Indonesian Journal of Applied Research IJAR

IN SILICO, TO DETERMINE THE ACTIVE COMPOUNDS OF BLACK TEA AND TURMERIC IN INCREASING THE ACTIVITY OF THE ENZYME SOD

Volume 3 Issue 1
(April 2022)
e-ISSN 2722-6395
doi: 10.30997/ijar.v3i1.187

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ARTICLE INFO

Article history:
Received: 14-03-2022
Revised version received: 26-03-2022
Accepted: 27-04-2022
Available online:12-05-2022

Keywords:
Antioxidants, Black tea, Molecular docking, Superoxide dismutase, Turmeric

How to Cite:
Hasan, A. E. Z., Safithri, M., Huda, A. S., & Kurniasih, R. (2022). IN SILICO, TO DETERMINE THE ACTIVE COMPOUNDS OF BLACK TEA AND TURMERIC IN INCREASING THE ACTIVITY OF THE ENZYME SOD. *Indonesian Journal of Applied Research (IJAR)*, *3*(1), 32-45. https://doi.org/10.30997/ijar.v3i1.187

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ABSTRACT

Damage to cells caused by stress can be reduced by the presence of antioxidants, one of which is Superoxide dismutase (SOD). The role of the active ingredients of black tea and turmeric will be studied using the *in-silico* method to identify the active compounds as components in SOD activation. The bioavailability and toxicity of the active compounds of black tea and turmeric were studied and followed by molecular docking and virtual games. The parameters studied are Gibbs's free energy (ΔG) and binding site similarity (BSS). The results were analyzed using Gibbs's free energy (ΔG) and binding site similarity (BSS) parameters. It was found that those that could increase the activity of Cu/Zn SOD enzymes were Epicatechin gallate (black tea) and curcumin (turmeric), with values of -9.5 and -7.4 Kcal/mol and the same BSS value of 81.8%. The control ligand used was beta amyrin. According to Lipinski's rules, Epicatechin gallate and curcumin compounds can be absorbed well and are safe for consumption. This study concludes that Epicatechin gallate, an active compound of black tea, and curcumin, an active compound of turmeric rhizome, have the best potential to increase the activity of Cu/Zn SOD enzymes based on the results of virtual screening and molecular docking. Epicatechin gallate and curcumin are predicted to be well absorbed by the body because they qualify Lipinski's rules and are not toxic and safe for consumption.

1. INTRODUCTION

Free radicals are molecules with one or more unpaired electrons in their outer orbits, making them relatively unstable. These reactive molecules look for their electron pairs to get stability, so they are also called Reactive Oxygen Species (ROS) (Ardhie, 2011). The harmful effect of free radicals causing potential biological damage, especially by ROS, is oxidative stress. This occurs in biological systems when ROS overproduction and a lack of antioxidants (Husain & Kumar, 2012).

Antioxidants are compounds that react by removing, taking, suppressing the formation, and suppressing the activity of radical species (oxidants) (Nirwana & Mutakin, 2019). Antioxidants can be divided into natural antioxidants and artificial antioxidants (Martono et al., 2016). Natural antioxidants are generally found in the body and can be enzymatic and non-enzymatic. Enzymatic antioxidants include enzymes such as superoxide dismutase or SOD, catalase, and glutathione peroxidase. Superoxide dismutase (SOD) is the most powerful antioxidant enzyme in cells. SOD catalyzes the cleavage of two molecules of superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2) and an oxygen molecule (O_2) so that the potentially dangerous superoxide anion becomes safer (Ighodaro & Akinloye, 2018).

Black tea is the most widely produced type of tea, about 78%, followed by green tea 20% and then white tea and oolong tea 2% (Rohdiana, 2015). The main polyphenol components in black tea are theaflavins and the arubigins, which are compounds produced by the oxidation of catechins (Rohdiana et al., 2013). Other compounds present in black tea are flavonoids in quercetin, kaempferol, and myricetin. Quercetin and kaempferol are very strong antioxidant compounds and have other benefits such as anticancer, antibacterial, and anti-inflammatory (Widiati, 2011).

