ISSN: 1410-8917
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 24 (5) (2021): 170–176

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Natural Compounds Activities against SARS-CoV-2 Mpro through Bioinformatics Approaches for Development of Antivirus Candidates

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https://doi.org/10.14710/jksa.24.5.170-176

Article Info

Article history:

Received: 19th May 2020
Revised: 28th June 2021
Accepted: 26th July 2021
Online: 31st July 2021

Keywords:

COVID-19; SARS-CoV-2 Mpro; ACE-2; natural compounds; bioinformatics study

Abstract

Coronavirus infection (COVID-19) caused by SARS-CoV-2 appears as a pandemic that has spread to almost all countries in the world. Antiviral therapy using natural compounds is one alternative approach to overcome this infectious disease. The therapeutic mechanism is proven effective against the main protease (Mpro) of SARS-CoV-2. This research aims to perform bioinformatics studies, including ligand-docking simulations and protein-protein docking simulations, to identify, evaluate, and explore five compounds' activity on SARS-CoV-2 Mpro and their effects against Angiotensin-Converting Enzyme 2 (ACE-2). Protein-ligand docking simulations show kaempferol, flavonol, and their glycosides (Afzelin and Juglanin) and other flavonoids (Quercetin, Naringenin, and Genistein) have a high affinity towards SARS-CoV-2 Mpro. These results were then confirmed using protein-protein docking simulations to observe the ability of five compounds to prevent the attachment of ACE-2 to the active site. Based on the results of the bioinformatics studies, Quercetin has the best affinity, with a binding free energy value of -33.18 kJ/mol. The five compounds are predicted to be able to interact strongly with SARS-CoV-2. The results in this research are useful for further studies in the development of novel anti-infective drugs for COVID-19 that target SARS-CoV-2 Mpro.

1. Introduction

The 2019 coronavirus infection (COVID-19) was first discovered in Wuhan City, Hubei Province, China, and has now become a pandemic due to its rapid spread to 25 countries worldwide [1]. For this incident, the World Health Organization (WHO) announced a global health emergency on 30 January 2020 [2, 3]. In its first emergency meeting, WHO estimated the COVID-19 mortality rate to be around 4% [4]. Collaborative efforts by researchers worldwide are underway to understand the characteristics of the virus that causes this disease, namely SARS-CoV-2 (originally named 2019-nCoV), and develop effective drug candidates to control and prevent it [5].

The SARS-CoV-2 genome consists of ~30,000 nucleotides and functions to encode two overlapping polyproteins, namely pp1a and pp1ab. These polyproteins are needed for virus replication and transcription [6]. Functional polypeptides are released from polyproteins through proteolytic processes involving the main protease (Mpro) [7]. Preliminary studies also show that SARS-CoV-2 has structural similarities to SARS-CoV based on complete phylogenetic analysis of the genome [8, 9]. Besides, both interact directly with Angiotensin-Converting Enzyme 2 (ACE-2) to enter the target cell [10].

ACE-2 can mediate the entry of SARS-CoV-2 into cells so that it acts as a functional receptor for coronavirus. SARS-CoV-2 involves ACE-2 with an affinity comparable to SARS-CoV [11]. The tight bonding in ACE-2 can explain part of the efficient transmission of SARS-CoV-2 in humans, as happened in SARS-CoV [12]. Therefore, the inhibition of SARS-CoV-2 attachment to ACE-2 is a pathway in developing inhibitors for COVID-19 infectious diseases.

