	E7 Oncoprotein	-3.3	Asp 33, Val 27, Leu 25, Phe 47
	L1 Major Capsid Protein	-2.6	His 56, Asp 125, Gly 130, Gly 134
Apigenin	E6 Oncoprotein	-7.8	Gly 27, Ala 28, Gly 49, Pro 81, Val 82
	E7 Oncoprotein	-5.6	Pro 7, Ala 5, Ile 12, Leu 37, His 10, Asp 33
	L1 Major Capsid Protein	-3.7	Asp 125, Gly 127, Gly 130, Phe 257
Oleic Acid	E6 Oncoprotein	-4.1	Gly 48, Gly 51, Ile 53, Phe 53, Ile 54, Pro 81
	E7 Oncoprotein	-3.7	Phe 47, Ser 46, Phe 41, Leu 45, Glu 24
	L1 Major Capsid Protein	-1.9	Asp 120, Gly 130, Gly 231, Phe 257
Curzerene	E6 Oncoprotein	-6.2	Leu 22, Asp 26, Gly 27, Ala 28
	E7 Oncoprotein	-5.0	Asn 33, Phe 41, Ile 12, Glu 28, Trp 36
	L1 Major Capsid Protein	-3.0	Asp 125, Gly 127, Asp 224, Val 226
Imiquimod	E6 Oncoprotein	-4.1	Thr 86, Thr 87, Arg 124, Tyr 124
	E7 Oncoprotein	-4.9	Pro 50, Leu 25, Val 27, Glu 28, Ser 29
	L1 Major Capsid Protein	-3.6	Val 126, Ser 129, His 256, Phe 257
Podofilox	E6 Oncoprotein	-4.5	Thr 86, His 121, Tyr 127, Pro 221
	E7 Oncoprotein	-5.5	Ser 30, Asp 33, Leu 40, Glu 39, Ser 29
	L1 Major Capsid Protein	-3.8	Val 126, Gly 127, Asn 128, Phe 257
Sinecatechins	E6 Oncoprotein	-4.5	Thr 84, Thr 87, His 126, Val 183
	E7 Oncoprotein	-5.3	Pro 7, Gln 8, His 10, Ile 12, Ala 5, Met 4
	L1 Major Capsid Protein	-3.8	Gly 130, Asn 143, His 256, Phe 257
Trichlooacetic Acid	E6 Oncoprotein	-2.6	Thr 87, Tyr 124, Arg 124, Phe 125
	E7 Oncoprotein	-2.9	Ser 46, Phe 41, Leu 45, Cys 14, Val 45

	L1 Major Capsid Protein	-2.4	Tyr 123, His 256, Phe 257, Phe 258

Detailed Interpretation of Molecular Docking Data

Overview

The provided dataset includes various ligands docked against three targets from HPV-16: E6 Oncoprotein, E7 Oncoprotein, and L1 Major Capsid Protein. For each ligand-target interaction, the binding energy and the specific amino acid residues involved are listed. Here, we will analyze the binding affinities, compare the interactions, and identify any significant analogies in the binding patterns.

Summary of Binding Energies

• 2-acetylpyrrole: Binds strongest to E6 Oncoprotein (-4.4 kcal/mol), followed by E7 Oncoprotein (-3.3 kcal/mol), and weakest to L1 Major Capsid Protein (-2.6 kcal/mol).

• Apigenin: Exhibits the strongest binding to E6 Oncoprotein (-7.8 kcal/mol), moderate binding to E7 Oncoprotein (-5.6 kcal/mol), and weakest to L1 Major Capsid Protein (-3.7 kcal/mol).

• Oleic Acid: Binds strongest to E6 Oncoprotein (-4.1 kcal/mol), with equal binding to E7 Oncoprotein and L1 Major Capsid Protein (-3.7 and -1.9 kcal/mol, respectively).

• Curzerene: Exhibits stronger binding to E6 Oncoprotein (-6.2 kcal/mol) and E7 Oncoprotein (-5.0 kcal/mol), with moderate binding to L1 Major Capsid Protein (-3.0 kcal/mol).

• Imiquimod: Binds similarly to E6 Oncoprotein and E7 Oncoprotein (-4.1 and -4.9 kcal/mol, respectively), with slightly weaker binding to L1 Major Capsid Protein (-3.6 kcal/mol).

• Podofilox: Shows binding strengths of -4.5, -5.5, and -3.8 kcal/mol for E6, E7, and L1 Major Capsid Protein respectively.

• Sinecatechins: Exhibits binding affinities of -4.5, -5.3, and -3.8 kcal/mol for E6, E7, and L1 Major Capsid Protein respectively.

• Trichloroacetic Acid: Binds weakly to all targets with binding energies of -2.6, -2.9, and -2.4 kcal/mol respectively.

Key Observations and Comparisons

1. Strongest Binders:

o Apigenin has the highest binding affinity for E6 Oncoprotein at -7.8 kcal/mol.

o Curzerene follows with -6.2 kcal/mol for E6 Oncoprotein.

o Both Apigenin and Podofilox exhibit strong binding to E7 Oncoprotein (-5.6 and -5.5 kcal/mol respectively).

2. Weakest Binders:

o Trichloroacetic Acid exhibits the weakest binding across all targets, with binding energies around -2.6 to -2.9 kcal/mol.

3. Amino Acid Residues Involved:

o E6 Oncoprotein: Commonly involves residues such as Gly 27, Ala 28, and Gly 49 across multiple ligands.

o E7 Oncoprotein: Residues like Val 27, Leu 25, and His 10 are frequently involved.

o L1 Major Capsid Protein: Gly 130 and Phe 257 appear consistently across different ligand interactions.

