Molecular docking of anthocyanins and ternatin in *Clitoria ternatea* as coronavirus disease oral manifestation therapy

**Abstract**

Herbal active compound with immunoregulator ability is considered a potential therapy for COVID-19 oral manifestation by downregulating pro-inflammatory cytokine storm. Meanwhile, anthocyanin and ternatin are the active compounds in *Clitoria ternatea*, which may act as a potential immunoregulator for COVID-19 therapy. The intention of this investigation was to investigate anthocyanin and ternatin as active compounds in *C. ternatea* that may be able to increase anti-inflammatory cytokine and inhibit pro-inflammatory cytokine and key proteins of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). This study implemented bioinformatic approach to analyze anthocyanin and ternatin as active compounds in *C. ternatea* with anti- and pro-inflammatory cytokines and antiviral examination history through blind molecular docking study (in silico). Moreover, anthocyanins and ternatin were obtained from PubChem database by minimizing ligand structure in PyRx software to increase the flexibility. RCSB database was employed for preparing the protein samples consisting of interleukin (IL)-6, SARS-CoV-2-ACE2 glycoprotein complex, tumor necrosis factor-α receptor (TNF-αR), matrix metalloproteinase-9 (MMP-9), IL-6, IL-10, and human beta defensin-2 (HBD-2). In addition, The PyMol sofware was used to sterilize the protein samples to obtain the molecular docking optimization. This investigation found that, in the molecular docking simulation, the anthocyanin and ternatin showed producing the negative binding affinity to the ACE2 domain which interacted with RBD glycoprotein SARS-CoV-2. Anthocyanin and ternatin were then predicted to be able to influence any inhibitory activity of TNF-αR, MMP-9, and IL-6; increase IL-10; and increase HBD2 binding affinity values negatively. It can be predicted through molecular docking that anthocyanin and ternatin as the active compounds in *C. ternatea* contribute as a potential agent for COVID-19 oral manifestation therapy.

**Key words:** Anthocyanins, *Clitoria ternatea*, COVID-19, infectious disease, medicine, ternatin

**INTRODUCTION**

Caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease 2019 (COVID-19) is...
a worldwide pandemic that has occurred since the early 2020 with 121 million cases and a reported mortality rate of 2.68% in a year. Indonesia is ranked 19th globally and 4th in Asia for the highest number of COVID-19 occurrences. After 1 year from the first identified suspected case of COVID-19, the number of COVID-19 cases in Indonesia has currently reached 1.4 million, with 2.7% of mortality rates.[1,2] The most common symptoms found in COVID-19 patients are dry cough, fever, and shortness of breath. However, different patients may exhibit different symptoms. As the time passed by, SARS-CoV-2 virus has mutated and exhibited another different manifestation. Moreover, oral manifestations are one of the manifestations which can be discovered in COVID-19 patients and cause morbidity. In this regard, dentists have a fundamental contribution to examine COVID-19 oral manifestations. The oral manifestations located in COVID-19 are not specific compared to oral candidiasis and oral ulcers which most often appear, but these manifestations are still controversial to be diagnosed as COVID-19.[3] A cause-and-effect relationship between coronavirus infection and the appearance of oral lesions due to immunosuppression in people with COVID-19 may provide a good signal. This is due to an increase of tumor necrosis factor-α (TNFα) in COVID-19 patients which causes chemotaxis of neutrophil in the oral mucosa and develops into aphthous lesions. The inflammatory response in COVID-19 patients increases so that immunoregulation during inflammation is considered as a potential therapy for the oral manifestations of COVID-19.[3,4]

In addition, the utilization of natural ingredients such as anthocyanin and ternatin as the active compounds in Clitoria ternatea for herbal-based COVID-19 therapy may be effective and potential as it has the ability to overcome the infectious diseases.[5] Natural compounds such as anthocyanins and ternatin are characterized by a large macromolecular specificity and less toxicity. However, any concerns regarding the biocompatibility and bioavailability pose a challenge in making the natural substances into drugs. Furthermore, the aim of this investigation was to examine anthocyanin and ternatin as the active compounds in C. ternatea which can advance mucosal immunity and anti-inflammatory cytokine and inhibit pro-inflammatory cytokine and key proteins of SARS-CoV-2 for COVID-19 oral manifestation therapy.

