



***In silico* studies of *Ruellia tuberosa* L. compounds as aldose reductase, dipeptidyl peptidase 4, and α -glucosidase inhibitors against type 2 diabetes mellitus**

[Estudios *in silico* de compuestos de *Ruellia tuberosa* L. como inhibidores de la aldosa reductasa, dipeptidil peptidasa 4 y α -glucosidasa contra la diabetes mellitus de tipo 2]

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Abstract

Context: The search for a safe and effective anti-diabetic medication has escalated due to the unfavorable side effects of synthetic drugs and the geometric rise in diabetes mellitus cases. *Ruellia tuberosa* is an important medicinal plant that can potentially reduce postprandial hyperglycemia.

Aims: To identify the inhibition of aldose reductase, dipeptidyl peptidase 4 (DPP-4), and α -glucosidase for anti-diabetic drug discovery from *Ruellia tuberosa* bioactive compounds using computational methods, including molecular docking, binding free energy estimates and ADMET predictions.

Methods: A molecular docking study of betulin, betulinic acid, cirsiolol, cirsimarin, cirsimaritin, and pedalitin with aldose reductase, DPP-4, and α -glucosidase inhibitors was done using Glide XP-docking module. The adsorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction was carried out by the QikProp module, and ligand binding energy was ascertained by the prime molecular mechanics with generalized born and surface area (MM/GBSA) module, Schrodinger suite 2020-2.

Results: The molecular docking and complexes' MM/GBSA show specific interactions and high binding free energies. The ADMET prediction demonstrates the excellent safety profile, pharmacokinetic characteristics, and favorable drug-likeness of betulin, betulinic acid, cirsiolol, cirsimarin, cirsimaritin, and pedalitin. This study shows the inhibition potential of *Ruellia tuberosa* compounds against aldose reductase, DPP-4, and α -glucosidase inhibitors.

Conclusions: Therefore, for this chemical to be developed further into novel pharmaceuticals for treating type 2 diabetes mellitus, optimization and experimental research are required.

Keywords: anti-diabetic; *in silico* ADMET; MM/GBSA; molecular docking; *Ruellia tuberosa*.

Resumen

Contexto: La búsqueda de una medicación antidiabética segura y eficaz se ha intensificado debido a los efectos secundarios desfavorables de los fármacos sintéticos y al aumento geométrico de los casos de diabetes mellitus. *Ruellia tuberosa* es una importante planta medicinal que potencialmente puede reducir la hiperglucemia postprandial.

Objetivos: Identificar la inhibición de la aldosa reductasa, la dipeptidil peptidasa 4 (DPP-4) y la α -glucosidasa para el descubrimiento de fármacos antidiabéticos a partir de compuestos bioactivos de *Ruellia tuberosa* mediante métodos computacionales, incluyendo acoplamiento molecular, estimaciones de energía libre de enlace y predicciones ADMET.

Métodos: Se realizó un estudio de acoplamiento molecular de betulina, ácido betulínico, cirsiolol, cirsimarina, cirsimaritina y pedalitina con inhibidores de aldosa reductasa, DPP-4 y α -glucosidasa utilizando el módulo de acoplamiento Glide XP. La predicción de adsorción, distribución, metabolismo, excreción y toxicidad (ADMET) se llevó a cabo mediante el módulo QikProp, y la energía de unión del ligando se determinó mediante el módulo de mecánica molecular primaria con área superficial y de nacimiento generalizada (MM/GBSA), Schrodinger suite 2020-2.

Resultados: El acoplamiento molecular y el MM/GBSA de los complejos muestran interacciones específicas y elevadas energías libres de enlace. La predicción ADMET demuestra el excelente perfil de seguridad, las características farmacocinéticas y el parecido favorable a fármacos de la betulina, el ácido betulínico, el cirsiolol, la cirsimarina, la cirsimaritina y la pedalitina. Este estudio muestra el potencial de inhibición de los compuestos de *Ruellia tuberosa* frente a los inhibidores de aldosa reductasa, DPP-4 y α -glucosidasa.

Conclusiones: Por lo tanto, para que esta sustancia química pueda seguir desarrollándose en nuevos fármacos para el tratamiento de la diabetes mellitus tipo 2, se requiere optimización e investigación experimental.

Palabras Clave: acoplamiento molecular; antidiabético; *in silico* ADMET; MM/GBSA; *Ruellia tuberosa*.

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INTRODUCTION

The metabolic or multiple risk factor syndrome, which includes conditions like obesity, hypertension, and hyperglycemia, has become a significant worry in today's culture due to the rapid changes in food habits and lifestyle patterns. Interestingly, diabetes ranks as the sixth most common cause of death worldwide. Over 90% of people with diabetes have type 2 diabetes mellitus, which has grown to be a severe health issue globally. Diabetes, once only seen in the elderly, is now prevalent in young people and has killed over 1.6 million people globally. The World Health Organization estimates that by 2030, there will be over 366 million diabetics worldwide, up from the current level of over 220 million (Saeedi et al., 2019).

