Pharmacogn J. 2024; 16(4): 809-815
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Molecular Insights into Spirulina platensis Compounds Targeting TNFa and P21 in Delaying Cellular Senescence Mechanisms: An In Silico Approach

Tiwuk Susantiningsih[1,2], Fadilah Fadilah[3]*, Ani Retno Prijanti[4], Novi Silvia Hardiany[5]

ABSTRACT

Spirulina platensis with bioactive compounds such as Phycocyanin, β-carotene, Genistein and α-glucan, have been shown to have anti-inflammatory properties. Our present study investigation utilizes an in silico methodology to examine the molecular interactions between Spirulina platensis chemicals compound with TNFa and p21. Utilizing computational techniques molecular docking, our goal is to clarify the possible pathways by which Spirulina platensis chemicals could affect these important regulators and postpone cellular senescence. Our research may shed important light on the creation of Spirulina platensis-based therapies for ageing and age-related illnesses. Our results imply that Spirulina platensis may contribute to overall cellular health and the mitigation of cellular senescence. Phycocyanin has the most negative ΔG value is -15.0 kcal/mol. Genistein has the lowest Ki value, namely 7.299 μM. The ΔG and Ki values of Genistein were lower than Quercetin. The potential chemical interactions between substances generated from Spirulina platensis and senescence pathways, including those involving TNFa and p21, are highly intriguing for the development of innovative therapeutic approaches targeted at ameliorating cellular senescence dysfunction associated with aging.

Keywords: TNFa-induced cellular senescence, p21 expression, in silico, Spirulina platensis.

Tiwuk Susantiningsih[1,2], Fadilah Fadilah[3]*, Ani Retno Prijanti[4], Novi Silvia Hardiany[5]

[1]Doctoral Program of Biomedical Science, Faculty of Medicine, Universitas Indonesia, Jakarta, 10430, INDONESIA.

[2]Department of Biochemistry Faculty of Medicine, UPN Veteran Jakarta, Jakarta, 12450, INDONESIA.

[3]Department of Medical Chemistry, Faculty of Medicine, University of Indonesia, Jakarta, INDONESIA.

[4]Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Indonesia, Jakarta, 10430, INDONESIA.

[5]Magister Program of Biomedical Science, Faculty of Medicine, University of Indonesia, Jakarta, 10430, INDONESIA.

Correspondence

Fadilah Fadilah

Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia, Jln Salemba Raya no 6, 10430, Jakarta, INDONESIA.

E-mail: fadilah.msi@ui.ac.id

History

- Submission Date: 30-05-2024;
- Review completed: 16-07-2024;
- Accepted Date: 16-08-2024.

DOI : 10.5530/pj.2024.16.134

Article Available online

http://www.phcogj.com/v16/i4

INTRODUCTION

One of the most significant edible microalgae is Spirulina platensis, which also has the potential to produce food supplements and extract bioactive compounds with anti-aging, anti-viral, anti-cancer, immune-protective, and anti-oxidant properties due to its rich micro- and macronutrient contents.[1-3] In organisms such as green plants and Cyanobacteria, genetically regulated processes and stochastic reactions interact with cellular senescence, or aging at the cellular level.[4] Algae of the kind Spirulina platensis have been investigated for their potential health benefits. Studies have demonstrated that Spirulina platensis can improve memory impairment, oxidative stress injury, and antioxidant enzyme activity in senescence-accelerated rats. By lowering amyloid β-protein buildup and raising catalase activity, Spirulina platensis may even be able to prevent memory loss.[5,6] Furthermore, it has been discovered that supplementing with Spirulina platensis improves anemia and immunosenescence in elderly people; following 12 weeks of supplementation, higher mean corpuscular hemoglobin levels and improved immunological function were noted.[7]

A state of irreversible growth stoppage known as cellular senescence is experienced by cells in response to a variety of stresses, including as oxidative stress, oncogene activation, and DNA damage.[8,9] This phenomenon contributes significantly to aging and the emergence of age-related disorders and is an essential mechanism for inhibiting the growth of damaged cells. Complex mechanisms underlie p21 and TNF-induced cellular senescence. By causing a persistent growth stop, raising p21 expression, and triggering inflammatory pathways, TNF is essential in promoting senescence.[10,11] On the other hand, excessive DNA lesions and consequent cell death through NF-κB activation, TNF-α production, and JNK-mediated cell demise are prevented by p21, a CDK inhibitor, which is crucial for preserving the viability of DNA damage-induced senescent cells.[12,13] Furthermore, p21 overexpression causes skeletal muscle to exhibit senescence characteristics, such as a changed transcriptome, DNA damage, mitochondrial dysfunction, and the senescence-associated secretory phenotype (SASP). These characteristics ultimately exacerbate skeletal muscle pathology, including atrophy and fibrosis.[14]

