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Pharmacology Analysis of The Active Compound of Makassar Fruit (*Brucea Javanica*) as Antidiabetic Via Bioinformatics Approach

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Abstract. Diabetes is one of the emergency diseases worldwide due to the large case each year. Therefore, searching the potential treatment become crucial to treating the disease. In this research, homology modeling and molecular docking were employed to analyze the active compounds of Makassar fruit (*Brucea javanica*) as antidiabetic. Alpha glucosidase (AG) is used as a target, and the ligands are javanicin and javanicisode B, both active molecules of *Brucea javanica*. According to molecular docking results, all ligands have the potential to bind to the receptor because of their negative energy scores. Also, javanicin has higher binding energy (-8.0 kcal/mol) than the control (-5.7 kcal/mol). Furthermore, hydrogen bonds and hydrophobic interactions allow these two active chemicals to attach to an important location on AG. Thus, our finding implied that two active compounds of *Brucea Javanica* could be inhibitors for the receptor, and javanicin may become a promising drug against diabetes.

Introduction

Diabetes is a chronic condition that arises when pancreas cells fail to produce the insulin hormone that the body requires, or when insulin is not utilised properly by the body. This hormone is vital for controlling the conversion of blood sugar to energy. Type 1 and type 2 diabetes are the two forms of diabetes that can be found in the general population. The patient's immune system targets and destroys pancreatic cells in type 1 diabetes. Insulin synthesis is disturbed; as a result, resulting in an unregulated rise in blood glucose levels. Type 2 diabetes affects roughly 90-95 percent of patients with diabetes worldwide. This type is caused by the body's cells being less sensitive to the hormone insulin. Therefore, the hormone produced cannot be used properly by the body (Dabelea et al., 2014; DeFronzo et al., 2015).

Based on data reported by the International Diabetes Federation (IDF), in 2021, around 19.46 million people in Indonesia had diabetes. This figure increased by 81.8% compared to 2019. From this data, Indonesia is the fifth-highest diabetic country after China, India, Pakistan, and the United States. For the Southeast Asian region, Indonesia is in the top 10 countries with the most diabetes cases (Handayani, 2021). Therefore, it is necessary to conduct an intense molecular investigation of the biomacromolecule involved in diabetics. In addition, the discovery and development of safe and effective drugs in treating diabetes need to be continued and improved, considering the number of cases is increasing every year.

In the last three decades, the use of natural ingredients from plants that have pharmacological activity has become the center of world attention and has become popular in almost all countries, especially in developing countries. This is because drugs from plants are easy to obtain, affordable prices, have no side effects, and can prevent and even treat various diseases. Because of this, the demand for medicinal plants is increasing every year (Ernst, 2005). In Indonesia,

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especially on the island of Sulawesi, various kinds of medicinal plants can be found easily which are believed by the community to be able to treat various diseases including diabetes. One type of medicinal plant commonly used as an anti-diabetic is *Brucea javanica* or better known as Makassar fruit (Ablat et al., 2014; NoorShahida et al., 2009). To determine the pharmacological activity of this plant in treating diabetes, in-depth molecular analysis is needed regarding the binding of secondary metabolites present in plants with proteins involved in the body of people with diabetes. Several active compounds from *Brucea javanica* such as Javanicin, Javanicoside B, Yadanzioside B, Yadanziolide A, Macedonic acid have been identified experimentally (Kim et al., 2003; Lin et al., 1990). However, further research is needed to determine the specific active compounds for the treatment of diabetes.

In this study, a bioinformatics approach using homology modeling combined with molecular docking can be applied to investigate active compounds of Makassar fruit. This plant is believed to have antidiabetic activity (A. Arwansyah et al., 2022). In the homology modelling, the target protein is constructed by several online tools to find the complete 3D structure. Then, molecular docking is carried out to identify the binding site of the ligand in the active site area of the alpha glucosidase (receptor). This enzyme has the function of breaking the polysaccharides and disaccharides to glucose. So, inhibition of this protein can prevent the excess glucose in the blood, and the potential ligands binding to the crucial pocket of this protein are predicted to have inhibitory activities as an antidiabeticIndonesia is a mega-biodiversity country that is rich in medicinal plants and has the potential to be developed, but it has not been managed optimally. The natural wealth of plants in Indonesia includes 30,000 plant species out of a total of 40,000 plant species in the world, 940 species of which are medicinal plants (this number is 90% of the total medicinal plants in Asia) (Ministry of Forestry, 2010).