One significant risk factor for type 2 diabetes is postprandial hyperglycemia (Kim, 2020). Managing blood glucose levels is one of the most effective strategies to prevent diabetes and hyperglycemia (Rines et al., 2016). Digestive enzymes like α -glucosidase and α -amylase act as catalysts for the hydrolysis of carbohydrates, resulting in blood sugars. The enzyme α -glucosidase is present in intestinal cell membranes and is in charge of hydrolyzing polysaccharides. Similarly, the salivary glands and pancreas secrete α -amylase, an enzyme that can hydrolyze starches and oligosaccharides into simple sugars. Inhibiting these enzymes can slow down the digestion of carbohydrates, reducing the rate at which glucose enters the bloodstream. Therefore, it is considered that one therapeutic strategy to manage diabetes is to reduce the activity of these enzymes in the digestive system (Ghosh et al., 2014; Li et al., 2022). In the polyol pathway, aldose reductase is an essential enzyme. It connects the various body parts impacted by diabetes and the onset of consequences from the disease by catalyzing the conversion of glucose to sorbitol. Intracellular sorbitol accumulation causes localized hyperosmotic situations that lead to the development of diabetic complications, including neuropathy, nephropathy, retinopathy, and cataracts (Tang et al., 2012). Therefore, aldose reductase has been a promising therapeutic target for treating diabetic complications (Dunlop, 2000). Furthermore, the gut's release of hormones in reaction to the absorption of nutrients, particularly glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are crucial in regulating postprandial hyperglycemia (Holst, 2019). Because the enzyme DPP-4 inactivates these hormones by cleaving the first two amino acids from their N-terminals, resulting in GIP (3-42) and GLP-1 (9-36), they have a limited circulation half-life.

For this reason, DPP-IV inhibitors are helpful when treating type 2 diabetes (Deacon, 2019).

Many anti-diabetic medications have been created over time, are readily available in the market, and successfully treat diabetes. These medications include miglitol, emiglitate, acarbose, and voglibose. Nevertheless, it has been noted that long-term use of these drugs can cause side effects like headache, nausea, and dizziness, which can worsen other health issues, such as cardiovascular events (Lee et al., 2014). For this reason, it's important to look for safer substitutes with good drug profiles and no negative side effects. There is a growing trend in the management and prevention of diabetes and obesity with functional meals and plant-based medications that change physiological effects. Many studies have shown that medicinal plants have anti-hyperglycemic characteristics, showing that they are rich in anti-diabetic chemicals, particularly in inhibiting aldose reductase, DPP-4, and α -glucosidase (Ansari et al., 2021; Kalita et al., 2018; Salehi et al., 2019).

One of the many medicinal plants in tropical nations like Indonesia is *Ruellia tuberosa* L. This plant has been used extensively in herbal medicine as an analgesic, anti-diabetic, antidiuretic, antihypertensive, antioxidant, and anti-inflammatory (Ratna Wulan et al., 2015; Thi Pham et al., 2022). Many flavonoids, including betulin, cirsiolol 4-glucoside, sorbifolin, pedalitin, cirsimarin, and cirsimaritin, were found to be separated from the ethyl acetate fraction of *Ruellia tuberosa* methanolic extracts (Lin et al., 2006). Based on an *In silico* analysis using chemicals from *Ruellia tuberosa*, betulin was the most potent inhibitor of both human and rat model α -amylase (Wulan et al., 2014). Moreover, *Ruellia tuberosa* has hypoglycemic potential by inhibiting pancreatic α -amylase (Ratna Wulan et al., 2015). Certain bioactive chemicals in anti-diabetic plants are thought to cause their inhibitory effects on aldose reductase, DPP-4, α -glucosidase, and other therapeutic targets. These compounds could be used as potential agents in creating medications that effectively cure type II diabetes (Lin et al., 2019; Tundis et al., 2010). It is essential to find potential aldose reductase, DPP-4, and α -glucosidase inhibitors in *Ruellia tuberosa* to investigate this plant's hypoglycemic potential in creating medications for treating diabetes mellitus. This study identifies therapeutic drugs for treating type 2 diabetes mellitus by assessing the inhibitory effectiveness of *Ruellia tuberosa* bioactive compounds against aldose reductase, DPP-4, and α -glucosidase through *in silico* tests.

MATERIAL AND METHODS

Ligand preparation

The ligands' two-dimensional (2D) structures were obtained in Spatial Data File (SDF) format from PubChem: <https://pubchem.ncbi.nlm.nih.gov/>. The ligands included pedalitin (CID 31161), betulin (CID 72326), betulinic acid (CID 64971), cirsiol (CID 44559837), cirsimarin (CID 159460), cirsimaritin (CID 188323), betulin (CID 64971), and standard anti-diabetic drug acarbose (CID 41774). The ligand preparation process was carried out using the supplied SDF files, which assigned the correct bond ordering and created a three-dimensional geometry. The Ligprep force field in Maestro Schrödinger Suite 2020-2 with optimized potential liquid simulations (OPLS-2005) was used to finish this. Moreover, ionization states were generated utilizing Epik 5.2 at pH 7.0 ± 2.0 in the Maestro Schrödinger Suite 2020-2 (Schrodinger, LLC, NY, USA). We generated fifteen possible stereoisomers for every ligand.

