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Computational Drug Design Study of Curcuma longa L. Compound as HPV-16 Antiviral Candidate against Cervical Cancer

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ABSTRACT
Human papillomavirus (HPV) is one of the causes of cervical cancer in women. These viruses have DNA-type genetic material, are not enveloped, and are divided into two types based on their ability to trigger cancer. High-risk HPV is the type of HPV that often causes cervical cancer cases, this is due to the high expression of oncoprotein E6. E6 works through the inhibition and degradation of p53 to trigger cancer. HPV drugs tend to be expensive and must use alternative solutions such as the herbal Curcuma longa L. *Curcuma longa* L. is an herbal plant that is widely used by the public for their daily needs, for example as an antiviral, antioxidant, and anti-inflammatory. This study aims to predict the molecular mechanism and screening of drug candidate compounds in *Curcuma longa* L. to inhibit the activity of the HPV E6 protein through a bioinformatics approach. This study used in silico methods such as docking simulations and molecular interaction analysis to identify potential antiviral candidate compounds. Curcuma longa L. can act as an antiviral through the binding activity of Curcumin compounds in preventing the E6-p53 complex through the specific domains and stable because it have a many number of hydrogen bonds. We recommend the E6-binding domain for further research as a target for HPV drug design and the results of this study are yet to be verified through wet lab testing.

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

INTRODUCTION

Human papillomavirus (HPV) has infected millions of women since 2018 and has caused cervical cancer since the early twentieth century[1]. HPV consists of many types, but the classification can be divided into two types based on the ability to cause cancer. The high risk (HR) type can trigger the formation of cervical cancer when it infects and many cases of benign tumors (warts) have been identified due to low risk (LR) infection. HR types consist of 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, & 82 and LR types are 6, 11, 40, 42, 43, 44, 54, 61, 72, 81, & CP6108. In addition to cervical cancer, HPV infection also causes genital warts in men and women. The majority of cervical cancer cases are caused by HPV-16 and 18[2].

† Footnotes relating to the title and/or authors should appear here.

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HPV is a DNA virus, non-enveloped, has a major and minor capsid. The HPV genomic region consists of LCR, E6, E7, L1, L2, E1, E2, E4, and E5. L1 and L2 play a role in the capsid assembly process, the viral DNA replication process is initiated by E1 and E2, then the assembly and release of viral particles is regulated by E4, the signal transduction activity of viral attachment to the host receptor is regulated by E5[3]. HPV has oncoproteins E6 and E7 to trigger increased cell proliferation and the degradation of tumor suppressor genes in host cells[4]. The host of HPV is epithelial cells, one of which is found in the genitals, but several studies have not been able to identify the actual type of entry receptor for HPV. However, HPV drug design studies are more likely to observe the characteristics of oncoproteins that cause cancer problems[5].

Oncoprotein E7 plays an important role in preventing host cells from carrying out the checkpoint process and preventing the expression of genes encoding apoptotic responses by retinoblastoma (RB). Oncoprotein E6 works in inhibiting the tumor suppressor protein or p53 activity, it causes host cells to lose homeostasis to regulate cell division stability and cause cancer[5]. E6 binds to the p53 region and then triggers its degradation via the ubiquitin proteasome pathway. *Curcuma longa* L. is an herbal plant that is widely used by the public for their daily needs, for example as an antiviral, antioxidant, and anti-inflammatory[6]. This study aims to predict the molecular mechanism and screening of drug candidate compounds in *Curcuma longa* L. to inhibit the activity of the HPV E6 protein through a bioinformatics approach.

METHODS

Sample retrieval

The bioactive compound *Curcuma longa* L. consists of Curcumin, Curcumenol, Ar-Curcumene, γ-curcumene, Zingiberene, and Ar-Turmerone obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/)[7]. This study used E6 of HPV (GDP ID: 4XR8) obtained from the protein databank (https://www.rcsb.org/). The ligand conversion process from *sdf* format to *pdb* was carried out using PyRx 0.9.9 version software and PyMol 2.5 version is used to remove water molecules in proteins[8].

Docking Simulation

This simulation is to identify the ability of molecular interaction between ligand-protein and refers to the value of binding affinity. This study used a docking screening method to determine potential domains in proteins for ligand binding targets. PyRx 0.9.9 version software was used in this study to simulate the docking of Curcuma longa L. compound with E6 HPV. The position of the docking grid in this study was set to cover the entire protein surface[9].