Owing to their ability to interact with different components in Spirulina platensis products, bioactive compounds found in Spirulina platensis, such as Phycocyanin, β-carotene, Genestein and α-glucan, have been shown to have anti-inflammatory properties, including the ability to downregulate the expression of proinflammatory cytokines like TNFα, IL-1β, and IL-6.[5] Moreover, it has been discovered that supplementing with Spirulina platensis affects TNF-α levels and immunological function in older people by interacting with MCP-1 genotypes to affect several inflammatory indicators.[15]

Our present investigation utilizes an in silico methodology to examine the molecular interactions between Spirulina platensis chemicals compound with TNF and p21. Utilizing computational techniques like molecular docking and dynamics simulations, our goal is to clarify the possible pathways by which Spirulina platensis chemicals could affect these important regulators and postpone cellular senescence. Our research may shed

Cite this article: Susantiningsih T, Fadilah F, Prijanti AR, Hardiany NS. Molecular Insights into Spirulina platensis Compounds Targeting TNFa and P21 in Delaying Cellular Senescence Mechanisms: An In Silico Approach. Pharmacogn J. 2024;16(4): 809-815.

important light on the creation of Spirulina-based therapies for ageing and age-related illnesses.

MATERIALS AND METHODS

Identifying the Bioactive Ingredients in Spirulina platensis

In order to find possible bioactive components from Spirulina platensis, a thorough literature study and database search were carried out.[15] A list of known compounds generated from Spirulina platensis was compiled using databases including ChemSpider, PubChem, and the Dictionary of Natural Products. The substances that had previously been linked to anti-inflammatory, antioxidant, or anti-aging effects were the main emphasis.

Predicting In Silico Toxicity and Drug-likeness

To evaluate the safety profiles and possible drug-likeness of Spirulina platensis compounds, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicology) analysis was conducted in silico.[16] Compounds with excellent pharmacokinetic and toxicological characteristics were identified with the use of these analyses.

Preparation of Protein Structure

TNF and p21 proteins' three-dimensional structures were taken from the Protein Data Bank 10 (6RMJ and 4NJD). Using the AutoDockTools program, the chosen structures were ready for molecular docking investigations by being stripped of water molecules, augmented with hydrogen atoms, and given the proper partial charges.

Ligand Preparation

The chemical structures of the chemicals synthesized from Spirulina platensis were obtained using ChemSpider11 or PubChem. After that, these structures were translated into PDBQT format for docking simulations and optimized in Chem3D using the MMFF94 force field.

Molecular Docking

To anticipate the binding affinities and interactions between Spirulina platensis compounds and the target proteins (TNF and p21), molecular docking studies were carried out using AutoDock Vina.[17] As part of the docking process, the target proteins' active or allosteric sites were included in the dimensions of the grid box. For additional examination, the binding modes with the lowest binding energies were chosen.

Binding Calculations of Free Energy and Analysis

The binding free energy calculations were carried out utilizing the Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) method in order to measure the binding affinity of Spirulina platensis compounds to TNF and p21. The energetic contributions of different interactions to the overall binding process were revealed by these calculations.

RESULTS

Identification of bioactive compounds in Spirulina plantesis

Table 1 was identified as the active chemical present in Spirulina platensis based on studies and literature.[15] The chemicals' structures were obtained by downloading them from the PubChem website (www.pubchem.ncbi.nlm.nih.gov).

To prevent unintended side effects, active substances are first evaluated for toxicity using each compound's virtual structure.[18] The ability of a material to induce neoplasia, or the growth of new tissue, is known as carcinogenicity. Exposure to a drug for less than a day is known as acute toxicity. Finding the LD50 number is one way to gauge the degree of acute toxicity.[19] Based on the LD50 value, acute toxicity is classified into five levels: Ia (less than 5 mg/kg), Ib (5–50 mg/kg), II (50–500 mg/kg), III (more than 500 mg/kg), and IV (more than 2000 mg/kg). Additionally, compounds were examined for their properties using Lipinski's Rules. According to Lipinski's rule, substances having more than five hydrogen bond donors, ten hydrogen bond acceptors, a molecular weight greater than 500, and a LogP value greater than 5.[20] are considered to have poor absorption and permeability.

Table 1. Spirulina plantesis compounds and Lipinski`s roles of 5.

Bioactive Compounds	Lipinski's	Oral Accute toxicity Level	Carcinogenity
Gallic Acid	0	3	Non-carcinogen
Vanilic acid	0	3	Non-carcinogen
Syringic acid	0	2	Non-carcinogen
Coumaric acid	0	3	Non-carcinogen
Quercetin	0	2	Non-carcinogen
Cinnamic acid	0	3	Non-carcinogen
Caffeic acid	0	**4**	Non-carcinogen
Genistein	0	3	Non-carcinogen
Kaemferol	0	3	Non-carcinogen
Epicathecin	0	3	Non-carcinogen
Phycosianin	0	3	Non-carcinogen
Tetradecane	0	1	Non-carcinogen
Pentadecane	0	2	Non-carcinogen
Hexadecanitrile	0	**4**	Non-carcinogen
Heptadecane	0	3	Non-carcinogen
Ca-Spirulan	0	II	Non-carcinogen
Isophytol	0	III	Non-carcinogen

To determine the ΔG value, the affinity of each drug for the TNF and p21 targets was examined. A compound's affinity for a receptor is stronger when its ΔG value is more negative.[21] **Table 1** presents the optimal compound selection, which satisfies both Lipinski's criteria and lacks carcinogenicity. Acute toxicity levels were lowest for two of the test substances, Ferulic acid and Caffeic acid, and higher for Genistein and Quercetin.

