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Identification of Compounds in the *Spirulina platensis* Microalgae as Antieczematic Using Molecular Docking Method

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A B S T R A C T

Spirulina platensis is a blue-green microalgae that serves as a natural source of nutrients and is rich in various compounds such as vitamins, minerals, proteins, and bioactive substances. This microalgae can be utilized as an antieczematic agent for the treatment of eczema or atopic dermatitis. Eczema is a skin disease commonly found among people living in highly industrialized areas. The compounds found in Spirulina platensis that have potential as antieczematic agents include Tetradecanoic Acid, Hexadecanoic Acid, Hexanoic Acid Methyl Ester, and 9-Octadecenoic Acid. These four compounds were tested using the in silico method, specifically molecular docking. Molecular docking is the process of combining two compounds that will act as a ligand and a receptor protein. Before testing, the four compounds underwent SwissADME and PassOnline analysis to assess their potential as drug candidates. The molecular docking process will yield two values: binding affinity and RMSD. The binding affinity value indicates the energy released by the ligand to interact with the target protein, while the RMSD value shows how much conformational change occurs in the ligand compound.

Keywords: Spirulina platensis, Molecular Docking, SwissADME, PassOnline, Binding Affinity, RMSD

Introduction:

Antieczematic are compounds used to treat or prevent the skin disease eczema/atopic dermatitis human desease. This skin disease occurs due to inflammatory disorders of the skin. Eczema or atopic dermatitis is usually characterized by itching and the appearance of a red, blistering rash on the sufferer's skin. Eczema triggers can be influenced by various factors, including exposure to allergens or irritants in the sufferer's environment. Antieczematic reduce inflammation that occurs in the skin layers and soothe irritated skin areas (Azizah, 2022).

Spirulina plantesis has many high nutritional values such as vitamins, pigments, proteins, essential fatty acids, etc. The highest constituent of microalgae is protein. The protein contained in *Spirulina platensis* reaches a value of 50% with essential and non-essential amino acids needed by the human body. Compound The pharmacological content of the microalgae is also in the high category so it can be used in various sectors such as Hexadecanoic Acid, Tetradecanoic Acid, Hexanoic Acid Methyl Ester, etc. The compounds of microalgae can be utilize various fields such as antieczematic, Anti-inflammatory, antioxidant, antimicrobial and many more (Riyadi *et al*., 2024).

Potential from Spirulina can be demonstrated with in silico method like molecular docking. Molecular docking method is a computational modelling procedure used to predict chemical bonds between macromolecules (receptors) and small molecules (ligands). This method aims to identify interactions between compounds to estimate binding affinity and potential biological activity. Using this method has several advantages, namely being able to use compounds virtually, reducing costs and time used in research. Molecular docking can also be used to discover inhibitors of enzymes, analyze genetic mutations that influence protein function, and study interactions between protein compounds. The level of validity and accuracy of the prediction results depends on the quality of the target structure chosen, along with the science literature or article to support the results (Frimayanti *et al.*, 2021).

Material & Methods:

This study aims to explore the potential of bioactive compounds derived from the microalga Spirulina platensis as candidates for antieczematic agents and inhibitors of atopic dermatitis or eczema. Protein target used in this research is 5IKQ (Baehaqi *et al.,* 2022), which represents the COX-2 (Cyclooxygenase) enzyme. The methodology begins with the preparation of the protein by removing water molecules to enhance the accuracy of the results. This step aims to prevent water molecules from interfering with the hydrophobic interactions formed between the ligand and the target protein. Subsequently, an in silico analysis is performed on the compounds identified in the HPLC results of Spirulina platensis using PASS Online and SwissADME. This in silico evaluation helps determine the likelihood of the microalgal compounds to act as potential antieczematic agents. The next step involves downloading the 3D structures of the ligands from the PubChem database. The ligands utilized in this study include Tetradecanoic Acid,

Hexadecanoic Acid, Hexanoic Acid Methyl Ester, and 9-Octadecenoic Acid. Molecular docking is conducted using PyRx software, followed by an analysis of the binding interactions using Discovery Studio.

