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In Silico Analysis of Bioactive Compounds in Red Betel Leaves to Glutathion Peroxidase Activity

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ABSTRACT

Glutathione peroxidase (GPx) is one of the antioxidants that protect organisms from oxidative stress. Several compounds in certain plants have been shown to increase GPx activity. Red betel leaves are known to contain antioxidant compounds that have the potential to increase GPx activity. The background issue of this research is that there needs to be a study that examines the increase of glutathione peroxidase activity by red betel leaves active compounds with *in silico* method. Therefore, 44 red betel leaf active compounds were tested *in silico*, starting with receptor and ligand preparation, grid box determination, virtual screening, and molecular docking. The virtual screening eliminated seven compounds; in the Lipinski test, two compounds were eliminated; the AdmetSAR test eliminated 21 compounds, so 14 compounds continued to the file preparation stage, molecular docking, and analysis of ligand-receptor interactions. The parameters of affinity energy and percentage of binding site similarity (%BSS) used in the molecular docking analysis showed that several compounds have the potential as antioxidant compounds by increasing the performance of GPx enzyme; the best compound identified is guanidine tartrate with an affinity energy of -4.8 Kcal/mol and BSS percentage of 62.5%, this compound is considered safe to be consumed based on its physicochemical and toxicity test. In the future, higher research, such as *in vitro* and *in vivo* tests, will be needed to determine the potential of red betel leaves as antioxidants.

1. INTRODUCTION

Free radicals are reactive chemical species with one unpaired electron in their outer orbit, so they are highly reactive and destructive. Humans are constantly exposed to free radical chains, which will increase the level of oxidative stress that will cause the oxidation of proteins, lipids, and nucleic acids. Free radicals can cause cell damage, genetic mutation, organ malfunction, or organ failure that can lead to death (Mathew et al., 2011). Antioxidants can donate electrons to highly reactive free radicals and neutralize them, thereby reducing their capacity to damage the body's cells. Antioxidants neutralize free radicals through two main mechanisms: the transfer of hydrogen atoms and the transfer of single electrons (Belinda et al., 2019). Three antioxidant enzymes work as the first line of defense against free radicals, including superoxide dismutase, catalase, and glutathione peroxidase (Ighodaro & Akinloye, 2018). Glutathione peroxidase (self) belongs to the family of pervasive antioxidant enzymes. This enzyme catalyzes the reduction of hydrogen peroxide (H2O2) into the water and other lipid hydroperoxides into alcohols, corresponding to reduced glutathione (GSH). Due to the broad spectrum of substrate specificity and higher affinity to H2O2, GPx has been the subject of numerous studies related to oxidative stress. It is essential in detoxifying stress-induced reactive oxygen species and thus protects cells from severe oxidative damage (Islam et al., 2015).

This research was conducted to provide information on alternatives to diseases caused by free radicals, one of which is by increasing the work of antioxidant enzymes such as glutathione peroxidase with natural compounds. Those natural compounds can be derived from red betel leaves and also aimed to study the molecular interactions between the ligands, which are bioactive compounds from red betel leaves, and their receptor, which is glutathione peroxidase enzyme using the molecular docking technique. Research from Safithri & Kurniawati (2016) showed that beverage formulation from red betel leaves, cinnamon, and red ginger had antioxidant activity and inhibitory activity of enzyme α-glucosidase in the intestines of 873.2 µg/mL and 88.7%, respectively. Its water extract also shows that red betel leaves contain flavonoids, tannins, and alkaloids, so it can be concluded that the formula of this drink has a high content of antioxidants and good health benefits for humans.

Another research done by Tonahi et al. (2014) also supports the statement that the compounds in red betel leaves have antioxidant activity because the DPPH test result from red betel leaves extracts showed an IC_{50} score of 47.45 ppm and classified in a solid antioxidant class. Regarding the effect of red betel leaves on GPx enzyme, research by Ramadhan et al. (2019) showed that giving rats red betel leaf extract at a dose of 400 mg/kg BW increased GPx level to 83.50 ± 2.40 U/mg, higher than the negative group which had GPx level of 23.15 ± 2.61 U/mg. Research conducted by Retnaningsih et al. (2013) showed that the seeds of mucuna beans contain phenolic compounds. Based on the results of in vitro study, it was also shown that the methanol extract of mucuna bean seeds (*Mucuna pruriens*) has antioxidant activity and can increase the activity of the enzyme superoxide dismutase (SOD). In addition, the skin of the mangosteen fruit (*Garcinia mangostana*), which has the antioxidant compound tricyclic isoprenylated polyphenol (xanthone), can increase the activity of the catalase enzyme of rats exposed to used cooking oil (Setiawan & Nugroho, 2018).

The active compounds found in the leaves of noni fruit (*Morinda citrifolia*), such as allantoin, caprylic acid, hexanoic acid, deanoic acid, sorbitol, mannitol, and γ-tocopherol can increase the activity of glutathione peroxidase (Halim et al., 2018). Aswani et al. (2015) added that administering a single extract of turmeric or centella asiatica extract rich in antioxidant compounds could increase the activity of the enzyme glutathione peroxidase in the liver of normal rats. Other information also showed that taurine-treated mice experienced an increase

in the activity of the enzyme glutathione peroxidase in the liver and digestive organs compared to the control mouse group (Anand et al., 2011). Therefore, certain compounds can increase the activity of the enzyme glutathione peroxidase.

2. METHODS

2.1. Receptor and Ligands Preparation

The structure of the human glutathione peroxidase I (GPx1) enzyme was downloaded from RCSB *Protein Data Bank* database with the code 1GP1. These receptors were selected based on previous molecular docking research and based on their crystallographic resolution. After that, it was tested for stability by using the PROCHECK web server. Structural preparation can be started if the structure is categorized as stable. Preparation was carried out by opening the 1GP1 structure in the YASARA (Yet Another Scientific Artificial Reality Application) program, water molecules were removed, and hydrogen atoms were added again, then stored in .pdb format. After preparation using YASARA, the structure of receptors and ligands needs to be reformatted using Autodock Tools 1.5.6 to the .pdbqt format to prepare the structure to be compatible during molecular docking using Autodock Vina software (Ali et al., 2010).

