Prediction of the mechanism of action of catechin as superoxide anion antioxidants and natural antivirals for COVID-19 infection with *in silico* study

Achmad Zainuddin, Ika Wiani Hidayat, Dikdik Kurnia, Zenika Febian Ramadhanty, Rizal Padilah

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Sumedang, Indonesia

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Abstract

Coronavirus disease-2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus-2 attacking the lungs, which contain the most oxygen. The involvement of oxidative stress in the body and the role of antioxidant compounds, namely catechins, are thought to be able to prevent various diseases, including the COVID-19 infection virus. An *in silico* approach was employed between the catechins and the protein NADPH oxidase (Nox), followed by the coronavirus protease protein, to limit the generation of reactive oxygen species. This research using the *in silico* method seeks to predict the mechanism of action of catechin as a superoxide radical anion inhibitor and as an antiviral for COVID-19. This study carried out molecular docking simulations of catechin compounds against Nox and coronavirus proteases and then compared them with positive controls GKT136901 and remdesivir. The binding energy of catechin and Nox in a docking simulation is − 8.30 kcal/mol, which is somewhat lower than GKT136901's binding value of − 8.72 kcal/mol. Catechin and coronavirus proteases had binding energy of − 7.89 kcal/mol, which was greater than remdesivir's binding energy of − 7.50 kcal/mol. Based on *in silico* data, catechin as an antioxidant compound can be antiviral for COVID-19.

Key words: Antioxidant, antiviral coronavirus disease-2019, catechin, *in silico*

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) causes coronavirus disease-2019 (COVID-19), an acute respiratory disease that has lately become a pandemic.[1] COVID-19 is spread through the respiratory tract, with the lungs being one of the organs that receive the most oxygen in the human body.[2] In this case, oxidative stress becomes an important factor that can overcome environmental conditions that are susceptible to this virus.[3] Hence, it is important to analyze the role of cellular organelles and substances directly related to oxidative stress.[4]

The main focus in the quest for alternatives to combat the COVID-19 virus's propagation is to boost the body's natural immune system and immunity by eating foods high in antioxidant bioactive substances.[5] The efficacy

Address for correspondence:
Prof. Dikdik Kurnia,
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Sumedang, Indonesia.
E-mail: dikdik.kurnia@unpad.ac.id

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of antioxidants may be very useful in preventing and reducing symptoms of COVID-19; catechins are natural polyphenol compounds derived from one of the plants *Uncaria gambir*, and they are widely known for their ability to prevent various diseases, including viral infections. Catechins act as antioxidants because they can generate and capture free radicals either directly (removal of reactive oxygen species [ROS] and metal chelating agents) or indirectly (induction of antioxidant enzymes and inhibition of prooxidant enzymes and antioxidant enzymes).[6]

In finding alternative techniques to avoid COVID-19, it may be the best solution to use an *in silico* method, where multi-target inhibition of coronaviral or coronaviral-2 enzymes and proteins is one of the most promising ways to produce highly effective COVID-19 treatments. The *in silico* approach is a computational method, such as molecular docking, that looks at the binding affinity of a tiny molecule (ligand as an active substance) and a potential receptor to determine how interactions occur (target protein).[7,8] This method can be carried out simply and quickly so that it is suitable for screening bioactive compounds before *in vitro* studies, and this *in silico* method can be used to obtain new drug targets.[9] The target receptor chosen in this publication to see the prediction of catechin's mechanism of action as an antidote to superoxide anions is Nox because it is the only enzyme that generates ROS. As a result of this target selection, catechin compounds are expected to decrease ROS signaling. Nox is a major role in cell differentiation, senescence, and apoptosis, according to various studies.[10,11] Flavin adenine dinucleotide (FAD) will be used as a native ligand to validate the docking simulation between Nox and catechin. GKT136901 is one of the inhibitors that can be used against Nox, and in this article, it is used as a positive control.[12]

In addition, new COVID-19 evasion strategies are being developed. Anti-COVID-19 medicines are being developed as a priority target for SARS CoV-2's primary protease, which is a key component of viral replication. The goal of this study was to explore if catechin might be used as an antiviral treatment for corona. The coronavirus protease protein was selected to investigate other bioactivities of catechin as coronavirus antiviral drugs.[13] Remdesivir as the currently used corona antiviral drug was used as a positive control.[14]

Nox activity has been shown to have a role in respiratory virus-induced ROS overproduction in several *in vitro* and *in vivo* studies.[15] Changes in ROS are also associated with the coronavirus. So that in this journal, with an approach through molecular docking, it is hoped that the prediction of the mechanism of action of catechin compounds as superoxide anion radical inhibitors can also be candidates for compounds that have the potential as COVID-19 antivirals.

