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In Silico Study Of Anti-Inflammatory Effect Of Jicama (Pachyrhizus Erosus L., Fabaceae) Tuber Fiber

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Abstract – Uncontrolled inflammation is a serious problem associated with various pathological conditions and must be treated appropriately. Jicama (Pachyrhizus erosus L., Fabaceae) fiber has been shown to exert an immunomodulatory effect and contain bioactive compounds that act as anti-inflammatory. However, the mechanism of jicama fiber in inhibiting the inflammatory signaling pathway is unknown. This study aimed to investigate the ability of bioactive compounds in jicama fiber as anti-inflammatory by molecular docking. The molecular docking was performed using PyRx 0.8 with the Vina algorithm and using BIOVIA Discovery Studio v21.1.0.20298 and PyMOL v1.7.4 to visualize the docking results and to analyze the conformation and interaction of ligands and receptor. This in silico test tethered eight bioactive compounds that have the potential as anti-inflammatory in Jicama fiber as ligands with toll-like receptor-4 (TLR4) as a receptor involved in the inflammatory response. The result demonstrated that Cycloartenol has the lowest binding affinity and the highest number of amino acid residues while interacting with TLR4 as compared to the other ligands namely Dexamethasone (a standard ligand), Stemphol, Astaxanthin, Farnesol, Benzoic Acid, 2.6-Dihydroxibenzoic Acid, Nerolidol, and 9-Octadecenoic Acid. Our recent study revealed that bioactive compounds in jicama fiber particularly Cycloartenol have a potential to inhibit inflammation through the inactivation of TLR4 which is the central signaling system in the inflammatory response.

Keywords – Cycloartenol; inflammation; molecular docking; PyRx; TLR4

I. INTRODUCTION

Inflammation is one of the body's mitigation processes that contribute to maintaining and restoring tissue homeostasis and defense mechanism against endogenous and exogenous stimuli that can harm the body [1]. However, when the self-limited nature of inflammatory response fails, inflammation can lead to a chronic condition which can cause various types of inflammation-related diseases and pathological conditions including metabolic disease, cardiovascular disease, cancer, alzheimer, inflammatory bowel disease (IBD), rheumatoid arthritis (RA) and asthma [2], [3], [4]. Several studies have been carried out on synthetic drugs and natural compounds to treat excessive inflammation [5]. One of the potential natural compound is Jicama (*Pachyrhizus erosus* L.) fiber which has been reported to act as an anti-inflammatory [6].

Jicama fiber has been shown to modulate the body's immune system by regulating macrophage activity, antibody production, and pro-inflammatory cytokines both in vitro and in vivo studies [7], [8]. Jicama fiber was also proven could prevent inflammation in the development of obesity [9], and prevent the accumulation of free radicals and macrophage infiltration in the liver of hyperglycemia-induced mice[10]. Jicama fiber has been identified to contain bioactive compounds that are thought to have anti-inflammatory activity [6]. However, until recently, the mechanism of jicama fiber in inhibiting the inflammatory signaling pathway is unknown. This study aimed to investigate the ability of bioactive compounds in jicama fiber as anti-inflammatory through molecular docking. The conformation of bioactive compounds in jicama fiber as the ligand and target protein can be predicted to analyze the potential of a compound that plays a role in the inflammation signaling pathway [11]. The

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result studies can be used to help identify and design new drugs and select the most effective drug candidates against inflammation.

Toll-like receptor-4 (TLR4) are receptors that plays a role important in initiating inflammatory signaling pathways. TLR4 can recognize lipopolysaccharide (LPS) and free fatty acids (FFA) and then induce several signaling pathways that lead to the expression of molecules for leukocyte differentiation and activation as well as the release of proinflammatory cytokines [12] According to Reference [13] that activation and dysregulation of TLR4 signaling may contribute to chronic disease states. Consequently, TLR4 inactivation can inhibit the excessive inflammatory response. Therefore, this study is expected to be able to explain which compounds in jicama fiber have the most potential to play a role in inhibiting the inflammatory responses through the TLR4 pathway.

