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Study of Molecular Docking of Alkaloid Derivative Compounds from Stem Karamunting (*Rhodomyrtus tomentosa*)Against α-Glucosidase Enzymes

Budi Mulyati[1*], Riong Seulina Panjaitan[2]

[1]Industrial Engineering, Engineering Faculty, Nurtanio University, Bandung
[2]Pharmaceutical science, Pharmacy Faculty, 17 Agustus 1945 University, Jakarta
[*]*Corresponding Author: b.mulyati@unnur.ac.id*

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Abstract

Karamunting plant (Rhodomyrtus tomentosa) is a traditional medicinal plant. The leaves, roots, stems, and fruits of Karamunting have been identified, and their biological activities are antioxidants, antibacterial, antidiabetic, anti-inflammatory and anticancer that contained alkaloids, tannins, and flavonoids. The types of alkaloids found in karamunting stems are homolycorine, ismine, lycorine, maritidine and tazetine. This study aims to determine the binding score of alkaloid-derived compounds with protein α-glucosidase and determine the protein's active site bound to the ligand. The method used in this research is Protein-Ligand ANT-System (PLANTS). The results showed that the anchoring score of homolycorine was -60.83 kcal/mol, ismine -64.42 kcal/mol, lycorine -71.20 kcal/mol, maritidine -61.82 kcal/mol, and tazetine -65.02 kcal/mol. The active sites used for binding are Glu526, Gly555, and Pro556. The average score for anchoring alkaloid-derived compounds with protein α-glucosidase is 83.84%. This number indicates that karamunting stems can be used as antidiabetic.

Keywords: Karamunting, alkaloids, antidiabetic, docking, PLANTS.

INTRODUCTION

Diabetes Mellitus is one of the non-contagious diseases and is one of the comorbid or accompanying diseases in patients infected with Covid-19. Based on data from the International Diabetes Federation (2020), Indonesia ranks 7th out of 10 countries with the highest number of diabetes patients. There are more than 10.8 million people with diabetes in Indonesia. The prevalence of diabetes mellitus shows an increase with the patient's increasing age, which reaches its peak at the age of 55-64 years (Widiastuti, 2020). Various efforts to prevent and treat diabetes continue to be made, including developing various types of treatment. One of them is to look for new sources of medicine from natural ingredients. Many herbal plants have antidiabetic activity because they have active compounds that play a role, including flavonoids and alkaloid.

Treatment of diabetes mellitus can be done medically or traditionally. Medical treatment can be done orally or by injection, namely insulin, but the cost of this treatment is relatively expensive if used continuously. Alternative medicine by taking traditional medicine is an option because it is obtained easily. One of the plants used as traditional medicine by the people of Kalimantan is karamunting (*Rhodomyrtus tomentosa*). This plant belongs to the Myrtaceae family and has the international name Rosemyrle (Ningrum, Purwanti, & Sukarsono, 2016).

Karamuntings' stem contains flavonoids, alkaloids, saponins, tannins, terpenoids, and phenolics. Alkaloid compounds are effective as anti-diarrhea, anti-diabetic, anti-microbial, and anti-malarial. Still, some alkaloid compounds are toxic, so it is necessary to identify alkaloid compounds whose benefits can be known. Furthermore, Ningrum, Purwanti, & Sukarsono (2016) research showed that the alkaloids found in the karamunting plant were Maritidine, Homolycorine, Ismine, Tazettine, and Lycorine (Ningrum et al., 2016). α-Glucosidase inhibitor is a type of drug for type 2 diabetes that works to block starch metabolism by inhibiting enzymes in the intestine to break down carbohydrates to slow down glucose absorption (Ak, Juliani, Sugito, & Abrar, 2019; Laily & Khoiri, 2016; Ouassou et al., 2018; Yin, Zhang, Feng, Zhang, & Kang, 2014).

There are two approaches in computational chemistry, namely molecular mechanics and electron structure theory. Molecular mechanics is an empirical method used to express the potential energy of molecules as a function of geometric variables.