MATERIALS AND METHODS

Materials
The Nox protein (Protein Data Bank ID: 5O0X) and the coronavirus protease structure (Protein Data Bank [PDB] ID: 5N5O) were obtained from the RCSB PDB (https://WWW.RCSB.org/). Catechin (CID: 9064), remdesivir (CID: 12134016), and GKT136901 (CID: 17027464) were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/), and the Open Babel tool was used to build three-dimensional (3D) structures in PDB format. Redocking with native ligands was carried out to validate the docking simulation for the respective proteins, including Nox-FAD [Figure 1] and the coronavirus protease-8O5/Query [Figure 2]. Discovery studio is used to view data analysis and docking visualization.

Methods
OPEN BABEL 2.4.1 software Arizona, USA was used to convert ligands in SDF format to 3D structure PDB format. The 3D protein structure was downloaded from the RCSB protein database. Redocking is done first with each native ligand with the AutoDock 1.5.6 program. Then, the simulation of docking with various ligands against Nox protein using a grid center and a grid box with X, Y, and Z coordinates of 71,100, − 3,398, 62,918, respectively. Meanwhile, for all COVID-19 ligands and proteins, grid centers of −23.005, 1.142, and 1.896 were used for the X, Y, and Z coordinates. Genetic algorithm is applied as parameter in docking simulation with 100 GA run. Analysis of the ligand–receptor complex can be visualized using Discovery Studio 2020 within a 5Å radius.[16,17]

RESULTS

Based on the results, the binding affinity of the Nox–catechin complex interaction is − 8.30 kcal/mol, which is greater
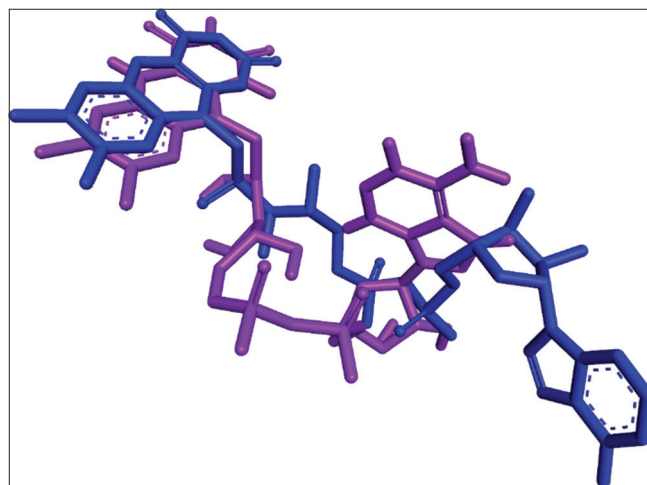

Figure 1: Results of FAD ligand redocking against Nox. FAD: Flavin adenine dinucleotide

Table 1: Binding affinity and hydrogen bond in complex Nox–flavin adenine dinucleotide

Ligand	Binding affinity (kcal/mol)	Binding of residues in ligand–protein complexes	
		Hydrogen interaction	Hyrdrophobic interaction
FAD	−7.62	Pro460, His459, His476, Arg478, Thr 462	Tyr445, Pro460, Trp695, Phe461, Ile538

**Table 2: Binding affinity and hydrogen bond in complex Nox–GKT136901**

Ligand	Binding affinity (kcal/mol)	Binding of residues in ligand–protein complexes	
		Hydrogen interaction	Hyrdrophobic interaction
Catechin	−8.30	Trp695, Pro460, Thr541, His476, Arg478	Ile538, Trp695, Pro460, Phe461
GKT136901	−8.72	Trp695, Pro461, His459	Ile538, Arg478, Val480, His459, Trp695, Phe461, Pro460