Several herbal antiviral agents have been developed to disrupt the viral life cycle [13]. At the beginning of the first appearance of SARS in 2002-2003, around 50% of patients in mainland China were successfully treated using herbal medicines [13]. Kaempferol flavonols and their glycosides (Afzelin and Juglanin), as well as other flavonoids (Quercetin, Naringenin, and Genistein), have been reported to have inhibitory activity against the main protease (Mpro) SARS-CoV or viral replication [14]. Interestingly, all of these compounds are contained in natural Indonesian herbs, such as Afzelin (from *Annona purpurea*, *Piper umbellatum*, *Zingiber zerumbet*, *Nymphaea odorata*, and *Ginkgo biloba*) [15], Juglanin (from *Polygonum aviculare*) [16, 17], Quercetin (from *Camellia sinensis*, *Moringa oleifera*, *Centella asiatica*, *Apium graveolens*, and *Coriandrum sativum*) [18], Naringenin (Citrus species, *Ficus carica*) [19], Genistein (*Flemingia vestita*, Rutaceae family, *Fortunella obovata*, *Erythrina variegata*, *Millettia reticulata*, *Tetracera scandens*, *Genista sessilifolia*, and *Amaryllidaceae species*) [20].

Nowadays, the need to design effective antiviral candidates against SARS-CoV-2 is increasing. This research aims to observe the molecular interactions between several natural compounds in the main protease (Mpro) of SARS-CoV-2 and their effects in inhibiting the binding of ACE-2. Bioinformatics approaches through ligand-protein, and protein-protein docking simulations can be used to identify, evaluate, and characterize potential components of SARS-COV-2 [21]. Specifically, SARS-CoV-2 Mpro is considered a target because it is a major part of forming the characteristics of the coronavirus. Thus, through this research, it is expected to obtain the structure of reference compounds to treat COVID-19 infections.

2. Methodology

2.1. Macromolecule Preparation

Macromolecules used in this research were the main proteases (Mpro) of SARS-CoV-2, and Angiotensin-Converting Enzyme (ACE-2) obtained from Protein Data Bank (http://www.rcsb.org/pdb) with PDB ID 6LU7 [22] and 2AJF [23], respectively. The preparation of these two macromolecules was carried out by removing water molecules and native ligands, adding polar hydrogen atoms, and calculating the Kollman charge using AutoDock 4.2 with MGLTools 1.5.6 [24, 25].