Computational Tools and Web Servers:

The docking process was conducted using a laptop equipped with an Intel Core i7-13700HX processor, RTX 4050 8gb, and 12GB of RAM. For in silico analysis, the PASS Online web server was accessed via https://www.way2drug.com while SwissADME analysis was performed using https://www.swissadme.ch/. The 3D structures of proteins were downloaded from the databases https://www.uniprot.org/ and https://www.rcsb.org/, whereas the 3D ligand structures were retrieved from the PubChem database at https://pubchem.ncbi.nlm.nih.gov/.

PassOnline

The in silico analysis results using the PASS Online method for each compound showed varying outcomes. Ligands with the highest Pa values were Tetradecanoic Acid and Hexadecenoic Acid. This was conducted to assess the potential of the ligand compounds to be used and to evaluate their properties as potential drug candidates, using SMILES structure. Based on these results, it can be concluded that these two compounds have a high probability of being potential Antieczematic candidates.

Ligand	SMILE Structure
Tetradecanoic Acid	CCCCCCCCCCCCCC(=O)O
Hexadecanoic Acid	CCCCCCCCCCCCCCCC(=O)O
Hexanoic Acid, Methyl Ester	CCCCCC(=O)OC
9-Octadecenoic Acid	CCCCCCCC/C=C/CCCCCCCC(=O)O

Table 1. SMILES Structure from Ligands *Spirulina platensis* Compound

Ligand	Pa Value	Pi Value
Tetradecanoic Acid	0,920	0,004
Hexadecanoic Acid	0,920	0,002
Hexanoic Acid, Methyl Ester	0,854	0,009
9-Octadecenoic Acid	0,720	0,012

Table 2. PASS Online Analysis Result Contains Probability from *Spirulina platensis* Compound

SwissADME

The parameters utilized in the in silico analysis results with the SwissADME method are highly variable. These parameters are selected based on the intended application of the drug, whether for dermal or oral use. The GI absorption parameter in the analysis results indicates whether the ligand can be absorbed during the digestive process. The outcome of this parameter is crucial when considering the ligand as a candidate for an orally administered drug. The Log Kp value is used to determine the suitability of a drug for epidermal application. A good standard for Log Kp is not less than -2.5 cm/s. The more negative the Log Kp value of a compound, the more difficult it will be for the compound to penetrate the skin, making it less suitable for use as an epidermal drug (Pratama *et al.,* 2023).

Ligand	Pharmacokinetic				
	GI *Absorption*	Pgp *Substrate*	CYP1A2 *Inhibitor*	CYP2D6 *Inhibitor*	Log Kp (cm/s)
Tetradecanoic Acid	High	No	Yes	No	-3,35
Hexanoic acid, methyl ester	High	No	No	No	-5,32
Hexadecanoic acid	High	No	Yes	No	-2,77
9-Octadecenoic Acid	High	No	Yes	No	-2,60

Table 3. SwissADME Pharmacokinetics Result from *Spirulina platensis* Compound

Ligand	Drug Likeness			
	Lipinski	Ghose	Veber	Bioavailability Score

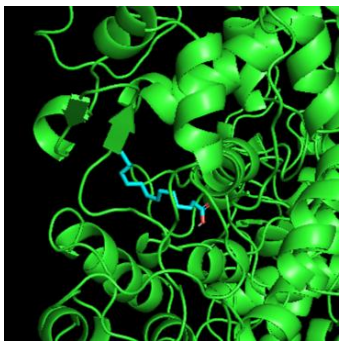
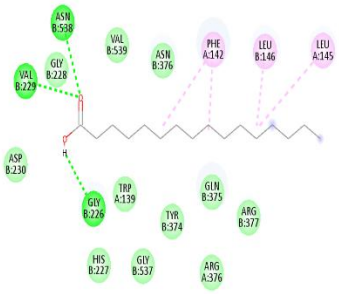
Tetradecanoic Acid	Yes	Yes	No	*0,85*
Hexanoic acid, methyl ester	Yes	No	Yes	*0,55*
Hexadecanoic acid	Yes	Yes	No	0,85
9-Octadecenoic Acid	Yes	No	No	0,85

