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Research Article

Molecular Docking Analysis of Volatile Compounds from Fraction of *Eichhornia crassipes* Herbs Ethanol Extract As α-Glucosidase Inhibitor

Diniatik*, Eka Retnowati, Asmiyenti D. Djalil
Pharmaceutical of Biology, Faculty of Pharmacy, Muhammadiyah University of Purwokerto, Central Java, and Indonesia

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*Corresponding author: diniatik@yahoo.com.au

Abstract
Diabetes mellitus is a degenerative disease characterized by an increase in blood sugar (hyperglycemia). Inhibiting it will prevent the uptake of glucose in the plasma. One of the plants that can be used as antidiabetic is *E. crassipes* herbs. This research aims to know the interaction between volatile chemical compounds from ethyl acetate fraction and ethanol fraction of ethanol extract of *E. crassipes* herbs with the α-glucosidase enzyme. This study used ethanol extract of *E. crassipes* herbs with a variation of ethyl acetate fraction and ethanol fraction then analyzed the fraction using GC-MS (Gas Chromatography-Mass Spectroscopy). The ethyl acetate fraction contained 2-pyridine-propanoic acid, alpha.-methyl-.beta.-oxo-,Ethyl ester;1-propanol-1-di; carbamic acid, methyl ester; acetic acid, aminooxy-, hydrazide; (2R) - [2-2H2] glycine methyl ester hydrochloride. The ethanol fraction contained semicarbazide hydrochloride; carbonic dihydrazide; hydroxy-acetaldehyde; 1-propanol-1-di; acetic acid, and hydroxyl. The result of docking with the α-glucosidase enzyme is 2-pyridine-propanoic acid, alpha.-methyl-.beta.-oxo-, the Ethyl ester of the ethyl acetate fraction having ΔG is -4.14 Kcal/mol. Semicarbazide hydrochloride from the ethanol fraction has ΔG of -3.17 Kcal/mol.
Keywords: Diabetes mellitus, docking molecular, (*Eichhornia crassipes* (Mart.) Solms Herbs, ethyl acetate fraction, α-glucosidase enzyme.

Analisis Penambatan Molekul Senyawa Kimia dari Fraksi Ekstrak Etanol Herba *Eichhornia crassipes* terhadap Enzim α-Glukosidase

Abstrak
Diabetes melitus adalah penyakit degeneratif yang ditandai dengan kenaikan gula darah (hiperglikemia). Inhibitor enzim α-glukosidase mencegah peningkatan glukosa dalam plasma. Salah satu tanaman yang berpotensi sebagai antidiabetes dengan mekanisme kerja golongan inhibitor enzim α-glukosidase adalah eceng gondok (*E. crassipes*). Penelitian ini bertujuan untuk mengetahui interaksi antara senyawa mudah menguap yang terdapat dalam fraksi etil asetat dan fraksi etanol ekstrak etanol herba eceng gondok (*E. crassipes*) dengan enzim α-glukosidase dengan metode penambatan molekul. Ekstrak etanol herba eceng gondok (*E. crassipes*) difraksinasi dengan etil asetat dan etanol kemudian di analisis menggunakan GC-MS (*Gas Cromatography-MS*). Hasil fraksi etil asetat diperoleh senyawa 2-*pyridinepropanoic acid,.alpha.-methyl-.beta.-oxo-,ethyl ester; 1-propanol-1-di; carbamic acid, methyl ester; acetic acid,aminooxo-,hydrazide; (2R)-[2-2H2] glycine methyl ester hydrochloride*. Senyawa dalam fraksi etanol adalah *semicarbazide hydrochloride; carbonic dihydrazide; hydroxy-acetaldehyde;1-propanol-1-diacetic acid, hydroxyl*. Hasil doking terhadap enzim α-glukosidase senyawa 2-*pyridinepropanoic acid, .alpha.-methyl-.beta.-oxo-,ethyl ester* dari fraksi etil asetat memiliki ΔG sebesar -4.14 Kkal/mol. Senyawa *semicarbazide hydrochloride* dari fraksi etanol memiliki ΔG sebesar -3.17 Kkal/mol.
Kata Kunci: Diabetes melitus, penambatan molekul, enzim α-glukosidase, herba eceng gondok (*Eichhornia crassipes* (Mart.) Solms), fraksi etil asetat.

