Original Article

Sirih (Piper betle) folium as new candidate for anti-herpes virus: In-silico study

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ABSTRACT

The benefits of betle leaf to treat diseases such as bad breath, cuts, inflammation, coughs, colds, digestive disorders have traditionally been known for a long time. Most of the health-related benefits of betle leaf come from the bioactive phenolic compounds found in the betle leaf, and polyphenol compounds have the potential as anti-virus. Herpes simplex virus (HSV) is a highly contagious pathogen that causes recurrent lesions. The process of herpes infection begins when the virion binds to the heparan sulfate portion present on the host cell surface. HSV is a highly contagious pathogen that causes recurrent lesions. The entry of the virus into cells by triggering the membrane lining or a combination of cells causes herpes infection. This study aims to determine the anti-herpes bioactive compounds contained in betle leaf against the HSV-1 receptor. This research used Discovery Studio for docking and ADMET methods. The computational results found that Guineensine was a bioactive compound that had activity against the HSV-1. It had a binding affinity of -9.09 Kcal/Mol and an inhibition constant of 0.217 µM that's better than acyclovir as a comparison drug. Despite having good ADME properties, this compound could not be used for oral treatment because it was mutagenic.

Keywords: Piper betle, Anti herpes, in-silico, Herpes simplex virus (HSV), ADMET

Introduction

Betle leaf (Piper betle L.) is a dark green heart-shaped leaf that grows vines and is a horticultural plant. Traditionally, herbal medicine has existed in Asian countries for a long time [1]. The benefits of betle leaf are usually to treat several diseases such as bad breath, cuts, wounds, inflammation [2], cough, cold, indigestion, etc. Various bioactive compounds, including polyphenols, terpenes, etc., have been identified from extracts and betle leaf essential oil (EO). Most of the health-related benefits of the betle leaf come from the bioactive phenolic compounds found in the betle leaf [2]. Piper betle has excellent potential for designing new drugs because of the compounds in the piper betle that exhibit high inhibition against opportunistic C.albicans infection [3].

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According to reports, every year, there are as many as 1.6 million cases. In the US, as many as 22% of adults have contracted herpes disease [2] caused by HSV-1 and HSV-2. HSV is a highly contagious pathogen that causes recurrent lesions. The entry of the virus into cells by triggering the membrane lining or a combination of cells causes herpes infection [4].

Infection with herpes simplex virus, commonly known as herpes, can be due to either herpes simplex virus type 1 (HSV-1) or herpes simplex virus type 2 (HSV-2). HSV-2 infection is widespread throughout the world and is almost exclusively sexually transmitted, causing genital herpes. HSV-2 is the leading cause of genital herpes, which can also be caused by herpes simplex virus type 1 [5, 6].

This study reports determining the anti-herpes bioactive compounds contained in betle leaf against the HSV-1 herpes receptor.

Materials and Methods

Materials

Hardware: LENOVO laptop with the 10th generation Intel® Core i3 processor specifications 2.3GHz, 4GB Random Access

Memory (RAM), 256GB SSD, Radeon Graphic, Microsoft Windows 10 operating system

Software: AutodockTools 4.0.1, Discovery Studio Visualizer 3.5, Chem3D Pro12.0,

Web Server: Protein Data Bank (https://www.rscb.org), https://preadmet.bmdrc.kr)

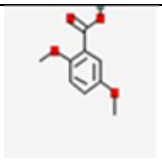
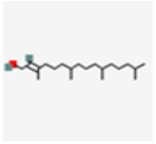
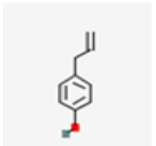
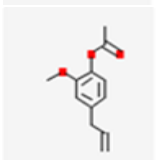
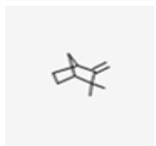
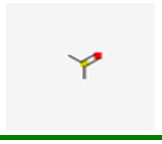
Receptor : 3D protein complex of HSV-1 (PDB code is 2KI5)

Ligand: structure of the bioactive compound of piper betle shown in **Table 1**. PubChem. (https://pubchem.ncbi.nlm.nih.gov/). and PreADMET (https://preadmet.bmdrc.kr)

Receptor : 3D protein complex of HSV-1 (PDB code is 2KI5)

Ligand: structure of the bioactive compound of piper betle show in **Table 1**.

Table 1. 2D structure of bioactive compounds of *Piper betle*

Name of ligand	2D structure
2,5- Dimethoxybenzoic_acid	
3,7,11,15-Tetramethyl-2- hexadecen-1-ol	
4-Allylphenol	
Acetyleugenol	
alpha-Pinene	
Camphene	
Dimethyl_sulfoxide	

Preparation and optimization of receptors

Downloaded 3D structure of receptor HSV-2 from Protein Data Bank with PDB ID is 2KI5 [7] and optimized by discovery studio to remove water molecules, separate from the native ligand, and select A chain of protein 2KI5.