2.2. Gridbox Validation

Docking of glutathione ligands was carried out to find the 3D conformation of glutathione ligand, which is γ-glutamylcysteinylglycine (reduced glutathione/GSH) against receptors by focussing on the coordinates of the center of the structure period and the magnitude of the gridbox of the binding site pocket in units of an angstrom (Vina) or several points (AutoDock). The stage of molecular docking was carried out between the active site of glutathione peroxidase, where the area is the area that interacts with the ligand. The determination of coordinates was based on the presence of essential residues from previous studies, namely Arg177 and His79, which are located on the catalytic site of enzymes interacting with GSH, and tested until obtaining the smallest root mean square deviation (RMSD) value, around $\leq$ 2.0 Å. (Ali et al., 2010; Saputri et al., 2016).

2.3. Virtual Screening

After the test and glutathione ligands have been prepared, they will be stored in the format (. GDP). Glutathione ligand, namely GSH, became the standard, and 44 other test ligands were assessed for their binding affinity energy using the PyRx Virtual Screening Tool application (Dallakyan & Olson, 2015).

2.4. Ligands Physicochemical and Toxicity Analysis

Glutathand test ligands used in this study are predicted for their solubility and permeability based on Lipinski's rules by accessing the scfbio-iitd.res.in/software/drugdesign/lipinski.jsp page. In addition, the toxicity of the test ligands

was predicted by accessing the http://lmmd.ecust.edu.cn/admetsar2/ page (Ochieng et al., 2017).

2.5. Molecular Docking Simulation

Molecular docking simulation was performed using AutoDock Vina with the 'targeted docking' technique. The ligand docking zone was limited by a grid box around the enzyme's active site using AutoDockTools. The vina folder was placed on the C:\Vina drive and filled in the CONF file.TXT. After the docking process is completed, a document with PDBQT format will be generated with a name corresponding to the output on the "conf" document in the Vina folder. Documents with PDBQT format can be converted into PDB on discovery studio visualizer software.

In addition, a txt-formatted "log" document will also be formed containing data on the change of gibbs free energy scores (ΔG). The equation of gibbs free energy used in AutoDock is shown here:

$$\Delta G^{\circ} = -RT \ln K$$

Description: R: gas constant with a value of 8,314 J $K^{-1}mol^{-1}$
T: temperature of the reaction in Kelvin
ln K: natural logarithm of the equilibrium constant K

Both documents were stored to analyze the energy and chemical bonds between the receptor and ligands. This method was validated by re-docking the GSH ligand attached to the 1GP1 enzyme structure according to a predetermined grid box. The GSH ligand structure is a cofactor of GPx and used in previous research taken from the PubChem website and docked to a receptor structure that has been prepared with the same procedure as the test ligand (Ali et al., 2010; Anitha et al., 2013).

2.6. Ligand-Receptor Interaction Analysis

Molecular interaction analysis, including hydrogen bond analysis and hydrophobic interaction in two dimensions (2D), can be performed using Ligplot+ software. The analysis was carried out by comparing the visualization of the ligand tethering region at the receptor with the GSH ligand, which is a cofactor of the GPx enzyme. The docking parameters were obtained from the affinity data of the test ligands smaller than the referenced GSH ligand and the percentage of Binding Site Similarity (BSS) of test ligands against the GSH ligand. BSS formula is shown here:

$$\text{BSS (\%)} = \frac{A}{B} \text{ x } 100$$

Description: A: number of residues from test ligand that interacted the same between GSH and GPx1
B: number of the total residues that interacted between GSH and GPx1. The total residues were 8.

3. RESULTS AND DISCUSSION

Red betel leaves contain active compounds; 44 compounds were tested by *in silico* method in this study which began with the receptor and ligand preparation stage, followed by the determination of the *grid box*, then proceeded to the virtual screening stage, molecular docking simulation, and interaction of ligands and receptor analysis. The ligand used as standard in this test is γ-glutamylcysteinylglicyne (GSH), known as GPx cofactor. The virtual screening stage eliminated seven compounds, while in the Lipinski test stage, there were two eliminated compounds, and the AdmetSAR test eliminated 21 compounds. The 14 remaining compounds proceeded to the file preparation, molecular docking, and analysis of ligand-receptor interactions stage. The stages from the method that have been carried out show that the guanidine tartrate compound has the best potential to increase the activity of the GPx enzyme.

3.1. Results

The 1GP1 receptor obtained from the web server research collaboratory for structural bioinformatics (RCSB) protein data bank is a crystal structure of GPx1 that has a crystallographic resolution of 2.00 Å. The resolution information obtained shows a high resolution, which is ≤ 2.00 Å. After these receptors are downloaded, a receptor stability analysis is performed with a Ramachandran plot from the PROCHECK web server. The results of the Ramachandran plot (Figure 1) show that 276 residues (89.0%) of amino acids are located in the most favored region. Then, 33 residues (10.6%) of amino acids were found in the additional allowed region. No amino acid residues were found generously allowed region, but one amino acid residue was found in the disallowed region. Based on the Ramachandran plot, it is known that most of the residues were located in a stable area. The result of the plot also shows that the total residues in these areas are 310 amino, neither glycine nor proline amino acids. There are also 4 terminal residues and a total of 28 glycine residues shown in a triangular shape and 28 proline residues in the plot.

