In Silico Study On S-Allyl Cysteine And Quercetin From Garlic (*Allium sativum* Linn) As Xanthine Oxidase Inhibitor

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ABSTRACT

Aim: The aim of this study was to predict the interaction S-Allyl Cysteine And Quercetin From Garlic (*Allium sativum* Linn) of receptors as xhantine oxidase inhibitor.

Methods: The method was Autodock 4.2.6. Molecular docking study is a computational method that aims to mimic the interaction of a ligand molecule with a target protein *in vitro* test. The receptor was xanthine oxidase with 1FIQ code.

Results: The interaction of S-allyl cysteine shows the amino acids residue: distance of GLU802 was 1,906 Å, ALA1078 was 2,134 Å, and free energy of binding was -4,89 kcal/mol, Quercetin shows the amino acids residue: distance of THR1010 was 2,215 Å, THR1010 was 1,759 Å, GLU1261 is 2,218 Å, SER876 was 2,224 Å, ALA1079 was 2,144 Å, and free energy of binding was -9,53 kcal/mol, Allopurinol shows the amino acids residue: distance of GLU802 was 1,959 Å, THR1010 as 2,092 Å, ARG880 was 2,140 Å, THR1010 was 2,141 Å, and free energy of binding was -5,75 kcal/mol.

Conclusion: Free energy of binding quercetin was -9,53 kcal/mol predicted more potential as a decrease in uric acid levels in xanthine oxidase receptor because it had a higher docking score than the comparative S-allyl cysteine and allopurinol.

Keywords: Garlic, allopurinol, xanthine oxidase, molecular docking

INTRODUCTION

Uric acid is the end result of purine catabolism. The occurrence of hyperuricemia, or an increase in uric acid in the blood with excessive amounts, will cause gout disease. Xanthine catalyzed by the xanthine oxidase enzyme will form uric acid. Xanthine oxidase, which plays an important role in purine catabolism, is found in liver and muscle cells, but can not be found in the blood. Normal values of uric acid levels are 3.5 to 7.0 mg / dL for men and 2.5 to 6.0 mg / dL for women [1].

Increased serum uric acid above the specific threshold as capital in the formation of uric acid crystals. Despite the fact that hyperuricemia is the main cause of damage to uric acid, many people with hyperuricemia do not cause uric acid or even form Uric Acid (UA) crystals. In fact, only 5% of patients with hyperuricemia above 9 mg/dL cause Gout. Therefore, it is estimated that other factors such as genetic predisposition occur in the incidence of gout[2].

Based on data from World Health Organization, the prevalence of gout is 2-5% of the

world population, generally suffered by men at the age of 40-50 years and women before menopause. In Indonesia, for rural areas, it reaches 1.7% and urban areas reach 4.8% [3]. In general, there is not many detailed data on the prevalence of gout both in the world and in Indonesia. The development of an increasingly sophisticated era and also economic progress was increasing, also changed the lifestyle that lived by society. One of the phenomena that accompany the progress of society is gout disease through the consumption of fast food and physical activity is lacking.

Another cause of hyperuricemia is in patients with malignancy due to very high purine and DNA turnover, alcohol consumption, leukemia, metastatic carcinoma, multiple myeloma, hyperlipoproteinemia, diabetes mellitus, kidney failure, stress, lead poisoning, and dehydration due to diuretic use. Several large studies have reported the effect of high uric acid risk of cardiovascular disease and myocardial infarction. Hyperuricemia caused by kidney disease, is often considered a sign of impaired renal function rather than some risk factor for kidney disease progression[2].

To prevent hyperuricemia, non-pharmacological therapy is needed by living with healthy life such as diet, drinking more water, and physical exercise, while pharmacological therapy uses uricostatic and uricosuric drugs. Allopurinol commonly used for xanthine oxidase inhibitor or uricostatics[4]. While probenecid is an example of uricosuric drugs that can increase uric acid excretion by reabsorption inhibitor in the renal tubules[2].

