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Modeling of Aqueous Root Extract Compounds of *Ruellia tuberosa* L. for Alpha-Glucosidase Inhibition Through in Silico Study

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Cover Page Footnote

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Modeling of Aqueous Root Extract Compounds of *Ruellia tuberosa* L. for Alpha-Glucosidase Inhibition Through in Silico Study

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Abstract

This study aims to analyze the inhibitory activities of aqueous root extract compounds of *Ruellia tuberosa* L. toward alpha-glucosidase protein by computational docking analysis. Three major compounds contained in the extracts (i.e., betaine, daidzein, and hispidulin) were selected as ligands; quercetin and acarbose were used as the reference. Computational docking analysis was performed using the HEX 8.0.0 program and visualized using the Discovery Studio Visualizer v19.1.0.18287 (2019 version) on the basis of the scoring functions. The interactions between ligands and alpha-glucosidase protein showed different binding patterns. The types of bonds involved in the interaction between the enzyme and these ligands were hydrogen and hydrophobic bonds. Energy generated from docking of betaine, daidzein, hispidulin, quercetin, and acarbose to alpha-glucosidase protein were -167.6 , -249.5 , -251.2 , -241.5 , and -322.1 cal/mol, respectively. Acarbose had the lowest energy, indicating that it has the strongest interaction with alpha-glucosidase, followed by hispidulin, daidzein, quercetin, and betaine. Amino acid residues that interacted with the ligands were His717, Met363, Arg608, Pro361, Phe362, Leu865, Glu869, Arg594, and Asp356. The current research shows that *R. tuberosa* L. aqueous root extracts have the potential to be used as an inhibitor for the alpha-glucosidase protein and as an antidiabetic agent. Nonetheless, further studies are needed to support this modeling study.

Keywords: alpha-glucosidase; betaine; daidzein; hispidulin; in silico; *R. tuberosa* L.

Introduction

Diabetes mellitus, a carbohydrate metabolic disorder characterized by high blood glucose levels, has become a major public health concern. In 2010, the International Diabetes Federation reported an onset rate of approximately 6.4% in adults and a total of 285 million cases of diabetes globally. This number is projected to increase to 439 million cases in 2030 [1]. There are two types of diabetes mellitus. Insulin-dependent diabetes mellitus or Type 1 DM is caused by the autoimmune destruction of insulin-producing pancreatic β cells. The hypoinsulinemic state when hyperglycemia ensues, which is a dangerous physiological state, must be countered by regular insulin injections for survival. In non-insulin-dependent diabetes mellitus or Type 2 DM, insulin-sensitive cells are resistant to the actions of insulin [2], [3]. As a result, the metabolic reactions that are stimulated in β cells to produce extra insulin are inadequate to maintain blood glucose homeostasis, and the elevated concentration of insulin secreted is a

major factor for the development of cardiovascular diseases and cancer [4].

Type 2 DM is prevalent with more than 90% of reported cases worldwide [3]. The pathogenesis of Type 2 DM remains unknown because several malfunctioning mechanisms occur concurrently that can contribute to the progress of the disease. Along with genetic factors in individuals that affect the progress of this disease, numerous aspects, including obesity, lack of physical exercise, and poor nutrition practices, can contribute to its development [4]. Type 2 DM can cause other chronic diseases, including cardiovascular diseases, nephropathy, retinopathy, and neuropathy [4], which will decrease life expectancy and increase disability. Consequently, the safe and effective management of Type 2 DM is a top priority for researchers and clinicians.

The established drugs for Type 2 DM treatment include dipeptidyl peptidase 4 (DPP-4) inhibitors, glucagon-like peptide 1 (GLP-1) receptor agonists, metfor-

min, sulphonylureas, thiazolidinediones, and alpha-glucosidase inhibitors [5]–[9]. Mild to severe adverse effects have been reported for some of these drugs. Upper respiratory tract infections have been increasingly reported among users of DPP-4 inhibitors compared with users of other antidiabetic drugs [10]. Animal studies indicated an association of GLP-1 receptor agonists with pancreatitis, pancreatic cancer, and thyroid cancer [11]. The use of thiazolidinediones or sulphonylureas may increase fracture risk, which can be attributed to an increased risk of hypoglycemia-induced falls [12]. Metformin has been reported to have a few side effects, including gastrointestinal symptoms, nausea, and vomiting [13]. Alpha-glucosidase inhibitors are reported to adequately control Type 2 DM. However, marketed drugs have different side effects and are expensive, which lead to poor patient compliance. Thus, alpha-glucosidase inhibitors need to be effective, safe, and affordable to ensure patient compliance [14–15].