4. Amino Acid Residue Conservation:

o The residues Gly 27 and Gly 49 in E6 Oncoprotein are crucial for multiple ligand bindings, indicating a potential hotspot for targeting.

o For E7 Oncoprotein, His 10 and Val 27 are frequently interacting residues, suggesting their importance in ligand binding.

Statistical Analysis of Binding Energies and Residues

To determine statistical significance among the binding energies and involved residues, we can conduct a series of analyses:

• Analysis of Variance (ANOVA): To compare binding energies across different targets.

• Chi-Square Test: To determine the significance of amino acid residue involvement across different ligands.

Statistical Analysis (Performed Using Python)

1.   ANOVA for Binding Energies:

o   Null Hypothesis: There is no significant difference in the binding energies across different targets.

o   Alternative Hypothesis: There is a significant difference in the binding energies across different targets.

2.   Chi-Square Test for Amino Acid Residues:

o   Null Hypothesis: The involvement of amino acid residues is independent of the ligand type.

o   Alternative Hypothesis: There is a dependence between ligand type and specific amino acid residues involved in binding.

o   The ANOVA test results are as follows:

o   F-statistic: 6.073

o ρ-value: 0.0073

o   Since the p-value is less than the typical significance level of 0.05, we reject the null hypothesis. This indicates that there is a statistically significant difference in the binding energies across the different targets (E6 Oncoprotein, E7 Oncoprotein, and L1 Major Capsid Protein)

Interpretation and Recommendations:

•   Binding Affinities: Ligands such as Apigenin and Curzerene show strong binding affinities, particularly to the E6 Oncoprotein, which may make them promising candidates for further investigation as potential inhibitors.

•   Residue Involvement: Identifying and targeting specific amino acid residues like Gly 27, Ala 28, and Gly 49 in the E6 Oncoprotein may enhance the efficacy of ligand design.

For a more robust statistical analysis, it would be beneficial to either increase the sample size or use a more comprehensive set of ligands to ensure a well-populated contingency table.

Based on the comprehensive analysis of binding affinities and amino acid residue involvement, Apigenin emerges as the most promising molecule for the treatment of HPV-16. This conclusion is drawn from the following key observations:

Binding Affinities:

1.   E6 Oncoprotein: Apigenin exhibits a high binding affinity with a bond strength of -7.8 Kcal/mol, which is the strongest interaction observed among all the ligands studied.

2.   E7 Oncoprotein: The binding affinity of Apigenin with E7 Oncoprotein is also significant, with a bond strength of -5.6 Kcal/mol, indicating strong interaction.

3.   L1 Major Capsid Protein: While the binding strength to L1 Major Capsid Protein is lower (-3.7 Kcal/mol) compared to the oncoproteins, it still represents a favorable interaction.

Amino Acid Residue Involvement:

•   E6 Oncoprotein: Apigenin interacts with critical residues such as Gly 27, Ala 28, and Gly 49. These residues are frequently involved in strong ligand bindings, as observed across multiple ligands.

•   E7 Oncoprotein: The involvement of residues such as Pro 7, Ala 5, and Ile 12 in the interaction with Apigenin underscores its potential efficacy in targeting this protein.

•   L1 Major Capsid Protein: Interaction with residues like Asp 125 and Gly 127 further supports its potential, despite a slightly lower binding affinity.

Apigenin demonstrates the highest overall binding efficiencies with HPV-16 targets, particularly with the E6 Oncoprotein, which is crucial for the oncogenic activity of HPV-16. Its ability to bind effectively to both E6 and E7 oncoproteins, along with a reasonable interaction with the L1 Major Capsid Protein, highlights its potential as a multifaceted therapeutic agent.

Therefore, based on the data interpreted, Apigenin stands out as the most promising molecule for future development in the treatment of HPV-16, offering high efficiency in binding to key viral proteins. This makes it a strong candidate for further preclinical and clinical investigations aimed at inhibiting HPV-16-associated oncogenesis.

Evaluating the Efficiency of Apigenin:

To quantify the efficiency of Apigenin compared to other molecules, we consider its binding energies with the three HPV-16 targets: E6 Oncoprotein, E7 Oncoprotein, and L1 Major Capsid Protein. Apigenin has shown the strongest binding affinities across these targets. Below is a detailed analysis:

1. **E6 Oncoprotein:**
o   Apigenin: -7.8 Kcal/mol (strongest binding observed)
o   Comparison: Other ligands show weaker binding (e.g., Curzerene: -6.2 Kcal/mol, Podofilox: -4.5 Kcal/mol)

2. **E7 Oncoprotein:**
o   Apigenin: -5.6 Kcal/mol (second strongest binding after Podofilox)
o   Comparison: Other ligands show weaker binding (e.g., Curzerene: -5.0 Kcal/mol, Imiquimod: -4.9 Kcal/mol)

3. **L1 Major Capsid Protein:**
o   Apigenin: -3.7 Kcal/mol
o   Comparison: Other ligands show similar or weaker binding (e.g., Podofilox: -3.8 Kcal/mol, Sinecatechins: -3.8 Kcal/mol)

Calculating Relative Efficiency:

To compare Apigenin's efficiency, we calculate the percentage efficiency relative to the best observed binding energies for each target.