The alternative search for gout is needed especially Indonesia is the Second Mega Biodiversity Country after Brazil with 2500 types of which are medicinal plants[5]. One alternative that is thought to reduce uric acid levels by garlic (*Allium sativum* Linn.). Health properties of garlic depending on its bioactive compounds, especially the organosulphur compounds, the phenolic acid and the flavonoid constituents[6]. S-allyl cysteine[7] and quercetin[8] as potent anti-gout.

The discovery of a drug is a linear process that begins with a target followed by *in vitro* and *in vivo* screening optimization to determine whether the compound meets the formulation criteria for initiation of clinical development. Previously, new drugs were developed by synthesizing compounds through a multistep and time consuming process, but now the use of computers as a tool in drug discovery by using molecular docking techniques. Molecular Docking is a computational technique that predicts the preferred orientation of one molecule (ligand) to another molecule (receptor) when bound to one another to form a stable complex which in turn predicts the strength of the affinity relationship or bond between molecules [9].

Thus, tt is necessary to develop xanthine oxidase inhibitors through study *in silico* of garlic content, namely quercetin and S-allyl cysteine as uricostatic alternatives.

MATERIALS AND METHODS

Materials

The hardware was HP Mini 110-4100 with BIOS Insydeh20 version CCB.03.61.46F.01, Intel(R) Atom(TM) CPU N2600 @1,60GHz, 2048 MB RAM, Windows 7 Ultimate 32-bit Operating System. Computer Software, ChemOffice 2010 (ChemDraw and Chem 3D Ultra). Then performed geometry optimization using Hyperchem version 12 with semi-empirical method (PM3). Preparation of ligand and docking receptors was done using Autodock 2.4.6, Autodock Tools, OpenBabel, Command Prompt. The test compounds were S-allyl cysteine, quercetin and allopurinol as the comparison. The 3D structural data of receptor crystals for molecular docking analysis was obtained from Protein Data Bank (PDB) at http://www.rcsb.org/pdb/. The receptors to predict the uric acid-lowering activity was the xanthine oxidase receptor with the 1FIQ PDB code.

Methods

Ligand Preparation

Ligands or xanthine oxidase test compounds were built using the 2004 Chem Office software (ChemDraw and Chem 3D Ultra). Then performed geometry optimization using Hyperchem version 12 with semi-empirical method (PM3).

Receptor Preparation

The receptor was downloaded from Protein Data Bank Receptors in the form of protein macromolecules were separated from other unnecessary molecules along with their ligands. Separation was done using AutodockTools-1.5.6rc3. Optimization was done with the addition of hydrogen atoms and Kollman charges.

Docking Method Validation

The docking method validation was performed by the redocking method using co-crystalline ligands contained in each receptor downloaded from Protein Data Bank. Parameters to assess the validity of RMSD value which was the value of docking ligand position space position compared with the position of ligand crystallographic results.

Ligand Compounds Matching Against Receptors

The grid box parameter setting was performed using AutodockTools1.5.6rc3. The dimensions were determined by the size of each ligand and the grid box coordinates are determined based on the coordinates of the co-crystal ligands of the receptor files used. The parameters were Genetic Algorithm with GA number of runs 10 times. One time the docking process produces 10 poses so that the end result docking obtained as many as 100 poses.

Analysis and Visualization of Docking Results

Determination of docking ligand conformation (best pose) was done by selecting the ligand conformation which had the lowest bond energy. Docking results with the best pose then analyzed using Autodock 4.2.6. Parameters analyzed included amino acid residues, hydrogen bonds, predicted inhibition constants, and bonded free energy.

RESULTS AND DISCUSSION

Data Analysis

Analysis was performed on free energy parameter binding; inhibition constants, hydrogen bonding, and interactions occurred between the ligand (quercetin, S-allyl cystein, and allopurinol) with amino acid residues at the active site receptors.