Protein preparation

The protein data bank (PDB) <https://www.rcsb.org/> provided the three-dimensional (3D) structures of aldose reductase (PDB ID: 3G5E), DPP-4 (PDB ID: 4A5S), and α -glucosidase (PDB ID: 3WEL). PDB structure files usually include water molecules, heavy atoms, and occasionally co-factors, metal ions, and co-crystallized ligands. Specific multimeric complexes might need to be broken down into a single unit. The placement of these groups needs to be confirmed because low resolution X-ray scans can make it difficult to distinguish between NH and O. Formal charges, bond ordering, and connectivity information that has to be assigned could not be included in PDB structures. The protein structure was prepared using the protein preparation wizard module from Maestro v12.4, Schrödinger, 2020-2 (Schrodinger, LLC, NY, USA) (preprocessed, optimized, and minimized). The chains were corrected to reflect the correct bond ordering and remove any missing hydrogens. Sample water orientations were used to maximize the H-bonds. Ultimately, the protein structure was minimized to the standard root mean square deviation (RMSD) value of 0.30 Å. The OPLS-2005 force field was utilized for molecular mechanics calculations to minimize energy.

Receptor grid generation

The prepared protein was used to generate receptor grids. The grids were generated with the OPLS-2005 force field. The polarity charge threshold was 0.25, and the protein atoms' van der Waals radii were scaled by 1.0. The receptor grid box was designed

with space for any ligand to fit inside the binding pocket, and it was placed in the center of the cognate ligands in each direction ($x = 14\text{Å}$, $y = 14\text{Å}$, and $z = 14\text{Å}$) (Khotimah et al., 2021). After grid generation, the dock main for each dimension (x , y , and z) was 14Å .

Molecular docking analysis

Following the grid-based docking procedure, ligand-receptor docking was carried out using the GLIDE module, Schrödinger, 2020-2 (Schrodinger, LLC, NY, USA) (Schrödinger Release 2020-2, 2020a). Suitable binders to the target receptor were thoroughly examined using Epik state penalties and Glide extra precision (XP) docking for precise and lucid details. There was no set of restrictions to protect ligand-receptor interactions. Ligand parameters were kept on default. The force field version utilized for calculations was OPLS_2005. The docked conformers were evaluated using the Glide (G) Score (Divyashri et al., 2021). The Maestro 12.3 Pose Viewer module was used to view and examine the output file produced by the docking pose.

Binding free energy calculation by Prime/MM-GBSA approach

The molecular mechanics-generalized born surface area (MM-GBSA)/Prime module, Schrödinger 2020-2 (Schrodinger, LLC, NY, USA), was utilized to calculate the binding free energy of the ligand-receptor complexes (Schrödinger Release 2020-2, 2020b). To evaluate the binding free energy of XP Glide docked output complexes, Prime MM/GBSA was employed. The relative energy of the complex field was assessed using the rotamer algorithm and the OPLS-2005 force field (Sun et al., 2014). The input structures for these calculations were taken from a Maestro 12.3 Pose Viewer file Glide output. The Prime MM/GBSA approach generated the following descriptors, according to equation [1].

$$\Delta G_{\text{Binding}} = \Delta G_{\text{complex}} - \Delta G_{\text{protein}} - \Delta G_{\text{ligand}} \quad [1]$$

Where the binding free energy is represented by $\Delta G_{\text{Binding}}$ and the complex, protein, and ligand-free energies are represented by $\Delta G_{\text{complex}}$, $\Delta G_{\text{protein}}$, and ΔG_{ligand} , respectively (Genheden and Ryde, 2015).

ADMET prediction

To assess the biochemical, molecular, physiological, and pharmacological impacts of the compounds, the adsorption, distribution, metabolism, excretion, and toxicity (ADMET) properties were calculated by the QikProp module of Maestro Schrödinger 2020-2 (Schrodinger, LLC, NY, USA) (Schrödinger Release 2020-2, 2020c). QikProp predicted the pharmacokinetic and physicochemical properties of the compounds.

Additionally, it assessed the tolerability of the analogs using Lipinski's rule of five, which stipulates that an analog cannot deviate from more than one of the following requirements: it can only have a maximum of five hydrogen bond donors and ten hydrogen bond acceptors, a molecular mass of less than 500 daltons, and a log P value for the octanol-water partition coefficient of less than five (Lipinski et al., 2012).

RESULTS

Molecular docking

Ruellia tuberosa bioactive compounds, including betulin, betulinic acid, cirsiol, cirsimarin, cirsimaritin, and pedalitin, exhibited a higher binding affinity to the receptors compared to the standard anti-diabetic drug acarbose or the control compound as determined by molecular docking results (Table 1). The docking scores for the aldose reductase receptor, DPP-4 receptor, and α -glucosidase receptor were found to be between -11.144 and -6.574 kcal/mol, -9.223 and -3.405 kcal/mol, and -7.082 and -0.898 kcal/mol, respectively. Similarly, the ligands in XP docking mode had the following glide energy, glide ligand efficiency, and glide emodel values: -57.188 to -18.804 kcal/mol, -0.056 to -0.459 kcal/mol, and -70.270 to -15.792 kcal/mol, respectively.

Fig. 1 shows betulin interacted with the active site of the aldose reductase inhibitor through one hydrogen bond with the amino acid residue Trp111. Betulinic acid is connected through two hydrogen bond interactions with residues Trp111 and Hie110. Cirsiol bonded to residues Asp43 and Gln183 via two hydrogen bond contacts and one pi-pi stacking interaction with residue Hie110. Three hydrogen bonds were formed between cirsimarin and the amino acid residues Trp111, Ser159, and Gln183. Two pi-pi stacking interactions between cirsimaritin and Trp111 and Phe122 were observed. Moreover, pedalitin connected with amino acid residues Trp20, Asn160, and Gln183 through three hydrogen bond interactions, one pi-cation interaction with residue Lys77, and one pi-pi stacking interaction with Hie110. For standard or reference anti-diabetic drugs, acarbose interacts with two hydrogen bonds with amino acid residues Asn160 and Gln183, one pi-pi stacking interaction with Trp20, and one pi-cation interaction with residue Lys77.

