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Screening Of α-Amylase Inhibitor *Rhodomyrtus tomentosa* (Aiton) Hassk. By Docking Method

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ABSTRACT

Diabetes cases in Indonesia are increasing along with economic growth and people's welfare, so the use of oral drugs for diabetes mellitus is also increasing. One of the drugs used is acarbose, this drug works as an α-amylase inhibitor. With the increasing use of drugs causing side effects on the body, it is necessary to screen natural ingredients for alternative treatments of diabetes mellitus. The natural ingredient used in this research is R. tomentosa. The docking method used to find interaction stability is pyrex. The result of this research are alpha-Tocopherol quinone (-5,1 kcal/mol); blumeatin (-7,3 kcal/mol); methyl cinnamate (-5,1 kcal/mol); myricetin (-7,7 kcal/mol); naringenin (-7,0 kcal/mol); quercetin (-7,5 kcal/mol); rhodomyrtone (-7,4 kcal/mol); rhodomyrtosone B (-7,3 kcal/mol); rhodomyrtosone C (-8,8 kcal/mol); tetrahydroxyflavanone (-7,2 kcal/mol); tocopherol A (-7,8 kcal/mol); and verimol K (-6,4 kcal/mol). Based on the screening of R. tomentosa content as an α-amylase inhibitor using the docking method, it can be concluded that the compound which has interaction stability is rhodomyrtosone C

1. Introduction

Diabetes is one of the diseases that is an important concern in Indonesia. Diabetes disorders increase along with the welfare of society. International Diabetes Federation (IDF) in 2019 stated that there were 463 million people who have type II diabetes and this will increase to 578 million by 2030 and 700 million by 2045. In Indonesia, there are 10,3 million people with diabetes, thus placing Indonesia in the sixth position after Mexico [1].

Diabetes is a chronic disease characterized by increasing sugar levels associated with abnormal protein, fat, and carbohydrate metabolism caused by low insulin sensitivity or decreased insulin secretion. Characteristics of people with diabetes are fasting sugar levels >160 mg/dL and sugar levels in normal conditions >200 mg/dL. The entry of glucose into the blood is mediated by GLUT 4 (transmembrane protein). Lack of insulin action causes an increase in blood sugar levels and this condition is occasionally asymptomatic. Complaints that are also experienced by patients are blurred vision, impaired body movement conditions, tingling in the legs and arms, and weight loss [2]. Inappropriate treatment of diabetes mellitus (DM) can cause macrovascular, microvascular complications of chronic neuropathy, stroke, nephropathy, retinopathy, cataracts, glaucoma, and skin infections [3].

The treatment for DM patients can be through education, food dietary regulation, exercise, and drug intervention [3]. This intervention can be carried out by various approaches, for example, α-glucosidase inhibitor and α-amylase inhibitor. α-amylase plays a role in the metabolism of carbohydrates into sugar monomers so these sugar monomers easily pass through the intestinal membrane into the blood [4]. α-amylase has the ability to hydrolyze starch into glucose by cutting α-1,4-glucoside [5]. If the action of this enzyme is inhibited, the sugar monomer that enters the blood will decrease and blood sugar levels will also decrease. The drug that acts by this mechanism is acarbose [6], [7]. However, the use of this drug in the long term can lead to tolerance, so natural ingredients that have a stable interaction with the α-amylase enzyme are needed. One of the plants that have been tested preclinically against diabetes is karamunting with the Latin name *Rhodomyrtus tomentosa* (Aiton) Hassk. The preclinical test is the water fraction at a dose of 280 mg/KgBB can reduce fasting blood levels and increase insulin levels [8]. Other uses are antipyretic, dysentery, snakebite, burns, and diarrhea [9]. The use of *R.tomentosa* has been around for a long time and is hereditary, thus the long-term use is relatively safe. Therefore, this research was conducted to screen the active compound of *R.tomentosa* which has the best interaction stability with the α-amylase enzyme.

2. Methodology

Tools and materials

The tools are used in this research are the Uniprot webserver, Swissmodel, prankweb and Pyrex software, and Discovery studio.

The materials are used in this research are the active compound of *R.tomentosa*, there are alpha-Tocopherol quinone, blumeatin, methyl cinnamate, myricetin, naringenin, quercetin, rhodomyrtone, rhodomyrtosone B, rhodomyrtosone C, tetrahydroxyflavanone, tocopherol A, and verimol K.

Procedures

Protein modeling followed the steps on the swiss-model server. The modeling stages are: determination of protein target sequences, identification of template proteins, model making and model evaluation. Selection of α-amylase protein target sequences from the website http://www.uniprot.org. The UniProt database shows the presence or absence of a 3D protein structure, if there is no RCSB PDB link, then the protein sequence does not yet have a 3D structure. Since the 3D structure is not yet available, it is necessary to look for the α-amylase sequence of Uniprot with the code P0DUB6. This sequence was selected as the target protein sequence. Identification of template proteins was carried out on a Swiss model server. The target protein sequence data from UniProt in FASTA format is submitted to the Swiss-model server. The template identification process will produce 25 protein templates with their parameters. Protein templates were selected according to the identity, QMEAN, and QSQE parameters.