II. Research Methods

2.1. Bioactivity Score Prediction

There were eight compounds contained in jicama fiber that have been reported as potential anti-inflammatories namely Stemphol, Astaxanthin, Farnesol, Benzoic Acid, 2,6-Dihydroxibenzoic acid, Cycloartenol, Nerolidol, and 9-Octadecenpic Acid [6] These eight bioactive compounds act as ligands whose 3D form downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). These eight bioactive compounds were further identified for their bioactivity spectrum as anti-inflammatory using PASS (https://way2drug.com/passonline/) in mol format [14].

2.2. Evaluation of Drug Likeliness Based on Lipinski's Rule of Five

The molecular properties of the ligands which are important for drug pharmacokinetics in the human body are evaluated using Lipinski's Rule of five test software website-based in SDF format (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). The results of the evaluation of the molecular properties of the ligands are then summarized and declared eligible if two of the five requirements of Lipinski's Rule of five tests are met [15].

2.3. Ligand Preparation

The selected ligands using PASS and Lipinski's Rule of five tests were then prepared using BIOVIA Discovery Studio v21.1.0.20298 software. The ligand that has been downloaded from the PubChem database is prepared and save in SDF format. The anti-inflammatory drug used as a standard ligand in this study is Dexamethasone (CID: 5743) [18].

2.4. Receptor Preparation

The receptor used in this study was downloaded from the Protein Data Bank database (http://www.rcsb.org) in pdb file format, namely toll-like receptor-4 (TLR4) (PBD ID: 3FXI) which is the protein involved in the inflammatory mechanism [16]. Then BIOVIA Discovery Studio v21.1.0.20298 was used to remove water molecules, native ligands and other non-standard residues from the receptor [17].

2.5. Molecular Docking Simulation

Molecular docking studies were performed using PyRx 0.8. The receptor was loaded to PyRx workplace windows and transformed into Macromolecules. Then the Open Babel tab used to select and transform ligands into Autodock Ligands. Vina was chosen as the docking algorithm. Molecular docking was finished by following the wizard's stage. The RMSD value 0.00 was selected for the docking results [19].

2.6. Visualization and Data Analysis

PyRx score data presented as kcal/mol were assessed based on the results of ligands interaction. Three-dimensional conformation of docking result in PDB format was visualized using PyMOL v1.7.4, meanwhile two-dimensional conformation was analyzed and visualized using BIOVIA Discovery Studio v21.1.0.20298 [17].

III. Result And Discussion

Based on previous studies, there were eight bioactive compounds that were known to have the potential as an anti-inflammatory [6]. Compounds that can act as anti-inflammatory in jicama fiber are sequentially based on the largest to the smallest area, namely Stemphol, Astaxanthin, Farnesol, Benzoic Acid, 2.6-Dihydroxibenzoic Acid, Cycloartenol, Nerolidol, and

9-Octadecenoic Acid. These eight compounds that have known potential as anti-inflammatory bioactivity spectrums were predicted using PASS software and analyzed for their molecular and physicochemical properties using Lipinski's Rule of five test software as a requirement in determining which bioactive compounds can be tested in silico. Prediction results with PASS as shown in Table 1.

All the compounds tested for PASS had an anti-inflammatory activity with varying probable activity (Pa) values. The Pa values of bioactive compounds with anti-inflammatory activity from the largest to the smallest were Astaxanthin, Nerolidol, 2.6-Dihydroxibenzoic Acid, 9-Octadecenoic Acid, Benzoic Acid, Cycloartenol, Farnesol, and Stemphol. Based on PASS (Table 1) it is confirmed that these eight compounds have a role in inhibiting the inflammatory response. In addition, based on the PASS results, this can be used as a reference for these eight compounds to be tested in silico with molecular docking as an anti-inflammatory agent.