Alpha-glucosidase inhibitors are regular antidiabetic drugs used to monitor carbohydrates converted into simple sugars and absorbed by the intestines. Drugs designed to diminish blood glucose levels and sustain glucose homeostasis derived from nature are attracting considerable attention. Many studies have been performed to discover substitute remedies from natural products that can lessen hyperglycemia through the downregulation of alpha-glucosidase activity [16, 8], [17–19]. Nevertheless, the exact mechanisms of action are still unknown.

The in silico molecular docking approach utilizes a simulation model that predicts molecular interaction, including The in silico molecular docking approach utilizes a simulation model that predicts molecular interaction, including protein–protein or ligand–protein interactions. Previous studies have investigated the interactions between flavonoid compounds and alpha-amylase protein, which is an important enzyme for the targeted treatment of diabetes [20–22]. Another study has also conducted in silico molecular docking of many natural compounds, such as curcumin, quercetin, berberine, catechin, and rutin, to both alpha-amylase and alpha-glucosidase enzymes [23]. Our previous study has also performed in silico molecular docking of *Ruellia tuberosa* L. aqueous root extracts to the alpha-amylase protein [24]. In summary, the aforementioned studies concluded that natural compounds showed inhibitory activities toward alpha-amylase and alpha-glucosidase proteins, thus having antidiabetic capacity.

The current study investigates the biological activity of the ligands contained in *R. tuberosa* L. aqueous root extracts using the in silico molecular docking approach. Our previous studies showed that *R. tuberosa*

L. contained mostly flavonoid and phytosterol compounds [24–25]. *R. tuberosa* L. aqueous root extracts also showed antidiabetic activity in in vivo studies [26–28]. LC-MS analysis revealed that betaine, daidzein, and hispidulin were detected in *R. tuberosa* L. aqueous root extracts [24]. Therefore, these compounds were selected as ligands and docked to alpha-glucosidase protein. Quercetin and acarbose are used as the positive ligand reference. Quercetin is a flavonoid compound that is found abundantly in vegetables and fruits and has many pharmacological activities [29], including antioxidant, anti-inflammatory, and anticancer activities [30]. Meanwhile, acarbose is one of the potent alpha-glucosidase inhibitors and has recently been recommended for the treatment of diabetes [31]. Furthermore, acarbose has received preferred status over other oral glucose-lowering drugs because of its proven capability to reduce cardiovascular diseases [31–33].

Methods

Ligand and Protein Preparation for Molecular Docking Analysis. The 3D structures of aqueous root extract compounds of *R. tuberosa* L., i.e., betaine (CID 247), daidzein (CID 5281708), and hispidulin (CID 5281628), were downloaded from the PubChem NCBI database. Acarbose (CID 41774) and quercetin (CID 5280343) were used as the positive ligand reference. The pharmacological activities of metabolite compounds from *R. tuberosa* L., in particular as an inhibitor for alpha-glucosidase, were calculated using the Prediction of Activity Spectra for Substance (PASS) analysis online software (<http://www.way2drug.com/passonline>). To minimize the energies of the compounds and convert the SDF format into PDB format, the PyRx Virtual Screening Tool software was operated. The human alpha-glucosidase protein was obtained from the RCSB Protein Data Bank, with PDB ID: 5kzx (<http://www.rcsb.org/pdb>). The Discovery Studio Visualizer v19.1.0.18287 program was used to remove other ligands or water that linked to the enzyme.

Simulations of Molecular Docking. Ligands (i.e., betaine, daidzein, hispidulin, acarbose, and quercetin) were docked to human alpha-glucosidase protein. The interaction and binding energy formed between betaine, daidzein, hispidulin, and quercetin to alpha-glucosidase enzyme were calculated using the blind docking HEX 8.0.0 software with Shape + Electro + DARS and root-mean-square deviation (RMSD) value ≤ 2 Å as docking parameter. A total of 10 runs were performed for each molecular docking. The visualization of the docking results was analyzed using the Discovery Studio Visualizer v19.1.0.18287 program. The molecular structures of the ligands are shown in Figure 1.