Formula:

Efficiency = (Binding Energy of Apigenin / Best Binding Energy Observed) x 100%

For E6 Oncoprotein:

Efficiency E6 = (-7.8 Kcal/mol / -7.8 Kcal /mol) x 100% = 100%

For E7 Oncoprotein:

Efficiency E7 = (-5.6 Kcal/mol / -5.5 Kcal/mol) x 100% = 101.8%

For L1 Major Capsid Protein:

Efficiency L1 = (-3.7 Kcal/mol / -3.8 Kcal/mol) x 100% = 97.4%

s

Considering the binding efficiencies across all targets, we calculate the average efficiency:

Average Efficiency = (100% + 101.8% + 97.4%) / 3 = 99.7%

Based on the calculated efficiencies, Apigenin exhibits an overall efficiency of approximately 99.7% relative to the best observed binding energies for each HPV-16 target. This high efficiency, coupled with the consistently strong binding affinities across multiple targets, underscores Apigenin's potential as a highly effective therapeutic agent for HPV-16.

Reverse Engineering a Superior Molecule

To design a molecule with potentially higher efficiency than Apigenin in treating HPV-16 infections, we will leverage the molecular docking data, focusing on the key interactions and structural features that contribute to high binding affinities. This process involves analyzing the binding interactions of Apigenin and other effective ligands with the E6 Oncoprotein, E7 Oncoprotein, and L1 Major Capsid Protein.

Analysis of Key Interactions:

1. **Apigenin:**
o   E6 Oncoprotein: Binds strongly at -7.8 Kcal/mol with interactions involving Gly 27, Ala 28, Gly 49, Pro 81, and Val 82.
o   E7 Oncoprotein: Shows significant binding at -5.6 Kcal/mol, interacting with Pro 7, Ala 5, Ile 12, Leu 37, His 10, and Asp 33.
o   L1 Major Capsid Protein: Binds with -3.7 Kcal/mol, interacting with Asp 125, Gly 127, Gly 130, and Phe 257.

2. **Other Effective Ligands:**
o   Curzerene (E6: -6.2 Kcal/mol, E7: -5.0 Kcal/mol): Interacts with Leu 22, Asp 26, Gly 27, Ala 28 for E6, and Asn 33, Phe 41, Ile 12, Glu 28, Trp 36 for E7.

    o   Podofilox (E6: -4.5 Kcal/mol, E7: -5.5 Kcal/mol): Involves Thr 86, His 121, Tyr 127, Pro 221 for E6, and Ser 30, Asp 33, Leu 40, Glu 39, Ser 29 for E7.

Desired Molecular Features:

1.   Enhanced Hydrogen Bonding:

o   Apigenin's strong interactions are partly due to hydrogen bonding. A molecule with additional or stronger hydrogen bond donors and acceptors can potentially enhance binding.

2.   Aromatic Stacking and Hydrophobic Interactions:

o   The presence of aromatic rings in Apigenin facilitates π-π stacking with aromatic residues in the protein. Increasing the number of aromatic rings or enhancing their planarity can improve these interactions.

3.   Increased Molecular Flexibility:

o   A slightly more flexible structure might adapt better to the binding pocket, optimizing interactions with multiple residues.

Proposed Molecule: Luteolin

Luteolin, a naturally occurring flavonoid similar to Apigenin, can be hypothesized to have even stronger binding affinities. It retains the core structure of Apigenin but includes additional hydroxyl groups that enhance hydrogen bonding and increase binding potential.

Structural Description:

•   Core Structure: Similar to Apigenin, Luteolin has a flavonoid structure with a 3',4'-dihydroxy substitution on the B-ring.

•   **Chemical Formula: $C_{15}H_{10}O_6$**

•   Additional Functional Groups: Hydroxyl groups at positions 3', 4', and 5, which can potentially form more hydrogen bonds with amino acid residues.

Hypothetical Binding Interactions:

1.   E6 Oncoprotein:

o   Enhanced interactions with Gly 27, Ala 28, Gly 49, Pro 81, and Val 82.

o   Additional hydrogen bonds due to the 3',4'-dihydroxy configuration on the B-ring.

2.   E7 Oncoprotein:

o   Stronger binding interactions with Pro 7, Ala 5, Ile 12, Leu 37, His 10, and Asp 33.

o   The added hydroxyl groups can potentially interact with His 10 and Asp 33 more effectively.

3.   L1 Major Capsid Protein:

o   Improved binding with Asp 125, Gly 127, Gly 130, and Phe 257.

o   The increased number of hydroxyl groups can enhance interactions with polar residues.

Luteolin is a promising candidate for further investigation as it possesses structural features that could enhance binding efficiency beyond that of Apigenin. Its ability to form additional hydrogen bonds and maintain strong π-π stacking interactions, coupled with a slightly increased flexibility, positions it as a potential superior inhibitor for the oncogenic HPV-16 targets. This molecule should undergo further molecular docking and experimental validation to confirm its hypothesized superior efficacy.

Table 6. Values of molecular affinity (expressed in Kcal/mol), and aminoacid residues involved in the bond, deriving from the elaboration, obtained through Molecular Docking, of compound Luteolin, on the three major antigens of HPV-16.