Experimental

Materials and Method

Homology Modelling and Assessment

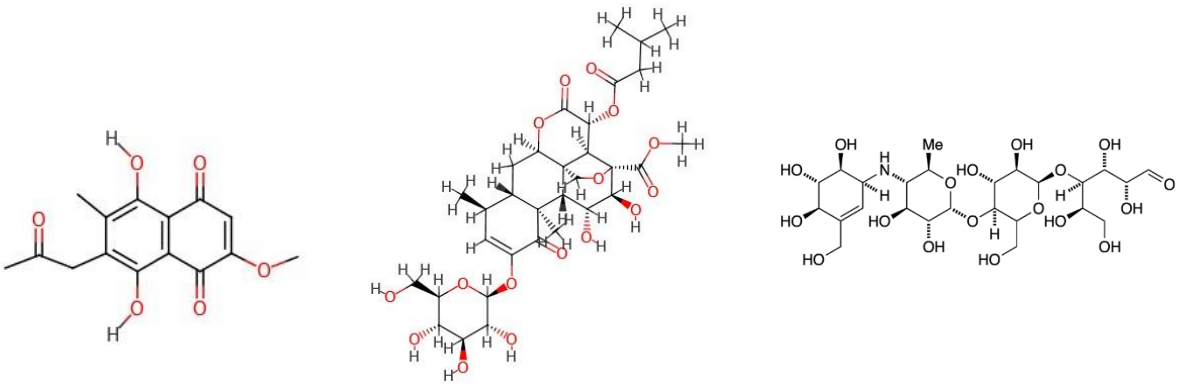
The sequence protein of alpha glucosidase was taken from National Center for Biotechnology Information (NCBI) database (accession: KAH8549454, GI: 2168653206). Using the Basic Local Alignment Search Tool, the FASTA file was retrieved to determine the similarity of the protein sequence (pBLAST). A total of 50 sequences were found, having a similarity of more than 30% to alpha glucosidase input. To identify which structure will be used as a reference template, we choose the top 5 PDB files, namely 3w37, 2qly, 5kzw, 5nn3, and 5nn4, that have a similarity percentage of > 90% of AG. On the reference template, homology modeling was accomplished using a comparable application (Modeller v10.0) (Fiser & Šali, 2003). The best probable structure among the ten structures was chosen based on the highest discrete optimized protein energy (DOPE) score. To confirm the protein sequence's similarity, the best-predicted model was matched to the reference template.

The DOPE per residue score as a function of residue position was determined to confirm the similarity of the protein sequences between the developed model of AG and the reference template (3w37). In addition, the predicted model was matched to the reference template to determine which amino acid residues were conserved between the two sequences. The Ramachandran diagrams of the produced AG model and references were also evaluated using the PROCHECK tool to confirm the streochemical accuracy of protein structure (Laskowski et al., 1993). In addition, we use the ProtParam tool of ExPASy on the selected model to determine its physicochemical properties (Gasteiger et al., 2005). The molecular weight, theoretical pI, total number of positively/negatively charged Asp + Glu and Arg + Lys residues, atomic composition, amino acid composition, aliphatic index, and aliphatic index are among these features.

