Original Research Article

Structure Based Docking of Secondary Metabolites against Alpha-amylase and Alpha-glucosidase Activities in Treating Diabetes

ABSTRACT

Diabetes mellitus (DM) is a long term disorder of metabolism characterized by high level of blood sugar (hyperglycemia) due to insufficient secretion of insulin, insulin resistance, or both, as well as poor lipid, protein and carbohydrate metabolism. These complications occur as a result of derangement in glucose storage for the regulatory system and metabolic fuel mobilization, including carbohydrate, protein and lipid anabolism and catabolism emanating from impaired action of insulin, secretion of insulin, or both. The *in silico* study was conducted with the help of molecular docking to treat diabetes to inhibit the activities of α-amylase and α-glucosidase by drug molecule. All the studies were based on docking with molecules. The docking was done using a docking software between all the ligands and the target protein receptors. Natural compounds, such as Conduritol A, Catechin and Quercetin were picked, and protein targets as α-amylase and α-glucosidase. Ligands were imported for visual screening into PyRx software while Biovia Discovery Studio Visualizer was used for protein preparation. Analysis of the properties of drug likeliness of the ligands was done via SwissADME online server according to Lipinski's Rule of Five. Final docking analysis was done through AutoDockVina and Biovia Discovery Studio client 2020. Molecular docking analysis of the ligands Conduritol A, Catechin and Quercetin showed strong binding interaction with both α-amylase and α-glucosidase. The test revealed different binding affinities, hydrogen bond interactions, hydrophobicity, solvent accessibility surface (SAS), root mean square deviation lower bound (RMSD LB) and root mean square deviation upper bound (RMSD UB). Conduritol A was the strongest compound against the protein targets, with its low binding strength, according to the PyRx test and Lipinski 's Rule of Five. The same molecules were further docked, and the interactions were visualized under PyMol Via Biovia Discovery Studio. According to the *in silico* study, we have found that these natural compounds can inhibit the activities of α-amylase and α-glucosidase which can be promising drugs for the treatment of diabetes after subjecting them to *in vitro* and *in vivo* studies.

Keywords: Diabetes, Conduritol A, catechin, Quercetin, α -amylase, α -glucosidase, *In silico* analysis.

1.0 INTRODUCTION

Diabetes mellitus (DM) is a long-term disorder of metabolism characterized by high level of blood sugar (hyperglycemia) due to insufficient secretion of insulin, insulin resistance, or both, as well as poor lipid, protein and carbohydrate metabolism [1]. These complications occur as a result of derangement in glucose storage for the regulatory system and metabolic fuel mobilization, including carbohydrate, protein and lipid anabolism and catabolism emanating from impaired action of insulin, secretion of insulin, or both [1,2]. As the condition developed, it causes vascular or tissue destruction, which can lead to serious complications of diabetes like renal disorder, ophthalmology, ulceration, coronary diseases and nervous disorder. Hence, diabetes encompasses a broad spectrum of diverse disorders [3].

Due to its high prevalence rates and high medical cost, diabetes has already become a concern to the global human population as well as individuals. Globally, diabetes has been reported as one of the generally known lifestyle-related non-infectious diseases with a permanent growth in the incidence. In most developed countries, it is one of the major causes of death and there is strong evidence that it is epidemic in many newly industrialized and economically developing countries, during the past two decades, the number of people diagnosed with diabetes has increased. In 2000, about 151 million people worldwide were diagnosed with diabetes [4, 5, 6], in 2010, more than 221 million people were reported to be diabetic and by 2025, about 324 million people have been projected to be diabetic with an estimated global prevalence of diabetes at 9% in 2014 [7]. By 2030, there is possibility that the total number of diabetics would rise to about 439 million worldwide [8].International Diabetes Federation (IDF) reported that, there are over 415 million adults have been estimated to be diagnosed with diabetes in 2015, with the possibility of the figure rising to 642 million adults in 2040, with type 2 diabetes (T2D) accounting for roughly 91% of all the incidences of diabetes. It has also been estimated that 193 million people with diabetes are undiagnosed and about 318 million adults with impaired glucose tolerance [9].