Fig. 2 shows betulin interacted with the DPP-4 inhibitor active site through hydrogen bonds with Asp1083 amino acid residues. Through single hydrogen bond interactions with residues Glu1048, cirsiol was linked to the DPP-4 active site. Two hydrogen bonds allowed cirsimarin to connect with Ser1006 and Asp1150 amino acid residues. Cirsimaritin interacted

with Lys1030 and Met1079 residues through a two-hydrogen bond interaction. Moreover, pedalitin is associated with residues Met1079 and Asp1150 through two hydrogen bond interactions. Meanwhile, one hydrogen bond and pi cation bond are residues Thr736 and Lys721, respectively, interacting with the standard anti-diabetic drug acarbose.

Fig. 3 shows betulinic acid interacted with the α -glucosidase inhibitor active site through one hydrogen bond with amino acid residue Arg814. Cirsiol connected through one pi-cation interaction with residue Arg699 and six hydrogen bond interactions with amino acid residues Glu301, Asp666, Arg670, Arg676, Asp684, and Glu792. Cirsimarin interacted through one pi-cation interaction with residue Arg699 and four hydrogen bond interactions with residues Asp666, Arg676, Arg699, and Glu792. Cirsimaritin interacted through one pi-cation interaction, Arg699, and five hydrogen bonds with residues Tyr659, Arg670, Arg699, Glu792, and Arg814. Furthermore, pedalitin interacted through two pi-cation interactions with Arg699 Arg814 and four hydrogen bond interactions with residues Tyr659, Arg670, Gly791, and Phe815. Meanwhile, the two hydrogen bonds of standard anti-diabetic drugs are connected to the amino acid residues Tyr662 and Asp698, and the two pi-cation bonds interact with Asn758 and Arg813.

Prime molecular mechanics with generalized born and surface area solvation (MM/GBSA)

The lead complexes were analyzed using the Prime MM/GBSA module, and their rescoring allowed for an estimation of the interaction binding's free energy. The Gibbs binding energy of the betulin, betulinic acid, cirsiol, cirsimarin, cirsimaritin, and pedalitin, as well as the standard anti-diabetic drug acarbose complexes from the molecular docking technique, was computed using the Maestro prime script. Cirsiol exhibited the most significant binding energy score of -66.94 kcal/mol when interacting with the aldose reductase receptor. Betulin had the highest Gibbs free energy (ΔG_{bind}) at -70.23 kcal/mol in its interaction with the DPP-4 receptor. With a binding energy value of -53.05 kcal/mol, pedalitin exhibited the most potent interaction with the α -glucosidase receptor. Furthermore, the ΔG_{bind} coulomb, ΔG_{bind} covalent, ΔG_{bind} vander, ΔG_{bind} HBond and ΔG_{bind} Lipophilic ligands in MM/GBSA mode were -0.26 to -20.44 kcal/mol, 0.01 to 15.81 kcal/mol, -3.70 to -51.50 kcal/mol, -0.19 to -6.57 kcal/mol, and -23.71 to -69.44, respectively (Table 2).

ADMET prediction

The QikProp module was used in this study to analyze the ADMET properties of betulin, betulinic acid,

Table 1. The results of a molecular docking study of *Ruellia tuberosa* compounds with anti-diabetic receptors.

Ligands	Receptor	Docking score (kcal/mol)	No of H-bonds	Glide energy	Glide ligand efficiency	Glide Emodel (kcal/mol)
Betulin	Aldose reductase	-6.767	1	-18.804	-0.211	-18.576
	DPP-4	-5.031	1	-36.559	-0.157	-43.661
	α -Glucosidase	-1.779	-	-19.299	-0.056	-23.251
Betulinic_acid	Aldose reductase	-6.574	2	-31.438	-0.199	-27.078
	DPP-4	-3.405	-	-25.157	-0.103	-15.792
	α -Glucosidase	-0.898	1	-21.135	-0.027	-18.044
Cirsiliol	Aldose reductase	-11.144	2	-52.382	-0.318	-63.704
	DPP-4	-6.726	2	-57.188	-0.192	-69.834
	α -Glucosidase	-5.493	6	-50.452	-0.157	-66.322
Cirsimarín	Aldose reductase	-10.106	3	-47.447	-0.297	-64.915
	DPP-4	-6.294	2	-55.471	-0.185	-70.270
	α -Glucosidase	-4.142	4	-44.085	-0.122	-54.423
Cirsimaritin	Aldose reductase	-8.927	-	-36.062	-0.388	-52.524
	DPP-4	-7.237	2	-40.967	-0.315	-54.512
	α -Glucosidase	-5.245	5	-34.545	-0.228	-42.947
Pedalitin	Aldose reductase	-10.554	4	-39.217	-0.459	-55.103
	DPP-4	-9.223	2	-44.939	-0.401	-55.513
	α -glucosidase	-7.082	4	-42.275	-0.308	-55.351
Acarbose*	Aldose reductase	-9.489	2	-36.679	-0.431	-49.179
	DPP-4	-1.615	1	-27.906	-0.073	-27.906
	α -Glucosidase	-6.096	3	-40.360	-0.277	-52.037

*Anti-diabetic reference drug.

