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Medicinal Uses of Single Garlic in Hyperlipidemia by Fatty Acid Synthase Enzyme Inhibitory: Molecular Docking

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Abstract. Lipid is a substance needed for the body as various activities, such as forming a plasma membrane. Lipid will be digested and absorbed through the digestive system in the form of fatty acids and glycerol. Metabolism of lipid into fatty acids and glycerol and the absorption process in the body involves various kinds of enzymes; one of them is Fatty Acid Synthase (FAS). Excess lipid in the body will cause various diseases, such as obesity and cardiovascular diseases. Treatment for excess in lipid level is usually by using synthetic drugs such as statins, but excessive consumption of drug causes various side effects. Single garlic (*Allium sativum*) (SG) is widely used as an herb that can treat diverse diseases. SG contains organosulfur compounds including Allicin, Alliin, and Ajoene (E-Ajoene and Z-Ajoene). This study aimed to determine the potential of organosulfur compounds in SG as inhibitors of fatty acid synthase (FAS) enzymes which play a role in the process of lipid metabolism. The molecular docking was used to determine the interaction of organosulfur compounds compared with controls (Statins) in the FAS enzyme. Molecular Docking began by taking organosulfur SG compounds and enzymes in PubChem online services and GDP in sequence. The enzyme was sterilized using PyMol software, followed by a docking process, visualization and interaction of ligands on enzyme were carried out using PyRx, PyMol, and LigPlot⁺ software consecutively. The results showed that organosulfur SG compounds had potential as inhibitor of FAS enzymes. The Allicin, E-Ajoene, and Z-Ajoene had the same binding site with Statins in the FAS enzyme. Based on molecular docking results, it is known that the active compounds found in SG could act as an inhibitor for FAS enzymes which play a role in de novo lipogenesis.

Keywords: Single garlic, hyperlipidemia, fatty acid synthase, molecular docking

1. Introduction

Lipids are biomolecules that play an important and varied roles in the body [1]. Lipids are transported in blood circulation in lipoproteins form. Lipoprotein contains apolipoprotein, cholesterol esters, free cholesterol, triglycerides (TG), and phospholipids [2]. Lipids in enterocytes bond with lipoproteins, specifically apo B-48, A-I, A-II, and A-IV, become chylomicrons. TG and cholesterol are synthesized endogenously in the liver and very low-density lipoprotein (VLDL) particles as a product. VLDL is released in the bloodstream. VLDL and CM interact with high-density lipoprotein (HDL), receive apo



C-II and apo E. Apo C-II then activates lipoprotein lipase (LPL) which has functions to hydrolyze TG in VLDL and release free fatty acids (FFA) and remnants CM. FFA will be transported to tissues and also can be stored in adipose tissue [3].

The homeostasis of lipid metabolisms was influenced by various lipogenic proteins that play a role in the process of lipogenesis and lipolysis, such as fatty acid synthase (FAS). FAS plays a role in de novo lipogenesis to catalyze the transformation of acetyl-CoA into malonyl-CoA [4]. The excess FAS results in impaired lipid metabolism and has an impact on uncontrolled lipid accumulation which causes various risks of diseases such as obesity, type 2 diabetes, cardiovascular disease, atherosclerosis, to liver steatosis [5]. One of the drugs used to treat diseases caused by hyperlipidemia is statin.

Statins are drugs that act as a competitive inhibitor of the HMG-CoA reductase enzyme in the liver. This inhibition results in the inhibited conversion of HMG-CoA to mevalonate, and the result is a reduction of cholesterol product [6]. HMG-CoA regulates carboxylase and FAS enzymes. Thus the activity of both enzymes can also be affected by statins [7]. Statins are generally tolerated in the body, but statins have side effects such as liver and muscle poisoning, liver dysfunction and renal insufficiency [1]. The current research aimed to find natural ingredients that can be used as an alternative medicine with the benefit that the natural compound has few side effects. One of the herbal medications is garlic (*Allium sativum*).

