ORIGINAL ARTICLE

Evaluation on the Potential of *Ganoderma lucidum* Bioactive Compounds as Alpha-Glucosidase Enzyme Inhibitor: A Computational Study

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ABSTRACT

Introduction: Computational simulation study was carried out on bioactive compounds of Ganoderma lucidum (G. lucidum). **Methods:** Molecular docking and molecular dynamics (MD) simulations were performed. The input files for protein and ligands were retrieved from Protein Data Bank (PDB) and PubChem database. Human maltase-glucoamylase (PDB ID: 3L4Y) is the protein (α-glucosidase enzyme). The ligands are thirteen compounds derived from G. lucidum together with acarbose and miglitol as controls. **Results:** Docking result showed the lowest binding energy is from Ganomycin B (-7.8 kcal/mol) compared to acarbose and miglitol (-5.0 kcal/mol and -4.4 kcal/mol) respectively. MD simulation showed interaction of 3L4Y-Ganomycin B achieved stable interaction and conformation as follows: root mean square deviation (RMSD) is ± 2.7 E, average distance of ±1.8 E and constant hydrogen bonds around 1 - 3. **Conclusion:** Ganomycin B was found to have good binding affinity, embarking its potential as a potent α-glucosidase inhibitor.
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Keywords: α-glucosidase inhibitor, Ganoderma lucidum, molecular docking, molecular dynamics, protein-ligand binding

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INTRODUCTION

Postprandial hyperglycaemia is a condition of high blood glucose level after taking a meal which defines the character of individuals with diabetes. It happened because the insulin in their body is unable to regulate their body's blood glucose level either due to failure of insulin production by the body (Type 1 Diabetes Mellitus) or desensitization of the body's insulin receptor (Type 2 Diabetes Mellitus). Furthermore, Type 2 Diabetes Mellitus (T2DM) has become a major contributor to the increase in non-communicable disease cases (1, 20). It has become a public concern as the rate of people diagnosed with T2DM kept increasing over the year.

Present therapeutic approaches suggest the use of acarbose and miglitol in reducing postprandial hyperglycaemia in T2DM management. The medications are oral anti-diabetic drug that serves as an inhibitor of the α-glucosidase enzyme. This enzyme helps break down complex carbohydrates into simpler molecules which can later be absorbed by the intestine. Hence, the blood glucose level able to be controlled. However, both medicinal drugs are reported to have adverse health effects on the human body such as diarrhoea and abdominal pain (1, 16, 21).

Hence, finding another alternative drug with lesser or no adverse effects is important. In this research, a computational study was carried out on the usage of bioactive compounds from a medicinal mushroom, *Ganoderma lucidum* (*G. lucidum*) to inhibit the α-glucosidase enzyme activity. The binding affinity of the bioactive compounds with α -glucosidase enzyme was measured using bioinformatics analysis. Acarbose and miglitol served as the positive control in the comparison of the binding affinity of the bioactive compounds from *G. lucidum*.

MATERIALS AND METHODS

Preparation of input files (protein and ligand)

The α-glucosidase enzyme was retrieved from PDB database. The three-dimensional (3D) crystal structure of human maltase-glucoamylase (PDB ID: 3L4Y), an enzyme belonging to the α-glucosidase family with 1.8 E resolution, was selected for its suitability for this study. The resolution value below 2.0 E indicates that the structure was considered acceptable for computational study (2). To eliminate nonessential ligands, 3L4Y enzyme was visualized in PyMOL (3) and all heteroatoms were removed to prepare an apoenzyme for molecular docking approach.