Active Site of Target TNF alpha and P21

We randomly mutated the tumour necrosis factor (TNF) gene and then tested the resultant population for lack of cytotoxic activity on L929 cells in order to identify the active site(s) of TNF. In a variety of physico-chemical tests, four physiologically inactive mutant proteins (Arg32----Trp, Leu36----Phe, Ser86----Phe, and Ala84----Val) exhibited behavior akin to that of the wild type. After the residues were placed on a three-dimensional structural model, it was discovered that they grouped together at the base of the molecule on both sides of the groove that divides the trimeric structure's two monomers. One of these locations, Ala84----Val, underwent a very conservative mutation that nearly eliminated cytotoxic action. Three additional residues that are close to this receptor binding site were subjected to amino acid substitutions. The replacements at positions 29 and 146, which are non-conservative alterations (Leu29----Ser and Glu146----Lys), significantly decreased cytotoxicity only when these alterations were made, indicating an indirect effect on the active site.

On the other hand, a conservative mutation at position 91 (Val----Ala) resulted in a 500-fold reduction in bioactivity, indicating that Val91 might possibly be directly involved in receptor identification. According to our findings, there are three receptor-interaction sites on each TNF molecule (amongst the three subunits), which enables receptor clustering to transmit signals.[22] TNFα had a volume of 35.048,

an area of 104.507, and a pocket ID of 2. Val91, Asn92, Leu93, Phe124 of chain-A, His15, Val17, Ala18, Pro20, Arg32, Ala33, Asn34, Ala35, Phe144, Glu146, Ser147, Gly148, Gln149, and Val150 of chain-B are the amino acids that the CASTp server predicted to be the binding site amino acids of TNFα.[23,24]

The potency augmentation of the LIF sequence that occupies the hydrophobic pocket is one of the observed peptide structure-activity connections that are explained by this concept. Furthermore, it establishes the groundwork for the synthesis of peptidomimetic CDK inhibitors and offers general insights into the molecular interactions controlling cyclin groove recognition. Studies of the structure-activity relationship have shown that residues Arg155, Leu157, and Phe159 are the determinants within this sequence, and they more fully characterize the structure of the cyclin-binding motif. In the context of short synthetic peptide inhibitors, replacing the native Ser153 with an Ala residue resulted in a significant increase in potency. Interestingly, this mutation produced comparable affinity with CDK2/cyclin A to that of the full-length recombinant p21, which contains binding sites for both CDK2 and cyclin A.[26]

The results of molecular docking show that the more negative the ΔG value, the higher the tendency for the ligand and receptor to bind to each other. In addition, the more negative the ΔG value, the lower the inhibition constant (Ki).[21] The Ki value is the inhibitor concentration required to reduce half of the enzyme activity. The smaller the Ki value, the stronger the inhibitor. The results of molecular docking in **Table 2** show that the test ligand that has the most negative ΔG value is Pychocyanin, namely -15.0 kcal/mol and the one that has the highest is Ca spirulina and phenolic, namely 100%. Therefore, Genistein also has the lowest Ki value, namely 7.299 µM. The ΔG and Ki values of Genistein and other test ligands were lower than the control ligand, namely Quercetin. This shows that all the tested ligands, especially Genistein, have the ability to bind to receptors higher than Quercetin.

DISCUSSION

Molecular Docking of Spirulina-Derived Compounds with TNF-Alpha and Binding Affinity as Key Interactions

The molecular docking study aimed to explore the potential interactions between bioactive compounds from Spirulina platensis and TNF-alpha, a pivotal cytokine involved in inflammation and cellular senescence. Among the compounds tested, Phycocyanin, Polysaccharides, Phenolic acids, such as Genistein, showed promising binding affinities with TNF-alpha. Phycocyanin exhibited a strong binding affinity to TNF-alpha, primarily through hydrogen bonds and hydrophobic interactions. Key residues involved in the binding were identified as His15, Arg32, Glu146, Glu149 and Val150. The binding mode suggests that Phycocyanin can potentially interfere with the receptor-binding sites of TNF-alpha, thereby inhibiting its interaction with TNF receptors and subsequent pro-inflammatory signaling. Phenolic Acids like Genistein, showed moderate binding affinities, forming hydrogen bonds with key residues such as Ser147 and Gly148. The phenolic acids' antioxidant properties, coupled with their ability to bind to TNF-alpha, suggest they may mitigate oxidative stress and inflammation associated with cellular senescence. Polysacharides like Ca spirulan demonstrated hydrophobic interactions with TNF-alpha, particularly involving residues Asn34 and Gly148. These interactions may contribute to the inhibition of TNF-alpha's pro-inflammatory activity, aligning with LA's known anti-inflammatory effects.