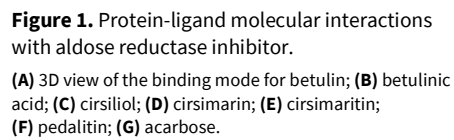
cirsiliol, cirsimarín, cirsimaritin, and pedalitin bioactive compounds from *Ruellia tuberosa* and standard anti-diabetic drug acarbose. Table 3 displayed the acceptable values for each parameter, the molecular weight, percentage of oral absorption, and number. Violations in the rule of five violations, and other kinetic parameters and toxicity profiles were compared with standard anti-diabetic drugs.

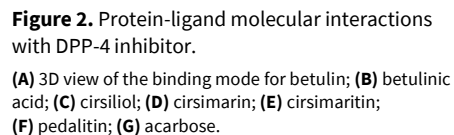
DISCUSSION

In the early stages of type 2 diabetes, reducing postprandial hyperglycemia is one possible treatment approach. Slowing the glucose absorption rate is achieved by blocking the digestive enzymes aldose reductase, DPP-4, and α -glucosidase, which break down carbohydrates. Therefore, inhibitors of these enzymes cause a decrease in the absorption of glucose, which in turn lessens the rise in plasma glucose following a meal (Tundis et al., 2010). Nevertheless, additional research into novel therapeutic approaches for the management and treatment of type 2 diabetes is now required due to some of the unfavorable side

effects of existing drugs, including diarrhea, lactic acidosis, liver issues, and hypoglycemia at higher dosages. Apart from the current treatments, numerous medicinal plants have been proposed as possible remedies for diabetes. Traditional medicines are widely used worldwide because of their effectiveness, lower cost, and lower risk of side effects (Ratna Wulan et al., 2015). Thus, research into medicinal plants' bioactive components is essential to identify potential therapeutic agents for developing anti-diabetic drugs. In the present study, possible aldose reductase, DPP-4, and α -glucosidase inhibitors for discovering anti-diabetic drugs were identified by evaluating the inhibiting activity of *Ruellia tuberosa* and its bioactive compounds using computational methods.

Finding compounds that may attach to biologically attractive targets quickly is essential for drug discovery. Conducting *in silico* analysis on compounds with high hits against a specific target is one method to accomplish this. Therefore, in the current study, computer-generated interaction images of bioactive compounds with target structures in different orientations