Turmeric (*Curcuma longa*) is one type of spice widely used for various kinds of cuisine. The most important major antioxidant component in turmeric is curcumin. Curcumin is a yellow pigment in turmeric which has a broad spectrum of biological activities, including antibacterial, antioxidant, and antihepatotoxic, which can increase the absorption of vitamins A, D, E, and K. Besides curcumin, turmeric also contains flavonoid, phenolic, saponin, and tannin compounds, which affect its antioxidant activity (Ikpeama et al., 2014). Turmeric rhizome can be used as an anti-inflammatory, antiprotozoal, antibacterial, antivenom, anti-HIV, antitumor, and for various diseases related to hepatoprotectors (Simanjuntak, 2012).

The antioxidant activity of black tea and turmeric have been proven by in vitro and in vivo method. The in vitro methods that have been used for analyzing black tea are 2,2-diphenyl-1-picryhydrazyl(DPPH), Hydroxyl Ion Scavenging, Nitric Oxide Radical Scavenging, Superoxide Radical Scavenging, Ferric Reducing Antioxidant Power (FRAP) (Hajiaghaalipour et al., 2016). Parameters used in the in vivo method are indicated by the increased activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) and the decreased levels of malonyl aldehyde (MDA) (Sun et al., 2012). In addition, the test of an antioxidant activity inside turmeric rhizome extract by in vitro method is only 2,2-diphenyl-1-picryhydrazyl (DPPH) and FRAP methods. Therefore, the in vivo parameters are indicated by the increased catalase and GPx activity and decreased MDA levels (Kodjio et al., 2016; Tanvir et al., 2017).

Antioxidant activity testing of black tea and turmeric was tested using crude extracts from each ingredient. The active compounds that play an important role in the antioxidant activity are not yet known. Therefore, it is necessary to identify the active compounds of black tea and turmeric, which play an important role in antioxidant activity, especially against the superoxide dismutase enzyme activity. According to Syahputra (2015), virtual screening selects certain compounds from the many active compounds in a material. Then, molecular docking is carried out to screen the literature regarding active compounds that have the strongest binding to certain receptor proteins (Fatmawaty et al., 2015). Based on Syahputra

(2015), the in silico was carried out to determine the activity of the drug candidate's active compounds to avoid unexpected compound activity. Through this method, the active compounds of black tea and turmeric can be determined for their activity, toxicity, and ability to increase the activity of the enzyme superoxide dismutase. This study aims to determine the bioactive compounds of black tea and turmeric that have the potential to increase the antioxidant activity of the superoxide dismutase enzyme.

2. METHODS

This research uses MarvinView, Discovery Studio Visualizer 2017 Client, PyRx, PyMOL, AutoDock Vina, AutoDock Tools, and Ligplot+ 1.5.4. The materials used are ligand file and SOD enzyme with fasta format, Protein Data Bank (PDB), Protein Data Bank, Partial Charge (Q), & Atom Type (T) (PDBQT), the chemical structure of SOD enzyme with PDB ID 1CB4, the chemical structure of active compounds of black tea and turmeric as test ligands, beta amyrin as comparison ligand. The preparation of the test ligand structure was carried out using Marvinview software and saved in PDB format. The preparation results are then optimized by removing water molecules and adding hydrogen ions and stored in PDBQT format. These ligands were then analyzed for bioavailability based on Lipinksi's rules via the website http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp. Ligands that have met the requirements of Lipinski's rule are then predicted for their toxicity. Prediction of ligand toxicity was carried out using the online software Admetsar 1.0 by uploading the SMILES structure of the ligand on the http://lmmd.ecust.edu.cn/admetsar1 page.

Validation was carried out by directional molecular belay using AutoDock Vina. The ligand anchorage zone is delimited by a grid box using AutoDockTools. The grid box is made based on the amino acid residues Val7, Lys9, Asp11, Asn51, Gly54, Cys144, Gly145, and Val146 so gridbox. Validation Gridbox on the SOD receptor was carried out by docking beta amyrin to determine grid box. The docking is done 20 times. Virtual screening was carried out on the comparison ligand, namely beta amyrin, and 40 test ligands that had passed the bioavailability and toxicity tests. The prepared test and comparison ligands are saved in (.PDB) format. The test and comparison ligands were assessed for their binding affinity using the PyRx-Virtual Screening Tool. The prediction results are then selected based on the magnitude of the affinity value and the value of Gibbs free energy (ΔG).

