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In-silico antidiabetic potential of phenolics identified from ethyl acetate fraction of *Ageratum conyzoides* methanol leaf extract

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Background: Different parts of *A. conyzoides* have been widely used in traditional medicine for therapeutic purposes, and it contains enormous secondary metabolites such as phenolics with varied biological activities. Poor druggability has caused many candidate compounds showing excellent *in-vitro* efficacy to be dismissed, which can be minimized in early drug discovery by *in-silico* drug-like prediction and virtual screening. Thus, this study was aimed at evaluating the antidiabetic potentials of phenolic compounds (furocoumarinic acid, liquiritin, isorhamnetin and syringin) identified from ethyl acetate fraction of *A. conyzoides* methanol leaf extract.

Methods: SwissADME and ADMETlab 2.0 software tools were used to predict the drug-likeness of the compounds, and AutoDock Vina and UCSF Chimera were used for docking studies. The compound that showed best interaction with receptors (target proteins) was then experimentally validated through fasting blood glucose (FBG) assay.

Results: Findings of this study indicated that all the four phenolics were found to have good drug-like potential according to the rule-based filter models, with oral bioavailability scores of 55% better than acarbose (17%). However, of the four phenolics, only isorhamnetin was able to demonstrate good interactions with target receptors, indicating an outstanding inhibitory effect on aldose reductase (AR), dipeptidyl peptidase 4, and glutamine fructose-6-phosphate amidotransferase (GFAT). Experimental validation indicated that FBG levels of the diabetic control (untreated) group, acarbose- and isorhamnetin-treated groups were 421.00±9.10, 232.40±6.15, and 239.60±8.56 mg/dL, respectively, with a corresponding percentage decrease of 10.65±3.20, 52.07±1.78, and 50.13±2.60 respectively.

Conclusion: This study has demonstrated that furocoumarinic acid, liquiritin, isorhamnetin, and syringin from ethyl acetate fraction of *A. conyzoides* methanol leaf exhibited good drug-likeness potentials with 55% oral bioavailability. Out of which, only isorhamnetin was able to inhibit AR, DPP-4, and GFAT activities, and activate glucokinase through docking studies.

Keywords: *Ageratum conyzoides, phenolics, druggability, molecular docking, isorhamnetin, and diabetes mellitus*

Introduction

The quest for lead compounds in drug design through traditional methods is a difficult and tedious process, and the apparently boundless choices one should filter through may deter, thus the need to incorporate early *in-silico* study. Computer-aided drug design (CADD) and discovery have been achieved through ligand-based drug advancement which gives an avenue to create new molecular entities interacting with specific biological targets utilizing a model of the said

targets (Batool et al., 2019). The advancement of structure-based drug design (SBDD) requires an in-depth comprehension of the natural molecules' 3-D structure. Then again, ligand-based drug design and discovery, also called indirect drug design, requires a profound comprehension of other compounds (ligands) that attach to the desired biological target (Anurak & Kesara, 2018). Fortunately, computational tools have saved the day and probably had a major impact on rationalizing the process of developing new drugs. Of all methods, molecular docking (MD) simulation has been significant in CADD and has quickly gained the rank to take an important place in current SBDD tactics. As a result, it has grown in significance as a method for drug discovery (Meng et al., 2011). The ligands or molecules of interest to be targeted towards the receptors could be isolated from plants such as *Ageratum conyzoides* with known ethno-medical and biological or pharmacological history.

Different parts of *A. conyzoides* has been widely used in traditional medicine for a number of therapeutic goals such as wound healing, malarial fever and diabetes management (Kaur & Dogra, 2014; Piero et al., 2012; Moura et al., 2005). Similarly, the plant has been demonstrated to contain enormous secondary metabolites such as phenolics which confers its antioxidant efficacy and other biological potentials (Ozioko et al., 2022; Atawodi et al. 2017; Fatema, 2013). Despite the large number of bioactive molecules, like phenolics, that are produced and deposited in different databases each year, there is no equivalent increase in new approved drugs by the US Food and Drug Administration (FDA) (Mullard, 2014). This may be attributed to efficacy and safety deficiencies that are related partly to ADME (absorption, distribution, metabolism and excretion) characteristics and numerous toxicity (T) effects which caused many candidate compounds with great *in-vitro* efficacy to be discarded due to poor drug-likeness. Unfortunately, the present techniques for evaluating ADME/T characteristics are time-consuming, expensive, and frequently necessitate extensive animal testing methods that are frequently insufficient for handling sizable chemical batches and ethical issues. Since it is becoming impractical to perform intricate and costly ADME/T experimental procedures especially at the academic research levels, *in-silico* druggability prediction and virtual screening (VS) then becomes method of choice in early drug discovery. Thus, the application of high-quality VS methods such as molecular docking will permit the parallel optimization of compound efficacy and drug-likeness potentials, which is anticipated to reduce overall costs because of a lower failure rate and increase the overall quality of drug candidates and their likelihood of success. Some examples of target proteins and enzymes implicated in diabetes mellitus (DM) include GFAT, AR, protein tyrosine phosphatase 1β (PTP1 β), alpha glucosidases, K_{ATP} channels, insulin receptor, glucokinase (GCK), sodium-glucose cotransporter 2 (SGLT2), and DPP-4 (Ngoc & Ly, 2012). Hence, this study was aimed at *in-silico* evaluation of the antidiabetic potentials of four phenolics (furocoumarinic acid, syringin, liquiritin, and isorhamnetin) identified from ethyl acetate fraction of *A. conyzoides* methanol leaf extract as reported by Ozioko et al. (2024) in search of cheap, novel and alternative oral antidiabetic drugs. The best docked candidate compound was then validated by *in-vivo* fasting blood glucose (FBS) assay on alloxan chemically-induced diabetic rats.