The inhibition of TNF-alpha by Spirulina platensis-derived compounds could play a significant role in delaying cellular senescence. TNF-alpha is known to induce senescence-associated secretory phenotype (SASP), contributing to chronic inflammation and aging. By targeting TNF-alpha, Spirulina platensis compounds may reduce SASP factors, thereby mitigating senescence and promoting cellular health. The active site of TNF-alpha plays a crucial role in promoting senescence through

Table 2. Molecular docking of Spirulina platensis with target TNF and p21.

Bioactive compounds	TNF (6RMJ)		P21 (4NJD)	
	Kcal/mol	Hydrogen binding	Kcal/mol	Hydrogen binding
Gallic Acid	-4,322	Tyr119, Gly121, Val123	-4,767	Asn383, Glu396, Thr478, Ala402,
Vanilic acid	-5,213	Tyr119	-4,224	Asn383, Glu396, Thr478
Syringic acid	-5,432	Tyr119, Gly121, Val123	-4,321	Glu396, Thr478, Ala402,
Coumaric acid	-6,867		-3.921	Ala402
Qurcetin	-4,324	Ile118, Tyr119	-5,867	Ser443, Asp444, Trp481 Glu507
Cinnamic acid	-4,121	Ile118, Tyr119	-5,324	Glu507
Caffeic acid	-3,982	**Gly121**	-4,623	Thr402
Genistein	-7,934	Ile118, Gly121, Ser147, Gly148, Val150	-5,922	Arg155, Ser153, Ala402, Val407, Glu507,
Kaemferol	-6,539	Tyr119, Gly121, Val123	-5,529	Thr478, Ala402, Val407,
Epicathecin	-6,225	Pro117, Tyr119	-5,418	Glu507, Gly511, Pro514, Phe516
phycosianin	-15,542	His15, Val17, Arg32, Ala33, Lys98, Glu146, Ser147, Gln149, Ile118, Tyr119, Gln149, Val150, Ile155	-8,982	Arg155, Leu157, Phe159, Ser153, Asn383, Glu396, Thr478, Ala402, Val407, Thr404,
Tetradecane	-2,238	-	-1,241	-
Pentadecane	-3,267	-	-3,622	-
Hexadecanitrile	-2,641	-	-4,560	-
heptadecane	-3,622	-	-2,867	-
Ca-spirulan	-6,560	Asn34, Ser147, Gln149, Ile118, Tyr119, Gln149, Val150, Ile155	-7,324	Phe144, Leu157, Ala402, Val407, Thr404
isophytol	-3,921	-	-2,121	-