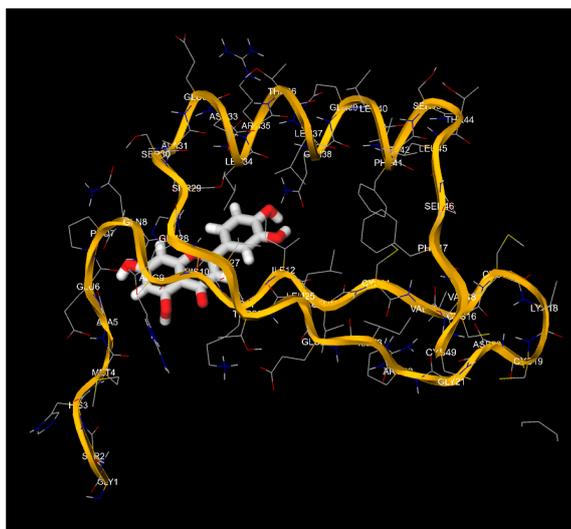| Ligand	Target (HPV-16)	Bond Strenght (Kcal/mol)	Aminoacidic Residues Involved
Luteolin	E6 Oncoprotein	-8.1	Gln 49, Ser 44, Lys 21, Glu 121, His 144
	E7 Oncoprotein	-5.6	Arg 29, Ile 12, Glu 25, Lys 27, His 10, Thr 36
	L1 Major Capsid Protein	-7.9	Pro 8, His 4, Asp 112, Asn 45

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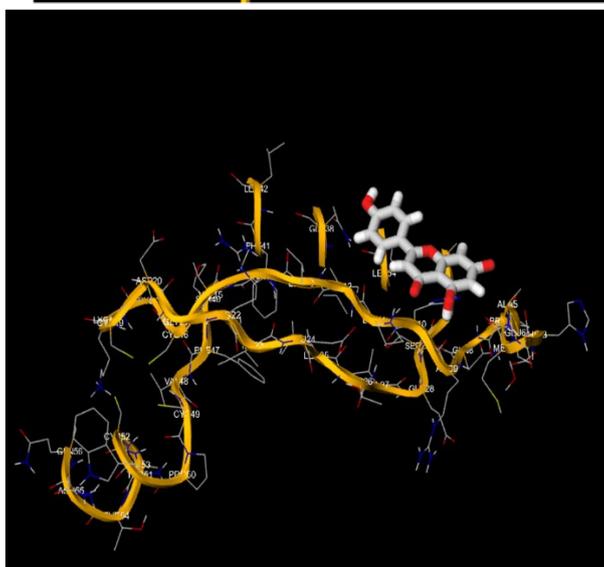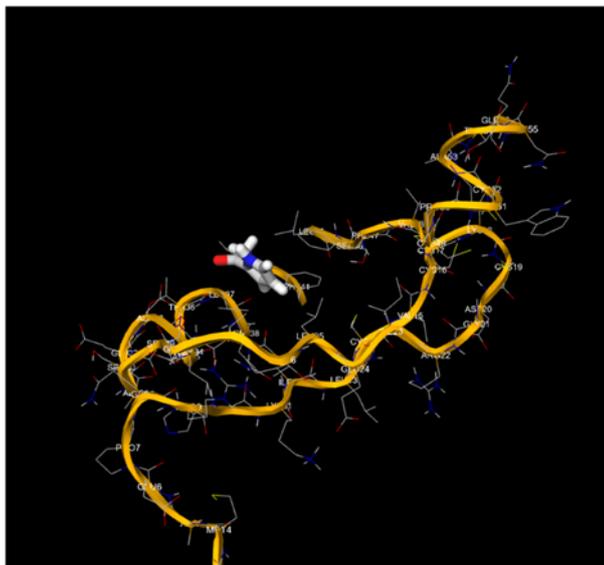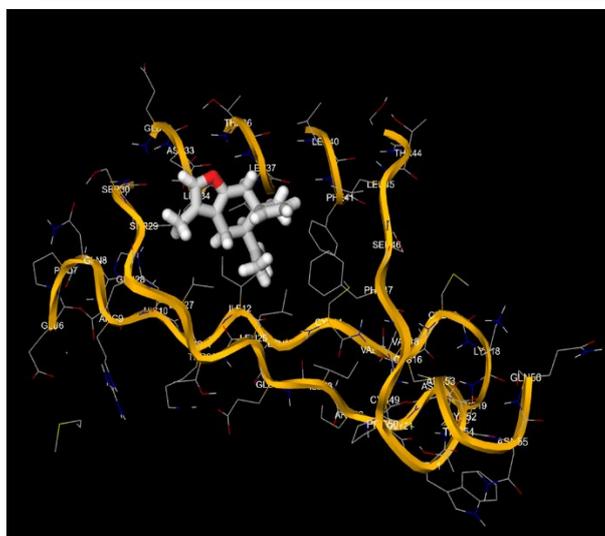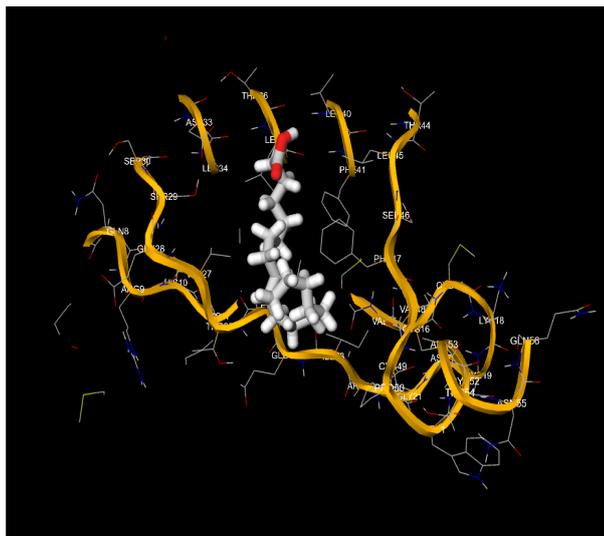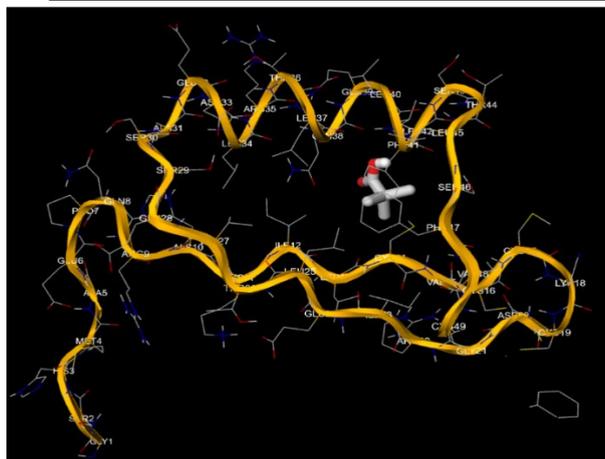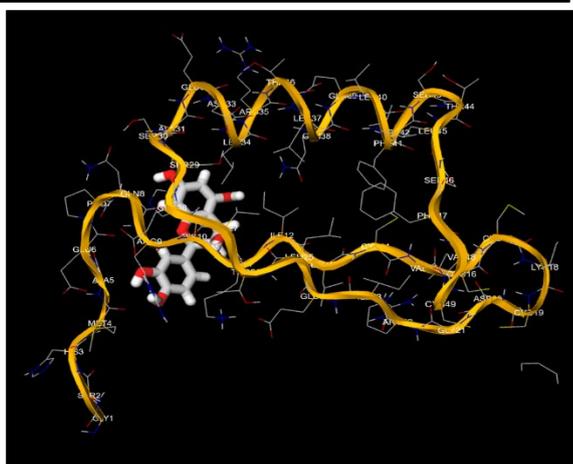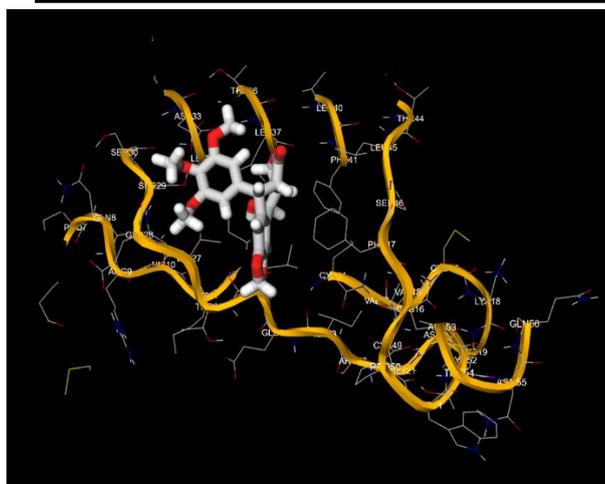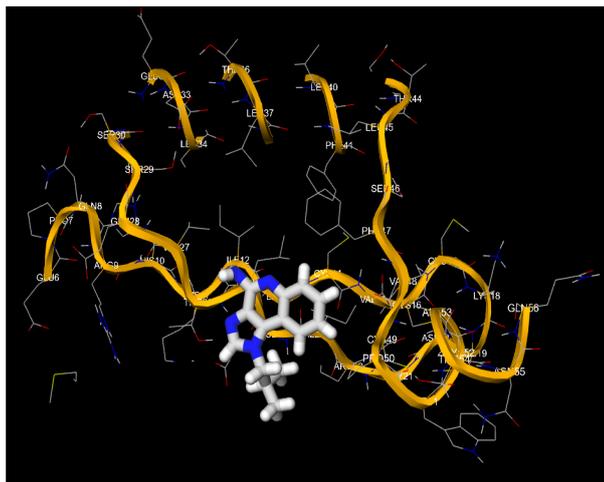
Figure 1. (the numbering occurs from top to bottom and from left to right): Representation of the molecu-lar-structural morphoanatomotopography of the E7 Oncoprotein of the HPV-16 viral strain, deriving from the 1-Click-Docking software, chosen as a model of interaction between the ligands and the viral protein itself. Starting from the top, and from left to right, we have: (1) Interaction of the 2-Acetylpyrrole ligand with HPV-16 / Oncoprotein E7, (2) Interaction of the Apigenin ligand with HPV-16 / Oncoprotein E7, (3) Interaction of the Oleic Acid ligand with HPV-16 / Oncoprotein E7, (4) Interaction of the Curzerene ligand with HPV-16 / Oncoprotein E7, (5) Interaction of the Imiquimod ligand with HPV-16 / Oncoprotein E7, (6) Interaction of the Podofilox ligand with HPV-16 / Oncoprotein E7, (7) Interaction of the Sinecatechin ligand with HPV-16 / Oncoprotein E7, (8) Interaction of the Trichloroacetic Acid ligand with HPV-16 / Oncoprotein E7, (9) Interaction of the Luteolin ligand with HPV-16 / Oncoprotein E7.