Molecular Interaction

Weak bonds play a role in ligands to produce an activity response to proteins. The ligand-protein interactions formed are weak bonds, weak bonds such as hydrogen, electrostatic, alkyl, van der Waals, and hydrophobic. The Discovery Studio 2016 software version was used in this study to identify the weak bonds formed in molecular complexes[10].

3D Visualization

Molecular visualization of the ligand-protein complex was carried out using PyMol 2.3 version software through staining and structural selection methods. The 3D structure shown consists of cartoons, surfaces, and sticks, staining is done on the protein constituent chains[11].

RESULT AND DISCUSSION

Binding affinity of *Curcuma longa* L. compounds

Curcuma longa L. in people's lives around the world is used for traditional medicine such as anti-inflammatory, antiviral, preventing free radicals, and others. Curcuma longa L. was identified to have chemical compounds consisting of Curcumin, Curcumenol, Ar-Curcumene, γ-curcumene, Zingiberene, and Ar-Turmerone[12,13]. This study used a chemical compound from *Curcuma longa* L. to identify candidate antiviral drugs against HPV by inhibiting the activity of oncoprotein E6 by molecular docking[13,14]. Curcumin has a binding affinity of -6.6 kcal/mol more negative than other compounds when it binds to E6 protein (Table 1), curcumin is predicted to inhibit the activity of E6 protein in HPV because it has more negative binding than other compounds. The molecular docking simulation aims to determine the activity of the ligand binding on the protein domain by referring to the binding affinity value[15,16,17,18]. The binding affinity value indicates the inhibitory ability of a

ligand on the activity of the target protein. Visualization of docking results is displayed through transparent surfaces structure and sticks with selected coloring (Figure 1).

Table 1. Binding affinity from docking simulation

Compounds	CID	Molecular Weight (g/mol)	Target	Binding Affinity (kcal/mol)
Curcumin	969516	368.4	E6	-6.6
Curcumenol	167812	234.33	E6	-6.2
Ar-Curcumene	92139	202.33	E6	-5.6
γ-Curcumene	12304273	204.35	E6	-5.7
Zingiberene	92776	204.35	E6	-5.2
Ar-Turmerone	160512	216.32	E6	-6.4

Position of chemical bond interactions at E6 protein

Chemical bond interactions in molecular complexes are identified to determine the type of bond and position[19,20,21,22]. All ligands have the same interaction position at Val60, Val38, Tyr39, Leu74, Gln114, Phe52, Val69, and Leu57, this position allows them to act as potential domains to lead inhibitory activity at E6 (Table 2). Weak bond interactions such as van der Waals, hydrogen, and alkyl are also formed in all ligands, curcumin has more hydrogen bond interactions than other compounds and this can strengthen the prediction that curcumin can act as a good drug candidate. Weak bond interactions consisting of hydrogen, hydrophobic, and alkyl can play a role in triggering the response of biological activity on the target protein[23,24,25]. The number of hydrogen bonds can be used as an indicator of the stability of a drug candidate molecule[26]. Thus, curcumin is predicted to become a drug molecule because it can affect the activity of E6 protein through weak bonds and has the highest number of hydrogen bonds. the visual results of molecular interactions in this study are displayed with a 2D structure (Figure 1).

Table 2. The positions of chemical interaction on E6 pocket binding domain

Compounds	Molecular Interaction
Curcumin	**van der Waals**: Val60, Ala68, Val38, Tyr39, Leu74, Gln114, Ser78, Leu107, Asp56, Lys18, Phe52 **Hydrogen**: Cys58, Arg109, Arg138, Ser81, Tyr77 **Alkyl**: Val69, Leu57
Curcumenol	**van der Waals**: Ser81, Ser78, Tyr77, Gln114, Phe52, Val60 **Alkyl**: Val38, Leu74, Tyr39, Val69, Leu57
Ar-Curcumene	**van der Waals**: Ala68, Gln114, Asp56, Arg109, Tyr39 **Alkyl**: Val60, Val38, Val69, Phe52, Leu74, Leu57, Cys58
γ-Curcumene	**van der Waals**: Ala68, Gln114, Arg109, Tyr39 **Alkyl**: Leu57, Val69, Val38, Val60, Phe52, Leu74, Cys58
Zingiberene	**van der Waals**: Phe52, Ala68, Tyr39, Gln114 **Alkyl**: Val69, Val38, Leu74, Leu57, Cys58, Val60
Ar-Turmerone	**van der Waals**: Arg138, Ser81, Tyr39, Tyr77, Cys58, Ala68 **Hydrogen**: Gln114 **Alkyl**: Val69, Phe52, Val38, Leu57, Val60, Leu74

