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Molecular Docking Approach of Potential Alpha Glucosidase Inhibitors from Extracts Compounds of *Ruellia tuberosa* L

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ABSTRACT. *The present study investigates anti-diabetic capacity of compounds enclosed in the Ruellia tuberosa L. root extracts by molecular simulation approach to examine the potential of those compounds acting as alpha glucosidase inhibitors. Compounds chosen were cirsimarin, cirsimaritin, and sorbifolin; quercetin was used for the reference. Those compounds were downloaded from PubChem database, and human alpha glucosidase 3D structure was obtained from Protein Data Bank. The protein was docked to the flavonoid compounds using HEX 8.0 software and visualized using Discovery Studio 4.1. The interactions of cirsimarin, cirsimaritin, sorbifolin, and quercetin on alpha-glucosidase showed similar binding patterns. They interacted with the active sites of the enzyme, causing inhibition on enzyme activity. The interactions between proteins and ligands were mostly through formation of hydrogen bonds and Van der Waals forces. The binding energy of cirsimarin cirsimaritin, sorbifolin, and quercetin to alpha glucosidase were comparable at -323.3, -279.4, -256.8, and -241.5 kJ/mol, respectively. These confirm that compounds contained in the extracts of R. tuberosa L have capacity to be used as inhibitor for alpha glucosidase.*

Keywords*: Ruellia tuberosa L., in silico, alpha glucosidase, cirsimarin, cirsimaritin, sorbifolin*

INTRODUCTION

Diabetes mellitus, a disease of sugar metabolism disorders characterized by high blood glucose levels, become the major public health concerns. A report from International Diabetes Federation (IDF) in 2013 shows an onset rate of around 8.4% in adults, and a total amount of 382 million cases of diabetes globally. The quantity is projected to grow to 592 million cases in 2035 [1]. There are commonly 2 types of diabetes mellitus [1]. Insulin-dependent diabetes mellitus or type 1 DM is caused by auto immunological damage of the insulin secreting pancreatic β-cells [2]. In the hypoinsulinemic state when hyperglycemia ensues, this 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 are major factors for development of cardiovascular diseases and cancer [4]

The diabetes prevalent predominantly links to type 2 diabetes, which reports for over 90% of all cases of diabetes worldwide [1]. The pathogenesis of type 2 diabetes remains unknown because there are several malfunctioning mechanisms that occur concurrently that can contribute to the progress of the disease [5]. Along with genetic factor, which affects individuals to progressing this disease, there are also numerous aspects which can be part of its development, including obesity, lack of physical exercise, and poor nutrition practices [5]. Type 2 diabetes can cause to other chronic diseases, including cardiovascular diseases, nephropathy, retinopathy, and neuropathy [6]. These will result in reducing life expectancy and increasing disability [6]. Consequently, attempts to seek for safe and effective managements for type 2 diabetes are top priority for researchers and clinician.

The established drugs for type 2 diabetes treatment, including dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide 1 (GLP-11) receptor agonists, metformin, sulphonylureas, thiazolidinediones, and alpha glucosidase inhibitors [7, 8]. Alpha glucosidase inhibitors used as anti-diabetic drugs since they regulate the hydrolysis of

carbohydrates, and ultimately decreasing the rate of sugar absorption from the gastrointestinal tract. These makes alpha-glucosidase inhibitors important drugs for inhibiting carbohydrates release in the intestines, and can be used to treat T2DM. The alpha-glucosidase inhibitors are generally applied to early diabetic patients or used in combination with other drugs [6].

Medicines devised to decrease blood glucose concentrations and to control glucose homeostasis derived from the nature are becoming more popular [9, 10]. Many studies have been performed in order to discover substitute treatments from natural resources that could lessen high blood sugar levels through the down-regulation of alpha glucosidase activity [10]. Nonetheless, the exact mechanisms of action are still unknown.

In silico molecular docking approach is a simulating modeling that predicts molecules interaction, including protein-protein or ligand-protein interactions [11]. Previous studies have investigated interactions of flavonoids compounds to alpha amylase protein, important enzyme for diabetic target treatment [12, 13]. Another study also conducted *in silico* molecular docking of many natural compounds, such as curcumin, quercetin, berberine, catechin, rutin, to both alpha amylase and alpha glucosidase enzymes [14]. In summary, those studies concluded that natural compounds showed inhibitory activities to alpha amylase and alpha glucosidase proteins, and thus, have anti-diabetic capacity.