Globally, diabetic people are at high risk for premature death as a result of macrovascular and microvascular diseases; diabetes is primarily a major cause of sightlessness owing to retinopathy, a leading cause of chronic nephropathy as well as end-stage nephropathy that calls for dialysis, as well as other severe morbid conditions including amputation of the lower limb. Diabetes, if left untreated or handled ineffectively, can lead to death. Greater action is needed to improve diabetes outcomes in order to reduce the global burden of diabetes which is today affecting over 425 million people worldwide [10]. Bioinformatics plays a significant role in the search for targets and compounds for the disease treatment. Computational docking is widely used to study the interactions between protein-ligand, and to discover and construct drugs. The procedure usually starts with a well-known target structure, like the crystallographic structure of a medicinal interest catalyst. The tying up is then used to predict small molecular conformation and bind free energy to the target. Single docking experiments are useful in testing target efficiency, and virtual screening is also used to classify new drug development inhibitors when an outsized compound library is docked and rated.

2.0 MATERIALS AND METHODS

2.1 Identification of Compounds

The X-ray crystal structures of α-Glucosidase (PDB ID:3WY1) and α -Amylase (PDB ID: 3BAW) with resolutions of 2.15Å and 2.00Å respectively were downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Database Bank (PDB) (http://www.rcsb.org/pdb).

2.2 Selection of Ligand Molecules

Ligands were selected from various phytochemical constituents of the plants. Such ligand molecules were obtained by PubChem (https:/pubchem.ncbi.nlm.nih.gov/). The ligands were downloaded as 3D structure in sdf format [11]. All downloaded ligand structures were further translated into pdb format through the online SMILES Converter (https:/cactus.nci.nih.gov/ translate/). The converted files are downloaded in the pdb format. These pdb files were used to run different resources and applications.

2.3 Drug Likeliness Property Analysis

The properties of drug likeliness were analyzed using SwissADME online server. The ligands screened were analyzed for their property on drugs. SMILE screened ligand notations were copied from PubChem and pasted on SwissADME online web server [12]. Drugs for the five-fold Lipinski rule were analyzed [13]. The five points of the Lipinski rule are as follows: -

1) The molecular weight should be less than five hundred (500) Dalton.
2) The LogP partition coefficient should be less than five (5).
3) The number of hydrogen bond donors should be less than five (5).
4) The number of hydrogen bond acceptors should be less than ten (10).
5) Not more than one (1) rule can be violated.

The ligands which followed the above Lipinski rule of five were selected for final docking through AutoDockVina and Biovia Discovery Studio Client 2020.

2.4 Ligand Structures

Optimization of all ligand structures in order to remove all strain from the molecular structure was done using the Merck Molecular Force Field (MMFF) and the semi-empirical Austin Model (AM1) methods, both of which are implemented in Discovery studio visualiser (v20.1.0.19295, BIOVIA Software, http://www.3dsbiovia.com/product/collaborative-science/biovia-discovery-studio/). Furthermore, this will ensure that the study's compounds have a well-defined conformer connection [21]. The calculation was set to equilibrium geometry at the ground state using density functional theory at B3LYP (Becke88 three-parameter hybrid exchange potentials with Lee-Yang-Parr correlation potential) level of theory and 6-311G (d) basis set for the geometrical optimization of the cleansed structures i.e. B3LYP/6-311G (d) level of theory using the setup calculation option on Discovery studio visualise v20.1.0.19295. The display-output and display-properties options on Discovery studio visualiserv20.1.0.19295 were used to obtain the Discovery studio visualiser descriptions after optimization. Through the file option on the Discovery studio visualiser v20.1.0.19295, the completely optimized 3D structure without symmetry restrictions, was saved as an SD file.

2.5 Docking Simulations

All proteins preparation and minimization were done with the Discovery studio visualizer's (v20.1.0.19295) tools and protocols. The structure was optimized using a force field developed by Harvard Macromolecular Mechanics (CHARMm). Hydrogen atoms were added to the complex throughout the protein preparation technique, after which water molecules were removed and the pH of the protein was set to nearly neutral value. To identify the binding site of the protein structure, a sphere binding site with a radius of nine Armstrong (A^o) was defined around the attached ligand. The ligands' SD files of were then imported into PyRx-virtual screening tool, where they were utilized to dock the receptors that have been prepared. The ligands are scored on the basis of biased probability Monte Carlo (BPMC) method, which selects a conformation in the internal coordinate space at random and then moves to a new random position that is independent of the previous one but follows a specified continuous probability distribution. The results of the best scored, binding energy and inhibition constants of all the ligands were reported on a table.

2.6 Structure Visualization through PyMOL

Structure visualization was done with the PyMOL method. PyMOL is an open-access instrument. The protein molecule in the form of pdbqt was loaded on PyMOL's graphical screen, followed by the output of the pdbqt file. The docked structure was visualised and converted to molecular surface [15].