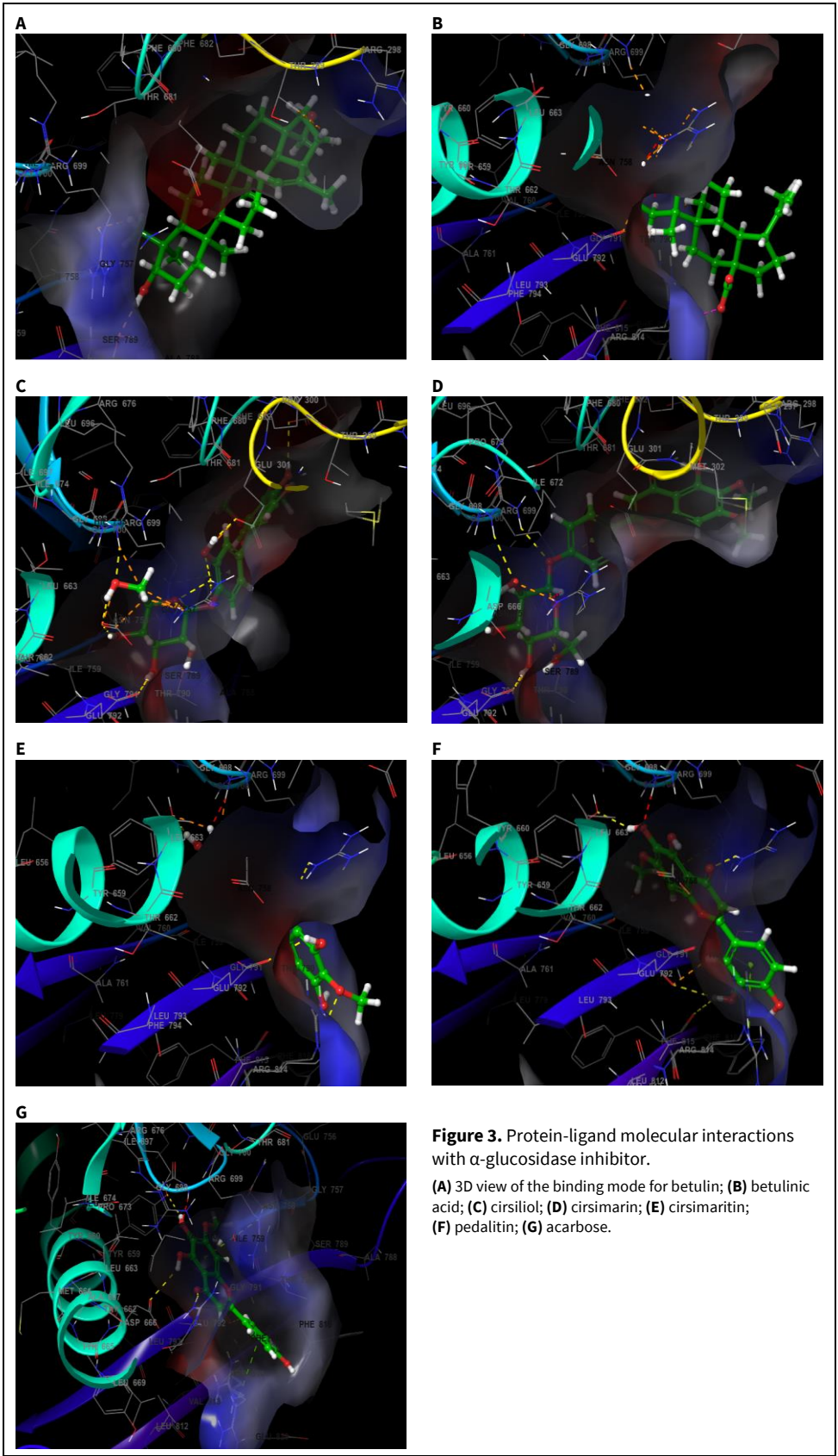


Table 2. Binding free energy calculations of *Ruellia tuberosa* compounds against the target's anti-diabetic receptors.

Ligands	Receptor	ΔG_{bind} (kcal/mol)	ΔG_{bind} Coulomb	ΔG_{bind} covalent	ΔG_{bind} Vander	ΔG_{bind} Hbond	ΔG_{bind} lipophilic
Betulin	Aldose reductase	-40.26	-10.43	15.81	-3.70	-0.25	-60.15
	DPP-4	-70.23	-7.94	5.30	-36.21	-0.29	-69.44
	α -Glucosidase	-29.85	-0.26	1.79	-19.67	-0.30	-32.82
Betulinic_acid	Aldose reductase	-58.40	-10.63	6.23	-25.84	-0.24	-53.97
	DPP-4	-9.85	-86.84	10.37	-11.51	-0.18	-66.82
	α -Glucosidase	-17.72	-5.33	5.42	-21.13	-0.83	-32.39
Cirsiliol	Aldose reductase	-66.94	-20.21	12.72	-51.50	-1.80	-41.29
	DPP-4	-44.68	-8.59	0.01	-51.10	-2.09	-37.53
	α -Glucosidase	-47.93	-37.75	12.93	-39.60	-6.57	-25.40
Cirsimarlin	Aldose reductase	-68.59	-15.51	7.91	-44.75	-1.05	-39.29
	DPP-4	-52.03	-21.80	5.25	-45.92	-1.46	-39.40
	α -Glucosidase	-40.92	-18.24	2.50	-33.86	-5.86	-23.71
Cirsimaritin	Aldose reductase	-63.33	-7.87	5.80	-40.20	-0.19	-31.67
	DPP-4	-53.34	-17.52	2.23	-37.05	-1.44	-29.15
	α -Glucosidase	-40.70	-16.67	6.53	-30.24	-1.39	-25.34
Pedalitin	Aldose reductase	-46.74	-19.27	4.41	-28.64	-1.08	-27.29
	DPP-4	-61.64	-31.67	6.97	-32.82	-1.94	-32.00
	α -Glucosidase	-53.05	-31.12	3.59	-31.16	-2.83	-24.24
Acarbose*	Aldose reductase	-46.09	-15.46	7.57	-27.56	-0.94	-27.55
	DPP-4	-10.17	-9.47	0.57	-16.27	-0.24	-7.85
	α -Glucosidase	-50.32	-26.20	3.00	-31.77	-2.47	-24.15

*Anti-diabetic reference drug.

were displayed by the Schrodinger software. The highest compounds had XP docking scores ranging from -11.144 to -0.898 kcal/mol, as shown in Table 1.

The high affinity that *Ruellia tuberosa* compounds have for cirsiliol implies that these compounds may be cirsiliol inhibitors and possible therapeutic agents against anti-diabetes. A three-dimensional representation of the interactions between the ligands and the amino acid residue of cirsiliol revealed that these interactions were caused mainly by hydrogen bonds with the carbonyl groups, hydroxyl groups, and oxygen atoms of the aromatic rings found in the ligands. Enzymatic catalysis and the structural stability of biomolecules depend on hydrogen bond interaction (Kikiowo et al., 2022). Tryptophan, tyrosine, histidine, and phenylalanine amino acid residues have aromatic interactions that are essential for drug design because they improve molecular recognition, interactions, and the specificity of drug-like compounds, which ultimately enhances efficacy (Chukwuma et al., 2022).

This work found the docking poses of *Ruellia tuberosa* bioactive compounds and their binding sites on

aldose reductase, DPP-4, and α -glucosidase by carefully analyzing the optimum conformation of the docking data, as illustrated in Figs. 1, 2, and 3, respectively. Various bonding interactions were involved, such as H-bonds, pi-cation interaction, and aromatic interaction (pi-pi stacking). According to published research, the strength of a hydrogen bond in an excited state is similar to that of the pi-pi interaction when stabilizing a structural complex (Deng et al., 2020). Although the active-site conformation remains unaffected in the ground state, the rate of chemical activity is reduced by a factor of 20–30 due to the lack of the pi-pi stacking interaction (Pecsi et al., 2010). On the other hand, van der Waals or hydrophobic interactions might increase ligand affinity for the target protein (Ferreira De Freitas and Schapira, 2017). Hydrophobic interactions and hydrogen bonding influence complex stability. The interactions that help proteins avoid water and cluster together in their globular form are known as hydrophobic interactions (Camilioni et al., 2016). The residues of non-polar amino acids that tend to form clusters inside proteins are the residues involved in hydrophobic interactions. This

Table 3. ADMET properties of *Ruellia tuberosa* compounds.