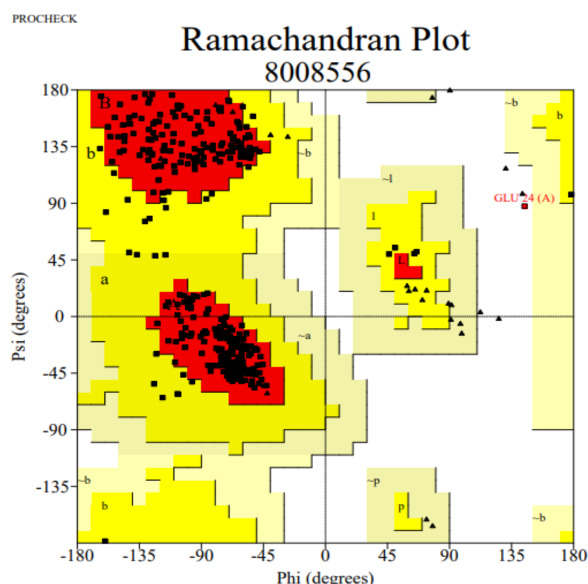

Figure 1 Ramachandran Plot of 1GP1

After the preparation of the receptor, the 1GP1 receptor, known to have no GSH ligand, was docked to its theoretical GSH ligand, which is reduced glutathione (GSH). Gridbox validation was carried out by docking the GSH ligand twenty times with a grid box centered

on the essential residues arg177 and His79 interacting with GSH on the catalytic site in the process of hydroperoxide reduction and enzyme reactivation so that coordinates x= 31.986; y= 43.207 were obtained and z= 51.084, as well as in dimensions x= 18; y= 31; and z= 21. Exhaustiveness was set at number 8 and num_modes at number 20. The validation results (Figure 2) show minimal ligand rotation, and the receptor has a high alignment value. The average score of Root means square deviation (RMSD) was 1.254 Å, and the best RMSD value was 1.0802 Å, while the average score of GSH ligand affinity with receptors obtained was -4.82 kcal/mol.

This Virtual screening was performed on 44 test ligands with 1GP1 receptor to select suitable ligands for docking. The average score of the binding affinity energy obtained from all test ligands was -4.82 kcal/mol. Test ligands that passed the virtual screening had an affinity value range of -4.5 kcal/mol to -8 kcal/mol, with 37 ligands that passed. The test ligand that has the highest affinity value (the most negative value) is a rutin compound with an affinity value of -8 kcal/mol. In comparison, the lowest affinity value is an α-thujene compound with an affinity value of -4.5 kcal/mol.

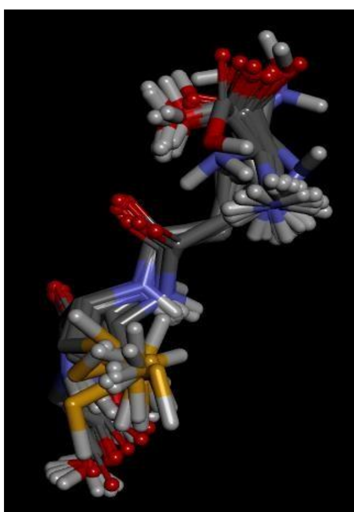

Figure 2 Grid Box Validation Result

A total of 37 test ligands that passed the virtual screening step were tested for their physicochemical with the Lipinski Rule of five. Only two rules violation out of a total of five Lipinski rules to become ligands that still can pass this ligand physicochemical analysis test; from this stage, two ligands did not pass, those ligands were kaempferitin and rutin (Table 1), where these two bioactive compounds violate more than two Lipinski rules, so that there were 35 ligands passed this stage.

The results of the toxicity test with admetSAR are shown in table (x). Several test ligands did not pass because they were categorized as toxic compounds in hepatotoxic parameters, some of them were isocaviunin 7-O-gentiobioside and Flemiphilippinin A. In addition, seven other ligands did not meet the requirements of AdmetSAR. Unlike the Lipinksi Rule of five stages, AdmetSAR is not allowed to violate even one rule. Therefore, in this stage, there were 14 other ligands categorized as non-toxic and have toxicity classes that can be accepted as oral drugs; those compounds were 3,4-(dimetoxyphenyl)-1-propena, benzofuran, humulene, a-thujene, a-terpinene, ß-phellandrene, γ-terpinene, ß-terpineol, α-terpineol, copaene, α-caryophyllene, Germacrene D, 3-(3,4-dimethoxyphenyl)propionic acid, and guanidine tartrate (Table 2).

Table 1 Ligands physicochemical analysis with Lipinski rule of five

Compound	Molecular Weight (Da)	Hydrogen Donor	Hydrogen Acceptor	Log P	Molar Refractivity
Isocaviunin 7-O-gentiobioside	400	0	6	4.4874	109.9449
Kaempferitin	578	8	14	-0.9079	134.5258
Rutin	610	10	16	-1.8788	137.4958
Guanidine Tartrate	150	4	6	-2.1226	27.2851

Table 2 Ligands toxicity analysis with admetsar

	Human Oral Bioavailability		carcino-genicity		hERG Inhibition		Hepato-toxicity		Oral Acute Toxicity	
Ligand	Cate gory	Score	Cate-gory	Score	Cate-gory	Score	Cate-gory	Score	Class	Score
isocaviunin 7-O-gentiobioside	+	0.5429	+	0.9429	+	0.8349	+	0.575	III	0.6141
Flemiphilippini A	-	0.5714	-	0.9143	+	0.692	+	0.775	III	0.6924
3,4-(dimetoxyph-enyl)-1-propena	+	0.7143	-	0.7429	-	0.4384	-	0.7	III	0.9019
Benzofuran	+	0.5143	-	0.9	-	0.7812	-	0.8	III	0.767
Humulene	+	0.7286	-	0.5	-	0.3698	-	0.85	III	0.6889
α-thujene	+	0.5571	-	0.7286	-	0.5821	-	0.8	III	0.6216
α-terpinene	+	0.8714	-	0.6857	-	0.5238	-	0.875	III	0.8506
ß-phellandrene	+	0.7714	-	0.6714	-	0.5171	-	0.85	III	0.7543
γ-terpinene	+	0.7571	-	0.5857	-	0.5486	-	0.9	III	0.8413
ß-terpineol	+	0.5571	-	0.8429	-	0.6281	-	0.775	III	0.8289
α-terpineol	+	0.6857	-	0.8429	-	0.5172	-	0.775	IV	0.6381
Copaene	+	0.7429	-	0.7286	-	0.462	-	0.825	III	0.776
α-caryophyllene	+	0.7286	-	0.5	-	0.3698	-	0.85	III	0.6889
Germacrene D	+	0.7571	-	0.6714	-	0.75	-	0.825	III	0.8141
3-(3-4-dimetox-yphenyl) propio-nic acid	+	0.5429	-	0.8064	-	0.4461	-	0.675	III	0.7668
Guanidine tartrate	+	0.6	-	0.8888	-	0.8445	-	0.75	III	0.4991