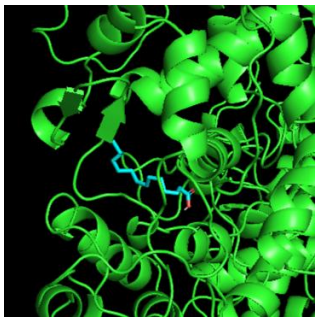
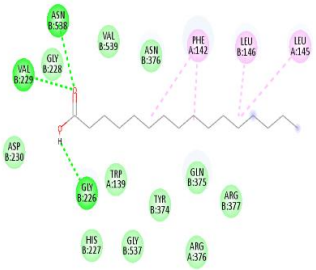
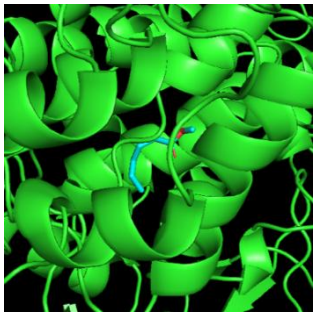
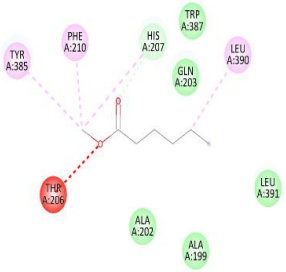
Table 4. SwissADME Drug-Likeness Result from *Spirulina platensis* Compound

Molecular Docking Analyze :

Molecular docking is a computational modeling procedure used to predict the chemical binding between macromolecules (receptors) and small molecules (ligands). This method aims to identify interactions between compounds to estimate binding affinity and potential biological activity (Frimayanti *et al.,* 2021). The PyRx software is an application used for virtual screening and molecular docking. This application features a user-friendly interface (UI) that simplifies research processes and offers various features that can be utilized for both molecular docking and virtual screening. One of the advantages of PyRx is its integration with other software such as AutoDock and AutoDock Vina (Dallakyan & Olson, 2015).

2D & 3D Structure

No	Ligand	3D Structure	2D Structure
1	Tetradecanoic Acid		

No	Ligand	3D Structure	2D Structure
2	Hexadecanoic Acid		
3	Hexanoic Acid, Methyl Ester		

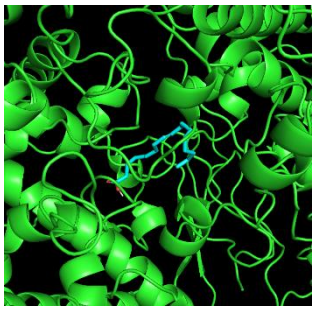
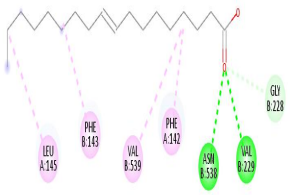
4	9-Octadecenoic Acid		

Table 5. 3D & 2D Structure Molecular Docking Visualization Results

The 2D visualization of interactions between the ligand and protein reveals the formation of various bonds, such as Van der Waals, carbon-hydrogen, conventional hydrogen, Pi-Alkyl, and others. These bonds influence the interactions between the ligand and protein. Hydrogen bonds in a compound play a significant role in stabilizing the interaction between the ligand and protein after binding (Gandu *et al.*, 2021). This indicates that hydrogen bonds help maintain the stability of the bond formed between the protein and ligand to ensure sufficient affinity.

Docking analysis reveals various bonds formed between the protein and ligand compounds tested. The table shows that the four compounds exhibit unfavourable interactions, carbon-hydrogen bonds, Van der Waals interactions, alkyl, and Pi-Alkyl bonds with varying distances. Amino acid residues within a distance of 5Å are considered to be part of the active site residues of the ligand used in the tests. Based on this, it can be concluded that all the amino acids listed in the table are active residues that interact with the ligand. A compound can be considered a successful inhibitor or reactant of a target protein in molecular docking if it forms a carbon or hydrogen bond within a maximum distance of 2.5Å (Vreven *et al.,* 2014).

Based on this criterion, the amino acid residues interacting with the ligand within the maximum distance include TRP388, HIS389, ILE409, PHE408, VAL229, ASN538, GLY226, ASP230, HIS227, ALA202, ALA199, LEU391, ASN538, VAL229, ASP230, and ARG376.