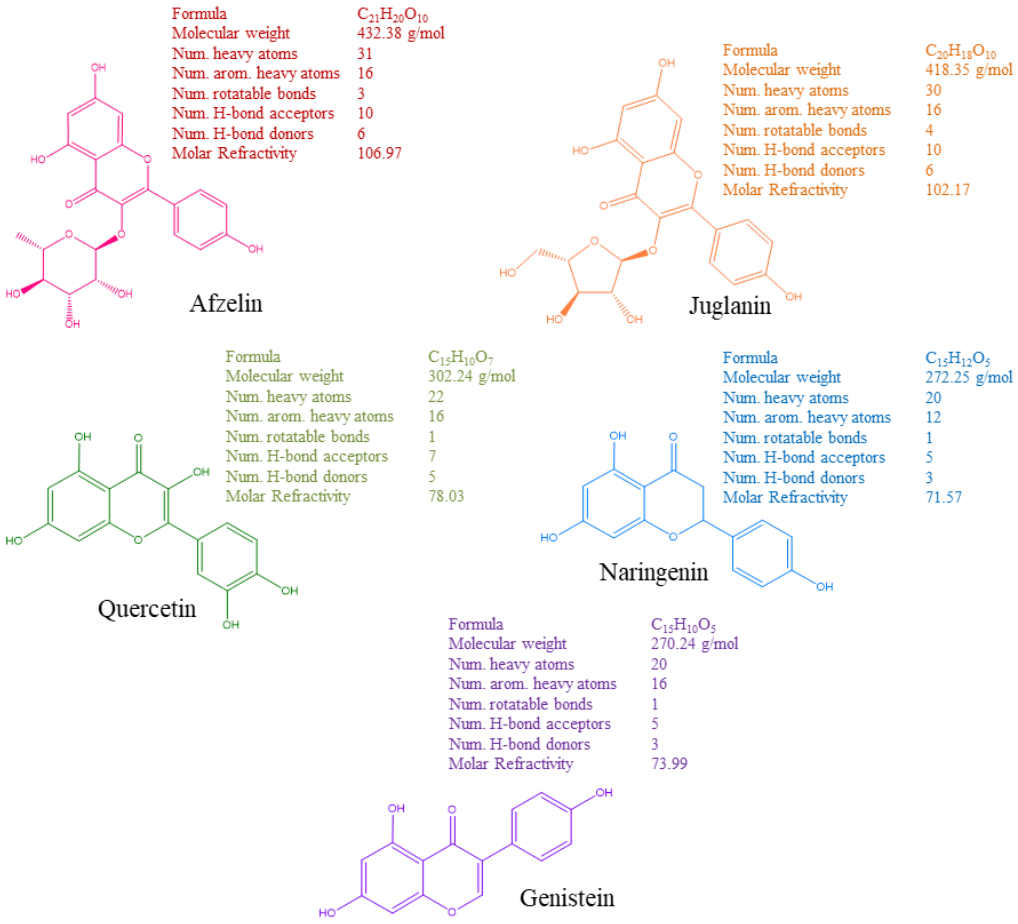

Figure 1. Two-dimensional structure and physicochemical properties of ligands

2.2. Ligand Preparation

The ligands used in this research were Kaempferol flavonol and their glycosides (Afzelin and Juglanin), as well as other flavonoids (Quercetin, Naringenin, and Genistein), obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Figure 1). The ligand structure was optimized using Quantum ESPRESSO 6.6 with Density Functional Theory (DFT) method based on the 3-21G basis set [26, 27]. Finally, all ligands were prepared by adding a hydrogen atom and calculating the Gasteiger charge using AutoDock 4.2 with

MGLTools 1.5.6 [24, 25]. Structural preparation aims to optimize compounds for use as input in ligand-protein docking simulations.

2.3. Identification of Ligand-Binding Sites in SARS-CoV-2 Mpro

Macromolecules of SARS-CoV-2 Mpro that have been prepared are then identified, evaluated, and explored binding sites that are most responsible for biological activity using BIOVIA Discovery Studio 2020 [28]. All amino acids that occur around the native ligand within a spherical radius distance set are prepared for the location of protein-ligand binding in ligand-protein docking simulations.

2.4. Ligand-Protein Docking Simulations

The ligand-protein docking simulations were performed using AutoDock 4.2 with MGLTools 1.5.6 [24, 25]. All ligand molecules for this simulation were added hydrogen atoms and partial charges data from the Density Functional Theory (DFT) calculations. Simulations were created by a grid box 64 × 60 × 60 points with 0.375 Å spacing to cover the binding cavity of the target [29]. The Lamarckian Genetic Algorithm with 100 conformations was chosen for each simulation, and other docking parameters were set as default. Observation of the docking simulation results was performed using Discovery Studio 2020 [28].

2.5. Protein-Protein Docking Simulations

Protein-protein docking simulations were performed using PatchDock for five complexes from the results of ligand-protein docking simulations in SARS-CoV-2 Mpro [30]. The simulations were accomplished against active sites of the Angiotensin-Converting Enzyme (ACE-2) macromolecules, including Gln24, Lys31, His34, Glu37, Asp38, Tyr41, Gln42, Met82, Tyr83, Glu329, Asn330, Lys353, and Gly354. The default clustering RMSD 4.0 Å was used, and the complex type was chosen to be a protein-protein. The Connolly dot surface representation of the molecules into different components such as convex, concave, and flat patches were generated through the PatchDock algorithm. PatchDock was optimized, refined, reshuffled, and rescored the side chain interface of the top 10 candidate solutions. It also amends the orientation of the relative molecules by confining the flexibility to the side-chains of the interacting surface and allow the movements of a small rigid body. The conformation of the systems was verified by visualization analysis with Discovery Studio 2020 [28].