Single garlic (SG) (*A. sativum*) is a plant that is widely used as a treatment for the various diseases. The active compounds (especially organosulfur) in an SG has a higher concentration than regular garlic. Active compounds in SG were Alliin (411.4 mg/mL), Allicin (268.2 mg/mL) and ajoene differentiated into E-ajoene (101.5 mg/mL), and Z-ajoene (251.4 mg/mL) [8]. Organosulfur compounds are the main active compounds which have hypolipidemic and hypocholesterolemic effects [9]. Other studies have shown that garlic extract had various inhibitory effects on several different stages of cholesterol biosynthesis pathways in human liver cells. Allicin is a compound that inhibits cholesterol biosynthesis in hepatocytes, thus contributes to lowering serum cholesterol [10,11]. Bhatt & Patel (2013) reported that raw garlic had scavenging activity in a case to decrease free radicals [12]. Garlic also significantly decreased malondialdehyde (MDA) in rats fed with high-cholesterol diet [13]. The treatment using single clove garlic also has the ability to increase endogenous antioxidants such as catalase (CAT) and superoxide dismutase (SOD) [14]. The studies of garlic showed that garlic has more capability as a treatment for hyperlipidemia compared with the statin.

Molecular docking is a method used in the process of found new compounds which has potential as a drug candidate. This method is the most significant theoretical method used to determine the orientation of the ligand on the binding site. The binding energy produced from ligand-receptor interactions plays a role in designing new candidates of drugs effectively [15]. Molecular docking is used to predict and design the drugs for various diseases; one of the diseases is caused by the imbalance of lipid metabolism. Allicin derivatives such as Ajoene have a beneficial effect on cardiovascular disease [16]. Based on this, this study aimed to determine the drug candidates from active compounds in SG (Alliin, Allicin, E-Ajoene and Z-Ajoene) and to inhibit over-expression of FAS using molecular docking methods.

2. Methods

The 3D structure of the organosulfur SG compounds (Alliin, Allicin, E-Ajoene, and Z-Ajoene) and Statins were obtained from the PubChem data collection (<https://pubchem.ncbi.nlm.nih.gov>). The 3D structure of the FAS enzyme was attained from a data set of Protein Bank Data or GDP (<http://www.rcsb.org>). The 3D enzyme structure was sterilized using PyMol software (Python Molecular Viewer) to remove water molecules and all the ligands in the enzyme.

Molecular docking processes were carried out between all organosulfur and statin compounds in the FAS enzyme. The molecular docking processes were in 3 stages, namely 1) molecular docking between organosulfur compounds and statin in FAS enzyme using PyRx 0.8 software Autodock Vina

program, 2) the visualization of binding positions in amino acid using PyMol software, 3) the display of hydrophobic interaction using LigPlot⁺ software. The data of molecular docking processes were used to determine the potential of organosulfur compounds as inhibitors of FAS enzyme. This potential was known through a comparison between organosulfur compounds in the SG with a statin (control). The comparisons included binding affinity values, amino acid residues, and hydrophobic interactions.

3. Results

The 3D structure of single garlic organosulfur compounds and Statins (control) in the PubChem online service (Figure 1).

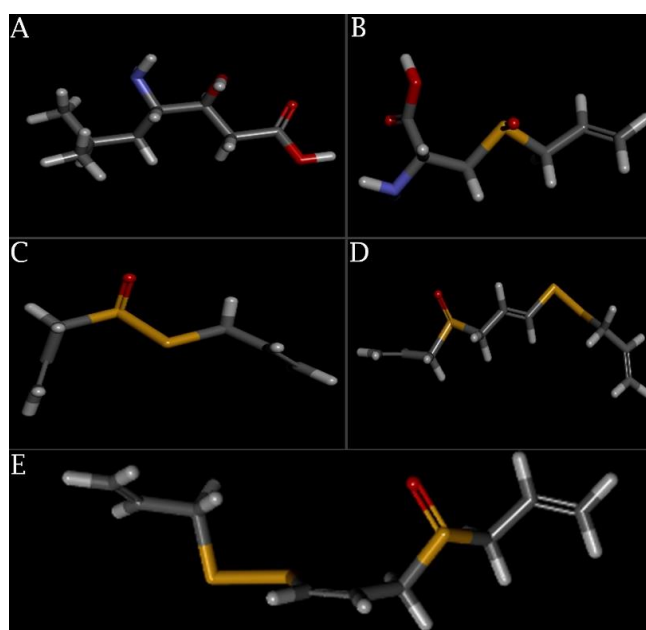


Figure 1. 3D structure of the organosulfur compound in SG and statin. A. Statin; B. Alliin; C. Allicin; D. E-Ajoene; E. Z-Ajoene (PubChem, 2017)

The 3D structure of FAS enzyme was taken in the RCSB GDP online service (Figure 2a). PyMol software was then used for removing water molecule and other ligands in FAS enzyme (Figure 2b).

