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Utilization of Quercetin Flavonoid Compounds in Red Onion (*Allium cepa*) as Inhibitor of Spike Sars-CoV-2 Protein against ACE2 Receptors

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Abstract. The world is facing the challenge of the COVID-19 disease, which is now stated as a pandemic. Inside the host cell, spike envelope protein (spike) of SARS-CoV-2 interact with the Angiotensin-converting Enzyme 2 (ACE2) receptor. It can be inhibited by bioactive compounds such as flavonoids which have anti-viral and broad pharmacological effect. This study aimed to determine the spike protein inhibitory activity by quercetin against the ACE2 receptor using the molecular docking method. This study focused on the inhibitory of the penetration activity of s proteins in ACE2 by utilizing natural material compounds that have the potential to be used as anti-SARS-CoV-2 drug development agents. The flavonoid compound quercetin was extracted by the maceration method. The quantitative analysis was carried out using a UV-Vis spectrophotometer to prove the presence of quercetin content. Molecular docking simulations were carried out to look for the binding affinity between the spike protein and quercetin. Docking was carried out using the Autodock, PyRx, and visualization using Discovery Studio. Indicators that prove that quercetin forms binding affinity and protein complexes with spikes are the Vina Score and RMSD. It is also supported by ADME analysis and conformity to Lipinski's rules of five. That matter becomes a success indicator of the spike activity inhibition by quercetin, which makes it possible to be used as an anti-SARS-CoV-2 drug development agent. The novelty from this study is molecular docking method that used to show that quercetin in red onion had inhibitory activity on the penetration process of protein spike in SARS-CoV-2. Results obtained from this study can be used as a recommendation for advanced research in invitro and invivo studies as a drug which has potential to inhibit protein spike of SARS-CoV-2.

Key words: SARS-CoV-2, quercetin, maceration, docking, anti-SARS

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INTRODUCTION

Acute respiratory infection, also known as SARS (Severe Acute Respiratory Syndrome), is an infectious disease that causes respiratory system failure. This disease has basically never been identified in humans, but in certain animals as reservoirs. The case of COVID-19 appeared and attacked humans for the first time in Wuhan Province, China, and entered Indonesia in mid-March 2020. The cause of COVID-19 is the SARS-CoV-2 virus or commonly called as the coronavirus, the virus will attack the respiratory system. Coronavirus transmission starts from the respiratory route in the form of droplets and in many cases is caused by physical contact with sufferers (Xu et al., 2020). Inside the host cell, the spike envelope (S) protein of SARS-CoV-2 can interact with the Angiotensin-converting Enzyme 2 (ACE2) receptor (Zheng et al., 2020). The homolog of Angiotensin-converting Enzyme (ACE), namely ACE2 can balance the function of ACE itself. The similarity of the nucleotide base sequence of the ACE2 receptor with the Angiotensin-converting Enzyme (ACE) is 42% (Zheng et al., 2020). In almost all tissues in the human body, ACE2 contained in type-1 membranes is expressed and activated (Long et al., 2020). The highest content of ACE2 is found in the respiratory, digestive, and heart tracts. Thus, an important role in the susceptibility and resistance to SARS-CoV-2 infection is held by genetic variations in the ACE2 receptor (Ikawaty, 2020; Magrone & Jirillo, 2020).

The role of ACE2 as a SARS-CoV-2 receptor has led many researchers to develop ways to inhibit the interaction between the SARS-CoV-2 S protein and ACE2 (Bourgonje et al., 2020). Therefore, through this research, we are trying to develop the use of natural ingredients that could be used as a solution by utilizing the flavonoid compound quercetin in red onion to inhibit the interaction of the SARS-CoV-2 S protein with ACE2 in the human body (Xu et al., 2020). This bioactive compound is believed to have anti-viral properties and based on its abundant

availability it has the potential to be developed as an anti-SARS drug agent (Fischer et al., 1997; Parthasarathy et al., 2021). Previous research found that quercetin inhibited influenza infection with a wide spectrum of strains, specifically the entry of the H5N1 virus (Wu et al., 2016). High levels of quercetin can be found in red onion plants (Petrus et al., 2011). Quercetin is classified as an antioxidant compound, so it could act as an inhibitor of the oxidation of other molecules (Williams et al., 2004). With the chemical substructure of polyphenols, quercetin can take free radicals in the oxidative chain reaction so that the oxidation reaction could be stopped. In addition, it was reported that quercetin could change the activity of a number of proteins to be active or inhibited (Bao & Fenwick, 2004).