Predictor	Active compounds						
	Betulin	Betulinic_acid	Cirsiliol	Cirsimarín	Cirsimaritin	Pedalitin	Acarbose
mol_MW	442.724	456.707	492.435	476.436	314.294	316.267	642.526
donorHB	2	2	5	4	1	3	9
accptHB	3	3.7	13.7	13	4.5	5.2	16.3
QPlogS	-6.27	-6.24	-3.77	-3.98	-4.11	-3.37	-6.40
QPlogPo/w	5.94	4.25	-0.01	0.61	2.69	1.15	-1.13
QPlogHERG	-3.766	-1.834	-5.831	-6.023	-5.237	-3.376	-5.482
QPlogBB	-0.376	-0.436	-3.465	-2.874	-1.036	-1.981	-5.127
#metab‡	4	3	8	7	4	5	10
CNS	+/-	-	--	--	--	--	--
QPPCaco	1765	320	11	33	424	51	0.31
RuleOfFive	1	1	2	1	0	0	3
RuleOfThre	1	1	2	1	0	0	0
HumanOralAbsorption	100	95	20	45	90	64	0
PercentHumanOralAbsorption	Low	Low	Medium	Medium	HIGH	HIGH	Low

A * indicates a violation of the 95% range. mol_MW: Molecular weight (recommended value: 130–725); donorHB: Estimated number of hydrogen bond donors (recommended value: 0–6); accptHB: Estimated number of hydrogen bond acceptors (recommended value: 2–20); QPlogPo/w: Predicted octanol/water partition coefficient (recommended value: –2–6.5); QPlogS: Predicted aqueous solubility (recommended value: 6.5–0.5); QPlogHERG: Predicted IC₅₀ value for blockage of HERG K⁺ channels (recommended concern below -5); QPlogBB: Predicted brain/blood partition coefficient (recommended value: –3–1.2); #metab‡: Number of likely metabolic reactions (recommended value: 1–8); CNS: Predicted central nervous system activity (recommended value: –2 (inactive), +2 (active)); QPPCaco: Predicted apparent Caco-2 cell permeability (recommended value: <25 poor, >500 great); QPPMDCK: Predicted apparent MDCK cell permeability (recommended value: <25 poor, >500 great); RuleOfFive: Number of violations of Lipinski's rule of five. The rules are MW < 500, logP < 5, DHB ≤ 5, AHB ≤ 10, Positive PSA value; RuleOfThre: Number of violations of Jorgensen's rule of three. The three rules are: logS > -5.7, PCaco > 22 nm/s, PM < 7; HumanOralAbsorption: Predicted human oral absorption percent (recommended value: >80% is high, <25% is poor); PercentHumanOralAbsorption: Predicted human oral absorption on 0 to 100% scale (>80% is high, <25% is poor).

discovery is critical for new drugs since most H-bond residues are acceptors and donors. The intermolecular properties discovered in this study could be explored to optimize protein–ligand complexes. Using this method, entirely new compounds with biological activity that can interact and inhibit the target can be developed both *in vitro* and *in vivo*.

The molecular docking result was confirmed by identifying the binding free energy with Maestro's Prime MM-GBSA module. This post-docking method is used to evaluate the stability of the protein–ligand complex following docking (Basnet et al., 2023). The MM-GBSA module calculated the ΔG_{bind} for aldose reductase, DPP-4, and α-glucosidase receptor-lead ligand complexes. Using advanced mechanics, ΔG_{bind} was utilized to compute the binding energy of the screened compounds after the docking investigation. Several researchers assert that the MM-GBSA methodology is a reliable post-docking technique for ascertaining the docked complexes' binding location (Genheden and Ryde, 2015). The result showed that *Ruellia tuberosa*'s bioactive compounds have high binding free energy values (-9.85 to -70.23 kcal/mol) toward aldose reductase, DPP-4, and α-glucosidase inhibitors. Studies have demonstrated a correlation

between a favorable binding free energy and a reliable molecular docking study, indicating that a low (more negative) binding free energy corresponds to a robust and more stable protein–ligand complex. Therefore, favorable interactions with the protein crystal structure can be inferred from the docking data of the bioactive compounds from *Ruellia tuberosa*. Moreover, the binding free energy estimates the intermolecular interactions between the protein and the ligand. Conversely, the docking score indicates the binding affinity between the protein and the ligand after docking. As the docking score demonstrates, the binding affinity is defined by the strength of the intermolecular interactions between the protein and the ligand, shown by the binding free energy values. Overall, betulin, betulinic acid, cirsiliol, cirsimarín, cirsimaritin, and pedalitin are potential inhibitors that have the affinity of the binding of MMGBSA, pi-pi intermolecules, and bond interactions with aldose reductase, DPP-4, and α-glucosidase amino acid residues.