Protein modeling is done by selecting the resulting protein template. Modeling is done on the swiss-model server. The choice of one protein template usually results in one or more models. The resulting model is selected according to the parameters GMQE, QMEAN, Oligo State, Ligands, and Similarity. Model evaluation is carried out on the model selected in the previous step. Model evaluation is carried out on the website with the "Structure Assessment" feature. Evaluation was carried out on parameters including: Ramachandran Plot, Mol Probity Results. The visualization of the protein model was carried out with the Yasara program. After getting the next 3D structure looking for ligand binding sites with the help of prankweb. With the predicted binding site, docking can be done with the Pyrx software. The last step is visualization of ligand bond with α-amylase to determine the bound amino acid residue.

Data Analysis

Data analysis is a docking score of at least 90% of acarbose (control ligand) and similarity of amino acid residues compared with acarbose.

3. The Result

Screening of the active compound of *R.tomentosa* against α-amylase was carried out by the docking method. However, this docking requires a 3D α-amylase structure. Since the 3D structure doesn't yet exist, a 3D structure model is made first. The first step in this research is to find the sequence of human α-amylase and do homology modeling. The result of homology can be seen in Figure.1. Figure.1 shows the addition of the missing amino acid residues as many as 15 amino acid residues to form a complete sequence. PDB ID: 1q4n is the template chosen because it has a % identity of 99,80% so it has a high similarity, QMQE 0.97 is also the highest among other templates. The next step is to build a 3D structure. After getting the 3D structure, the next step is the analysis of the 3D structure of α-amylase using MOLPROBITY. The assessment of the 3D structure is the QMEAN value, that is Z score between 0-1, so this 3D structure is good because the z score describes the degree of authenticity of the 3D structure [10]. Based on Figure.2, the ramachan favoured value is bigger than outliers, which is 97,17%, so this value is good [11]. MolProbity Score value is 1.3, this value is the predicted crystallographic resolution value. If the MolProbity score is lower than the template resolution, then this model is said to be good, in this case, the crystal resolution is 2.07Å. After getting the 3D structure of α-amylase, the next step is to determine the binding site using prankweb. The predicted binding sites of this structure are GLN56, GLY300, PRO303, SER304, ASP305, ARG306, ALA307, LEU308, VAL309, and PHE310.

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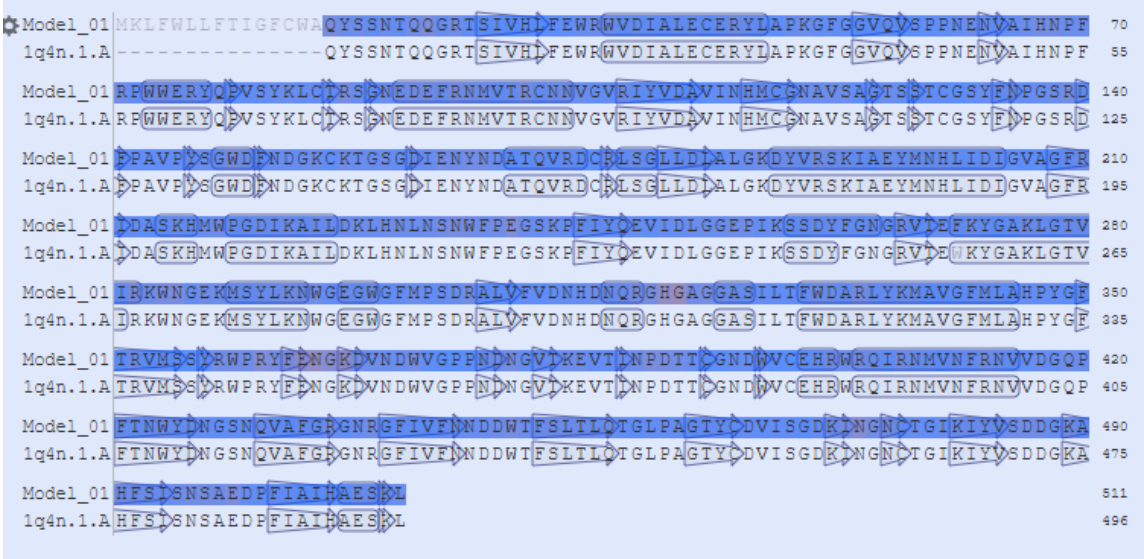

