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Virtual Screening of *Kaempferia galanga* L. Bioactive Compounds as HPV-16 Antiviral Mechanism through E6 Inhibitor Activity

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ABSTRACT
Human papillomavirus (HPV) infection is caused by a virus with a type of DNA genetic material from the Papillomaviridae family, about 90% of HPV infections have no definite symptoms. HPV infection is spread through sexual intercourse, multiple sexual partners, anal sex, and from mother to child during pregnancy. HPV has oncoproteins E6 & E7 which affect the ability to trigger cancer cases. The role of E6 is very crucial and is able to cause cells to lose homeostasis in the cell cycle and transform into cancer, this shows that E6 has the potential to be a binding target for HPV drug candidate compounds. Kaempferia galanga L. is used as a cooking spice in Indonesia and known as kencur, this plant is often used by Javanese and Balinese people to make jamoe. The potential of Kaempferia galanga L. as an antiviral only has little scientific evidence as previously done, the plant has high protease inhibitory activity of HIV-1 and HCV viruses. This study aims to identify chemical compounds from Kaempferia galanga L. which have potential as HPV antivirals through inhibition of E6 activity. Chemical compounds from Kaempferia galanga L. are predicted to be antiviral candidates through the activity of Ethyl cinnamate and γ-Cadinene with an E6 inhibition mechanism in the Val69 & Leu57 domains, the results of this study must be further analyzed through a wetlab approach to strengthen scientific evidence.

Keywords: Antiviral, Bioinformatics, *Curcuma longa*, HPV

INTRODUCTION

Human papillomavirus (HPV) infection is caused by a virus with a type of DNA genetic material from the Papillomaviridae family, about 90% of HPV infections have no definite symptoms. Some cases of infection only trigger the formation of warts

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which have an effect on increasing the risk of cancer such as cervix, vagina, vulva, penis, mouth, and anus1. Cancer cases in the cervix are caused by HPV-16 and HPV-18 with a percentage reaching 70%, but in cases of genital warts it is caused by infection with HPV-6 and HPV-11. HPV infection is spread through sexual intercourse, multiple sexual partners, anal sex, and from mother to child during pregnancy. The HPV vaccine has been found to prevent infection but not everyone gets it because of the high cost2.

The host cell of HPV is a stratified epithelium, the process of viral infection is slow because it takes about 12-24 hours for transcription initiation, this may occur due to antibody defenses on the cell surface3. HPV lesions originate from the basal layer of keratinocytes, HPV enters the host cell through receptors such as integrins, annexin A2, and laminis, these receptors can trigger the process of transporting viral molecules to the nucleus by an unknown mechanism. HPV has oncoproteins E6 & E7 which affect the ability to trigger cancer cases. E6 binds to p53 to initiate the process of immortalization in these cells, then E7 binds to pRb to stop the process of checking points in cells. The role of E6 is very crucial and is able to cause cells to lose homeostasis in the cell cycle and transform into cancer, this shows that E6 has the potential to be a binding target for HPV drug candidate compounds4.

Kaempferia galanga L. is used as a cooking spice in Indonesia and known as kencur, this plant is often used by Javanese and Balinese people to make jamoe5. Chemical compounds in the rhizome Kaempferia galanga L. consist of α-Pinene, Camphene, δ-Carene, 1,8-Cineole, Borneol, Ethyl cinnamate, γ-Cadinene, and Linoleoyl chloride. Kaempferia galanga L. has health benefits such as anti-inflammatory, anti-oxidant, anti-bacterial, anti-tumorous, anti-angiogenesis5. The potential of Kaempferia galanga L. as an antiviral only has little scientific evidence as previously done, the plant has high protease inhibitory activity of HIV-1 and HCV viruses6,7. This study aims to identify chemical compounds from Kaempferia galanga L. which have potential as HPV antivirals through inhibition of E6 activity.