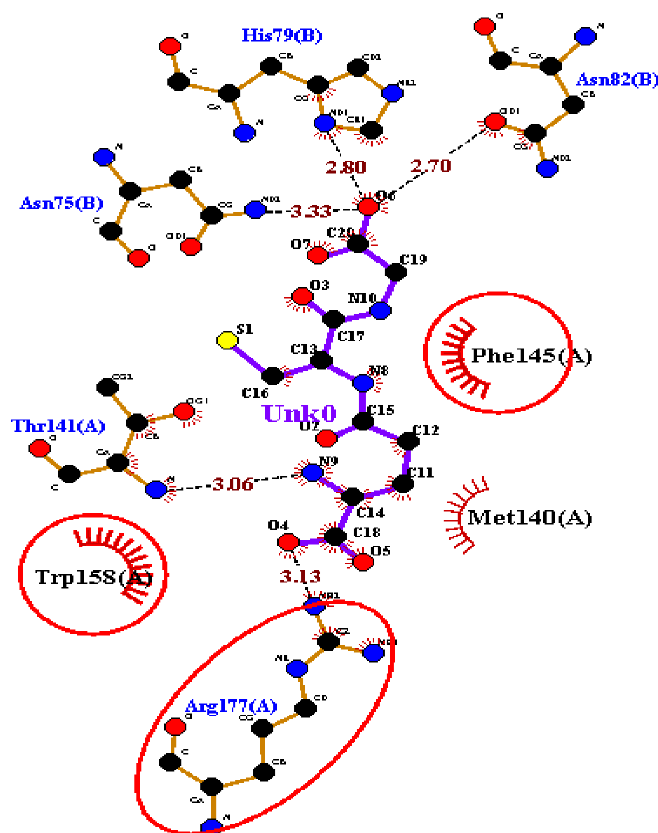

Figure 3 Visualization of GSH docking

GSH ligand has average affinity energy of -4.8 kcal/mol and had interactions with eight amino acid residues, as shown in Figure 3, including His79, Asn82, Asn75, Arg177, and Thr141, which had hydrogen bonds, where the functional groups in this ligand were located at a distance of 2.8 Å at O6 for His79, 2.7 Å at O6 for Asn82 residues, 3.33 Å at O6 for Asn75, 3.13 Å at O4 for Arg177, and 3.06 Å at N9 for Thr141. This ligand also had hydrophobic interactions with Phe145, Met140, and Trp158 residue.

The interaction of red betel compound test ligands with the 1GP1 receptor was visualized with LigPlot+ software, shown in Table 3, to look at the residues that interact with the ligand and the interaction distance of these residues. The ligand with the most negative affinity value and passed the Lipinski and admetSAR test was β-terpineol with an affinity value of -5.7 Kcal/mol. This ligand has no hydrogen bonds but hydrophobic interactions with the catalytic residue Arg177 and Thr141, Phe145, and Trp158 residue. This ligand has a BSS value of 50%.

Table 3 Result of Ligplot+ visualization

Ligand	Binding Affinity Energy (Kkal/mol)	Hydrogen Bonds	H-bounds Distance (Å)	Hydrophobic Interactions	BSS Percentage (%)
GSH (Glutathione)	-4.82	His79	2.8 Å at O6	Phe145, Met140, Trp158	100
		Asn82	2.7 Å at O6		
		Asn75	3.33 Å at O6		
		Arg177	3.13 Å at O4		

		Thr141	3.06 Å at N9		
3,4-(dimetoxyph-enyl)-1-propena	-5	Arg177	3.06 Å at O1	Met140, Trp158, Phe145	62.5
		Thr141	3.08 Å at O2		
β-terpineol	-5.7	-	-	Thr141, Phe145, Trp158, Arg177	50
α-terpineol	-4.8	Arg177	3 Å at O1	Phe145, Gln80, Trp158	37.5
Guanidine tartrate	-4.8	Arg177	2.9 Å at O4	Phe145, Met140, Trp158	62.5
		Asp142	2.84 Å at O3		
		Thr141	3.09 Å at O3		

3.2. Discussion

Glutathione is a cofactor and source of electrons used to reduce H_2O_2 to water, and organoperoxides turn into alcohol (Hall et al., 2014) in the GPx enzyme. GPx-1 activity can be increased by mechanisms such as phosphorylation or protein interactions. The concept shows that GPx-1 can be a target for kinase phosphorylation, and GPx-1 was shown to be a substrate for the kinases c-Abl and Arg, which are essential in the cellular response to oxidants (Cao et al., 2003). Increased GPx activity can also be caused by increased intracellular GSH levels (Ma et al., 2011). The research of Ramadhan et al. (2019) showed that the administration of red betel leaf significantly increased GPx production. The red betel leaf extract causes this contains bioactive compounds such as flavonoids, alkaloids, saponins, and tannins, so it is known that these substances can work as antioxidants that play a role in reducing oxidative stress in hyperglycemic rats after alloxan induction (Parildar et al., 2011).

The action of glutathione peroxidase is also assisted by superoxide dismutase (SOD) and catalase enzyme. SOD enzyme will convert superoxide radicals (O_2^{-}) generated from respiration, and the environment then converts them into hydrogen peroxide (H_2O_2), which is still reactive. SOD is found in the cytosol and mitochondria. Peroxide is catalyzed by the enzymes catalase and glutathione peroxidase (GPx). Catalase can use one H_2O_2 molecule as an electron donor substrate and another H_2O_2 molecule as an electron acceptor substrate so that for two H_2O_2 molecules, two molecules of H_2O and O_2 will be made. In erythrocytes and other tissues, GPx catalyzes the destruction of H_2O_2 and lipid hydroperoxides by using a reduced form of glutathione (GSH) to protect membrane lipids and hemoglobin from oxidation by H_2O_2, as well as preventing hemolysis caused by peroxide attack. GSH will be oxidized to GS-SG. For GSH to be available again to help the GPx enzyme work, this GS-SG must be reduced to GSH again. The glutathione reductase enzyme carries this role (Werdhasari, 2014).