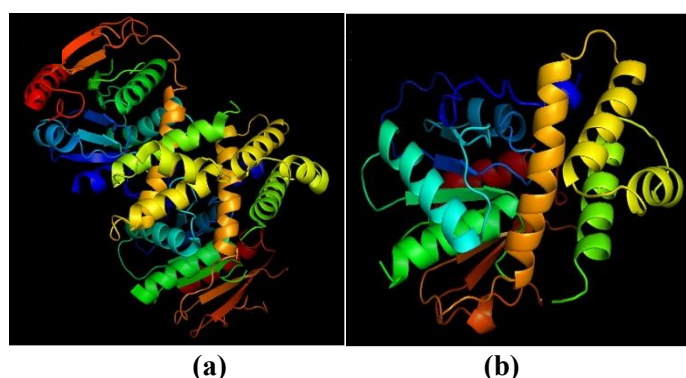


Figure 2. (a) 3D structure of FAS enzyme from RCSB GDP; (b) 3D structure of FAS enzyme after removing water molecules and other ligands

The results of molecular docking between organosulfur compounds in SG (Alliin, Allicin, E-Ajoene, and Z-Ajoene) with FAS enzyme (after removing water molecules and other ligands) used AutoDock Vina program on the PyRx software with the following results (Table 1).

Table 1. The results of molecular docking between the organosulfur compounds in SG and statin with FAS enzyme.

Compound	Binding affinity (kcal/mol)	Amino acid residues	The distance of hydrogen bonding (Å)	Hydrophobic interaction
Statin	-5.0	Glu2251	3.20	Phe2423,
		Arg2482	2.99	Gln2374,
			3.17	Phe2371,
				Leu2222
Alliin	-4.5	Gln2272	2.87	Met2232,
		Thr2254	2.93	Ser2253,
			3.08	Val2224,
			3.24	Ser2221,
		Thr2255	2.87	Leu2223,
			3.09	Arg2220
E-Ajoene	-4.4	Ser2308	2.88	Phe2371,
		Asp2338	2.51	Leu2222,
		His2481	3.08	Phe2375,
		Arg2482	3.05	Glu2251,
				Gln2374, Ile2250
Z-Ajoene	-4.2			Phe2370,
				Phe2423,
				Leu2427,
				Ala2367,
				Arg2428,
				Tyr2424,
				Ala2363, Glu2366
Allicin	-3.8	Ser2308	2.96	Phe2370, Ile2250,
		Asp2338	2.51	Phe2423,
		His2481	3.03	Leu2427,
		Arg2482	3.04	Tyr2309

The results of molecular docking showed that statin had the highest binding affinity for FAS enzymes compared to organosulfur compounds (-5.0 kcal/mol). The visualization of binding position using PyMol software showed that the organosulfur compounds (E-Ajoene, Z-Ajoene, and Allicin) had the same binding site with statin in FAS enzyme. The binding site of ligands and protein is shown in Figure 3. Ligands are shown in red (Alliin), magenta (Allicin), blue (E-Ajoene), orange (Z-Ajoene), and white (Statin).