Materials and Methods

All reagents were of analytical grade. Materials and reagents used include *Ageratum conyzoides* leaves, Alloxan, $C_4H_2N_2O_6.H_2O$ (KEM LIGHT Laboratory Put Ltd, Mumbia, India. CAS No. 2244-11-3), 1% glucose, Acuu-chek glucometer, glucose test strips, and acarbose (5mg). Collection and Extraction of *Ageratum conyzoides* leaves: It was as described by Ozioko et al., 2022.

In-Silico drug-likeness study

The prediction of drug-likeness of the phenolic compounds and some oral hypoglycemic drugs in use were carried out using SwissADME online tool (Daina et al., 2017) and the ADMETlab 2.0 tool (Xiong et al., 2021). The drug similarity filter test was performed for the ligands using these web servers which evaluate the compound's drug-likeness according to the Lipinski's Rule of 5 (Ro5) (Lipinski et al., 2001) and other parameters which confirms the property of an oral drug candidate.

Molecular docking studies

Protein-ligand docking has been widely used to predict binding modes and affinities of ligands. Using the AutoDock Vina 1.2.0 docking software program (Trott & Olson, 2010) and UCSF Chimera 1.16 (Pettersen et al., 2004), docking studies were performed to examine the structural interactions between the receptors and ligands. Blind docking approach was adopted in which the Grid Boxes were chosen to cover the whole probable binding pocket of the respective targets.

Preparation of targets and ligands

Prior to molecular docking, the molecular targets were selected and downloaded from the protein data bank (PDB) (Burley et al., 2021; Rose et al., 2020), and subsequently prepared. The targets were selected based on the scientific reports implicating them in the pathophysiology or molecular mechanism of DM regulations (Ngoc & Ly, 2012). The proteins of interest that were explored and targeted in this study included: AR, PTP 1β, alpha glucosidase, SUR1, GCK, PPAR-γ, GFAT, DPP-4, free fatty acid receptor 1 (FFAR 1), and G-protein-coupled receptor 119 (GPR119). The targets were prepared following the protocols described by Shapovalov et al. (2011) and Jain & Nicholls, (2008) using different interfaces of the AutoDock Vina integrated in UCSF Chimera 1.16. Besides, the native ligands and non-standard residues attached to the main target chains were deleted. The grid box coordinate of each protein targets was set to accommodate sufficient binding pockets through blind docking approach. The ligands (the 4 phenolics from *A. conyzoides* methanol leaf extract and an example each of respective class of oral antidiabetic drugs in market circulation) were selected and downloaded from PubChem Database of National Center for Biotechnological Information (NCBI) (Kim et al., 2016) (Table 1), and prepared for docking, which involves the addition of hydrogen atoms, desolvation of water molecules, addition of partial charges, and structural minimization through the "Dock Prep" interface of AutoDock Vina integrated in UCSF Chimera 1.16. Both the prepared protein and ligand structures were saved in the PDBQT file format.

Table 1. The Chemical formula and PubChem ID of ligands

S/No	Ligands	PubChem CID	Molecular Formula	Anti-diabetic Class
1	Glibenclamide	3488	$C_{23}H_{28}ClN_3O_5S$	Sulfonylurea
2	Metformin	4091	$C_4H_{11}N_5$	Biguanides
3	Rosiglitazone	77999	$C_{18}H_{19}N_3O_3S$	Thiazolidinediones(TZD)
4	Acarbose	41774	$C_{25}H_{43}NO_{18}$	α-Glucosidase Inhibitors
5	Sitagliptin	4369359	$C_{16}H_{15}F_6N_5O$	DPP-4 Inhibitor
6	Repaglinide	65981	$C_{27}H_{36}N_2O_4$	Meglitinides
7	Liraglutide	16134956	$C_{172}H_{265}N_{43}O_{51}$	Incretin Memetics
8	Furocoumarinic acid	31750885	$C_{17}H_{18}O_9$	-
9	Liquiritin	503737	$C_{21}H_{22}O_9$	-
10	Isorhamnetin	5281654	$C_{16}H_{12}O_7$	-
11	Syringin	5316860	$C_{17}H_{24}O_9$	-

DPP= Dipeptidyl peptidase-4.
One each of the class of anti-diabetic agent was represented.

Visualization and analyses of docking interactions

Using Bovia Discovery Studio v21.10.20298 (Biovia, 2021), the target-ligand docked complexes were visualized and interactions analyzed. It is an open-source program for the interactive visualization and analysis of molecular structures and related data. So, these molecular visualization tools were used to examine the hydrogen bond interactions and other basic parameters between the protein targets and the ligands.

Validation of docked results by *in-vivo* fasting blood glucose assay

The sample containing the phenolic compound (i.e., isorhamnetin-containing extract) with relative best docked result were validated using *in-vivo* FBG assay. This *in-vivo* antidiabetic activity was carried out according to de Carvalho et al. (2003) using alloxan-induced diabetic rats.

Induction of diabetes in experimental rats

Experimental diabetes was induced by an intraperitoneal injection of freshly prepared alloxan monohydrate (de Carvalho et al., 2003) at the dose of 180 mg/kg to at least 12 h-fasted rats. The alloxan mechanism of action involves the destruction of the beta cells of the pancreas. The uric acid derivative initiates free radical damage to DNA in the beta cells of the pancreas, causing the cells to malfunction and die. This would cause the liberation of its content in the blood, especially insulin. The high insulin level in the blood would cause a hypoglycemic shock to the rats which may lead to death. To avoid this shock, 1% glucose solution was orally administrated to the animals. In this study, both male and female (non-pregnant) rats were selected randomly with body weight between 180-240g. After 48hours of induction of diabetes, the blood tested using strips of Acuu-chek glucometer for glycemic value, and also after 72hours. Rats that showed blood glucose levels greater than 250mg/dl were selected and used for the study. The standard oral hypoglycemic drug used was acarbose (5mg/kg body weight).