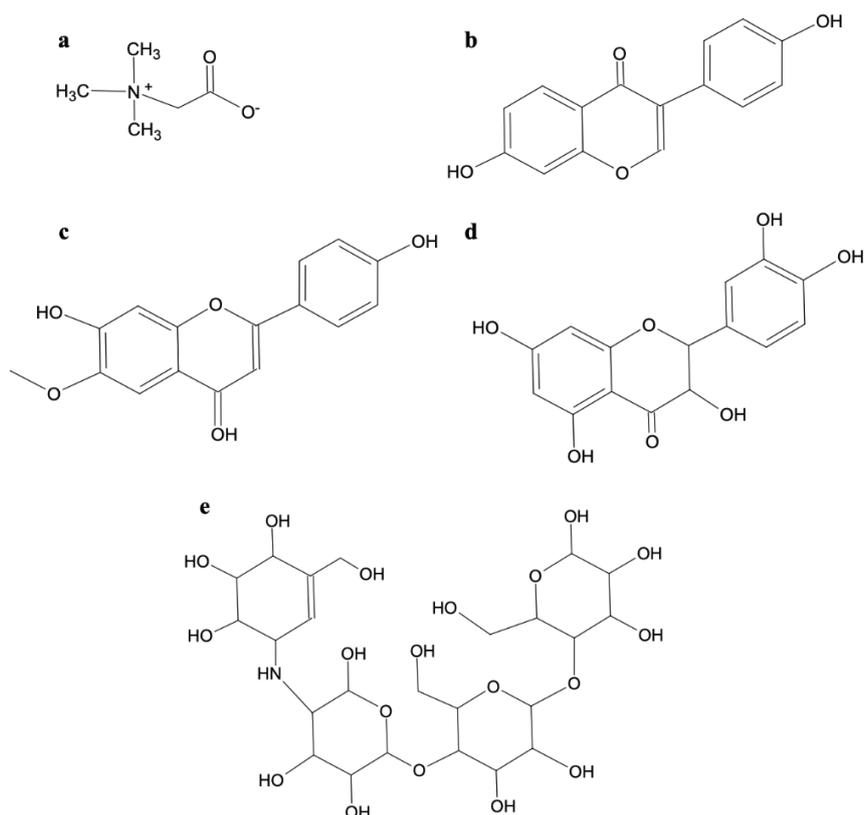


Figure 1. Molecular Structures of (a) Betaine (CID 247), (b) Daidzein (CID 5281708), (c) Hispidulin (CID 5281628), (d) Quercetin (CID 5280343), and (e) Acarbose (CID 41774). The Molecular Structures were Adapted from the PubChem NCBI Database

Results and Discussion

The validation of the docking results was conducted using the RMSD value. All docking results have an RMSD value ≤ 2.0 Å. The docking accuracy is based on the RMSD value of the locations of all heavy atoms of the ligand in the docked pose in the crystal structure. An RMSD value < 2.0 Å is acceptable [34]. The molecular docking of betaine, daidzein, hispidulin, quercetin, and acarbose to human alpha-glucosidase protein has been conducted to examine the interaction between these ligands and the protein. The ligand–protein interactions are denoted by the chemical bonds formed and the binding sites of amino acid residues (Table 1). The molecular docking results are presented in Figures 2 to 6.

The three amino acid residues in human alpha-glucosidase that interacted directly with betaine (Figure 2) were His717, Val867, and Leu868. These interactions were facilitated by carbon and conventional hydrogen bond formation. The amino acid residues that interacted with betaine through Van der Waals forces were Met363, Arg594, Ser864, Leu865, Glu866, and Glu869. The binding energy of the betaine–human alpha-glucosidase complex was -167.6 cal/mol.

By contrast, daidzein was observed to bind to the different binding positions of human alpha-glucosidase, with the binding energy of 249.5 cal/mol (Figure 3). The daidzein–human alpha-glucosidase complex had a higher number of interactions than the betaine–human alpha-glucosidase complex (Table 1). On the basis of the molecular docking results shown in Figure 3, Ser265 was the amino acid residue that bonded to daidzein by establishing a conventional hydrogen bond, whereas Pro266, Leu269, and Ile276 mediated pi-alkyl through hydrophobic interactions. The amino acid residues that interacted with daidzein through Van der Waals forces were His263, Leu264, Met268, Ser270, Thr274, Arg275, Thr277, Gly288, Ala289, and Asn290.

Four hydrogen bonds formed during the interaction between hispidulin and alpha-glucosidase protein (Figure 4). The amino acid residues involved in hydrogen bond formation were Tyr360, Met363, Ser864, and Glu866. Two amino acid residues, namely, Arg608 and His717, formed hydrophobic interactions with hispidulin. Other residues, including Gly359, Pro361, Phe362, Arg594, Leu865, Val867, Leu868, and Glu869, interacted with hispidulin through Van der Waals forces. The binding energy of the hispidulin–human alpha-glucosidase complex was -251.2 cal/mol.