3.0 RESULTS

The X-ray crystal structures of α-Glucosidase (PDB ID:3WY1) and α -Amylase (PDB ID: 3BAW) with resolutions of 2.15Å and 2.00Å respectively were downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Database Bank (PDB) (http://www.rcsb.org/pdb) as shown in figure 1.

A **B**

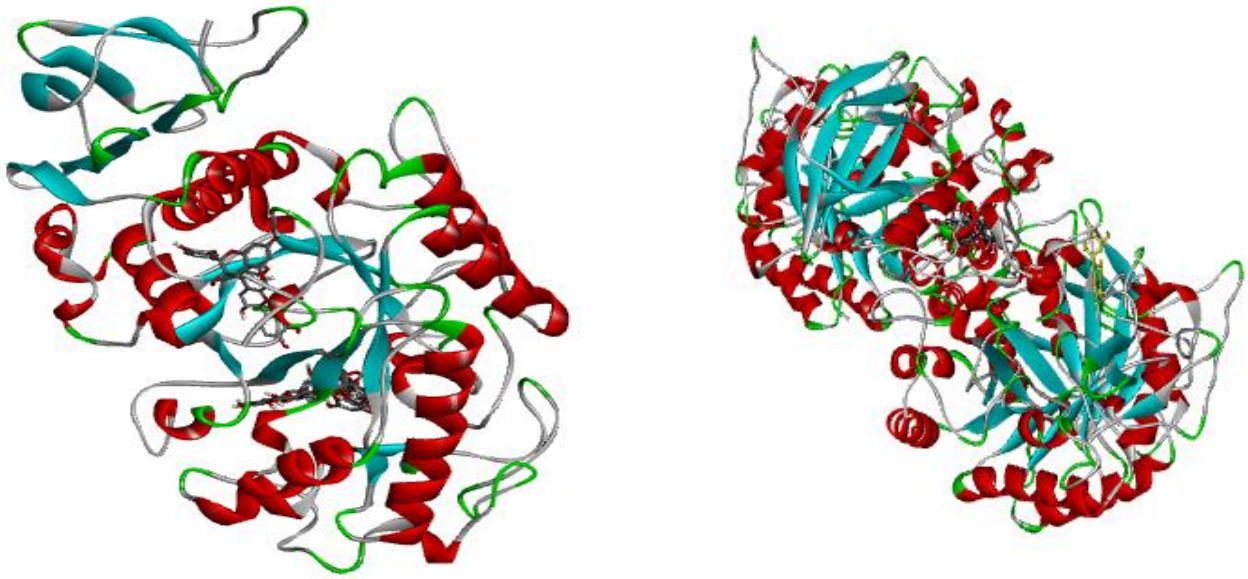
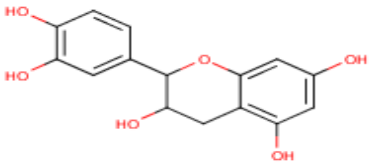
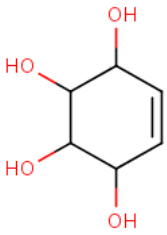
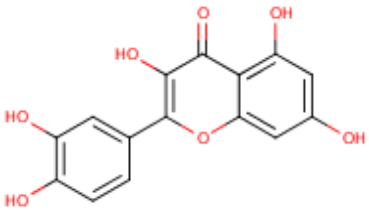


Figure 1: (A) The crystal structure of α –Amylase, (B) The crystal structure of α-Glucosidase

Secondary metabolites from different plants were retrieved from PubChem online database. The structures of Conduritol A, Catechin and Quercetin were downloaded in sdf format as shown in Table 1. The downloaded structures were converted into pdb format.

Table 1:Structure of Ligand

Compound Name	Molecular Formula	Molecular Structure	PubChem ID
Catechin	$C_{15}H_{14}O_6$		73160
Conduritol A	$C_6H_{10}O_4$		10290861
Quercetin	$C_{15}H_{10}O_7$		5280343

The compounds were analysed for drug likeliness property analysis using the pkCSM and SwissADME online servers and screened using the qualifying Lipinski Rule of five. The compounds were further analysed for its Hydrogen bond acceptor, Rotatable Hydrogen bonds, Hydrogen bond donor, Molecular weight, LogP and Surface area as shown in table 2.