Preparation and optimization of ligands

The 3D structure of ligands was downloaded from PubChem and then prepared using Chemdraw 3D by minimizing the energy [8].

Prediction of ADME and toxicity

Prediction of Adsorption Distribution and metabolism (ADME) and toxicity using a preADMET web server (https://preadmet.bmdrc.kr/) by uploading the 2-dimensional structure of the ligands in molfile (*.mol). The ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of molecules were of vital importance. Therefore, the ability to quickly and accurately predict these properties simply from the 2D structure of the molecule was beneficial in making decisions that could determine the success of our work. ADMET Predictor was state-of-the-art ADMET property prediction software [9].

Validation methode

Validation using the Autodock program (Analyze > RMSD > Molecule) inserted specific ligands and receptors in the .mol2 format. RMSD stood for the root mean square distance. The RMSD values heavy atom was less than 2.0 [10].

Molecular docking and visualization

How two or more molecular structures (e.g., drug and enzyme or protein) fit together was seen in Molecular docking [11]. Docking can be simply defined as a molecular modeling technique used to predict how a protein (enzyme) interacts with small molecules (ligands). Using notepad software, the molecular docking results could be seen in the output document. For Visualization, applied the Discovery Studio Visualizer application. The parameters considered are the value of Gibbs free energy (ΔG / binding affinity), the inhibition constant (Ki), and the amino acid bond between the ligand and the receptor.

Results and Discussion

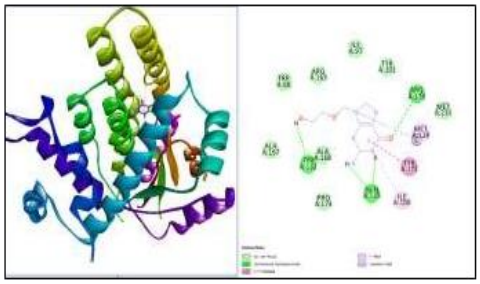
Herpes simplex virus (HSV) belongs to the Alphaherpesvirus, which could cause herpes. There were two types of HSV so far, namely HSV-1 and HSV-2. Both viruses could infect neurons and cause disease. HSV infection began with the entry of the virus into susceptible human cells. This entry process involved regulated interactions between glycoproteins in the viral envelope and host surface molecules and receptors. HSV-1 mainly caused herpes labialis and was transmitted through intimate oral contact, while HSV-2 caused genital herpes and was due to sexual intercourse. Therefore, HSV glycoproteins were critical molecular targets in molecular docking studies, and receptors with PDB code 2ki5 as the control ligand was native (Acyclovir)

After the ligand and receptor preparation process, the analysis method validation found the RMSD value was 1.9, then visualized using the Discovery Studio.

This result showed that the method could use for molecular docking between the bioactive compound of Piper betle as a ligand and receptor 2ki5. The molecular docking process uses twelve ligands. Molecular docking results can be seen in **Table 2**, and visualization amino acid bond of ligand and receptor can be seen in **Figure 1**.

Table 2. Result of Molecular Docking

Compounds	ΔG (Kcal/Mol)	Ki (μM)	Amino Acid Bond
Acyclovir	**-4.69**	**363.89**	**TYR132;GLN125;ARG176;PRO173;MET231; ALA168;ALA167;TRP88;ARG163;ILE97;TYR101**
2,5- Dimethoxybenzoic_acid	-6.12	32.51	ARG176;MET121;HIS58;MET231; TYR101;ALA168;GLN125
3,7,11,15-Tetramethyl-2- hexadecen-1-ol	-7.83	1.83	ARG176;MET231;GLN125;GLY129;HIS58;GLU225; ALA167;GLU83;LYS62
4-Allylphenol	-7.79	1.96	ARG163;TYR132;GLU83;GLU225;ARG176;MET231
Acetyleugenol	-6.77	10.93	ARG176;MET231;TYR101;GLN125;TYR172;ARG163
alpha-Pinene	-5.44	103.07	ARG176;MET231;GLN125
Camphene	-5.44	102.63	ARG176; MET231
Dimethyl_sulfoxide	-6.73	11.62	GLU225;ILE97;TYR101;MET231;ARG176;GLN125; ILE100;GLN83;ARG163;HIS58;ARG222
Ethyl_oleate	-7.34	4.15	ALA167;ALA168;GLN125;MET231;ILE100;ARG222; THR63;ASP162;LYS62;GLU83;ARG163;HIS58;GLU225; ILE97;TYR101;TYR132;ARG176
Guineensine	**-9.09**	**0.217**	ARG163;TRP88;HIS58;GLU225;ARG222;ARG176;MET231; GLN125;TYR101;TYR;172;ILA97;ALA167;LYS62;TYR132; GLU83;PRO173;ALA168;GLY129
Palustrol	-7.79	1.96	TYR132;ARG163;GLU83;GLU225;MET231;ARG176
Phenol, 2-methoxy(2-propenyl)	-5.58	81.24	HIS58;TYR101;MET231;GLN125
Phytol	-7.76	2.03	TRP88;GLN125;ALA175;ARG176;TYR172;PRO173