From the literature, 13 bioactive compounds from *G. lucidum* were identified as good and potent α-glucosidase inhibitors. Bioactive compounds (with PubChem ID) such as Ganomycin B (ID: 10246918), Ganomycin I (ID: 75079690), Lucidenic acid E (ID: 23247892), Lucidenic acid F (ID: 23247893), Ganoderlactone D (ID: 134715244), Ganoderol B (ID: 13934286), Ganoderiol F (ID: 471008), Dihydroagnosterol (ID: 11826075), Ganoderic acid DF (ID: 57402147), Ganoderenic acid A (ID: 6442088), Ganoderenic acid B (ID: 71457627), Ganoderic acid A (ID: 471002) and Ganoderic acid B (ID: 471003) were retrieved from PubChem database. For comparison, the control compound, acarbose (ID: 41774) and miglitol (ID: 441314) were also referred to and downloaded from the same database.

Molecular docking

The initial preparation of files for the docking analysis involved using AutoDock Tools (ADT) version 4.2 (4). Polar hydrogen atoms were added to the protein structure to address the absence of hydrogen atoms in the PDB file format. These polar hydrogen atoms play a crucial role in accurately representing the spatial and chemical properties of the protein, particularly its ability to form hydrogen bonds with ligands during docking simulations. Furthermore, the partial atomic charges, including Kollman Charges and Gasteiger Charges, were assigned to both the protein and ligand molecules, facilitating the calculation of the scoring function. Kollman Charges are derived from the CHARMM force field and provide a realistic description of the electrostatic properties of the molecules. Gasteiger Charges, on the other hand, are widely used to estimate partial atomic charges based on the atom's local environment. These charges help in quantifying the extent to which atoms participate in bonding and non-covalent interactions. The entire structures, now complete with atom type information, were saved in the PDBQT format.

In their study, Ibrahim et al. (5) identified that the protein 3L4Y had five active site residues, namely Asp203, Asp327, Arg526, Asp542, and His600. The binding region for this protein was specified as a grid map of dimensions 22 Ч 20 Ч 24 points, with a grid spacing of 1.0 E. The center of the grid box was precisely determined at coordinates X= 2.129, Y= -15.311, and Z= -23.182. As a result, all subsequent docking operations were confined within the boundaries of this grid box.

Given the extensive number of compounds requiring analysis, employing ADT version 4.2 for molecular docking became a time-consuming task, as it only permitted the use of one protein and one ligand at a time. To streamline the process, the molecular docking analyses for all proteins and ligands were conducted using AutoDock Vina software.

The molecular docking process was configured to generate ten of the best-docked conformations for each protein-ligand interaction, providing a comprehensive view of potential binding configurations. The assessment included the determination of binding energy, the number of hydrogen bonds, the distances of hydrogen bond interactions, and the identification of interacting residues. Subsequently, the structure resulting from the docking analysis with the lowest binding energy level was extracted for use in molecular dynamics simulations.

Molecular dynamics simulation

Preparation of protein and ligand topology

The protein-ligand complexes, namely 3L4Y-Ganomycin B, 3L4Y-Acarbose, and 3L4Y-Miglitol, were subjected to molecular dynamics simulations using the Gromacs 2016 software. The choice of 3L4Y-Ganomycin B was due to the lowest binding energy when compared to other bioactive compounds from *G. lucidum*. In contrast, 3L4Y-Acarbose and 3L4Y-Miglitol served as control compounds for comparative analysis.

Prior to initiating the molecular dynamics simulation, topology files for the protein and ligand were prepared individually. The GROMOS54a7 force field was applied to the protein preparation, resulting in the creation of three essential files: complex.gro, system.top, and posre.itp, each serving distinct functions.

It is worth noting that GROMACS 2016 software encountered limitations in generating a topology file for the ligand. Consequently, an external tool, specifically the Automated Topology Builder (ATB), was employed to address this issue. To ensure the accuracy of the ligand's representation, hydrogen atoms were incorporated into the ligand structure before its submission to the ATB server for further processing. This step was crucial in comprehensively and reliably describing the ligand's behavior during the subsequent molecular dynamics simulation.