Molecular mechanics uses the classical mechanics' approach. Molecular mechanics methods are helpful for modeling macromolecular systems but cannot study electron distribution systems such as bond formation or breaking and electron excitation processes (Male, Sutapa, & Ranglalin, 2015). The application of computational chemistry methods in discovering and developing new drug compounds has become popular. This reason is that in silico method offers an economic strategy and an effective effort to find new drugs by utilizing computer capabilities in performing simulations and calculations such as optimization of activity, geometry, and reactivity before compounds are experimentally synthesized (Kilo, Aman, Sabihi, & Kilo, 2019). Docking is currently the most widely used molecular modeling method.

Gaspersz & Sohilait (2019) have used molecular docking. They have studied about the interaction between mangostin compounds and α-Amilase (Gaspersz & Sohilait, 2019). The binding has three main objectives: predicting the binding of the active site of a new ligand using virtual screening and predicting the binding affinity between the compound and the active site of the known ligand. One of the software that supports protein ligand-receptor binding is PLANTS. PLANTS is a free application that has the same quality as other paid docking applications. In addition, the advantages of the PLANTS software are that it is simple and easy. However, PLANTS does not provide the function of protein preparation, ligands, or visualization, so additional applications are needed (Purnomo;, 2011). Research conducted by (Dewijanti, Mangunwardoyo, Dwiranti, Hanafi, & Artanti, 2020) on the activity of bay leaf extract, which contains alkaloids, flavonoids, tannins, steroids, and phenols, can interact with proteins, inhibiting the work of the α-glucosidase enzyme (Dewijanti, Mangunwardoyo, Astari Dwiranti, Muhammad Hanafi, & Nina Artanti, 2020)

The purpose of this study was to determine the binding score of the ligands of alkaloid-derived compounds with protein α-glucosidase and to find the active site of binding between alkaloid-derived compounds that can bind to protein α-glucosidase. By knowing the percentage of the anchorage score, it can be concluded that the tendency of alkaloid-derived compounds to bind to α-glucosidase protein can be concluded.

METHODOLOGY

Hardware

The hardware used in this research is a set of computers with processor chips Core™ i5-1135G7 Processor 2.4GHz, 8.00 GB DDR4, VGA Card Intel® Iris® Xe Graphics.

Software

The software used in this research is *Marvinsketch* version 5.2.5.1, *Marvinspace* version 5.2.5.1, *PLANTS (Protein-Ligand ANT-System)*, YASARA, and Discovery Studio 2021. The online database program used by PDB (Protein Data Bank) with code 2JKE (https://www.rcsb.org/structure/2JKE), online PASS (*Prediction of Activity Spectra for Substances*).

Procedures

Protein Validation.

The study started with protein preparation. The protein used was α-Glucosidase from Thetaiotamicron bacteria with deoxynojirimycin ligand. This protein structure was taken from the protein data bank with the code 2JKE (https://www.rcsb.org/structure/2JKE). The validation process is intended to predict the position and interaction of the protein α-glucosidase and the natural ligand deoxynojirimycin. This validation process begins by removing the ligands and metal calcium (Ca) that are not needed. Then the natural ligands were prepared using Marvin Sketch version 5.2.5.1 by protonating at pH 7.4. Determination of pH 7.4 because the pH is the pH of the human body.

Furthermore, the natural ligands were searched for the best conformation of the ten conformations in the Marvin Sketch application. The best conformation is the conformation of the ligand, which has the lowest energy, then is stored in the form of .mrv (Ruswanto, 2015). Then the ligands with ten confirmations were optimized with the protein crystal structure using the PLANTS software program to obtain a docking score. Then the best score is selected and saved (in mole) after that, calculated the value of the RMSD pose of the optimization result regarding the experimental results or protein crystal structure with the Yet Another Scientific Artificial Reality Application (YASARA) program, which aims to test the validation of sample proteins, ligands and device performance hard.

Validation of Alkaloid Derivative Compounds

In this case, the alkaloid derivative compounds, homolycorine, ismine, lycorine, maritidine, and tazettine were tested first on PASS online. Each ligand

was prepared using Marvin Sketch version 5.2.5.1 and conditioned at pH 7.4 according to body human pH with a two-dimensional structure. Then the ligands are stored in the form of 2D ligands. The five ligands were searched for the ten best conformations for the following bonding process.