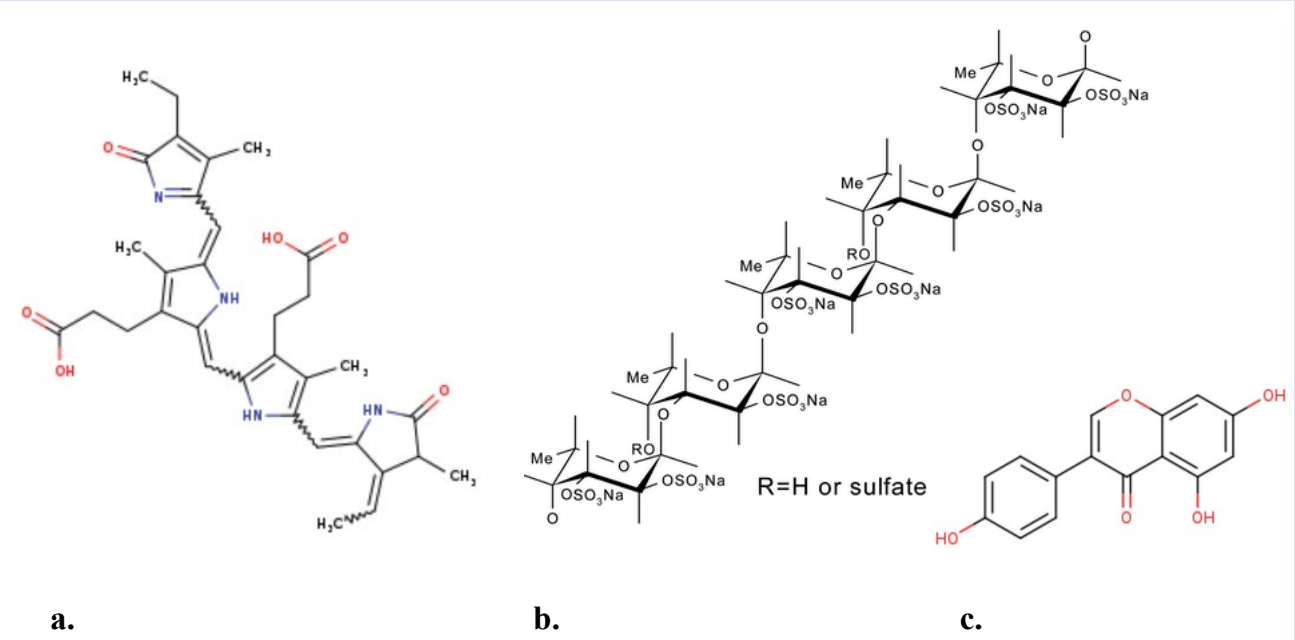

Figure 1. Marker compounds in Spirulina plantesis a) Phycocyanin, b) Polisakarida, c) Polyphenol (Genistein).

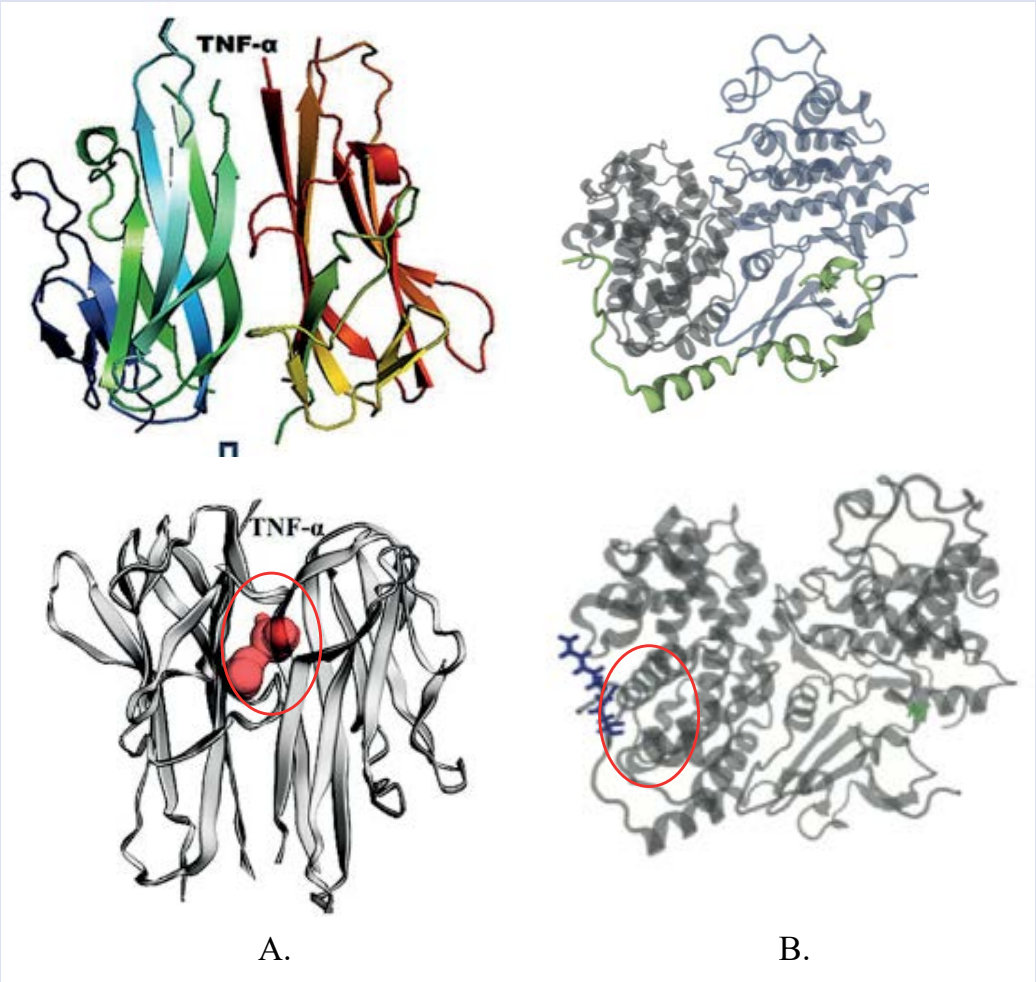

Figure 2. Active site of target; A. TNF[22] and B. p21[25]