MATERIALS AND METHODS

Sample retrieval
Anthocyanin and ternatin chemistry components were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The sample was identified with ID, formula, weight, and canonical smile, and the ligand structure was minimized by employing PyRx 0.8.1 version, SourceForge, California, US to increase its flexibility.[6,7] RCSB database (https://www.rcsb.org/) in this study was applied for the preparation of protein samples consisting of interleukin (IL)-6, SARS-CoV-2-ACE2 glycoprotein complex (Glyc-ACE2), tumor necrosis factor-α receptor (TNF-αR), matrix metalloproteinase-9 (MMP-9), IL-10, and human beta defensin-2 (HBD-2). Meanwhile, data collection on protein samples was arranged consisting of ID, visualization method, resolution, atom count, weight, chain, and sequence length. At last, the sterilization of protein samples by utilizing PyMol 2.5 version, Schrödinger Inc, California, US was carried out for the optimization of molecular docking.[8]

Virtual screening
In this study, the ability of the ligands from anthocyanin and ternatin in the binding activity within the targeted protein domain in this study was identified through molecular docking simulations. The goal of molecular docking simulations was to determine the types of binding, enhancing, and inhibitor activities. Furthermore, the binding energy produced by the ligand when binding to the targeted protein site was able to trigger a specific biological response; the more negative the binding score, the higher the effect on the targeted protein activity.[6] LIGAND–PROTEIN INTERACTION

The process of identifying the type of target protein activity was triggered by the two ligands, which was known through the analysis of position and chemical bonds from the molecular docking molecular complex in Discovery Studio Version 2016, BIOVIA, California, US. In addition, the hydrophobic, hydrogen, pi, and Van der Waals bonds that were formed in molecular complexes can be seen through these servers, and the types of weak bond interactions had a contribution in the context of biological activity of a protein.[9,10]

MOLECULAR VISUALIZATION

This research implemented PyMol software to visualize 3D molecular docking results. The 3D structure of ligand–protein molecule complex was demonstrated in the form of cartoons, surfaces, sticks, and spheres.[9] RESULTS

Ligand samples consisting of anthocyanin and ternatin were obtained with the information of ID, formula, weight, and canonical smile [Table 1]. Protein samples consisting of IL-6, Glyc-ACE2, TNF-αR, MMP-9, IL-10, and HBD-2 were generated from RCSB PDB database attained with ID information, visualization method, resolution, atom count, weight, chain, and sequence length [Table 2].
Based on the results of molecular docking simulations, anthocyanin produced more negative binding affinity than ternatin in ACE domain that interacted with RBD glycoprotein SARS-CoV-2, which allowed the inhibition of viral attachment because RBD could not interact with ACE. Meanwhile, ternatin compounds interacted to produce a more negative binding affinity than anthocyanins when they bound to TNF-αR. Ternatin was predicted to be able to influence TNF-αR inhibitory activity and triggered the formation of an anti-inflammatory response. The inhibition of MMP-9 activity is also played by ternatin with a more negative binding affinity than anthocyanins and inhibits collagen degradation. Anthocyanin was predicted to be able to suppress the occurrence of cytokine storm by inhibiting IL-6 and IL-10 activity with a more negative binding affinity value than ternatin. Anthocyanins are predicted to initiate an increase in innate immune response in the oral cavity through increased HBD-2 activity, thereby preventing the occurrence of oral manifestation [Table 3].

Meanwhile, the identification of molecular interactions and the binding positions of docking protein–ligand complexes revealed that the bonds of anthocyanins in ACE were able to produce a total of eight interactions consisting of Van der Waals, hydrogen, and pi bonds [Figure 1]. The bonds of ternatin compounds in TNF-αR were capable to produce 10 interactions in total which included Van der Waals, hydrogen, unfavorable, and pi bonds [Figure 2]. Moreover, the bonds of the ternatin compound in MMP-9 successfully created a total of 11 interactions and contained with Van der Waals, hydrogen, unfavorable, and pi bonds [Figure 3]. Similarly, the bonds of anthocyanin compounds in IL-10 were also competent to create 11 interactions in total but only consisted of Van der Waals and pi bonds [Figure 4]. In addition, the bonds of anthocyanin in HBD-2 were accomplished to produce 8 interactions in total composed with Van der Waals, unfavorable, hydrogen, and pi bonds [Figure 5]. The bonds of anthocyanin in IL-6 were able to produce 13 interactions in total and contained with Van der Waals, unfavorable, and pi bonds [Figure 6].

**DISCUSSION**

COVID-19 may trigger an inflammatory response also involving immunopathogenesis so that it can cause various kinds of manifestations. The manifestations of COVID-19 may arise due to the cytokine storm. Cytokine storm is a condition when pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α significantly increase. Based on previous research, oral manifestation in COVID-19 patient
Anthocyanins and Ternantin in Clitoria ternatea in silico

was well known as a COVID tongue such as aphthous stomatitis, geographic tongue, and petechiae. Furthermore, immunomodulation as antiviral COVID-19 response is necessary. Some of drug such as Pegylated Interferon-2a and 2b can be used COVID-19 therapy through immunomodulation action.\textsuperscript{[11]}