The PASS prediction results are interpreted as follows: (i) if Pa>0.7, the probability of activity of the compound experimentally is quite high and there is a possibility that the compound being tested is an analog of the existing drug, (ii) if 0.5<Pa< 0.7, the possibility of activity of the experimental compound is relatively low and the substance tends to be different from the existing drugs, (iii) if Pa<0.5, the probability of finding the activity of the compound experimentally is very small [14].

Table 1. Possible bioactivity of bioactive compounds contained in jicama fiber based on PASS test

No	Compounds	Bioactivity	Probable Activity	Information
1	Stemphol	TNF expression inhibitor	0.528	Low
		Anti-inflammatory, intestinal	0.518	Low
2	Astaxanthin	Anti-inflammatory	0.985	High
		Immunosuppressant	0.870	High
3	Farnesol	TNF expression inhibitor	0.840	High
		Anti-inflammatory	0.643	Low
		Immunosuppressant	0.595	Low
4	Benzoic Acid	Anti-inflammatory, intestinal	0.717	High
		TNF expression inhibitor	0.486	Very Low
5	2.6-Dihydroxibenzoic Acid	Anti-inflammatory, intestinal	0.736	High
		TNF expression inhibitor	0.506	Low
6	Cycloertanol	Anti-inflammatory	0.702	High
		Immunosuppressant	0.624	Low
7	Nerolidol	Anti-inflammatory	0.800	High
		TNF expression inhibitor	0.559	Low
		Immunosuppressant	0.485	Very Low
8	9-Octadecenoic Acid	Leukotriene-C4 synthase inhibitor	0.720	High
		TNF expression inhibitor	0.724	High
		Anti-inflammatory, intestinal	0.685	Low
		Immunosuppressant	0.505	Low

Furthermore, the test results of bioactive compounds contained in jicama fiber using Lipinski's Rule of five test software are presented in the following table.

Table 2. Evaluation of physicochemical properties of bioactive compounds contained in jicama fiber based on Lipinski's Rule of Five Test

No	Compound Name	Characteristics					
		Molecular Weight (Da)	***Log P***	***H Donor***	***H Acceptor***	***Molar Refraction***	***Justification***
1	Stemphol	312	0.053	5	6	77.145	Valid
2	Astaxanthin	596	8.905	2	4	184.951	Valid
3	Farnesol	222	4.398	1	1	72.499	Valid
4	Benzoic Acid	122	4.260	1.4	2	33.401	Valid
5	2.6-Dihydroxibenzoic Acid	154	0.796	3	4	36.730	Valid
6	Cycloartanol	426	8.169	1	1	130.720	Valid
7	Nerolidol	222	4.396	1	1	72.477	Valid
8	9-Octadecenoic Acid	282	6.108	1	2	87.088	Valid

Note: Valid (meets requirements for in silico test based on Lipinski rules)

Based on Table 2, it is known that the molecular weight of the eight compounds is less than 500 Da, which means that they meet the requirements except for astaxanthin compounds. Furthermore, Astaxanthin, Cycloartenol, and Nerolidol had log P values that exceeded the standard. The H donor, it appears that the Stemphol does not meet the requirements where the value of the H donor must be less than 5. The H acceptor value shows that all compounds meet the requirements with a value of less than 10 H acceptors. Astaxanthin compounds do not appear to meet the requirements for a molar refraction value that exceeds the value of standard molar refraction. Based on Lipinski's Rule of five tests, it was found that the eight compounds were valid or eligible for the in silico test with molecular docking because the eight compounds had met at least two of the five physiochemical properties based on Lipinski's rules.

Lipinski's Rule of Five is a rule of thumb for evaluating whether a compound with a particular pharmacological activity has physical or chemical properties that are important for its pharmacokinetics in the human body. This rule explains that a compound has properties similar to drugs if the compound has a molecular weight of <500 Da, has a number of hydrogen bond donors <5 and a number of hydrogen bond acceptors <10, and is related to passive diffusion capabilities which include lipophilicity (Log P) <5 and the molar refractivity should be between 40-130. Ligands weighing <50 Da more easily through the cell membrane than ligands weighing >50 Da [15], [20].