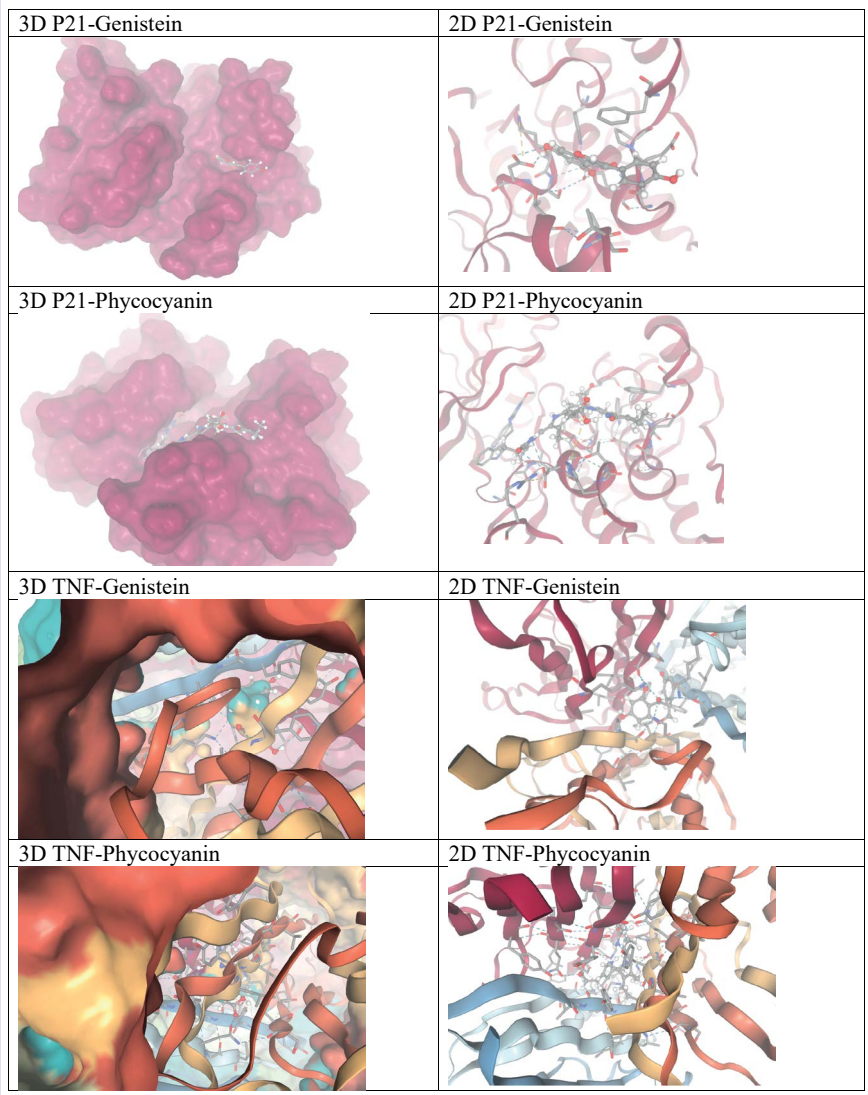

Figure 3. Molecular docking p21 ada TNF with Genistein and Phycocyanin.

various mechanisms. TNF-alpha stimulates the secretion of soluble factors from activated microglia, with TNF-alpha being the most potent stimulator, leading to the cell-to-cell propagation of α-synuclein and triggering cellular senescence.[27] Additionally, TNF-alpha induces permanent growth arrest in human umbilical vein endothelial cells (HUVECs) by activating a STAT-dependent autocrine loop, sustaining cytokine secretion, and promoting an interferon signature that locks cells into senescence.[12] Furthermore, TNF-alpha upregulates miR-155, which targets SIRT1, suppressing its expression and driving endothelial cell senescence, a process that can be disrupted by simvastatin via the miR-155/SIRT1/FoxO-1/p21 pathway signaling.[12] Overall, the active site of TNF-alpha plays a pivotal role in orchestrating senescence through various signaling pathways and mechanisms, highlighting its significance in the progression of age-related diseases.

From **Figure 3** our findings from this in silico study suggest that Spirulina platensis compounds, particularly Phycocyanin and polysaccharides, hold promise as natural inhibitors of TNF-alpha. These compounds could be further developed into supplements or pharmaceuticals aimed at reducing inflammation and delaying age-related cellular senescence. Future in vitro and in vivo studies are warranted to validate these interactions and assess the therapeutic potential of Spirulina platensis in age-related diseases and inflammatory conditions.

Molecular Docking of Spirulina platensis-Derived Compounds with P21 and Binding Affinity as Key Interactions

The molecular docking study investigated the interactions between bioactive compounds from Spirulina platensis and the cyclin-dependent kinase inhibitor p21, a key regulator of cell cycle arrest and cellular senescence. The selected compounds included Phycocyanin, polysaccharides (Ca-Spirulan), phenolic acids (such as Caffeic acid and Ferulic acid). Phycocyanin exhibited a strong binding affinity to p21, forming stable interactions primarily through hydrogen bonds and hydrophobic interactions. Key residues involved in binding were identified as Leu157, Phe159, and Ser153. These interactions suggest that Phycocyanin could stabilize the structure of p21, potentially enhancing its inhibitory effects on cyclin-dependent kinases (CDKs) and promoting cell cycle arrest. Polysaccharides, such as calcium spirulan, showed significant binding interactions with p21 through multiple hydrogen bonds. Notable interacting residues included Arg155 and Leu157. These interactions might reinforce the inhibitory function of p21 on CDKs, thereby contributing to its role in maintaining cell cycle arrest and preventing uncontrolled cell proliferation. Phenolic Acids, like Caffeic acid, Genistein and Ferulic acid demonstrated moderate binding affinities with p21, forming hydrogen bonds with residues like

Ser153. The antioxidant properties of these phenolic acids, combined with their ability to bind p21, may contribute to reducing oxidative stress-induced senescence and supporting p21's function in cell cycle regulation.