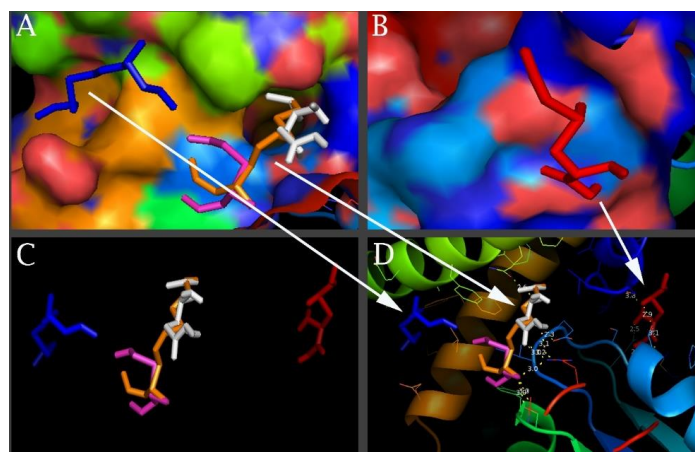


Figure 3. A & B. The visualization of the binding site in organosulfur compounds and statin to FAS enzyme; C. The magnification of the visualization of an organosulfur compound and statin binding site; D. The visualization of all compounds. The white arrow shows the position of organosulfur compounds and statin in the FAS enzyme.

The interactions visualized using LigPlot⁺ software showed that Allicin and Z-Ajoene were in one site with statins through amino acid residues and the same hydrophobic interactions (Figure 4). The same amino acid residue was Arg2482, while the same hydrophobic interactions, i.e Phe2423, Leu222, Gln2374, Ile2250. The hydrophobic interaction Phe2433 indicated the same binding site between E-Ajoene and Statins. The visualization of the interactions in the FAS enzyme did not show any similar site between Alliin and Statins but Alliin had several amino acid residues and hydrophobic interactions in the FAS enzyme. Amino acid residues and hydrophobic interactions indicated that Alliin had the potential to inhibit the FAS enzymes (Figure 4).

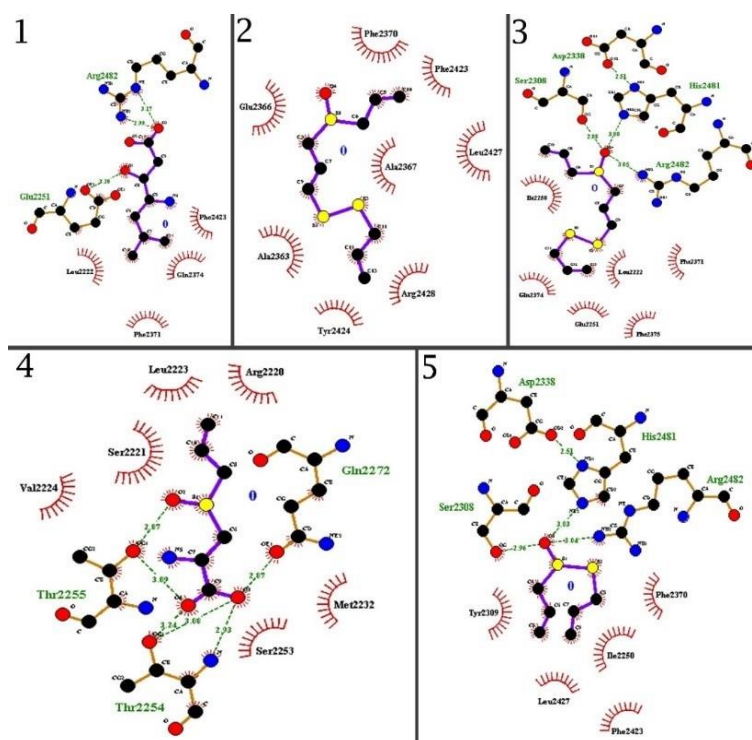


Figure 4. The visualization of the interaction of each organosulfur compound and statin in the FAS enzyme. 1. Statin; 2. E-Ajoene; 3. Z-Ajoene; 4. Alliin; 5. Allicin

4. Discussion

Lipids were obtained from food or through de novo synthesis in the liver. The fatty acids were produced in lipid metabolism mainly stored as triglycerides. Excess production and accumulation of triglycerides harm the metabolism and can cause various diseases [17]. The synthesis of triglycerides in the body involves a variety of proteins with different mechanisms; one of them is FAS. FAS is a treatment target which is responsible for plaque development and inflammation of atherosclerosis [18,19].