Preparation of receptors

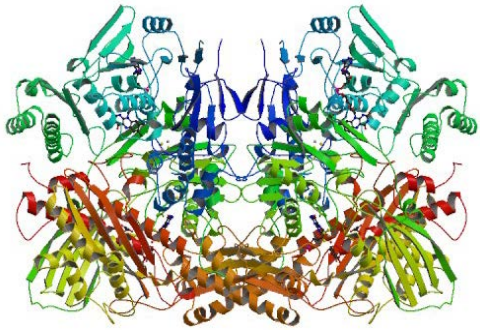

Figure 1. Structure of xanthine oxidase (PDB code: 1FIQ)
Source: RCSB PDB (2018)

The macromolecule was optimized using Autodock tools.1.5.6rc3. This optimization was done so that macromolecules can adjust to the existence of the environment, that is by adding the hydrogen atom is intended to adjust the atmosphere of docking to approach the

atmosphere at pH 7, the physiological pH in the body, while setting the grid box to determine the ligand space will be docked. The ligand afinity was determined by heading to the original ligand that has been chain with protein macromolecules when downloaded.

Ligands Preparation

The ligands in this study were S-allyl cysteine and quercetin from garlic. The two-dimensional structure of ligands and Allopurinol as comparative drugs were downloaded from the PubChem Compound site. Then the ligands were transformed into three-dimensional shapes using ChemDraw 3D and ligands stored in .mol format. Figure 2 shown 3D structure of quercetin, S-allyl cystein, and allopurinol with different structure. Molecular docking simulations in this study were conducted to analyze the xanthine oxidase enzyme inhibitory activity by predicting the affinity and interaction between quercetin, S-allyl cystein, the xanthine oxidase enzyme, and compared with allopurinol. It was done through energy predictions bond conformation and orientation of molecules in the active site to target receptors and modeling the interactions between the receptor and the ligand. The crystal structure of bovine xanthine oxidase in complex with quercetin and S-allyl cystein used as the target receptors obtained from the PDB (http://www.rcsb.org/pdb/.) with PDB code 1FIQ.

A B C

Figure 2. 3D of Quercetin (A), S-allyl cystein (B), Allopurinol (C)

Docking Method Validation

The validity of the docking method parameters evaluated based on the value root-mean-square deviation (RMSD) and declared valid if the value of RMSD smaller than 2.0 Å[10]. The validation results into the xanthine oxidase receptor in the 1FIQ PDB code indicating that methods and calculation parameter settings meet the criteria of validity docking methods. Fig. 3 shown the validation results the docking process was done on the 40x40x40 grid box, the value of x 26.659, y=10.228, z=113.34, and spacing at 0.622 À.

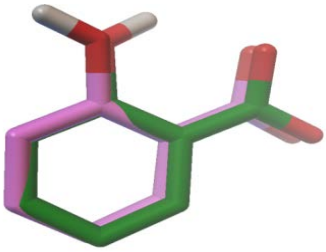

Figure 3. Validation results the docking process is done using a grid box that is 40x40x40

Analysis and Visualization of Docking

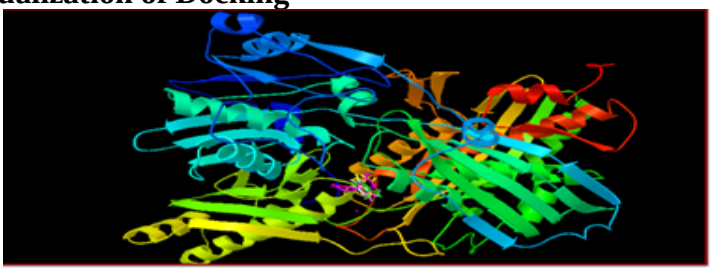

Figure 4. Result visualization of Ligands, Allopurinol, Quercetin, dan SAC into the xanthine oxidase

Receptor: Rainbow Ribbon
Ligands: Green Sticks and ball
Allopurinol: White Sticks and ball
Quercetin: Pink Sticks and ball
S-allyl cysteine: Yellow Sticks and ball