The main objective of this study was to prove the presence of quercetin content in red onion using the maceration method and analyze the inhibition process of the SARS-CoV-2 spike protein by quercetin in red onion (*Allium cepa* L.). This is a new innovation related to the supplements or drugs making based on natural ingredients from red onion as a preventive and therapeutic effort for SARS-CoV-2 in handling the pandemic. With this research, we expected to be able to provide information regarding on the use of quercetin compounds in red onion as an inhibitor of the SARS-CoV-2 spike protein which has a high affinity for the ACE2 receptor in the human body. The expected implication is the benefits that could be felt by community, such as disclose opportunities for the use of natural materials as a preventive action against coronavirus infection in humans and encouraging creations that could be developed for further research. This study was also expected to show that there is a scientific evidence that quercetin in red onions has the potential to be developed as a drug agent against SARS-CoV-2.

METHODS

Equipment and material research

The tools used in this research included scientific journals, laboratory tools such as; dehydrator, chopper, rotavator, beaker, funnel, stirring rod, and spectrophotometers. In silico studies used AutoDock applications, Vina Wizard, PyRx, PyMol, and the Discovery Visualizer 2021 application. The materials used were 98% methanol solvent, 96% ethanol, 2% aluminum chloride, potassium acetate, a yield of a red onion quercetin extract, the 3D structure of spike (S) protein (GDP-ID: 6VSB), and quercetin (CID: 5320844).

Research procedure

The research model used was a blended research model that combines virtual and laboratory, research models. Virtual research using the in silico method, namely molecular docking and laboratory research was conducted at the Diponegoro University Integrated Laboratory. The time required for this research was 4 months.

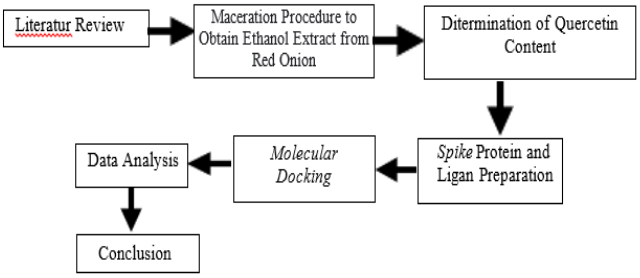

Figure 1. Research stages procedure

Literature review

A literature review or literature studies in this research were carried out on references from journals, both national and international journals that support the research needs. The literature taken was literature related to the interaction of quercetin compounds with spike proteins in SARS-CoV-2. Journals or scientific articles used as study material in the literature study were obtained from accredited journal publishers such as Elsevier, Springer, and the National Center for Biotechnology Information (NCBI). The literature study was conducted by reviewing, comparing, and reviewing previous research related to the search for viral glycoprotein inhibitors and flavonoid compounds that have the potential for drug development. This literature study was a source of support for laboratory research activities.

Maceration procedure to obtain ethanol extract from red onion

The red onion maceration process used 96% methanol and 98% ethanol as solvents. This process aimed to extract the quercetin contained in red onion using the solvent without a heating process. A total of 1 kg of red onions were roughly chopped using a chopper and then dehydrated using an oven at 46 ºC for 16 hours to maintain the flavonoid content in it. The simplicia powder obtained was filtered using a 100 mesh sieve and a dry powder with 300 grams net weight was obtained. The simplicia powder was soaked in a solvent and stirred periodically for 5 days. The liquid extract was collected and then concentrated using a rotary evaporator to obtain a thick extract (Aminah et al., 2017).

Determination of quercetin content of methanol extract of red onion samples

The measurement of quercetin levels used a quercetin standard solution with concentration series of 6, 8, 10, 12, and 14 ppm. The absorbance was determined by the measurement results on a UV-Vis spectrophotometer. The standard curve was made to obtain a linear equation that will be used to determine the percent content. The wavelength absorption measurement used a wavelength of 435 nm. This wavelength was used to measure the absorption of the ethanol extract of the red onion samples.