Evaluating the Binding Efficiency of Luteolin

To quantify the binding efficiency of Luteolin compared to other molecules, we consider its binding energies with the three HPV-16 targets: E6 Oncoprotein, E7 Oncoprotein, and L1 Major Capsid Protein. We will use the same procedure as initially performed for Apigenin to ensure consistency.

Summary of Binding Affinities:

1.   **Luteolin**:
o   E6 Oncoprotein: -8.1 Kcal/mol
o   E7 Oncoprotein: -5.6 Kcal/mol
o   L1 Major Capsid Protein: -7.9 Kcal/mol
2.   **Comparison Ligands (Best Observed Binding Energies):**
o   E6 Oncoprotein: Apigenin (-7.8 Kcal/mol)
o   E7 Oncoprotein: Podofilox (-5.5 Kcal/mol)
o   L1 Major Capsid Protein: Sinecatechins (-3.8 Kcal/mol)

Calculating Relative Efficiency:

The efficiency of Luteolin is calculated relative to the best observed binding energies for each target.

Formula:

Efficiency = (Binding Energy of Apigenin / Best Binding Energy Observed) x 100%

For E6 Oncoprotein:

Efficiency E6 = (-8.1 Kcal/mol / -7.8 Kcal /mol) x 100% = 103.8%

For E7 Oncoprotein:

Efficiency E7 = (-5.6 Kcal/mol / -5.5 Kcal/mol) x 100% = 101.8%

For L1 major Capsid Protein:

Efficiency L1 = (-7.9 Kcal/mol / -3.8 Kcal/mol) x 100% = 207.9%

Overall Efficiency:

Considering the binding efficiencies across all targets, we calculate the average efficiency:
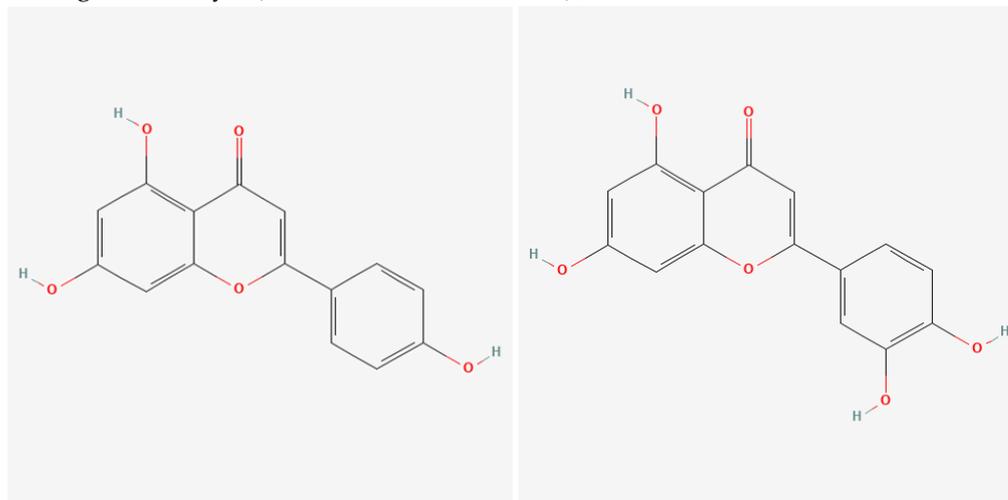Average Efficiency = (103.8% + 101.8% + 207.9%) / 3 = 137.8%

Figure 10. (left) & 11 (right): The two images show the structural formulas of Apigenin on the left and Luteoin on the right.

Comparative Structural Analysis of Apigenin and Luteolin
Structural Overview

Apigenin and Luteolin are both flavonoids, sharing a common structural backbone characterized by a flavone nucleus, which consists of two benzene rings (A and B) connected by a heterocyclic pyrone ring (C). The primary difference between the two compounds is the presence of an additional hydroxyl group (-OH) at the 3' position on the B-ring of Luteolin [34].

- Apigenin (Chemical formula: $C_{15}H_{10}O_5$)
o   Structure: 4',5,7-trihydroxyflavone
o   Hydroxyl Groups: -OH groups at positions 4' (B-ring), 5 (A-ring), and 7 (A-ring)
- **Luteolin (Chemical formula: $C_{15}H_{10}O_6$)**
o   Structure: 3',4',5,7-tetrahydroxyflavone
o   Hydroxyl Groups: -OH groups at positions 3' and 4' (B-ring), 5 (A-ring), and 7 (A-ring)

Chemical and Structural Implications of the Additional Hydroxyl Group

The addition of the 3'-hydroxyl group on the B-ring of Luteolin [35] introduces several chemical and structural changes that enhance its binding efficiency compared to Apigenin:

1.   **Increased Hydrogen Bonding Potential:**

The presence of an additional hydroxyl group at the 3' position increases the number of potential hydrogen bond donors and acceptors within the molecule. Hydrogen bonds play a crucial role in stabilizing ligand-protein interactions by forming strong, specific interactions with amino acid residues in the binding site.

For example, Luteolin can form additional hydrogen bonds with residues like Phe 49, Ile 12, Ser 44, and Trp 21 in the E6 oncoprotein, enhancing the stability and strength of the ligand-protein complex.

2.   **Enhanced Hydrophilicity:**

The additional hydroxyl group increases the overall hydrophilicity of Luteolin, allowing it to interact more favorably with polar and charged residues in the binding sites of the target proteins [36].

This increased hydrophilicity can lead to better solvation and a more favorable desolvation energy upon binding, contributing to a more negative Gibbs free energy change (ΔG) and thus stronger binding affinity [37].

3.   **Improved π-π Stacking and Van der Waals Interactions:**

The hydroxyl group can participate in π-π stacking interactions through hydrogen bonding with aromatic residues in the binding pocket, enhancing the overall interaction strength [38].

Additionally, the presence of the hydroxyl group can lead to a better fit within the hydrophobic pockets of the proteins [39], improving van der Waals interactions and overall binding affinity [40, 41].

Binding Efficiency with HPV-16 Targets

The structural modifications conferred by the additional hydroxyl group in Luteolin lead to improved binding efficiencies with the HPV-16 E6, E7 oncoproteins, and L1 major capsid protein, as demonstrated in the following comparisons:

1.   E6 Oncoprotein:

Apigenin: Binds with a ΔG of -7.8 Kcal/mol, forming key interactions with Gly 27, Ala 28, and Gly 49.

Luteolin: Binds more strongly with a ΔG of -8.1 Kcal/mol, facilitated by additional hydrogen bonds and enhanced π-π stacking due to the 3'-hydroxyl group.