The value of free energy can be used to predict the binding affinity and ability of a compound to inhibit the enzyme molecule. In the Allopurinol compounds, there are amino acid residues that interact: ALA1078, ALA1079, PHE1009, SER1008, ARG880, THR1010, THR1010, VAL1011, SER876, PHE914, LEU1014, LEU873, GLU802, and PRO1076. Free-bond energy in compounds of Allopurinol -5.65 kcal/mol and 61.24 µM. Allopurinol has four bonds, namely the bond between H atoms and O at amino acid residues ARG880, THR1010, with the respective bond distance of -2.214 Å, 2.012 Å and the bond between O atoms with H at amino acid residues GLU802, THR1010 with a bonding distance of -1,931 Å and 1,933 Å. These results clearly indicate that garlic have binding interactions with xanthine oxidase. Further investigations on the allopurinol.

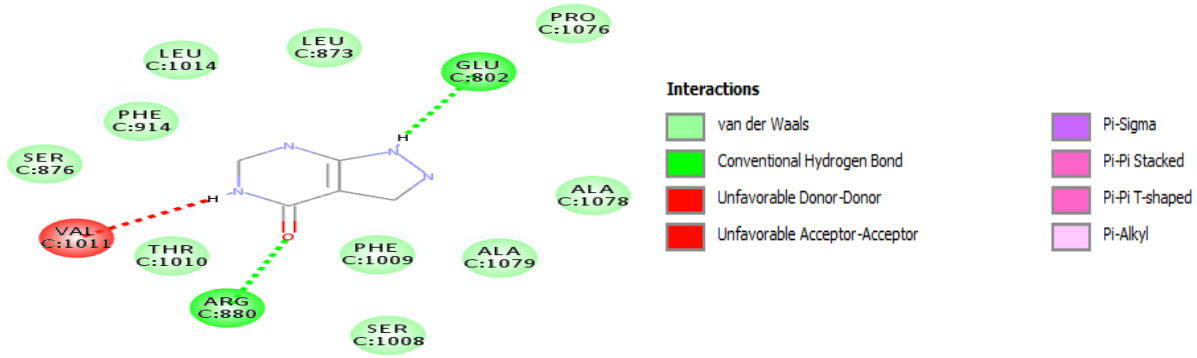

Figure 5. Result visualization of Allopurinol

The interaction of S-allyl cysteine compound with the receptor shows the residue of amino acids: ALA1078, ALA 1079, PHE 1009, PHE914, THR1010, and ARG880. Free energy bonding in compound one -4,89 kcal/mol with Ki (inhibitory constant) 259.03 µM. The interaction of S-allyl cysteine shows the amino acids residue: distance of GLU802 is 1,906 Å, ALA1078 is 2,134 Å. S-allyl cystein had indicated that garlic have binding interactions with xanthine oxidase, but in the below of quercetin and allopurinol.

