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Interaction Affinity Between Flavonoids of *Moringa Oleifera* Leaves Against Cytokines Interleukin 12 in Diabetes Mellitus

Afinitas Interaksi Antara Flavonoid Terhadap Sitokin Interleukin 12 Pada Penyakit Diabetes Militus

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ABSTRACT

Background: Interleukin 12 can destroy insulin-producing cells, suppresses IL4 production, and can stimulate the formation of Thelper1. Quercetin is a flavonoid suitable the lead compound for development of safe anti-diabetic agent because its anti-diabetic effect and protective effect in pancreas track.
Objective: This research aims to study the docking models of certain flavonoids and to predict the quercetin and triterpene derivatives inhibition activity on Interleukin12.
Method: The molecular docking method was used using the PyRx program with the Autodock Vina menu.
Results: IL-12 bond affinity with Dextromethoorpene results -7 kcal/mol as the highest affinity value energy while the lowest energy is -6.1 kcal/mol, IL-12 bond affinity value with Quercetin with the highest affinity value energy is -9 kcal/mol, and the lowest energy is -7.8kcal/mol. The affinity value IL-12 bond and triterpene with the highest affinity value is energy -7 .9 kcal/mol, and the lowest energy is -7.4 kcal/mol
Conclusion: Quercetin, has hydrogen bonds and hydrophobic bonds, where the ligand of the Quercetin compound is the ligand that has the ability to form the best interactions and bonds with IL-12 receptors (4OG9)

Keywords: ***IL-12, quercetin, diabetes millitus, docking***

ABSTRAK

Latar Belakang: Interleukin 12 dapat menghancurkan sel penghasil insulin, menekan produksi IL4, dan dapat merangsang terbentuknya T helper1. Quercetin adalah flavonoid sebagai senyawa utama untuk pengembangan agen antidiabetes yang aman, karena efek antidiabetes dan efek perlindungan pada jalur pankreas
Tujuan: Untuk mempelajari model docking flavonoid tertentu dan memprediksi aktivitas penghambatan turunan quercetin terhadap Interleukin12
Metode: Molekuler Docking dengan menggunakan PyRx menggunakan program Autodock Vina
Hasil: Afinitas ikatan IL-12 dengan Dekstrometoorpen afinitas tertinggi energi seminimum mungkin yaitu -7 kcal/mol dan yang paling rendah atau energi maksimum yaitu -6,1 kcal/mol, nilai afinitas ikatan IL-12 dengan Quercetin dengan nilai afinitas tertinggi yaitu energi seminimum mungkin yaitu -9 kcal/mol dan yang paling rendah atau energi maksimum yaitu -7,8kcal/mol dan Nilai afinitas ikatan IL-12 dengan Triterpene nilai ikatan afinitas antara ikatan IL-12 dengan triterpene dengan nilai afinitas tertinggi yaitu energi seminimum mungkin yaitu -7,9 kcal/mol dan yang paling rendah atau energi maksimum yaitu -7,4 kcal/mol
Kesimpulan: Quercetin, memiliki ikatan hidrogen dan ikatan hidrofobik, dimana ligan dari senyawa Quercetin merupakan ligan yang memiliki kemampuan untuk membentuk interaksi dan ikatan terbaik dengan reseptor IL-12 (4OG9)

Kata kunci: IL-12, Quercetin, Diabetes Millitus, Docking

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INTRODUCTION

The small proteins, cytokines, are mediators and regulators of immunity, inflammation and hematopoiesis. They are one of a number of substances produced by certain cells of the immune system that carry signals between local cells, resulting in effects on other cell as a result of the immune system's stimulus response. Interleukin 12 is a cytokine secreted by macrophage cells, dendrite cells, and neutrophils that is directly triggered by the presence of lipopolysaccharides or pathogenic microorganisms that enter the body.[1] The role of the cytokine interleukin 12 is proven to be an interleukin that regulates the intra-cellular immunity center that activates NK cells, which are the main essential mediators to convert Thelper 0 into Thelper 1, and can directly trigger the production of IFN-Ÿ by Thelper1 cells and NK cells.[2] IFN-Ÿ is a glycoprotein; an activator for monocular phagocytes known to contain antiviral effects, produced by NK cells. While NK cells as triggers are cytokines produced by macrophages in the form of TNF-α and interleukin 12 and IFN-Ÿ itself.[3]

Diabetes is a metabolic disease classified by hyperglycemia due to effects on insulin secretion, insulin action, or both. Chronic hyperglycemia in diabetes is strongly associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, innervation, heart, and blood vessels.[4] At the same time, elevated blood glucose levels can affect changes in estimated Glomerular Filtration Rate (GFR) in patients with diabetes mellitus.[5]

IFN-Ÿ seems to have an important role in the development of insulin dependent diabetes mellitus (IDDM). Interleukin 12 is an interleukin which has the nature of destroying insulin-producing cells that can affect glycose levels in human blood. In addition, IL-12 can also suppress the production of IL4, and it can stimulate the formation of Thelper1 which produces pro-inflammatory cytokines.[6',7,8] Interleukin-12 (IL-12) is a pro-inflammatory cytokine and increased in type 2 DM contributes to the occurrence of inflammation to the development of atherosclerotic plaques.[9,10]