Molecular Docking

The molecular docking method is used to determine the molecular interactions between drug candidates (ligands) and target proteins (receptors) (Natsir et al., 2022; Sumaryada et al., 2016; Fitrasari et al., 2022). The 2D structure of the active plants of *Brucea javanica* used as ligands were presented in Figure 1. Meanwhile, the receptor was taken from the constructed protein obtained by homology modeling. In this research, AutoDock software version 4.2 was used to test the pharmacological activity of the *Brucea javanica* plant as an anti-diabetic. The software developed by Morris and his team is one of the popular docking programs used for molecular docking analysis (Morris et al., 2009). In carrying out the simulation, hydrogen atoms and charges (Kollman's united atomic charges) are added to the ligand-receptor molecule. The extension of the ligand and receptor (*.pdb) is converted to *. pdbqt through the Auto Dock Tools 1.5.6 program. Then, docking is done by making a grid box size in the simulation system so that each ligand with flexible conditions can move freely in the target protein area. The box size was set at the center of x, y, and z: 8.663, 0.183, and -25.033, respectively. The number of point dimensions was computed as 24, 24, and 20, with a grid spacing 1 angstrom. After determining the grid box size, the Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm was used to look for stable conformations of the ligands when they bind in the protein binding pocket area. The other parameters in docking are adjusted to the default from AutoDock. The simulation results from docking were analyzed and visualized

using the PLIP program (Salentin et al., 2015) and PyMOL software. The docking protocols were computed according to our previous work presented in Ref. (A. Arwansyah, 2015; M. S. Arwansyah et al., 2019; Syahputra et al., 2018).



(a) (b) (c)

Figure 1. 2D structure of the active compounds of *Brucea javanica* (a) Javanicin and (b) Javanicoside B. Meanwhile, (c) Acarbose is used as control.

Result and Discussion

Protein model and evaluation

Because the X-ray structures contained in the PDB database are not always complete of the amino acid sequence, the homology modeling approach is frequently used to produce a comparison model of protein structure from the query protein. This method was implemented using the Modeller v10.0 program packages, which uses the developed modules to construct 3D protein models. To determine the tertiary structure of alpha glucosidase (AG, the protein sequence from National Center for Biotechnology Information (NCBI) database with accession: KAH8549454 and GI: 2168653206) was used as query protein. 50 AG protein templates from the PDB database were retrieved and stored in FASTA format using the BLAST database. The top 5 PDB files, namely 3w37 (1.7 Å), 2qly (2.0 Å), 5kzw (2.0 Å), 5nn3 (1.9 Å), and 5nn4 (1.8 Å), that have a similarity percentage of > 90% of AG was selected. PDB: 3w37 is selected as the reference template of AG because of its better crystallographic resolution (1.7 Å). L. leaves contain alkaloids, flavonoids, and tannins, saponins, and terpenoids. The result of phytochemical test can be seen in the Table 1.

Table 1. The value of DOPE score.

Alpha Glucosidase Model	Score	score
Model1	-93904.46	1
Model2	-96379.25	1
Model3	-96260.17	1
Model4	-97322.55	1
Model5	-95647.75	1
Model6	-96420.12	1
Model7	-95781.14	1
Model8	-95593.72	1
Model9	-96190.37	1
Model10	-95898.52	1

In order to develop a protein model of AG, the sequence of 3w37 (reference template) was retrieved for homology modeling. Ten protein models were created using the Modeller v.10 tools. The AG tertiary structure with the lowest DOPE score was chosen. Model 3 is chosen for AG because it has the lowest DOPE score of 97322.55, as shown in Table 1. In addition, the DOPE per residue score is compared between the AG template and model 4 shown in Figure 2. The contour

of the trend graph for those scores is similar. This finding indicated that the produced model has a structure that is nearly identical to the reference template. However, the amino acid gaps are not clearly visible. As a result, the model is aligned to the sequence of 3w37 (reference template) amino acid residues illustrated in Figures 3 and 4, respectively. There are some gaps in the template and filled by constructed model by homology modeling. Figure 5 shows the AG model's tertiary structure using the reference template 3w37. This model is used for further analysis using molecular docking.