1. Introduction

Diabetes mellitus (DM) is a disease of a chronic metabolite disorder characterized by hyperglycemia. DM is one of the most dangerous conditions which is the fourth most significant cause of death in the world[1]. DM sufferers experience an increase every year. According to WHO data, in 2030, it is estimated that 21,3 million Indonesians will suffer from DM. Indonesia is in fifth position with a total of 19,47 million people with diabetes. With a population of 179,72 million, this means that the prevalence of diabetes in Indonesia is 10,6%. Indonesia is in fifth position with a total of 19,47 million people with diabetes. With a population of 179,72 million, this means that the prevalence of diabetes in Indonesia is 10,6%[2]. Based on these data, Indonesia ranks fifth with the most DM patients after the United States, China, and India[3]. DM can also result in sufferers having complications from other diseases such as kidney failure, blindness, stroke, and the risk of amputation[1], so antidiabetic is needed. There are various kinds of antidiabetic effective mechanisms, including stimulating insulin secretion and beta cells in the pancreas, stimulating insulin-responsive genes, inhibiting the enzyme alpha-glucosidase, inhibiting DPP-4[4].

Indonesia is a tropical country, so it has various types of plants. Every plant has different chemical compounds. Not only plants that live on land, but plants that live in the waters also have chemical compounds as well. One of the plants that grow in water is the water hyacinth (*E. crassipes*). Water hyacinth is a plant that lives floating in the water. Water hyacinth has thick green leaves and bubbles that make it float. This water hyacinth plant is one of the weed plants. This water hyacinth plant can spread to the entire surface of the water so that it will cause sunlight cannot enter the inside. It creates the oxygen levels dissolved in the water to decrease, and dead plants will cause siltation. Currently, water hyacinth is widely used for handicrafts and organic fertilizers[5].

Based on research conducted by Rorong and Suryanto in 2010, stated that water hyacinth extract contains chemical compounds, among others: phenolic content, flavonoids, and tannins[6]. Other research shows that ethanol extract from water hyacinth has the chemical content of alkaloids, flavonoids, sterols, terpenoids, anthraquinones, proteins, and phenols[7]. Other research proves that the ethanol extract of herb water hyacinth which was tested using Gas Chromatography-Mass Spectroscopy (GC-MS) contains stigmasterol and 1-monolinoleoyglycerol compound trimethylsilyl ether8. According to Tyagi and Mala (2017), stigmasterol from the ethanol extract of herb water hyacinth has the potential as a hypoglycemic agent[8].

Based on the above studies, plants that have a chemical content of steroids have antidiabetic activity. Steroid compounds are one component of sterol compounds. Sterol compounds can be detected higher in extracts with ethanol, methanol, and ethyl acetate solvents[9]. Steroids are compounds that are soluble in semipolar solvents[10]. It is also evidenced in the study of the isolation of steroid compounds in sea cucumbers that steroid compounds are semipolar compounds and can dissolve well in ethyl acetate solvents[11]. Therefore, the researcher wanted to examine the chemical compounds of ethyl acetate fraction and ethanol fraction from the ethanol extract of water hyacinth herbs using GC-MS. The volatile chemical compounds from the GC-MS results, then do the docking with the α-glucosidase enzyme to determine its potential as a hypoglycemic agent with the mechanism of inhibiting the α-glucosidase enzyme.

2. Method

2.1. Instrument

The tools used for extraction and fractionation include a set of soxhlet tools, a blender, a set of glassware, a 40 mesh size sieve, filter paper, a porcelain cup, a pipette, a separating funnel, water bath. The tool used for the analysis of compound content in fractionation is a set of GC-MS tools. While the tools used for docking consist of hardware and software. The hardware consists of a computer with a Compaq Presario CQ43

laptop specification. The software used consists of autodock Vina, ADT 1.5.6 (The Scripps Research Institute, America) VMD 1.9.2, Discovery SV 3.5, Ligplot + 4.5.3 (Rowan Lawkowski), and OS. Ubuntu 14.04 lts.

2.2. Material

The materials used for this study include water hyacinth plants obtained from the swampy area, Ambarawa, and Semarang Regency. The solvent by using 96% ethanol extraction and fractionation was 70% ethanol and technical quality ethyl acetate. The material used for molecular docking was a two-dimensional structure from the compound in the fraction ligand from ethanol and ethyl acetate fractionation which was analyzed using GC-MS. While the macromolecule file chosen as a receptor is the α-glucosidase enzyme downloaded in the Protein Data Bank (GDP) database at the HTTP// www.rcsb.org site with the code 2QMJ.

2.3. Procedure

2.3.1. Determination of plants Eichhornia crassipes (Mart.) Solms

Plants obtained from the swampy area, Ambarawa, which is thought to be a plant of *Eichhornia crassipes* (Mart.) Solms were determined at the Environmental Laboratory, Faculty of Biology, Jenderal Sudirman University. The certificate number of determination is 2409/UN23.02.8/ PP.08.00/2018.

2.3.2. Stage of preparing plant powder

Material preparation. The material needed is water hyacinth (*Eichhornia crassipes*). Herbs of water hyacinth (roots, stems, leaves) are separated from the damaged part, and then wet sorting is done to separate the freshwater hyacinth and those that have been destroyed and rotten. The water hyacinth is cleaned with running water to remove dirt and cut to reduce size. Then dried in the sun by covering the black cloth until dry Simplicia is obtained. After drying, water hyacinth is powdered using a blender and sifted using a 40 mesh sieve until powder is obtained with uniform smoothness.