Figure.1. Homology modeling with 1q4n

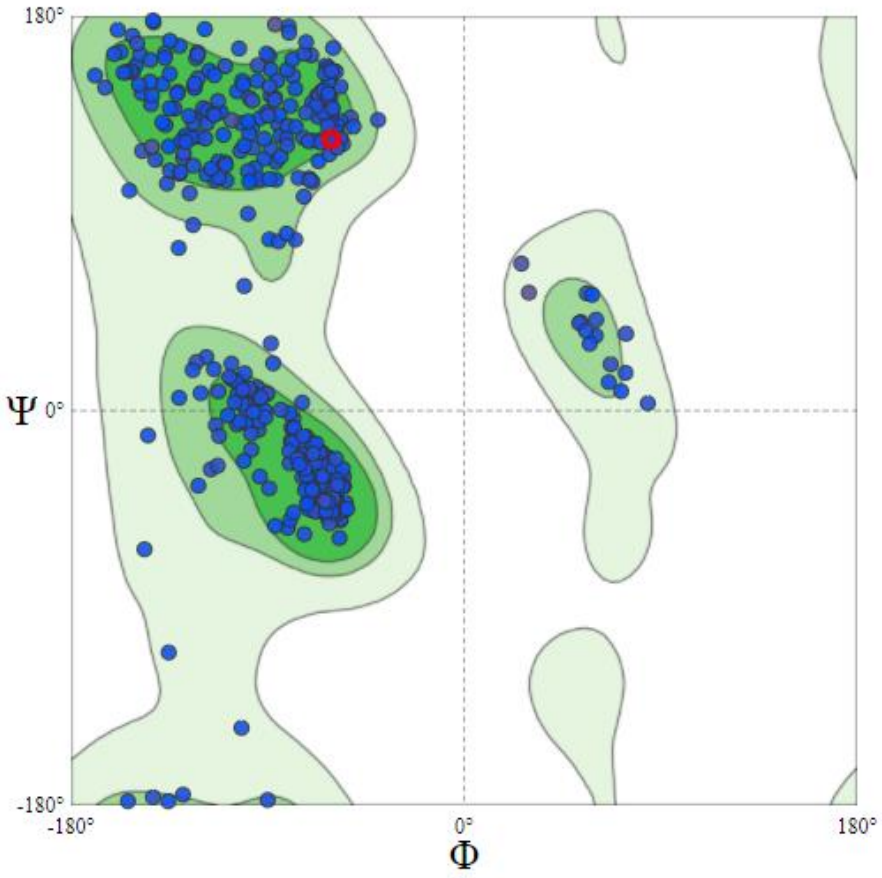

Figure 2. Ramachandran Plot

The docking result of the compounds contained in Karangmunting and the residues involved are shown in table 1. From table 1, the compounds that have minimum stability of 90% with the control ligand was acarbose against α-amylase are bluemetin (92%), myrecitin (97%), quercetin

(94%), rhodomyrtone (93%), rhodomyrtosone B (92%), rhodomyrtosone C (111%), tetrahydroxyflavanone (91%), and tocopherol A (98%). Based on the similarity of the interacting amino acid residues, the alpha-Tocopherol quinone is GLN56, ARG210, ARG352, MET354, MET343, LEU308, and PHE310. Blumeatin is HIS30, LEU308, ARG210, ARG352, and MET354. Methyl cinnamate is ARG352 and ARG 210. Myricetin is MET354, ARG210, HIS30, TYR246, ARG352, and MET354. Naringenin is ARG352, MET354, and PHE310. Quercetin is GLN56, MET354, ARG210, HIS30, and ARG352. Rhodomyrtone is VAL353, ARG352, and MET354. Rhodomyrtosone B is ARG352, MET354, ARG210, ARG352, and PHE310. Rhodomyrtosone C is HIS30, VAL57, ARG352, HIS30, TYR246, LEU308, PHE310, GLN56, VAL353, and MET354. Tetrahydroxyflavanone is HIS30, TYR246, ARG210, ARG352, MET354, and ARG352. Tocopherol A is TYR246, ARG352, LEU308, PHE310, and HIS30. Verimol K is MET354, HIS30, and ARG210.