Spike and ligand protein preparation

The 3D structure of the ligands was downloaded from the PubChem database page in .sdf format and converted to .pdb format with the PyMol application. The ligand used was quercetin (quercetin 4'-O-beta-glucoside) with CID code: 5320844. The receptor structure of SARS-CoV-2, namely S protein, was taken from the 3D structure from the Protein Data Bank page with the code PDB- ID: 6VSB. The preparation of protein receptors and ligands was carried out with the AutoDock application by removing residues in the polypeptide chain, removing water, and adding polar hydrogen along with Gasteiger charges. The protein strand used was the A strand which is the active site of SARS-CoV-2 binding. After that, through the same application, the receptor proteins and ligands were converted into pdbqt extensions for docking.

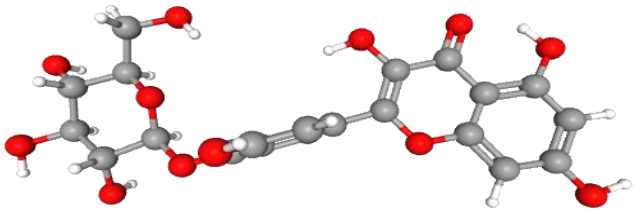

Figure 2. Quercetin visualitation

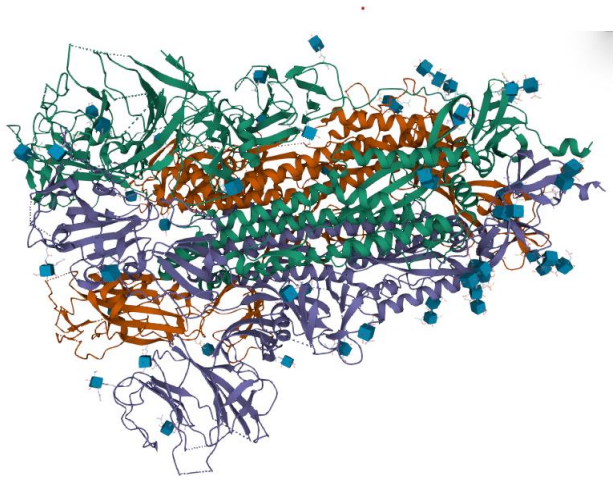

Figure 3. Spike protein visualization

Molecular docking

This study used a flavonoid class of phytochemicals, namely quercetin which was selected as a ligand to investigate its binding affinity to the SARS-CoV-2 spike protein A chain as a target receptor protein (Ge et al., 2013). The preparation of ligands and receptors was carried out using the AutoDock application, both the ligand and the receptor were removed from the water and then the polar poles and Gasteiger charge were added. The docking process was carried out using the PyRx application which is integrated with the Vina Wizard. Grid set was automatically by app with grid dimensions 82.89 Å , 79.91 Å , 168.06 Å (Kiran *et al.*, 2020; Pandey et al., 2020).

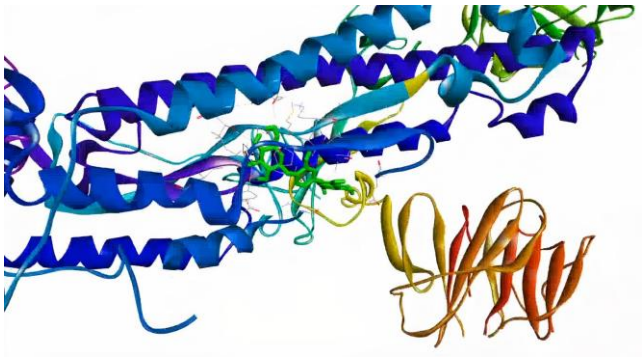

Figure 4. Visualization of spike proteins with ligands

Data analysis

Data analysis was carried out by reviewing the results of quantitative analysis of red onion flavonoid extracts that had been tested through the spectrophotometric stage. The indicators analyzed were wavelength, absorbance, and standard curves. Analysis of molecular docking data was carried out by reviewing the docking results in the form of Vina Score and RMSD values. The Vina Score value states the binding affinity value between spike protein and ligand interactions. The lower the bond energy, the greater the bond affinity. In this case, it could be concluded that the bond energy is inversely proportional to the bond affinity. Another thing that is also become a success indicator of molecular docking is by looking at the RMSD value. The RMSD value indicates the accuracy or validity of the molecular docking results (Kiran et al., 2020; Pandey et al., 2020). If the RMSD value is less than 2.0, then the docking result is declared valid.