2.3.3. Making ethyl acetate fraction and ethanol fraction from the ethanol extract of water hyacinth herb

Ethanol extract of herb water hyacinth is made by soxhletation. Extraction is done by using the soxhlet tool. A total of 35 g of water hyacinth dried powder (roots, stems, and leaves) were included in the soxhlet device and given 400 ml of solvent. The solvent was used 96% ethanol. The soxhlet device is set between 50°C-70°C with a circulation of 1.5 to obtain the extract. The extract was evaporated by using a water bath with a temperature of 60°C-70°C.

Fractionation of ethanol extract from water hyacinth herbs was carried out using the liquid-liquid partition method using n-hexane, chloroform, ethyl acetate, and 70% ethanol technically. The fractionation process is carried out repeatedly until the results of fractionation are clear to maximize the content of the active compounds which are expressed in the fraction. The remaining ethyl acetate fraction and ethanol fraction were evaporated using a water bath to obtain ethyl acetate fraction (EAF) and ethanol fraction (EF) ethanol extract of water hyacinth herb. Furthermore, EAF and EF are put into a glass container covered with aluminum foil and stored in the desiccator until when it is to be used.

2.3.4. Analysis of chemical compounds content of ethyl acetate fraction and ethanol fraction from the ethanol extract of herb water hyacinth by GC-MS

After obtaining EAF and EF analysis was carried out using GC-MS to analyze the content of active volatile compounds in it. A total of the 1µL fraction is injected in GC-MS.

The GC-MS analysis was carried out using GCMS-QP2010 SE from Shimadzu. The instrument is set to an initial temperature of 110ºC and maintained at this temperature for 2 minutes. At the end of this period, the oven temperature is increased to 280ºC, with a rate of increase of 5ºC / minute, and maintained for 45 minutes. The port injection temperature

is ascertained as 250ºC, and the helium flow rate is 1 ml/minute. The ionization voltage is 70V; the ion source temperature is 250ºC, temperature interface is 280ºC. The range of mass spectral scanning is set at 30-450 (m/z). The unknown component spectrum is compared to the known component spectrum in the wiley[9]. Lib data library. The results of the analysis are in the form of names, molecular weights, and structural components of the test material.

2.3.5. Molecular docking

The results of EAF and FE analysis by GC-MS are ligands that will be anchored with α-glucosidase receptors. Financing is done using the AutoDockTools 1.5.6 software. Besides using other software, Chem3D pro 12.0.2, Gaussian 03W, GaussView, and Discovery Studio 3.1 client. The protein used as a receptor is an α-glucosidase enzyme with code 2QMJ obtained online through the site http://www.rcsb.org/pdb (Setiawan, 2015). The following are the stages of docking performed:

Molecular structure description of GC-MS results: Compounds of GC-MS results were selected in 5 compounds that had the highest area of each fraction (EAF and EF). The structure of the compounds chosen is drawn using the Chem3D Pro 12.0.2 application.

Optimization of GC-MS results structure: The molecular structure that has been made with Chem3D Pro 12.0.2 made an optimization using Gaussian 03W. The optimization method chosen is the density functional theory (DFT) method. The optimization results are seen using GaussView.

Preparation of α-glucosidase enzyme protein molecules: The α-glucosidase enzyme protein molecule with 2QMJ code is prepared by searching the bank's protein data with the site http://www.rscb.org/pdb/. This site is managed by the Research Collaboratory for Structural Biology. The data search on this site is done by typing the keyword in the form of a protein name or GDP code if you already know the GDP id code used. The GDP file contains the names of proteins, research titles on proteins used, names of researchers, and research journal publications.

Preparation of the structure of the α-glucosidase enzyme protein using Discovery studio 3.1 client software. The α-glucosidase enzyme obtained was prepared using the Discovery Studio 3.1 client. Protein preparation aims to get the desired protein so that the docking that is done gets optimal results. Protein preparation carried out include: the removal of water, separation of receptors and natural ligands, and the addition of hydrogen.

Ligand preparation: The preparation of ligands aims to get the desired sample. Developments made include: adding hydrogen, adding charge, and regulating rotatable root. The prepared sample is expected to do the docking process well.

Validation of the docking method: Validation of the docking method was carried out by documenting the natural ligands. Financing is done using the ADT 1.5.6 program. The docking process is done by blind docking. Blind docking is a docking process where mining is done without knowing the exact active side of the compound. Blind docking can be used for unbiased mapping of the binding patterns of drug candidates. The location of the active team that is unknown is sure to cause the grid box parameters to be set not to the specific area but to all parts of the compound molecule. The grid boxes that have been specified in the validation method are used to document sample compounds. The determination of the validation method is based on the RMSD value. If the RMSD produced is <2 A, then the way is declared valid and can be used for research.