Moringa oleifera is a plant that contains many active compounds that have a function as an antidote to Reactive oxygen species by increasing endogenous antioxidant enzymes which have a central role in dealing with oxidative stress.[3,11]Moringa oleifera can also inhibit proinflammation through the TLR3 and TLR4 pathways while restoring naïve T reg cells.[12]

DM patients require pharmacotherapy such as injected insulin or oral antidiabetic drugs, but unfortunately all of these drugs can have dangerous side effects.[6',7]

Some flavonoids including quercetin-3-glycoside derived from plants have a function to regulate blood sugar levels. 4OG9 affects glucose intake in the small intestine mucosa leading the absorption time of glucose to the blood is longer which in turn can reduce blood sugar levels.[13,14]

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The affinity interaction between flavonoids and interleukin 12 can be observed using Structure-Based Drug Design (SBDD) where the three-dimensional structure of the drug target is used as a reference for developing herbal drugs. Recently, computer-aided drug design (CADD) techniques have developed rapidly.

Predicting the three-dimensional structure of the complex between the target protein and the drug candidate plays an important role in SBDD. Currently, many computer programs can be used to predict protein-ligand interactions, known as docking, such as GOLD, FlexX, Glide, DOCK, and AutoDock.[15,16]

The affinity interaction between the Interleukin 12 Protein macromolecule and (4OG9) with each activation ligand, namely quercetin, triterpenes. As a control using the drug Dextrometoorpen, it can be known through docking results using the PyRx program with the Autodock Vina menu, Specific Docking. It aims to predict whether the compound has activity before being tested.[17].

RESEARCH MATERIALS AND METHOD

This study was molecular docking method using PyRx program with Autodock Vina menu.

Materials

The structure of IL-12 protein that has binded with ligands from Protein Databank (PDB), various quercertin and triterpene ligands test, and dextroometorpane control were the materials of the study.

INSTRUMENT

A processor speed of 1.86 GHz and a memory capacity of 2 GB with ArgusLab 4.0.1 software of computer was used as the instrument of this study.

ANALYSIS RESULT

Analysis of ducking results includes free energy and hydrogen bonding between the ligand and the enzyme.

RESULT AND DISCUSSION

Table 1. IL-12 binding affinity with Dekstrometoorpen

Ligand	Binding Affinity	rmsd/ub	Rmsd/lb
4OG9_Deks	-7	0	0
4OG9_Deks	-6.7	32.916	30.339
4OG9_Deks	-6.4	4.95	2.648
4OG9_Deks	-6.3	18.617	16.24
4OG9_Deks	-6.3	36.345	33.645
4OG9_Deks	-6.2	45.997	42.897
4OG9_Deks	-6.2	19.492	17.874
4OG9_Deks	-6.1	51.734	49.534
4OG9_Deks	-6.1	17.885	15.001

The table presents there are 9 predicted bond affinity values between IL-12 and triterpene bonds with the highest affinity value being the minimum possible energy of -7.9 kcal/mol and the lowest or maximum energy being -7.4 kcal/mol.

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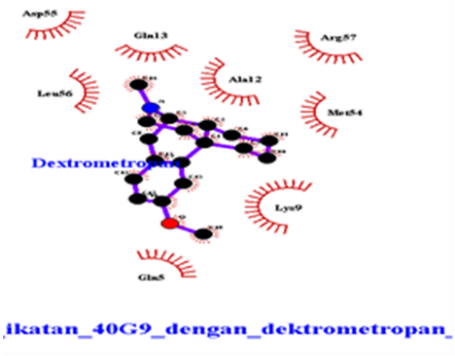

Figure 1. Binding visualization between IL-12 and dextroomethorpene

Table 2. Binding affinity of IL-12 with Quercetin

Ligand	Binding Afinity	rmsd/ub	Rmsd/lb
4OG9_Quercetin	-9	0	0
4OG9_Quercetin	-8.7	3.644	2.288
4OG9_Quercetin	-8.3	7.227	2.026
4OG9_Quercetin	-8.2	3.514	1.665
4OG9_Quercetin	-8	7.222	5.244
4OG9_Quercetin	-7.9	6.152	3.532
4OG9_Quercetin	-7.8	6.514	4.208
4OG9_Quercetin	-7.8	7.96	3.301
4OG9_Quercetin	-7.8	8.617	3.75

From the table, it shows there are 9 predicted bond affinity values between IL12 and Quercetin with the highest affinity value being the minimum possible energy of -9 kcal/mol and the lowest or maximum energy being 7.8kcal/mol