The rules that define a bioactive can be categorized as having the opportunity to be developed as a drug development agent, namely in the form of absorption, distribution, metabolism, and excretion (ADME). According to Pandey et al, (2020), in order to ensure that the results of the docking analysis between S protein and quercetin which have been carried out

have potential as an anti-SARS drug development, the results have to meet Lipinski's five rules, namely; (a) molecular mass of < 500 Daltons, (b) degree of lipophilicity expressed as LogP ≤ 5, (c) less than 5 hydrogen bond donors, (d) less than 10 hydrogen bond acceptors, (e) molar refractivity between 40 and 130. These parameters matched the results of the ADME analysis.

RESULT AND DISCUSSION

Literature studies or narrative studies were carried out by reviewing articles from journals and references that are related to the interaction of quercetin compounds with spike protein in SARS-CoV-2. This literature study is a source of support for laboratory research activities. The results of our narrative study showed findings in the form of facts and secondary data, that quercetin is capable and potentially useful as an anti-SARS drug development agent because of its inhibitory properties. To prove this, we conducted a molecular docking study and laboratory experiments to collect primary data.

In the measurement of the quercetin flavonoid compound, AlCl3 was added to the sample solution which can form a complex, resulting in a shift in wavelength towards the visible (visible) which is indicated by the solution producing a more yellow color. The addition of potassium acetate which aims to maintain the wavelength in the visible region (visible). Incubation treatment for 1 hour before measurement is intended so that the reaction runs perfectly, so that the intensity of the resulting color is more maximal. The absorbance value of the red onion extract was entered into the quercetin standard curve equation, namely y = 0.0088x - 0.0954. The flavonoid quercetin ethanol extract of red onions obtained was 4.268 mgQE/gram. The result of quercetin content measurement is presented in Table 1.

Table 1. Quercetin content in thick methanol extract

Parameter analysis	Score	Unit
Total flavonoid	4.245	mg QE/g
	4.268	

After that, a graph of the relationship between absorbance (A) vs concentration (c) is made, so that a straight line equation is obtained y = mx + c , where y = A (absorbance), m = ab (absorbivity times the thickness of the cuvette 10 mm) and x = c (concentration). The straight line equation obtained is y= 0.0088x + 0.0954. The results are shown in Table 2.

Table 2. Results of quantitative analysis of quercetin samples with spectrophotometer.

Absorbance	Weight (g)	Volume add (mL)	QE Concentration (ug/mL)	DF (Dilution Factor)	ug QE/g	mg QE/g	Mean
0.265	0.106	1	45.0	10	4245.283	4.24528	4.25708
0.267	0.106	1	45.3	10	4268.868	4.26887	

Quercetin chemically has the name of 2-(3,4-dihydroxy phenyl)-3,5,7-trihydroxy4H-chromen-4-on Quercetin, which is a flavonol compound or is a flavonoid derived from plants, such as red onions (Patil & Salunkhe, 2012). Based on Suharyanto, et al (2020), the quercetin obeys Beer Lambert's law, which is in the concentration range between 45.0-45.3 g/ml at a wavelength of 435 nm. Quercetin showed good linearity between concentration and absorbance. It is evidenced that the UV Spectrophotometry method used for the determination of quercetin in red onion samples is simple, precise, and accurate.

According to Pandey et al (2020), the quercetin has been known to have various biological activities, such as antioxidant, anti-inflammatory, antiulcer, and neuroprotective effects. Red onions are a major source of quercetin and other flavonoid compounds. Quercetin is mostly bound to the sugar moiety in the form of quercetin glycosides, and its presence will increase when hydrolysis occurs by acidic compounds. When the time used for the hydrolysis process is increased, there will be an increase in the total quercetin obtained. Acid hydrolysis was carried out in this study to convert the glycosidic form of quercetin into aglycone form. The total amount of quercetin increased in the extract after acid hydrolysis.