The 1GP1 receptor was selected based on a previous study by Ali *et al.* (2010). Diffraction resolution category is divided into four categories; including small (<3.00 Å), medium (2.70-2.00 Å), high (2.00-1.50 Å), and very high (< 1.5 Å); it is known that the smaller the crystallographic value, the more specific to image generated visualization (Made & Pathni,

2018). Based on this statement, the resolution of the 1GP1 receptor is high because it has a value of 2.00 Å. Related to receptor structure accuracy, Rai & Rieder (2012) stated that the result of Ramachandran diagram of a structure with >90% residue in the favored region indicates an accurate structure. From Figure 1, the percentage of total residues of the receptor found in the most favored region was 89%. However, it is still proper for docking analysis because it has a high crystallographic value.

GSH ligands and test ligands were prepared to ensure that the ligands were in their active form and had flexibility. This was performed so the ligands could bind to the receptor's binding site (Miteva et al., 2011). According to Sastry et al. (2013), another goal for ligand preparation is to focus the test results so that the ligands do not interfere with other variables and can provide good virtual screening results.

The coordinate selection of the grid box used is based on the research of Ali et al. (2010), which states that the amino acids His79 and Arg177 play a role as catalytic acids/bases in molecular docking, and these residues interact directly with GSH which is the standard ligand of the enzyme GPx1. Therefore, the grid box was centered on those essential residues. The docking and molecular dynamic simulation studies results also showed that Arg177, His79, Phe145, and especially Trp158 played a crucial role in binding and showed the proper orientation of the GSH thiol group to the cellenocysteine reaction center, which is the active site of the enzyme GPx1. GPx1 enzyme is known to be present in the gastrointestinal and is the most abundant type of GPx in the body (Mansourian et al., 2018).

Selection of these coordinates was also because the 1GP1 receptor does not have natural ligands, so it must be done based on previous research on the enzyme 1GP1, then by trying the coordinates several times until the RMSD score ≤ 2.00 Å was found because docking results are considered good if it has an RMSD score less than 2 Å (Purnomo, 2013). Based on Figure 2 visualization and the RMSD score obtained, which was 1.0802 Å. The docking result is known to be excellent and suitable for docking simulation.

Virtual screening is a computational method that aims to increase the effectiveness of the search for medicinal compounds. In addition, this method can also reduce the number of compounds that must be simulated during molecular docking based on several parameters, including affinity energy, RMSD lower bound, and RMSD upper bound (Syahdi, 2011). The 44 test ligands selection was based on the ligands' affinity energy, which was then compared with the average affinity value of the GSH ligands, which was -4.82 Kcal/mol.

Physicochemical properties can be an essential benchmark in determining drug safety. The Lipinski test is carried out to determine the physicochemical properties of a ligand in drug discovery when it crosses cell membranes in the body. The conditions that must be met by a ligand based on the Lipinski rules are the molecular weight of < 500 Da, LogP value < 5, hydrogen donor < 5, hydrogen acceptor < 10, and the molar refractivity between 40-130. LogP value is related to the polarity of ligands in fat solvents, oils, and non-polar solvents. Ligands with a log value of P > 5 will interact more quickly through the lipid layer of the bilayer on the cell membrane and are widely distributed in the body. In addition, ligands with a molecular weight of < 500 Da will more easily pass through the cell membrane than ligands whose molecular weight is> 500 Da (Dwi & Abdul, 2011).

The molar refractivity allowed in Lipinski's rules ranges from 40 to 130. The molar refractivity indicates the steric value of a compound, which is commonly used as a simple parameter of the volume occupied (Almi et al., 2014). The number of hydrogen donors and acceptors indicates that the higher the hydrogen bond capacity, the higher the energy required for the absorption process. Lipinski's rules generally describe the solubility of certain

compounds to penetrate cell membranes by passive diffusion (Syahputra et al., 2014). Besides that, compounds that can be declared to pass the Lipinski rule are only compounds that violate a maximum of 2 rules of Lipinski as an excellent oral drug. Based on this, kaempferitin and rutin did not pass because these compounds violated four rules.

The in silico toxicity analysis used in this study was admetSAR. The prediction categories of toxicity analysis are carcinogenicity analysis, HumanEther-À-Go-Go-Related Gene (hERG) inhibition analysis, and acute oral toxicity dose (Pannindriya et al., 2021). Oral acute toxicity is the parameter to see the side effects of oral consumption of a compound with a specific dose within 24 hours (Astri et al., 2012). According to El-Din et al. (2016), oral acute toxicity values are divided into six classes, namely class I, fatal if swallowed, with a range of LD50 5 mg/kg mass of consumers; class II, fatal if swallowed (5 < LD50 50 mg/ kg consumer mass), class III toxic if swallowed (5 < LD50 300 mg/kg consumer mass), class IV harmful if swallowed (300 < LD50 2000 mg/kg consumer mass), class V may be harmful if swallowed (2000 < LD50 5000 mg/kg consumer mass), and class VI, non-toxic with an LD50 value > 5000 mg/kg consumer mass. Compounds with toxicity classes I and II are classified as toxic and cannot be used as oral drugs (Guan et al., 2019).

Hepatotoxicity is a category of toxicity to the liver caused by drug-induced liver injury (DILI) in the case of drugs (Bhat & Chatterjee, 2021). This hERG category is one of the vital things in new drug discovery. If this gene is inhibited, arrhythmia in the liver will occur, which can be fatal. Therefore, analysis of hERG inhibition is mandatory in the new drug development process (Di & Kerns, 2016). The parameter which was also used in this research was carcinogenicity. Carcinogenicity is the ability of a material to cause neoplasia (new tissue growth) (Pannindriya et al., 2021). Carcinogenic compounds should be avoided because they can harm the body long-term (Banerjee et al., 2018). Twenty-one compounds were eliminated from this test, leaving 14 that passed to the next stage.