Subsequently, in silico test was performed with molecular docking using Stemphol, Astaxanthin, Farnesol, Benzoic Acid, 2.6-Dihydroxibenzoic Acid, Cycloertanol, Nerolidol, 9-Octadecenoic Acid on receptor involved in inflammation namely TLR4. The molecular docking results are shown in the following table.

Table 3. Results of molecular docking of ligands to TLR4

No	Ligand	Pubchem ID	Binding Affinity (kcal/mol)
1	Dexamethasone (Standard)	5743	-8.4
2	Stemphol	170949	-7.0
3	Astaxanthin	5281224	-8.4
4	Farnesol	445070	-7.1
5	Benzoic Acid	243	-5.8
6	2.6-Dihydroxibenzoic Acid	9338	-5.9

7	Cycloartenol	92110	-10.3
8	Nerolidol	5284507	-7.0
9	9-Octadecenoic Acid	445639	-6.3

The results of docking eight compounds to the TLR4 showed that the lowest binding affinity value was in Cycloartenol with a lower value than the standard ligand, namely -10.3 kcal/mol while the highest binding affinity value was in Benzoic Acid, namely -5.8 kcal/mol. Table 3 presents Cycloartenol have the lowest binding affinity with TLR4 than other bioactive compounds and standard ligand, indicating an excellent affinity.

Visualization of the docking result is presented in Figure 1-9 and Table 4 which represents the conformation, amino acids recidues, and interaction between the ligands and TLR4.

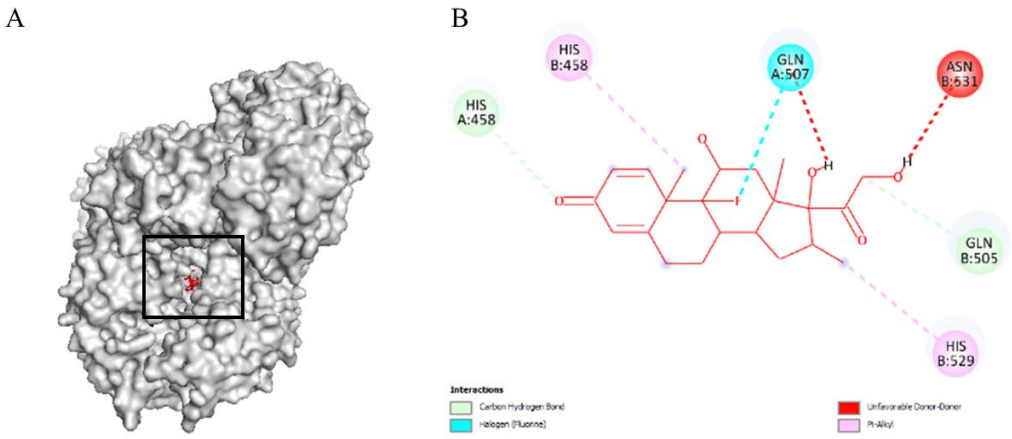

Figure 1. Interaction between TLR4 and Dexamethasone (A) 3D interaction (B) 2D interaction