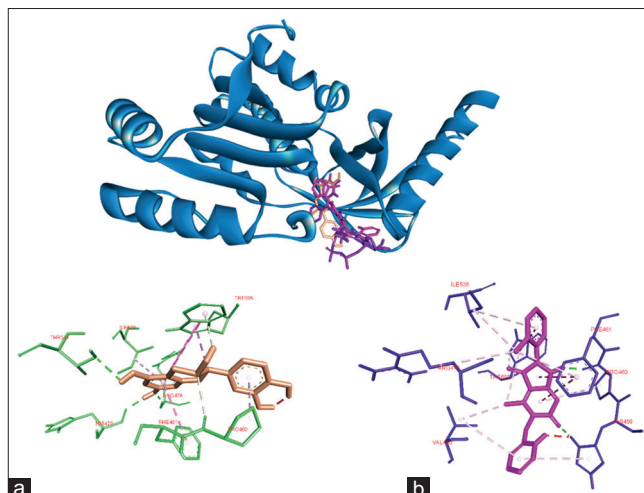**Table 3: Binding affinity and hydrogen bond in complex coronavirus protease–ligands**

Ligand	Binding affinity (kcal/mol)	Residues binding at ligand–protein complex	
		Hydrogen Interaction	Hyrdrophobic Interaction
8O5/Query	−8.46	Gly145, Ser144, Cys145, Gln189, Leu141, Ser144, His41, Thr26	Met49, His41, Pro168

Table 4: Binding affinity and hydrogen bond in complex coronavirus protease–ligand

Ligand	Binding affinity (kcal/mol)	Residues binding at ligand–protein complex	
		Hydrogen interaction	Hyrdrophobic interaction
Catechin	−7.89	Gly143, Ser144, Glu166, Arg188, Leu141, Cys145	His41, Met49, Met165
Remdesivir	−7.50	Gly143, Ser144, Cys145, Glu166, Gln189	Met165, Leu167, Pro168
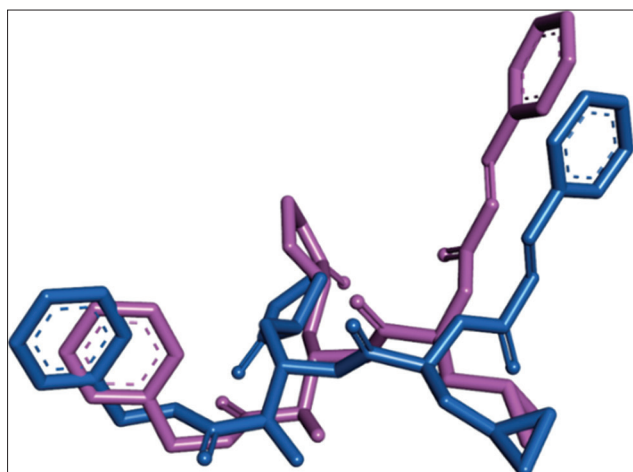
Figure 2: Results of ligand redocking against coronavirus protease

Figure 3: Ligand positions on Nox: catechin (a) and GKT136901 (b)

than the native ligand FAD's binding affinity of −7.62 kcal/mol. The positive control, GKT136901, on the other hand, has a greater affinity value of −8.72 kcal/mol, as seen in Tables 1 and 2. Then, Tables 3 and 4 show the results, the catechin–protease coronavirus has a binding affinity of −7.89 kcal/mol, which is higher than the positive control remdesivir, which has a binding affinity of −7.50 kcal/mol, according to the results of the docking simulation between the ligand and the coronavirus protease receptor. However, following redocking [Figure 2], the native ligand in this coronavirus protease protein has a relatively high binding affinity value of −8.46 kcal/mol.