The active site of p21, a crucial cyclin-dependent kinase inhibitor, plays a significant role in regulating cell proliferation, survival, and motility.[28] It is involved in cell-cycle arrest by interacting with various molecules and transition factors, acting as both a tumor-suppressor gene and an inhibitor of apoptosis.[29] Additionally, the phosphorylation of p21 at Thr 145 through the PI3K/Akt pathway influences its functions, including inducing endoreduplication and protecting against apoptosis.[25] The active site of p21 is essential for its regulatory functions in cellular senescence, where it inhibits cyclin-dependent kinases to maintain cell cycle arrest and regulate tissue integrity.[30] Understanding the conformational changes and activity patterns of p21 at its active site is crucial for developing novel therapeutic strategies targeting p21 for the treatment of cancer and other diseases.[31] The enhancement of p21 activity by Spirulina platensis-derived compounds could play a significant role in delaying cellular senescence. p21 is crucial for enforcing cell cycle arrest in response to DNA damage and other stressors, thereby preventing the proliferation of damaged cells. By stabilizing p21, Spirulina platensis compounds may enhance its protective role against cellular damage and senescence, promoting cellular health and longevity.

Our results demonstrate the complex interactions among p21, TNF, and processes of cellular senescence. Together, our results highlight the various chemical interactions that Spirulina platensis compounds have interactions with proteins that are involved in cellular signaling cascades and inflammation, highlighting the potential therapeutic effects of these compounds of Spirulina platensis.

CONCLUSION

Our results imply that Spirulina platensis may contribute to overall cellular health and the mitigation of cellular senescence. The potential chemical interactions between substances generated from Spirulina platensis and senescence pathways, including those involving TNF and p21, are highly intriguing for the development of innovative therapeutic approaches targeted at ameliorating cellular senescence dysfunction associated with aging.

ACKNOWLEDGMENTS

None.

FUNDING

This research has received funding from Hibah Riset UI 2022 PUTI PASCASARJANA Universitas Indonesia.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