3. Results and Discussions

3.1. Ligand-Protein Docking Simulations

Kaempferol flavonol and their glycosides (Afzelin and Juglanin) and other flavonoids (Quercetin, Naringenin, and Genistein) were docked into SARS-CoV-2 Mpro as a macromolecular target. Moreover, molecular docking of natural ligands was also performed as a comparison against the five tested ligands. All complexes from the results from protein-ligand docking were selected for further studies using protein-protein docking methods against ACE-2. Five ligands have a larger negative binding free energy than natural ligands to the active site of SARS-CoV-2 Mpro (Table 1). This phenomenon shows a promising sign that these ligands have an excellent affinity to the target macromolecules. Quercetin has the best binding at the active site of SARS-CoV-2, with a binding free energy value of -33.18 kJ/mol better than other ligands.

Table 1. Binding Free Energy of Ligands to SARS-CoV-2 Mpro

Ligand	Binding Free Energy (kJ/mol)
Native ligand	-18.58
Afzelin	-27.49
Juglanin	-32.05
Quercetin	-33.18
Naringenin	-30.46
Genistein	-29.33

All the ligands have related interactions against SARS-CoV-2 Mpro (indicated by the binding energy value, which is almost similar) (Figure 2). In general, the interaction of each ligand with SARS-CoV-2 Mpro was dominated by ten hydrogen bonds (with Phe140, Gly143, His163, His164, Met165, Glu166, His172, Gln189, and Thr190) and seven hydrophobic interactions (with His41, Met49, Leu141, Asn142, Met165, Pro168, and Ala191). Interestingly, Quercetin, as the ligand with the best binding free energy, has more molecular interactions than natural ligands. Quercetin can form twelve interactions, including seven hydrogen bonds (with Gln192, Thr190, Thr190, Glu166, His164, Tyr54, and Asp187) and five hydrophobic interactions (with His41, Met165, Met165, Met165, and Pro168). Meanwhile, natural ligands could only form ten interactions consisting of seven hydrogen bonds (with Asn142, Phe140, Glu166, Gln189, Thr190, Asn142, and Leu141) and three hydrophobic interactions (with Met165, Pro168, and Ala191).

