Potential of betacyanin as inhibitor of SARS-CoV-2 revealed by molecular docking study

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Abstract. Covid-19 is a global pandemic where an effective drug has yet to be found. A new coronavirus species, SARS-CoV-2 causes this disease. Several studies have been conducted on medicinal plant-based lead compounds to find antidotes for this virus. One of the fruits that with a high betacyanin content is super red dragon fruit produced by plant Hylocereus costaricensis. Betacyanin, besides having anti-inflammatory and immunomodulatory activities, also has antiviral activity. Therefore, this study aimed to evaluate betacyanin’s interaction with several SARS-CoV-2 receptors by observing its binding affinity and compared it with the nelfinavir and hydroxychloroquine sulfate that have been recommended for treating COVID-19. This research was an in silico study using computer software to assess binding affinity simulations based on molecular docking. The results of this study indicated that betacyanin had a good affinity with several receptors so that it has the potential to be developed as a lead compound to overcome COVID-19. Based on its binding affinity value, betacyanin’s ability was comparable to nelfinavir and hydroxychloroquine sulfate recommended by WHO as a therapeutic agent for COVID-19.

1. Introduction
The COVID-19 pandemic is still a global problem because there has been no cure for this disease until now. Several countries are in a race to find a vaccine against this disease. So far, this disease has caused a high global mortality rate, so drug discovery is being carried out on various lines, one of them is from the natural product of metabolite from a plant. The metabolite is produced either by itself
[1–3] or by endophyte [4] and available in almost all plant parts [5]. As the third-highest biodiversity country, Indonesia provides many types of plants that have potential as medicinal plants. However, there are only more or less 300 types of plants that have been investigated and used, and even then are as herbal medicine. Ethnobotany, ethnomedicine as well as laboratory studies have been conducted on the ability of local Indonesian plants that have potential as an antioxidant [6], antimicrobial and antiviral [2,5,15,16,7–14]. Tallei et al. [17] have studied the potency of several plants as SARS-Cov-2 inhibitors.

The plant *Hylocereus costaricensis* produces a fruit called super red dragon fruit. This fruit, which has a dark red color, is rich in antioxidants, which is caused by the pigment betacyanin, a red-violet pigment belongs to a class of plant pigment called betalain [18]. Betalain from the plant vacuoles has been accepted as a food additive [19] Betacyanin is also often used as a natural dye [20]. The peel contains the betacyanin pigment ranging from 115.61 to 118.97 g/kg [21], so it is a potential source of antioxidants.

Several studies have shown that the betacyanin pigment from red dragon fruit has antiviral [22–24] and immunomodulatory [25] activities. Zang et al. [26] reported that antioxidant exhibit potential antiviral agents for Japanese encephalitis viral infection. Referring to several important benefits of betacyanin, then this study aimed to evaluate the potential of betacyanin as an inhibitor of SARS-CoV-2, causing COVID-19 disease.

2. Material and Methods

2.1. Determination of Receptors

Four essential proteins from SARS-CoV-2 were selected as receptors in this study were Main Protease (Mpro) (also called 3C-like protease - 3CLpro) (PDB code: 6LU7), Spike Glycoprotein (S) (closed state) (PDB code: 6VXX), spike ectodomain structure (open state) (PDB code: 6VYB), and receptor binding domain (PDB code: 6YLA).

2.2. Ligand and Receptor Preparation

Three-dimensional structures of the receptors were retrieved from Protein Data Bank (http://www.rcsb.org/pdb). BIOVIA Discovery Studio Visualizer 2020 was used to open the receptors files. After removing the water molecules and native ligands, the receptors were stored in .pdb format. Then polar hydrogen atoms were added to the receptors using Autodock Tools, and saved in .pdbqt format.

Betacyanin acted as a ligand, and its structure was obtained from PubChem (http://pubchem.ncbi.nlm.nih.gov) (Figure 1). Using Open Babel, the .sdf format of the ligand was converted into .pdb format. After adjusting the torque, the file was saved in .pdbqt format.

![Chemical structure of betacyanin](image-url)
2.3. Receptor-Ligand Docking and Visualization

Autodock Vina was used for the docking process. The .pdbqt formats of the ligands and receptors were copied into the Vina folder. The vina configuration was typed in notepad and saved as conf.txt. The Vina program was subsequently run through the command prompt. Evaluation of the docking results was performed by selecting the best pose which showing the most negative Gibbs’ free energy of binding (-ΔG). Visualization was done in Biovia Discovery Studio 2020.

3. Results and Discussion

Biological research has experienced the enormous surge in the last century, including the structure of several macromolecules unleashed, chemical nature of DNA has been discovered, and the genome sequences of many organisms were entirely or partially determined. Bioinformatics is regarded as a groundbreaking, improvised area of biological science, which uses computational approaches for answering questions related to molecular and structural biology. With the availability of sequences and structures, biomedical parameters are now largely predicted by implying bioinformatics attributes [27–29]. Molecular docking is considered as a crucial tool in the field of bioinformatics, which predicts the predominant binding modes of small molecules, which are termed as a ligand, with a three-dimensional protein structure [30].