Quercetin showed the lowest and identical binding energy yield of -7.5 kcal/mol. According to this result, quercetin formed hydrogen bonds with amino acids PRO 1057, SER 730, THR 778, and GLN 853. In addition, it also formed Pi-Alkyl bonds with amino acids VAL 860 and ALA 956. Pi-cation bonds were also found in amino acids HIS 1058 on the active group of the A strand of the SARS-CoV-2 spike protein.

Hydrogen bonding interactions are essential for stable ligand binding at protein binding sites. The average number of hydrogen bonds between protein-ligand, ligand-water, and residue-water binding sites is visualized in figure C. In addition to hydrogen

bonding between protein and ligands, water-mediated hydrogen bonding provides extra stability to the protein-ligand system. Although similar hydrogen bond interactions were observed for all complexes, the difference in hydrogen bond occupancy values might be due to the possibility of obtaining various conformations during the simulation (Venugopal & Chakraborty, 2020). The concept of pi-cation interactions offers the use of systems in small molecules to increase binding affinity, specificity, selectivity, lipophilicity, bioavailability, and metabolic stability, which are desirable physiochemical features for drugs and pesticides (Liang & Li, 2018). Whereas pi-alkyl interactions are relationships that help increase the electrostatic contribution to binding affinity. During molecular dynamics simulations, H-bonds are rarely encountered with no major influence on bond energies. In general, pi interactions play a role in inhibitor binding because they are not affected by olvation/desolvation. Therefore, the enthalpy/entropy of punishment on the free binding energy can be minimal (Bernaldez et al., 2018).