Production run: simulation box setup, solvation, neutralization, energy minimization and equilibration

A simulation box with a cubic shape was generated to

enclose the protein-ligand complex, and this system was solvated with water. Subsequently, the water molecules within the box were replaced with Na+ and Cl- ions to achieve the neutralization of electric charges within the simulation complex (6). This step was pivotal in balancing the overall charge of the protein-ligand complexes, resulting in a neutral system.

The next phase involved conducting an energy minimization process for the entire system, ensuring the absence of steric clashes and guaranteeing the appropriate geometric arrangement of the complex. Following this, an equilibration step was executed, with constraints applied to the ligand to maintain its fixed position. The system was gradually adjusted to a consistent temperature of 303K and pressure of 1 atm over a period of 5000 ps (picoseconds). The integration time step used for these calculations was 2 fs (femtoseconds).

Finally, a molecular dynamics simulation was initiated, employing periodic boundary conditions while keeping the temperature and pressure constant at 300K and 1 atm, respectively. This dynamic simulation spanned a duration of 200 ns (nanoseconds). Throughout this simulation, various data were collected and analyzed, including graphs depicting changes in root-mean-square deviation (RMSD), atomic distance and hydrogen bond as documented in the study (2).

Data analysis

The generated structures resulting from the docking and molecular dynamics simulations were visualized and subjected to analysis using PyMOL. PyMOL was employed for displaying and measuring the hydrogen bond distances between interacting atoms. Moreover, the active sites of the enzymes exhibiting hydrogen bonding interactions were identified and distinguished through the use of appropriate color representations.

Additionally, LigPlot+ (7) software was utilized to present a two-dimensional representation of the molecular interactions occurring between the ligand and the active sites of 3L4Y. This representation included a detailed depiction of hydrophobic interactions and the precise identification of the involved residues.

To further assess the system's dynamic behaviour, graphs illustrating the changes in distance, hydrogen bond formation, and root-mean-square deviation (RMSD) during the molecular dynamics simulation were scrutinized. These plots were generated and analyzed using an application within GROMACS called GRACE (8), facilitating the comprehensive examination of the collected data.

RESULTS

Molecular Docking Analysis

Binding Energy and Hydrogen Bonds

Table I shows the result of molecular docking between protein 3L4Y and ligand compounds from *G. lucidum*. Compounds number 1 (acarbose) and 2 (miglitol) were the control compounds of the study while compounds number 3 until 15 were the bioactive compounds derived mainly from *G. lucidum*. All compounds comprise different values of binding energy after docking at the binding site of the target protein ranging from -3.5 to -7.8 kcal/mol. The binding energy also shows that all of them were in negative values. From the result (Table I), Ganomycin B recorded the lowest binding energy with a scoring value of -7.8 kcal/mol while Ganoderiol F has the highest binding energy value of -3.5 kcal/mol. Ganomycin B also has lower binding energy than the present drugs acarbose and miglitol, with values measured at -5.0 kcal/mol and -4.4 kcal/mol, respectively.

Table I: Binding energy resulted from molecular docking using AutoDock Vina

NO.	COMPOUND	BINDING ENERGY ΔG (kcal/mol)
1.	Acarbose	-5.0
2.	Miglitol	-4.4
3.	Ganomycin B	-7.8
4.	Ganoderic acid A	-7.2
5.	Ganoderlactone D	-7.2
6.	Lucidenic acid F	-7.1
7.	Ganoderol B	-6.7
8.	Ganomycin I	-6.7
9.	Ganoderenic acid A	-6.2
10.	Ganoderic acid B	-6.1
11.	Ganoderenic acid B	-6.0
12.	Dihydroagnesterol	-6.0
13.	Lucidenic acid E	-5.9
14.	Ganoderic acid DF	-5.7
15.	Ganoderiol F	-3.5

The formation of hydrogen bonds within the protein-ligand complex, involving Acarbose, Miglitol, and Ganomycin B, is presented in Table II. The results reveal that Acarbose establishes hydrogen bonds with three interacting residues: Thr205, Arg334, and Asp542. The distances for these bonds are 2.1 E, 2.8 E, and 2.2 E, respectively. When it comes to Miglitol, four hydrogen bonds are observed with interacting residues, specifically Arg202, Thr205, and Asp542, at stable distances of 3.1 E, 2.1 E, 2.1 E, and 2.0 E, respectively. Notably, Asp542 participates in two hydrogen bonds with the ligand.