The current study is investigating the activity from secondary metabolites compounds contained in *R. tuberosa* L. root extracts using *in silico* molecular docking approach. Based on our previous studies, *R. tuberosa* L. mostly contained flavonoids and phytosterol compounds [15, 16]. In addition, the *R. tuberosa L*. hydroethanolic extracts have showed anti-diabetic activity in an *in-vivo* study [17-19]. The LC-MS analysis revealed that flavonoids compounds, such as cirsimarin, cirsimaritin, and sorbifolin, were shown contained in the extracts [20]. Therefore, those compounds were chosen as ligands, and were docked to alpha-glucosidase protein. The binding energy from the interactions of the protein-ligand complex was then calculated, and the interactions of protein-ligands were analyzed.

METHODS

Preparation of Protein and Ligands for Molecular Docking Analysis

The extracts compounds of *R. tuberosa* L. in the 3D structures format, cirsimarin (CID159460), cirsimaritin (CID188323), and sorbifolin (CID3084390), were downloaded from PubChem NCBI database. Quercetin (CID 5280343) was used for a positive reference. In order to minimize energies of the compounds and to convert the SDF format into PDB format, 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). Discovery studio visualizer v19.1.0.18287 program (http://3dsbiovia.com/products/) was used to remove other ligands or water that linked to the enzyme.

Simulations of Molecular Docking

Ligands (cirsimarin, cirsimaritin, sorbifolin, and quercetin) were docked to human alpha-glucosidase protein. The interaction and energy binding formed between cirsimatin, cirsimaritin, sorbifolin, and quercetin to alpha-glucosidase enzyme were calculated using HEX 8.0 software. The visualization of the docking results was analyzed using Discovery studio visualizer v19.1.0.18287 program (http://3dsbiovia.com/products/). The molecular structures of the ligands are shown in Figure 1.

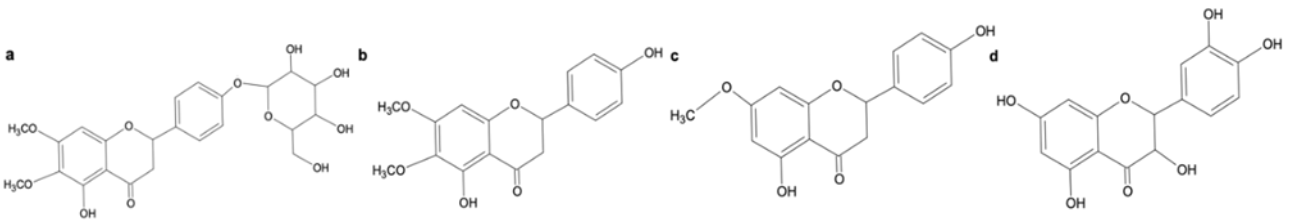

Figure 1. The molecular structures of: (a) cirsimarin: (b) cirsimaritin: (c) sorbifolin; and (d) quercetin. The molecular structures were adapted from PubChem databases [21-24].

RESULTS AND DISCUSSION

The molecular docking between cirsimarin, cirsimaritin, sorbifolin, and quercetin to human alpha-glucoside protein has been carried out in order to examine interaction between those ligands and protein. The interaction of ligand-protein was shown by the types of chemistry bond formed and the binding sites of amino acid residues (Table 1). The molecular docking results are presented in Figures 2-5.

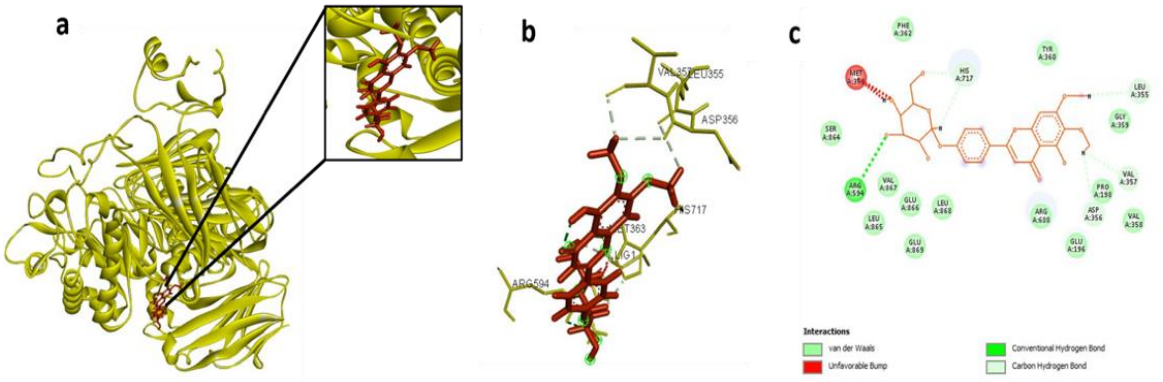

Figure 2. Molecular docking simulation results of human alpha glucosidase protein with cirsimarin: (a) overview; (b) the 3D structure; and (c) the 2D structure of cirsimarin-human alpha glucosidase complex