Molecular docking is a method used to predict the orientation of one molecule to another during electrostatic interactions with each other to form a stable bond. It is based on the technique of placing the ligand into the receptor's active site, followed by an evaluation of the molecule based on the conformational structure and electrostatic properties (Syahputra et al., 2014). Docking simulation can be used to define better the action mechanism of a chemical compound or macromolecule, such as a protein or peptide, on a molecular scale so that it is possible to design structural-based drugs (Ali et al., 2008). The targeted docking method is used in this research; a docking process carried out with the exact location of the active site known from a receptor to be used (Syahputra et al., 2014). It is necessary to explore the docking site because it can predict the intermolecular relations of a protein and its ligand by showing steric, hydrophobic, and electrostatic interactions and calculating the affinity energy value (Sethi et al., 2019).

The parameters observed for determining the energy affinity of the ligand with the receptor were free bond energy (ΔG), amino acid residue interactions, including the number of hydrogen bonds, and hydrophobic interactions (Pratama, 2016). The more hydrophobic bonds of a drug compound, the more active the compound will be (Patil et al., 2010). This is consistent with the study's results showing the interaction of ß-terpineol when compared with α-terpineol. ß-terpineol had hydrophobic interactions with residues Thr141, Phe 145, and with two other catalytic residues, namely Trp158 and Arg177, so that it had binding affinity energy of -5.7 Kcal/mol, more harmful than α-terpineol which interacted hydrophobicly with three residues, namely Phe145, as well as with the catalytic residue Gln80, Trp158 so that it had more positive affinity energy, which was -4.8 Kcal/mol.

After the molecular docking stage was performed, a 2D visual analysis was carried out using Ligplot+. This program can generate information on hydrogen bonds and residual contacts between the ligand and the receptor. Chemical bonds other than hydrogen bonds can also occur due to flexible ligands interacting with receptors. Interactions can be non-covalent or non-binding interactions which can increase the binding affinity. The most common bonds are electrostatic interactions and van der Waals bonds (Syahputra et al., 2014). Before performing the visualization, the characteristics of the test ligand results must be considered, such as the affinity value and the upper bound RMSD, which is the value of the suitability of each atom from a conformation with other conformations of the ligand (Ferencz & Muntean, 2015). Binding affinity measures the amount of energy required for a molecule to bind; binding affinity describes the intermolecular activity and the strength of the bonds between proteins and compounds. The smaller the binding affinity, the stronger the bond between proteins and compounds (Hanif et al., 2020).

Critical residues known to contribute to the activation of the GPx1 enzyme are Arg177, the site of free radical (ROOH) reduction in the enzyme, which will be converted into alcohol compounds (ROH). His79 residue work as a site for water formation and glutathione oxidation (Ali et al., 2010). Sec52 (SE745A) is an active redox component of the catalytic triad, acting as a nucleophile that attacks hydrogen peroxide and will later be regenerated into glutathione. Trp165 (Trp158A) acts as a general acid or base (proton traffic). The hydrogen bonding distance of selenium from peroxidatic Sec helps to activate selenol, which facilitates nucleophilic attack on hydroperoxides, and Gln87 (Gln80A) forms a catalytic triad acting similarly to the Trp165 residue but more towards an electrostatic stabilizer of the enzyme (Orian et al., 2015; Pannala et al., 2014; Tosatto et al., 2008).

Based on these catalytic sites, a test ligand is said to be able to activate and is suitable as a drug compound when interacting with these catalytic residues. GSH ligands had hydrogen bonds with His79, Asn82, Asn75, Arg177, Thr141 and had hydrophobic interactions with Phe145, Met140, Trp158 (shown in Figure 3). All test ligands interacted with most of these residues, particularly Arg177 and His79. This corresponds with the research by Mansourian et al. (2018) that showed the docking between GPx1 (PDB:1GP1) and metformin, an anti-diabetic drug, had hydrophobic interaction with Arg177 that almost surrounded the methyl group of metformin. All the test ligands that interacted with these residues and had the same interaction residues found in the GSH ligand with a larger number also have a high %BSS value, indicating a large percentage of docking site similarity, which is ≥ 50% (Ehrt, 2019). Binding site similarity percentage (% BSS) is known as the percentage of similarity of bonds formed between receptors and ligands (Sinurat et al., 2021).

According to Ali et al. (2010), the catalytical active form of GPx1 is selenolate anion (E-Se$^-$). In the first redox step, E-Se$^-$ is oxidized to selenic acid (E-SeOH), and at the same time, the hydroperoxide is reduced to an alcohol, which occurs in the Arg177 residue. In the second step, E-SeOH reacts with GSH to produce acelanyl sulfide adduct (E-SeSG); at this stage, the first GSH molecule enters, then is reduced to GS$^-$ and transformed into the water at the His79 residue. In the third step, the second molecule of GSH attacks E-SeSG to regenerate the active form of the enzyme, and an oxidized form of GSH (GSSG) is formed as a byproduct; this event occurs at His79 residue.

The potential of a compound as a drug must be calculated based on the affinity energy value; this affinity energy is influenced by compound interactions and the similarity of its docking sites (Patil et al., 2010). Based on this, the ligand that has the most significant potential as an activator of glutathione peroxidase (GPx1) enzyme is guanidine tartrate which had an

affinity value of -4.8 Kcal/mol, similar to the affinity energy of the GSH ligand; it had a total three hydrogen bonds and hydrophobic interactions with 1GP1. Other ligands that also had potential as activators due to their excellent affinity, hydrogen bonds, and hydrophobic interactions with critical residues include 3,4-(dimethoxy phenyl)-1-propene (-5 Kcal/mol) and α-terpineol, which has affinity energy of -4.8 Kcal/mol.

4. CONCLUSION

Tests conducted on 44 compounds found in red betel leaves to see their potential in the activation of the glutathione peroxidase (GPx1) enzyme showed that guanidine tartrate is the compound that has the highest potential to increase GPx1 enzyme activity in the body based on its binding affinity energy, hydrogen bonds, and hydrophobic interactions with the 1GP1 receptor. Physicochemical and toxicity test results also showed that the ligand had effective absorption in the body and was considered safe for use as oral drugs. Research at a higher level will be needed, such as *in vitro* and *in vivo* tests to determine the potential of the compounds contained in red betel leaves as antioxidants, and to increase the performance of glutathione peroxidase (GPx1) enzyme, also to get more information of the side effects that these compounds can cause.