DISCUSSION

One of the events of changing the redox state of the oxidant state in infected cells is associated with respiratory viral infections, and also plays a role in COVID-19 infection.[18,19] Based on data obtained from several research journals, it has been shown that oxidative stress induced by Nox activation is associated with thrombotic events in COVID-19 patients.[20] Docking simulations were carried out to see the relationship between antioxidant inhibition and COVID-19 antiviral inhibition, by looking at the molecular receptor and ligand
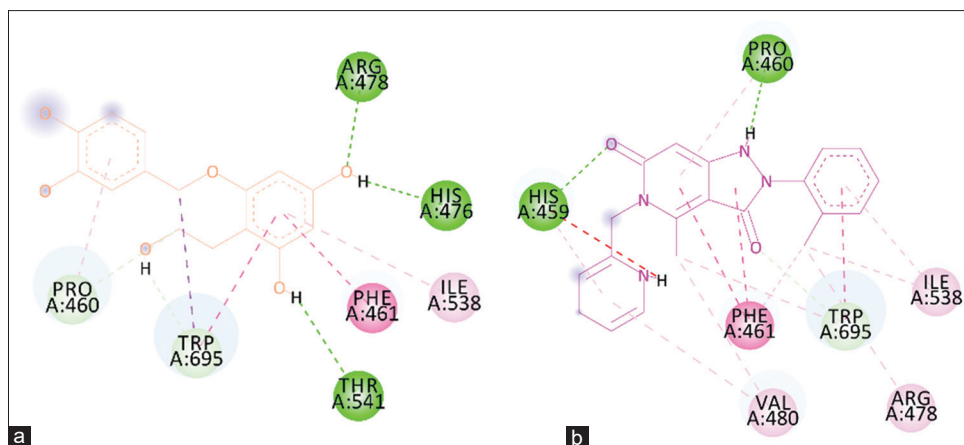
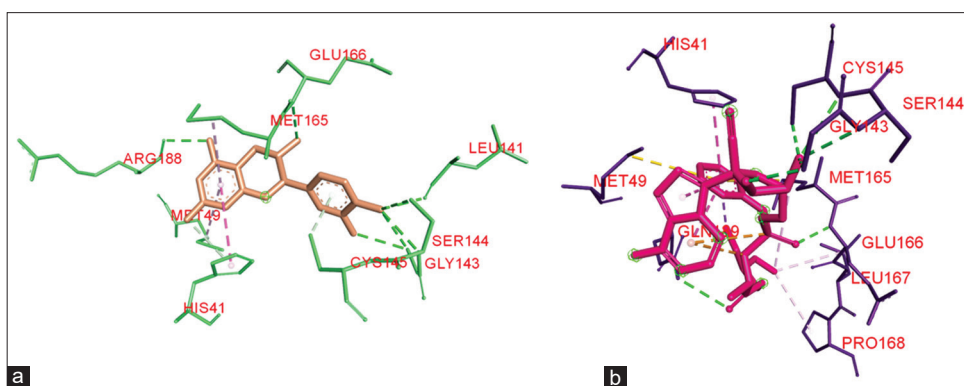**Figure 4:** Interaction Nox with ligands: catechin (a) and GKT136901 (b)

Figure 5: Ligand positions on coronavirus protease: catechin (a) and remdesivir (b)
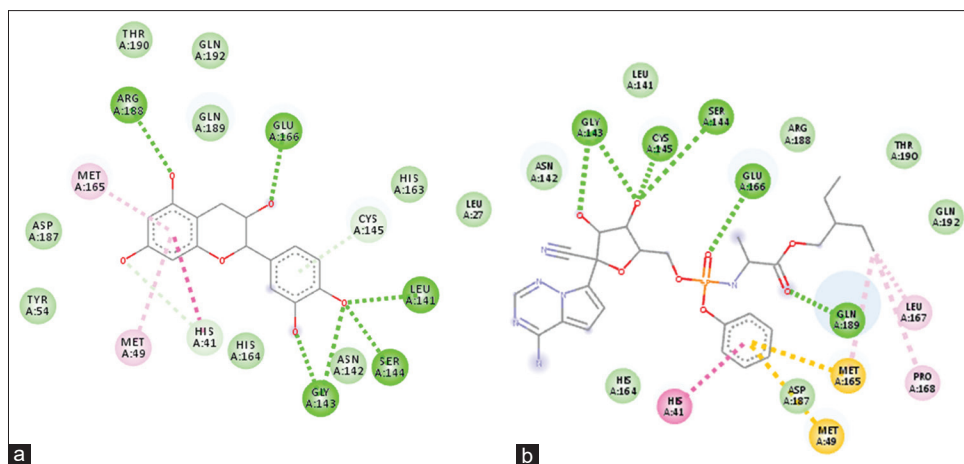

Figure 6: Interaction coronavirus protease with ligand: catechin (a) and remdesivir (b)

interactions, both the potential for catechin compounds to be compared with native ligands that had been redocked from each receptor and positive control from each receptor.