Binding of ligand and protein molecules

The ten conformational alkaloid derivatives were then docked with α-Glucosidase protein using PLANTS software on grid box x: 24.5376, y: 42.5666, and z: 68.8685, and binding site radius of 8.03809. Furthermore, the results of ligand and protein docking obtained the value of the docking score. The lowest score represents the best conformation of the ligand. Moreover, the proteins and ligands with the lowest score were combined to form new GDP using the YASARA application.

Data analysis

The new PDB from the YASARA application was then analyzed by processing the data obtained from YASARA and then calculated by comparing the anchoring scores between the ligands of alkaloid-derived compounds and proteins and then comparing the protein docking scores with natural ligands. Furthermore, the visualization of the docking results to see the protein's active site that binds to the ligand, the type of bond, and the distance of the bond is visualized in 2 dimensions and three dimensions using Discovery Studio 2021.

RESULTS AND DISCUSSION

Validasi Protein.

α-glucosidase is an enzyme that breaks down complex carbohydrates into simple sugars. The action of the α-glucosidase enzyme can be inhibited by a compound that functions as an inhibitor. Inhibition of the α-glucosidase enzyme is expected to reduce the breakdown of complex carbohydrates into sugar, so blood sugar levels become normal and improve the function of pancreatic cells (Pujiyanto, Sunarno, & Widyasari, 2015). Inhibition of the action of this enzyme can effectively reduce the breakdown of complex carbohydrates and the process of glucose absorption to reduce the increase in postprandial glucose levels in people with diabetes (Afandy, 2017).

This research was started by simulating the binding of the new ligand molecule with the sample protein (α-glucosidase enzyme) by validating the protein and calculating its RMSD value using the YASARA software. Then the results of protein validation with the reference ligand (deoxynojirimycin) were visualized using Pymol software version 2.4.1. The results of the visualization can be seen in Figure 2.

Figure 2. Visualization of the validation results of α-glucosidase and reference ligand

Before docking the ligands of alkaloid-derived compounds, validation was carried out first by re-docking the original ligand with the 2JKE origin receptor. The parameter used is RMSD (Root Mean Square Deviation). The docking method is valid if it has an RMSD 2Å (Puratchikody, Sriram, Umamaheswari, & Irfan, 2016). The smaller the RMSD value, the more similar the position of the docked ligand to the crystallographic ligand (Purnomo, 2011).

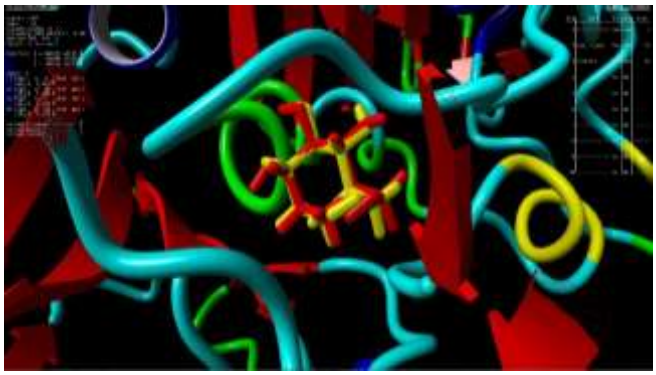

Figure 3 a. RMSD result visualization 0.2990

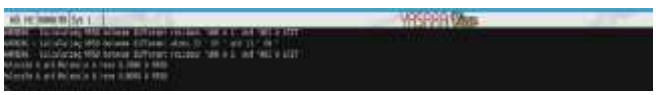

Figure 3b. RMSD value of α-Glucosidase

In this study, the result of 2JKE re-docking with a natural ligand, namely deoxnojirimycin, was 0.2990, indicating that the validated ligands and proteins have met the valid theory criteria. The results of the visualization of the RMSD value of the natural ligand deoxynojirimycin can be seen in Figure 3a. In this picture, the red ligand is the native ligand. At the same time, the yellow one is the ligand that has been

separated from its receptor, and we have tested its molecular bonding with the α-glucosidase receptor. The results of the RMSD value of α-Glucosidase protein as shown in Figure 3b.