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REFERENCES

Ali, S. T., Jahangir, S., Karamat, S., Fabian, W. M., Nawara, K., & Kóňa, J. (2010). Theoretical Study on the Redox Cycle of Bovine Glutathione Peroxidase GPx1: P K a Calculations, Docking, and Molecular Dynamics Simulations. *Journal of Chemical Theory and Computation*, *6*(5), 1670–1681.

Almi, Z., Belaidi, S., Lanez, T., & Tchouar, N. (2014). Structure Activity Relationships, QSAR Modeling and Drug-like calculations of TP inhibition of 1, 3, 4-oxadiazoline-2-thione Derivatives. *International Letters of Chemistry, Physics and Astronomy*, *18*, 113-124.

Anand, P., Rajakumar, D., Jeraud, M., Felix, A., & Balasubramanian, T. (2011). Effects of taurine on glutathione peroxidase, glutathione reductase and reduced glutathione levels in rats. *Pakistan Journal of Biological Sciences: PJBS*, *14*(3), 219–225.

Anitha, K., Gopi, G., & Girish, S. K. P. (2013). Molecular docking study on dipeptidyl peptidase-4 inhibitors. *International Journal of Research and Development in Pharmacy and Life Sciences*, *2*, 602–610.

Astri, Y., Sitorus, T., Sigit, J. I., & Sujatno, M. (2012). Toksisitas Akut per Oral Ekstrak Etanol Daun Dewa (Gynura pseudochina (Lour.) DC) terhadap Kondisi Lambung Tikus Jantan dan Betina Galur Wistar. *Majalah Kedokteran Bandung*, *44*(1), 38-43.

Aswani, T., Manalu, W., Suprayogi, A., & Rahminiwati, M. (2015). POTENSI EKSTRAK PEGAGAN (Centella Asiatica) DAN KUNYIT (Curcuma longa) UNTUK MENINGKATKAN AKTIVITAS ENZIM GLUTATION PEROKSIDASE (GSH Px) PADA JARINGAN HATI TIKUS. *Berita Biologi*, *14*(3), 259–265.

Banerjee, P., & Eckert, O. (2018). A.; Schrey, AK; Preissner, R. ProTox-II: A webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res*, *46*, W257-W263.

Belinda, N. S., Swaleh, S., Mwonjoria, K. J., & Wilson, M. N. (2019). Antioxidant activity, total phenolic and flavonoid content of selected Kenyan medicinal plants, sea algae and medicinal wild mushrooms. *African Journal of Pure and Applied Chemistry*, *13*(3), 43–48.

Bhat, V., & Chatterjee, J. (2021). The use of in silico tools for the toxicity prediction of potential inhibitors of SARS-CoV-2. *Alternatives to Laboratory Animals*, *49*(1-2), 22-32.

Cao, C., Leng, Y., Li, C., & Kufe, D. (2003). Functional interaction between the c-Abl and Arg protein-tyrosine kinases in the oxidative stress response. *Journal of Biological Chemistry*, *278*(15), 12961–12967.

Dallakyan, S., & Olson, A. (2015). *Chemical Biology; Hempel, JE, Williams, CH, Hong, CC, Eds*.

Di, L., & Kerns, E. (2015). *Drug-like properties: concepts, structure design and methods from ADME to toxicity optimization*. Academic press.

Dwi, P. H., & Abdul, K. (2011). Pengantar Kimia Komputasi. *Lubuk agung*.

El-Din, H. M. A., Loutfy, S. A., Fathy, N., Elberry, M. H., Mayla, A. M., Kassem, S., & Naqvi, A. (2016). Molecular docking based screening of compounds against VP40 from Ebola virus. *Bioinformation*, *12*(3), 192.

Ferencz, L., & Muntean, D. L. (2015). Identification of new superwarfarin-type rodenticides by structural similarity. The docking of ligands on the vitamin K epoxide reductase enzyme's active site. *Acta Universitatis Sapientiae, Agriculture and Environment*, *7*(1), 108–122.

Guan, L., Yang, H., Cai, Y., Sun, L., Di, P., Li, W., ... & Tang, Y. (2019). ADMET-score–a comprehensive scoring function for evaluation of chemical drug-likeness. *Medchemcomm*, *10*(1), 148-157.

Halim, A. M., Prajitno, A., & Chang, C. C. (2018). Enhancement glutathione peroxidase activity and α2-macroglobulin gene expression of Macrobrachium rosenbergii Fed With Aqueous Morinda citrifolia Leaves Extract-Supplemented Diet. *Indonesian Journal of Tropical Aquatic*, *1*(1), 9–16.

Hall, M. D., Marshall, T. S., Kwit, A. D., Jenkins, L. M. M., Dulcey, A. E., Madigan, J. P., Pluchino, K. M., Goldsborough, A. S., Brimacombe, K. R., & Griffiths, G. L. (2014). Inhibition of glutathione peroxidase mediates the collateral sensitivity of multidrug-resistant cells to tiopronin. *Journal of Biological Chemistry*, *289*(31), 21473–21489.

Hanif, A. U., Lukis, P. A., & Fadlan, A. (2020). Pengaruh minimisasi energi MMFF94 dengan MarvinSketch dan open Babel PyRx pada penambatan molekular turunan oksindola tersubstitusi. *Alchemy*, *8*(2), 33–40.

Ighodaro, O., & Akinloye, O. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, *54*(4), 287–293.

Islam, T., Manna, M., & Reddy, M. K. (2015). Glutathione peroxidase of Pennisetum glaucum (PgGPx) is a functional Cd2+ dependent peroxiredoxin that enhances tolerance against salinity and drought stress. *PLoS One*, *10*(11), e0143344.