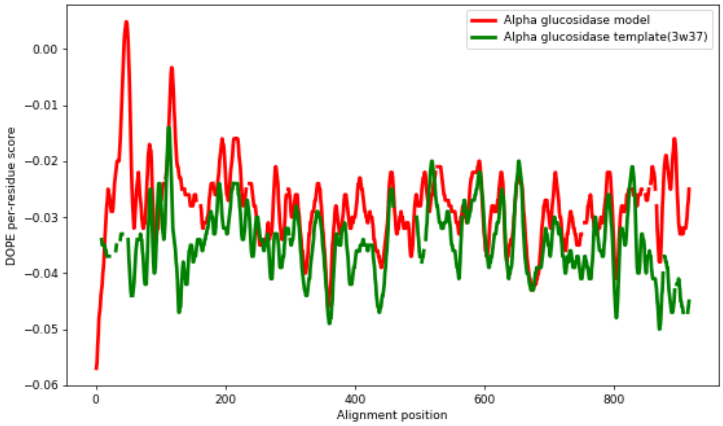


Figure 2. The DOPE per residue score between Xanthine oxidase template (3w37) and model 4.

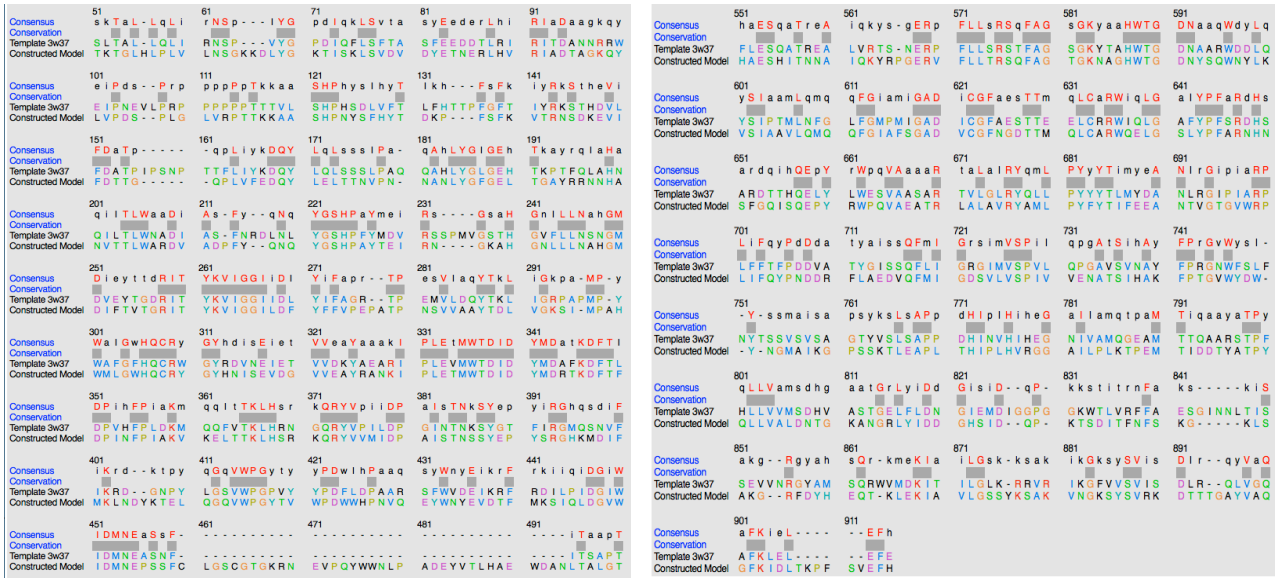


Figure 3. The sequence alignment of alpha glucosidase betweena reference template(3w37) and constructed model.

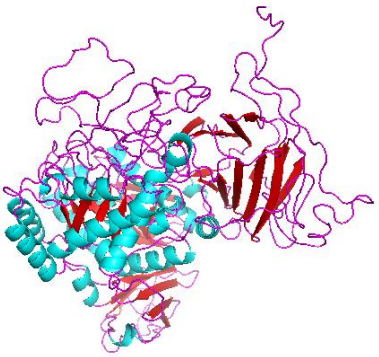


Figure 5. The tertiary structure of crated model of alpha glucosidase (model 4) utilizingthe reference template 3w37. The structures of α-helix and β-sheet are presented by cyan and red colors in cartoon models, respectively.