Molecular docking is done using the command prompt “cmd." The "cmd" program is opened, then the programming commands are executed until they are in the Vina folder. The programming command to run the molecular docking program is "*C:vina --config conf.txt --log log.txt*." The molecular docking results can be seen in the output file with PDBQT format and the log file, which can be opened using notepad software. Analysis of molecular docking data can be determined by looking at the Gibbs free energy (ΔGo). The test ligand is combined with the receptor by copying the selected model on the ligand and pasting it to a macromolecule. Ligands are pulled (drag) towards the receptor until it fuses. The combined results are stored in PDB format and then analyzed for energy, hydrogen bonding, and hydrophobic interactions using Ligplot+ 1.5.4 software, a program to generate schematic diagrams of protein-ligand interaction.

3. RESULTS AND DISCUSSION

The test ligands in this study amounted to 49 ligands derived from 20 active compounds of black tea and 29 active compounds of turmeric rhizome. The comparison ligand used is beta amyrin. This study uses a stepwise selection system so that the test ligands that do not pass one test stage are not included in the next test (eliminated). The tests carried out were ligand bioavailability, ligand toxicity, virtual screening, and molecular docking. The first test was

ligand bioavailability which resulted in 46 available ligands and three ligands declared unavailable. The second test was ligand toxicity, where there were seven ligands declared toxic so that only 39 ligands were included in the next test. Virtual screening resulted in 6 ligands having the best G values from each material. The six ligands were continued in the last test, namely molecular docking with beta amyrin as a comparison. The molecular docking results showed that there were two ligands with the best potential in increasing the Cu/Zn SOD enzyme activity. The two ligands are epicatechin gallate derived from black tea and curcumin derived from turmeric.

3.1. Result

Table 1 shows Lipinski's rule, a requirement rule for a test league in terms of bioavailability. From the r parameters of the Lipinski rule, a test ligand or drug substance must have three or more of the Lipinski rules. Three ligands from black tea compounds did not meet the requirements of 3 of 5 Lipinski rules, which were declared unavailable for use as an oral drug (Table 1). All ligands of the active compound of turmeric meet Lipinski's three or more so that they are available as oral drugs.

Table 1 Ligands bio availability

Ligands	Molecular Mass (Da)	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Log P	Molar refractivity
Beta amyrin (Comparison)	426	1	1	8,169	130,719
Theaflavin	564	9	12	2.344	139.541
Theaflavin_3-gallate	716	10	16	2.177	158.577
Theaflavin 3 O-gallate	704	10	15	3.285	172.988

Prediction of acute oral toxicity showed nine ligands in category IV and 30 ligands in category III. In addition, there were six ligands in category II and one ligand in category I. The test ligands with acute oral toxicity categories I and II were declared toxic and could not be used as drugs (Table 2). The Ramachandran plot is one method that can be used to analyze protein quality. The Ramachandran plot produces output in the form of a Ramachandran plot diagram and plot statistics. The Ramachandran plot contains the distribution of residues in four different quadrants: the most favored region, additional allowed region, generously allowed region, and disallowed region. Each quadrant has a different color.

Table 2 Ligands Toxicity

Ligand	Inhibition of Human Ether-A-Go-Go Related Gene (herG)		Carsinogenicity		Acute Oral Toxicity	
	Category	Score	Category	Score	Category	Score
Black tea						
Caffeine	Weak Inhibitor	0,8925	Non-Carcinogenic	0,9359	II	0,7405
Kaempferol	Weak Inhibitor	0,9795	Non-Carcinogenic	0,9363	II	0,6238
Myricetin	Weak Inhibitor	0,9781	Non-Carcinogenic	0,945	II	0,7348
Quercetin	Weak Inhibitor	0,9781	Non-Carcinogenic	0,945	II	0,7348

Theaflavin-3,3'-digallate	Weak Inhibitor	0,9903	Non-Carcinogenic	0,9636	II	0,3538
Turmeric						
Dehydrocurdione	Weak Inhibitor	0,6613	Non-Carcinogenic	0,8223	II	0,4719
Zedoarondiol	Weak Inhibitor	0,9739	Non-Carcinogenic	0,9077	I	0,3915