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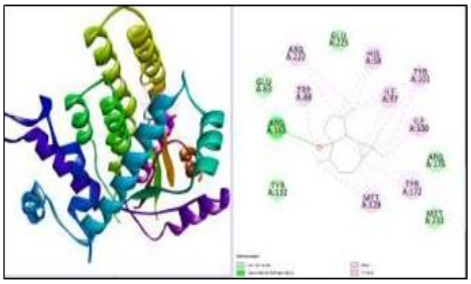

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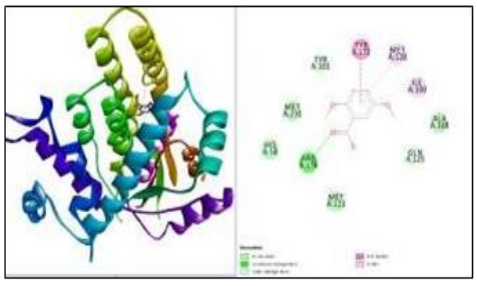

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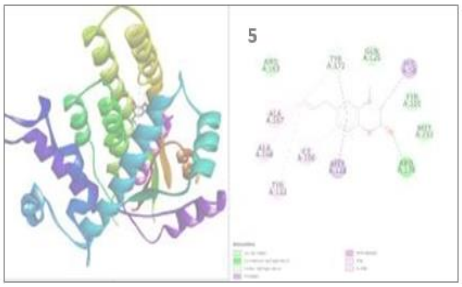

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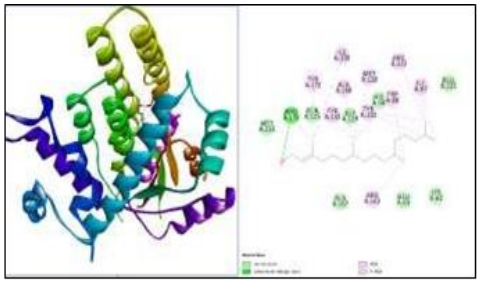

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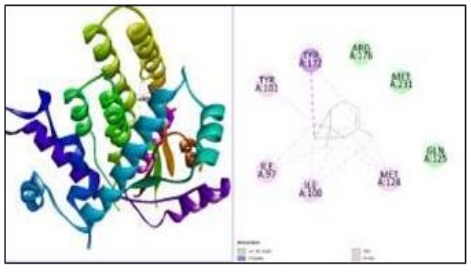

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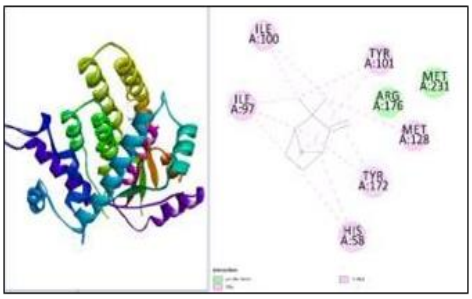

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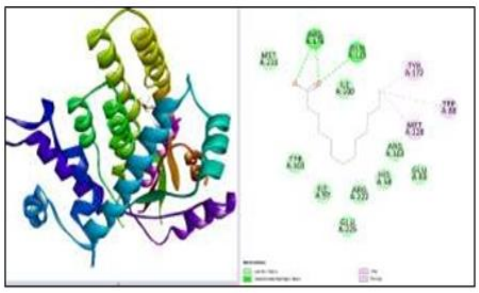

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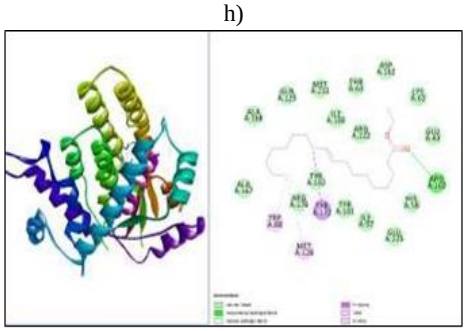
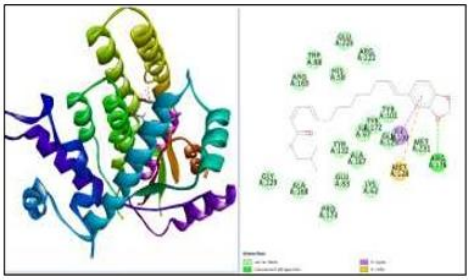


i)