Binding Affinity & RMSD *(Root Mean Square Deviation)*

Based on the analysis table, the binding affinity results indicate the energy required by the ligand to interact. Tetradecanoic Acid has the strongest binding affinity value of -5.2 kcal/mol, indicating that this ligand exhibits a relatively strong interaction with the target protein (receptor). The RMSD values of 31.028 and 26.539 suggest a very high conformational change or rotational displacement from the ideal position. Hexadecanoic Acid, with a binding affinity of -4.8 kcal/mol and RMSD values of 2.907 and 1.477, shows more stable micromolecular changes compared to Tetradecanoic Acid, as indicated by its lower RMSD values. A smaller RMSD value suggests lower and more favorable conformational changes of the tested ligand compound. This implies that the ligand undergoes minimal changes when interacting with the target protein, making it more stable upon binding (Kaharudin *et al.,* 2022). The optimal RMSD (Root Mean Square Deviation) standard is < 2 Å. This indicates that the smaller the conformational changes during testing, the more accurate and stable the resulting binding affinity values. Smaller RMSD values signify minimal deviations from the ideal molecular configuration, reflecting a higher precision and reliability of the docking results (Masula *et al.,* 2018). Hexanoic Acid exhibits a binding affinity of -4.5 kcal/mol, with RMSD values of 3.260 and 2.353. Meanwhile, 9-Octadecenoic Acid demonstrates a strong binding affinity of -5.0 kcal/mol, second only to Hexadecanoic Acid, with lower RMSD values of 7.960 and 4.584. However, these results indicate that 9-Octadecenoic Acid has less stable macromolecular conformations despite its relatively good binding affinity.

The binding affinity analysis above reveals that fatty acid compounds exhibit the lowest binding affinity values, making them the best candidates for potential anti-eczematic agents. The mechanism by which fatty acids inhibit the formation of the COX2 enzyme begins with their competitive nature against pro-inflammatory compounds (eicosanoids), which support arachidonic acid activity. Pro-inflammatory compounds involved in COX2 enzyme formation include prostaglandin E2 (PGE2) and leukotriene (LTB4). Fatty acids substitute and integrate into the cell membrane, disrupting the synthesis of prostaglandin E2, a pathogenic compound and a structural component of the COX2 enzyme. Fatty acids also modulate the transcriptional activity of other pathogenic compounds, such as NF-κB and TNF-α. Additionally, fatty acids function as Specialized Pro-Resolving Mediators (SPMs), activating the clearance of eczematic inflammatory cells (Setiawan & Ernawati, 2024). This mechanism further supports their role in reducing inflammation and promoting healing in eczematic conditions.

Ligan	*Binding Affinity (kcal/mol)*	RMSD/ub (Å)	RMSD/lb (Å)
Tetradecanoic Acid	-5,2	31,028	26,539
Hexadecanoic Acid	-4,8	2,907	1,477
Hexanoic Acid, Methyl Ester	-4,5	3,260	2,353
9-Octadecenoic Acid	-5,0	7,960	4,584

Table 6. Binding Affinity & RMSD Result from Molecular Docking

Conclusion:

The conclusions derived from the study on the identification of bioactive compounds in *Spirulina platensis* microalgae as anti-eczematic agents using the molecular docking method are as follows:

1. Bioactive Compounds as COX2 Inhibitors found in *Spirulina platensis* microalgae have the potential to inhibit the pathogenic protein COX2, which is associated with eczema. The selected compounds include Tetradecanoic Acid, Hexadecanoic Acid, Hexanoic Acid Methyl Ester, and 9-Octadecenoic Acid. These compounds were analyzed using molecular docking, focusing on binding affinity and RMSD values. The results indicate that all four compounds have the potential to act as inhibitors of the target protein for anti-eczematic treatment. However, not all compounds exhibit stable inhibition due to variations in their binding affinity and RMSD values.
2. Best Performing Compounds with the lowest binding affinity was Tetradecanoic Acid, with a value of -5.2 kcal/mol. This result is supported by the formation of a conventional hydrogen bond with a bond distance of 2.5 Å. The compound with the best RMSD values, meeting the standard (< 2 Å), was Hexadecanoic Acid, with RMSD values of 2.907 Å and 1.477 Å. These results indicate that the conformation of Hexadecanoic Acid is the most balanced and stable among the tested compounds.

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