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MOLECULAR DOCKING OF SHALLOT *(Allium ascalonicum)* ACTIVE COMPOUNDS TO LANOSTEROL ENZYM 14-ALPHA DEMETHYLASE AND SQUALENE MONOOXYGENASE FOR ANTIFUNGI POTENTIAL ACTIVITY

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ABSTRACT

Shallots *(Allium ascalonicum*) are one of the important horticultural export commodities in Indonesia, but the productivity of shallots has decreased due to the attack of pathogenic fungi. This study was aimed to determine the potential of active shallot compounds as ligands for inhibiting the activity of the enzyme lanosterol 14-alpha demethylase (ID: 4LXJ) and squalene monooxygenase (ID: 6C6R) enzymes which play an important role in the biosynthesis of ergosterol or fungal cell membranes. The molecular docking used the Autodock Vina (PyRx) program, an analysis of molecule interaction used PyMol, and Discovery Studio 2019 to analyze the types of bonds between molecules. Thirty-nine ligands from shallot compounds are docked to the anti-fungal target protein. The results showed ascalonicoside A1, ascalonicoside A2, ascalonicoside B, quercetin, isorhamnetin, quercetin di glucoside, quercetin tri glucoside, ethyl palmitate and benzyl salicylate have the potential to be anti-fungal to the enzyme lanosterol 14-alpha demethylase and squalene monooxygenase enzyme that responds to the synthesis of fungi cell wall. These ligand compounds bind to the target protein's amino acid residues with hydrogen and hydrophobic bonds. This research showed that shallot was the potential to be replicated as an anti-fungus for many purposes.

Keywords: *Shallots, Antifungi, Molecular Docking*

INTRODUCTION

Shallots (*Allium ascalonicum*) are a horticultural crop consumed by people. They have high economic value regarding national consumption as a source of farmer income and their role in increasing the country's foreign exchange. The increase in the use of shallots is due to uses such as traditional medicine and kitchen spice [1]. One of the ways to increase the production of shallots is by suppressing pathogenic fungi such as Trotol caused by the fungus *Alternatia porri*, fusarium wilt disease caused by the fungus *Fusarium oxysporum* (Hanz.), anthracnose disease caused by the fungus *Colletotrichum gloeosporioides* (Penz.), and dew disease caused by the fungus *Peronospora destructor* (Berk.) Casp [2] .

Agriculture usually uses chemical fungicides to prevent the growth of pathogenic fungi. However, synthetic fungicides raise

concerns because of possible toxin residues, carcinogenicity and environmental pollution. Another concern is the possibility of developing new resistant fungal strains, which is also of great concern. Therefore, using natural compounds can be a solution [3]. But, again, bioactive compounds derived from plant metabolism are included in a very diverse group of chemical compounds and have different biochemical and physiological roles. Hence, they are considered versatile molecules [4-7] and have identified the active compound in shallots using GC-MS, which can then be tested as an anti-fungal candidate.

One of the anti-fungal mechanisms of action is the inhibition of ergosterol synthesis as a component of fungal cell membranes [8]. The target protein of the anti-fungal in ergosterol synthesis is the enzyme lanosterol 14-alpha-demethylase which plays a role in metabolic functions such as membrane permeability, membrane fluidity, enzyme activity, cell morphology, and cell cycle development [9]. Another target protein for anti-fungal is the enzyme squalene monooxygenase which, together with (2,3)-oxidosqualene cyclases, plays a role in the cyclization of squalene to lanosterol [10].

Several studies have revealed the potential activity of shallot extract as an anti-fungal [11-14]. Shallot extract can inhibit the growth of fungi, and saponins are known to have anti-fungal activity. However, this research still uses the total extract containing all the shallot compounds. Therefore it is necessary to study the specific compounds that act as anti-fungal from shallots.