Table 2: Drug Likeliness Property Analysis of the Ligands

Compound Names	Molecular Weight (g/mol)	Number of Hydrogen Donor	Number of Hydrogen Acceptor	Number of Rotatable Bonds	LogP	Surface Area	Violations
Catechin	290.27	5	6	1	1.5461	119.662	No violation
Conduritol A	146.14	4	4	0	-2.0002	58.045	No violation
Quercetin	302.23	5	7	1	1.9880	122.108	No violation

All the ligands were subjected for virtual screening through PyRx software. Docking analysis showed the compounds to have inhibitory activities against α-amylase (PDB ID = 3BAW) with Catechin having a total score of binding affinity of –8.8Kcal/mol, root mean square deviation (RMSD) lower bound of 1.719 and RMSD upper bound of 2.147, the binding affinity of Conduritol A was -5.4Kcal/mol with root mean square deviation (RMSD) lower bound of 1.756 and RMSD upper bound of 2.606, while that of Quercetin was –8.1Kcal/mol with root mean square deviation (RMSD) lower bound of 2.044 and RMSD upper bound of 3.219 as shown in Table 3.

Table 3: Docking Results of Receptor (α-amylase) with Ligands Catechin, Conduritol A and Quercetin

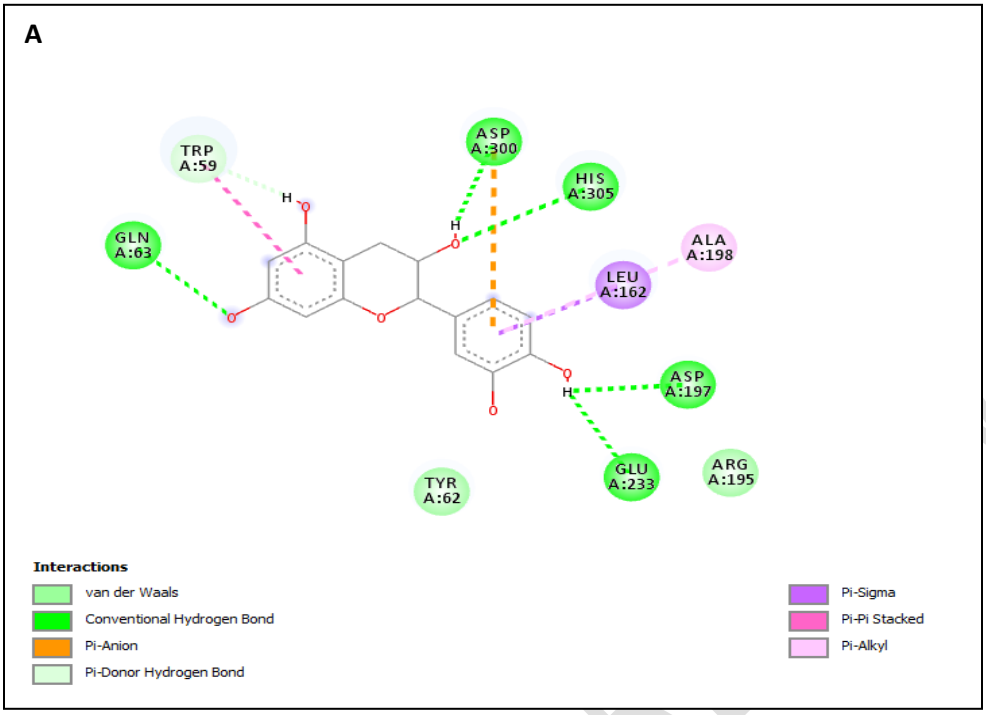
Ligands	Binding affinity (Kcal/mol)	Number of Hydrogen bond between ligand and receptor	Hydro-phobicity	Interpolated charge	Solvent accessibility surface (SAS)	RMSD lower bound	RMSD upper bound
Catechin	-8.0	5	-1.00	-0.033	12.5	1.719	2.147
Conduritol A	-5.4	4	-1.00	-0.033	10.0	1.756	2.606
Quercetin	-8.1	5	-2.00	-0.033	12.5	2.044	3.219