Square dots and black triangles show the distribution of amino acid residues in the Ramachandran plot. Square dots indicate amino acids other than glycine, while triangular dots indicate the amino acid glycine. Amino acid residues located in the *most favored region* were 210 residues (89.0%). The amino acid residues located in the *additional allowed region* are 24 residues (10.2%). Amino acid residues located in the *generously allowed region* were two residues (0.8%), and there were no residues in the *disallowed region* (Figure 1). Gridbox validation aims to ensure that the molecular docking corresponds to the active site of the receptor used. The superoxide dismutase receptor used has no natural ligand tethered in its structure. Gridbox validation was carried out by making a grid box around the residue of the ligand-binding site at the receptor. The grid box with coordinates center-x = 13.21, center-y = 70.06, center-z = 5.803; and with a grid box of size-x = 25, size-y = 40, size-z = 25 and spaced 0.375 Å.

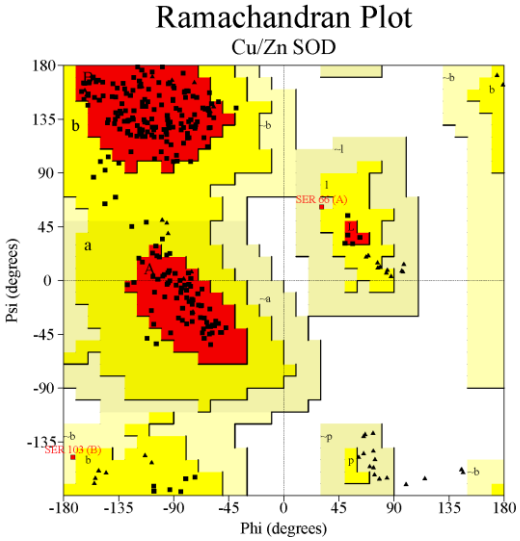


Figure 1 Ramachandran plot of Cu/Zn Superoxide dismutase

The validation results show that the RMSD ranges from 0.0156 Å to 0.3227 Å with an average value of 0.1699 Å. The RMSD value is a benchmark for the docking algorithm to predict the protein-ligand conformation. The RMSD value is good if it is worth <2.0 (Firdayani et al., 2017). According to Pratama (2016), molecular tethering gives results close to experimental experiments if it has an RMSD value of <2.0. The evaluation was carried out by aligning the ligands that were bonded together and showing the positions of the 20 beta amyrin close together (Figure 2).

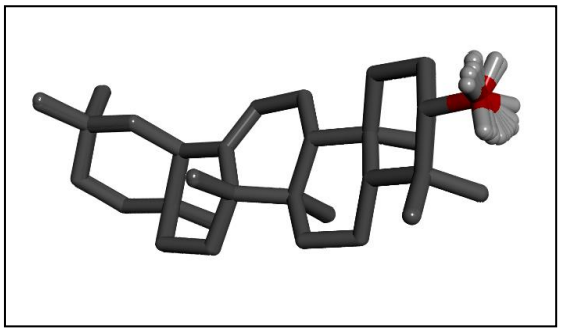
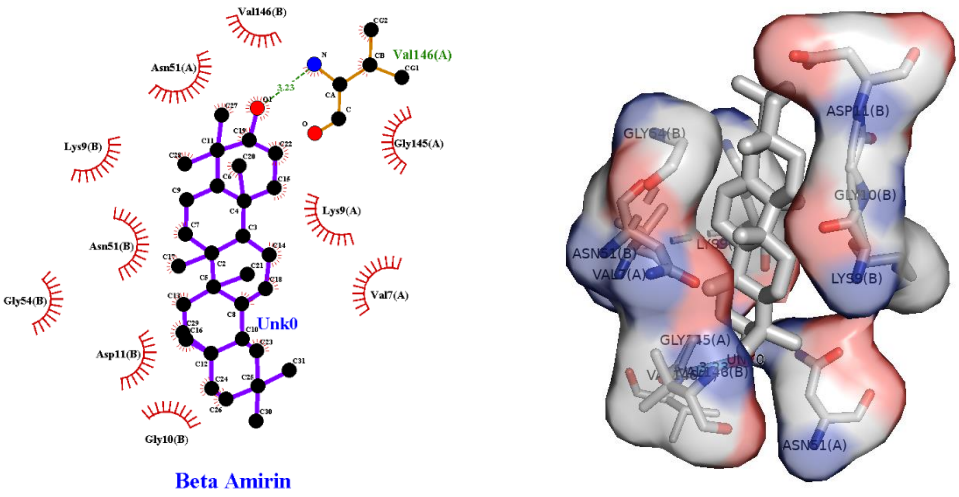