The potential of compounds from the onion plant as an anti-fungal in inhibiting ergosterol synthesis can be predicted by molecular docking. Study molecular docking alilin compound from garlic showed potential to inhibit 1,3-beta-glucan synthase enzyme. Molecular docking is a predictive mode of binding target compounds and proteins in evaluating the energy values of different bond conformations [15]. Molecular docking is a method for filtering compounds based on the principle of structure (structure-based) with computational assistance. During tethering, there are enthalpy and entropy changes in the protein-ligand system related to the structure between the protein and the ligand. This method has been widely used in early research to discover bioactive compounds that can be used as drug candidates [16]. Based on the prediction of the potential of onion plant compounds as an anti-fungal was carried out by tethering the active compounds obtained from previous studies on proteins that play a role in ergosterol synthesis, *lanosterol 14-alpha-demethylase* and squalene.

METHODS

Tools

The tools used in this study are hardware in the form of a laptop with specifications: Intel® Celeron® CPU N2840 type processor and 2.00 GB RAM. The application for docking used Autodock Vina (PyRx) software, PyMol and Discovery Studio 2019 Client.

Material

The active compounds used are allyl propyl trisulphate, 2-methyl-2-propanol, allyl propyl disulfide, dipropyl trisulfate, methyl isopropyl disulfide, and methyl propyl trisulfide, methanethiol, propanethiol[6]. *S*-

allyl cystein, diallyl monosulfide, diallyl disulfide [7]. *(Z) -3-hexenal, hexanal, linalool, borneol, methyl chavicol, 3-ethyl-5-methyl-1,2,4-trithiolane, dimethyl tetrasulfide, butyl thiocyanate, methyl 1- (methylthiopropyl) disulfide, 2-undecanone , 3-methoxyoctane, methyl eugenol, 2-tridecanone, 2-methyl-3,4-dithiaheptane, benzyl salicylate, methyl palmitate, ethyl palmitate, ethyl linoleate, ethyl oleate* [5]. *Ascalonicoside A1, ascalonicoside A2, ascalonicoside B, quercetin, isorhamnetin, quercetin-di-glucoside* and quercetin-tri-glucoside[4]. The receptors used were lanosterol 14-alpha-demethylase (ID: 4LXJ) and squalene monooxygenase (ID: 6C6R) enzymes obtained from the Protein Data Bank (http://www.rscb.org/pdb/).

Ligand preparation

The 2D structure of shallots (Allium ascalonicum) used as ligands and control ligands were obtained from the database PubChem (http://pubchem.ncbi.nlm.nih.gov). The preparation was carried out using the Pyrex program, minimized to minimize the ligand activation energy, and converted into a .pdbqt file format. They were then saved in .pdb form.

Protein Preparation with Discovery Studio 2019

Protein files downloaded from the Protein Data Bank (http://www.rcsb.org/pdb/) remove the water molecules, natural ligands and other residues attached to the protein. After that, the protein is stored in the .pdb file format.

Molecular Docking with AutoDock Vina (PyRx)

The molecular docking process begins with the insertion of selected ligands and 4LXJ macromolecules (receptors) and 6C6R protein, and then the *grid box* includes the active site on the protein. Then the program will start docking by pressing the forward button and calculating the scoring value of the receptor-ligand. To save the docking complex, click on the docking result in the navigator, then save it as PDB.

Analysis and Visualization of Docking Results

The docking ligand is then complexed with the receptor, so molecular interactions can be analyzed using the Pymol program. The method that can be done is to enter the ligand and receptor files from the docking results in the Pymol program and then save the molecular complexes in a .pdb file format. Furthermore, to analyze the types of bonds between molecules that interact in 2D using the Discovery Studio 2019 program

RESULTS AND DISCUSSION

Molecular docking (molecular docking) is a chemical method in silico that is to find new drug compounds at a low cost and shorter time [13]. But, before tethering, the ligand molecule and protein to be tethered must be prepared. The ligand preparation is carried out in the PyRx program by optimizing (minimizing) the energy of the ligands. According to [13], optimising the geometry of the ligands aims to produce ligands with a lower energy structure so that the chemical structure of the ligands becomes stable.

