THE POTENTIAL OF BUTTERFLY PEA FLOWER METHANOL EXTRACT AS AN ANTIOXIDANT BY IN SILICO

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Abstract: Butterfly pea flower (*Clitoria ternatea* L.) is a flowering plant from the Fabecea family that can grow vines. Butterfly pea flower are known to have chemical components that can act as antioxidants. This study aims to predict the potential of active compounds from methanol extract of butterfly pea flower in inhibiting reactive oxygen species (ROS) based on bond affinity (ΔG), the value of Root Mean Square Deviation (RMSD) and their interactions. The method used was a computational method with in silico technique. The software used was Autodock Vina with visualization using the Biovia Discovery Studio Visualizer 2020. The enzyme receptor was Nicotinamide Adenine Dinucleoide Phosphate (NADPH) Oxidase (NOX) obtained from Protein Data Bank and the test ligands were a chemical compound from methanol extract of butterfly pea flower. The results of the in silico study showed that the NO had a innate ligand, namely the GTP ligand which has a ΔG value of -7.3 kcal/mol, an RMSD value of 3.1111 Å and the interaction with the receptor that involves the presence of hydrogen bonds. Based on the results of the analysis of 11 test ligands, the chemical component of caffeine was predicted to have the most potential in inhibiting ROS compounds with a value of ΔG -5.4, RMSD value of 1.328 Å and had the same amino acid residue in hydrogen bonding, namely ASP^{118}, and GLY^{15}. The test ligand had the ability to inhibit ROS compounds with a lower level of stability than the innate ligand.

Keywords: antioxidant; butterfly pea flower; in silico; NADPH oxidase, ROS

1. INTRODUCTION

Butterfly pea flower is a flower that has a distinctive color and produces green peas. Butterfly pea flowers grew with the way vines in the yard of the house or in wild places. According to Dalimartha (2008), this flower has a name that varies depending on the region such as blue flowers, kelenit flowers and telang flowers for Sumatra, teleng flowers for Java, Talang flowers for Sulawesi and bisi for Maluku. Usually butterfly pea flowers are used as ornamental plants because of their attractive colors. In addition, butterfly pea flower has also been used as a family medicinal plant for healing various diseases. Butterfly pea flowers contain tannin compounds, saponins, triterpenoids, flavonoids, alkaloids, phenols, flavanol glycosides, anthraquinones, anthocyanins,

essential oils, proteins and carbohydrates which have health benefits (Al-Sanafi, 2016). One of the pharmacological effects of butterfly pea flower is as an antioxidant.

Antioxidants are compounds with small molecular weights that can ward off free radicals in the body so that cell damage can be inhibited (Winarsi, 2007). The human body can produce free radicals from the metabolic process and also produce endogenous antioxidants as a form of defense. According to Werdhasari (2014), free radicals are destructive if there is an oxidative stress condition, which is a condition where there is an imbalance between free radicals and antioxidants in the body. Reactive oxygen compounds (ROS) are a form of oxygen-derived free radicals which, if produced in excess, can cause many diseases. According to Panday *et al.*, (2015), many ROS in cells are produced by NADPH oxidase. The potential methanol extract of butterfly pea flower in reducing ROS compounds was studied in silico.

In silico method is a computer simulation-based approach by docking molecules. According to Jensen (2007), molecular docking can align ligands which are small molecules and receptors in the form of proteins by paying attention to the properties of both. Based on this background, it is necessary to conduct research using computational techniques to predict the tethering of active compounds of butterfly pea flowers to ROS.

2. METHODS

2.1. Preparation of Reseptor Stucture

NADPH Oxidase (NOX) macromolecules was downloaded from the Protein Data Bank at http://www.rscb.org. The molecular identity was 1E96. Macromolecule data was downloaded in *.pdb format, with a ligand and water molecule bound form. Protein macromolecules was separated from the solvent (water molecule) and non-standard ligands or residues. The separation of macromolecules from unnecessary molecules was carried out using the Biovia Discovery Studio Visualizer 2020. The separation results was used for tethering, and the results was saved in *.pdb format. The NOX macromolecules that had been separated from the residue were optimized using Autodock Tools 1.5.6 software. The optimization includes: adding hydrogen atoms and setting the grid box parameter. Results are saved in *.pdbqt format.