Validation of Alkaloid Derivative Compounds

Ligands are organic or inorganic compounds that play a role in interactions in the binding domain of proteins and can affect the activity of these proteins. Based onNingrum, Purwanti, & Sukarsono (2016), karamunting stems contain alkaloid-derived compounds, namely, Maritidine, Homolycorine, Lycorine, Ismine and Tazetine, where homolycorine and maritidine are ligands in large quantities (Ningrum, Purwanti, & Sukarsono, 2016). Ligands will be used for the anchoring process adjusted to human conditions, namely pH 7.4. Figures 4a to 4e are the chemical structures of the ligands of alkaloid-derived compounds used in this study. We have tested the ligands with PASS online. PASS online is a software product designed to evaluate the general biological potential of organic drug-like molecules. PASS provides a simultaneous prediction of various types of biological activity based on the structure of organic compounds. Thus, PASS can estimate the biological activity profile for virtual molecules before their chemical synthesis and biological assays. If the Pa value is more than 0.7, then the compound we are studying is included in the sub-class of active compounds. The online PASS test results for these five ligands are > 0.8.

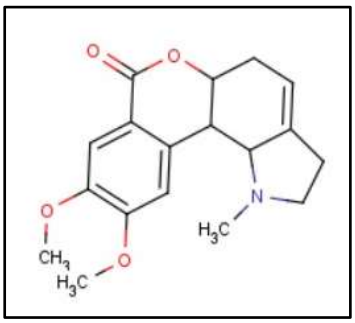

Figure 4.a. Homolycorine Structure

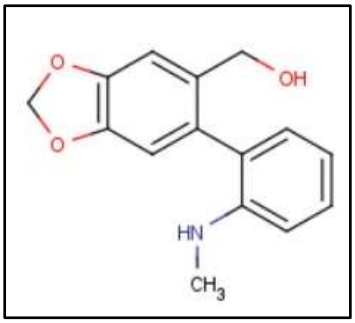

Figure 4.b. Ismine Strucuture

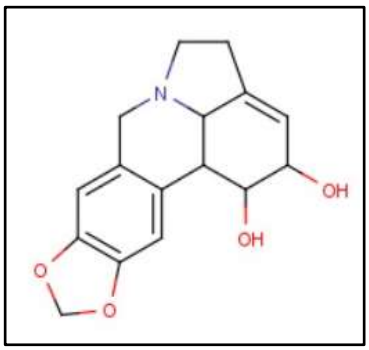

Figure 4.c. Lycorine Structure

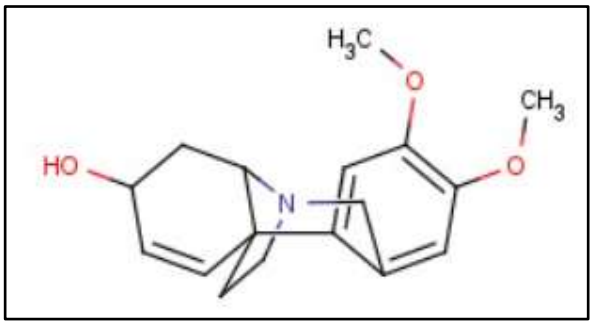

Figure 4.d. Maritidine Structure

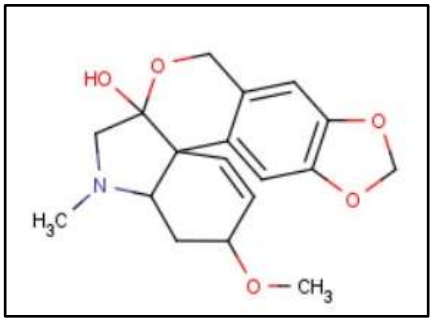

Figure 4.e. Tazetine Structure

Data Analysis

The docking is carried out between the target protein with the test ligand and the target protein with the comparison ligand. The result of docking is a grid score. The grid score is the energy required by the ligand bind to the protein. This energy is Gibbs energy. More negative energy, means the bond between the ligand and protein is getting stronger.