Docking the α-glucosidase enzyme against the GC-MS yield ligand: The docking process for sample compounds was carried out after the docking validation process. The structure of the optimized sample compound was prepared by adding hydrogen, removing water and adding the charge, and then stocking it with an α-glucosidase enzyme protein. The documenting and grid box arrangement process follows during the

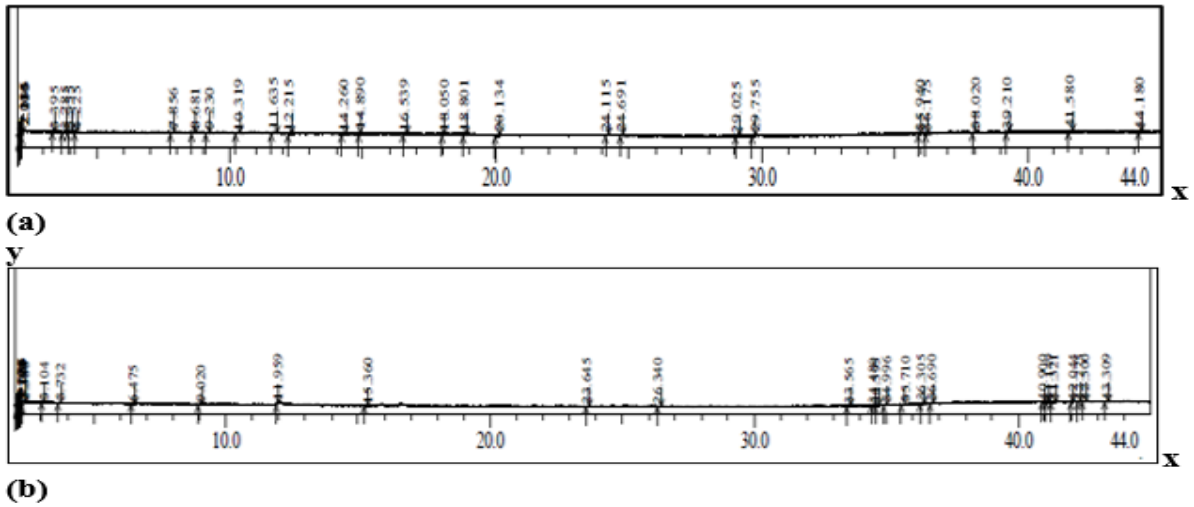

Figure 1. Results of the chromatogram of ethyl acetate fraction (a) and ethanol fraction (b) of ethanol extract of water hyacinth

validation process. Furthermore, analysis of the results of docking based on ΔG or free energy test compounds and binding sites of amino acids in the α-glucosidase enzyme protein.

3. Result

GC-MS test results from EAF are expressed in chromatogram Figure 1. Based on the results of the EAF chromatogram obtained 30 peaks can be seen in table 1 and selected five peaks with the largest peak area to be anchored with the α-glucosidase enzyme. GC-MS EF test results are expressed in chromatogram Figure 1. Based on the results of the EF obtained 29 peaks can be seen in table 1 and selected five peaks with the largest peak area.

4. Discussion

The GC-MS chromatogram of EAF and EF from the ethanol extract of herb water hyacinth recorded a total of 59 peaks. All of the peaks were corresponding to the bioactive compounds that were recognized by relating their peak retention time, peak area (%), height (%), and mass spectral fragmentations to obtain the compounds described by the Wiley[9] library. Results revealed that 30 and 29 compounds were identified in EAF and EF from the ethanol extract of herb water hyacinth, respectively (Tables 1 & 2). The five largest peak areas of phytoconstituents in the EAF from the ethanol extract of herb water hyacinth were found in 2-pyridine-propanoic acid, alpha.-methyl-.beta.-oxo-, ethyl ester, 1-propanol-1-D1, Carbamic acid, methyl ester, Acetic acid, aminooxo-, hydrazide, (2R)-[2-2H2] glycine methyl ester hydrochloride. They were at retention times of 2,036; 2,100; 2,145; 2,214; and 3,785 minutes. The five largest peak areas phytoconstituents in the EF from the ethanol extract of herb water hyacinth were found semicarbazide hydrochloride; carbonic dihydrazide; hydroxy-acetaldehyde; 1-propanol-1-di; acetic acid, hydroxyl. They were at retention times of 2,055; 2,120; 2,175; 2,280; and 2,386 minutes. The peak area shows the abundance of compounds obtained from the analysis. The greater the peak area, the greater the abundance of the compound.

