Microalgae pigments as a promising immunomodulating food ingredient: In silico study

D Widyaningrum1*, R A Oktafika1 and D Cecilia1
1Food Technology Department, Faculty of Engineering, Bina Nusantara University, Jakarta, Indonesia, 11480

*Corresponding email: dwiyantari.widyaningrum@binus.ac.id

Abstract. Microalgae pigments attract the commercial market as functional food ingredients because of their potential as an antioxidant and anti-inflammatory agents. Through in vitro and in vivo studies, microalgae pigments showed a potential therapeutic effect to reduce the expression of pro-inflammatory cytokines by inhibiting inflammation signaling. Our study explored the potency of microalgae pigments as an immunomodulator by modeling the direct interaction between pigments and pro-inflammatory proteins by molecular docking. The docking study was carried out using AutoDock Vina. At the same time, the binding visualization was obtained by using Discovery Studio Visualizer. The result showed all investigated microalgae pigments (i.e., phycocyanobilin, astaxanthin, β-carotene, 9-cis-β-carotene, and violaxanthin) docked to pro-inflammatory proteins (i.e., IL-6, TNF-α, and NIK), respectively in various binding energy. The binding between pigment compounds and the target protein is mostly attributed to the Van der Waals interaction. Notably, the pigments docked in crucial residues in pro-inflammatory proteins, suggesting the effect of the protein interaction on its receptor and cytokines activity. The results showed a therapeutic potency of microalgae pigment to support immune system modulation that could prevent and attenuate chronic inflammation.

1. Introduction
Microalgae are unicellular organisms living in marine and freshwater environments. Microalgae include prokaryotic cyanobacteria and eukaryotic microalgae. Microalgae has a vast diversity with more than 70,000 species within 16 classes [1]. The microalgae groups that widely explore for a commercial application are green algae (Chlorophyceae), cyanobacteria (Cyanophyceae), and diatom (Bacillariophyceae) [1,2]. A ten-years bibliometric study showed that very few species have been studied well and have a commercial importance such as Spirulina, Chlorella, Haematococcus, and Dunaliella [2].

Microalgae are well-known as the source of highly valuable products with health potencies such as polyunsaturated fatty acids (PUFAs), proteins, vitamins, minerals, and pigments. Therefore, microalgae have been utilized as supplements, pharmaceutical products, cosmetics, and functional food ingredients. Among the valuable compound from microalgae, natural pigments, such as carotenoids and phycobilin, are attractive to explore. Those compounds widely used as natural food colorants besides pigment also consume as supplements because of health benefits for antioxidant, anti-inflammatory, and anticancer [1,2]. There are three major classes of pigment found in microalgae: phycobilin protein, carotenoids, and chlorophylls. [2].

Recently, in the COVID-19 pandemic, people in the community and researchers have tried to find the best way to prevent and cure the infection by exploring the bioactive plant compound for
alternative medicine and immune-boosting. Since the immune system has an essential role in preventing infection, a recent trend showed an increment in the consumption of herbal containing certain active compounds that have antioxidant, anti-inflammatory, and immunomodulatory activities [3]. Those bioactive compounds are suggested to have a health potency by modulating the human immune system. Therefore, herbal consumption is possible to prevent virus infection and work as therapeutic agents [3,4]. Studies in COVID-19 and influenza infection suggested that virus infection stimulates inflammation by inducing pro-inflammatory protein activity such as interleukin (IL)-1β, IL-2, IL-6, IL-11, tumor necrosis factor (TNF)-α, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kβ). Moreover, high expression and activity of pro-inflammatory protein are associated with the severity of patients from virus infection [5,6].

This study aimed to explore the immunomodulating potency from microalgae pigments. The pigments were phycoerythrin, astaxanthin, β-carotene, 9-cis-β-carotene, and violaxanthin. Those pigments have a commercial value and are widely utilized in the pharmaceutical industry [2]. The in-silico study by molecular docking aimed to predict the activity of microalgae pigments against several pro-inflammatory proteins: IL-6, TNF-α, and NF-kβ inducing kinase (NIK). Thus, the initial study is to inspect the immunomodulatory activity of microalgae pigment to be developed as a functional food ingredient. Moreover, the study also proposes an initial clue in the mechanism of microalgae pigments to modulate the human immune system.