The results obtained from the test ligand and target protein binding and the comparison ligand with the assumption that the interaction of α-Glucosidase complex formation with validated deoxynomirijin is 100%, so the percentage of α-Glucosidase interaction is shown as shown in Table 1.

Table 1. The score of ligand binding with α-Glucosidase with natural ligands as comparison

Ligand	Docking Score (Kkal/mol)	Percentage (%)
Deoxynojirimycin	-77.12	100
Homolycorine	-60.83	78.87
Ismine	-64.42	83.53
Lycorine	-71.20	92.32
Maritidine	-61.82	80.16
Tazzetine	-65.02	84.31

In binding homolycorine molecules and protein α-Glucosidase, the binding score is -60.83 kcal/mol compared to standard ligands, which is a 78.87% tendency for homolycorine to form bonds with proteins, which is quite large. There are hydrogen bonds in Arg 551, Asn 525, Glu526, Pro 556, and hydrophobic interactions at residues Pro 512, Gly555, Pro 556, Ala 528, Arg 551, and Met 557. The O1 atom of the homolycorine ligand and the protonated N atom that positively charged Arg 551 protein has an "attractive charge" or a salt bridge of 2.83. The hydrogen bonding of the salt bridge causes the stability of the bond between the homolycorine ligand and the -glucosidase receptor. Visualization results between -Glucosidase protein and homolycorine ligand can be seen in Figure 5.

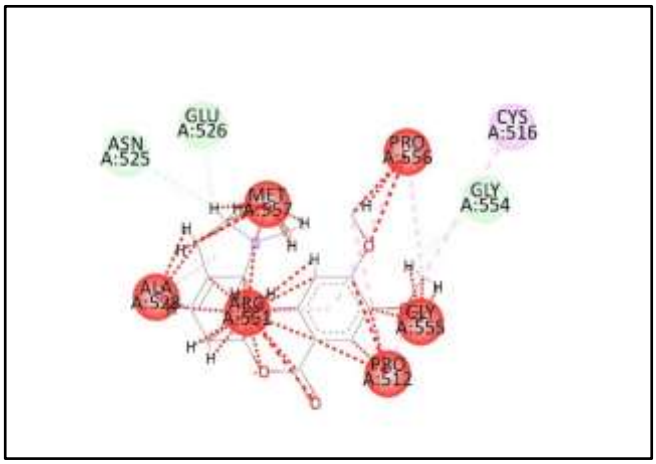

Figure 5. Visualization of α-Glucosidase and homolycorine

In the binding of protein molecules, α-Glucosidase and ismine have a binding score of -64.42 kcal/mol. Ismine and protein scores are lower than homolycorine and protein scores due to the presence of the OH functional group so that it is easy to form hydrogen bonds with amino acids in α-Glucosidase proteins. There are two hydrogen bonds with amino acid residues in the visualization of the binding between the protein α-Glucosidase and the ismine ligand (Figure 6). The hydrogen bond between Val 553 and Arg 551 and the hydrophobic interaction of the ligand with amino acid residues, namely Pro512, Pro556, Gly 555, Ala 528, Arg 55, and Met 557.