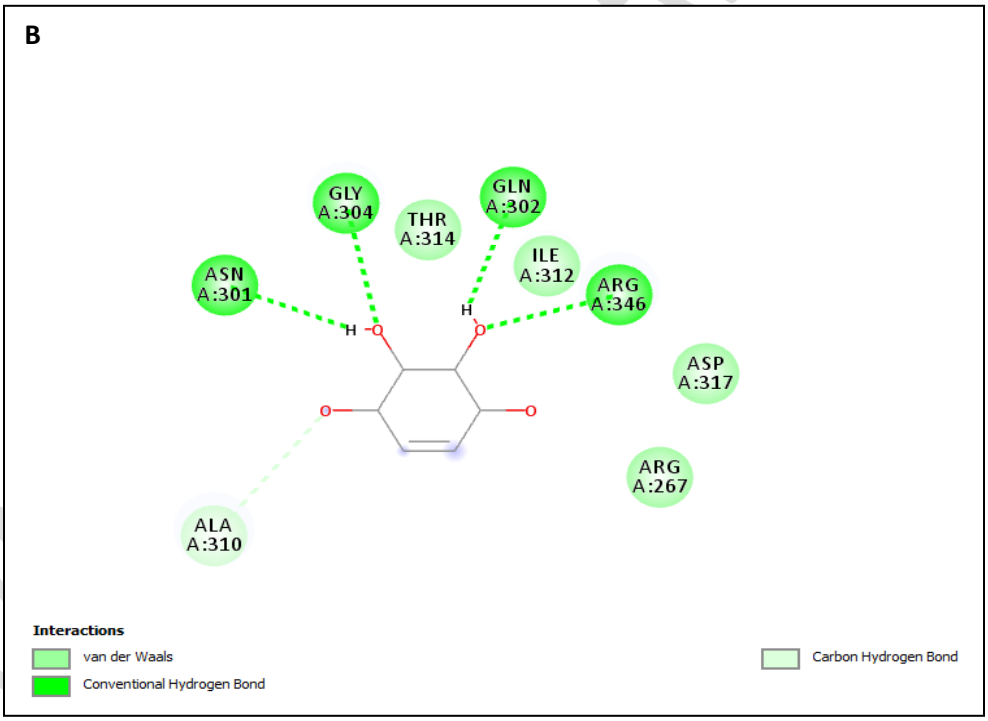
Docking with the protein α-glucosidase (PDB ID= 3WY1) revealed the total binding affinity for Catechin –8.3Kcal/mol, the root mean square deviation (RMSD) lower bound was 1.352 and RMSD upper bound was 7.047,the total binding affinity for Conduritol A was –5.4Kcal/mol, the root mean square deviation (RMSD) lower bound was 55.827 and RMSD upper bound was 56.681, while the total binding affinity for Quercetin was –8.5Kcal/mol, the root mean square deviation (RMSD) lower bound was 1.630 and RMSD upper bound was 7.027 as shown in Table 4.

Table 4: Docking Results of Receptor (α-glucosidase) with Ligands Catechin, Conduritol A and Quercetin

Ligands	Binding affinity (Kcal/mol)	Number of Hydrogen bond between ligand and receptor	Hydro-phobicity	Interpolated charge	Solvent accessibility surface(SAS)	RMSD lower bound	RMSD upper bound
Catechin	-8.3	5	-2.00	-0.033	12.5	1.352	7.047
Conduritol A	-5.4	4	-1.00	-0.033	12.5	55.827	56.681
Quercetin	-8.5	3	-2.00	-0.033	12.5	1.630	7.027

Molecular docking has shown the binding affinities of all the ligands to α-amylase catalytic residues. Other interactions such as hydrogen bonds, van der Waals interactions, hydrophobicity as well as pi-bonds cannot be discarded in the inhibitory activities of the compounds against alpha-amylase. Three hydrogen bonds interaction were found between catechin and α-amylase catalytic residues: ASP197, GLU 233 and ASP300 were present. Conduritol A was found to interact with ASN301 and GLN302 through two hydrogen bonds interaction. Three hydrogen bonds interaction were found between Quercetin and the α-amylase catalytic residues: GLY334, GLN7 and THR6 (Figure 2).


A
TRP A:59
ASP A:300
HIS A:305
GLN A:63
ALA A:198
LEU A:162
ASP A:197
GLU A:233
ARG A:195
TYR A:62
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Anion
Pi-Donor Hydrogen Bond
Pi-Sigma
Pi-Pi Stacked
Pi-Alkyl


B
GLY A:304
THR A:314
GLN A:302
ILE A:312
ASN A:301
ARG A:346
ASP A:317
ARG A:267
ALA A:310
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond

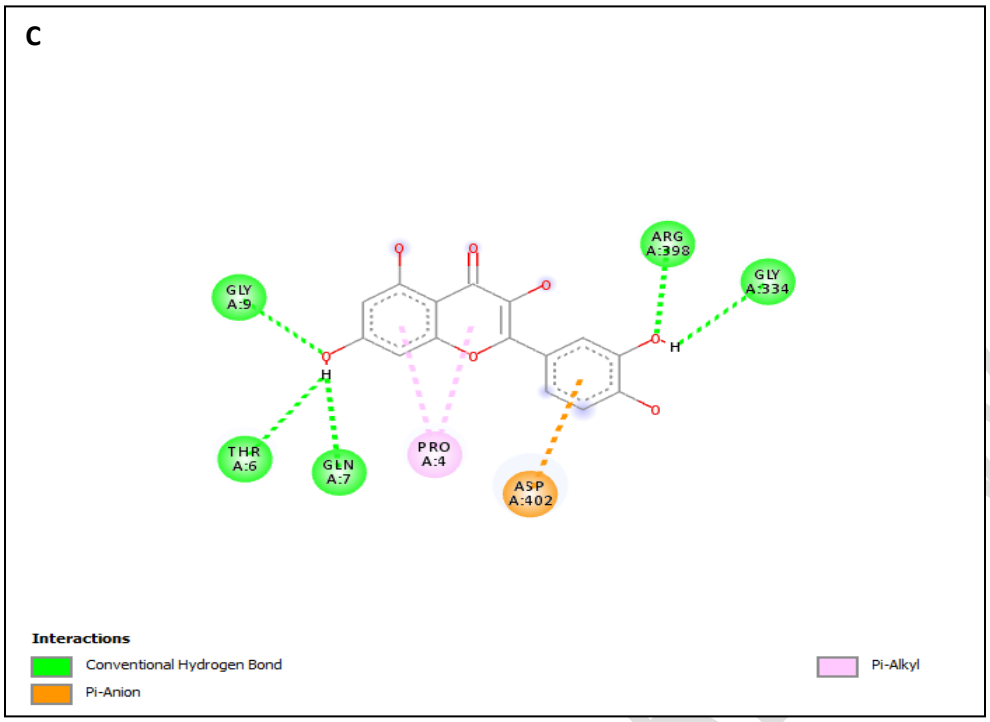
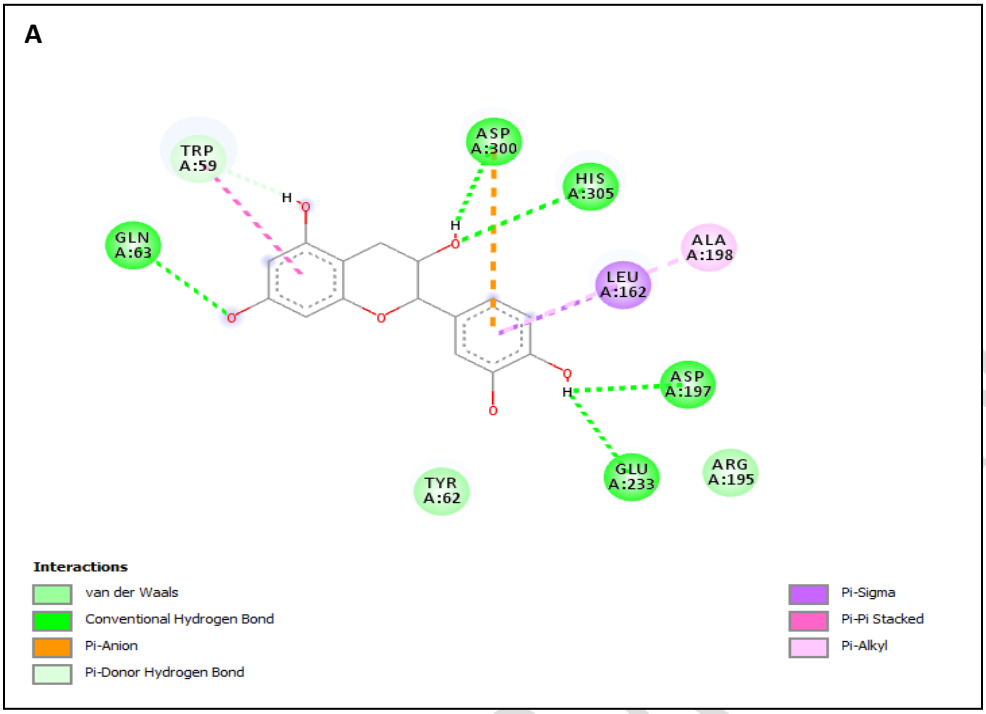