2. Methods
2.1 Retrieving the protein structures
Four X-ray crystal structures of protein IL-6 (PDB ID: 1ALU) at 1.90 Å resolution, TNF-α (PDB ID: 2AZ5) at 2.10 Å resolution, and NIK (PDB ID: 4IDV) at 2.90 Å resolution used in this study were retrieved from RSCB Protein Data Bank (https://www.rcsb.org/). The protein structure was prepared for molecular docking by removing water molecules, added polar hydrogen, and added charges. The prepared protein structures were saved in PDBQT format using Molecular Graphic Laboratory (MGL) tools and AutoDock 4.2 [7].

2.2 Retrieving the Ligand
Ligand molecules structures of 9-cis-β-Carotene (CID: 9828626), astaxanthin (CID: 5281224), β-carotene (CID: 5280489), phycoerythrin (CID: 6438349), and violaxanthin (CID: 448438) were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and downloaded as SDF format [8]. First, ligands were converted to PDB format using PyMol. After that, the ligands were set flexible torsion angles at all rotatable bonds using MGL tools and saved in PDBQT format.

2.3 Preparing Grid and Molecular docking
The Molecular Docking process was carried out using AutoDock Vina [9]. Configuration files were created for all protein receptors by setting suitable Cartesian coordinates to generate a Grid box around the native ligand, considered the protein active site. The exhaustiveness was set on 100. Each protein molecule was re-docked using the native ligand and calculate the Root Mean Square Deviation (RMSD) value to validate and detect any error before the docking process with the acceptable RMSD value is 2.

All pigments were docked two times to all target protein receptors. As a result, ten best poses of the complex structure of protein-ligand were generated with hydrogen bonds and parameters like intermolecular energy (Kcal/mol). All of the complexes were ranked based on the docking energy. The complexes with the lowest binding energies were utilized as research data in the first and second docked. Averages, SDs, and coefficients of variation were computed for the two assays to identify the best compounds and false-positive findings. The docking results were saved as a PDB file and visualized in the Discovery Studio Visualizer to obtain a 3D image and binding interaction of the protein-ligand structure.
3. Result and Discussion

*Haematococcus pluvialis*, *Spirulina platensis* (Arthospira platensis), *Dunaliella salina*, and *Chlorella* sp. are cultivated mainly for biomass and pigment production following the pigments utilization for feed, supplements, and natural food coloring [1,2]. *Haematococcus pluvialis* primary pigment is astaxanthin which belongs to the xanthophylls class [10]. *Spirulina platensis*, cyanobacteria, contains major pigment phycocyanin that composes phycocyanobilin, a tetrapyrrole chromophore [11]. While, *Dunaliella salina* is rich with carotenoids in the form of β-carotene and 9-cis β-carotene [12]. *Chlorella* species contain chlorophyll and carotenoids such as lutein, violaxanthin, and zeaxanthin. Violaxanthin is a primary carotenoid found in *Chlorella ellipsoidea* that widely apply to human food and supplements [13]. Recently, there has been a growing interest in microalgae pigments such as astaxanthin, phycocyanin, and carotenoids. The pigments have been credited with antioxidant and inflammatory activities, potentially being utilized as functional food ingredients [2].

Several in-vitro and in-vivo studies suggested the potency of anti-inflammatory activity from microalgae pigments, besides the compounds also suggested working as an antioxidant to support the immune system. Astaxanthin was suggested to inhibit the mRNA and protein expression of several inflammatory mediators such as chemokines and cytokines [10]. The in-vitro study demonstrated that astaxanthin blocked the NF-κB-dependent signaling pathway that led to the repression of the expression of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α [14]. Moreover, astaxanthin is also suggested to stimulate higher expression of antioxidant enzyme genes [10]. Other microalgae carotenoids such as β-carotene and violaxanthin decreased the secretion of pro-inflammatory cytokines such as cyclooxygenase (COX), TNF-α, and IL-1β by suppressing the NF-κB pathway [15].