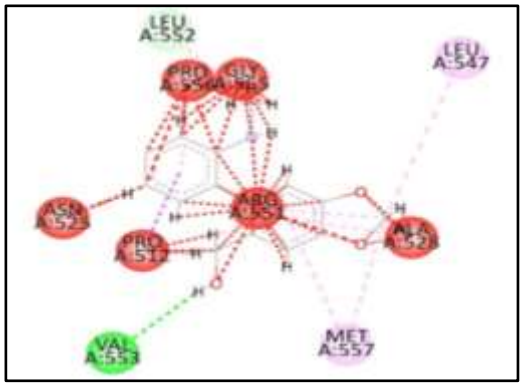

Figure 6. Visualization Protein with ismine

In the binding of protein molecules, α-glucosidase and lycorine have a binding score of -71.20 kcal/mol. This anchoring score is the best bonding score of all ligands derived from alkaloid compounds because the chemical structure of lycorine ligands has two OH groups. It is easy to form hydrogen bonds with amino acids. As shown in Figure 7, the ligands include five hydrogen bonds with the amino acids Gly 555, Asn 525, Arg 551, Ser 527, and Gly 524, with bond distances ranging from 1.61Å - 3.04. The presence of a lone pair of electrons from oxygen, as an electron donor binds Gly 555 to form good stability so that the highest docking score reaches 92.32%. Hydrophobic interactions are with Glu 526, Ala 528, Arg 551, Pro 512, Gly 555, Pro 556, and Met 557.

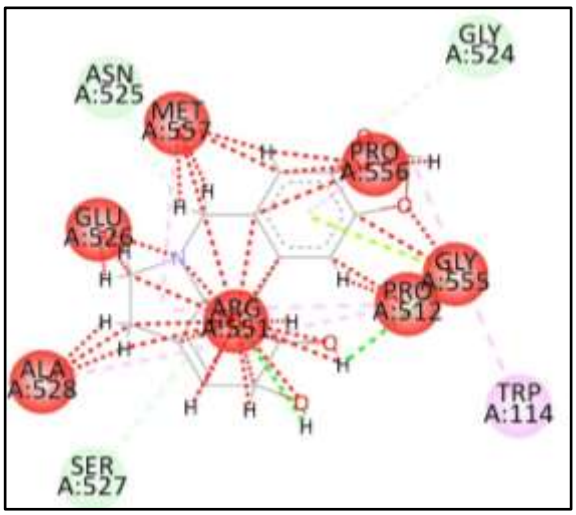

Figure 7. Visualization of -Glucosidase and lycorine.

In binding protein molecules, -Glucosidase and maritidine have a binding score of -61.82 kcal/mol. The binding score between maritidine and protein α-glucosidase is good because there are two hydrogen bonds, Glu 526 and Leu 552. Hydrophobic interactions are with Pro 512, Gly 555, Pro 556, Asn 525, Arg 551, Met 557, Glu 526, and Ala 528. The bonding scores between maritidine and protein are not as good as lycorine. This scores because the structure of maritidine only has three oxygen atoms. The hydrogen bonds formed between the oxygen atoms of the amino acid Glu 526 and lycorine. This oxygen atom binds to three carbon atoms of the lycorine (ligand), namely C14, C15, and C16. The Oxygen atoms of Leu 552 attaches to the C9 and N1 of lycorine. The presence of one OH group on maritidine resulted in a better maritidine score than homolycorine. Visualization of the binding of maritidine ligands and α-Glucosidase protein can be seen in Figure 8.

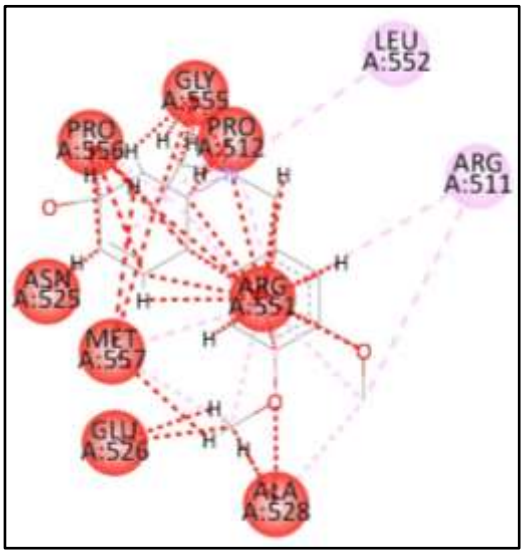

Figure 8. Visualization of α-Glucosidase and Maritidine

The binding score between tazzetine and protein-glucosidase is low at -65.02 kcal/mol because there are four amino acids, namely Glu526, Asn525, Gly555, and Pro556, which form bonds with ligands and nine amino acids that form hydrophobic bonds, namely Arg511, Pro512, Ile523, Gly524, Ser527, Ala528, Leu552, Val553, and Gly554. The binding score of tazzetine and protein is better than the other three ligands because tazzetine has four oxygen atoms and one OH group in its structure. The oxygen atom has a lone pair of electrons to become an electron donor to the surrounding amino acids and an OH group that can form hydrogen bonds with amino acids. Two nitrogen atoms of Asn525 bind to the O4 atom of the ligand with bond lengths of 2.51Å and 3.24Å. The oxygen atom of Ser527 binds to the C6 atom of the ligand. The Oxygen atom of Gly555 binds to C16 of the ligand. The oxygen atom of Pro556 attaches to the C13 atom. The visualization results of -Glucosidase protein and Tazzetine ligands can be seen in Figure 9.