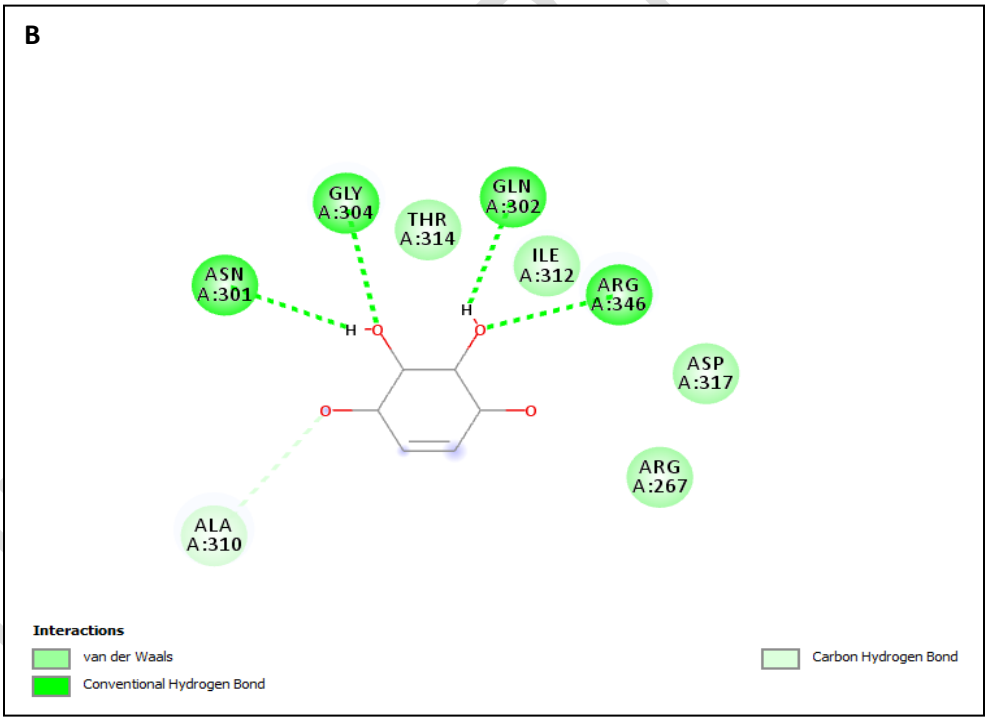