METHODS

Sample retrieval

Kaempferia galanga L. contains chemical compounds consisting of -Pinene, Camphene, δ-Carene, 1,8-Cineole, Borneol, Ethyl cinnamate, γ-Cadinene, and Linoleoyl chloride[5]. PubChem (https://pubchem.ncbi.nlm.nih.gov/) was used to extract CID, canonical SMILE, molecular formula, and 3D structure of the target compound. The ligand minimization process is carried out on the target compound that has been isolated from the database through OpenBabel 3.1.1 software, it aims to change the *sdf* format to *pdb*[8,9]. The RCSB PDB (https://www.rcsb.org/) in this study was used for sampling E6 HPV-16 (PDB ID: 4XR8).

Bioactivity Prediction

Kaempferia galanga L. compound must have activity as an inhibitor of the E6 protein on HPV-16, the activity was predicted through Molinspiration v2018.03 (https://www.molinspiration.com/cgi-bin/properties) with Canonical SMILE. The prediction refers to the positive value of the probability to show the level of activity in the target compound[10,11].

Blind Docking Simulation

Docking indicates the level of activity of a ligand when interacting with the target protein domain according to the research objective. The blind docking method in this study aims to determine the level of activity of the *Kaempferia galanga* L. Autogrid compound directed at the entire target surface because the functional site on the protein domain has not been identified. The blind docking simulation was carried out through the Vina Wizard on the PyRx 0.8 software[12,13].

Ligand-protein Interaction

The chemical bond interactions in the docking complex were identified through the Discovery Studio 2016 version. The binding position of the

compound containing *Kaempferia galanga* L. in the E6 HPV-16 domain was determined to determine the similarity of the interactions, then the types of chemical bonds displayed by the Discovery Studio 2016 version were conventional hydrogen bonds, pi-alkyl, hydrophobic, Van der Waals, and electrostatic[14,15].

Structural Visualization

3D structures are displayed through PyMol 2.5 version, models are displayed through transparent surfaces, cartoons, and a selection of coloring based on structures. 3D visualization makes it possible to identify the location of the binding position of the ligand in the protein domain with a representative view[16,17].

RESULT AND DISCUSSION

Kaempferia galanga L. compound bioactivity

Kaempferia galanga L. has chemical compounds consisting of α-Pinene, Camphene, δ-Carene, 1,8-Cineole, Borneol, Ethyl cinnamate, γ-Cadinene, and Linoleoyl chloride which have anti-inflammatory, anti-oxidant, anti-inflammatory properties. anti-bacteria, anti-tumorous, anti-angiogenesis[19,20,21]. The potential of Kaempferia galanga L. as an antiviral only has little scientific evidence as previously done, the plant has high protease inhibitory activity of HIV-1 and HCV viruses[22,23]. This study aims to identify chemical compounds from Kaempferia galanga L. which have potential as HPV antivirals through inhibition of E6 activity. Information on chemical compounds of *Kaempferia galanga* L. such as CID, formula, and Canonical SMILE was obtained from the PubChem database (Table 1).

Table 1. Chemical compounds of *Kaempferia galanga* L. from PubChem Database

Compounds	CID	Formula	SMILE Canonical
α-Pinene	6654	$C_{10}H_{16}$	CC1=CCC2CC1C2(C)C
Camphene	6616	$C_{10}H_{16}$	CC1(C2CCC(C2)C1=C)C
δ-Carene	26049	$C_{10}H_{16}$	CC1=CCC2C(C1)C2(C)C
1,8-Cineole	2758	$C_{10}H_{18}O$	CC1(C2CCC(O1)(CC2)C)C
Borneol	64685	$C_{10}H_{18}O$	CC1(C2CCC1(C(C2)O)C)C
Ethyl cinnamate	637758	$C_{11}H_{12}O_2$	CCOC(=O)C=CC1=CC=CC=C1
γ-Cadinene	92313	$C_{15}H_{24}$	CC1=CC2C(CC1)C(=C)CCC2C(C)C
Linoleoyl chloride	9817754	$C_{18}H_{31}ClO$	CCCCCC=CCC=CCCCCCCCC(=O)Cl

Prediction of compound bioactivity in *Kaempferia galanga* L was identified for the determination of compounds with inhibitory properties, the results of this study showed that α-Pinene, δ-Carene, Borneol, Ethyl cinnamate, γ-Cadinene, and Linoleoyl chloride were predicted as inhibitors and Camphene & 1,8-Cineole as a modulator (Table 2). Inhibitor compounds will be used for further analysis because it is predicted to inhibit E6 activity[24,25].