Table 1. Results of the Interaction between Human Alpha-glucosidase and Betaine, Daidzein, Hispidulin, Quercetin, and Acarbose by Molecular Docking Analysis

Compounds	Energy (cal/mol)	Interaction*	Distance (Å)	Chemistry Bond	Types
Betaine	-167.6	A:VAL867:HN - :LIG1:O	2.199	Hydrogen Bond	Conventional Hydrogen Bond
		A:LEU868:HN - :LIG1:O	2.830	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:H - A:HIS717:NE2	2.594	Hydrogen Bond	Carbon Hydrogen Bond
		:LIG1:H - :LIG1:O	2.432	Hydrogen Bond	Carbon Hydrogen Bond
Daidzein	-249.5	A:SER265:HG - :LIG1:O	2.128	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1 - A:LEU269	5.038	Hydrophobic	Pi-Alkyl
		:LIG1 - A:PRO266	4.400	Hydrophobic	Pi-Alkyl
		:LIG1 - A:ILE276	4.453	Hydrophobic	Pi-Alkyl
Hispidulin	-251.2	A:TYR360:HN - :LIG1:O	2.228	Hydrogen Bond	Conventional Hydrogen Bond
		A:MET363:HN - :LIG1:O	2.052	Hydrogen Bond	Conventional Hydrogen Bond
		A:GLU866:HN - :LIG1:O	2.294	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:H - :LIG1:O	2.214	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:H - A:SER864:O	2.933	Hydrogen Bond	Carbon Hydrogen Bond
		A:HIS717 - :LIG1	3.478	Hydrophobic	Pi-Pi T-shaped
Quercetin	-241.5	:LIG1 - A:ARG608	4.399	Hydrophobic	Pi-Alkyl
		A:HIS717:HD1 - :LIG1:O	2.509	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:H - :LIG1:O	2.216	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:H - A:GLU196:O	2.897	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:H - A:ARG608:O	2.749	Hydrogen Bond	Conventional Hydrogen Bond
		A:PRO198:C - :LIG1:O	3.331	Hydrogen Bond	Carbon Hydrogen Bond
		A:GLY359:CA - :LIG1:O	3.451	Hydrogen Bond	Carbon Hydrogen Bond
		A:ARG608:NH1 - :LIG1	3.938	Electrostatic	Pi-Cation
		A:ARG608:NH2 - :LIG1	3.577	Electrostatic	Pi-Cation
		:LIG1 - A:ARG608	4.149	Hydrophobic	Pi-Alkyl
A:VAL357:O - :LIG1:O	2.092	Unfavorable	Unfavorable Bump		
Acarbose	-332.1	A:ARG178:HH21 - :LIG1:O	2.581	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:H - :LIG1:O	2.294	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:H - A:ASP356:O	2.944	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:H - A:LEU355:O	2.434	Hydrogen Bond	Conventional Hydrogen Bond
		:LIG1:H - A:HIS717:O	2.811	Hydrogen Bond	Conventional Hydrogen Bond
		A:ASP356:CA - :LIG1:O	3.311	Hydrogen Bond	Carbon Hydrogen Bond
		:LIG1:H - A:ASP356:OD2	2.445	Hydrogen Bond	Carbon Hydrogen Bond
		:LIG1:H - A:ASP356:O	2.436	Hydrogen Bond	Carbon Hydrogen Bond
		:LIG1:H - A:LEU355:O	2.940	Hydrogen Bond	Carbon Hydrogen Bond
		:LIG1:H - :LIG1:O	2.420	Hydrogen Bond	Carbon Hydrogen Bond
		:LIG1:H - :LIG1:O	2.779	Hydrogen Bond	Carbon Hydrogen Bond
		:LIG1:H - :LIG1:O	2.618	Hydrogen Bond	Carbon Hydrogen Bond
:LIG1:O - A:TYR360	2.963	Other	Pi-Lone Pair		

*The H-donors in betaine–human alpha-glucosidase interactions; H-donors and pi-orbitals in daidzein–human alpha-glucosidase interactions; H-donors and pi-orbitals in hispidulin–human alpha-glucosidase interactions; H-donors, positive ion, pi-orbitals, and steric ligand in quercetin–human alpha-glucosidase interactions; and H-donors and pi-orbitals in acarbose–human alpha-glucosidase interactions are rendered in bold.