2.2. Preparation of Ligands Structure

The ligands used were GTP ligands as a comparison ligand and 11 active compounds of Buterfly Pea Flower methanol extract identified by Neda *et al*. (2013) downloaded on the PubChem website (https://pubchem.ncbi.nlm.nih.gov/) in the form of a 2D structure with *.sdf format. The format of the ligands were converted into 3D to *pdb format using Marvin Sketch 20.11. The ligands structure that has been made was optimized using AutoDock Tools 1.5.6 by increasing the gaisteiger load and setting the number of active torsion. These results were saved in *.pdbqt format. All ligand compounds were filtered according to Lipinski's rules using online access http://www.scbio-iitd.res.in/software/utility/lipinskifilters.jsp (Lipinski, 2004).

2.3. Molecular Docking with Autodock Vina

The ligands and proteins that had been stored in the *.pdbqt format were copied into the Vina folder. Then type the vina configuration into notepad, saved as 'config_rigid.txt'. Vina was run via the Commond Prompt and collected docking data in Vina folder.

2.4. Analysis

The docking result files were analyzed for Lipinski filter results, bond energy (ΔG), root mean square deviation (RMSD) values and hydrogen bonding. The value of ΔG, RMSD and interaction between ligand and receptor obtained were comared with validation ligand and other journals to determine the potential of ligands as antioxidants.

3. RESULTS AND DISCUSSION

3.1 Identification of Receptor Structure

The NO receptor structure downloaded from the Protein Data Bank contains natural ligands and H_2O molecules. These ligands and H_2O molecules must be removed from the receptors because they can interfere with the docking process (Fikry, 2014). The optimization process is carried out by adding hydrogen atoms and arranging the grid box as the ligand mooring space. According to Droe (2005), hydrogen atoms are added to adjust the tethering atmosphere.

3.2 Identification of Ligand Structure

The test ligands used were the results of GC-MS analysis of methanol extract of butterfly pea flower conducted by Neda *et al*., (2013) and the natural ligands found in the NOX receptor. There were 11 types of chemical compounds that were tested, namely acetic acid, cyano-; pyridine-2-d, 6-methyl; Hirsutene; pyrimidine, 4-hydroxy-; butane, 2-isothiocyanate; bicyclo- [4.1.0] hept-3-ene, 3,7,7-trimethyl-; cyclohexen, 1-methyl-4- (1-methylethylideme); 1,3-benzodioxole, 5- (2-propenyl); 1-nitro-2-acetamido-1,2-dideoxy-d-monnitol; caffeine; and hexadecanoic acid. The optimization of the ligands was carried out by adding a gaisteiger charge and setting the number of active torsion. Adjusting the amount of active torsion can reduce the performance and time required to determine the active bonds (Morris *et al*., 2012). All ligands attached to the receptor must comply with Lipinski's rule (Table 1).

Table 1 Lipinski's Rule of Ligand

Ligand	Lipinski's rule				
	A	B	C	D	E
GTP ligand (validation)	507	-4,737250	0	18	82,351898
acetic acid, cyano-	85	-0,0155320	1	3	17,865801
pyridine-2-d, 6-methyl-	109	1,095620	1	2	30,638794
Hirsutene	204	4,414999	0	0	64,582985
pyrimidine, 4-hydroxy-	96	-0,341800	1	3	25,774696
butane, 2-isothiocyanate	115	1,887700	0	1	34,778992
bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl-	312	-0,053101	5	6	77,145782
cyclohexen, 1-methyl-4-(1-methylethylideme)-	138	3,532899	0	0	46,005985
1,3-benzodioxole, 5-(2-propenyl)	162	2,143800	0	2	46,465992

1-nitro-2-acetamido-1,2-dideoxy-d-mannitol	252	-3,157201	5	8	54,572292
Caffeine	194	0,061900	0	5	49,100494
hexadecanoic acid	256	5,552299	1	2	77,947777

Information :
A : Relative atomic mass <500 Da   D : Bond Acceptor <10
B : Log P <5   E : Molar refractifity 40 – 130
C : Bond donors H <5

All test ligands comply with Lipinski's rules. This shows that the ligand can interact with the NOX receptor.

3.3 Bond Affinity (ΔG) and RMSD Value

The ligand and receptor bond affinities were expressed in the form of Gibbs free energy (ΔG). Gibbs free energy shows the energy required for the ligand to bind to the receptor at the binding site (Karim, 2018). A small ΔG value indicates that the conformation is more stable. Apart from ΔG, an analysis was also carried out on the RMSD value. This value determines the success rate in predicting the mode of bonding.