Recently, it has been demonstrated that molecular docking is more accurate than high-throughput screening, which is also a dominant approach for lead discovery [31]. In the current experiment, we have utilized molecular docking simulation to predict the interaction amongst betacyanin with four respective targets of SARS-CoV-2, namely Main Protease (M<sup>pro</sup>) (6LU7), Spike Glycoprotein (S) (closed state) (6VXX), spike ectodomain structure (open state) (6VYB), and receptor binding domain (6YLA). The results of this study indicated the strong interaction between betacyanin and receptors of SARS-CoV-2. The results for the binding affinity are presented in Table 1. Figures 2-5 A display the 3D results of the docking analysis showing the interacting amino acid of the receptors with betacyanin, while Figure 2-5B displays 2D results showing the type of contact formed between the receptors and the ligands. All receptors show a variety of interactions with ligands, both in the form of van der Walls interactions and hydrogen bonds.

Table 1. Binding analysis of ligand betacyanin with SARS-CoV-2 receptors

| Ligands          | PubChem CID | Binding Free Energy kcal/mol |
|------------------|-------------|------------------------------|
|                  |             | 6VXX | 6LU7 | 6VYB | 6YLA |
| Nelfinavir       | 64143       | -8.8 | -8.2 | -8.5 | -8.9 |
| Hydroxychloroquine sulfate | 12947 | -7.3 | -6.6 | -7.2 | -6.9 |
| Betacyanine      | 6324775     | -9.2 | -7.4 | -8.6 | -9.5 |

The current experiment’s findings delineated that betacyanin had shown a greater interaction towards the spike glycoprotein receptor-binding domain, possessing a docking score of -9.5kcal/mol and less interaction was represented for M<sup>pro</sup> (docking score: -7.4kcal/mol). Chella Perumal et al. [32] stated that the least binding energy shown by ligands and receptors indicates a strong interaction. Betacyanine had an excellent binding affinity with 6VXX and 6YLA, with docking scores of -9.2 and -9.5 kcal/mol.

Three parameters that must be taken into account when calculating the interaction results including binding affinity, H-bond, and residual interaction [33–35] Interactions between ligands and macromolecular amino acid residues are formed as hydrogen bonds, hydrophobic interactions, and electrostatic interactions (Tabel 2). Hydrophobic interactions are in the form of Akyl / Pi-Alkyl bonds, and electrostatic interactions are in the form of Van der Waal's interactions [36].

The electrostatic interaction in the form of a salt bridge is a salt bond between groups of opposite charges in the amino acid side chains and ligand groups [37]. Whereas van der Waal's is a relatively
weak electric attractive force due to permanent or induced polarity of molecules [38]. Table 2 shows that each ligand forms several interactions with its receptor, where each ligand has hydrogen bonds with the receptor. The more hydrogen bonds formed with amino acid residues, the bonds will be stronger, and the energy score will be lower and more stable [39]. Table 2 also shows that the most hydrogen bonds were between betacyanin and 6YLA at residues Gln-H:1, Tyr-A:369, Gly-L:63, Thr-B:0, Val-L:64 and 6LU7 at residues Lys-A:137, Asn-A:238, Thr-A:199, Glu-A:288, dan Leu-A:287. However, binding affinity showed that the best poses were with 6YLA and 6VXX. Betacyanin has four hydrogen bonds with 6VXX at residues Thr-C:912, Arg-B:1091, Thr-B:1117, dan Arg-A:1091).

Hydrophobic interactions play a role in determining the stability of ligands against receptors. Hydrophobic interactions are interactions that avoid the liquid environment and tend to cluster together in the globular structure of proteins [40]. Residues involved in hydrophobic interactions are residues of nonpolar amino acids. Nonpolar (hydrophobic) amino acid residues tend to form clusters in the interior of proteins.

There was also an unfavorable bond in the interaction of betacyanin and these four receptors, which can harm the bond’s strength between interacting molecules, indicating that there is a repulsive force between the two molecules. The formation of these bonds influences the stability of the ligands that will be used as drug candidates, because this type of bond can reduce the stability of other types of bonds [41].

Spike Protein is a potential receptor target for the discovery of new types of drugs [42]. In Spike Protein (closed state), betacyanin had amino acid residue bonds in the form of seven van der Waal's interactions, four hydrogen bonds, while Spike Ectodomain Structure (open state) had 12 van der Waal's interactions, two hydrogen bonds, and one Pi-Alkyl bond. Receptor Binding Domain is a class of Spike Glycoprotein receptors that have heavy chains and light chains. The binding of Betacyanin ligands to these receptors contained nine van der Waal's amino acid residues, five hydrogen bonds, three attractive charge and pi-cation bonds, and three unfavorable positive-positive and unfavorable donor-donor.

According to research by Tahir ul Qamar et al. [43], the binding site area of M\(^\text{pro}\) was located on the active side of Cys-145, Lys-137, and His-41. The ligands that bind this receptor’s active sites can significantly inhibit the performance of the receptor. The interaction in the betacyanin and M\(^\text{pro}\) exactly occurred in one of these amino acid residues, namely the Lys-137 residue, where the residue is included in the type of hydrogen bond. This shows that betacyanin binds strongly to M\(^\text{pro}\) right on the active site.