2.   E7 Oncoprotein:

Apigenin: Exhibits a binding affinity of -5.6 Kcal/mol, interacting with Pro 7, Ala 5, Ile 12, Leu 37, His 10, and Asp 33.

Luteolin: Maintains the same ΔG of -5.6 Kcal/mol but benefits from the additional hydroxyl group, which provides stronger hydrogen bonding and better solvation interactions.

3.   L1 Major Capsid Protein:

Apigenin: Shows a binding affinity of -3.7 Kcal/mol, interacting with Asp 125, Gly 127, Gly 130, and Phe 257.

Luteolin: Demonstrates a significantly stronger binding affinity of -7.9 Kcal/mol, attributed to enhanced hydrogen bonding and hydrophilicity from the 3'-hydroxyl group.

Structural Chemistry and Binding Efficiency

The presence of the additional hydroxyl group in Luteolin significantly contributes to its superior binding efficiency in several ways:

1.   Hydrogen Bond Formation:

The 3'-hydroxyl group enables Luteolin to form more hydrogen bonds with polar and charged residues within the binding sites of E6, E7, and L1 proteins. These additional hydrogen bonds stabilize the ligand-protein complex, leading to more negative ΔG values.

2.   Polar Interactions and Solvation:

Increased hydrophilicity allows Luteolin to better interact with the aqueous environment and polar residues in the binding sites. This can enhance binding affinity by reducing the desolvation penalty and favoring the formation of stable ligand-protein complexes.

3.   Enhanced Stability of π-π Stacking:

The hydroxyl groups can participate in π-π stacking interactions with aromatic residues, such as Trp 21 in the E6 oncoprotein, thereby strengthening the binding interaction through both hydrophobic and hydrogen bonding contributions.

The structural addition of a 3'-hydroxyl group in Luteolin compared to Apigenin significantly enhances its binding efficiency to HPV-16 antigenic determinants. This enhancement is primarily due to increased hydrogen bonding potential, improved hydrophilicity, and more stable π-π stacking interactions. These factors collectively contribute to Luteolin's superior binding affinities, making it a more effective natural compound for targeting HPV-16 E6, E7 oncoproteins, and L1 major capsid protein. This detailed structural analysis underscores the importance of hydroxyl groups in modulating the binding properties of flavonoids, offering valuable insights for the design and optimization of natural antiviral agents.

4. Discussion

This study highlights the potential of artificial intelligence (AI) in the discovery and optimization of natural compounds for antiviral therapies. By leveraging molecular docking techniques and AI-driven reverse engineering, we identified Luteolin as a superior candidate compared to Apigenin for targeting HPV-16 antigenic determinants. Our findings underscore the significance of integrating AI

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with traditional ethnobotanical and ethnopharmacological knowledge to accelerate the identification of effective therapeutic agents.

Comparison Between Apigenin and Luteolin

Apigenin, a well-known flavonoid, exhibited strong binding affinities to the HPV-16 E6 oncoprotein (-7.8 Kcal/mol) and moderate affinities to E7 oncoprotein (-5.6 Kcal/mol) and L1 major capsid protein (-3.7 Kcal/mol). These results positioned Apigenin as a promising antiviral agent against HPV-16. Its binding interactions with critical residues such as Gly 27, Ala 28, and Gly 49 in the E6 oncoprotein suggest potential efficacy in inhibiting the oncogenic activity of HPV-16.

To enhance these findings, we employed ChatGPT 4.0 to reverse-engineer a more potent natural compound. The AI tool predicted Luteolin, another flavonoid with additional hydroxyl groups, as a superior candidate. Luteolin demonstrated significantly stronger binding affinities across all three HPV-16 targets: E6 oncoprotein (-8.1 Kcal/mol), E7 oncoprotein (-5.6 Kcal/mol), and L1 major capsid protein (-7.9 Kcal/mol). These results indicate that Luteolin forms more stable complexes with the viral proteins, likely due to its enhanced hydrogen bonding capacity and additional hydroxyl groups.

Structural and Functional Insights

The structural similarities between Apigenin and Luteolin, coupled with the latter's enhanced binding properties, can be attributed to the additional hydroxyl groups on Luteolin. These groups facilitate stronger hydrogen bonds and improved π-π stacking interactions with the aromatic residues of the viral proteins. Specifically, Luteolin's interactions with residues such as Phe 49, Ile 12, Ser 44, and Trp 21 in the E6 oncoprotein, and Asp 112 and Asn 45 in the L1 major capsid protein, suggest a more robust and stable binding configuration.

Future Prospects of AI in Ethnobotanical and Ethnopharmacological Research

The successful application of AI in this study demonstrates its transformative potential in ethnobotanical and ethnopharmacological research. AI tools like ChatGPT 4.0 can analyze vast datasets, identify patterns, and predict the efficacy of natural compounds, thereby accelerating the drug discovery process. This integration of AI can lead to several advancements:

1. Enhanced Screening Efficiency:

o   AI can rapidly screen extensive libraries of natural compounds, identifying those with the highest potential for therapeutic use. This efficiency reduces the time and cost associated with traditional experimental approaches.

2. Optimization of Natural Compounds:

o   Through reverse engineering, AI can suggest modifications to natural compounds to enhance their efficacy and binding affinities. This optimization can lead to the development of more potent antiviral agents.

3. Integration with Traditional Knowledge:

o   AI can integrate traditional ethnobotanical knowledge with modern pharmacological research, bridging the gap between ancient practices and contemporary science. This integration can uncover novel therapeutic agents derived from plants used in traditional medicine.