Experimental design

The rats were distributed into four groups (n=5) and treated accordingly once a day for one week except group one that was only administered with vehicle of administration. The FBG level was measured using strips on Acuu-chek glucometer every 24hrs for 7 days.

Group I: Normal control administered with 0.9% normal saline (vehicle of administration).
Group II: Diabetic control administered with 0.9% normal saline
Group III: Diabetic rats administered with acarbose (5 mg/kg/day body weight).
Group IV: Diabetic rats administered with isorhamnetin-containing sample (5 mg/kg/day body weight).

Results

The drug-likeness analyses of the ligands

The drug-likeness potentials of the ligands as predicted using SwissADME and ADMETlab 2.0, according to different rule-based filters (like Lipinski, Amgen, GSK, Pharmacia, and Bayer), were presented in Table 2, with diverse ranges of properties within which a compound is defined as drug-like. Two web tools were used side-by-side to compare if their result outputs were similar when juxtaposed since they were built using different algorithm. The SwissADME tool gave access to five different rule-based filters (Lipinski, Amgen, Veber, Egan, and Muegge), with diverse ranges of properties inside of which a compound is defined as druggable or drug-like. The output values of "0-5" connotes the number of violations of the affected rule. Any compound that have >1violation of any rule is considered be less druggable. From the result, acarbose violated three rules (Lipinski, Amgen and Bayer), while metformin violated two rules (Amgen and Bayer). However, other ligands had zero or 1 violation including all the four phenolics identified in this research. Besides this five rule-based filter, SwissADME also employed another model, Abbot Bioavailability (BA) Score. This semi-quantitative rule-based score defined four classes of compounds with oral bioavailabity probabilities of 11%, 17%, 56% or 85%. Here, all the ligands have BA score above 10% with acarbose having the least oral bioavailability (17%) (Table 2). ADMETLab 2.0 tool on the other hand employed four different rule-based filters models to define the druggable status of a candidate drug. These rules were the Lipinski (MW≤500; logP≤5; NHBA≤10; NHBD≤5), (logP > 3; TPSA <75), GSK (MW ≤ 400; logP ≤4), and Golden triangle (GT) (200 ≤MW ≤500; -2 ≤ logD ≤5). The output of these rules is either 'Accepted (Ac)' or 'Rejected (Rj)', depending on whether the rule was obeyed or violated respectively. From the results obtained, acarbose violated three rules (Lip., GSK and GT), which is similar to the result output using SwissADME tool. Furocoumarinic acid, isorhamnetin, syringin, and rosiglitazone obeyed all the rules, while glibenclamide, metformin, sitagliptin, repaglinide, and liquiritin had one violation each (Table 2).

Table 2. Predicted drug-likeness potential of the ligands

Ligands	Swiss ADME						ADMETLab 2.0			
	Lip.	Amgen	GSK	Pharmecia	Bayer	BA Score	Lip	Pfizer	GSK	GT
Glibenclamide	0.00	1.00	1.00	0.00	0.00	0.55	Ac	Ac	Rj	Ac
Metformin	0.00	3.00	0.00	0.00	2.00	0.55	Ac	Ac	Ac	Rj
Rosiglitazone	0.00	0.00	0.00	0.00	0.00	0.55	Ac	Ac	Ac	Ac
Acarbose	3.00	4.00	1.00	1.00	5.00	0.17	Rj	Ac	Rj	Rj
Sitagliptin	0.00	0.00	0.00	0.00	0.00	0.55	Ac	Ac	Rj	Ac
Repaglinide	0.00	1.00	1.00	0.00	1.00	0.56	Ac	Ac	Rj	Ac
Furocoumarinic acid	0.00	0.00	1.00	1.00	1.00	0.56	Ac	Ac	Ac	AC
Liquiritin	0.00	0.00	1.00	1.00	0.00	0.55	Ac	Ac	Rj	Ac
Isorhamnetin	0.00	1.00	0.00	0.00	0.00	0.55	Ac	Ac	Ac	Ac
Syringin	0.00	1.00	0.00	1.00	0.00	0.55	Ac	Ac	Ac	Ac

BA= Bioavailability; Lip.=Lipinski rule; GSK= GlaxoSmith Klin; Ac=accepted; Rj=rejected.
Note that 0-5 categorical values which connote the number of violations.

The molecular docking studies

The target proteins that were implicated in etiology and pathophysiology of DM with their respective PDB codes and 3D structures were as presented in the Appendix. Tables 3 and 4 summarized the basic properties of the docked ligand-protein

complexes as deduced using Discovery Studio. Table 4 presented the targets with primary inhibitory mechanism of action. The basic properties of each ligand-protein complexes, which comprises of their binding energies (BE) or binding affinities or docking scores, inhibition constant (Ki) (calculated from the BE), number of interacting bonds (NBs) and binding site interacting amino acids, were analyzed and recorded as shown in the table. According to this results, isorhamnetin, metformin and acarbose have a respective BE of -12.75, -5.65 and 36.08 kcal/mol when docked with aldose reductase (AR), and isorhamnetin had very high inhibitory effect on the target protein with a very low calculated Ki (0.05nM) value. D44, K78, Q184 and Y210 were the common interacting amino acid residues at both the binding sites of isorhamnetin- and metformin-AR complexes unlike that of acarbose. Also analyzing docking interactions with α-glucosidase, acarbose, isorhamnetin and metformin have respective BE of -9.86, -9.49 and -4.87 kcal/mol, with acarbose having the best inhibition effect (calculated Ki of 58.10nM). Among the phenolics identified in this study, only isorhamnetin was able to dock with AR, α-glucosidase, DPP-4, SGLT2 and GFAT. Table 4 on the other presented the docking results for the targets with stimulatory mechanism of actions. Similar to Table 3 result, metformin, acarbose and isorhamnetin interacted relatively well with some targets of which only acarbose and isorhamnetin exhibited stimulatory effects on glucokinase, while only glibenclamide exhibited same to SUR-1.