Prepare proteins by removing water molecules, ligands or other residues attached to the target protein structure using the

Discovery Studio 2019 program [17]. The separation of the residue from the target protein structure aims to prevent the slow docking process because the macromolecular or protein structure contains the original ligand and the solvent.

At the beginning of the docking process, a grid box is made with coordinate points covering the active side of the protein so that it becomes the binding space between the ligand and the target protein. Grid box coordinates were determined by knowing the location of the target protein's active site using discovery studio 2019. The grid box is the place or position of the ligand-residue interaction on the target protein and is represented like a cube [17]. The grid box size of the target protein at molecular docking is shown in Table 1.

Table 1. Target protein grid box size

Target Protein	Center			Dimensions (Angstroms)		
	X	Y	Z	X	Y	Z
4LXJ	27,1486	17,7528	21,6206	50.6730	49,6607	43,6805
6C6R	-17.0789	-74,0007	3,6647	58.3592	49,0603	56,7303

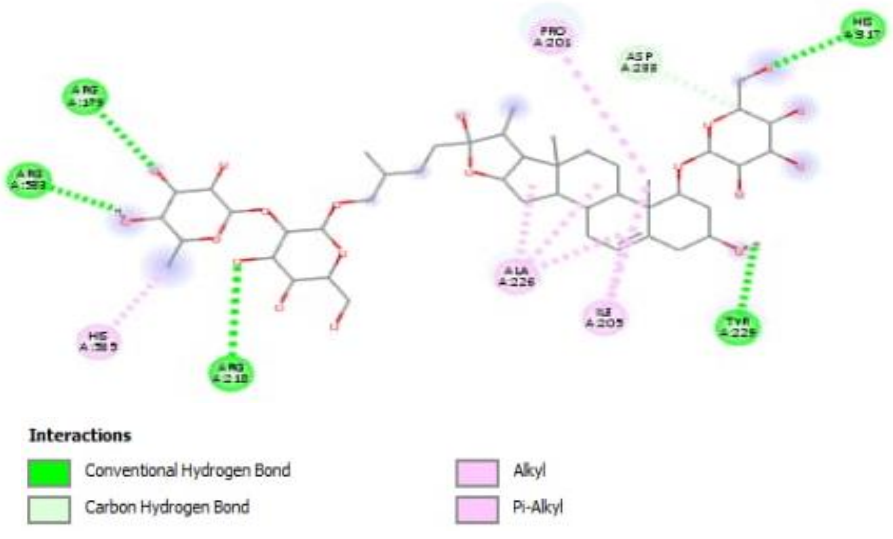
Of the 39 ligands tethered with 4LXJ protein, there were 8 ligands with the higher ΔG bind value compared to the control ligands, namely ascalonicoside A1 ligand, ascalonicoside A2, ascalonicoside B, quercetin, isorhamnetin, quercetin-di-glucoside, quercetin-tri-glucoside, and benzyl salicylate. Meanwhile, the docking results of 6C6R protein showed 2 ligands with ΔG bind value were better than the control ligands, ascalonicoside A1 and ascalonicoside A2 ligands. In addition, there were 6 ligands with ΔG bind values which were still smaller than the control protein 6C6R, but were close to the control ligands, ascalonicoside B, quercetin, isorhamnetin, quercetin-di-glucoside, quercetin-tri-glucoside, and ethyl palmitate.