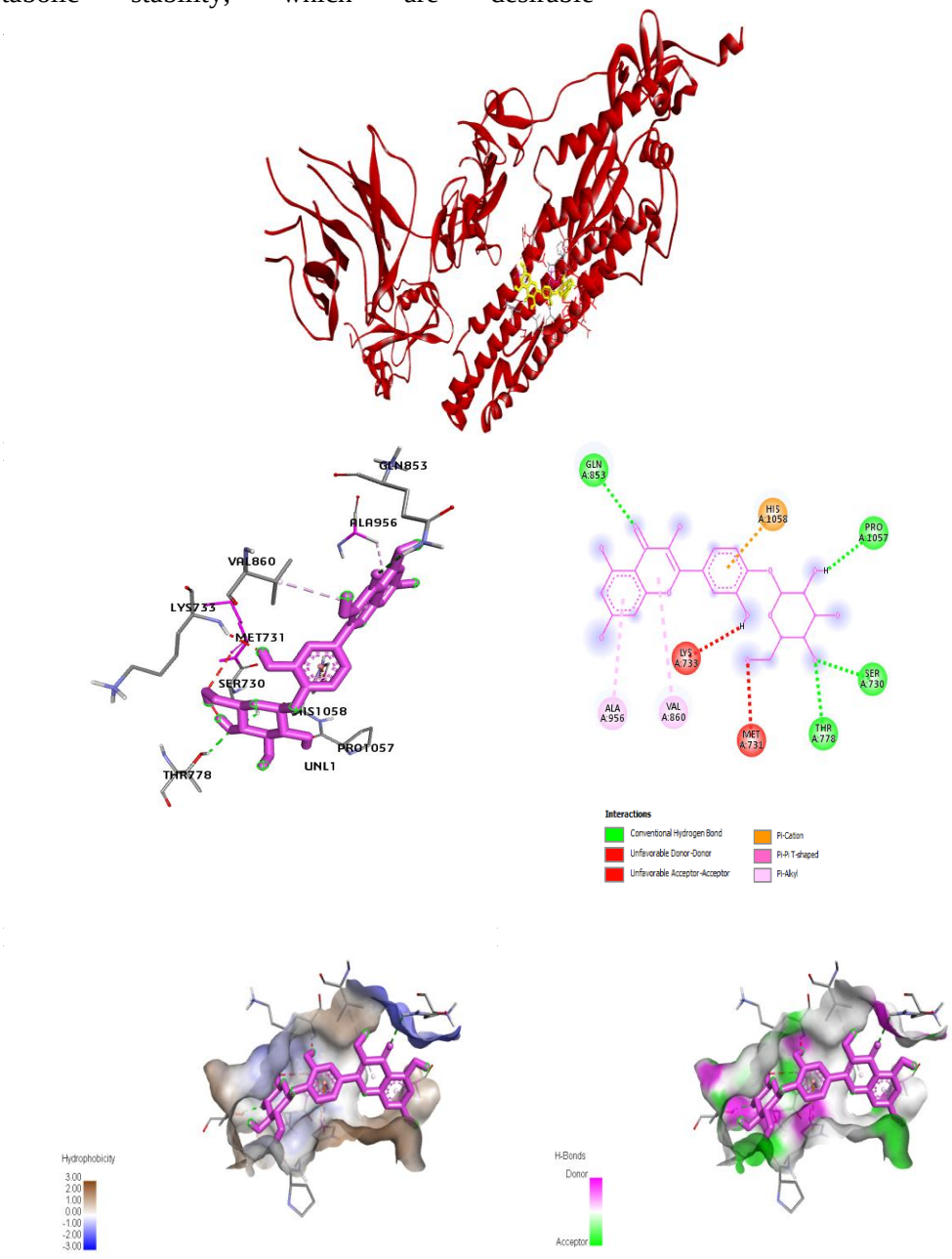

Figure 5. Visualization of MD results. (A) 3D visualization of the protein-ligand complex showing the binding site chain A. (B) 3D diagram of the protein-ligand complex showing the binding of the ligand to the amino acid in chain A. (C) 2D diagram showing the type of bond between the amino acid and the ligand. (D) Visualization of the degree of surface hydrophobicity of protein-ligand complexes. (E) Surface visualization of protein-ligand complexes based on donor-acceptor hydrogen bonds.

Table 3. Vina score between spike protein and quercetin

Ligand	Binding Affinity (kcal/mol)	Mode	RMSD Lower Bound	RMSD Upper Bound
Ligand complex_1	-7.7	0	0.0	0.0
Ligand complex_2	-7.6	1	3.04	5.167
Ligand complex_3	-7.6	2	53.459	56.784
Ligand complex_4	-7.6	3	51.061	54.975
Ligand complex_5	-7.5	4	1.896	10.009
Ligand complex_6	-7.4	5	66.8	68.356
Ligand complex_7	-7.4	6	1.602	10.019
Ligand complex_8	-7.3	7	46.83	48.736
Ligand complex_9	-7.3	8	52.977	55.64

Molecular docking analysis in tracing the bioactive compound quercetin shows promising activity against the ligand-protein S protein complex. Thus, in order to get a better understanding of the conformational stability of ligand binding and the contribution of the active site in terms of binding free energy, we performed molecular docking simulations in the dissolved state at physiological temperature for 100 ns. The RMSD value obtained was 1.896, so it can be concluded that the results of the molecular docking are valid. The bioactive compound selected as the ligand, namely quercetin, is found to meet the criteria required by Lipinski's five rules based on the results of ADME analysis; (a) a molecular mass of 302 Daltons, (b) a degree of lipophilicity expressed as LogP 2, (c) 5 hydrogen bond donors, (d) 7 hydrogen bond acceptors, and (e) a molar refractivity of 74.

The novelty from this study is a molecular docking method which used to determine the inhibitory activity of quercetin on protein S. The formation of a complex between quercetin and protein S indicated that in the docking simulation, quercetin could be an inhibitor of the SARS-CoV-2 protein S. The benefit of this study is providing baseline data which can be used for in vivo and in vitro tests for the development of anti-SARS-CoV-2 drugs from natural compounds. Quercetin in red onion that has been extracted can form a complex with protein S of SARS-Cov-2 so it could not bond with ACE2 receptor, penetrated and proliferated in target cell.

CONCLUSION

Quercetin from red onions is one of bioactive compounds that have the potential to be developed as anti-SARS-CoV-2 drugs. The results of molecular docking analysis showed a good binding affinity between spike protein and quercetin. The bioactive of quercetin fulfills Lipinski's five rule and ADME criteria so it can be used as an anti-SARS-CoV-2 drug development agent.

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