Figure 2 The result validation of beta amyrin

The results of the tethering are visualized to see the bound residue. The results show that beta amyrin is tethered to the allosteric site of the receptor. This is known through the residues that interact with the ligands. The total interactions are 11, with one hydrogen bond and ten hydrophobic bonds (Figure 3). The validation results also show an average G of -8.4 kcal/mol. In addition, the interacting residues at the validation stage have similarities with the interacting residues in the previous test, namely Val7, Lys9, Asp11, Asn51, Gly54, Cys144, Gly145, and Val146 (Hatai & Banerjee, 2019).



(a) (b)

Figure 3 Result of binding of beta amyrin to receptors. (a) 2D; (b) 3D

The virtual screening results show that the value of ΔG beta amyrin as the comparison ligand was -8.4 kcal/mol.values -5.1 kcal/mol to -9.5 kcal/mol. 3 black tea ligands have lower ΔG values than the comparison ligands, namely epicatechin gallate, epigallocatechin gallate, and the rubigin. The three ligands were continued for molecular bonding. All turmeric ligands have ΔG values greater than the comparison ligands, so two ligands with the best affinity values are taken, namely procurcumadiol, cyclocurcumin, and germacrone-4,5-epoxide, and one ligand as a compound characteristic of turmeric rhizome, namely curcumin for molecular anchoring (Table 3).

Table 3 The result of virtual screening

Ligand	ΔG (Kkal/mol)
Beta amyrin	-8,4
Black tea	
Epicatechin Gallate	-9,5

Epigallocatechin gallate	-8,9
Thearubigin	-8,5
Turmeric	
Procurcumadiol	-7,7
Cyclocurcumin	-7,6
Curcumin	-7,3

Molecular docking is done to see the interaction between the ligand and the receptor. Beta amyrin was used as a comparison ligand, while the test ligand was the ligand that was filtered by virtual screening. Molecular docking visualization displays the amino acid residues of the receptor-interacting with the ligand. The letter A or B at the end of the residue indicates the chain of the receptor residues circled in red are the same amino acid residues as the comparison. The residue accompanied by a dotted green line indicates the interaction formed in the form of hydrogen bonds. The residue contained in the red eyelash-like symbol indicates that the interaction formed is a hydrophobic bond (Figure 3).

The percentage amyrin (%BSS) or the similarity of the binding site between the control ligand and the test ligand to the receptor is shown in Table 4. 11 amino acid residues interact with beta amyrin. The residue was used as the %BSS reference for the test ligand. Beta amyrin as a comparison ligand has a total interaction of 1 hydrogen bond and ten hydrophobic bonds. The hydrogen-bonded residue is Val146(A) (3.23Å). The ten residues that interact with hydrophobic bonds are Val7(A), Lys9(A), Lys9(B), Gly10(B), Asp11(B), Asn51(A), Asn51(B), Gly54(B), Gly (145(A), and Val146(B) (Table 4).

Table 4 Ligand interactions with amino acid residues

	Ligand						
	1	2	3	4	5	6	7
Val7(A)	√	H(3.00;3.03; 3.14)	H(2.99;3.04; 3.14)	H(3.06)	H(3.09)	-	√
Lys9(A)	√	H(2.70)	H(2.71)	√	√	√	√
Lys9(B)	√	√	√	H(2.70)	√	√	√
Gly10(B)	√	√	√	-	-	√	√
Asp11(B)	√	-	-	-	-	H(2.93)	-
Asn51(A)	√	√	√	H(2.93)	√	-	H(2.91)
Asn51(B)	√	√	√	H(3.10)	√	√	√
Gly54(B)	√	-	-	√	-	-	-
Gly145(A)	√	√	√	-	√	-	√
Val146(A)	H(3.23)	H(2.91)	H(2.92)	H(2.71)	√	H(2.86;3.14)	H(2.92, 3.09)
Val146(B)	√	H(3.07)	H(3.09)	H(2.70)	H(3.35)	H(3.20)	H(3.01)
∑ Amino acid	11	9	9	8	8	7	9
BSS (%)	100	81,8	81,8	72,7	72,7	63,6	81,8