The GC-MS analysis revealed that the ethanol extract of herb water hyacinth contained 59 bioactive compounds (Tables 1 & 2). The five largest peak areas phytoconstituents each from EAF and EF were analyzed for activities against diabetic target proteins. The docking studies were carried out for phytoligands using the ADT 1.5.6 (AutoDock Tools 1.5.6) program to elucidate the binding affinities to the target proteins. The description of the molecular structure of the test compound was carried out using the Chem3D Pro 12.0.2 application. Compounds that have been drawn are saved in the form of an a.pdb file. The molecular structure that was drawn using Chem3D was then optimized using the Gaussian application. The purpose of optimizing the structure is to get a structure with a more stable and optimal conformation.

Table 1. The compound content fraction of water hyacinth ethanol extract

Compounds of ethyl acetate fraction of water hyacinth ethanol extract				
RT (min)	**Compounds**	**Molecular Formula**	**Molecular weight**	**Peak area %**
2.036	2-pyridine-propanoic acid, .alpha.-methyl-. beta.-oxo-, ethyl ester	C11H13NO3	207	17.87
2.100	1-propanol-1-D1	C3H7DO	60	11.88
2.145	Carbamic acid, methyl ester	C2H5NO2	75	11.05
2.214	Acetic acid, aminooxo-, hydrazide	C2H5N3O2	103	5.23
3.395	1-(ethoxycarbonyl-1-fluoro-2-(methoxycarbonyl)-1,3-butadiene	C9H11FO4	202	2.02
3.785	(2R)-[2-2H2] glycine methyl ester hydrochloride	C3H6D2ClNO2	125	3.02
3.955	N-2-keto derivative of methapyrilene	C3H6D2ClNO2	125	2.23
4.225	Metadrenaline	C14H17N3OS	275	1.78
7.856	semicarbazide hydrochloride	CH5N3O	75	2.26
8.681	1-(ethoxycarbonyl-1-fluoro-2-(methoxycarbonyl)-1,3-butadiene	C9H11FO4	202	2.54
9.230	6-methylhept-5-en-1-nitrile	C8H13N	123	1.76
10.319	2-n-butyl-5-cyclooctene	C12H22O	182	1.93
11.635	1-(ethoxycarbonyl-1-fluoro-2-(methoxycarbonyl)-1,3-butadiene	C9H11FO4	202	2.79
12.215	semicarbazide hydrochloride	CH5N3O	75	2.31
14.260	Air	O2	32	1.95
14.890	methanone, diphenyl	C13H10O	182	2.75
16.539	Pentadeuteropropionitril	C3D5N	55	2.27
18.050	1-propanol-1-di	C3H7DO	60	1.78
18.801	2-pyridine-propanoic acid, .alpha.-methyl-. beta.-oxo-, ethyl ester	C11H13NO3	207	1.68
20.134	2-pyrrolidinone, 1 methyl	C5H9NO	99	2.28
24.115	acetic acid, hydrazide	C2H6N2O	74	1.80
24.691	2-pyridine-propanoic acid, .alpha.-methyl-. beta.-oxo-, ethyl ester	C11H13NO3	207	1.78
29.025	4-bromo-n-(piperidinomethyl) phthalimide	C14H15BrN2O2	322	1.94
29.755	L-alanine, N-(3-methoxy-1,3-dioxopropyl)-, methyl ester	C8H13NO5	203	1.86
35.940	semicarbazide hydrochloride	CH5N3O	75	1.77
36.175	2-pyridine-propanoic acid, .alpha.-methyl-. beta.-oxo-, ethyl ester	C11H13NO3	207	1.89
38.020	1,25-dihydroxy vitamin D2	C28H44O3	428	1.79
39.210	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-hexasiloxane	C12H38O5Si6	430	1.79
41.580	hydrazine, (1,1-dimethyl ethyl)-, monohydrochloride	C4H12N2	88	1.80
44.180	norepinephrine-pentatms	C23H51NO3Si5	529	2.19
The compound content of ethanol fraction of water hyacinth ethanol extract				
2.055	Semicarbazide hydrochloride	CH5N3O	75	18.63
2.120	Carbonic dihydrazide	CH6N4O	90	10.58
2.175	Hydroxy-acetaldehyde	C2H4O2	60	7.89
2.195	1-butanol. 2-nitro	C4H9NO3	119	3.02
2.221	1-propanol-1-di	C3H7DO	60	5.40