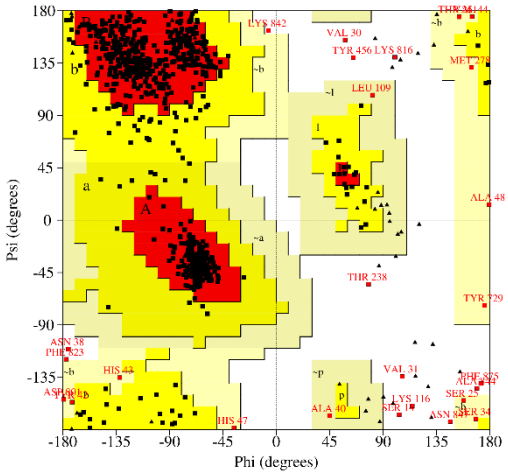


Figure 6. The ramachandran diagrams of constructed model from the reference template (PDB: 3an1) of alpha glucosidase.

Since the model will be used for further research, its geometrical conformations and streochemical quality must be assessed. The PROCHECK program developed by Laskowski and coworkers was used to assess the Ramachandran diagram of the AG model illustrated in Figure 6. According to the diagram, 84.1% of residues were found in the most favoured regions, 12.5% and 1.7% in additional and generously allowed regions, and 1.7% in the disallowed regions. Furthermore, Table 2 shows the physiochemical parameters of the xanthine oxidase (AG) model built using the ProtParam (ExPASy) tool citeGasteiger2005. Xanthine oxidase has a molecular weight of 98978.76 Da and includes 880 amino acids. The amino acids Leu (7.8%), Thr (7.5%), and Gly (7.4%) are the most common residues in the sequence. The protein's isoelectric point (pI) is predicted to be 6.6, indicating that it prefers neutral pH. The GRAVY score was found to be -0.358, indicating that this protein has better interaction with the surrounding water molecules. The protein's stability index of 26.95 suggested that it could be a stable structure (Gasteiger et al., 2005).

Table 2. Physiochemical identities of created model of AG

Physiochemical identities	AG model	Amino acid composition	No.	Composition (%)
Number of amino acids	880	Ala (A)	64	7.3
Molecular weight	98978.76	Arg (R)	31	3.5
Theoretical pI	6.6	Asn (N)	50	5.7
No. of negatively charged (Asp + Glu)	86	Asp (D)	50	5.7
No. of potively charged (Arg + Lys)	80	Cys (C)	5	0.6
Atomic composition:		Gln (Q)	31	3.5
Carbon (C)	4494	Glu (E)	36	4.1
Hydrogen (H)	6781	Gly (G)	65	7.4
Nitrogen (N)	1185	His (H)	31	3.5
Oxygen (O)	1304	Ile (I)	43	4.9
Sulfur (S)	22	Leu (L)	69	7.8
Formula	$C_{4494}H_{6781}N_{1185}O_{1304}S_{22}$	Lys (K)	49	5.6
Total number of atoms	13786	Met (M)	17	1.9
Aliphatic index	77.01	Phe (F)	38	4.3
Grand average of hydropathicity	-0.358	Pro (P)	50	5.7
Instability index	26.95	Ser (S)	53	6
		Thr (T)	66	7.5
		Trp (W)	20	2.3
		Tyr (Y)	51	5.8
		Val (V)	61	6.9

Molecular docking analysis

The Pubchem database is used to get the active compounds of *Brucea javanica*, i.e., javanicim and javanicisode B to treat alpha glucosidase (AG). When the ligand-receptor combination has a negative binding energy, the potential of ligand binding to the receptor site is attained.

Table 3. The binding energy of ligands in complex with receptor obtained by molecular docking.

No.	Compound	Binding energy (kcal/mol)
1	Javanicin	-8
2	Javanicisode B	-4.5
3	Control	-5.7

Table 3 lists three complexes obtained from our docking with a different binding energy. Acarbose is used as a positive control. Because all binding energy scores are negative, all ligands have the potential to bind to the receptor. However, from the table, only javanicin (-8.0 kcal/mol) has larger binding energy than control (-5.7 kcal/mol). This finding implied that it has the potential to become a promising drug against diabetes.