Table 3. Basic properties of the docked ligand-protein complexes for targets with inhibitory action

Ligands	Parameters					Targets
	BE (Kcal/mol)	Ki(nM)	TNBs	NHBs	A.As. Involved in Interaction	
Metformin	-5.65	71.41	8	4	D44, K78, Q184 and Y210.	AR
Acarbose	36.08	$30.02x10^{35}$	24	4	V264, G265, P267, F268, P270, A510, G511, H512, G513, G544, R514, L543, V545, P546, L547 and F619.	
Isorhamnetin	**-12.72**	0.05	13	8	G19, T20, W21, K22, D44, Y49, K78, WP80, W112, S160, N161, Q184, Y210, S211, P212, S215, W220, I261, P262, K263 and C299.	
Metformin	-4.87	266770	7	4	V106, P107, L109, F217, L219, V264, L480, L483, I487, F509, A510, G511, H512, R514 and Y515.	α-Glucosidase
Acarbose	-9.86	58.10	11	1	D188, A190, W282, L289, W387, S429, R506, D522, F555, L556, G557, S582, L583 and L584.	
Isorhamnetin	**-9.47**	108.58	11	3	D188, D310, W282, I347, W387, W422, D424, M425, R506, D522, D551, F555 and H580.	
Metformin	-5.11	177830	12	5	E167, E168, Y509, Y624 and Y628.	DPP-4
Acarbose	-6.67	12741	11	4	E167, E168, Y509, Q515, and Y624.	
Isorhamnetin	**-7.83**	1790.40	11	6	R87, E167, Y509, S592, Y628 and N672	
Metformin	-5.10	180860	10	4	C353, L399, S400, S402, and T405.	GFAT
Acarbose	-6.00	39526	5	3	T405, G450 and V451.	
Isorhamnetin	**-7.79**	1919.90	16	5	G354, T355, S400, Q401, S402, T405, A542 and S453.	
Metformin	-5.39	110800	8	4	N55, F78, Y270 and K301.	SGLT-2
Glibenclamide	-9.57	94.85	9	3	N55, H60 and Y270.	
Isorhamnetin	**-9.29**	152.23	12	3	N55, K301 and Q437.	

BE= binding energy; TNBs= total number of bonds; NHBs= number of hydrogen bonds; A.A.= amino acid; Ki= inhibition constant; AR= aldose reductase; GFAT= glutamine fructose-6-phosphate amidotransferase; SGLT-2=. Sodium glucose co-transporter-2; DPP-4= dipeptidyl peptidase-4; PTP-1β= protein tyrosine phosphatase 1β.
Ki= $e^{\Delta G/RT}$; where ΔG = free energy change (binding energy in $Kcal.mol^{-1}$), R= universal rate constant ($1.987x10^{-3}Kcal.mol^{-1}.K^{-1}$), and T= absolute temperature (298K).

Table 4. The Basic properties of the docked ligand-protein complexes for targets with stimulatory action

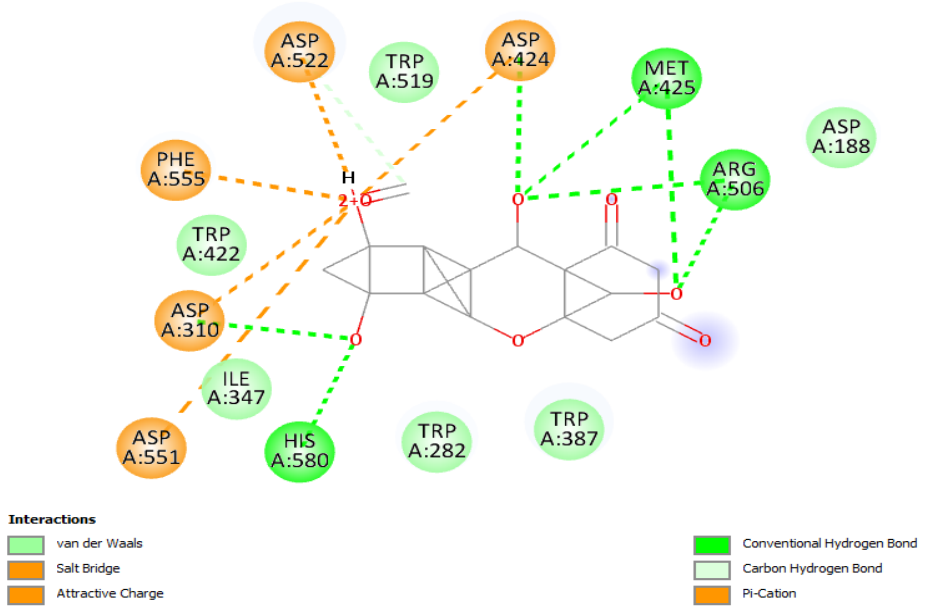
Ligands	Parameters					Targets
	BE (Kcal/mol)	Ki(nM)	TNBs	NHBs	A.As. Involved in Interaction	
Metformin	-2.70	$10.44x10^{6}$	2	2	E13, H343, S389, and R392.	Glucokinase
Acarbose	**11.37**	$22.08x10^{16}$	16	5	T169, I188, G192, C193, S194, E219, and Q250, E253.	
Isorhamnetin	**19.43**	$18.15x10^{22}$	12	4	M187, C196, T218, G348, L349 and V352.	
Metformin	-4.83	285400	6	2	K60, E63, C159, and E161.	FFAR-1
Acarbose	-3.19	4560400	5	1	V82, L133, L136, F140, R172 and R247.	
Isorhamnetin	-10.05	42.15	12	3	V82, F85, R172, and R247.	
Metformin	-5.5	83133	5	2	F153, E250 and W254.	GPR 119
Acarbose	-9.22	171.34	6	3	F53, Q61, A85, S152, F153, F161, V162, W227, R251, W254	
Isorhamnetin	-9.97	48.25	6	3	F161, E250 and W254.	
Metformin	-4.40	590260	6	4	I147 and F151.	PPAR-γ
Acarbose	-8.68	426.73	3	5	C68, L113, V117, L122, V124, N126, G127 and K150	
Isorhamnetin	-8.68	426.73	5	3	F151 and H232.	