Potential compounds from *Curcuma longa* L. for E6 protein inhibition activity

Curcuma longa L. is an HPV antiviral candidate to prevent the development of cervical cancer through the binding of curcumin to the E6 domain. Curcumin compounds can produce a more negative binding affinity than others and produce weak binding interactions to trigger an E6 inhibitor response through the Val60, Val38, Tyr39, Leu74, Gln114, Phe52, Val69, and Leu57 domains. The inhibition of the E6-p53 complex by the curcumin compound causes a decrease in E6 activity and an increase in p53 protein activity, then normal cells will not develop into cancer[27]. Inhibition of the E6-p53 complex will also trigger apoptosis in cells that have been infected with HPV[28,29].

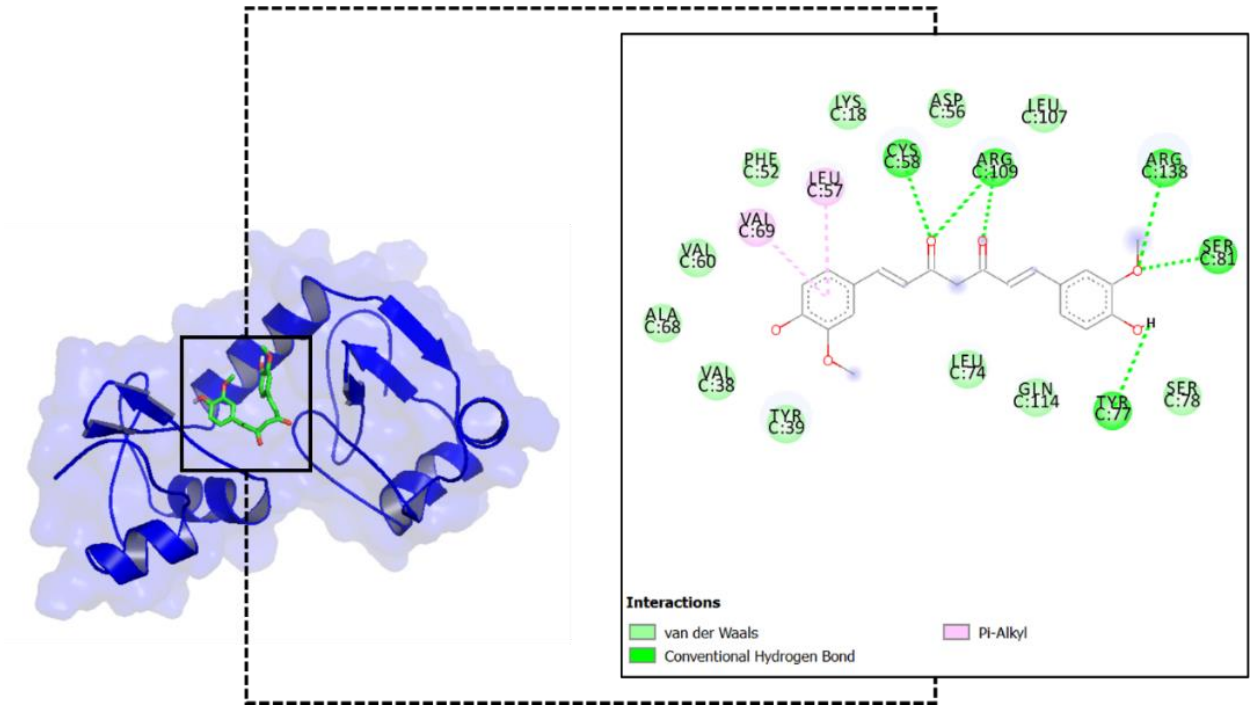

Figure 1. 3D results of molecular docking simulation and analysis of chemical bond interactions on the Curcumin-E6 complex. Ligands are shown with green sticks and blue transparent surfaces on proteins.

CONCLUSION

Curcuma longa L. can act as an antiviral through the binding activity of Curcumin compounds in preventing the E6-p53 complex through the specific domains Val60, Val38, Tyr39, Leu74, Gln114, Phe52, Val69, and Leu57 through weak bond interactions and are stable because Curcumin have a large number of hydrogen bonds. We recommend the E6-binding domain for further research as a target for HPV drug design and the results of this study are yet to be verified through wet lab testing.

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