Figure 2: Molecular docking of a receptor α-amylase and the identified ligands, (A) 2D structural interaction of α-amylase with catechin, (B) 2D structural interaction of α-amylase with conduritol A, (C) 2D structural interaction of α-amylase with Quercetin.

The subjects under study have indicated a significant inhibitory mechanism against α-glucosidase. Catechin docked with α-glucosidase produced four hydrogen bond interactions involving ARG437, ALA 451, SER 44 and GLU432. Conduritol A was found with three hydrogen bond interactions involving ARG437, ALA451 and ASP441 of α-glucosidase binding site, Quercetin was found with two hydrogen bond interactions involving ALA451 and ARG437 of α-glucosidase binding site (Figure 3).


A
TRP A:59
ASP A:300
HIS A:305
GLN A:63
ALA A:198
LEU A:162
ASP A:197
GLU A:233
ARG A:195
TYR A:62
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Anion
Pi-Donor Hydrogen Bond
Pi-Sigma
Pi-Pi Stacked
Pi-Alkyl


B
GLY A:304
THR A:314
GLN A:302
ILE A:312
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ARG A:346
ASP A:317
ARG A:267
ALA A:310
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond

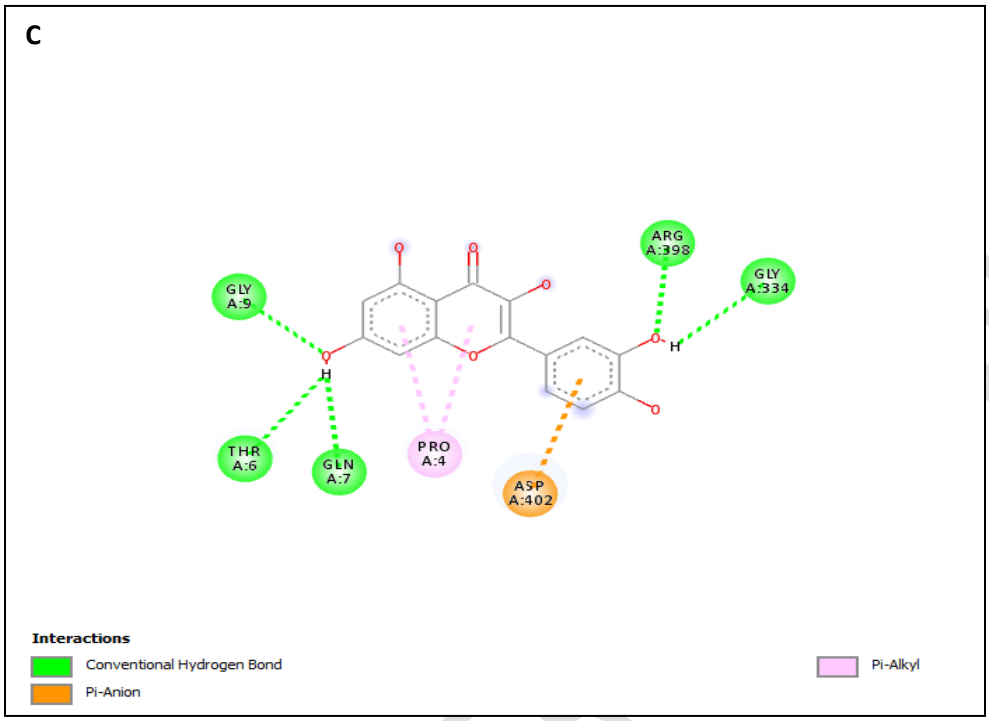

Figure 3: Molecular docking of a receptor α-glucosidase and the identified ligands, (A) 2D structural interaction of α-glucosidase with catechin, (B) 2D structural interaction of α-glucosidase with conduritol A, (C) 2D structural interaction of α-glucosidase with Quercetin.

4.0 DISCUSSION

The docking analysis in this study revealed many hydrogen bond interactions, hydrophobic interactions, interpolated charges, solvent accessibility surface, binding energy and bond length, all of which are critical for optimizing the activity of the active compounds upon or against any biological targets between all the subject under study [16]. The proposed action's mechanism of the alpha-amylase inhibitory activity is connected to its ability to produce a sliding barrier by establishing a hydrogen bond interaction with the residues of the active or substrate binding (catalytic) region [17]. The subjects under study were found to have inhibitory mechanism towards α-amylase.

In ligand-protein binding, hydrophobic interactions are critical [18]. The majority of ligand binding sites have at least one hydrophobic (nonpolar) region, and many of them show a distinct preference for non-polar ligands. Hydrophilicity and loss of hydrophobicity are indicated by the negative values of logP (Table 2). As a result of which there must be a relationship between pharmacological activity and the hydrophobicity (logP). The hydrophobicity (logP) of the compounds has a direct relationship with their activity, as log P declines, activity also diminishes [19].

The solvent accessible surface (SAS) is also a valuable tool for determining the overall extent of a hydrophobic region on a molecule or at the binding site of a protein, but does not take into account the specific atom types that make up the binding site or their relative positions [19]. There is a direct link between the activity of the compounds and SAS, and as the SAS declines, so does the activity. It has become impossible to sustain a hydrogen-binding network in the proximity of a huge hydrophobic item, causing the structure of water to be disrupted as well as a stronger hydrophobic interaction. The change that occurs from the hydrophobic hydration of small non-polar solutes to a high tendency for water depletion on extended nonpolar nanometer-scale length surfaces, such as those in proteins can be accounted by the Lum-Chandler Weeks theory of hydrophobicity [20,21].

The computer simulations and subsequent theoretical advancements have consequently revealed that capturing the increased hydrophobic attraction that would exist between a ligand and a protein with a broad or concave nonpolar surface is necessary. The shape and extent of the exposed molecule surface, as well as the polarity, determine the strength of the hydrophobic interaction. For many drug-receptor interactions, hydrogen bonding is very certainly an essential requirement. A single hydrogen bond is relatively weak and would not be anticipated to support a drug-receptor interaction alone; nevertheless, when many hydrogen bonds are established between drugs and receptors, as is usually the case, the drug-receptor interaction gain a significant amount of stability [19, 22].Therefore, the molecular docking study radically confirms the inhibition of α-glucosidase and α-amylase enzymes and their binding affinity [23] and further substantiates the insulin-mimicking ability of secondary metabolites [24, 25, 26, 27].

5.0 CONCLUSION

The findings of this study revealed that plant secondary metabolites could reduce glucose level by inhibiting the activity of α-amylase and α-glucosidase, two important enzymes involved in the digestion of complex carbohydrates into absorbable monosaccharides units. Molecular docking was performed to determine the interactions between the different compounds and the target protein. Studies of docking have shown that the compounds have strong affinity to diabetes-related protein. These compounds can thus act as inhibitors according to the *in-silico* analysis and can be used in a type of drug that can regulate diabetes and can be used as a potential antidiabetic agent for the treatment of diabetes.

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