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Alliin as a Natural Bioactive from Single Bulb Garlic (*Allium sativum*) for Nitric Oxide (NO) Increasing in Atherosclerotic Process Based on Insilico Screening

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Abstract

Atherosclerosis is a chronic inflammatory disease in the arterial wall mediated by proinflammatory factor. Atherosclerosis cause heart disease and stroke is generally believed to be preventable. The single bulb garlic is one of the local plants known as the medicinal plant in Indonesia. Garlic contains *alliin* compounds as antiatherosclerotic agent. This study aimed to determine the potential of active compound of single bulb garlic as an antioxidant that improve endothelial function by increasing nitric oxide (NO) to increase vasodilation. The bioinformatics webserver used in this study are: Pubchem, Pharmmapper, Swiss Target Prediction, Superpred, Uniprot, and Protein Data Bank. Docking software using PyRx, PyMOL and Discovery Studio. Based on these steps, it was found that *alliin* interacts with nitric oxide synthase (NOS) through hydrogen and alkyl bonds. *Alliin* is effective as an inhibitor of NOS based on binding afinity (-5.4 kcal / mol) although no more negative than an aspirin inhibitor based on binding afinity (-6.5 kcal / mol). Based on the docking results, it is found that *alliin* is effective as a potential drug to decreased atherosclerosis.

Keywords: *alliin*; bulb garlic; nitric oxide; reverse docking; atherosclerosis.

INTRODUCTION

Indonesia has many medicinal plants containing therapeutic properties and have beneficial pharmacological effects on animal and human body (Motalib et al., 2010). Garlic is remarkable for the number of compounds it contains, including seventeen amino acids, at least 33 sulphur compounds, 17 amino acids (Josling, 2010; Febyan et al., 2015), eight minerals (germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc) and the vitamins A, B and C (Josling, 2010).

In 1944 an Italian chemist, C. J. Cavallito, as an inventor the isolated an unstable, odorous sulphur-containing compound with antibacterial properties from extracts of fresh garlic. The name of the substance is *allicin*, based on the generic name for the plant *Allium sativum*. Four years later researchers Stoll and Seebeck, also working with garlic, discovered an odourless sulphur-containing compound called *alliin* (Josling, 2010). They found that this compound would be converted by a second garlic constituent, an enzyme called *allinase*, to form allicin. The researchers made an additional, remarkable discovery: When they studied the cloves in cross-section they found that alliin and allinase were stored in different compartments. In an undamaged clove they remained completely separate, but once its structure was ruptured- typically by cutting-the two substances came into contact and formed *allicin*.

Atherosclerosis is a chronic inflammatory disease of the arterial wall, especially oxidation of lipoproteins that stimulate the innate immune response and adaptive immune response (Tedgui & Mallat, 2006; Ait-Oufella et al., 2011). Atherosclerosis cause stroke, hypertension, and coronary heart (Tanuwijaya, 2003). Atherosclerotic cause by free radicals (Sumarno et al., 2012) and unhealthy lifestyle (Rahman, 2012). The high free radicals in the body cause degenerative diseases (Winarsi, 2007). Unhealthy lifestyle like high fat diet cause endothelial disfunction. Endothelial disfunction causes decrease production of vasodilation compounds such as *nitric oxide* (NO) (Oparil et al., 2003; Devlin, 2002). NO is the smallest signal molecule produced by three isoforms of nitric oxide synthase (NOS) (Forstermann & Sessa, 2011). The NO formation enzymatic of L-arginine is catalyzed by nitric oxide synthase (NOS) (Schrijvers et al., 2004).

Therapy used to reducing inflammation and improving endothelial function or rupture of plaque is done by aspirin. Drug use to reduce inflammation has side effects. Aspirin cause gastrointestinal irritation and bleeding (Majid, 2008), therefore safe therapies are required without harmful side effect in patients with atherosclerotic. Safe therapy using herbal drug that

contain biochemical compounds, one of them is garlic in Indonesia such as single bulb garlic. *Alliin* compounds are potential as antioxidant (Febyan et al., 2015) and antiatherosclerotic (Hernawan & Setyawan, 2003). Endothelial disfunction causes a decrease in eNOS activation resulting in reduced NO production (Danuyanti et al., 2014). *Alliin* contained in *Allium sativum* lowers blood pressure through complex pathways resulting in vasodilation so that decreasing of plaque atherosclerotic. NO is synthesized by nitric oxide synthase (NOS) from L-arginine. After synthesis, NO diffuses from endothelial cells to smooth muscle cells of the blood vessels and causes an increase in intracellular cyclic guanosine monophosphate (cGMP) to relaxation of smooth muscle of blood vessels so that vasodilation occurs (McKeever, 2009).

OBJECTIVE

This study aimed to discover *alliin* compound from single bulb garlic for antiatherosclerotic agent based on insilico screening.

METHODOLOGY

Ligand Preparation

The chemical structure of 3D and SMILES ligands (*alliin*) is taken from Pubchem compound database (https://pubchem.ncbi.nlm.nih.gov/) with ID number: 87310.

Target Selection

Input *alliin*'s SMILES on Pharmmapper (http://lilab.ecust.edu.cn) to identify potential target candidates using mapping approaches Swiss Target Predictions (http: //www.swisstargetprediction.c/) and chemical structure associations with molecular 3D (Gong et al., 2013).

Molecular Docking

Molecular docking *alliin*, target protein, and known inhibitors of target protein used PyRx 0,8 software.

Visualization of Molecule and Small Molecule Interaction

The interactions between *alliin*, target protein, and inhibitors were analyzed using PyMol.