Table II: Docking analysis for the control compound and the best rank of the binding compound.

Ligand	No. of H-bond	Interacting residues	Distance (E)	Binding Energy (kcal/mol)
Acarbose	3	Thr205	2.1	-5.0
		Arg334	2.8	
		Asp542	2.2	
Miglitol	4	Arg202	3.1	-4.4
		Thr205	2.1	
		Asp542	2.1	
		Asp542	2.0	
Ganomycin B	3	Asp327	1.9	-7.8
		Arg526	2.9	
		Asp542	3.1	

In the case of Ganomycin B, three residues are found to engage in hydrogen bonding with the ligand, namely Asp327, Arg526, and Asp542. The distances for these interactions are 1.9 E, 2.9 E, and 3.1 E, respectively. Notably, all of these interacting residues are situated in the protein's binding site.

Hydrophobic interaction of the protein-ligand binding

As shown in Table III, Ganomycin B has the highest number of hydrophobic interactions compared to Acarbose and Miglitol with a total of fifteen amino acids involved. The control compounds formed hydrophobic interactions with eight and two residues, respectively. Miglitol formed more hydrogen bonds than any other compound tested but it has the least hydrophobic interaction. There are six identical amino acid residues formed hydrophobic interaction in the protein-ligand binding of 3L4Y-Acarbose and 3L4Y-Ganomycin B. The similar interacting residues are Met444, Phe450, Phe575, Ala576, Gln603 and Tyr605.

Molecular dynamics analysis

Molecular dynamics simulation is necessary for computational drug discovery to replicate the conditions of aqeous experiments, in vivo or in vitro (12). The 3L4Y-Ganomycin B complex is used to further analysed in molecular dynamics simulation.

Table III: Residues that formed hydrophobic contact with 3L4Y

Compound	No. of residues	Interacting residues
Acarbose	8	Trp406, Met444, Phe450, Phe575, Ala576, Gly602, Gln603, Tyr605
Miglitol	2	Thr204, Lys480
Ganomycin B	15	Asp203, Thr205, Tyr299, Ile364, Trp406, Trp441, Asp443, Met444, Phe450, Trp539,Asp542, Phe575, Ala576, Gln603, Tyr605

Overall stability

Root mean square deviation (RMSD)

RMSD is the mean atom displacement at a moment of simulation (13). During the molecular dynamics simulation, the target protein frequently deviated from its initial conformation to achieve stable interaction with the ligand. The value on average of atom deviate during the interaction can be observed in RMSD (14). Based on Figure 1a, the RMSD value begin around ± 2.7 E at the initial time of the simulation. At approximately 150 ns, it is clear that the RMSD value remains constant and reach plateau around 2.7 ± 0.2 nm until the end of the simulation.

Local binding conformations

Minimum distance

To ensure the physical validity of the binding in the protein-ligand complex, the analysis of atomic distances is a critical step. In the case of the 3L4Y-Ganomycin B complex, the average minimum distance observed is approximately ±1.8 E. Figure 1b, as referenced in the analysis, provides a visual representation of how the distance within this complex behaves over the course of the simulation. The data from this figure demonstrates that the distance between the atoms remains stable and consistently maintained throughout the simulation. This stability of the atomic distance in the complex strongly supports the notion that the binding interaction between Ganomycin B and the 3L4Y protein is not only physically valid but also robust and enduring over the simulated period.