Figure 7 shows the binding location and geometrical pose of the ligands in complex with the receptor utilizing molecular docking. Model 1 and Model 2 were referred to javanicin and javanicisode B in complex with AG. Meanwhile, Model 3 was denoted to the control in binding with AG. The binding of ligands to the receptor is aided by hydrogen bond and hydrophobic interactions shown in Tables 4 and 5, respectively. Some residues, including GLU-471, ASN-459, and LYS-207, are contributed to at least two ligands, indicating these residues are important for the binding site of the receptor. On the other hand, TYR-203 and TRP-457 were identified to have contributed to the stability via hydrophobic interactions shown in Table 5. We demonstrated that each ligand that can bind to the crucial site of AG might become a promising drug for treating diabetes. Our finding implied that two active compounds of *Brucea javanica* could become inhibitors for the AG enzyme.

Table 4. Hydrogen bonds of ligands in complex with receptor.

Model	Residue	AA	Distance H-A (Å)	Distance D-A (Å)	Donor Angle	Donor Atom	Acceptor Atom
Model 1	459	ASN	3.26	3.6	100.86	4500 [Nam]	8568 [O3]
	473	ASP	2.99	3.58	120.5	8568 [O3]	4637 [O2]
	475	ASN	2.08	2.8	125.87	4652 [Nam]	8570 [O2]
	500	GLY	2.05	2.7	122.53	8572 [O3]	4875 [O2]
	507	LYS	2.21	2.85	119.13	4932 [N3+]	8562 [O2]
Model 2	199	ALA	2.19	3.18	162.27	1900 [Nam]	8593 [O3]
	459	ASN	2.44	3.01	116.88	8582 [O3]	4504 [O2]
	459	ASN	2.14	2.97	137.08	4500 [Nam]	8566 [O2]
	471	GLU	2.54	3.03	112.93	4612 [O3]	8600 [O3]
	473	ASP	2.67	3.28	121.48	8593 [O3]	4637 [O2]
Model 3	196	ARG	3.11	3.62	112.2	1875 [Ng+]	8582 [O3]
	196	ARG	2.33	2.98	120.36	1878 [Ng+]	8582 [O3]
	203	TYR	2.16	2.95	140.09	1944 [O3]	8590 [O3]
	459	ASN	2.86	3.82	157.3	4500 [Nam]	8586 [O3]
	459	ASN	2.14	3.05	147.86	4494 [Nam]	8579 [O3]
	459	ASN	2	2.88	149.1	8579 [O3]	4499 [O2]
	471	GLU	3.46	3.99	117.11	8605 [O3]	4611 [O2]
	471	GLU	2.06	2.98	157.37	8601 [O3]	4612 [O3]
	475	ASN	3.05	3.58	113.23	4652 [Nam]	8579 [O3]
	499	ASN	2.23	3.02	137.73	8582 [O3]	4870 [O2]
	507	LYS	2.81	3.78	159.4	4925 [Nam]	8575 [O3]
	507	LYS	1.63	2.5	140.34	4932 [N3+]	8575 [O3]

Table 5. Hydrophobic interaction of ligands in complex with receptor.

Model	Residue	AA	Distance	Ligand Atom	Protein Atom
Model 1	198	VAL	3.63	8574	1897
	203	TYR	3.78	8576	1941
	203	TYR	3.46	8566	1942
	457	TRP	3.36	8563	4474
	504	LEU	3.94	8559	4903
Mocel 2	202	PHE	3.87	8608	1931
	572	TYR	3.51	8608	5579
Model 3	203	TYR	3.35	8561	1942
	457	TRP	3.03	8561	4475
	457	TRP	3.58	8571	4474