Metformin	-7.78	1952.6	9	4	Q130, V337, N638, N641, and W642.	SUR-1
Glibenclamide	**13.48**	$92.41x10^{17}$	11	5	Q130, E599, D603, W642, N638, N641, W642, and	
Isorhamnetin	-7.63	2515.9	8	3	F183, R596 and E599.	

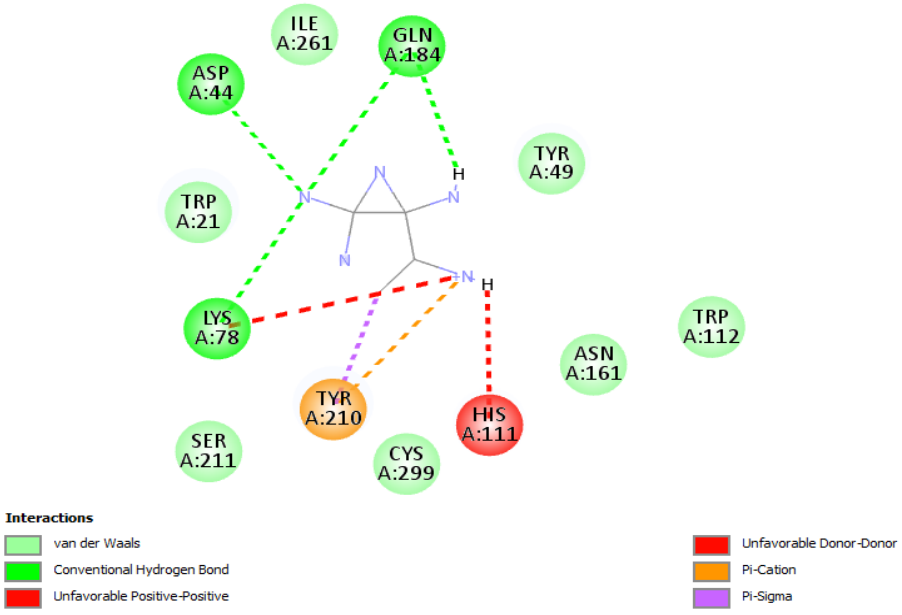
BE= binding energy; TNBs= total number of bonds; NHBs= number of hydrogen bonds; A.A.= amino acid; Ki= inhibition constant; FFAR1= free fatty acid receptor 1; PPAR- γ= peroxisome proliferator-activated receptor; GPR= G-protein receptor; SUR-1= sulfonylurea receptor 1.

Ki= $e^{\Delta G/RT}$; where ΔG = free energy change (binding energy in $Kcal.mol^{-1}$), R= universal rate constant ($1.987x10^{-3}Kcal.mol^{-1}.K^{-1}$), and T= absolute temperature (298K).

Figures 1 to 7 presented some of the representative ligand-receptor (target) complex interactions in either 2D or 3D showing the types of bonding interactions and amino acid residues as well as the respective bond distances and types. Others were included in the supplementary data.