2.280	1-propanol-1-di	C3H7DO	60	5.62
2.386	Acetic acid, hydroxyl	C2H4O3	76	6.77
3.104	1H-benzotriazole-1-sulfonamide, N, N-dimethyl	C8H10N4O2S	226	1.95
3.732	imidazole-4-propionic acid, ethyl ester	C8H12N2O2	168	1.55
6.475	isobutyl phthalate	C16H22O4	278	1.52
9.020	Semicarbazide hydrochloride	CH5N3O	75	1.66
11.959	N, N-dimethyl-3-button-1-amine	C6H13N	99	3.52
15.360	2-n-butyl-5-cyclooctenol	C12H22O	182	1.60
23.645	n-butylamine-D9	C4H2D9N	73	1.61
26.340	methyl D-glycolate	C3H5DO3	90	1.78
33.565	trimethyl-ethyl-butyl-hexahydro-indene	C18H32	248	1.65
34.480	N-amino32-hydroxypropanamidate	C3H8N2O2	104	1.96
34.595	N-(N-methylformamidyl)-semithiocarbazide	C3H8N4OS	148	1.79
34.996	4-dimethylamino but-2-in-1-ol	C6H11NO	113	1.56
35.710	2-pyridine-propanoic acid, .alpha.-methyl-. beta.-oxo-, ethyl ester	C11H13NO3	207	1.69
36.305	Semicarbazide hydrochloride	CH5N3O	75	2.37
36.690	Butylsemithiocarbazide	C5H13N3S	147	1.51
40.900	thiourea, 1,1-diethyl-3-(2-mercapto-5-benzoxazolyl)	C12H15N3OS2	281	1.50
41.149	2-pyridine-propanoic acid, .alpha.-methyl-. beta.-oxo-, ethyl ester	C11H13NO3	207	1.89
41.321	cyclotetrasiloxane, octamethyl	C8H24O4Si4	296	1.89
42.044	ethyl N-methylcarbamate	C4H9NO2	103	1.50
42.275	cyclotetrasiloxane, octamethyl	C8H24O4Si4	296	1.78
42.500	hexane, 1,1-diethoxy	C10H22O2	174	1.81
43.309	(2,4-dichloro-6-nitrophenoxy) acetic acid	C8H5C12NO5	265	1.61

Optimization is carried out using the density functional theory (DFT) method. The DFT method is a fairly good optimization method, but the time required is relatively long depending on the shape of the structure to be optimized. From the optimization of table 2, it can be seen the results of the molecular structure and energy-optimized by the DFT method. The smaller the power obtained, the better the conformation and the more stable it is[12] (Male et al., 2015). It proves that if the energy value becomes more negative, the higher the strength in forming the bond. The optimization results obtained are stored in the form of a file.gjf to see the results of the energy gained. The optimization results are also saved as a file.mol2 so that the optimization results can be opened in the form of images. These ligands will be docking with an alpha-glucosidase enzyme to determine its binding affinity

Sequence data of the α-glucosidase enzyme protein were prepared by searching the protein data bank with the website http://www.rscb.org/pdb/. This site is maintained by the Research Collaboratory for Structural Biology. Searching for data on this site can be done by typing in keywords in the form of protein name or PDB id code if you already know the PDB id code used. The PDB file contains the name of the protein, the title of the research on the protein, the name of the researcher, and where the researcher's journal is published. The PDB id code used is 2QMJ. 2QMJ is a PDB code that is often used by researchers for molecular documentation of the α-glucosidase enzyme. 2QMJ is the crystal structure of the α-glucosidase enzyme obtained from x-ray diffraction. 2QMJ consists of two molecules, namely a protein molecule called amylase glucoamylase and a water molecule[13](Sim et al., 2008). Before docking, the ligands were prepared first. The ligand prepared is alpha acarbose a

Table 2. Structure of the compound test for EF and EAF which has been optimized by the DFT method

EF (Energi Kcal/mol)	Fraksi EAF (Energi Kcal/mol)
Semicarbazide Hydrochloride (-225.9600497)	2-pyridinepropanoic acid, .alpha.-methyl-.beta.-oxo-,ethyl ester (-698.3638271)
Carbonic dihydrazide (-331.3146752)	1-propanol-1-di (-191.8749976)
Hydroxy-acetaldehyde (-225.9600497)	Carbamic acid, methyl ester (-280.6357419)
1-propanol-1-di (-191.8749976)	Acetic acid, aminooxo-, hydrazide (-388.5911573)
Acetic acid. hydroxyl (-300.1523521)	(2R)-[2-2H2] glycine methyl ester hydrochloride (-775.7890932)

natural ligand used for docking validation. The preparation of GC-MS ligands was also carried out as test compounds in the docking process. Ligand preparation is carried out by adding hydrogen. The addition aims to add a partial charge to the ligand molecule. The compound that has been added with hydrogen is stored in the file.pdbqt. The compound with a file.pdbqt is determined by the number of rotatable used. The more rotatable bonds in a ligand, the more flexible the ligand will be. After the rotatable bond is determined, select the output, and the file is saved as file. pdbqt. File.pdbqt will be used for the docking process. The.pdbqt file shows that the ligand molecule already has a partial charge on each of its atoms.