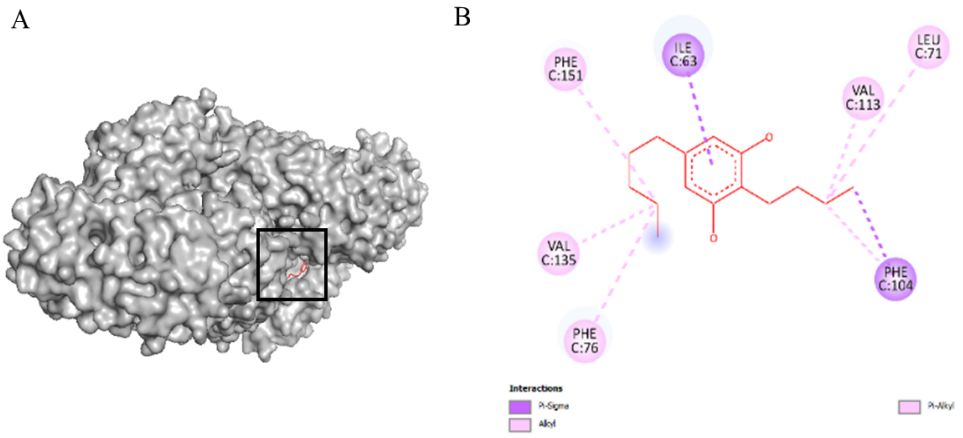

Figure 2. Interaction between TLR4 and Stemphol (A) 3D interaction (B) 2D interaction

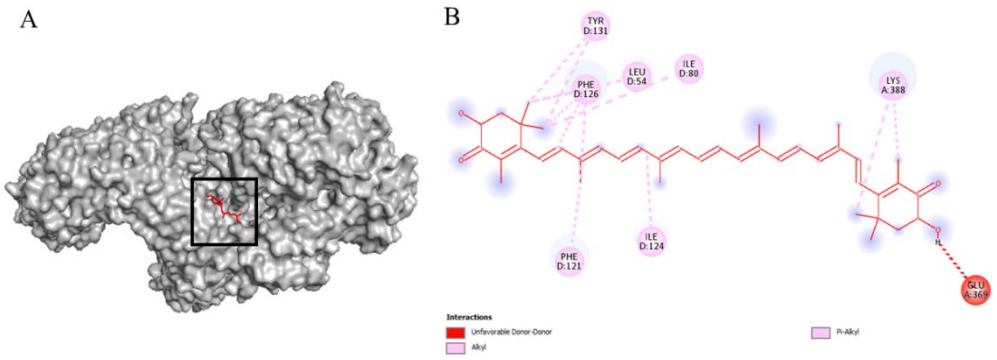

Figure 3. Interaction between TLR4 and Astaxanthin (A) 3D interaction (B) 2D interaction

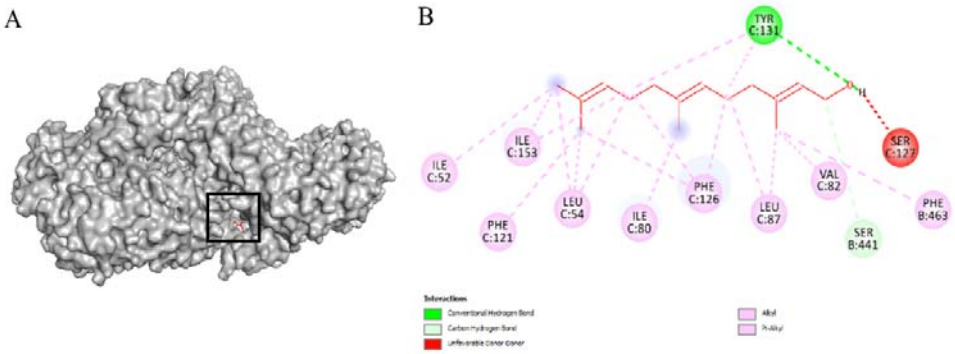

Figure 4. Interaction between TLR4 and Farnesol (A) 3D interaction (B) 2D interaction