A



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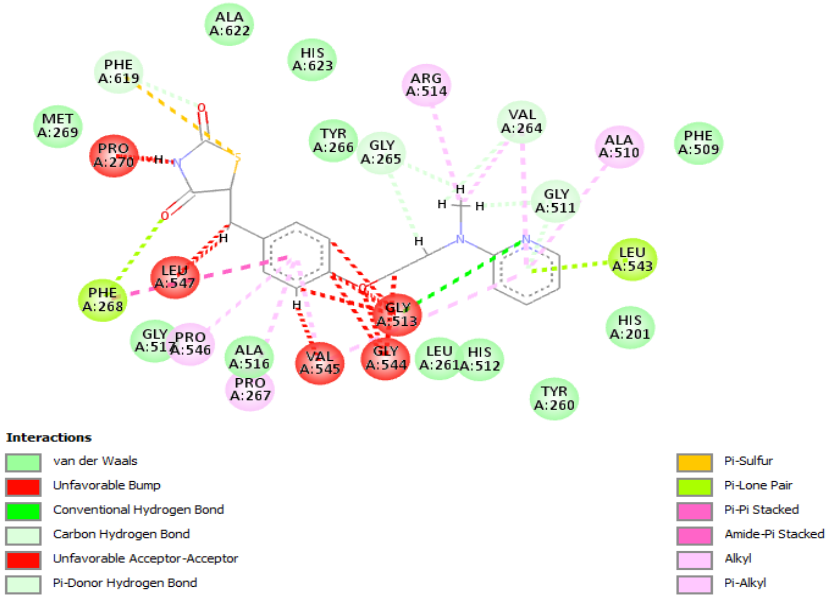
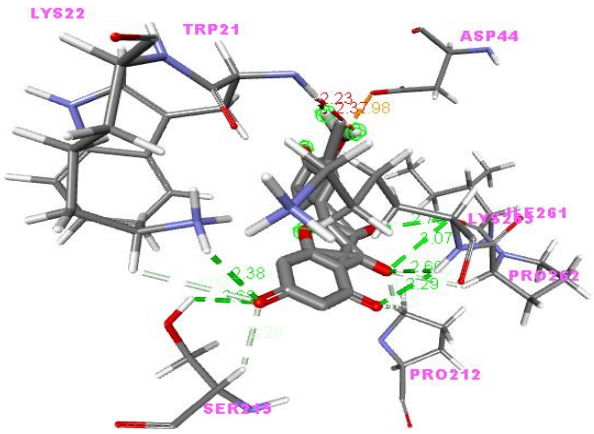
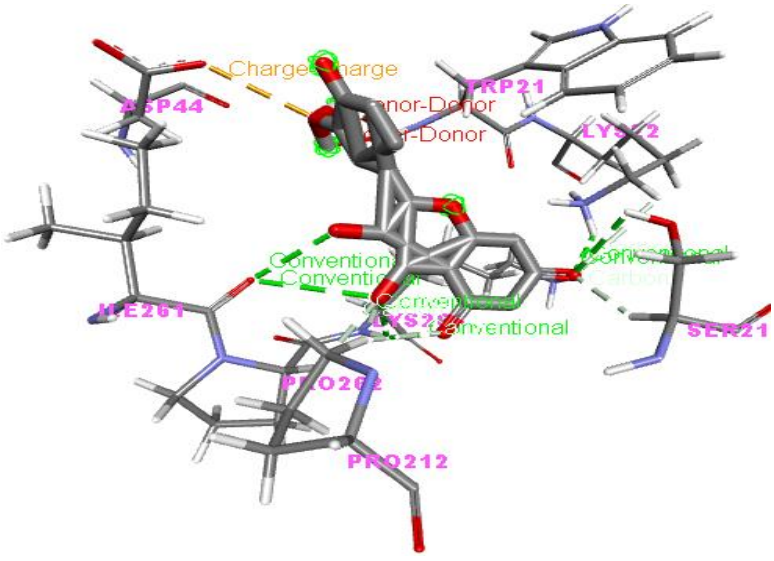


C

Figure 1. 2-D Binding interactions of the isorhamnetin (A), metformin (B) and acarbose (C) with aldose reductase receptor



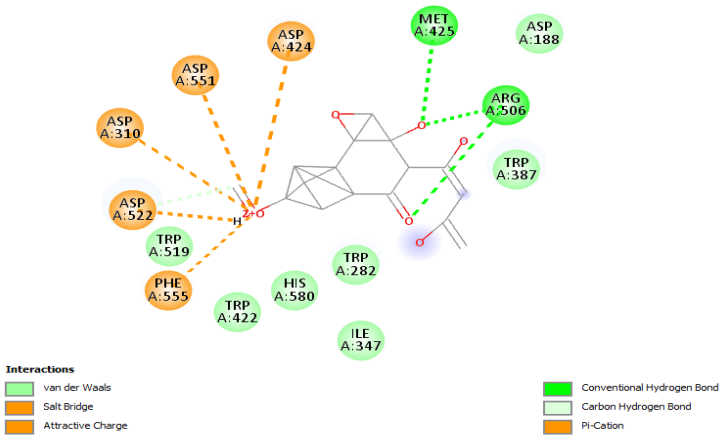
A



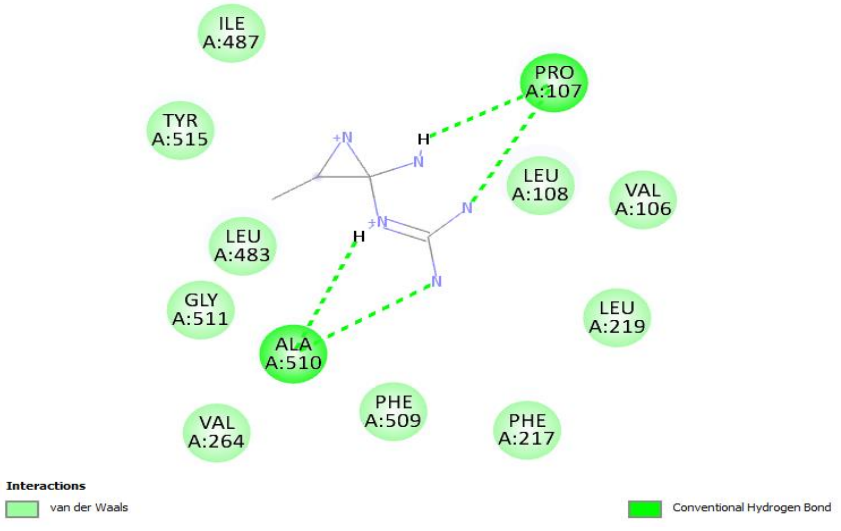
B

Figure 2. 3-D Binding interactions of isorhamnetin with AR showing the bond distances (A), and bond types (B) of the receptor's active site amino acid residues