Validation in the autodock method, by re-tethering the natural ligands to the α-glucosidase enzyme receptor. Experimentally the two are known to interact with each other. The validation results are seen based on the RMSD value. The RMSD value is used to evaluate the similarity of two structures based on the distance of similar atoms. If the resulting RMSD value is <2 A, then the method can be declared valid[14]. The validation process of the docking method is carried out using autodock because this program is free without requiring registration of licenses. This program is also quite often used as a media for docking research. During the documentation process, protein and ligand files must be available in the form of a file.pdb, file.pdbqt, and file.pdbqt that have been prepared previously. The protein and ligand files must be made into a folder with the installed autodock program. After the ligands and proteins are in the form of an a.pdbqt file, then the grid preparation is carried out by adjusting the grid box. The grid box is one of the parameters used to adjust the area when docking. The purpose of the grid box arrangement is to provide space for the ligands to rotate and find the active side. The grid box parameters used in the method validation between natural ligands and the α-glucosidase enzyme can be seen in Table 3.

After the grid box settings are set, they are saved. The resulting grid output is file.gpf. File.gpf is run autogrid to get file.glg. This file.glg is data that contains the peak area by the predefined grid boxes. Furthermore, the docking process is carried out to determine the location of the active side of the ligand which can interact with the α-glucosidase enzyme. The docking process in the validation method is carried out by preparing natural ligands and α-glucosidase enzyme proteins in the form of an a.pdbqt file. The results are stored in the.dpf file. File.dpf is performed on autodock to get the.dlg file. This.dlg file is data that contains information on the RMSD value and binding energy. The following is

Table 3. RMSD and Grid Box Parameters

Grid Box	Natural ligand to α-glukosidase enzym	RMSD
x-dimension	28	1,41
y-dimension	22	
z-dimension	46	
X center	-22,097	
Y center	-6,491	
Z center	-5,222	

Table 4. Binding energies of the EAF and EF against the α-glucosidase enzyme

Fraction	Compounds	ΔG (Kcal/mol)
EAF	2-pyridine-propanoic acid, .alpha.-methyl-.beta.-oxo-, ethyl ester	-4.14
	1-propanol-1-DI	-1.64
	carbamic acid, methyl ester	-2.55
	acetic acid, aminooxy-, hydrazide	-3.43
	(2R)-[2-2H2] glycine methyl ester hydrochloride	-3.10
EF	Semicarbazide hydrochloride	-3.17
	Carbonic dihydrazide	-3.12
	Hydroxy-acetaldehyde	-1.56
	1-propanol-1-di	-1.66
	Acetic acid, hydroxyl	-1.28
	Natural ligan (alpha acarbose)	-3.37

Table 5. Amino acid bonds formed between α-glucosidase enzymes towards ligand

Fraction	Compounds	Amino acids that bind to
EAF	2-pyridine-propanoic acid, .alpha.-methyl-.beta.-oxo-, ethyl ester	Asp 452, Gly 457
	1-propanol-1-DI	Asp 452
	carbamic acid, methyl ester	Asp 452, Ser 454
	acetic acid, aminooxy-, hydrazide	Asp 452, Ser 454, Val 455
	(2R)-[2-2H2] glycine methyl ester hydrochloride	Asp 452, Ser 454
EF	Semicarbazide hydrochloride	Asp 452, Ser 454, Val 455
	Carbonic dihydrazide	Asp 452, Ser 454, Val 455
	Hydroxy-acetaldehyde	Asp 452
	1-propanol-1-di	Asp 452
	Acetic acid, hydroxyl	Asp 452, Gly 457, Ser 457
	Natural ligan (alpha acarbose)	Asp 203, Asp 327, Asp 542, Arg 526, His 600, Phe 575

the RMSD value from the validation results (Table 3). The results of the average RMSD obtained were 1,41 A. These results state that the validation method used is valid because the RMSD value is <2 A. The smallest bond energy captured is -3.37 which is at run 8 with RMSD 1.4 A. Selection of the best conformation based on the value of RMSD <2 A and the smallest binding energy. After getting the best conformation results, select write current to save the best conformation files for the ligands.

Visualization of docking results can be analyzed using the autodock program. Docking analysis is done by opening the dlg file in the autodock program so that the results of the docking natural ligands can be seen. Then activate the receptors in the form of an a.pdbqt file. Then choose the best conformation from the run results obtained. Write complex to store files containing the best ligands and their receptors. The validation process that has been carried out and obtaining valid results is then carried out with the support of the test compound. The test compounds that were blocked from FEA were 2-pyridine-propanoic acid and .alpha.-methyl-.beta.-oxo-, ethyl ester; 1-propanol-1-di; carbamic acid, methyl ester; acetic acid, aminooxo-, hydrazide; (2R) - [2-2H2] glycine methyl ester hydrochloride. Meanwhile, the test compounds that were blocked from FE were semicarbazide hydrochloride; carbonic dihydrazide; hydroxy-acetaldehyde; 1-propanol-1-di; acetic acid, and hydroxyl-. The test compound used is a compound that has been optimized in advance using the DFT method.

The results ΔG binding energy of the EAF compounds showed that from the higher to lower were 2-pyridine-propanoic acid, .alpha.-methyl -beta.-oxo-, ethyl ester at -4.14 Kcal/mol and acetic acid, aminooxy-, hydrazide amounting to -3.43 Kcal/mol.