Description: 1. Beta amyrin (Comparison), 2. Epicatechin gallate, 3. Epigallocatechin gallate, 4. Thearubigin, 5. Procurcumadiol, 6. Cyclocurcumin, 7. Curcumin. √ = hydrophobic bonds, H= hydrogen bonds

The molecular tethering results can be analyzed by looking at the Gibbs free energy, or ΔG. Beta amyrin has a ΔG of -8.4 kcal/mol, one hydrogen bond, and ten hydrophobic bonds. Epicatechin gallate is a ligand from black tea with the best G of -9.5 kcal/mol and five hydrogen bonds, and six hydrophobic bonds. Cyclocurcumin is a ligand from turmeric which has the best G of -7.6 kcal/mol and has there are three hydrogen bonds and four hydrophobic bonds (Table 5).

Table 5 Gibbs free energy and chemical bonds

Compound	ΔG (Kkal/mol)	Number of Hydrogen Bonds	Number of Hydrophobic Bonds
Beta amyrin (Comparison)	-8,4	1	10
Black tea			
Epicatechin gallate	-9,5	5	6
Epigallocatechin gallate	-9,4	5	7
Thearubigin	-8,7	9	5
Turmeric			
Cyclocurcumin	-7,6	3	4
Procurcumadiol	-7,5	2	8
Curcumin	-7,4	4	6

3.2. Discussion

All ligands tested for black tea complied with Lipinski and were declared available as oral drugs except for three ligands that did not comply with Lipinski's rule. This follows Anitha et al. (2014), which state that all catechin compounds and their derivatives derived from tea leaves have complied with Lipinski regulations and can be used as oral drugs. Three compounds that do not comply with Lipinski are Theaflavin, Theaflavin-3-gallate, and Theaflavin-3-O-gallate. The three compounds are derivatives of Theaflavin. Kanbarkar & Mishra (2021) stated that theaflavin does not qualify Lipinski's rules and could not be used as oral drugs. Compounds that do not comply with Lipinski's rules are most likely to have problems with oral activity. However, many drugs are given orally but do not qualify Lipinski (Chagas et al., 2018).

The test ligands derived from the active compound of turmeric rhizome have complied with Lipinski and are declared to be used as oral drugs. The test ligand is an active compound of turmeric rhizome which belongs to the curcuminoid and terpenoid groups. Suryawanshi & Kulkarni, (2020) stated that seven curcuminoid compounds complied with Lipinski. Dongare et al. (2019) stated that as many as ten terpenoid compounds complied with Lipinski. In addition, Balaji & Chempakam, (2009) stated that 20 compounds of the diphenylheptanoid group from turmeric rhizome meet Lipinski and can be used as oral drugs.

All test ligands, both the active compound of black tea and turmeric rhizome, were classified as weak inhibitors of herG, so they were said to be non-toxic. This is because inactivation or a reduction in herG activity can increase the potential for long QT syndrome (LQTS), electro cardiac abnormalities that can lead to torsades de pointes, and even sudden death (Lamothe et al., 2016).

All test ligands are non-carcinogenic compounds. Carcinogens are compounds that can induce tumors or increase tumor malignancy when they enter the body either through inhalation, injection, or consumption (Mulware, 2013). The International Agency of Research on Cancer (IARC) completed a literature review of more than 100 compounds consisting of chemical compounds, physical agents, biological agents, and other compounds. IARC then classifies more than 100 of these compounds into 4 classifications, namely compounds that are carcinogenic to humans (class 1), compounds that are highly likely to be carcinogenic to humans (class 2A), compounds that are low likely to be carcinogenic to humans (class 2B), compounds whose carcinogenicity cannot be determined (class 3), and compounds that are not carcinogenic to humans (class 4) (Cogliano et al., 2011).