Meanwhile, medicinal plants have great potential to be utilized as an alternative medicine and are the basis for the discovery of natural compounds for the development of therapeutic agents in pharmacology. It is known that lungs are the organ most severely affected by COVID-19, similar to SARS. Therefore, a special attention is paid to herbs that can protect the lungs and mucosal areas by supporting the immune system.\textsuperscript{[9]} One of the plants whose parts have the functional benefits for the human body is the butterfly pea flower (\textit{C. ternatea}).\textsuperscript{[12]} \textit{C. ternatea} or \textit{flower telang} has the active compounds of anthocyanin and ternatin, which has the potential to act as immunoregulator therapy for SARS-CoV-2 infection. The immunoregulatory activity of anthocyanin and ternatin in \textit{C. ternatea} petals has been tested in mice, resulting in the indication of regulated immune response.\textsuperscript{[13]}

The immunoregulation mechanism of \textit{C. ternatea} occurs due to the decreased immune cell sensitization, immune cell presentation, and phagocytosis. Anti-inflammatory and antioxidant properties have a major contribution in immunoregulation activity. Moreover, \textit{C. ternatea} also

### Table 3: Results of molecular docking simulation

| Protein      | Ligand   | Center | Grid positions | Dimensions | Binding affinity (kcal/mol) |
|--------------|----------|--------|----------------|------------|-----------------------------|
| IL-6         | Anthocyanin | X: −0.20 | X: 25.00 | −6.9 |
|              | Ternatin  | Y: 0.30 | Y: 25.00 | −6.6 |
| Glyc_ACE-2   | Anthocyanin | X: 179.92 | X: 43.31 | −6.0 |
|              | Ternatin  | Y: 163.98 | Y: 25.00 | −5.3 |
|              |           | Z: 124.42 | Z: 31.38 |     |
| TNF-\alpha   | Anthocyanin | X: 21.15 | X: 25.00 | −5.6 |
|              | Ternatin  | Y: 14.45 | Y: 25.00 | −5.7 |
|              |           | Z: 35.67 | Z: 25.00 |     |
| MMP-9        | Anthocyanin | X: 36.88 | X: 25.00 | −6.3 |
|              | Ternatin  | Y: 38.84 | Y: 25.00 | −6.4 |
|              |           | Z: 34.62 | Z: 25.00 |     |
| IL-10        | Anthocyanin | X: 13.02 | X: 25.00 | −7.5 |
|              | Ternatin  | Y: 38.84 | Y: 25.00 | −5.6 |
|              |           | Z: 4.39 | Z: 25.00 |     |
| HBD-2        | Anthocyanin | X: 0.23 | X: 25.00 | −5.5 |
|              |           | Y: −4.75 | Y: 25.00 |     |
|              |           | Z: 3.08 | Z: 25.00 |     |

\(\text{IL: Interleukin, TNF-\alpha: Tumor necrosis factor-alpha, MMP: Matrix metalloproteinases, HBD-2: Human beta-defensin 2, Glyc-ACE2: Glycoprotein angiotensin-converting enzyme 2}\)

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**Figure 2**: Molecular docking visualization results of tumor necrosis factor-\(\alpha\) receptor ((lime) docking complex with ternatin (green). The ligands are displayed in the form of sticks, transparent surfaces and cartoons for the target protein, then the types of molecular interactions formed from Ternatin on the tumor necrosis factor-\(\alpha\) receptor (binding site are presented in 2D)

**Figure 3**: Molecular docking visualization results of matrix metalloproteinase-9 complex (lime) with ternatin (green). The ligands are displayed in the form of sticks, transparent surfaces and cartoons for the target protein, then the types of molecular interactions formed from Ternatin on the matrix metalloproteinase-9 binding site are illustrated in 2D.
mediates its inhibitory effect on pro-inflammatory immune responses through the modulation of the expression of chemokines and/or adhesion molecules, as well as inhibition of humoral antibody formation and phagocytosis.[13,14]

In addition, anthocyanins have anti-inflammatory activity by inhibiting the expression and biological activity of several pro-inflammatory substances such as COX-2, TNF-α, nuclear factor kB, IL-1b, and IL-6.[13] The anthocyanin and ternatin may act as an antiviral by inhibiting the binding of the SARS-CoV-2 RBD spike 1 protein to the cell’s ACE2 receptor. Meanwhile, the inhibition of TNF-αR and TNF-α results in a decrease in TNF-α cytokines which can increase IL-10 secretion and inhibit storm cytokines through IL-6 inhibition. Furthermore, anthocyanins can increase the innate immunity of the oral mucosa by increasing HBD-2; thus, oral manifestation did not occur. At last, anthocyanins also have fundamental contribution in increasing the tissue regeneration by inhibiting the secretion of MMP-9; therefore, collagen degradation does not occur.

CONCLUSION

Based on our study, it can be predicted through molecular docking that anthocyanin and ternatin as the active compounds in C. ternatea are the potential agents for COVID-19 oral manifestation therapy in silico. However, further studies are needed to confirm anthocyanin and ternatin in C. ternatea activity in vitro and in vivo.

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Conflicts of interest
There are no conflicts of interest.

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