Table 2. Bond Affinity and RMSD Value of Ligand

Ligand	Bond Affinity (ΔG) (kcal/mol)	RMSD (Å)
GTP ligand (validation)	-7,3	3,111
acetic acid, cyano-	-4,0	1,637
pyridine-2-d, 6-methyl-	-4,4	1,131
Hirsutene	-6,2	9,988
pyrimidine, 4-hydroxy-	-3,9	7,423
butane, 2-isothiocyanate	-3,7	2,486
bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl-	-5,0	0,918
cyclohexen, 1-methyl-4-(1-methylethylideme)-	-5,8	1,617
1,3-benzodioxole, 5-(2-propenyl)	-5,7	0,868
1-nitro-2-acetamido-1,2-dideoxy-d-mannitol	-5,2	5,210
Caffeine	-5,4	1,328
hexadecanoic acid	-6,2	6,156

Based on the Table 2, the ΔG value of the GTP ligand was greater than that of the test ligand. This shows that the GTP ligand has a better level of stability in binding to the receptor. According to Setiawan (2015), the difference in ΔG between the test ligand and the validation ligand is due to differences in the characteristics of the interactions in the bonds. The RMSD value for the test ligand ranged from 0.868 Å to 13.296 Å, while for the GTP ligand was 3.111 Å. The RMSD value received has a limit of ≤ 2.5 Å (Jain & Nicholls, 2008). The RMSD value ≤ 2.5 Å indicates that the compound is a competitive inhibitor. Phrueksanan *et al.*, (2014) reported butterfly pea flower extracts could protect canine erythrocytes from hemolysis and oxidative damage induced by 2,20-azobis-2-methyl-propanimidamide dihydrochloride (AAPH).

3.4. Interaction of Ligand and Receptors

The interaction of ligands and receptors can be determined by observing the interaction of amino acid residues. GTP ligands had hydrogen bonds with amino acid residues ALA[159], LYS[116], CYS[18], VAL[18], GLY[15], GLY[12], PRO[34], TYR[32], THR[35], ALA[13], THR[17], GLY[30], ASP[118]. Meanwhile, the test ligands that had the same binding to amino acid residues as the GTP ligand were caffeine compounds, namely ASP[118] and GLY[15]. Caffeine is predicted to inhibit ROS because it has an RMSD value of 1.328 Å, where the RMSD value was <2.5 Å with a ΔG value of -5.4 kcal/mol, and has the same residual residues in hydrogen bonds with validation GTP ligands. Based on research by Sukoha *et al.* (2011), caffeine in Robusta coffee beans in Lampung had an antioxidant activity of 21.41 ppm using the DPPH method. In another study results show that there is an effective protection of caffeine against oxidative degradation of adenine (Viera et al., 2020). The ligand and receptor interactions can be seen in Figure 1.
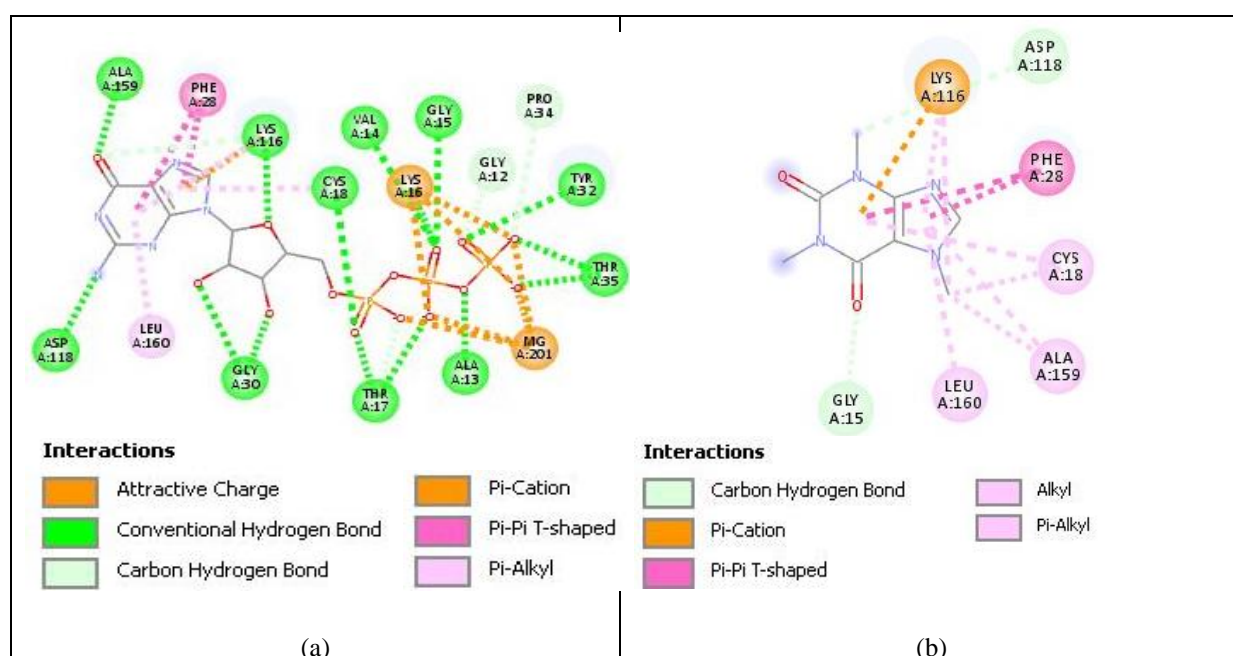