Hydrogen bond

The examination of hydrogen bonds provides crucial

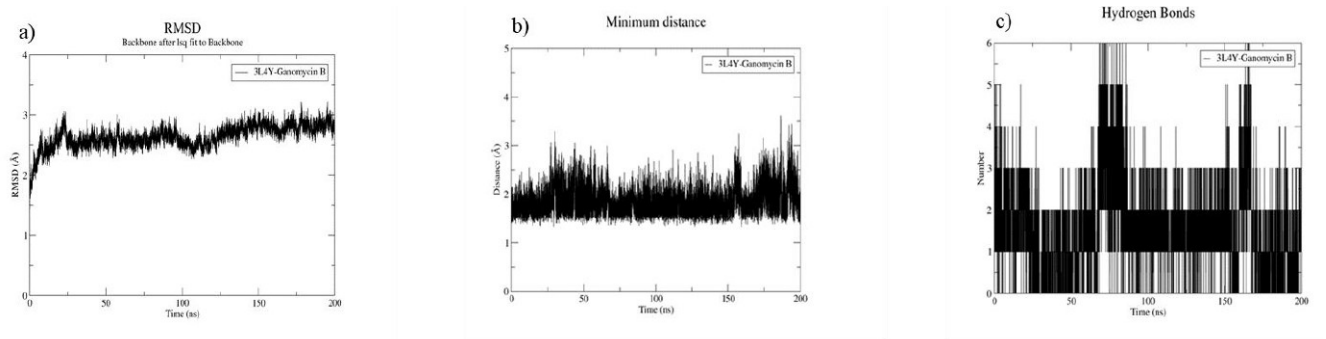

Figure 1: Molecular dynamics simulation analysis. a) Root Mean Square Deviation (RMSD) b) Minimum Distance c) Hydrogen Bonds

insights into the strength of binding. These hydrogen bonds are pivotal in ensuring the proper association between the protein and the ligand. Figure 1c presents a graphical representation to ascertain whether the protein structure can consistently identify and bind with Ganomycin B throughout the simulation.

As depicted in Figure 1c, the number of hydrogen bonds remains relatively constant, typically fluctuating between 1 and 3 bonds. This observation signifies a positive and consistent affinity of the 3L4Y protein towards Ganomycin B. It suggests that the protein's structure effectively recognizes and interacts with Ganomycin B throughout the simulation, reinforcing the notion of a stable and specific binding interaction between the two entities.

DISCUSSION

Molecular docking between protein 3L4Y and ligand compounds from *G. lucidum* was performed by using AutoDock Vina. AutoDock Vina is a program that utilized a specific scoring function and global optimization algorithm for predicting the most likely conformation when ligands bind to a protein (9). Docking result from AutoDock Vina usually presents the binding energy (ΔG) from the interaction of protein-ligand interaction. As confirmed by Afriza et al. (10) protein-ligand binding can occur naturally when the binding energy is negative and the binding energy value represents the stability of the protein-ligand interaction. Hence, all tested compounds from *G. lucidum* for docking have the potential to establish potent and stable complexes with the target 3L4Y protein. Among all compounds, Ganomycin B recorded the lowest binding energy. The lower the value indicates a higher binding affinity of Ganomycin B compared to all compounds. Both protein folding and protein-ligand binding are likely to occur when binding energy is low (10). The complex's conformation will also be at the most stable state without much interference from the molecular interaction. Since Ganomycin B has the best docking score compared to other compounds, it is chosen further for simulation analysis in this study.