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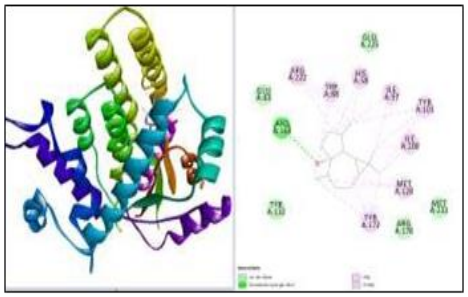

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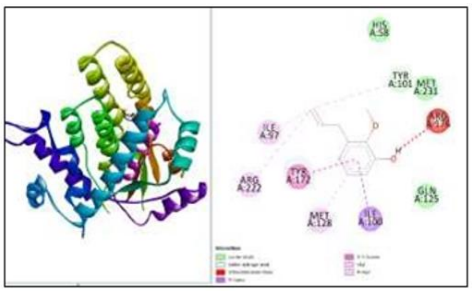
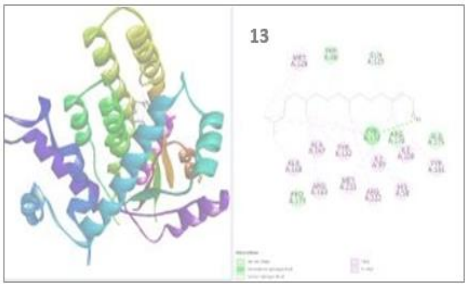


l)



m)

Figure 1. Visualization of Amino Acid Bond
(a) Acyclovir (b) 2,5-Dimethoxybenzoic_acid (c) 3,7,11,15-Tetramethyl-2- hexadecen-1-ol (d) 4-Allylphenol (e) Acetyleugenol (f) alpha-Pinene (g) Camphene (h) Dimethyl_sulfoxide (i) Ethyl_oleate (J) Guineensine (k) Palustrol (l) Phenol, 2-methoxy(2-propenyl) (m) Phytol.

The value of Gibbs free energy (ΔG) was significant from the molecular docking results. Only when the system free energy change was negative could the protein-ligand binding occur spontaneously [12, 13]. Since the degree of protein-ligand association depends on the magnitude of the negative G, it can assume that G determines the stability of a given protein-ligand complex or the binding affinity of the ligand to a particular acceptor [13].

The constant inhibition value (Ki), the smaller value of Ki, means better it to become a drug candidate. For example, **Table 2** showed the ΔG value of acyclovir as a receptor was -4.69 Kcal/Mol with a Ki value of 363.89 μM micromoles. In contrast, the test ligand has a smaller value of ΔG and Ki, especially Gueneensine with ΔG -9.09 Kcal/Mol and Ki 0.217 μM. In line with the amino acid bond Gueneensine has amino acid bonds similar to acyclovir shown in **Figure 1**, number 10, meaning equivalent to herpes medicine.

ADME and toxicity predictions were also carried out on bioactive compounds from betle plants using the preADMET website

In the ADME prediction to determine the absorption properties, the% Human Internal Absorption (HIA) and In Vitro CACO-2 cell permeability values are considered good absorption. if the% HIA is 70% - 100% moderate absorption if 20% - 70% and low absorption 0% - 20% and high permeability if>70 nm sec Medium permeability if 4-70 nm sec Permeability is low if <4 nm sec. The value of % plasma protein binding is strong if protein binding > 90% and weak binding <90% [14].

The carcinogenicity prediction method utilizes the results of such short-term tests to screen for chemicals that are most likely to cause cancer [15, 16]. If the mutagenic result is positive, it means no change of population (vs. blank plate). Suppose negative means change of population, more than double of blank plate's change. If the carcinogenicity test is positive, it means there is no evidence of carcinogenic activity. Negative means there is carcinogenic activity [17, 18]. From the result, we know that betle leaf has mutagenic and carcinogenic properties.

Conclusion

The bioactive compound from (Piper betle) has activity against the HSV-1 receptor, especially the Guineensine compound, which has a binding affinity -9.09 Kcal/Mol and inhibition constant 0.217 μM that's better than acyclovir as a comparison drug and has good ADME properties. The ADMET test showed that this leaf contains mutagenic and carcinogenic compounds, making it only suitable for topical treatment.

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Conflict of interest: None

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Ethics statement: None

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