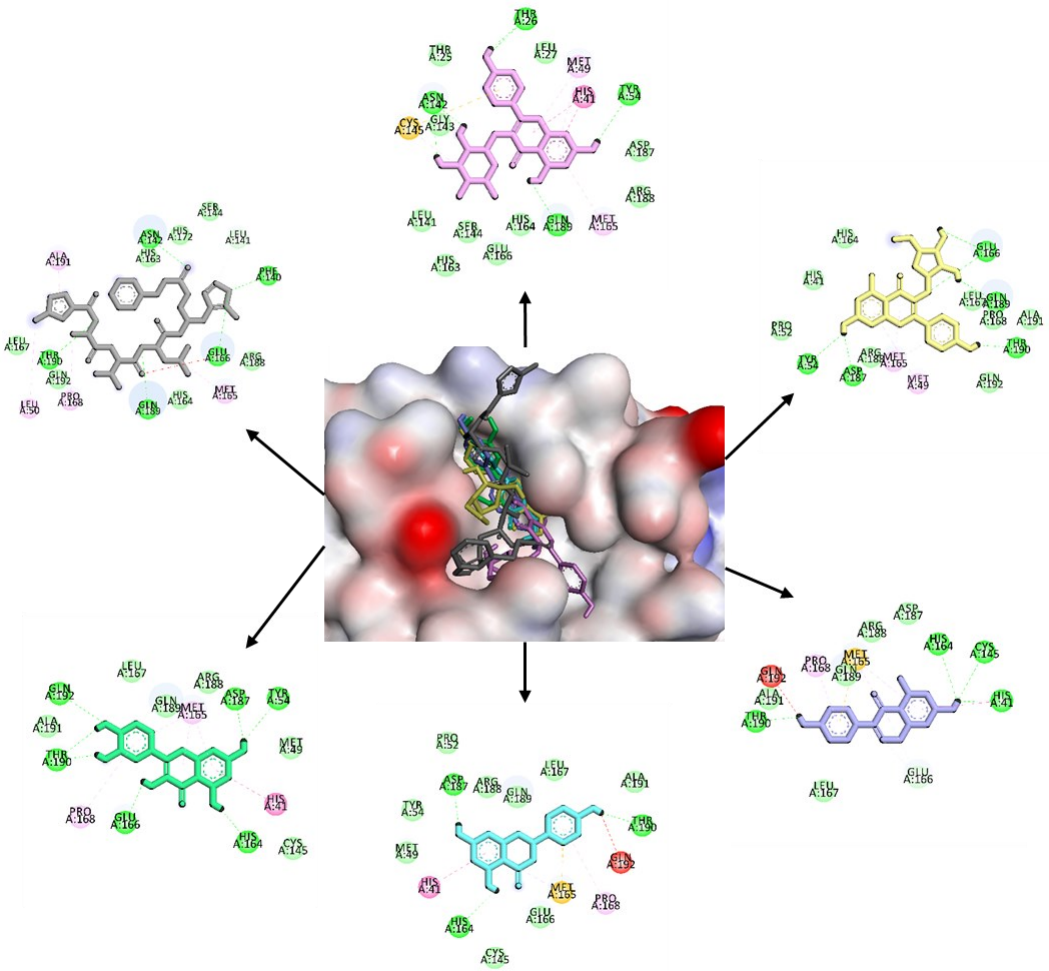

Figure 2. Overlay the docking pose of Afzelin (pink), Juglanin (yellow), Quercetin (green), Naringenin (blue), Genistein (purple), and natural ligand (gray) in the binding site of SARS-CoV-2 Mpro

The molecular interactions that form between molecular ligands and the active site of the macromolecular target consisted of many hydrogen bonds. These ligands act as donor hydrogen bonds and protein amino acid residues as hydrogen bond acceptors. Most hydrogen bonds between protein-ligands were quite strong, with an average bond length below 3 Å. Due to the presence of hydrogen bonds, the interaction between five ligands and SARS-CoV-2 Mpro was also dominated by hydrophobic interactions [31]. It can be predicted that hydrogen bonds and hydrophobic interactions that contribute against macromolecules protein play an important role in stabilizing the complex. The best conformation of ligands to the SARS-CoV-2 Mpro receptor was chosen for performing the protein-protein docking simulations.

3.2. Protein-Protein Docking Simulations

After ligand-docking simulations, further identifications were conducted using protein-protein docking methods. The atomic contact energy evaluated the ACE-2 (ACE) score's binding affinity into the PatchDock [30]. The purpose of such docking simulations was to examine the bonding effect of each ligand with the target protein in preventing the attachment of ACE-2 to the active site of SARS-CoV-2 Mpro. The preparation of ACE-2 macromolecules was demonstrated by removing water molecules and native ligands, adding polar hydrogen atoms, and calculating the Kollman charge using AutoDock 4.2 with MGLTools 1.5.6 [24, 25]. A strong ligand bond to the target macromolecule is predicted to tend to inhibit the entry of coronavirus into cells due to the inability of SARS-CoV-2 Mpro to reach ACE-2 to forward the signal [32]. Therefore, the exploration of amino acid residues responsible for arresting the formation of molecular interactions between the binding sites of SARS-CoV-2 Mpro and ACE-2 is also needed.