Given that 60% of drugs fail to achieve pharmacokinetic properties during the drug development process, the ADMET analysis could help predict pharmacokinetic properties early on and reduce late-stage

compound attrition during the drug discovery process, lowering the cost of drug development research (Bocci et al., 2017). A set of physicochemically significant descriptors was generated in the current study using the QikProp module to aid in further evaluating ADMET characteristics. As a general guideline, Lipinski's rule of five can be used to assess drug-likeness or to ascertain whether a chemical molecule possessing a specific biological or pharmacological activity has characteristics that would probably make it an orally active drug in humans. A drug's ADMET and other molecular characteristics that are crucial to its pharmacokinetics in the human body are described in the rule. This parameter indicates how many property descriptors for 95% of the drugs listed that are not within the optimal range of values were obtained using QikProp. Molecular weight <500 , QP log P o/w <5 , donor HB ≤ 5 , and acceptor HB ≤ 20 were among the parameters computed for each *Ruellia tuberosa* compound in the present investigation. The capacity of oral drugs to dissolve in intestinal fluid is one important characteristic. When a medication is required to have systemic effects, its inability to dissolve could hinder the absorption by the intestine through the portal vein system. The main reason why water-soluble chemicals make medication research so much easier is that they are simple to handle and formulate. Solubility is a key factor regulating absorption when taken orally. Similarly, drugs intended for parenteral delivery must be highly soluble in water to deliver a sufficient active compound concentration (Daina et al., 2017). The solubility of substances in water was represented by the QP log S value (log S). The water solubility (QP log S) ranged from -6.273 to -3.771 , and all readings were within the suggested range, indicating that *Ruellia tuberosa* compounds have adequate absorption and distribution potential. This is important for drug absorption and distribution inside the body.

The partition coefficient (log P o/w) between water and octanol is another widely used descriptor to evaluate lipophilicity. The permeability of the compounds through biological membranes is correlated with their lipophilicity. Very hydrophilic substances generally cannot passively diffuse through them, but they can be lowered when lipophilicity is too low (Lagorce et al., 2017). Another important indicator of the effective absorption and distribution of *Ruellia tuberosa* compounds throughout the body is the partition coefficient (QP log P o/w), which ranged from 0.017 to 6.253 . All of the measurements were within the recommended range. One of the key elements influencing a drug's efficacy in the central nervous system is its permeability across the blood-brain barrier. The blood/brain partition coefficients (log B/B) were calculated and used to predict central nervous

system (CNS) access in the current investigation. The log B/B ranged from -3.465 to -0.436 . These values were within the recommended ranges, indicating that *Ruellia tuberosa* compounds had excellent permeability across the CNS.

The permeability of compounds in the gut-blood barrier was investigated using the Caco2 cell permeability assay. The current study confirms that each compound in the gut-blood barrier has enhanced permeability. This parameter, which access to biological membranes varied from 11 to 1765 in the recent investigation, is critical to drug metabolism. In addition, QikProp evaluated the quantity of potential metabolic processes to determine whether the molecules might easily reach the intended location after entering the bloodstream. All compounds' metabolic reactions in the current study are within permissible limits. Numerous pharmaceuticals seem to cause cardiac toxicity; the HERG K⁺ channel, most known for its function in the heart's electrical activity that controls heartbeat, is the molecular target (Vandenberg et al., 2001). A significant number of drugs have been withdrawn from the market due to hERG-related cardiotoxicity. Therefore, HERG K⁺ channel blockers have the potential to be toxic. During the initial phases of drug discovery, the predicted IC₅₀ values usually provide acceptable projections for the cardiac toxicity of drugs. (Lee et al., 2019). To inhibit HERG K⁺ channels, predicted log IC₅₀ values (logHERG) should be in the range of >-5 . Given that the expected range of values for the analysis was between -1.834 and -6.023 , all the compounds are safer to use. Overall, both the *Ruellia tuberosa* compounds and the standard anti-diabetic drug acarbose demonstrated the excellent value of Lipinski's rule of five and predicted pharmacokinetic parameters without too much violation of drug-likeness features so that these results can be taken further for preclinical evaluation.

CONCLUSION

By a computational approach, we could identify potent therapeutic compounds derived from *Ruellia tuberosa* that target specific inhibitors associated with anti-diabetes. The potential of *Ruellia tuberosa* compounds as a treatment against diabetes mellitus is suggested by their good interaction and binding energy to aldose reductase, DPP-4, and α -glucosidase inhibitors compared to the standard anti-diabetic drug acarbose. Further, all six of the evaluated *Ruellia tuberosa* compounds showed drug-likeness, and many of their pharmacokinetic and physicochemical parameter values were within the permissible range established for human usage during ADMET analysis, suggesting that they may have the potential to be drug-like molecules. Thus, this work supports the

traditional medicine practice of using *Ruellia tuberosa* as an anti-diabetic agent and highlights the plant in the quest for novel and secure anti-diabetic medicines for drug development.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Retnaningtyas E	Setiawan A	Susatia B	Hariyanto T	Sudiwati NLPE
Concepts or ideas	x				
Design	x	x			
Definition of intellectual content	x	x			
Literature search	x	x			
Experimental studies	x	x			
Data acquisition	x	x	x	x	x
Data analysis	x	x	x	x	x
Statistical analysis	x	x			
Manuscript preparation	x	x	x	x	x
Manuscript editing	x	x	x	x	x
Manuscript review	x	x	x	x	x

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