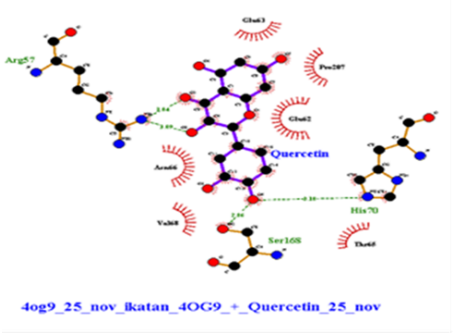

Figure 2. Binding visualization between IL12 with Quercetin

Table 3. Binding Affinity of IL-12 with Triterpene

Ligand	Binding Afinity	rmsd/ub	Rmsd/lb
4OG9_Quercetin	-9	0	0
4OG9_Quercetin	-8.7	3.644	2.288
4OG9_Quercetin	-8.3	7.227	2.026
4OG9_Quercetin	-8.2	3.514	1.665
4OG9_Quercetin	-8	7.222	5.244
4OG9_Quercetin	-7.9	6.152	3.532
4OG9_Quercetin	-7.8	6.514	4.208
4OG9_Quercetin	-7.8	7.96	3.301
4OG9_Quercetin	-7.8	8.617	3.75

Table 3 presents there are 9 predicted bond affinity values between IL-12 and triterpene bonds with the highest affinity value being the minimum possible energy of -7.9 kcal/mol and the lowest or maximum energy being -7.4 kcal/mol. From the results, it can be shown in the interaction between IL-12 with quercetin where there is the highest affinity value which is the minimum possible energy of -9 kcal/mol and the lowest or maximum energy of -7.8 kcal/mol as the energy used higher than other ligands

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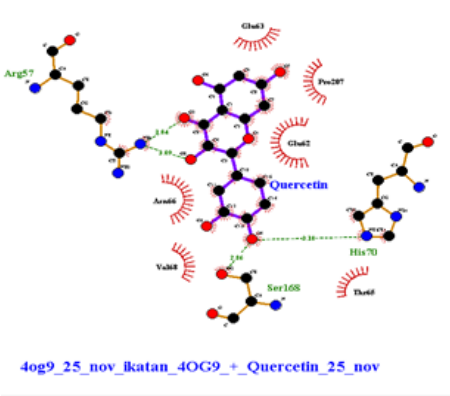

Figure 3. Binding visualization between IL12 with Triterpene

The fewer bond interactions that occur, the lower the bond affinity value formed. Hydrogen bonds are weak bonds formed as interactions of attraction between atoms that have electronegative properties with hydrogen atoms located on other atoms that have the same properties (electronegative).

Hydrogen bonds occur in a molecule with other molecules, an example that occurs in proteins is the bond between N and H. While hydrophobic bonds are bonds in molecules insoluble in water. Such bonds will occur in polypeptide chains that form folding such that non-polar groups will go inside, while polar groups will be on the outside so that the protein will be more stable in water. Thus, protein stability depends on hydrophobic bonds. Hydrophobic interaction occurs in the bond between the R-group with non-polar compounds to form a folding fold.[18]

The visualization results of the bond between IL-12 and dextroomethorpene found no hydrogen bonds, whereas the number of hydrophobic interactions are 8, namely, Asparagine no 55, Glutamine no 5, Glutamine no 13 Arginine no 57, Alanine no 2, Leucine no 56 Methionine no 54, Lysine no 9. However, the visualization of the bond between IL-12 and quercetin found the number of hydrogen bonds as much as 4 bonds, with bond distances of 3.04 Å, 3.09 Å, 3.10 Å, 2.86 Å. The position of hydrogen interaction is Arganin 37, Histidine 70, and serine 168. There are 6 hydrophobic interactions, namely Glutamine no 62, Glutamine no 63, Proline no 207, Asparagine no 66, Valine no 68, and Threonine no 65. Furthermore, the visualization of the bond between IL-12 and triterpene found no hydrogen bonds, while the number of hydrophobic interactions is 12, namely, Tryptophan no 45, Tyrosine no 314, Tyrosine no 315, Serine no 316, Aspartic Acid no 312, Aspartic acid no 292, Arginine no 313, Cystine no 41, Glutamine no 311 Glutamine no 38, Threonine no 42, and Threonine at no 272.

CONCLUSION

In conclusion, the most improved interaction between the ligand and the Interleukin 12 receptor of the three compounds above is quercetin, as it has hydrogen bonds and hydrophobic bonds, where the ligand compound Quercetin is a ligand that has the ability to form the best interaction and bond with the IL-12 receptor (4OG9) because the quercetin compound has the highest affinity bond value of -9 kcal/mol. Meanwhile, the lowest bond is with the IL-12 inhibitor forming the lowest affinity value of -7.8 kcal/mol which is the energy used higher than other ligands. Thus, the bonds that make up the interaction

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can be seen. The interaction between IL-12 receptor and quercetin compound has 4 hydrogen bonds, and 6 hydrophobic bonds. The fewer bond interactions that occur, the lower the affinity bond value formed. Hydrophobic bonds affect the stability of the protein. So that quercetin compounds can be used as a candidate for herbal medicine for therapy of patients with Diabetes Millitus.[10'19] Hence, quercetin compounds in Moringa plants are proven to be therapeutically active as anti-inflammatory based on the results of molecular docking.[20]

THANK YOU

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