Ganomycin B and the control compounds were further analyzed in ADT and PyMOL software to locate the hydrogen bond formed between protein-ligand and identify the distance of hydrogen bond interaction. Hydrogen bonding is necessary to be studied in protein-ligand binding as hydrogen bonds are also known as protein-ligand promoters (11). Hydrogen bonds are one type of molecular interaction that form with selective protein amino acid residues to determine the preferable conformation of an interaction (10). The distance of the hydrogen bond formed is vital in determining the stability and conformation of protein-ligand binding. The acceptable range or distance of hydrogen bond is within 1.0 E to 3.5 E meanwhile good and stable hydrogen bonds are at a distance of 2.8 E (11). As shown in Table 2, all hydrogen bonds formed in these three compounds were still within the acceptable range for hydrogen bonding regulation. Arg334 that form bonding with Acarbose has the most stable interaction with 2.8 E. Besides, Thr205 formed bonds consistently in Acarbose and Miglitol specifying that it is stable in both interactions. Specifically, the amino acid residue that is compatible with three of the compound studied is Asp542. The most interesting part of the findings, all amino acid residues that form hydrogen bonds with Ganomycin B are part of the active site cited by the literature. In literature, five active sites were listed for protein 3L4Y which were Asp203, Asp327, Arg526, Asp542and His600 (5). Three of the active sites which are Asp327, Arg526 and Asp542 form hydrogen bonding with the bioactive compound Ganomycin B. In addition, Ganomycin B also shows the highest number of hydrophobic interactions compared to Acarbose and Miglitol. As asserted by Afriza et al. (10), hydrophobic interaction is the predominant contributor to the stability of proteins. Even though hydrogen bonding also enhances protein stability, but the impact is supported by hydrophobic interaction. This hydrophobic is among major determinant of folding configuration stability in many protein molecules. In our study the finding strengthens the hypothesis that Ganomycin B is a potent compound in new drug discoveries to treat T2DM. It has the lowest binding energy supported by the evidence of stable three hydrogen bonding and fifteen hydrophobic interaction formed during the binding with protein receptor, which also the highest compared to the controls.

The 3L4Y-Ganomycin B complex undergoes a comprehensive examination using molecular dynamics simulation as a means to validate the results obtained from the initial molecular docking analysis. This extended analysis includes an assessment of overall stability, as measured by Root-Mean-Square Deviation (RMSD), and a localized evaluation of conformational changes. The RMSD analysis is particularly revealing. It demonstrates that there are no significant spikes or abrupt deviations within the defined time intervals, indicating that the protein remains remarkably stable throughout the simulation. Beyond the 150 ns mark, the graphical representation displays minimal deviations, signifying that the protein's conformation has achieved a state of definitive stability. This suggests that the protein structure has successfully reached its equilibrium within the simulation timeframe.

Furthermore, the investigation extends to the minimum distance involved in the binding interaction of the complex. This distance demonstrates binding stability and validity across the entire 200 ns simulation duration. The corroborating factor for this stability is the analysis of hydrogen bond lengths. This analysis reveals that the hydrogen bonds remain consistently intact throughout the simulation. Such constancy suggests that the protein-

ligand complex remains stable, with the ligand securely bound to the protein's active site.

CONCLUSION

In summary, molecular docking and dynamics simulations are valuable computer-based tools for assessing potential inhibitors and improving lead compounds. In a previous study, it was found that compounds from Ganoderma lucidum have the potential to slow down the activity of the α-glucosidase enzyme in type 2 diabetes patients. Our molecular docking results indicate that Ganomycin B has the strongest binding with a low energy of -7.8 kcal/mol, surpassing acarbose and miglitol. Ganomycin B also forms more hydrophobic contacts, enhancing its binding in the active site. Additionally, in the 3L4Y-Ganomycin B complex, three key residues (Asp327, Arg526, and Asp542) create hydrogen bonds within the active site. Furthermore, six common interacting residues are involved in hydrophobic interactions between 3L4Y-acarbose and 3L4Y-Ganomycin B.We further assessed the 3L4Y-Ganomycin B complex to validate our docking results using molecular dynamics simulation. This simulation confirms our initial findings and provides a detailed understanding of the complex's stability and consistent binding over time. As a result, Ganomycin B shows promise as a potent compound with potential anti-diabetic effects, making it a potential candidate for further drug development as an alternative to acarbose and miglitol for treating type 2 diabetes.

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