TABLE 1
DOCKING SCORE AND AMINO ACID RESIDUE

Ligand	Binding Affinity (Kcal/mol)	Interacting Residue	
		Hydrogen	Hydrophobes and other interactions
acarbose	-7.9	HIS30, TYR109, MET354, MET343, LEU308, VAL57, HIS30, ARG210, VAL353	GLN56, PHE310, HIS30, VAL57, ARG352, TYR246
alpha-Tocopherol quinone	-5.1	GLN56, ARG210, ASN313	ARG352, MET354, MET343, LEU308, ILE28, PHE310
blumeatin	-7.3	-	HIS30, LEU308, ILE28, ARG210, ARG352, MET354
methyl cinnamate	-5.1	ARG352	ARG352, ARG210
myricetin	-7.7	MET354, ASP312, VAL55, ARG210	HIS30, TYR246, ARG352, MET354
naringenin	-7	VAL311, ASP312	ARG352, MET354, PHE310
quercetin	-7.5	GLN56, MET354, ASP312, ARG210	HIS30, ARG352
rhodomyrtone	-7.4	HIS314, VAL353,	ILE28, ARG352, MET354
rhodomyrtosone B	-7.3	ASN313	ARG352, MET354, ARG210, LEU308, ARG352, PHE310
rhodomyrtosone C	-8.8	HIS30, VAL57, THR269	ARG352, HIS30, TYR246, LEU308, PHE310, GLN56, VAL353, MET354
tetrahydroxyflavanone	-7.2	-	HIS30, TYR246, ARG210, ARG352, MET354, ARG352
tocopherol A	-7.8	PHE32, TYR246, VAL353	VAL311, ARG352, LEU308, TYR273, PHE310, VAL311, HIS30

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verimol K	-6.4	MET354	HIS30, LEU308, ARG210

4. Discussion

α-amylase inhibitor is one of the targets of diabetes mellitus drugs. This enzyme plays a role in the metabolism of carbohydrate polymers into glucose, thus this enzyme has a major effect on increasing blood sugar in people with diabetes mellitus. An example of an α-amylase enzyme inhibitor is acarbose. Long-term use of this drug causing various side effects including flatulence, fart frequently, abdominal pain, diarrhea, severe constipation, bloody diarrhea, easy bruising or bleeding, and impaired liver function. Therefore, this research was conducted to predict the active compound of karamunting which has stable interaction with α-amylase receptors as indicated by free binding energy (ΔG). The more negative the value of ΔG, the more spontaneous interactions that occur without the releasing energy of protein [12], [13]. The stronger ligand interaction with α-amylase changes the conformation of the enzyme to be inactive. This inactive form reduces the ability of enzymes to metabolize carbohydrates into glucose so that the blood sugar decrease [14].

Based on the docking results in table 1, the compound that has the potential as an α-amylase inhibitor is rhodomyrtosone C. This ligand has the same residue of 66%. It happens because of the involvement of hydrogen bonds, hydrophobic, and other interactions, that is Pi-Sulfur as shown in Figure.3. This negative binding affinity value is contributed by the bond between the lone pair O of the ligand and the H atom of HIS30 with a distance of 2.07512 A^0, the H atom of VAL57 with a distance of 2.86387 A^0, and the H atom of THR269 with a distance of 1.94552 A^0. Another type of bond is Pi-sulfur which is an aromatic system of ligands with MET354. The interaction with residues LEU308 and PHE310 is important because these residues play a role in flexible loops and are involved in the formation of water bridges as well as acarbose [15]. The existence of O, N, or halogen groups (F, Cl, Br, I) increases the stability of the interaction between because it has a lone pair of electrons which facilitates the formation of hydrogen bonds with the α-amylase enzyme, so that the bond distance becomes closer [16]. The presence of π/π interaction also increases the stability of the interaction, so these two interactions are a priority in virtual drug screening, either in the synthesis or bioassay-guided from plants, fungi, bacteria, or marine animals [17]. This π/π bond occurs due to the presence of an aromatic structure [18]. All compounds of Karangmunting that were tested using docking have O atoms which have lone pair electrons and aromatic groups that help the π/π interaction occur.

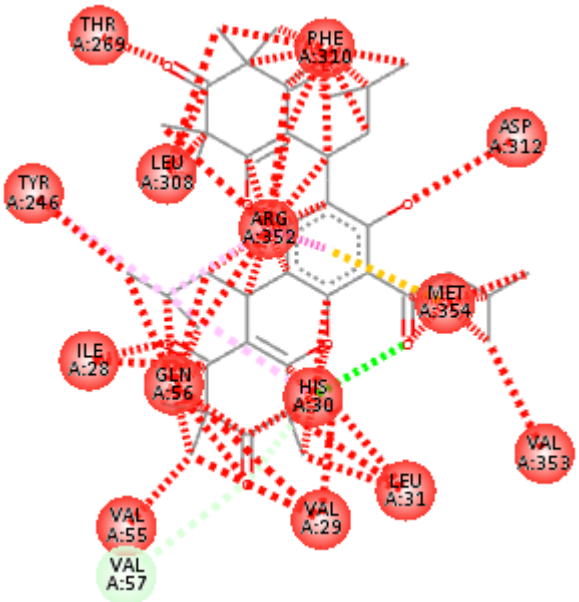

Figure 3. Interaction of Rhodomyrtosone C and Amino Acid Residue α-amylase.

5. Conclusion

Based on the screening of *R. tomentosa* content as an α-amylase inhibitor using the docking method, it can be concluded that the compound which has interaction stability is rhodomyrtosone C.

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