AR= aldose reductase



A



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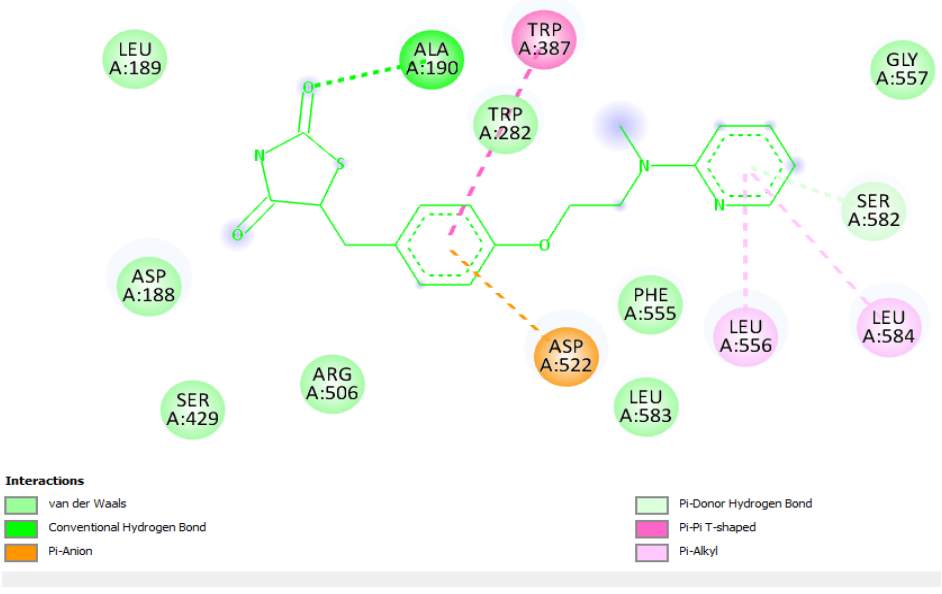


C

Figure 3. 2-D Binding interactions of the isorhamnetin (A), metformin (B) and acarbose (C) with α-glucosidase

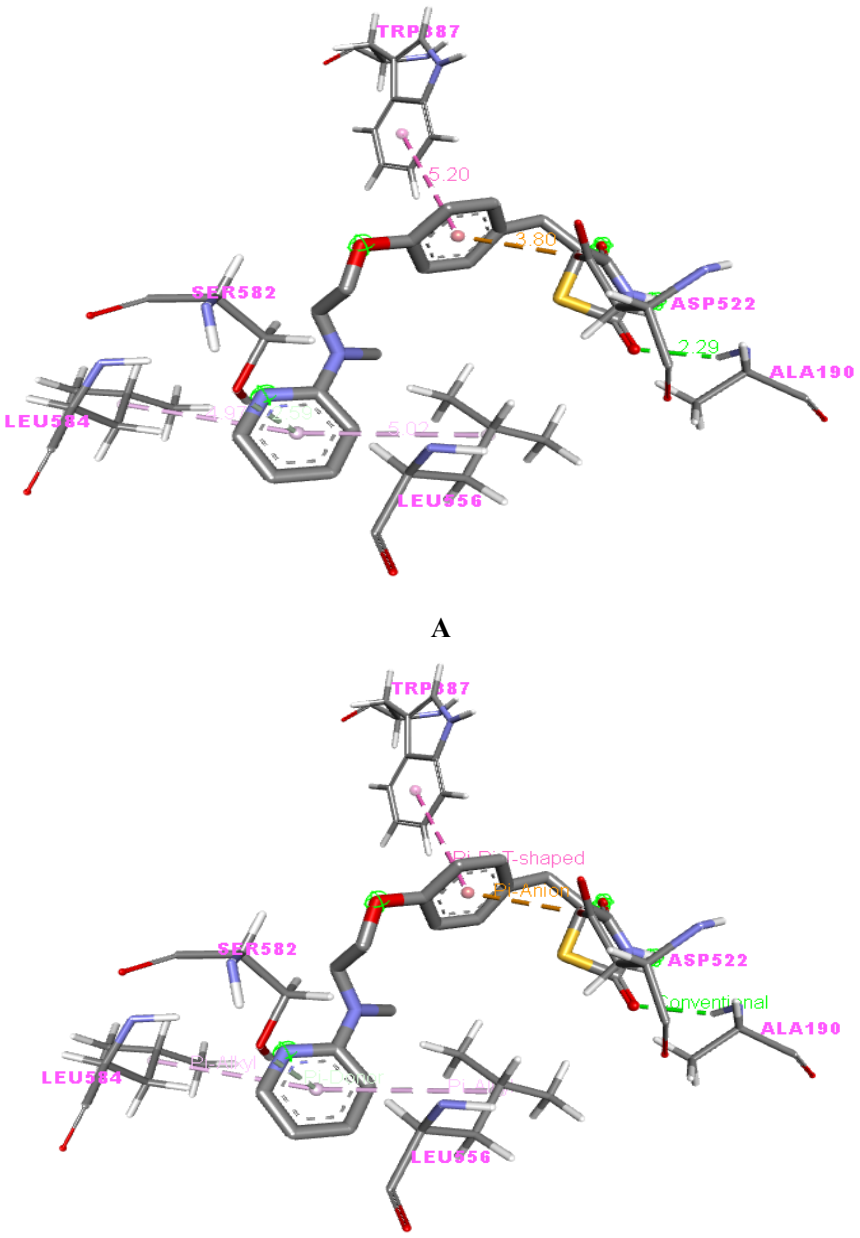
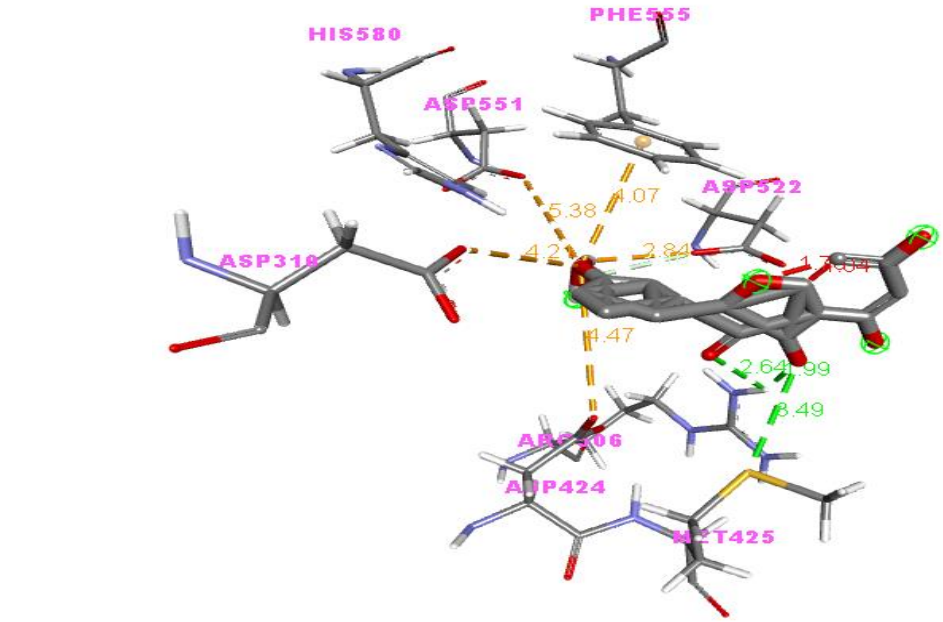
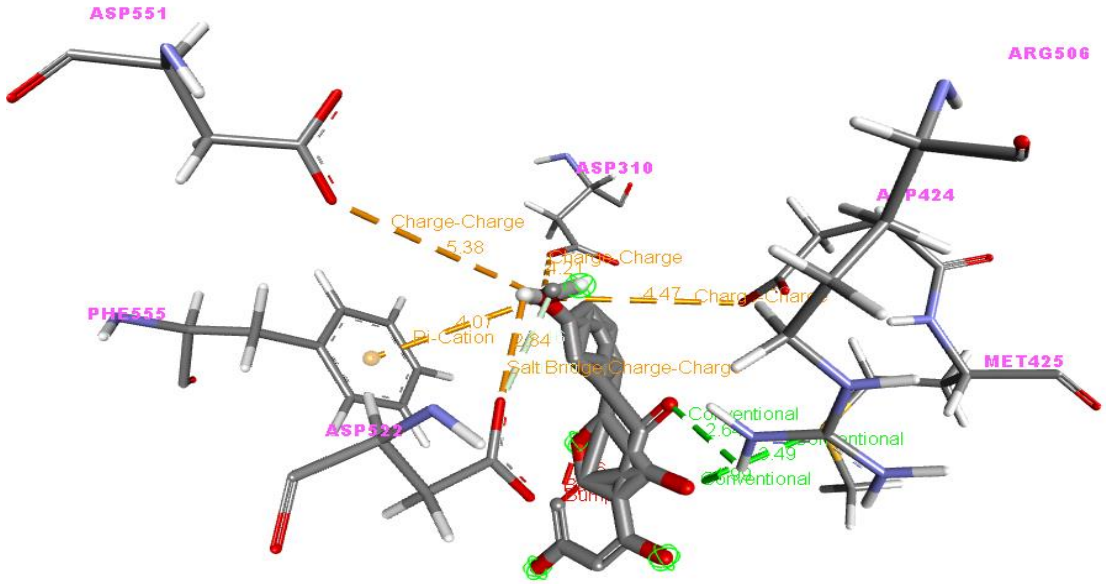