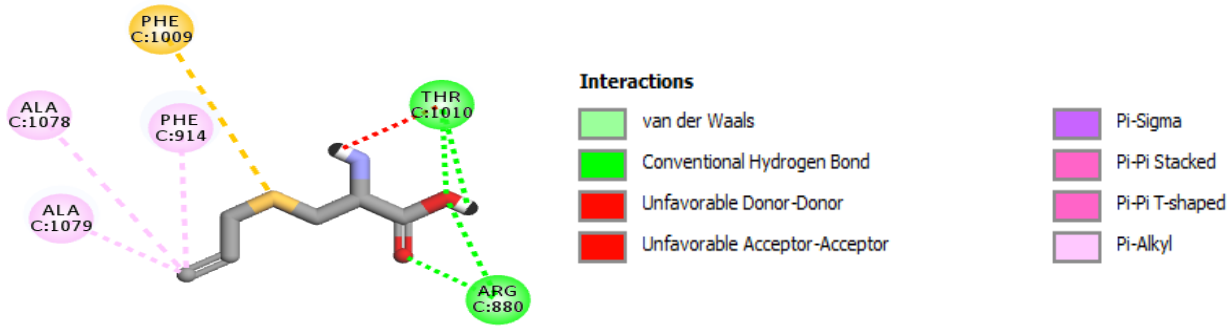

Figure 6. Result visualization of S-allyl cysteine

Quercetin shows the amino acids residue: distance of THR1010 is 2,215 Å, THR1010 is 1,759 Å, GLU1261 is 2,218 Å, SER876 is 2,224 Å, ALA1079 is 2,144 Å, and free energy of binding is -9,53 kcal/mol. The interaction of Quercetin compound with the receptor shows the residue of amino acids: ALA 1079, ALA910, ALA1078, PHE914, PHE1009, GLU802, PHE1009, LEU1014, PRO1076, LEU873, LYS771, LEU648, SER876, VAL1011, THR1010, ARG880, GLY913, and GLU1261. Negative free energy of binding inhibition constants indicates good affinity between ligands and receptors. Quercetin in lowest conformation energy had free energy binding -9,53 kcal/mol, more negative than that of S-allyl cystein -4,89 kcal/mol and allopurinol -5.65 kcal/mol. This suggests the affinity of quercetin stronger than that of S-allyl cystein and allopurinol so that it could be predicted that quercetin was more potential to inhibit xanthine oxidase enzyme. Thus, the garlic containing the active compound quercetin was a potential use as antihyperuricemia.

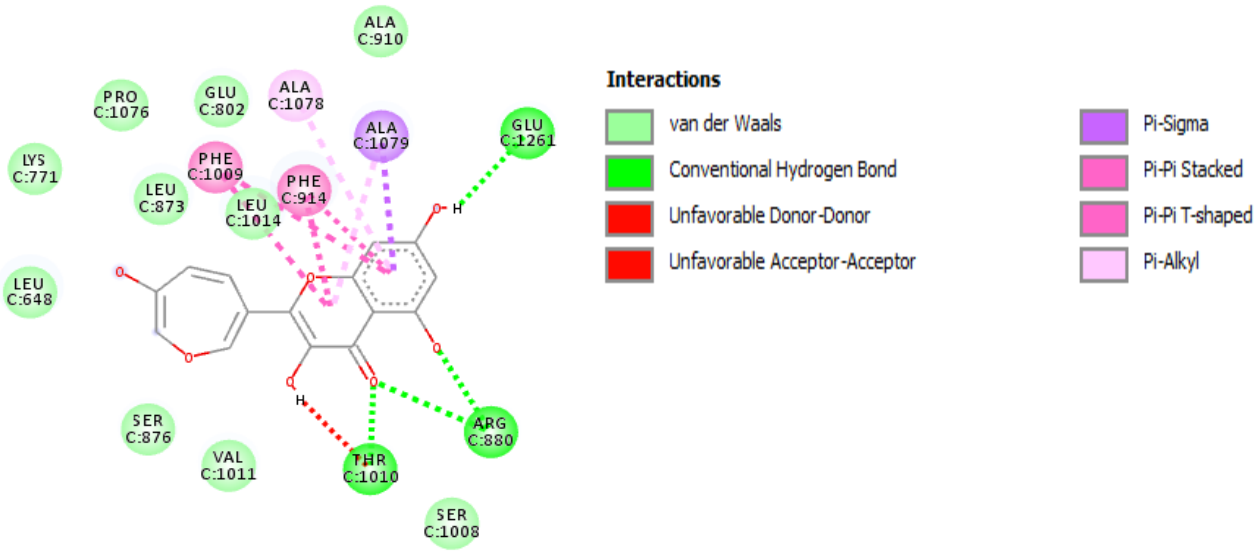

Figure 7. Quercetin visualization

CONCLUSION

Quercetin with free energy of binding was -9,53 kcal/mol predicted more potential as xanthine oxidase inhibitor than S-allyl cysteine and allopurinol.

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