Table 2. The result of compound bioactivity

Compounds	GPCR Lingand	ICM	KI	NRL	PI	EI	Bioactivity Type
α-Pinene	-0.48	-0.43	-1.50	-0.62	-0.85	-0.34	Inhibitor
Camphene	-1.02	-0.55	-1.85	-1.15	-1.40	-0.82	Modulator
δ-Carene	-1.29	-0.79	-1.51	-1.30	-1.28	-0.53	Inhibitor
1,8-Cineole	-0.93	0.01	-1.60	-1.07	-0.90	-0.15	Modulator
Borneol	-0.47	-0.51	-1.57	-0.84	-0.80	-0.23	Inhibitor
Ethyl cinnamate	-0.88	-0.46	-1.09	-0.58	-0.94	-0.45	Inhibitor

γ-Cadinene	-0.21	0.11	-0.80	-0.27	-0.73	0.27	Inhibitor
Linoleoyl chloride	0.11	0.07	-0.12	0.06	-0.07	0.18	Inhibitor

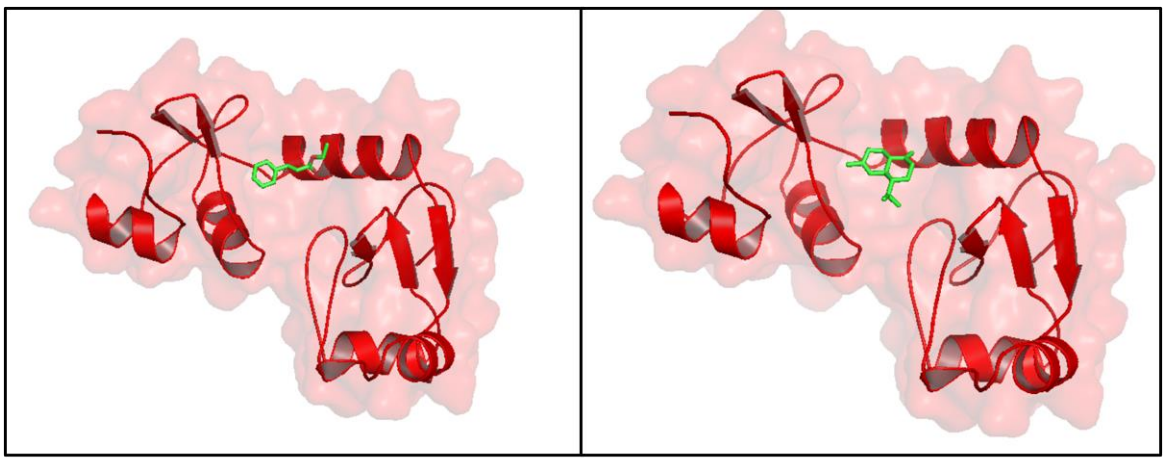
ICM: Ion channel modulator, KI: Kinase inhibitor, NRL: Nuclear receptor ligand, PI: Protease inhibitor, EI: Enzyme inhibitor

The comparison of *Kaempferia galanga* L. compound binding affinity

Molecular interaction simulation through 3D structure with bioinformatics approach is molecular docking[26]. This simulation aims to identify the chemical bonding activity of ligands in protein-specific domains[27]. This study used ligands consisting of α-Pinene, δ-Carene, Borneol, Ethyl cinnamate, γ-Cadinene, & Linoleoyl chloride and E6 as targets. The docking results showed that Ethyl cinnamate and -Cadinene had the most negative binding affinity (Table 3). 3D visualization of the two compounds was carried out through cartoon structures, surfaces, and sticks (Figure 1). Thus, Ethyl cinnamate and γ -Cadinene compounds from *Kaempferia galanga* L. are predicted to act as potential inhibitors of E6 activity because they have a more negative binding affinity.