For phycocyanobilin, the in-vivo study in the mice model suggested that oral administration of the compounds helped to inhibit the expression of NAD(P)H oxidase components. A study reported that NAD(P)H expression stimulated the transcription of the ROS production enzyme [14]. Grover [16] reported that C-phycocyanin increased superoxide dismutase (SOD) activity and IL-10, an anti-inflammatory cytokine, expression in a concentration-dependent manner. Besides, a higher concentration of C-phycocyanin consumption resulted in a reduction of TNF-α expression.

The inflammation process describes as signaling cascades consists of cytokines, chemokines, and receptor proteins. Cytokines and chemokines bind to their specific target receptor to activate the target cell and target protein functionality. The interaction between cytokines and its receptor occurs at cytokines-specific binding sites and receptor active sites [17]. Therefore, when ligand (i.e., bioactive compound) binds to the specific binding site at cytokine structure, it could be resulted inhibition of the binding interaction between cytokines and target receptor.

In molecular docking, binding energy demonstrates the affinity between ligand and receptor. The degree of binding of the ligand with the protein refers to the binding affinity. The interaction between ligand and protein results in the binding energy because of the bond formation, which releases the energy. The free energy of the favorable reaction is negative, contributed by the non-covalent interaction such as hydrogen bond and van der Waals interaction. Therefore, the lesser the binding energy, the better is the binding of the ligand and protein. [18]. Our study investigated the interaction between microalgae pigments, as the ligands, to the pro-inflammatory cytokines, as the target protein through molecular docking. Moreover, we also predicted the common binding residues in cytokines structure to microalgae pigments.

### 3.1 Docking interaction between microalgae pigments and IL-6

Table 1 shows the result of molecular docking between microalgae pigments to IL-6 cytokines. The lowest binding energy resulted from β-carotene with binding energy -7.9 Kcal/mol. The common binding residues were ARG30, LEU33, ASP34, GLN175, LEU178, and ARG179. Moreover, the result suggested that microalgae pigments constantly docked to ARG179, the crucial residue affecting IL-6 and IL-6r interaction. The interaction through hydrogen bond appeared between astaxanthin and IL-6 in ARG30 and ASP34 residues. Phycocyanobilin bind with three hydrogen bonds in IL-6 in
residue ASP26, GLN175, and ARG182. The interaction between β-carotene and IL-6 was mostly attributed to van der Waals interaction (Fig. 1).

**Table 1.** Docking score and binding residues of microalgae pigments against IL-6 cytokines

| Molecules       | Binding energies (Kcal/mol) | AVG  | SD  | CV (%) | Binding Residues                                 |
|-----------------|-----------------------------|------|-----|--------|--------------------------------------------------|
|                 | Assay 1 | Assay 2 |     |        |                                                  |
| Astaxanthin     | -7.2    | -7.3    | -7.25 | 0.05   | 0.69%                                           |
|                 |          |          |      | ARG30; LEU33; ASP34; LYS66; ALA68; GLU69; PHE74; GLN175; LEU178; ARG179 |
| Phycocyanobilin | -7.3    | -7.5    | -7.4 | 0.1    | 1.35%                                           |
| 9-cis β-Carotene| -7.4    | -7.5    | -7.45| 0.05   | 0.67%                                           |
|                 |          |          |      | ARG30; LEU33; ASP34; SER37; LYS66; MET67; PHE74; LYS171; GLU172; GLN175; SER176; LEU178; ARG179 |
| β-Carotene      | -7.9    | -7.8    | -7.85| 0.05   | 0.64%                                           |
|                 |          |          |      | LYS27; ARG30; TYR31; LEU33; ASP34; MET67; PHE74; GLU172; GLN175; SER176; LEU178; ARG179 |
| Violaxanthin    | -7.5    | -7.6    | -7.55| 0.05   | 0.66%                                           |
|                 |          |          |      | LYS27; ARG30; TRY31; LEU33; ASP34; PHE74; GLU172; GLN175; SER176; LEU178; ARG179 |