Docking simulations were carried out first on catechin ligands as the target compound compared with GKT136901 as a Nox-positive control to see the potential of the compound in inhibiting the Nox enzyme which is the

main producer of ROS in many cells. The interactions that will be obtained are binding energy, hydrogen bonds, and hydrophobic interactions. Observations were made to obtain a score that can indicate the strength of the protein–ligand complex in relation to intermolecular interactions between the receptor and the ligand.[21] Tables 1-4 show the binding energy of the compound representing the Gibbs free energy (ΔG) in kcal/mol. The

stability of the receptor–ligand complex can be obtained with negative ΔG which will form a spontaneous reaction to the binding of the receptor–ligand complex.[22] The more negative ΔG, the more stable the interactions in the complex formed.[23] Catechin has a higher binding energy value than native ligand (FAD) but slightly lower than the positive control (GKT136901). In Figures 3 and 4, Nox-catechin complex showed hydrophobic interactions with Ile538, Trp695, Pro460, and Phe461. The Nox–FAD complex exhibits hydrophobic interactions of Tyr445, Pro460, Trp695, Phe461, and Ile538. Meanwhile, the Nox–GKT136901 complex showed hydrophobic interactions with Ile538, Arg478, Val480, His459, Trp695, Phe461, and Pro460. Hydrogen bonding is usually considered a facilitator for receptor–ligand binding.[24] Donors and acceptors establish hydrogen bonds, and hydrogen bond donors are typically found in receptors.[25] Nox–catechin has five hydrogen bonds at residues Trp695, Pro460, Thr541, His476, and Arg478. Arg478 and Trp695 residues act as hydrogen bond donors, while Thr541, His476, Pro460, and Trp695 act as hydrogen bond acceptors. The Nox–FAD complex has five hydrogen bonds at residues Pro460, His459, His476, Arg478, and Thr 462. The residues Arg478 and Thr462 act as hydrogen bond donors, whereas Pro460 and His476 act as hydrogen bond acceptors. The Nox–GKT136901 complex has three hydrogen bonds Trp695, Pro460, and His459, with residues His459 and Trp695 acting as hydrogen bond donors, while Pro460 as hydrogen bond acceptors.

Hydrogen interactions and hydrophobic contacts are two types of receptor and ligand interactions that can show bond stability; the more the number of bonds formed, the higher the complex bond affinity.[26,27] In the interaction between the receptor and the compound, hydrogen bonds will be represented by a green dotted line, while hydrophobic bonds are purple. The existence of competitiveness between ligands and receptors can be observed through the residues bound to the ligands, which indicates that if there are the same or more residues, the ligands will compete with each other.[28]

On the other hand, the interaction of the ligand and the COVID-19 receptor was also seen with a docking simulation. The binding affinity of catechin and remdesivir to the coronavirus protease is shown in Table 2 where the binding affinity of catechin to the coronavirus protease protein is lower than that of remdesivir. Binding affinity is a parameter of protein binding strength to ligands. The smaller the binding affinity, the stronger the ligand and protein bonds. The binding strength or low binding affinity of catechin compared to remdesivir is due to the interaction between the amino acid residues of the coronavirus protease more than remdesivir. Figures 5 and 6 showed six hydrogen interactions (Gly143, Ser144, Glu166, Arg188, Leu141, and Cys145) and three hydrophobic interactions (His41, Met49,

and Met165) of catechin with amino acid residues of corona proteases, whereas remdesivir has only five hydrogen interactions (Gly143, Ser144, Cys145, Glu166, and Gln189) and three hydrophobic interactions (Met165, Leu167, and Pro168). The overlapping contacts of remdesivir with native ligands in the corona protease active site area with amino acid residues Gly143, Ser144, Cys145, Glu166, and Gln189 through hydrogen interactions have led to research on remdesivir as a coronavirus protease inhibitor.Similar interactions observed when catechin was docked against coronavirus proteases, specifically with amino acid residues Gly143, Ser144, Glu166, Arg188, Leu141, and Cys145, suggest catechin's potential as coronavirus protease inhibitors. Figure 5 depicts catechin's contact with coronavirus protease amino acid residues, while Figure 6 depicts remdesivir's interaction with coronavirus protease amino acid residues.

CONCLUSSIONS

According to a docking simulation, catechin, which has antioxidant activity against Nox proteins, which are ROS producers, can also inhibit the coronavirus protease protein, where the inhibition occurs when compared to remdesivir, a drug commonly used as a COVID-19 drug, catechin can help COVID-19 patients'respiratory cells.

Conflicts of interest
There are no conflicts of interest.

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