Table 3. Binding affinity comparison

Compounds	CID	Target	Binding Affinity (kcal/mol)
α-Pinene	6654	E6 HPV-16	-5.4
δ-Carene	26049	E6 HPV-16	-5.1
Borneol	64685	E6 HPV-16	-5.5
Ethyl cinnamate	637758	E6 HPV-16	-6.3
γ-Cadinene	92313	E6 HPV-16	-6.3
Linoleoyl chloride	9817754	E6 HPV-16	-5.3



(A) (B)

Figure 1. Molecular visualization of 3D docking result. (A) Ethyl cinnamate_E6 (B) γ-Cadinene_ E6

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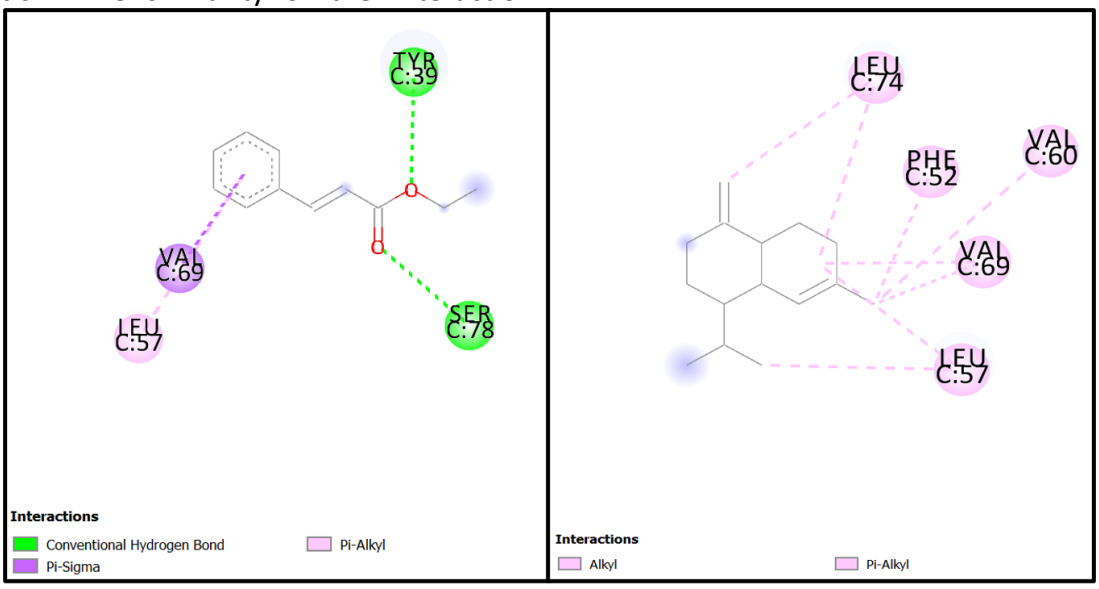
Molecular interaction on E6 protein domain

Chemical interactions are formed in molecular complexes from the docking results, these interactions play a role in triggering biological responses such as activation and inhibition of target proteins[28]. These types of bonds are hydrogen, van der Waals, alkyl, hydrophobic, and electrostatic[29]. The similarity of the interaction positions on the ligands can be used to recommend potential sites in proteins. Ethyl cinnamate and γ-Cadinene have similar interactions in the E6 domain consisting of Val69 & Leu57, the two ligands can form hydrogen and alkyl bond interactions to trigger an E6 inhibitory response (Figure 2).



(A) **(B)**

Figure 2. Visualization of 2D molecular interaction analysis results. (A) Ethyl cinnamate_E6 (B) γ-Cadinene_E6.

CONCLUSION

Chemical compounds from *Kaempferia galanga* L. are predicted to be antiviral candidates through the activity of Ethyl cinnamate and γ-Cadinene with an E6 inhibition mechanism in the Val69 & Leu57 domains, the results of this study must be further analyzed through a wetlab approach to strengthen scientific evidence.

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