*Bold residues reflect amino acid involved in hydrogen bond*

**AVG:** Average

**CV:** Coefficient of Variation;

**SD:** Standard Deviation

**Fig 1.** β-carotene docked with IL-6 cytokine (PDB ID: 1ALU). A) 3D structure of the complex, β-carotene (ligand) is represented in sticks, IL-6 (receptor) is in blue. B) Interaction map of the ligand/receptor complex

IL-6 is a cytokine that takes account of broader physiological events in the immune and metabolic system. In the immune system, IL-6 functioned as an early signal of inflammation due to tissue damage or pathogen invasion. Furthermore, IL-6 activated the acute phase response, stimulated the antibody production, supported the B cell maturation, and promoted the CD4+ T-cell associated with
the innate immune system. However, the IL-6 dysregulation could promote chronic inflammation and autoimmunity [19, 20]. Therefore, exploration and development of compound as IL-6 inhibitor beneficial to treat the autoimmune disease and prevent cytokine storm.

IL-6 signal activation starts by binding of IL-6 to IL-6 receptor (IL-6r) following by the attachment of gp130 to form a heterotrimer. Somers et al. [21] reported several binding sites in IL-6 structure. The first site is the location of interaction between IL-6 and IL-6r. ARG179 is the critical residue in which the mutation of ARG179 to ALA reduced the affinity resulted in the 100-fold decrement of IL-6 activity. Mutation in GLN175 in IL-6 cytokine also resulted in the reduction of cytokine activity. Other mutations in the first site in SER177, ALA180, LEU178, and LEU181 proposed to alter the IL-6 conformational structure cause an indirect effect of the binding between IL-6 and IL-6r. The second site is the location of interaction between IL-6 and gp130. The mutation in the second site in the residues of TYR31, GLY35, SER118, and VAL121 suggested reducing the affinity between IL-6 to gp130.

3.2 Docking interaction between microalgae pigments and TNF-α
Table 2 shows the result of molecular docking between microalgae pigments to TNF-α cytokines. The lowest binding energy resulted from 9-cis β-carotene with binding energy -7.9 Kcal/mol. In contrast, the highest binding energy resulted from phycocyanobilin with 6.85 Kcal/mol. All investigated microalgae pigments docked to TYR59 and TYR151. Astaxanthin, 9-cis β-carotene, β-carotene, and violaxanthin bind to TYR119 but none in phycocyanobilin. The interaction between pigments and TNF-α mainly was attributed to van der Waals, as illustrated in the interaction between 9-cis β-Carotene and TNF-α (Fig. 2).

| Molecules                  | Binding energies (Kcal/mol) | Binding Residue                                                                 |
|----------------------------|-----------------------------|---------------------------------------------------------------------------------|
|                            | Assay 1 | Assay 2 | AVG | SD | CV (%)     |                                                                 |
| Astaxanthin                | -7.2    | -7.2    | -7.2 | 0  | 0.00%      | ASP10; LYS11; VAL13; LEU36; ASN39; TYR59; SER60; GLN61; TYR119; TYR151; ILE155 |
| Phycocyanobilin            | -6.9    | -6.8    | -6.85| 0.05| 0.73%      | TYR59; SER60; GLN61; LEU63; TYR110*; TYR115; GLU116; PRO117; LEU120; GLY121; GLN149; **TYR151** |
| 9-cis β-carotene           | -7.9    | -7.9    | -7.9 | 0  | 0.00%      | LYS11; PRO12; VAL13; H1S15; LEU36; TYR59; TYR119; LEU120; GLY121; TYR151; ILE155; ALA156 |
| β-Carotene                 | -7.6    | -7.5    | -7.55| 0.05| 0.66%      | LEU57; TYR59; SER60; GLN61; LEU63; PRO117; TYR119; GLY121; TYR145; TYR151; ILE155 |
| Violaxanthin               | -7.1    | -7.1    | -7.1 | 0  | 0.00%      | VAL13; LEU36; TYR59; LEU94; ALA96; TYR119; LEU120; TYR151; ILE155 |

Bold residues reflect amino acid involved in hydrogen bond
(*) unfavorable interactions between microalgae pigments and TNF-α receptor
AVG: Average
CV: Coefficient of Variation;
SD: Standard Deviation
TNF-α is one of the cytokines that have a role in the regulation of the immune system. TNF signaling contributes to the activation of the NF-κβ pathway and mitogen-activated pathway kinase (MAPK) cascade. To start the TNF signaling, TNF-α can bind to TNF receptor (TNFR) type 1 or TNFR type 2. TNFR1 acts as a pro-inflammatory factor and promotes apoptosis. Therefore, blocking the attachment of TNF-α to TNFR1 and reducing the TNF-α activity become a potential treatment of autoimmune disease and work to alleviate the cytokine storm [22].