Figure 1 Interaction of Ligand and Reseptor. (a) GTP-NOX; (b) Caffeine-NOX

4. CONCLUSION

Based on the analysis of antioxidant with in silico methode, the methanol extract of butterfly pea flower had a ΔG value greater between -3.6 kcal/mol to -6.2 kcal/mol compared to the GTP ligand as the comparative ligand of -7.3 kcal/mol. So that the test ligand of methanol extract of the butterfly pea flower compound can act as an antioxidant in inhibiting ROS with predicted activity is to be lower than the GTP ligand. The active compound in butterfly pea flower, which was predicted to have the most potential to inhibit ROS, was caffeine.

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REFERENCES

Al Sanafi, A.E. (2016). Pharmacologial importance of *Clitoria ternatea*. IOSR Journal of Pharmacy, 6(3), 57-67.

Dalimartha,S. (2008). Atlas Tumbuhan Obat Indonesia. Jakarta. [Internet]. Jilid 5, 86-87. Tersedia pada : http//books-google.co.id/books?id [01 Mei 2020].

Droe, J. H. (2005). Pharmacophore-based virtual screening: a practical perspective. In J. Alvarez & B. Shoichet Virtual in drug discovery. Boca Raton: CRC Press.

Fikry, M. A. (2014). Studi penambatan molekul senyawa-senyawa flavonoid dari buah mengkudu (*Morinda citrifolia* L) pada peroxisome proliferator-activated receptor (PPARγ) [skripsi]. Program Studi Farmasi, Fakultas Kedokteran dan Ilmu Kesehatan, UIN Syarif Hidayatullah, Jakarta.

Jain, A.N., & Nicholls, A. (2008). Recommendation for evaluation of computatioanl methods. Journal of Computer-Aided Molecular, 22, 133-139.

Jensen, F. (2007). Introduction to computional chemistry, 2nd Ed. Odense : John Wiley & Sons.

Karim, M. A. 2018. Analisis docking molekuler senyawa flavonoid dan steroid terhadap enzim siklooksigenase dan fosfolipase [skripsi]. Fakultas Farmasi. Universitas Seia Budi, Surakarta.

Morris, G. M., Goodsell, D. S., Pique, M.E., Lindstrom, W., & Huey, R. (2012). AutoDock Version 4.4: automated docking of flexible ligands receptors. La Jolla, California, USA: The Scripps Research Institute

Neda, G.D., Rabeta, M.S., & Ong, M.T. (2013). Chemical composition and anti-poliferative properties of flowers of *Clitoria ternatea*. International Food Research Journal, 20(3), 1229-1234.

Panday A, Sahoo M.K., Osorio D, & Batra S. (2015). NADPH oxidases: an overview from structure to innate immunity-associated pathologies. Cellular & Molecular Immunology, 12, 5-123.

Phrueksanan, W., Yibchok-anun, S., & Adisakwattana, S. (2014). Protection of Clitoria ternatea flower petal extract against free radical-induced hemolysis and oxidative damage in canine erythrocytes. Research in veterinary Science, 97, 357–363.

Setiawan, T. (2015). Studi molecular docking ekstrak kurkuminoid asal Wonogiri sebagai inhibitor enzim DNA Topoisomerase II [tesis]. Departemen Biokimia. Sekolah Pascasarjana. Institut Pertanian Bogor, Bogor.

Sukoha, A., Setiawan, F.F., & Wirakusumah, H. (2011). Isolasi dan karakteristik senyawa sitotoksik kafein dan asam klorogenat dari biji kopi robusta Lampung. Jurnal Medika Planta, 1(4), 366-368.

Lipinski, C. A. (2004). Drug discovery today. Technologies, 1(4): 337-341.

Viera A.J.S.C., Gaspar E.M., & Sanyos P.M.P. (2020). Mechanisms of potential antioxidant activity of caffeine. Radiation Physics and Chemistry, 174(108968)

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

Winarsi, H. (2007). Antioksidan alami dan radikal bebas. Yogyakarta : Kanisius.