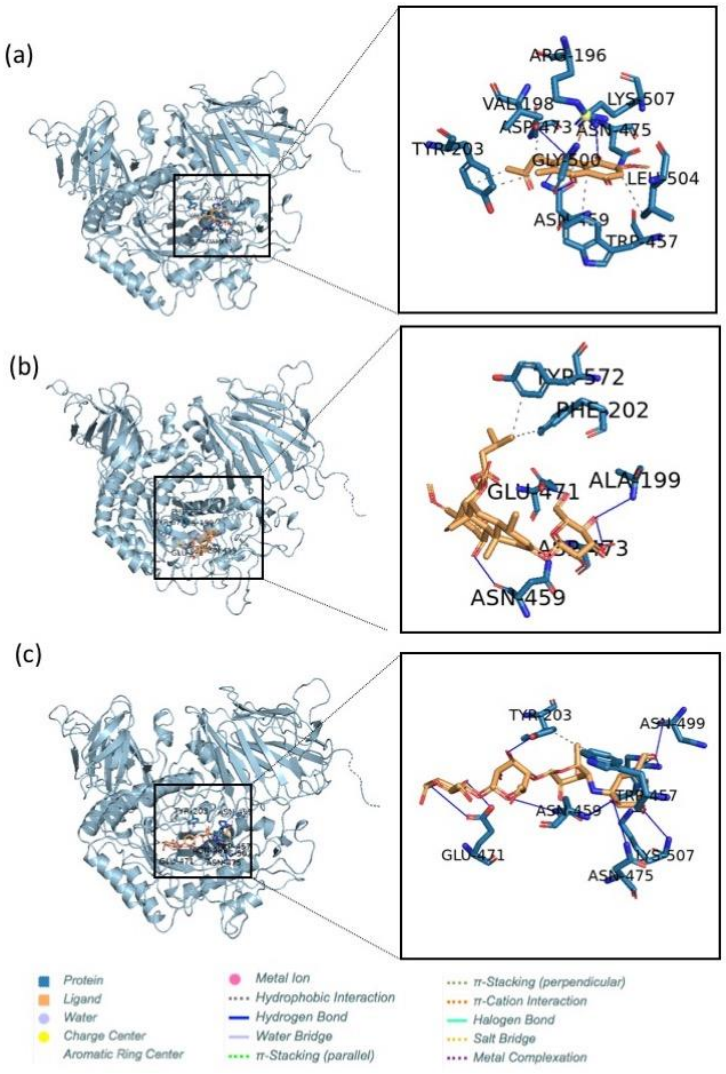


Figure 7. The binding pocket of the ligands into the site of receptor (alpha glucosidase). (a) Model 1, (b) Model 2, and (c) Model 3. The 3D conformation of the complex was visualized by PLIP program combined with PyMOL v.2.3 software.

Conclusion

In this study, a bioinformatics approach using homology modeling combined with molecular docking was applied to investigate active compounds of Makassar fruit (*Brucea javanica*) as antidiabetic. Alpha glucosidase is used as a receptor, while two active compounds, including javanicin and javanicisode B of *Brucea javanica*, are used as ligands. From our results, all ligands have the potential to bind to the receptor due to their negative energy scores. Besides, javanicin (-8.0 kcal/mol) has larger binding energy than control (-5.7 kcal/mol), indicating strong binding to the AG. Furthermore, these two active compounds can participate in the binding site of AG via hydrogen bonds and hydrophobic interactions. Thus, our finding implied that two active compounds of *Brucea javanica* could become inhibitors for AG enzyme, and javanicin may become a promising drug against diabetes.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

References

Ablat, A., Mohamad, J., Awang, K., Shilpi, J. A., & Arya, A. (2014). Evaluation of antidiabetic and antioxidant properties of *Brucea javanica* seed. *The Scientific World Journal*, *2014*.

Arwansyah, A. (2015). Simulasi Molecular Docking Senyawa Kurkumin dan Analognya Sebagai Selective Androgen Receptor Modulators (SARMs) pada Kanker Prostat. *Dinamika*, *5*(2).

Arwansyah, A., Arif, A. R., Ramli, I., Hasrianti, H., Kurniawan, I., Ambarsari, L., Sumaryada, T. I., & Taiyeb, M. (2022). Investigation of Active Compounds of *Brucea Javanica* In Treating Hypertension Using A Network Pharmacology-Based Analysis Combined with Homology Modeling, Molecular Docking and Molecular Dynamics Simulation. *ChemistrySelect*, *7*(1), e202102801.