Ma, L., Ohyagi, Y., Nakamura, N., Iinuma, K. M., Miyoshi, K., Himeno, E., Soejima, N., Yanagihara, Y. T., Sakae, N., & Yamasaki, R. (2011). Activation of glutathione peroxidase and inhibition of p53-related apoptosis by apomorphine. *Journal of Alzheimer's Disease*, *27*(1), 225–237.

Mansourian, M., Sadeghi, H., & Doustimotlagh, A. H. (2018). Activation of the glutathione peroxidase by metformin in the bile-duct ligation-induced liver injury: in vivo combined with molecular docking studies. *Current Pharmaceutical Design*, *24*(27), 3256-3263.

Mathew, B. B., Tiwari, A., & Jatawa, S. K. (2011). Free radicals and antioxidants: A review. *Journal of Pharmacy Research*, *4*(12), 4340–4343.

Miteva, M. A., Robert, C. H., Maréchal, J. D., & Perahia, D. (2011). Receptor flexibility in ligand docking and virtual screening. *In-silico lead discovery. Bentham Science Publishers*, 99-117.

Ochieng, P. J., Sumaryada, T., & Okun, D. (2017). Molecular docking and pharmacokinetic prediction of herbal derivatives as maltase-glucoamylase inhibitor. *Asian J Pharm Clin Res*, *10*(9), 392–398.

Orian, L., Mauri, P., Roveri, A., Toppo, S., Benazzi, L., Bosello-Travain, V., De Palma, A., Maiorino, M., Miotto, G., & Zaccarin, M. (2015). Selenocysteine oxidation in glutathione peroxidase catalysis: An MS-supported quantum mechanics study. *Free Radical Biology and Medicine*, *87*, 1–14.

Pannala, V., Bazil, J., Camara, A., & Dash, R. (2014). A mechanistic mathematical model for the catalytic action of glutathione peroxidase. *Free Radical Research*, *48*(4), 487–502.

Pannindriya, P., Safithri, M., & Tarman, K. (2021). Analisis In Silico Senyawa Aktif Sprirulina platensis sebagai Inhibitor Tirosinase. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *24*(1), 70-77.

Parildar, H., Serter, R., & Yesilada, E. (2011). Diabetes mellitus and phytotherapy in Turkey. *JPMA-Journal of the Pakistan Medical Association*, *61*(11), 1116.

Pathni, P. M. S. D. (2018). Terapi Diabetes dengan GLP-1 Receptor Agonist. *Cermin Dunia Kedokteran*, *45*(4), 291-296.

Patil, R., Das, S., Stanley, A., Yadav, L., Sudhakar, A., & Varma, A. K. (2010). Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing. *PloS One*, *5*(8), e12029.

Pratama, M. R. F. (2016). Studi Docking Molekular Senyawa Turunan Kuinolin terhadap Reseptor Estrogen-α (Molecular Docking Study of Quinoline Derivatives Towards Estrogen-α Receptor). *Jurnal Surya Medika*, *2*(1).

Ramadhan, S., Iswari, R., & Marianti, A. (2019). Effect of red betel (Piper crocatum Ruiz & Pav.) leaves extract on blood glucose levels and glutathione peroxidase levels in hyperglycemic male rats. *Biotropika*, *7*(1), 1–10.

Rai, D. K., & Rieder, E. (2012). Homology modeling and analysis of structure predictions of the bovine rhinitis B virus RNA dependent RNA polymerase (RdRp). *International journal of molecular sciences*, *13*(7), 8998-9013.

Retnaningsih, C., Darmono, D., Widiarnoko, B., & Muis, S. F. (2013). Peningkatan aktivitas antioksidan superoksida dismutase pada tikus hiperglikemi dengan asupan tempe koro benguk (Mucuna pruriens L.). *Agritech*, *33*(2).

Safithri, M., & Kurniawati, A. (2016). Formula of Piper crocatum, Cinnamomum burmanii, and Zingiber officinale extracts as a functional beverage for diabetics. *International Food Research Journal*, *23*(3), 1123.

Saputri, K. E., Fakhmi, N., Kusumaningtyas, E., Priyatama, D., & Santoso, B. (2016). Docking molekular potensi anti diabetes melitus tipe 2 turunan zerumbon sebagai inhibitor aldosa reduktase dengan autodock-vina. *Chimica et Natura Acta*, *4*(1), 16–20.

Setiawan, J., & Nugroho, T. (2018). Pengaruh Ekstrak Kulit Manggis terhadap Enzim Katalase Hepar Tikus Terpapar Minyak Jelantah. *DIPONEGORO MEDICAL JOURNAL (JURNAL KEDOKTERAN DIPONEGORO)*, *7*(1), 263–272.

Sinurat, M. R., Rahmayanti, Y., & Rizarullah, R. (2021). Uji Aktivitas Antidiabetes Senyawa Baru Daun Yakon (Smallanthus sonchifolius) sebagai Inhibitor Enzim DPP-4: Studi in Silico. *Jurnal IPA & Pembelajaran IPA*, *5*(2), 138–150.

Syahdi, R. R., Mun'im, A., Suhartanto, H., & Yanuar, A. (2012). Virtual screening of Indonesian herbal database as HIV-1 reverse transcriptase inhibitor. *Bioinformation*, *8*(24), 1206.

Syahputra, G. (2014). Simulasi docking kurkumin enol, bisdemetoksikurkumin dan analognya sebagai inhibitor enzim12-lipoksigenase. *Jurnal Biofisika*, *10*(1).

Tonahi, J. M. M., Nuryanti, S., & Suherman, S. (2014). Antioksidan dari daun sirih merah (Piper crocatum). *Jurnal Akademika Kimia*, *3*(3), 158–164.

Tosatto, S. C., Bosello, V., Fogolari, F., Mauri, P., Roveri, A., Toppo, S., Flohé, L., Ursini, F., & Maiorino, M. (2008). The catalytic site of glutathione peroxidases. *Antioxidants & Redox Signaling*, *10*(9), 1515–1526.

Werdhaswari, A. (2014). Peran antioksidan bagi kesehatan. *Jurnal Biotek Medisiana Indonesia*. 3(2), 59-68.