He et al. [23] suggested the potency of a small-molecule compound made from trifluoromethylphenyl indole and dimethyl chromone moieties as a TNF-α inhibitor by hampering the attachment between TNF-α and TNFR. The compound bound to TNF-α active site contained sixteen residues: LEU57, TYR59, SER60, GLN61, TRY119, LEU120, GLY121, GLY122, and TYR151. In addition, TYR119 was suggested as a notable residue to accommodate the TNF-α binding to its receptor [22,23].

A previous study identifying the potential plant natural compound as TNF-α inhibitor reported four potential compounds [22]. The compounds bound to TNF-α with binding energy in the range of -6.6 kcal/mol to -8.4 kcal/mol. Similar to our study, the compounds also attached to residue TYR59, TYR119, and TYR151. In addition, in the previous study, a benzophenone derivative compound showed potent inhibitory activity with the IC₅₀ value of 32.5 ± 4.5 µM [22].

3.3 Docking interaction between microalgae pigments and NIK

Table 3 shows the result of molecular docking between microalgae pigments to NIK. The lowest binding energy resulted from phycocyanobilin with binding energy -9.9 Kcal/mol. The common binding residues were ARG408, GLY409, VAL414, ALA427, MET469, LEU471, LEU472, GLY475, SER476, GLN479, LEU522, and CYS533. The interaction through hydrogen bond appeared between astaxanthin and NIK in GLU470 and LEU472 residues. Phycocyanobilin bound to NIK through hydrogen bond in residue SER410, GLU413, SER476, and ASP534. The interaction between pigments and NIK mainly was attributed to van der Waals, as illustrated in the interaction between phycocyanobilin and NIK (Fig. 3).

NIK regulates the NF-κβ pathway and supports the TNF-α signaling cascade. Therefore, exploration of natural compounds as NIK inhibitors is beneficial to inhibit the NF-κβ pathway that leads to help the immune system modulation [24]. A structural study of NIK suggested several notable residues: LYS429, GLU440, MET469, and PHE535. Moreover, LYS429 is the catalytic site, while
MET469 is a gatekeeper that regulates the binding specificity between ligand and NIK [25]. Therefore, the interaction between ligand in MET469 residue possibly affect the NIK activity.