REFERENCES

1. Akbarizare M, Ofoghi H, Hadizadeh M. Dual effect of sapogenins extracted from spirulina platensis on telomerase activity in two different cell lines. *Mol Biol Res Commun.* Mar 2021;10(1):1-4.
2. Brito AF, Silva AS, de Oliveira CVC, et al. Spirulina platensis prevents oxidative stress and inflammation promoted by strength training in rats: dose-response relation study. *Sci Rep.* Apr 14 2020;10(1):6382.
3. Tamtaji OR, Heidari-soureshjani R. The effects of spirulina intake on clinical and metabolic parameters in Alzheimer's disease: A randomized, double-blind, controlled trial. *Phytotherapy ….* // 2023.
4. Beck E, Scheibe R. Senescence and Ageing in Plants and Cyanobacteria. *Physiologia Plantaum.* 2003;119:1-4.
5. Rahmawati S, Hidayatulloh S, Suprayatmi M. Extraction of Phycocyanin From Spirulina platensis For Biopigment and Antioxidant. *Jurnal Pertanian.* 2017;8:36-45.
6. Zhou T, Liu Y, Wang Q, et al. Spirulina platensis alleviates high fat diet-induced cognitive impairment in mice via the gut-brain axis. *Journal of Functional Foods.* 2021;86.
7. Hwang J-H, Lee I-T, Jeng K-C, et al. Spirulina prevents Memory Disfunction, Reduces Oxidative Stress Damage and Augments Antioxidant Activity in Senescence-Accelerated Mice. *J Nutr Sci Vitaminol.* 2011;57:186-191.
8. Zhang L, Pitcher LE, Yousefzadeh MJ, Niedernhofer LJ, Robbins PD, Zhu Y. Cellular senescence: a key therapeutic target in aging and diseases. *J Clin Invest.* Aug 1 2022;132(15).
9. Zhang L, Pitcher LE, Prahalad V, Niedernhofer LJ, Robbins PD. Targeting Cellular Senescence with Senotherapeutics: Senolytics and Senomorphics. *FEBS J.* Jan 11 2022.
10. Homann L, Rentschler M, Brenner E, Bohm K, Rocken M, Wieder T. IFN-gamma and TNF Induce Senescence and a Distinct Senescence-Associated Secretory Phenotype in Melanoma. *Cells.* Apr 30 2022;11(9).
11. QIU1 H-B, W-GB, L-JZ, et al. Inhibition of p53/p21 by TWIST alleviates TNF-α induced nucleus pulposus cell senescence in vitro. *European Review for Medical and Pharmacological Sciences.* 2020;24.
12. Kandhaya-Pillai R, Miro-Mur F, Alijotas-Reig J, Tchkonia T, Kirkland JL, Simo Schwartz J. TNF α - senescence initiates a STAT-dependent positive feedback loop, leading to a sustained interferon signature, DNA damage, and cytokine secretion. *Aging.* 2017;9.
13. Zheng QH, Ma LW, Zhu WG, Zhang ZY, Tong TJ. p21Waf1/Cip1 plays a critical role in modulating senescence through changes of DNA methylation. *J Cell Biochem.* Aug 1 2006;98(5):1230-1248.
14. Yosef R, Pilpel N, Papismadov N, et al. p21 maintains senescent cell viability under persistent DNA damage response by restraining JNK and caspase signaling. *EMBO J.* Aug 1 2017;36(15):2280-2295.
15. Tan KC, Pham TX, Lee Y, Lee JY, Balunas MJ. Identification of Apocarotenoids as Chemical Markers of In Vitro Anti-Inflammatory Activity for Spirulina Supplements. *J Agric Food Chem.* Nov 3 2021;69(43):12674-12685.
16. Guan L, Yang H, Cai Y, et al. ADMET-score – a comprehensive scoring function for evaluation of chemical drug-likeness. *MedChemComm.* 2019;10(1):148-157.
17. Sarkar A, Concilio S, Sessa L, Marrafino F, Piotto S. Advancements and novel approaches in modified AutoDock Vina algorithms for enhanced molecular docking. *Results in Chemistry.* 2024;7.
18. Roncaglioni A, Toropov AA, Toropova AP, Benfenati E. In silico methods to predict drug toxicity. *Curr Opin Pharmacol.* Oct 2013;13(5):802-806.
19. Aleksunes LM, Eaton DL. Principles of Toxicology. *Access Biomedical Sciences.* 2013;McGraw-Hill Education.
20. Lipinski CA, Ombardo FI, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings *Advanced Drug Delivery Reviews.* 1997;23:3-25.
21. Liljefors BT, K S, P K-L. Molecular Recognition. . *Madsen U (Eds.).* 2016;Textbook of Drug Design and Discovery(5th ed.). CRC Press. .

22. Xaveer Van Ostade, Jan Tavernier, Prange T, Fiers W. Localization of the active site of human tumour necrosis factor (hTNF) by mutational analysis. *The EMBO Journal.* 1991;10:827-836.

23. Molly M He, Annemarie Stroustrup Smith, Johan D. Oslob, et al. Small-Molecule Inhibition of TNF-a. *Science.* 2005;30:1022-1025.

24. Kaistha SD, Sinha R. Homology modeling of phosphoryl thymidine kinase of enterohemorrhagic Escherichia coli OH: 157. *Bioinformation 3(6): 240-243 (2009)* 2009.

25. Rudolph J, Crawford JJ, Hoeflich KP, Chernoff J. p21-activated kinase inhibitors. *Enzymes.* 2013;34 Pt. B:157-180.

26. Swadling JB, Warnecke T, Morris KL, Barr AR. Conserved Cdk inhibitors show unique structural responses to tyrosine phosphorylation. *Biophys J.* Jun 21 2022;121(12):2312-2329.

27. Bae EJ, Choi M, Kim JT, et al. TNF-alpha promotes alpha-synuclein propagation through stimulation of senescence-associated lysosomal exocytosis. *Exp Mol Med.* Jun 2022;54(6):788-800.

28. Gul M, Fakhar M, Najumuddin, Rashid S. Phosphorylation-dependent activity-based conformational changes in P21-activated kinase family members and screening of novel ATP competitive inhibitors. *PLoS One.* 2019;14(11):e0225132.

29. Moon DO, Choi YH, Kim GY. Role of p21 in SP600125-induced cell cycle arrest, endoreduplication, and apoptosis. *Cell Mol Life Sci.* Oct 2011;68(19):3249-3260.

30. Levi N, Papismadov N, Majewska J, et al. p21 facilitates chronic lung inflammation via epithelial and endothelial cells. *Aging.* 2023;15:2395-2417.

31. Parveen A, Akash MSH, Rehman K, Kyunna WW. Dual Role of p21 in the Progression of Cancer and Its Treatment. *Critical Reviews™ in Eukaryotic Gene Expression, 26(1):49–62 (2016).* 2016.

Cite this article: Susantiningsih T, Fadilah F, Prijanti AR, Hardiany NS. Molecular Insights into Spirulina platensis Compounds Targeting TNFa and P21 in Delaying Cellular Senescence Mechanisms: An In Silico Approach. Pharmacogn J. 2024;16(4): 809-815.