Table 2. The docking results of the ligand molecule with 4LXJ protein and 6C6R protein

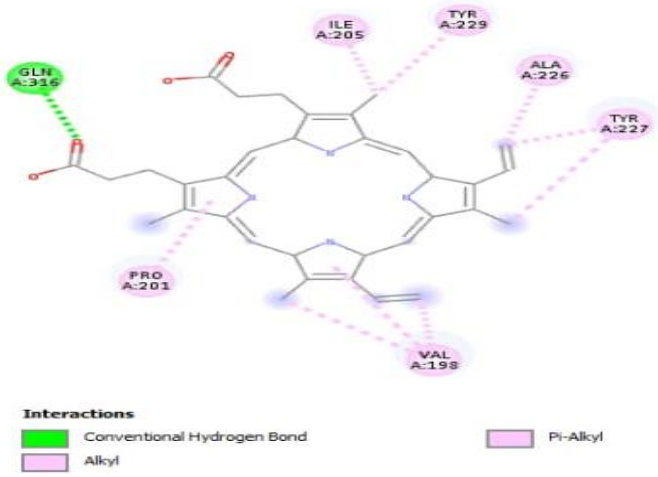
No.	Name of ligands	ΔGº Binding Affinity (kcal / mol)	
		4LXJ	6C6R
1.	Control ligands	-7.8	-9.5
2.	Ascalonicoside A1	-9.5	-9.6
3.	Ascalonicoside A2	-11.3	-9.6
4.	Ascalonicoside B	-10.9	-9.2
5.	Quercetin	-9.9	-7.5
6.	Isorhamnetin	-9.1	-7.7
7.	Quercetin-di-glucoside	-9.1	-8.2
8.	Quercetin-tri-glucoside	-8.9	-8.1
9.	Benzyl salicylate	-8.3	-6.8
10.	Ethyl palmitate	-6.3	-7

The molecular docking results can be seen from the binding affinity value. The conformation between protein and ligand from molecular docking can be determined from one ligand conformation with the best or the smallest ΔGº binding affinity [18]. The

binding energy of the scoring result is a function of Gibbs free energy (ΔG) as the translation of the third law of thermodynamics. These data indicate the stability of the interaction (bonding) of the ligand with protein at the binding site. ΔG <0, the reaction goes spontaneously (the reaction goes to the product). ΔG = 0 The reaction is reversible. ΔG> 0 reaction does not occur (reaction goes towards the reactants). The smaller the ΔG value, the stronger the bonds between the ligand and the receptor, and the more stable it is [19]. If the bond-free value (ΔG) <0, the reaction proceeds spontaneously and produces a product. The bond formed spontaneously between atoms is proportional to the decrease in free energy. Therefore, the value of free energy is negative. So the smaller the value of free energy produced, the better the stability or balance of the drug-receptor bond [20].



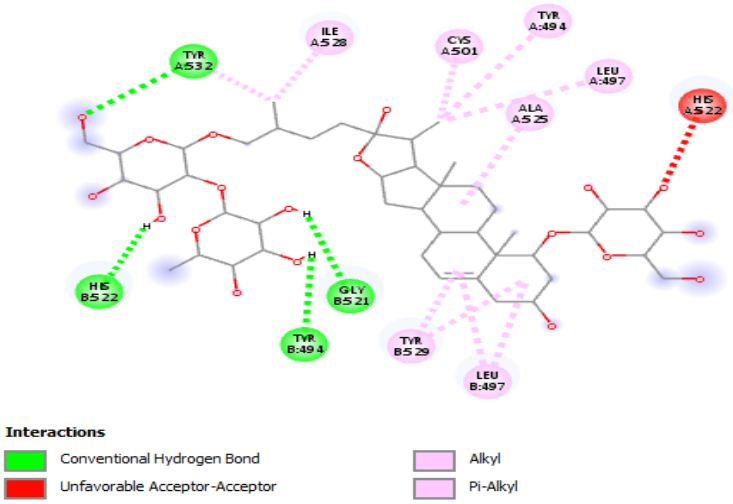
(a)