**Table 3. Docking score and binding residues of microalgae pigments against NIK**

| Molecules          | Assay 1 | Assay 2 | AVG | SD | CV (%) | Binding Residue                                                                 |
|--------------------|---------|---------|-----|----|--------|--------------------------------------------------------------------------------|
| Astaxanthin        | -9.7    | -9.7    | -9.7| 0  | 0.00%  | ASP159; ARG408; GLY409; VAL414; ALA427; VAL453; MET469; GLU470; LEU471; **LEU472**; GLY475; SER476; GLY478; GLN479; LYS482; LEU522; CYS533; HIS588; GLY592; CYS593; HIS594; THR597; GLN598; PRO675 ARG405; ARG408; GLY409; **SER410**; PHE411; GLU413; VAL414; HIS415; ARG416; ALA427; LYS429; MET469; LEU471; LEU472; GLY475; **SER476**; GLYN478; GLN479; ASP519; ASN520; LEU522; CYS533; **ASP534**; PHE535 ARG408; GLY409; VAL414; ARG416; ALA427; MET469; GLU470; LEU471; LEU472; GLY475; SER476; GLN479; ASP519; LEU522; CYS533; ASP534; HIS588 |
| Phycocyanobilin    | -9.9    | -9.9    | -9.9| 0  | 0.00%  | ARG408; GLY409; VAL414; ALA427; LYS429; MET469; LEU471; LEU472; GLY475; SER476; GLYN478; GLN479; ASP519; ASN520; LEU522; CYS533; **ASP534**; PHE535 ARG408; GLY409; VAL414; ARG416; ALA427; MET469; GLU470; LEU471; LEU472; GLY475; SER476; GLN479; ASP519; LEU522; CYS533; ASP534; HIS588 |
| 9-cis β- Carotene  | -9.3    | -9.1    | -9.2| 0.1| 1.09%  | ARG408; GLY409; VAL414; ALA427; MET469; GLU470; LEU471; LEU472; GLY475; SER476; GLY478; GLN479; LYS482; ASP519; LEU522; CYS533; HIS588; GLY592; CYS593; HIS594; GLN598; PRO672 ARG408; GLY409; VAL414; ALA427; MET469; GLU470; LEU471; LEU472; GLY475; SER476; GLY478; GLN479; LYS482; ASP519; LEU522; CYS533; HIS588; GLY592; CYS593; HIS594; GLN598; PRO672 |
| β-Carotene         | -9.3    | -9.4    | -9.35| 0.05| 0.53%  | ARG408; GLY409; VAL414; ALA427; MET469; GLU470; LEU471; LEU472; GLY475; SER476; GLY478; GLN479; LYS482; ASP519; LEU522; CYS533; HIS588; GLY592; CYS593; HIS594; GLN598; PRO672 ARG408; GLY409; VAL414; ALA427; MET469; GLU470; LEU471; LEU472; GLY475; SER476; GLY478; GLN479; LYS482; ASP519; LEU522; CYS533; HIS588; GLY592; CYS593; HIS594; GLN598; PRO672 |
| Violaxanthin       | -9.1    | -9.1    | -9.1| 0  | 0.00%  | ARG408; GLY409; VAL414; ALA427; MET469; GLU470; LEU471; LEU472; GLY475; SER476; GLY478; GLN479; LYS482; ASP519; LEU522; CYS533; HIS588; GLY592; CYS593; HIS594; GLN598; PRO672 |

*Bold residues reflect amino acid involved in hydrogen bond*

**AVG:** Average  
**CV:** Coefficient of Variation  
**SD:** Standard Deviation
Fig 3. Phycocyanobilin docked with NIK (PDB ID: 4IDV). A) 3D structure of the complex, phycocyanobilin (ligand) is represented in sticks, NIK (receptor) is in blue. B) Interaction map of the ligand/receptor complex.

3.4 The potency of microalgae pigments for treating COVID-19

Recent studies suggested that imbalance conditions in the immune system of COVID-19 patients affect the infection severity [26-30]. During Sars-Cov-2 infection, the host immune system releases inflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-α to stimulate the innate immune system, leading to macrophages activation [27]. However, the imbalance condition may cause dysregulation in the immune modulatory system, failing to tune the protective inflammation appropriately. Moreover, the dysregulation possibly induces a cytokine storm, a hyperactive inflammation response, leading to cell damage and organ dysfunction [26,27,29]. Therefore, nowadays, researchers investigate a prospectus bioactive compound that has a potential effect as a pro-inflammatory inhibitor to alleviate the cytokine storm.

Pro-inflammatory cytokines become a biomarker for severe Sars-CoV-2 infection in the lung system [26,27]. For example, several clinical studies suggested that critically ill COVID-19 patients have a higher level of IL-6 compared to COVID-19 patients with mild to moderate symptoms [31]. Other studies also suggested elevation in pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, marked the initial chronic inflammation stage. Thus, the inflammation in the epithelial cell due to virus infection may activate the NF-κB signaling pathway, which promotes the transcription of TNF-α, IL-6, and other cytokines. Moreover, the virus can induce elevated expression levels of IL-6 and other pro-inflammatory factors that are likely to lead to a cytokine storm outbreak [27].