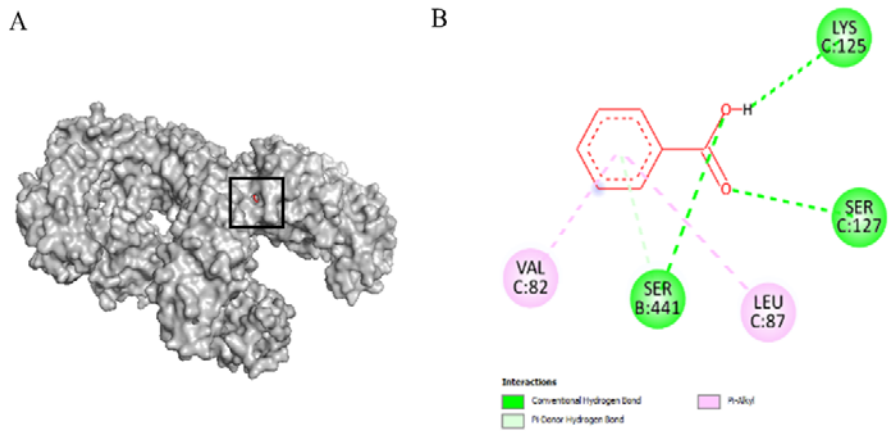

Figure 5. Interaction between TLR4 and Benzoic Acid (A) 3D interaction (B) 2D interaction

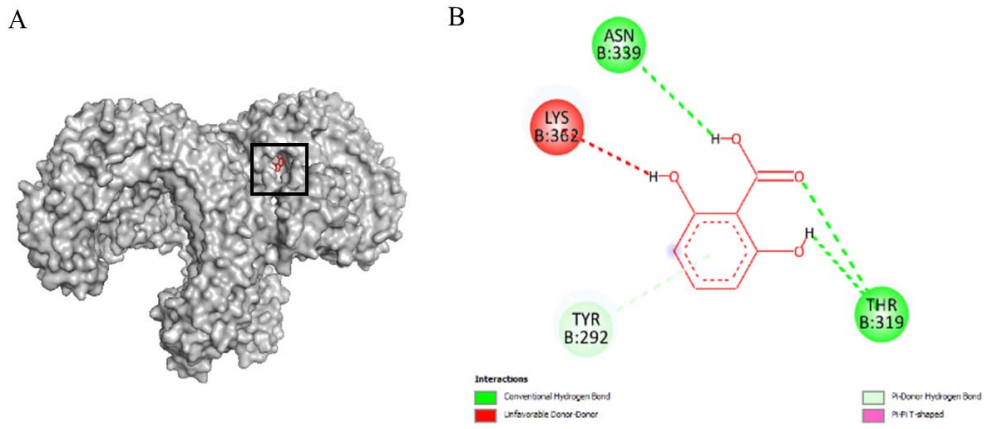

Figure 6. Interaction between TLR4 and 2.6-Dihydroxibenzoic Acid (A) 3D interaction (B) 2D intera

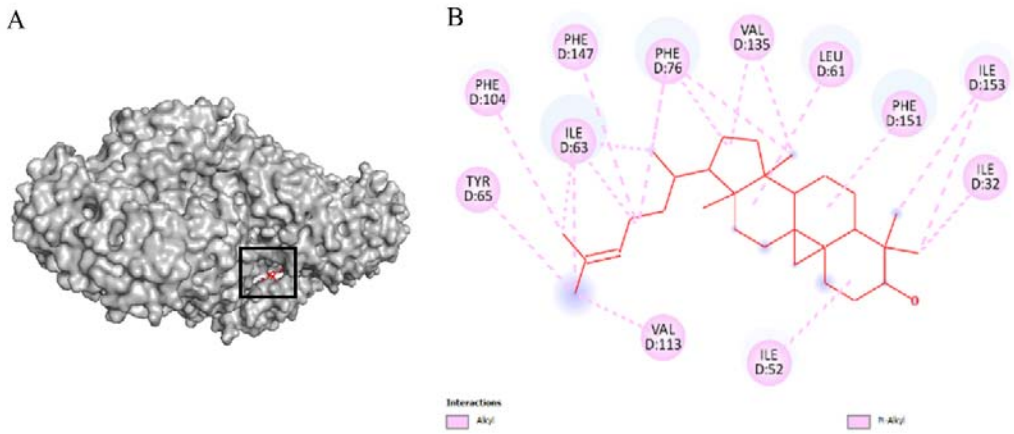

Figure 7. Interaction between TLR4 and Cycloartenol (A) 3D interaction (B) 2D interaction