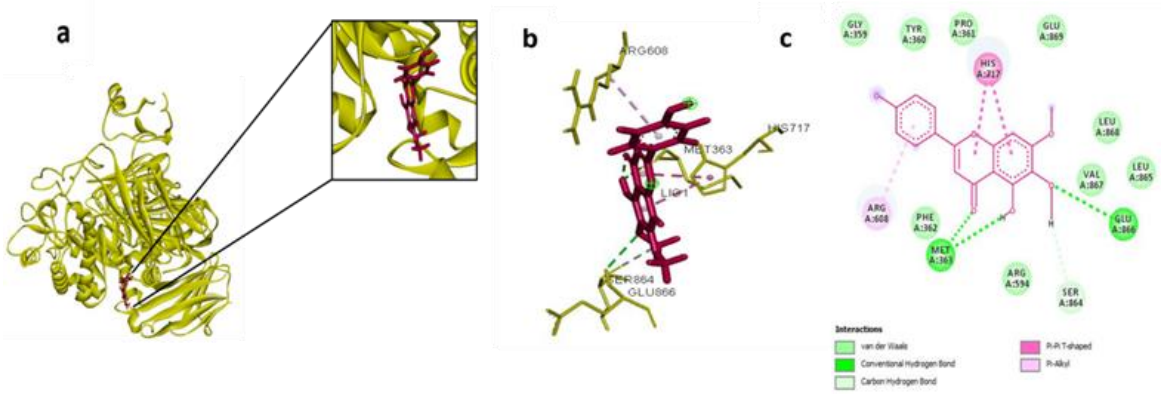

Figure 3. Molecular docking simulation results of human alpha glucosidase protein with cirsimaritin: (a) overview; (b) the 3D structure; and (c) the 2D structure of cirsimaritin-human α-glucosidase complex.

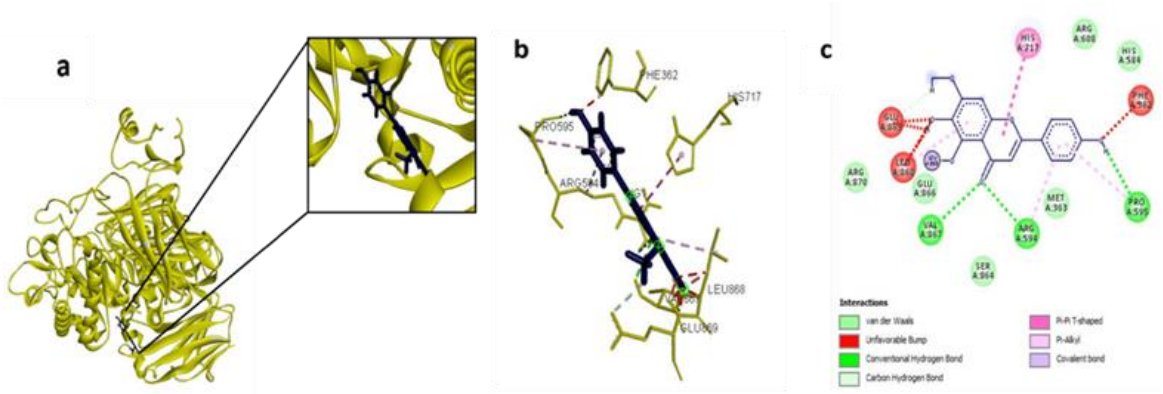

Figure 4. Molecular docking simulation results of human alpha glucosidase protein with sorbifolin: (a) overview; (b) the 3D structure; and (c) the 2D structure of sorbifolin-human alpha glucosidase complex.