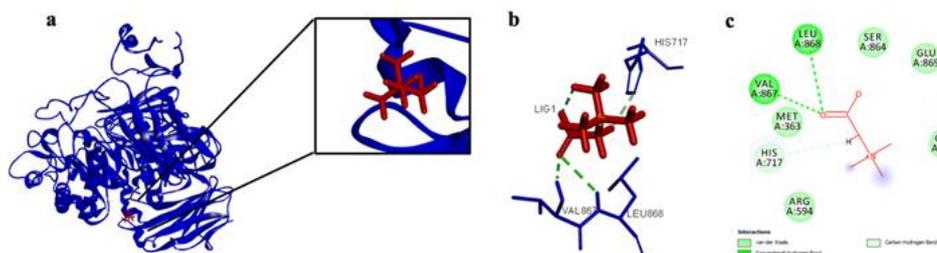


Figure 2. Model of the Interaction between Human Alpha-glucosidase Protein and Betaine: (a) Overview; (b) the 3D Structure of the Betaine–human Alpha-glucosidase Complex; and (c) the 2D Structure of the Betaine–human Alpha Glucosidase Complex

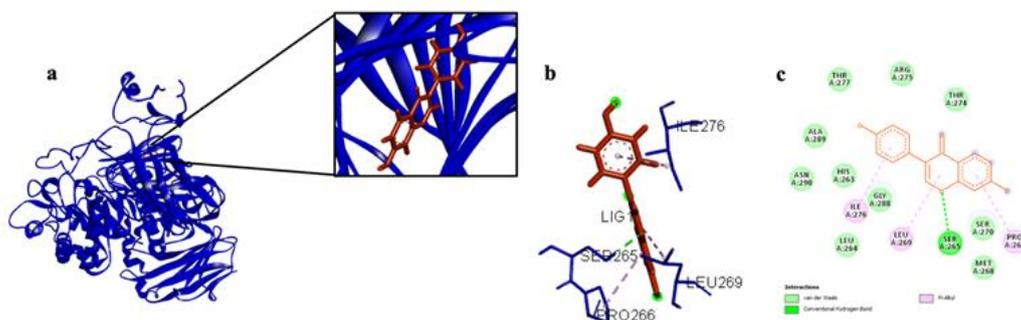


Figure 3. Model of the Interaction between Human Alpha-glucosidase Protein and Daidzein: (a) Overview; (b) the 3D Structure of the Daidzein–human Alpha-glucosidase Complex; and (c) the 2D Structure of the Daidzein–human Alpha Glucosidase Complex

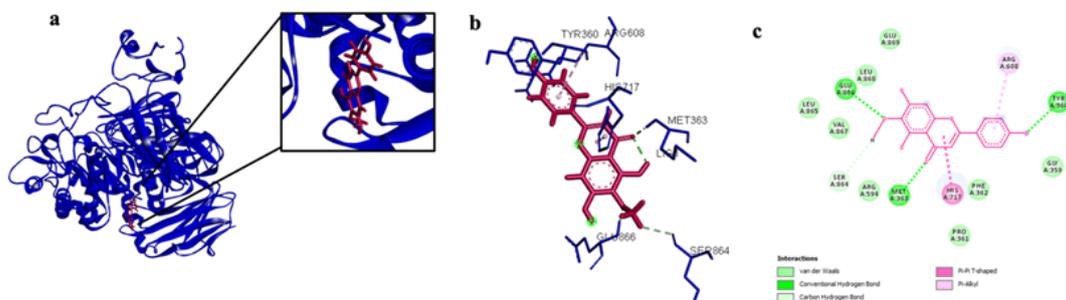


Figure 4. Model of the Interaction between Human Alpha-glucosidase Protein and Hispidulin: (a) Overview; (b) the 3D Structure of the Hispidulin–human Alpha-glucosidase Complex; and (c) the 2D Structure of the Hispidulin–human Alpha Glucosidase Complex

By contrast, daidzein was observed to bind to the different binding positions of human alpha-glucosidase, with the binding energy of 249.5 cal/mol (Figure 3). The daidzein–human alpha-glucosidase complex had a higher number of interactions than the betaine–human alpha-glucosidase complex (Table 1). On the basis of the molecular docking results shown in Figure 3, Ser265 was the amino acid residue that bonded to daidzein by establishing a conventional hydrogen bond, whereas Pro266, Leu269, and Ile276 mediated pi-alkyl through hydrophobic interactions. The amino acid residues that interacted with daidzein through Van der Waals forces were His263, Leu264, Met268, Ser270, Thr274, Arg275, Thr277, Gly288, Ala289, and Asn290.