![Figure 2](image-url) **Figure 2.** (A) The AutodockVina output which shows the interacting amino acid residues Spike Glycoprotein (closed state) (6VXX) with the ligand betacyanin (B) 2D diagram showing the types of contacts formed between receptor and ligand.
Figure 3. (A) The AutodockVina output which shows the interacting amino acid residues Spike Ectodomain Structure (open state) (6VYB) with the ligand betacyanin (B) 2D diagram showing the types of contacts formed between receptor and ligand.

Figure 4. (A) The AutodockVina output which shows the interacting amino acid residues Receptor Binding Domain (6YLA) with the ligand betacyanin (B) 2D diagram showing the types of contacts formed between receptor and ligand.

Figure 5. (A) The AutodockVina output which shows the interacting amino acid residues Mpro(6LU7) with the ligand betacyanin (B) 2D diagram showing the types of contacts formed between receptor and ligand.
When evaluating the drug-likeness of a lead compound, what needs to be considered are Lipinski's rule of five (Ro5) which includes: (1) less than five hydrogen-bond donors, (2) less than ten hydrogen-bond acceptors, (3) molecular mass less than 500 Dalton, and (4) log P not greater than 5 [44]. Table 3 shows the results of the Ro5 analysis of the compounds evaluated. This research suggested that nelfinavir and chloroquine met the Ro5 criteria, whereas betasianin showed three violations: eight hydrogen bonds, fourteen hydrogen acceptors, and a molecular weight of 567.78 g / mol. However, the logP of betacyanin is -2.735. Log P is a measure of the relative solubility of a compound in the aqueous and organic phase [45] The higher the logP, the more nonpolar the compound, or the lower the polarity of the compound, so that the drug/compound will pass through the permeable cell membrane more easily.

Villaño et al. [46] stated that betalain is a bioavailable molecule and will be present in its intact form at systemic levels, although it is quite low [47]. Betacyanin from red dragon fruit only experienced minor loss (<25%) at a gastric-like environment, but experienced greater loss during the intestinal phase digestion Choo et al. [18]. Further suggested by Choo et al. [18] that the bioavailability of betacyanin increased when it was fermented, so that it would be more durable after intestinal digestion.

Betalain as a drug candidate can work effectively in maintaining serum lipid profiles, reducing LDL concentrations, normalizing blood glucose, and improving the performance of NAD-dependent histone deacetylase [48]. According to research by Karthika et al. [49], apart from being an antiviral, betalain can inhibit the structure of apoptotic proteins so that they have the potential to also act as an anti-cancer. Even according to a study by Khan et al. [50], betalain can be used as a drug candidate to fight diseases such as diabetes, diuretics, and inflammation. Therefore, apart from its antiviral activity, betacyanin also has the potential to relieve the symptoms of comorbidities that accompanies covid-19 patients.

Table 2. Amino acid residues of the receptors showing different interactions with the ligand

| Receptor                     | Amino acid residues showing different interactions | Van der Waal’s | Conventional Hydrogen Bond | Salt Bridge, Attractive Charge and Pi-Cation | Unfavorable Positive-Positive and Unfavorable Acceptor-Acceptor |
|------------------------------|---------------------------------------------------|----------------|---------------------------|---------------------------------------------|---------------------------------------------------------------|
| Spike Glycoprotein (closed state) (6VXX) | Gln-C:1106, Gly-C:1093, Thr-C:1105, Val-C:1104, Gln-C:1113, Arg-C:1091, Asn-C:1119 | Gln-C:1106, Gly-C:1093 | Thr-C:912, Arg-B:1091 | Asp-C:1118, Glu-C:1092 | Asp-B:1118, Asp-A:1118 |
Table 3. Lipinski’s Rule of Five (RO5) of SARS-CoV-2 M\textsuperscript{pro}/3CL\textsuperscript{pro} and S protein potential inhibitors

| Compounds     | Molecular Formula | Molecular weight (<500 g/mol) | LogP (<5) | H-bond donor (<5) | H-bond acceptor (<10) | Violations | Meet RO5 criteria |
|---------------|-------------------|------------------------------|-----------|------------------|------------------------|------------|-------------------|
| Nelfinavir    | C\textsubscript{12}H\textsubscript{15}N\textsubscript{5}O\textsubscript{4}S | 567.78 | 4.41 | 4 | 5 | 1 | Yes |
| Chloroquine   | C\textsubscript{18}H\textsubscript{26}ClN\textsubscript{3} | 319.87 | 4.15 | 1 | 2 | 0 | Yes |
| Betacyanin    | C\textsubscript{21}H\textsubscript{26}N\textsubscript{2}O\textsubscript{13} | 550.5 | -2.735 | 8 | 14 | 4 | No |

4. Conclusion
The development of new medicinal plant products cannot be ignored in the search for drugs in overcoming covid-19. This current research suggests that betacyanin can be used as a candidate for the covid-19 antidote based on the evaluation of docking results.
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