From these results, it states that 2-pyridine-propanoic acid, alpha-methyl-beta-oxo-, ethyl ester, and acetic acid, aminooxy-, hydrazide have the strongest bond among the five EAF compounds. The bond energy possessed by 2-pyridine-propanoic acid,. alpha.-methyl-.beta.-oxo-, ethyl ester and acetic acid, aminooxo-, hydrazide is greater than the bond energy possessed by natural ligands, which is -3.37 Kcal/mol (table 4). This shows that 2-pyridine-propanoic acid,.alpha.-methyl-.beta.-oxo-, ethyl ester and acetic acid, aminooxo-, and hydrazide compounds can shift the position of natural ligands because they have stronger bonds with the α-glucosidase enzyme. Based on the results of the visualization, it can be seen that the amino acids α-glucosidase enzymes that can interact with the EAF compound ligands are listed in Table 5.

The results of the ΔG binding energy of the EF compounds showed that the high G energy binding was semicarbazide hydrochloride of -3.17 kcal/mol and carbonic dihydrazide of -3.12 Kcal/mol. These results stated that semicarbazide hydrochloride and carbonic dihydrazide have a strong bond through the five EF compounds. However, the energy of semicarbazide hydrochloride and carbonic dihydrazide has not been able to defeat the bond energy possessed by natural ligands at 3.37 Kcal/mol. Based on the results of the visualization, it can be seen that the amino acids α-glucosidase enzymes that can interact with the EF ligands were listed in Table 5.

The binding interaction and conformation of each compound (EAF and EF) with the target protein were predicted and ranked based on the ADT 1.5.6-computed lowest energy and total binding energy among its different conformations (table 4). In addition to seeing the results of ΔG, analysis was carried out based on the amino acid bonding of the α-glucosidase enzyme with the compound. Based on the results of the visualization, it can be seen that the amino acid α-glucosidase enzyme which can interact with the ligand of the compounds (EAF & EF) were shown in Table 5. The amino acid that interacts with all the compound of EAF and EF were different from the natural ligand, which indicates the different mechanism of interaction. Based on the results of the amino acid bonding of the α-glucosidase enzyme with the compound, the EAF, and EF showed no similarities in amino acids that bind to their natural ligands.

The results of this study indicate that the EAF contains 2-pyridinepropanoic acid, alpha-methyl-beta-oxo-, ethyl ester, and acetic acid, aminooxy-, hydrazide which has molecularly significant activity as an inhibitor of alpha-glucocidation enzymes compared to natural ligands. From the other research, the 17.5 mg/ kg of water hyacinth and Sintrong leaves were able to reduce blood sugar levels for 7 days reducing blood glucose levels in alloxan-induced experimental animals [15]. The methanolic extract of aerial parts of Eichhornia crassipes (MEEC) possesses antihyperglycemic effects as demonstrated through the oral glucose tolerance test (OGTT) in mice with a 21.5% reduction in blood glucose level [16]. Water hyacinth extract contains phenolic content, flavonoids, and tannins[6]. Other studies have shown that ethanol extract from water hyacinth contains alkaloids, flavonoids, sterols, terpenoids, anthraquinones, proteins, and phenols[17]. Another study proved that the ethanol extract of the water hyacinth herb which was tested using Gas Chromatography-Mass Spectroscopy (GC-MS) contained stigmasterol and 1-monolinoleoyglycerol trimethylsilyl ether compounds 8. Stigmasterol from water hyacinth herb ethanol extract has the potential as a hypoglycemic agent[8]. In the ecological study, the beneficial use of water hyacinth herb is phytoremediation, animal feed, biofertilizer, bioenergy (biogas, bioethanol, and briquettes), carbon source for enzyme production, and biopolymers[18,19,20].

5. Conclusion

This research concludes that EAF compounds have the higher value were 2-pyridine-propanoic acid,.alpha.-methyl-.beta.-oxo-, ethyl ester ΔG was -4.14 kcal/mol, and acetic acid, aminooxo-, hydrazide

at -3.43 Kcal/mol greater than the bond energy possessed by natural ligands, which is -3.37 Kcal/mol. This shows that 2-pyridine-propanoic acid, .alpha.-methyl-.beta.-oxo-, ethyl ester and acetic acid, aminooxo-, and hydrazide compounds can shift the position of natural ligands because they have stronger bonds with the α-glucosidase enzyme. The results of the EF compounds on the α-glucosidase enzyme which had the highest ΔG energy values were semicarbazide hydrochloride of -3.17 kcal/mol and carbonic dihydrazide of -3.12 kcal/mol lower than natural ligands. Based on the results of the amino acid bonding of the α-glucosidase enzyme with the compound, both the EAF and EF showed no similarities in amino acids that bind to their natural ligands.

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