Four hydrogen bonds formed during the interaction between hispidulin and alpha-glucosidase protein (Figure 4). The amino acid residues involved in hydrogen bond formation were Tyr360, Met363, Ser864, and Glu866. Two amino acid residues, namely, Arg608 and His717, formed hydrophobic interactions with hispidulin. Other residues, including Gly359, Pro361, Phe362, Arg594, Leu865, Val867, Leu868, and Glu869, interacted with hispidulin through Van der Waals forces. The binding energy of the hispidulin–human alpha-glucosidase complex was -251.2 cal/mol.

The binding energy of the interaction between alpha-glucosidase protein and quercetin used as the positive

ligand reference was -241.5 cal/mol. The quercetin–human alpha-glucosidase complex had a slightly higher number of interactions than the hispidulin–human alpha-glucosidase complex (Table 1). On the basis of the molecular docking results shown in Figure 5, Glu196, Gly359, Arg608, and His717 were the amino acid residues that bonded to quercetin by establishing hydrogen bonds. The only amino acid residue that interacted with quercetin through pi-cation electrostatic and pi-alkyl hydrophobic bonds was Arg608. The rest of the amino acid residues, i.e., Leu195, Thr197, Asp356, Val358, Tyr360, Pro361, Phe362, and Met363, interacted with hispidulin through Van der Waals bonds. An unfavorable interaction was observed between quercetin and Val357 residue.

Another ligand used as a reference is acarbose. The four amino acid residues that interacted with acarbose through hydrogen bonds are Arg178, Leu355, Asp356, and His717, as shown in Figure 6. The pi-lone pair interaction was observed in the interaction between Tyr360 and acarbose. Van der Waals forces were

formed between alpha-glucosidase and acarbose through the following amino acid residues: Met146, Met172, Glu174, Thr175, Thr197, Pro198, Arg608, and Val718. The binding energy of -332.1 cal/mol of the acarbose–human alpha-glucosidase complex was the lowest among all of the ligands used, indicating that acarbose had the strongest binding affinity to alpha-glucosidase protein.

The biological potential of the compounds belonging to particular classes of active compounds as inhibitors for alpha-glucosidase was predicted using PASS analysis. PASS analysis estimates the probable biological activity profiles for the investigated compounds on the basis of their structural formulas presented in MOL or SD file format. The average accuracy of prediction estimated in the leave-one-out cross-validation procedure for the entire PASS set is approximately 96%. The scores obtained by PASS analysis for betaine, daidzein, hispidulin, quercetin, and acarbose as alpha-glucosidase inhibitors are listed in Table 2. Acarbose and quercetin as control ligands showed the highest and second

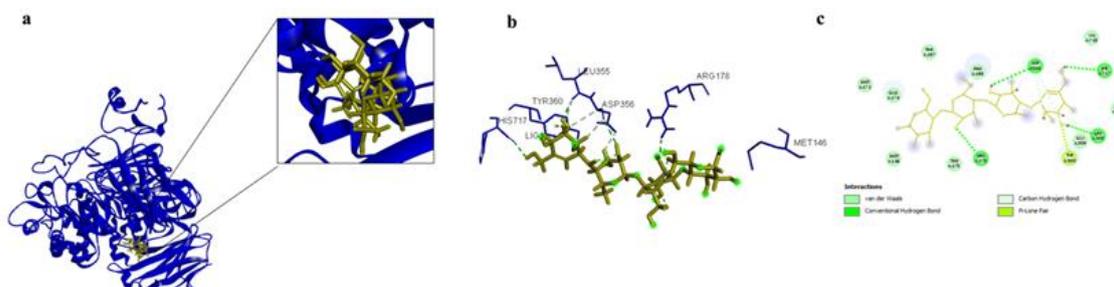


Figure 5. Model of the Interaction between Human Alpha-glucosidase Protein and Quercetin: (a) Overview; (b) the 3D Structure of the Quercetin–human Alpha-Glucosidase Complex; and (c) the 2D Structure of the Quercetin–human Alpha-Glucosidase Complex