Table 2. Atomic Contact Energy of Each Ligand-Protein Complex to ACE-2 in Protein-Protein Docking

Ligand-Protein Complex	Atomic Contact Energy (kJ/mol)
SARS-CoV-2 Mpro	-1172.98
Afzelin + SARS-CoV-2 Mpro	1321.39
Juglanin + SARS-CoV-2 Mpro	1532.14
Quercetin + SARS-CoV-2 Mpro	1684.48
Naringenin + SARS-CoV-2 Mpro	1676.91
Genistein + SARS-CoV-2 Mpro	1654.90
Afzelin + SARS-CoV-2 Mpro	1321.39

Data from protein-protein docking simulation shows that five ligand-protein complexes have positive ACE scores (Table 2). This occurrence can be caused by unfavorable interactions between each ligand-protein

complex against ACE-2 [33]. Interestingly, Quercetin effectively inhibited the attachment of ACE-2 to the binding area of SARS-CoV-2 Mpro because it has the largest positive ACE, with a score of 1684.48 kJ/mol. Most inhibitors work by binding firmly to the active sites of the target receptor and competing with its native ligand, as well as stabilizing the structure of the receptor macromolecules and preventing the conformational changes needed to progress the signal further.

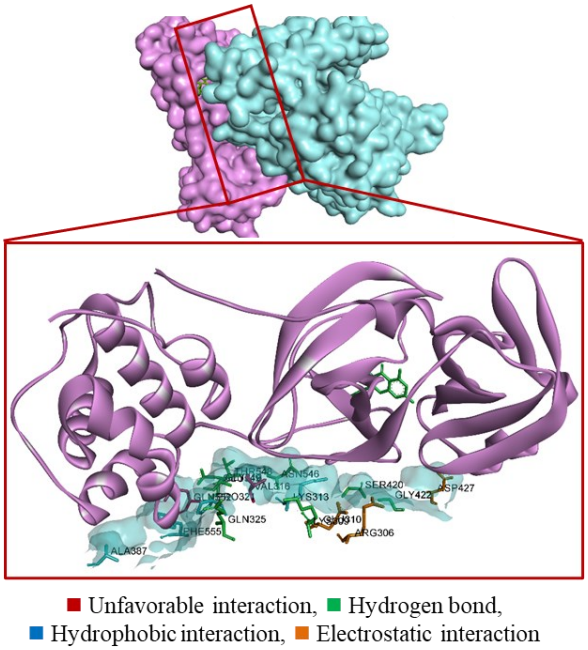

Figure 3. Protein-Protein Docking Pose of Quercetin Complexes (pink) against ACE-2 (blue)

Subsequent analysis was performed on the conformation of the Quercetin + SARS-CoV-2 Mpro complex with ACE-2 (Figure 3). Ten unfavorable bonds were formed, namely the amino acid residues Lys313, Val316, Val316, Ala387, Gln552, Gln552, Gln552, Gln552, Phe555, and Pro321. Besides, other interactions that contribute include thirteen hydrogen bonds (with Arg306, Asp427, Glu310, Lys309, Thr548, Gln325, Thr548, Glu549, Asn546, Gly319, Gly319, Ser420, and Gly422), six hydrophobic interactions (with Lys313, Pro321, Ala387, Lys313, Pro321, and Phe555), and nine electrostatic interactions (with Arg306, Asp427, Asp427, Glu310, Glu310, Asp427, Arg306, Lys313, and Phe555). Eventually, it can be predicted that Quercetin is a potential candidate for SARS-CoV-2 Mpro inhibitor and can prevent attachment to ACE-2, which acts as an entry point into human cells.

4. Conclusion

Several compounds of kaempferol, flavonol, and glycoside derivatives (Afzelin and Juglanin) and other flavonoids (Quercetin, Naringenin, and Genistein) can bind stably against SARS-CoV-2 Mpro. However, Quercetin has the strongest interaction with the target macromolecular active site, with the binding free energy value of -33.18 kJ/mol. The five ligands are also able to inhibit the formation of interactions with ACE-2. Thus, the study results indicate that the compound can be further developed as a SARS-CoV-2 Mpro inhibitor in COVID-19 infection therapy.

Acknowledgment

The authors thank the LPPM (Institute for Research and Community Service), Universitas Islam Bandung, for the research financially supported by the Special Research Grant Program 2020, No.039/B.04/LPPM/IV/2020.

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