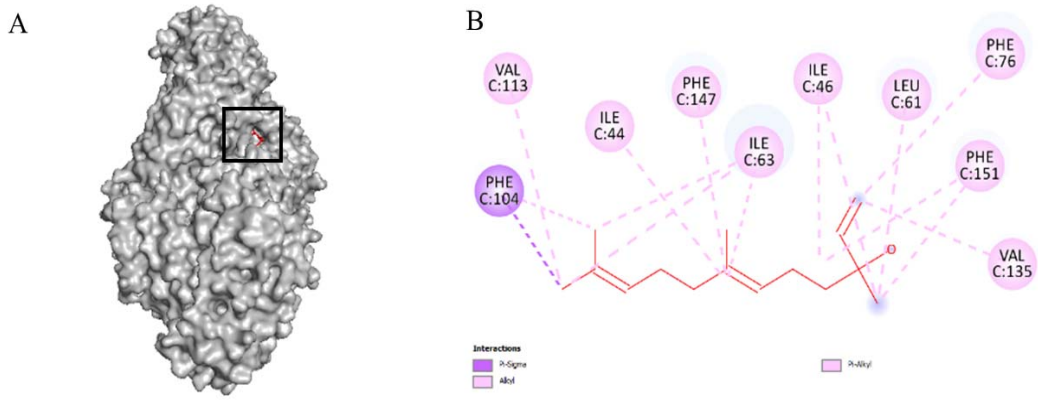

Figure 8. Interaction between TLR4 and Neroldol (A) 3D interaction (B) 2D interaction

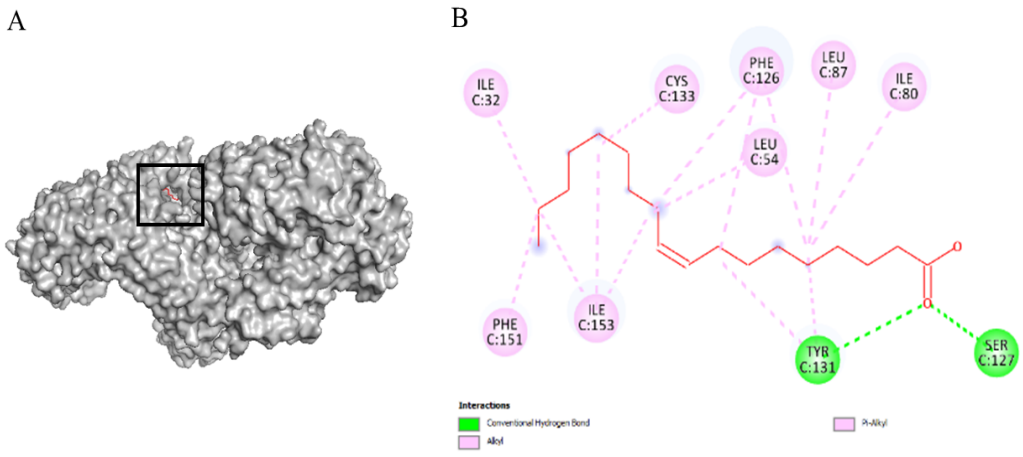

Figure 9. Interaction between TLR4 and 9-Octadecenoic Acid (A) 3D interaction (B) 2D interaction

The most amino acid residues on the TLR4 receptor were found in the Cycloartenol ligand interaction, which was as many as 20 bonds, and the least was found in the interaction of the 2.6-Dihydroxybenzoic acid ligand, which was as many as six bonds. The highest number of hydrogen bonds formed was in the interaction of TLR4 with Benzoic Acid while the highest number of hydrophobic bonds formed was in the interaction of TLR4 with Cycloartenol.

Based on the results of molecular docking visualization of Stemphol, Astaxanthin, Farnesol, Benzoic Acid, 2.6-Dihydroxibenzoic Acid, Nerolidol and 9-Octadecenoic Acid on TLR4 represent that there are no similar amino acid residues involved in interactions between these eight ligands and Dexamethasone on TLR4. In addition, there is no similarity in the molecular structure and binding site between the eight bioactive compounds in the jicama fiber and Dexamethasone on TLR4.