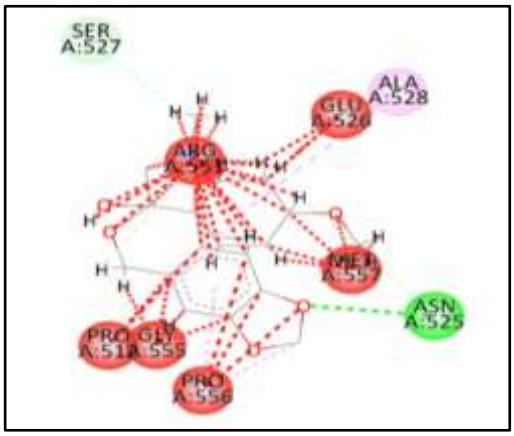

Figure 9. Visualization of α-Glucosidase and Tazzetine

The results summary of binding to the α-Glucosidase receptor and each ligand regarding the type of bond and the bond distance can be seen in Table 2.

Table 2. Ligand interactions with amino acid residues in α-Glucosidase

Ligand	Interaction with Amino Acid residues			
	Amino Acid Residu	Bond Type	Bonding Distance	Hydrophobic
Homolycorine	Asn 525	Hydrogen Bond	2.84 Å	Pro 512
	Glu 526	Hydrogen Bond	1.57 Å	Cys 516
	Pro 556	Hydrogen Bond	1.42 Å	Ala 528
	Arg 551	Hydrogen Bond	2.79 Å	Arg 551
			2.32 Å	
Ismine	Val 553	Hydrogen Bond	2.61 Å	Pro512
	Val 553	Hydrogen Bond	2.02 Å	Pro556
	Arg 551	Hydrogen Bond	2.86 Å	Gly 555
				Ala 528
				Arg 551
				Met 557
Lycorine	Asn 525	Hydrogen Bond	2.46 Å	Glu 526
	Arg 551	Hydrogen Bond	2.20 Å	Ala 528
	Gly 524	Hydrogen Bond	2.75 Å	Arg 551
	Ser 527	Hydrogen Bond	3.04 Å	Pro 512
	Gly 555	Hydrogen Bond	1.61 Å	Gly 555
				Pro 556
				Met 557
Maritidine	Glu 526	Hydrogen Bond	1.27 Å	Pro 512
	Leu 552	Hydrogen Bond	1.33 Å	Gly 555
				Pro 556
				Asn 525
				Arg 551
				Met 557
				Glu 526
				Ala 528

Tazetine	Asn 525	Hydrogen Bond	2.50 Å	Arg511
	Ser 527	Hydrogen Bond	2.03 Å	Pro512
	Arg 551	Hydrogen Bond	2.38 Å	Ile523,
	Gly 555	Hydrogen Bond	1.95 Å	Gly524,
	Pro 556	Hydrogen Bond	1.65 Å	Ser527,
				Ala528,
				Leu552,
				Val553
				Gly554.

CONCLUSION

Based on the discussion results, the anchoring scores of alkaloid-derived compounds with-glucosidase are as follows: homolycorine -60.83 kcal/mol, ismine -64.42 kcal/mol, lycorine -71.20 kcal/mol, maritidine -61.82 kcal/mol and tazetine -65.02 kcal/mol. The active sites used for binding were Arg 551, Gly 555, and Pro 556. The average score for binding of alkaloid-derived compounds to -glucosidase protein was 83.84%, indicating that karamunting stems can be used as candidates for diabetes drugs in silico.

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