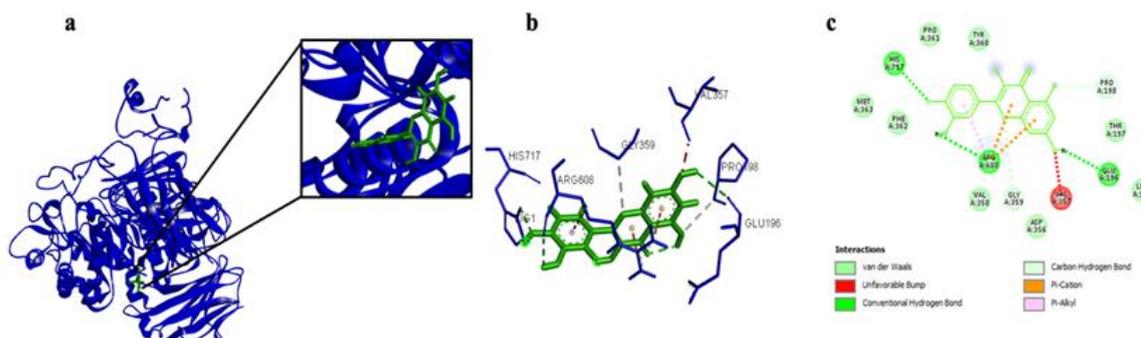
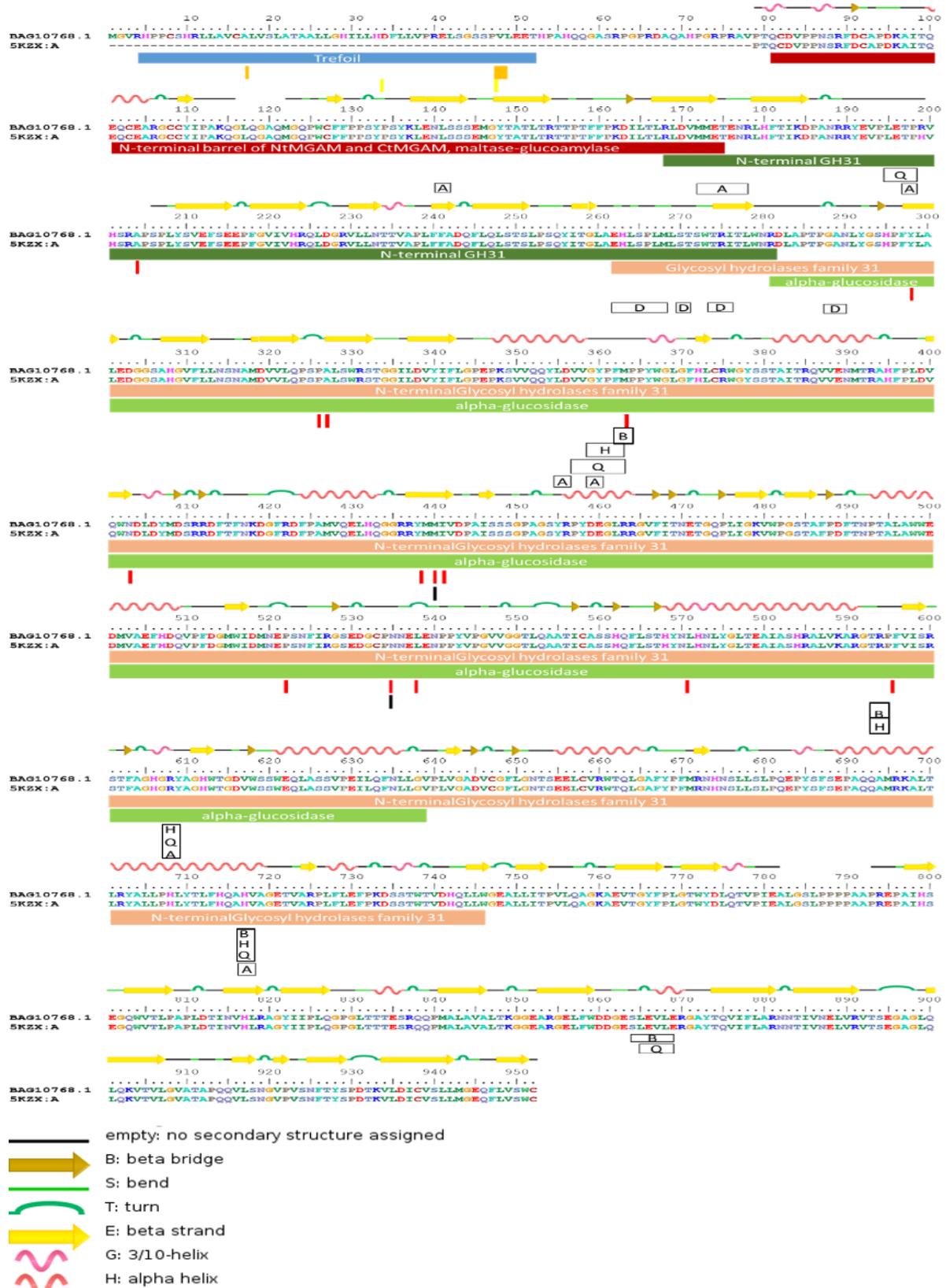