Table 4. Interaction between ligands and TLR4

No	Ligands	Amino acid residues involved in the interaction	Total Hydrogen Bond	Total Hydrophobic Bond
1	Dexamethasone (Standard)	GLN505; HIS458; HIS458; GLN507; HIS531; HIS529	2	2
2	Stemphol	PHE104, ILE62, VAL135, LEU71, VAL113, PHE76, PHE104, PHE151	0	8
3	Farnesol	TR131, SER441, LEU87, ILE80, VAL82, LEU87, LEU54, ILE153, ILE52, LEU54, ILE153, LEU54, PHE463, PHE121, PHE126, PHE126, PHE126, TRY131, TRY131	2	17
4	Benzoic Acid	LYS125, SER441, SER127, SER127, SER441, VAL82, LEU87	5	2
5	2,6-Dihydroxibenzoic Acid	ASN339, THR319, THR319, TYR292, TYR292	5	1
6	Cycloertanol	LEU61, ILE52, VAL135, ILE153, ILE32, ILE153, ILE63, ILE63, ILE63, VAL113, ILE63, VAL113, ILE63, TRY65, PHE76, PHE76, PHE76, PHE104, PHE147, ,PHE, 151	0	20
7	Nerolidol	PHE104, ILE63, ILE146, ILE46, LEU61, VAL135, ILE63, ILE63, VAL113, PHE76, PHE104, PHE147, PHE147, PHE151, PHE151,	0	15
9	9-Octadecenoic Acid	SER127, TRY131, ILE80, LEU87, LEU54,	2	14

		ILE153, ILE153, ILE32, ILE153, CYS133, PHE126, PHE126, PHE126, TRY131, TRY131, TRY131		

According to the value of binding affinity and ligand interactions obtained from the molecular docking results, revealed that of all the ligands tested, Cycloartenol is the most stable compound that binds to the TLR4, Thus predicting it can prevent inflammation by inhibiting inflammatory signaling. The molecular docking results showed that Cycloartenol was the best bioactive compound because it was found to have the lowest binding affinity value and compared to eight other bioactive compounds against the TLR4 even lower than the standard ligand binding affinity value. According to Reference [21] that the lower the binding affinity value of the docking of a ligand to macromolecules, the stronger and more stable the bond between the two is.

According to the interaction of ligands with macromolecules, it appears that Cycloartenol only has ligand interactions with the type of hydrophobic binding to the TLR4. Cycloartenol ligands have a higher number of bonds than other ligands, although these hydrophobic bonds are weaker than hydrogen bonds. Based on Reference [22] that the stability of the bond between the ligand and its target can be influenced by the number and type of ligand interactions. The more bonds formed, the more complex the conformation formed between the ligand and the macromolecule so that the interaction becomes more stable.

The in silico test in this study indicated that eight bioactive compounds in jicama fiber particularly Cycloartenol can inhibit inflammation through the inactivation of TLR4 which is the central signaling system in the inflammatory response. Cycloartenol was reported contained in the triterpene fraction extracted from *Crataegus monogyna* Jacq has anti-inflammatory activity by preventing leukocyte migration and inhibiting phospholipase A2 (PLA2) [23]. Cycloartenol isolated from Euphorbia neriifolia leaves has proven play a role in increasing antioxidant activity and preventing lipid peroxidation[24]. Thus, it can prevent inflammatory response signaling due to oxidative stress. Cycloartenol was also reported to had various pharmacological activities such as antidiabetic, antitumor, antioxidant, anti-inflammatory, and anti-alzheimer [25].

IV. Conclusions

Bioactive compounds in jicama fiber particularly Cycloartenol have a potential to inhibit inflammation through the inactivation of TLR4 which is the central signaling system in the inflammatory response. Cycloartenol is the best compounds that has excellent interaction to TLR4 because it has the lowest binding affinity value and highest number of amino acids residues than standard ligand and seven other bioactive compounds.

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