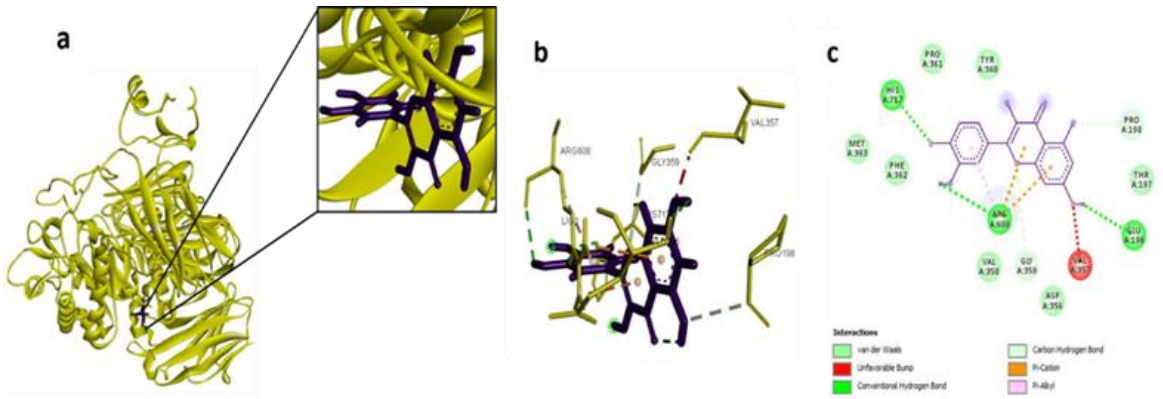

Figure 5. Molecular docking simulation results of human alpha glucosidase protein with quercetin: (a) overview; (b) the 3D structure; and (c) the 2D structure of quercetin-human alpha glucosidase complex

Table 1. Results of interaction of human alpha glucosidase with cirsimarin, cirsimaritin, sorbifolin, or quercetin by molecular docking analysis

Compound	Interaction*	Chemistry bond	Types	Energy (kJ/mol)
Cirsimarin	**A:ARG594:HE** - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	-323.3
	:LIG1:H - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:HIS717:CE1 - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - A:HIS717:NE2	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - A:ASP356:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - A:VAL357:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:LIG1:H - A:LEU355:O	Hydrogen Bond	Carbon Hydrogen Bond	
	A:MET363:CB - :LIG1:O	Unfavorable	Unfavorable Bump	
	A:MET363:CB - :LIG1:H	Unfavorable	Unfavorable Bump	
Cirsimaritin	**A:GLU866:HN** - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	-279.4
	:LIG1:H - A:MET363:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - A:SER864:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	
	A:HIS717 - :LIG1	Hydrogen Bond	Carbon Hydrogen Bond	
	A:HIS717 - :LIG1	Hydrophobic	Pi-Pi T-shaped	
	:LIG1 - A:ARG608	Hydrophobic	Pi-Pi T-shaped	
Sorbifolin	**A:GLU866:HN** - :LIG1:O	Hydrophobic	Pi-Alkyl	-256.8
	A:ARG594:HH22 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:VAL867:HN - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - A:PRO595:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - A:GLU869:OE1	Hydrogen Bond	Carbon Hydrogen Bond	
	A:HIS717 - :LIG1	Hydrophobic	Pi-Pi T-shaped	
	:LIG1 - A:LEU868	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:ARG594	Hydrophobic	Pi-Alkyl	
	:LIG1 - A:PRO595	Hydrophobic	Pi-Alkyl	
	A:PHE362:CD2 - :LIG1:O	Unfavorable	Unfavorable Bump	
Quercetin	**A:HIS717:HD1** - :LIG1:O	Unfavorable	Unfavorable Bump	-241.5
	:LIG1:H - :LIG1:O	Unfavorable	Unfavorable Bump	
	:LIG1:H - A:GLU196:O	Unfavorable	Unfavorable Bump	
	:LIG1:H - A:ARG608:O	Unfavorable	Unfavorable Bump	
	A:PRO198:C - :LIG1:O	Unfavorable	Unfavorable Bump	
	A:GLY359:CA - :LIG1:O	Unfavorable	Unfavorable Bump	
	A:ARG608:NH1 - :LIG1	Unfavorable	Unfavorable Bump	
	A:ARG608:NH2 - :LIG1	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1 - A:ARG608	Hydrogen Bond	Conventional Hydrogen Bond	
	A:HIS717:HD1 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	

*The H-donors and steric ligands in cirsimarin-human alpha glucosidase interaction; H-donors and pi-orbitals in cirsimaritin-human alpha glucosidase interaction; H-donors, steric ligands, and pi-orbitals in sorbifolin-human alpha glucosidase interaction; and H-donors, positive ions, and pi-orbitals in quercetin-human alpha glucosidase interaction were typed in bold letter.