Several reports suggested that inhibiting the pro-inflammatory cytokine activity and blocking the inflammatory cytokines signaling pathway can resume immune system modulatory activity. Therefore, the treatments may contribute to preventing hyper inflammation and overcoming the cytokine storm [26,27]. A clinical study on drugs targeting IL-6 receptors showed a therapeutic effect to reduce the severity of the COVID-19 cytokine storm. At the same time, the therapeutic potential of the IL-6 inhibitor is still evaluated in the clinical study [26,30]. A pre-clinical study of etanercept, TNF-α blocker, suggested that the treatment showed a progressive recovery from COVID-19 chronic inflammation [32]. Another study proposed that the NF-κB pathway is essential to initiate the conversion to the severe symptom of COVID-19. Therefore, NF-κB inhibition may be a possible potential treatment against COVID-19 [33].

In our in-silico study, the investigated pigments can interact with pro-inflammatory cytokines: IL-6 and TNF-α. The interaction occurs in crucial residues in cytokines structure, proposing microalgae pigments as cytokines inhibitors. Furthermore, microalgae pigments also interacted with gatekeeper residue in NIK protein, suggesting the blocking activity to NF-κB signaling. Thus, based on molecular docking results, the microalgae pigments may have a potential therapeutic effect to maintain immune...
system modulation, suggesting a potential treatment to prevent and reduce chronic inflammation due to the COVID-19 cytokine storm.

It is worth noting that astaxanthin and phycocyanobilin showed the hydrogen bond interaction with IL-6, TNF-α, and NIK, although the bonding occurs in non-notable residues. In the drug-receptor study, the hydrogen bond is also interesting to be investigated because hydrogen bonds are suggested to play a crucial role in facilitating the protein-ligand binding that affects molecular recognition, structural stability, protein activity, and compound permeability. Moreover, several simultaneous hydrogen bonds are suggested to increase the strength and stability of ligand-protein interaction [18,34]. Therefore, the hydrogen bonds between astaxanthin and phycocyanobilin to the investigated pro-inflammatory proteins may result in better stability interaction that provides higher immunomodulatory potency.

Several recent reports suggested the potential of astaxanthin to alleviate the cytokine storm. Talukdar [35] reported a literature review study consisting of clinical studies that suggest the anti-inflammatory and immunomodulatory effects of astaxanthin. The study suggested that the consumption of astaxanthin as an adjunctive supplement could alleviate chronic inflammation. The study proposes that astaxanthin regulates the expression of pro-inflammatory factors IL-1β, IL-6, IL-8, and TNF-α that may positively attenuate the inflammatory response by regulating the signaling pathways like NF-κB, NLRP3, and JAK/STAT [35].

Moreover, several in-silico studies reported the potency of microalgae pigments as the antiviral agents against Sars-Cov-2. Xanthophylls, such as astaxanthin and violaxanthin, interacted with the main protease (Mpro) and papain-like protease (PLpro) of Sars-Cov-2 with binding energy similar to ivermectin, the control drug. Mpro and PLpro facilitate viral replication. Thus, the proteases are a potential target for anti-Sars-Cov-2 drugs. Therefore, the study suggested that astaxanthin may inhibit the Sars-Cov-2 virus replication [36]. Another in-silico study reported that phycocyanobilin is bound to spike receptor-binding domain (spike-RBD) of Sars-Cov-2, which potentially inhibits the binding between Sars-Cov-2 to angiotensin-converting enzyme-2 (ACE-2) [37].

4. Conclusion

In summary, the interaction between microalgae pigments with pro-inflammatory proteins IL-6, TNF-α, and NIK had been assessed by in silico study through molecular docking. Beta-carotene has the lowest binding energy to IL-6. 9-cis beta-Carotene has the lowest binding energy to TNF-α. At the same time, phycocyanobilin has the lowest binding energy to NIK. Moreover, the hydrogen bonds that occur between astaxanthin and phycocyanobilin to pro-inflammatory proteins may provide better stability. By molecular docking, all investigated microalgae pigments can bind to the essential residues in the pro-inflammatory structure, which affects the cytokines activity and protein interaction to its receptor. The results proposed the immunomodulatory potency of microalgae in the immune system. However, further investigation through in vitro and in vivo studies is crucial to prove the therapeutic potency of microalgae pigments as immunomodulatory agents.

Acknowledgement

We would like to acknowledge the Research and Technology Transfer Office, Bina Nusantara University for supporting the publication fee.

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