RESULTS AND DISCUSSION

The results of target selection using Pharmmapper (job ID: job ID: 170.228.034.654) dan Swiss Target Prediction, found that *alliin* interact with nitric oxide synthase (NOS) in human vascular endothelium cells. NO accumulation induces the formation of a strong oxidizing agent, peroxinitrite (Schwartz et al., 2002). NOS became an interesting object of this study for the development of drugs as inhibitors of atherosclerotic plaque reduction. *Alliin* as a drug effectively inhibit atherosclerotic disease (Zhang et al., 2001; Borek, 2001; Seo et al., 2012). Garlic extract can lowering cholesterol levels significantly, reducing the thickening of the aortic wall and reducing fat accumulation in macrophage cultures. The mechanism is related to the garlic on cholesterol metabolism (Campbell et al., 2001).

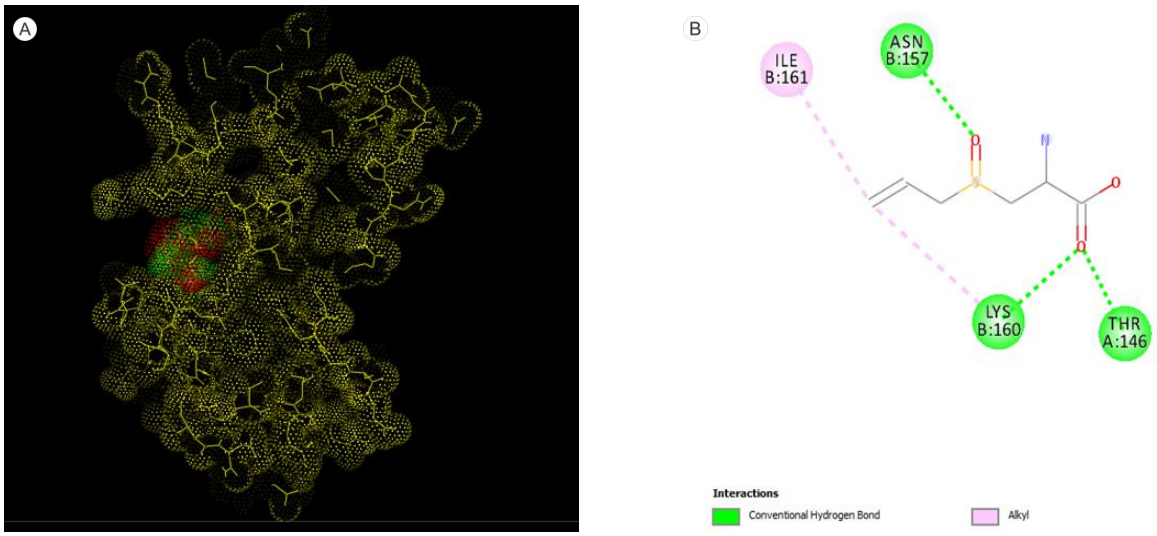

Figure 1. (A) The interaction between target protein (NOS) with *alliin* and aspirin inhibitor suggests that binding target protein on the same site. Green (*alliin*), red (aspirin), yellow (NOS); (B) Interactions through hydrogen and alkyl bonds of ILE B: 161, ASN B: 157, LYS B: 160, and THR A: 146.

Reverse docking is a method used to predict the interaction activity between ligand (compound / drug) with receptor (protein/target) (Kharkar et al., 2014). Based on a reverse docking of NOS (GDP ID: 5UNR) with a resolution of 1.95 Å, aliin is known as an inhibitor of NOS informing that *alliin* has binding affinity (-5.5 kcal/ mol). The result of molecular visualization and molecular interaction using PyMOL software found that *alliin* bind to target protein (NOS) at the same site as aspirin inhibitor via hydrogen and alkyl bond of ILE B: 161, ASN B: 157, LYS B: 160, and THR A : 146 as shown in Figure 1. So it can be said that *alliin* has the same function as aspirin as NOS inhibitor in atherosclerotic.

Decreased levels of NO cause vasoconstriction of blood vessels and endothelial becomes more proaterogenic and proinflammation (Kuzkaya et al., 2003). NO plays an important role in blood vessels such as vasodilation, inhibits smooth muscle cell proliferation, platelet aggregation, monocyte and platelet adhesion, low density lipoprotein oxidation (LDL), expression of molecular adhesion and endothelin production (Behrendt & Ganz, 2002; Yetik-Anacak & Catravas, 2006).

Alliin as inhibitors of atherosclerotic plaque reduction with improves component of vasodilation. NO is synthesized by nitric oxide synthase (NOS) from L-arginine. This arginine will be changed by the enzyme nitrite oxidase becomes NO. NO stimulate guanylate cyclase to change GTP (guanosine triphosphate) becomes cyclic-GMP to activates protein kinase G which causes the re-taking Ca2+ and opening potassium channel which is activated by calcium. Decrease of Ca2+ concentrations myosin light-chain kinase (MLCK) cannot phosphorylates myosin longer, thus stopping the cycle cross bridge and cause relaxation smooth muscle cells of the blood vessels so that vasodilation occurs (Ishimura et al., 1998 & McKeever, 2009).

Allium sativum with water will break down into diallyl sulfide, diallyl disulfide and diallyl trisulfide which then merge into the organic polysulfide that causes red blood cells to produce H_2S (hydrogen sulfide). H_2S will bind and activate channel K_{ATP}, so the concentration of $Ca2^+$ cell will decrease and hyperpolarization occur vascular smooth muscle cell that cause vasodilation of blood vessel (McKeever, 2009).

The result of reverse docking show that *alliin* interact with NOS that can increasing NO product, so it can be seen that NOS acts as an atherosclerotic inhibitor.

CONCLUSION

This study proved that *alliin* has potential as an NOS inhibitor based on binding affinity and intermolecular interaction. *Alliin* is a potential antiatherosclerotic drug.

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