Arwansyah, M. S., Kodama, K., Kurniawan, I., Kataoka, T., Kawaguchi, K., & Nagao, H. (2019). Molecular dynamics study of free energy profile for dissociation of ligand from CA I active site. *Sci. Rep. Kanazawa Univ. ARTICLES*, *63*(63), 15–28.

Dabelea, D., Mayer-Davis, E. J., Saydah, S., Imperatore, G., Linder, B., Divers, J., Bell, R., Badaru, A., Talton, J. W., Crume, T., & others. (2014). Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. *Jama*, *311*(17), 1778–1786.

DeFronzo, R. A., Ferrannini, E., Groop, L., Henry, R. R., Herman, W. H., Holst, J. J., Hu, F. B., Kahn, C. R., Raz, I., Shulman, G. I., & others. (2015). Type 2 diabetes mellitus. *Nature Reviews Disease Primers*, *1*(1), 1–22.

Ernst, E. (2005). The efficacy of herbal medicine--an overview. *Fundamental \& Clinical Pharmacology*, *19*(4), 405–409.

Fiser, A., & Šali, A. (2003). Modeller: generation and refinement of homology-based protein structure models. In *Methods in enzymology* (Vol. 374, pp. 461–491). Elsevier.

Fitrasari, D., Arwansyah, M. S., Kawaguchi, K., Purqon, A., & Nagao, H. (2022). Theoretical Study of Dissociation Process of Plastocyanins by PaCS-MD Simulation. *Journal of Physics: Conference Series*, *2207*(1), 12021.

Gasteiger, E., Hoogland, C., Gattiker, A., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005). Protein identification and analysis tools on the ExPASy server. *The Proteomics Protocols Handbook*, 571–607.

Handayani, I. (2021). *Data IDF Sebut 19,46 Juta Orang Indonesia Mengidap Diabetes*.

Kim, I. H., Suzuki, R., Hitotsuyanagi, Y., & Takeya, K. (2003). Three novel quassinoids, javanicolides A and B, and javanicoside A, from seeds of *Brucea javanica*. *Tetrahedron*, *59*(50), 9985–9989.

Laskowski, R. A., MacArthur, M. W., Moss, D. S., & Thornton, J. M. (1993). PROCHECK: a program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography*, *26*(2), 283–291.

Lin, L.-Z., Cordell, G. A., Ni, C.-Z., Clardy, J., & others. (1990). A quassinoid from *Brucea javanica*. *Phytochemistry*, *29*(8), 2720–2722.

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*(16), 2785–2791.

Natsir, H., Arif, A. R., Wahab, A. W., Budi, P., Arfah, R. A., Arwansyah, A., Fudholi, A., Suriani, N. L., & Himawan, A. (2022). Inhibitory effects of Moringa oleifera leaves extract on xanthine oxidase activity from bovine milk. *Pharmacia*, *69*(2), 363–375.

NoorShahida, A., Wong, T. W., & Choo, C. Y. (2009). Hypoglycemic effect of quassinoids from *Brucea javanica* (L.) Merr (Simaroubaceae) seeds. *Journal of Ethnopharmacology*, *124*(3), 586–591.

Salentin, S., Schreiber, S., Haupt, V. J., Adasme, M. F., & Schroeder, M. (2015). PLIP: fully automated protein--ligand interaction profiler. *Nucleic Acids Research*, *43*(W1), W443--W447.

Sumaryada, T., Roslia, A. W., Ambarsari, L., & Kartono, A. (2016). Molecular docking simulation of mangostin derivatives and curcuminoid on maltase-glucoamylase target for searching anti-diabetes drug candidates. *2016 1st International Conference on Biomedical Engineering (IBIOMED)*, 1–4.

Syahputra, G., Arwansyah, W. K., & Kusharyoto, W. (2018). Molecular Docking and Molecular Dynamics Study of Alcoholic Compounds as Mycobactericidal Agents Using InhA, MabA and PanK as Receptors. *Annales Bogorienses Vol*, *22*(2), 101.