Figure 6. Model of the Interaction between Human Alpha-Glucosidase Protein and Acarbose: (a) overview; (b) the 3D Structure of the Acarbose–human Alpha-glucosidase Complex; and (c) the 2D Structure of the Acarbose–human Alpha-glucosidase Complex

Table 2. Results of the Pharmacological Activities of Betaine, Daidzein, Hispidulin, Quercetin, and Acarbose as Alpha-glucosidase Inhibitors

Alpha glucosidase inhibitor	Acarbose		Quercetin		Hispidulin		Daidzein		Betaine	
	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
	0.956	0	0.36	0.093	0.191	0.005	0.244	0.004	nd	nd

*Pa is potentially active, Pi is potentially inactive. The Pa and Pi values were obtained by PASS analysis, <http://www.way2drug.com/passonline>.



highest activities as alpha-glucosidase inhibitors, respectively. Daidzein had a higher score as alpha-glucosidase inhibitor than hispidulin. Nonetheless, betaine was not active as an inhibitor for alpha-glucosidase, as the score was undetected.

Figure 7 shows that some of the ligands used in this study bonded to the same amino acid residues. For instance, betaine, hispidulin, quercetin, and acarbose interacted with His717; betaine, hispidulin, and quercetin interacted with Met363; and hispidulin, quercetin, and acarbose interacted with Arg608. Moreover, Pro361 and Phe362 bonded to hispidulin and quercetin; Leu865, Glu869, and Arg594 bonded to betaine and hispidulin; and Asp356 bonded to quercetin and acarbose. Notably, the amino acid residues that bonded to daidzein did not bind to other ligands (Table 1).

Nonetheless, the 2D structure map of the alpha-glucosidase ligands showed that all of the compounds did not interact with the active sites or catalytic residues of the alpha-glucosidase enzyme. This phenomenon was observed for all ligands, even for acarbose, which is an effective alpha-glucosidase enzyme inhibitor. This finding is somewhat different from previous findings that acarbose bonded to the active sites of human alpha-glucosidase [35]. However, in [35], acarbose was docked directly to the active sites of alpha-glucosidase and bound to amino acid residues mostly through hydrogen bonds [29] because acarbose is a competitive inhibitor for the alpha-glucosidase enzyme [36]. Other ligands, including quercetin, betaine, daidzein, and hispidulin, are proposed to have a noncompetitive inhibition model. Hence, these ligands did not bind to the active site of the enzyme. To define the mode of inhibition of the ligands, further studies of the enzyme kinetics of alpha-glucosidase should be conducted.

The distinctive interaction affected the binding energy. The important contributors to the structure and protein–protein or ligand–receptor interaction are generally hydrogen bonds. Moreover, the hydrogen bond is critical to the assessment of the efficacy and specificity of the protein target and proposed drug [37–39]. In this study, the acarbose–alpha-glucosidase interaction had the highest number of hydrogen bonds compared with all other ligands. Hence, the acarbose–alpha-glucosidase interaction had the lowest binding energy (i.e., –332.1 cal/mol).

Therefore, on the basis of the interaction types, energy scores, and binding energies, acarbose is the most potent antidiabetic drug through alpha-glucosidase inhibition, followed by hispidulin, daidzein, and quercetin; meanwhile, betaine has the lowest potential to be used as an alpha-glucosidase inhibitor. Therefore, the order of activity of the ligands based on the binding energy is acarbose > hispidulin > daidzein > quercetin > betaine.

Nevertheless, the order of activity of the investigated compounds based on the PASS analysis is acarbose > quercetin > daidzein > hispidulin > betaine.

Conclusion

Through in silico molecular docking studies, the models of the interactions between the ligands and the alpha-glucosidase protein were obtained. The interactions between the protein and the ligands used in this study help us understand the potential mechanisms of their interactions. Compounds contained in *R. tuberosa* L. aqueous root extracts, i.e., hispidulin and daidzein, have the potential to be used as an inhibitor for alpha-glucosidase protein, with the binding energies of –251.2 and –249.4 cal/mol, respectively. By contrast, betaine has the least potential to be used as an inhibitor for the enzyme, with the binding energy of –167.6 cal/mol. The inhibition types of the ligands should be determined further through in vitro enzyme kinetics studies.

Conflict of Interest

The authors of the work have no conflict of interest.

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