The molecular docking showed that organosulfur compounds in SG such as Alliin, Allicin, E-ajoene, and Z-ajoene had the potential as FAS inhibitors. These potentials were determined by the binding affinity, amino acid residues, and hydrophobic interactions between each ligand-receptor. The amino acid residues formed and linked to the presence of hydrogen bonds indicated that the organosulfur compounds in SG were bond to the active side of the receptor. These interactions proved that ligands play an important role in inhibiting protein function [20]. Hydrophobic interactions formed between ligand-receptors serve to stabilize ligands in conformation to protein structure [21] and increase ligand-receptor binding affinity and increase biological activity from ligands [22]. SG (*A. sativum*) has an ability as a therapeutic agent because it can inhibit the invasion of carcinoma, provide cardiovascular protection, lower cholesterol and blood pressure, anti-platelet activity, and thromboxane formation [23]. FAS encoded by *Fasn* gene is an enzyme that catalyzed the biosynthesis of saturated fatty acids from the de novo lipogenesis pathway. The first product in the FAS reaction is palmitate. FAS substrates are acetyl-Co-A, malonyl-Co-A, and NADPH. The fatty acid extends from the initial acetyl-CoA by repeated condensation with malonyl-CoA, which gives two carbons in each condensation cycle. Palmitic synthesis requires seven cycles of the addition of malonyl-Co-A to primary acetyl-Co-A to produce saturated lipid, i.e. 16 carbon fatty acids [24]. FAS is a 273 kDa homodimer subunit. Each monomer contains seven domains of proteins needed for the synthesis of fatty acids, namely acyl carrier, acyltransferase, β -ketoacyl synthase, -ketoacyl reductase, β -hydroxylakyl dehydratase, enoyl reductase, and thioesterase. However, enzymatic FAS is only active in the form of dimers [25,26]. FAS dissolves protein and is localized in the cytoplasm, although specifically, its subcellular localization is largely unknown. The distribution of FAS happens in tissues with the highest level, such as liver, adipose tissue, and lungs [26,27].

FAS is categorized as a protein in the liver involved in the synthesis of lipid for energy storage. Recent research has shown that liver FAS is also involved in the signaling process which includes the activation of the Peroxisome Proliferator-Activated Receptor (PPAR α) [24]. The activation of PPAR α mediates an adaptive response by promoting the transcription of genes involved in the absorption and catabolism of fatty acids [4]. FAS removal can cause the death of mice suggesting that de novo lipogenesis is needed early during development [28] and perhaps FAS is necessary to provide lipids in the embryo's growing cell membranes.

The activity of FAS in the liver has increased along with the presence of obesity and fatty liver [29], but the mechanism of occurrence of the two related things has not been clearly explained. $26 \pm 7\%$ of liver triglycerides is originated from de novo lipogenesis in non-alcoholic fatty liver disease (NAFLD) patients [30]. Another research reported that elimination of specific FAS in the liver rats showed that it was not protected from lipid accumulation, but there was hepatic steatosis while rats were fed with non-fat diet [25]. This mechanism occurred when the deficiency of FAS decreased the expression of PPAR α and dietary fat caused the hypothesis that the "new" fat derived from de novo lipogenesis could activate PPAR α , while the "old" fat originated from peripheral tissue or stored in the liver could not activate PPAR α . The inhibition of Carbohydrate Response Element Binding Protein (ChREBP) as a transcription factor for FAS enzyme in the obese model, showed the decrease in lipid accumulation and lipogenesis in the liver [31]. That research showed that the activation FAS as a lipogenic enzyme is closely related to obesity. The organosulfur compounds in SG had an inhibitory activity of FAS enzyme; thus, it could decrease synthesis of fatty acid.

5. Conclusion

The results of molecular docking method showed that organosulfur compounds in SG had potential as drug candidates by inhibiting the FAS enzyme. The visualization of the binding site showed that the organosulfur compounds (E-Ajoene, Z-Ajoene, and Allicin) in SG had the same binding site with Statins in FAS enzyme. FAS is an enzyme which plays a role in the biosynthesis of lipid. The inhibition of FAS could reduce the production of fatty acid. Therefore, the SG could be used as an alternative medicine for various diseases caused by hyperlipidemia.

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