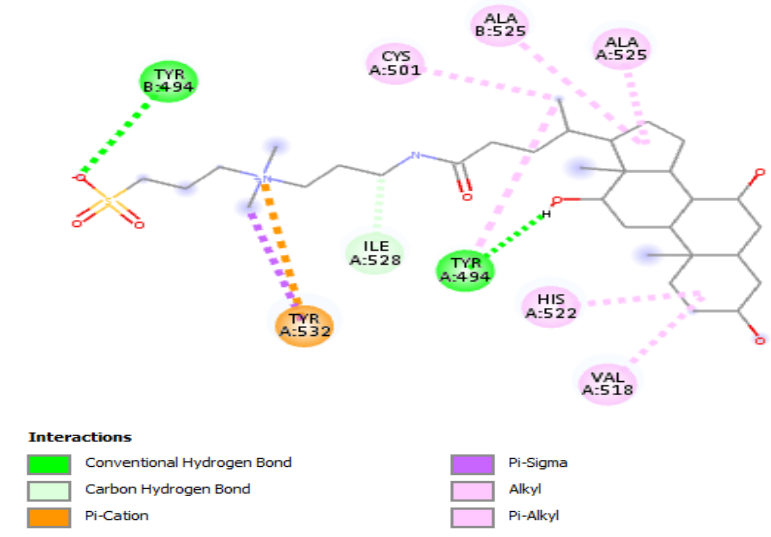
(b)

Figure 1.Visualization of 4LXJ protein docking results with (a) ascalonicoside A2 ligand and (b) 4LXJ protein control ligand

Another factor that can determine the formation of a stable conformation of ligand-protein is the presence of hydrogen and hydrophobic bonds. Hydrophobic bonds are non-polar molecules that are insoluble in water and are important in combining non-polar regions of drug molecules with non-polar regions of receptors [21]. And the presence of hydrogen bonds can affect the activity of physical-chemical properties such as boiling point, melting point, solubility in water, and the ability to form chelates. In addition, these changes can affect biological activity, such as inhibition of the tyrosinase enzyme [22]. The results of visualization with the Discovery Studio results of ligand and protein docking with the best ΔG bind values are in Figures 1 and 2.



(a)



(b)

Figure 2. Visualization of 6C6R protein docking results with (a) ascalonicoside A2 ligand and (b) 6C6R protein control ligand

Ascalonicoside A2 is a ligand of value. The best ΔG bind results from tethering with the lanosterol 14-alpha demethylase enzyme and the squalene monooxygenase enzyme with ΔG bind values of 11.3 kcal/mol and 9.6 kcal/mol. Figure 1 shows that the number of hydrogen bonds formed in ascalonicoside A2 is more than the control ligand. The ΔG bind value is negative or decreased, so ascalonicoside A2 has more potential as anti-fungal. The residual equation that is tethered between the test ligand and the control ligand is one of the determining factors that allow the test compound to bind to the test receptor (protein), namely the tethering with the enzyme lanosterol 14-alpha-demethylase is tethered to the residue ile 205, ala 226, pro 201. Similar residues in the squalene monooxygenase enzyme are tethered to tyr 494, tyr 532, his 522, ala 525, cys 501 and ile 528.

The docking of the molecule between the test ligand as an inhibitor with the protein forms hydrogen and hydrophobic bonds, which cause changes in biological activity and give certain pharmacological effects. Two mechanisms of action of drug molecules (ligands) as inhibitors against enzymes can occur. First, ligands act as analog substrates, which react as competitive inhibitors for enzymes to inhibit enzyme activity; second, ligands act as wrong substrates so that drug molecules undergo chemical transformation. As a result, enzymes produce unwanted (abnormal) products, so metabolic pathways are disrupted [23].

CONCLUSION

Active compounds that can act as antifungal properties of shallots are ascalonicoside A1, ascalonicoside A2, ascalonicoside B. quercetin, isorhamnetin, quercetin diglucoside, quercetin triglucoside, ethyl palmitate and benzyl salicylate. The ΔGº bind value is more or closer to the ΔGº bind value of the control ligand and the interactions formed between the anti-fungal target protein ligands. The ligands forming hydrogen bonds and hydrophobic bonds with the target protein residues increase the bond-free energy (ΔGº binding affinity) so that the target proteins' conformational bonds are more stable.

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