There were 18 amino acid residues in human α-glucosidase amylase interacted with cirsimarin (Figure 2): Arg594, Arg608, His717, Asp356, Val 357, Val358, Val867, Leu355, Leu868, Phe362, Tyr360, Gly359, Pro198, Arg608, Glu169, Glu866, Glu869, and Ser864. Those interactions were facilitated by hydrogen bond formation, either carbon-hydrogen bonds or conventional hydrogen bonds, and also van der Walls forces. The binding energy of the cirsimarin human α-glucosidase complex was -323.3 kJ/mol. There was also unfavorable interaction between cirsimarin and Met363 residue.

Cirsimaritin bound to the same binding sites to human alpha glucosidase (Figure 3). The cirsimaritin-human alpha glusosidase complex had slightly fewer number of interactions compared to those of cirsimarin-human alpha lgucosidase complex (Table 1). Based on the molecular docking results in Figure 2, His717 was the amino acid residue that bound to cirsimaritin by establishing by Pi-Pi T-shaped, while Arg806 mediating by Pi-alkyl, both interactions were formed through hydrophobic interaction. The carbon and conventional hydrogen bonds formed between amino acid residues Glu866 and Met363 and cirsimaritin. These amino acid residues interacted with cirsimaritin through van der Waals force: Gly359, Tyr360, Pro361, Glu869, Leu865, Leu868, Val867, Arg594, and Phe362. The cirsimaritin-human alpha glucosidase complex resulted in the binding energy of -279.4 kJ/mol.

Sorbifolin and human alpha glucosidase reacted at similar binding locations with those of cirsimarin and cirsimaritin (Figure 4). The binding energy of the sorbifolin-human alpha glucosidase complex was -256.8 kJ/mol. Sorbifolin and human alpha glucosidase molecular docking resulted in the higher number of interactions. There were three amino acid residues interacted through hydrogen bonds with sorbifolin, including Arg594, Val867, and Pro595. Five amino acid residues established van der Walls when interacted with sorbifolin: Arg608, His584, Met363, Ser864, and Arg870. The hydrophobic interaction was shown in the interaction of His717 to sorbifolin, using Pi-Pi T-shaped bond. There were unfavorable interactions between Phe362, Leu868, Glu869 residues and sorbifolin.

Quercetin, used for the positive reference, flavonoid compound that found abundantly in vegetables and fruits, has many pharmacological activities [25]. These are including anti-oxidant, anti-inflammatory, and anti-cancer [25]. Interestingly, interaction between quercetin and human α-glucosidase also occurred in the same binding sites to those of the previous ligands to the protein (Figure 5). The binding energy of the quercetin-human α-glucosidase complex resulted in the comparable number with those of cirsimarin, cirsimaritin, and sorbifolin, at -241.5 kJ/mol. There were seven amino acid residues involved in the van der Waals interactions, Pro361, Tyr360, Thr197, Asp356, Val358, Phe362, and Met363. The hydrogen bonds formed in the quercetin-human α-glucosidase complex showed that His717, Gly359, Pro198, and Glu196 were the amino acids residues involved in the interactions. An unfavorable interaction was shown between quercetin and Val357 residue.

The various classes of interaction may influence to the binding energy. It is understandable that hydrogen bonds formation is significant contributor for the interaction of ligand-receptor or protein-protein [26]. Moreover, hydrogen bonds are crucial to gain the specificity of the drug to protein target. The order of the number of hydrogen bonds formed between the ligands and protein from the lowest to the highest is quercetin (3) <sorbifolin (5) <cirsimaritin (6) <cirsimarin (7). Therefore, it is reasonable that the binding energy between ligands and alpha glucosidase has also resulted in the same order as quercetin (-256.8 kJ/mol) < sorbifolin (-241.5 kJ/mol) <cirsimaritin (-279.4 kJ/mol) <cirsimarin (-323.3 kJ/mol).

CONCLUSION

The current work implies that *R. tuberosa* L. root extracts possess a capacity as inhibitor for alpha glucosidase protein. Alpha glucosidase protein inhibitory activity directs to a new breakthrough of plant-based medicinal products, specifically for diabetes. The binding energy of cirsimarin, cirsimaritin, sorbifolin and quercetin to alpha glucosidase were -323.3, -279.4, -241.5, and -256.8 kJ/mol, respectively. Molecular docking simulation could be applied as a resourceful supportive instrument for designing a new remedy. Further study on *in vitro* and *in vivo* methodologies are necessary to support the present work.

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