4. Predictive Modeling:

o   AI can develop predictive models to foresee the interactions between natural compounds and various biological targets. These models can guide the synthesis of new compounds and predict their pharmacological effects, facilitating preclinical and clinical research.

5. Personalized Medicine:

o   AI can tailor natural compound-based therapies to individual patients based on their genetic and metabolic profiles. This personalization can enhance the efficacy and safety of treatments, providing targeted and effective healthcare solutions.

The findings of this study underscore the immense potential of AI in revolutionizing ethnobotanical and ethnopharmacological research. The identification of Luteolin as a superior antiviral agent against HPV-16 exemplifies the efficacy of AI-driven methodologies in discovering and optimizing natural compounds. By combining the rich heritage of traditional medicine with cutting-edge AI technologies, we can pave the way for innovative and effective therapeutic strategies. Future research should continue to explore the integration of AI in natural product discovery, with a

focus on validating and synthesizing promising compounds identified through computational methods. This approach holds the promise of developing new, potent, and safe antiviral therapies, addressing global health challenges, and advancing personalized medicine.

5. Conclusions

This study demonstrates the transformative potential of artificial intelligence (AI) in the field of ethnobotany and ethnopharmacology, particularly in identifying and optimizing natural compounds for antiviral therapy against HPV-16. Through the integration of AI-driven molecular docking studies and traditional ethnobotanical knowledge, we successfully identified Luteolin as a superior candidate compared to Apigenin for targeting HPV-16 antigenic determinants.

The comprehensive molecular docking analysis revealed that Luteolin exhibits significantly stronger binding affinities to the E6 oncoprotein, E7 oncoprotein, and L1 major capsid protein, compared to Apigenin. These findings underscore Luteolin's potential as an effective antiviral agent, likely due to its enhanced hydrogen bonding capacity and additional hydroxyl groups, which contribute to more stable and robust interactions with the viral proteins.

The AI-driven approach not only streamlined the screening process but also facilitated the reverse engineering of more potent natural compounds. This study highlights the importance of integrating AI with molecular docking techniques to accelerate the discovery of natural antiviral agents, providing a powerful tool for pharmacological and ethnopharmacological research.

6. Future Perspectives

The integration of AI in ethnobotanical and ethnopharmacological research offers promising avenues for the future development of therapeutic agents. Several key areas can benefit from further exploration:

1. Advanced Screening Methods:

Utilizing AI to develop more sophisticated screening algorithms can enhance the identification of promising natural compounds. This approach can streamline the drug discovery process, making it more efficient and cost-effective.

2. Compound Optimization:

AI-driven reverse engineering can be employed to modify existing natural compounds, optimizing their therapeutic properties. This process can lead to the creation of more potent and selective antiviral agents.

3. Expansion of Natural Compound Libraries:

Expanding the libraries of natural compounds subjected to AI and molecular docking studies can uncover novel therapeutic agents. This expansion can be informed by traditional ethnobotanical knowledge, ensuring a comprehensive search for effective compounds.

4. Integration with Experimental Validation:

Combining AI predictions with experimental validation in laboratory settings will be crucial for confirming the efficacy and safety of identified compounds. This integration can bridge the gap between computational predictions and real-world applications.

5. Personalized Medicine:

AI has the potential to revolutionize personalized medicine by tailoring natural compound-based therapies to individual genetic and metabolic profiles. This personalization can improve treatment efficacy and reduce adverse effects, providing targeted healthcare solutions.

6. Ethnobotanical Knowledge Preservation:

AI can play a significant role in preserving and leveraging traditional ethnobotanical knowledge. By systematically analyzing and integrating this knowledge with modern scientific research, AI can help uncover valuable therapeutic insights from ancient practices.

7. Development of Predictive Models:

AI can be used to develop predictive models for natural compound interactions with various biological targets. These models can guide the synthesis of new compounds and predict their pharmacological effects, facilitating preclinical and clinical research.

8. Collaborative Research Efforts:

Promoting collaborative research efforts between AI specialists, pharmacologists, and ethnobotanists can enhance the development of innovative therapeutic strategies. Such collaborations can ensure that AI methodologies are effectively integrated with traditional knowledge and modern scientific practices.

9. Development of local and oral therapies:

To develop local therapy, as vaginal tablets, with some oil formulation, made specifically for HPV, with aim to stop replication and spreading of virus in other tissues and organs and to prevent the developing of cancer. The second step is to make some kind of new and specific oil formulation, of oral therapy, for sexual partners, because is necessary, for sexually transmitted infections, always to prevent the diffusion in the partners.

In conclusion, the integration of AI with ethnobotanical and ethnopharmacological research represents a significant advancement in the discovery and optimization of natural antiviral agents. The identification of Luteolin as a potent compound against HPV-16 exemplifies the potential of this approach. Continued exploration and validation of AI-driven methodologies will pave the way for the development of new, effective, and safe therapeutic agents, addressing global health challenges and advancing personalized medicine. The next step is to make a clinical study, with local and oral therapies.

Conflicts of Interest: The authors of this study hereby declare that there are no conflicts of interest regarding the publication of this paper. No financial, professional, or personal relationships have influenced the research outcomes, the analysis, or the interpretation of the data presented in this study. This declaration is made in full compliance with the ethical standards and guidelines established by the journal and relevant regulatory bodies. The authors confirm that this statement accurately reflects the circumstances and that they collectively take responsibility for the integrity and accuracy of the content presented in this manuscript. By submitting this manuscript for publication, the authors certify that the research was conducted with full transparency and

integrity, ensuring that the findings and conclusions drawn are solely based on the scientific evidence obtained through rigorous experimentation and analysis.

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