All tested ligands were classified as non-toxic except for seven caffeine, kaempferol, quercetin, myricetin, theflavin-3,3'-digallate, dehydrocurdione, and Zedoarondiol. The seven

compounds are classified as toxic because they are in category II except for Zedoarondiol, which is included in category I. This follows previous studies that stated that the catechin and curcuminoid compounds belong to categories III and IV, so they are not included in toxic compounds (Anitha et al., 2014; Awaluddin et al., 2017; Reshad et al., 2020). Quercetin, myricetin, kaempferol are toxic compounds because they belong to category II (Ferdian et al., 2021). Compounds with acute oral toxicity categories I and II are toxic and dangerous compounds when they enter the human body (Guan et al., 2019).

The quality matrix commonly used in quality validation of protein atom models is the Ramachandran plot. The Ramachandran plot depicts the two-dimensional distribution of the main protein chain at the torsion angles of phi (φ) and psi (ψ) (Sobolev et al., 2020). The Ramachandran plot provides a simplified view of the protein conformation. The -ψ angle groups the amino acids into distinct regions in the Ramachandran plot, where each region corresponds to a specific secondary structure (Ho & Brasseur, 2005). The Ramachandran diagram of the Cu/Zn superoxide dismutase receptor shows that the enzyme Cu/Zn superoxide dismutase (PDB ID: 1CB4) has high quality and structural stability because the distribution of amino acids in the most favored regions is more than 80%. The distribution of amino acids in the disallowed regions is less than 15%. The protein structure is good and stable if the distribution of amino acids in the most favored regions is higher than in the disallowed regions (Rahman et al., 2020). According to Suhadi et al. (2019), the protein structure is good and stable if the distribution of amino acids in the most favored regions is more than 80% and the distribution of amino acids in the disallowed regions is less than 15%.

The screening results in the form of 3 test ligands derived from the active compound of black tea had a better ΔG than beta amyrin as a comparison ligand. The three ligands are epicatechin gallate, epigallocatechin gallate, and thearubigin, where epicatechin gallate is the ligand with the best G value. The results of the research by Bhandari et al. (2015) showed that catechin-derived compounds have a good affinity for Cu/Zn SOD enzymes. Epicatechin gallate is a compound with the best affinity compared to other catechin compounds. Procurcumadiol, and cyclocurcumin are ligands of the active compound of turmeric, which have the best G value, although not better than beta amyrin as a comparison ligand. Curcumin is a compound that characterizes turmeric rhizome, with a good affinity energy value. No studies discuss the affinity of turmeric rhizome compounds for the Cu/Zn SOD enzymes. Suprihatin et al. (2020) stated that the active compounds in turmeric rhizome, curcumin, and terpenoids, have potential as antioxidants based on probably activity (Pa) analysis.

Beta amyrin, as a comparison ligand, has a total interaction of 1 hydrogen bond and ten hydrophobic bonds. The hydrogen-bonded residue is Val146(A) (3.23Å). The ten residues that interact with hydrophobic bonds are Val7(A), Lys9(A), Lys9(B), Gly10(B), Asp11(B), Asn51(A), Asn51(B), Gly54(B), Gly (145(A), and Val146(B). This is in accordance with previous studies which stated that the interaction of beta amyrin with Cu/Zn SOD enzymes involved residues Val7, Lys9, Asp11, Asn51, Gly54, Cys144, Gly145, and Val146 (Hatai and Banerjee, 2019; Liu et al., 2019; Wu et al., 2019). Beta amyrin has been shown to increase SOD enzyme activity in CCL_4-induced mice (Sunil et al., 2014). All the test ligands have a negative ΔG value which indicates the reaction occurs spontaneously and does not require energy (Chairunnisa & Runadi, 2016). The ΔG value of black tea ligands was lower than that of turmeric rhizome ligands. One of the factors that affect the value of ΔG is the hydrogen bond and hydrophobic bond formed between the ligand and the receptor. Ligands with more hydrogen bonds can hypothetically bind to the receptor more strongly and more easily (Rollando, 2018). At the same time, hydrophobic bonds are thought to contribute to stabilizing the ligand and receptor interactions. When the protein folds, most of the non-polar residues will be buried in the interior of the protein and protected so that the protein structure can be maintained. This phenomenon is known as the hydrophobic effect (Malau & Sianturi, 2017).

Epicatechin gallate is the best black tea ligand in increasing Cu/Zn SOD enzyme activity based on G value and binding site similarity (%BSS) (Figure 4). Epicatechin gallate has the most negative affinity value (Gibbs free energy). According to Chairunnisa & Runadi, (2016), the smaller the value of Gibbs free energy, the more stable the conformation formed and the more spontaneous chemical reactions that occur so that the reaction no longer requires energy or is exothermic and the ligand-protein complex and its activity will be better.

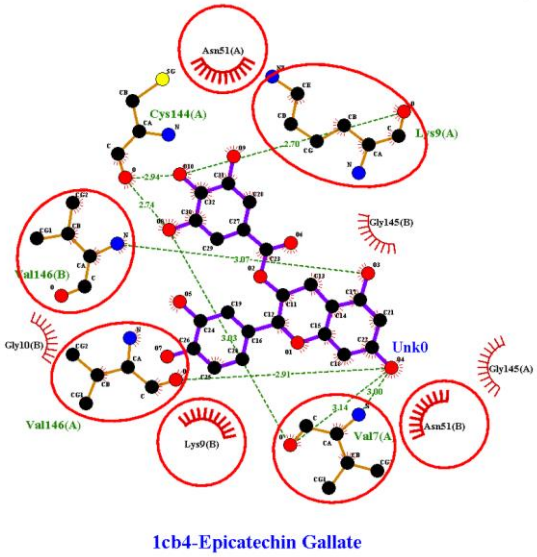


Figure 4 Epicatechin gallate-receptor interactions

Epicatechin gallate has the highest binding site similarity value compared to other black tea compounds. The higher the similarity of the interacting residues, the similarity of interaction type and activity similarity between the test ligands and the comparison ligands (Cosconati et al., 2010). The presence of hydrogen bonds with the amino acid Val7 can improve the quality of the epicatechin gallate complex with the Cu/Zn SOD enzyme (Hatai & Banerjee, 2019). Epicatechin gallate has been shown to increase the Cu/Zn SOD enzyme activity in vitro in Human Brain Microvascular Endothelial Cells (Fu et al., 2019). Li et al. (2007) stated that Epicatechin gallate increased the activity of Cu/Zn SOD and prolonged the life of hydrogen peroxide-induced cells.

Curcumin has the lowest ΔG value compared to other turmeric rhizome compounds. However, curcumin was the best turmeric rhizome ligand in increasing the activity of Cu/Zn SOD enzymes based on the value of similarity of binding sites (%BSS) (Figure 5). This is supported by the research of Manogaran et al. (2015), who showed that the administration of curcumin could increase the activity of the SOD enzyme in rabbits on a high-fat diet. Curcumin can also increase SOD enzyme activity in nicotine-induced mice and D-galactose-induced mice (Kumar et al., 2011; Motaghinejad et al., 2017).

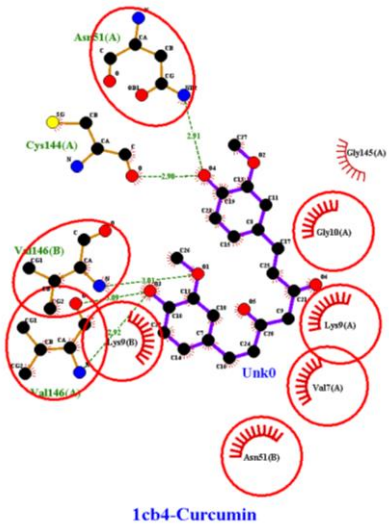


Figure 5 Curcumin-Receptor Interaction

4. CONCLUSION

Epicatechin gallate, an active compound of black tea, and *curcumin*, an active compound of turmeric rhizome, have the best potential to increase the activity of Cu/Zn SOD enzymes based on the results of virtual screening and molecular docking. *Epicatechin gallate* and *curcumin* are predicted to be well absorbed by the body because they qualify Lipinski's rules and are not toxic and safe for consumption.

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