Figure 4. 3-D Binding interactions of acarbose with α-glucosidase showing the bond distances (A), and bond types (B) of the receptor's active site amino acid residues

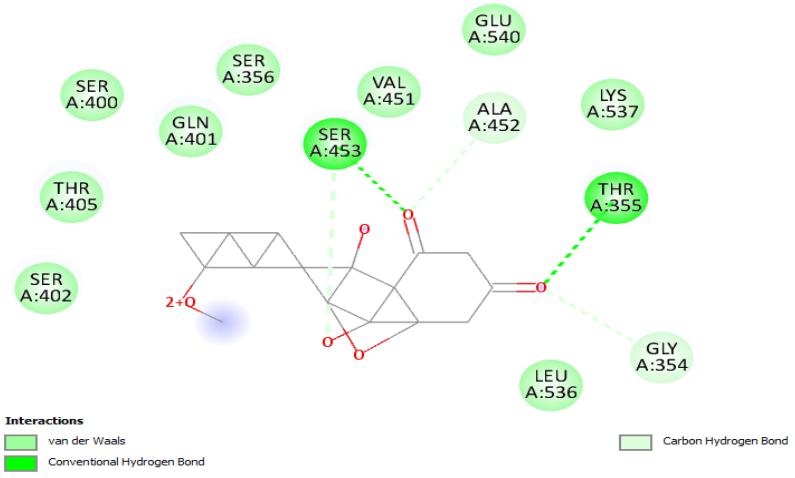


A

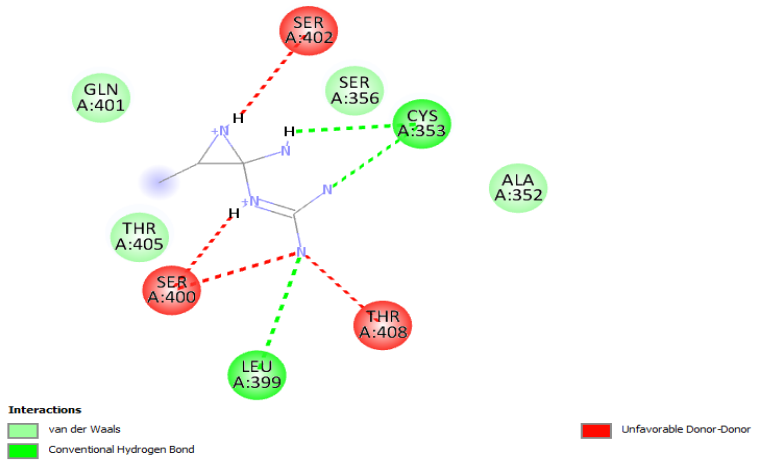


B

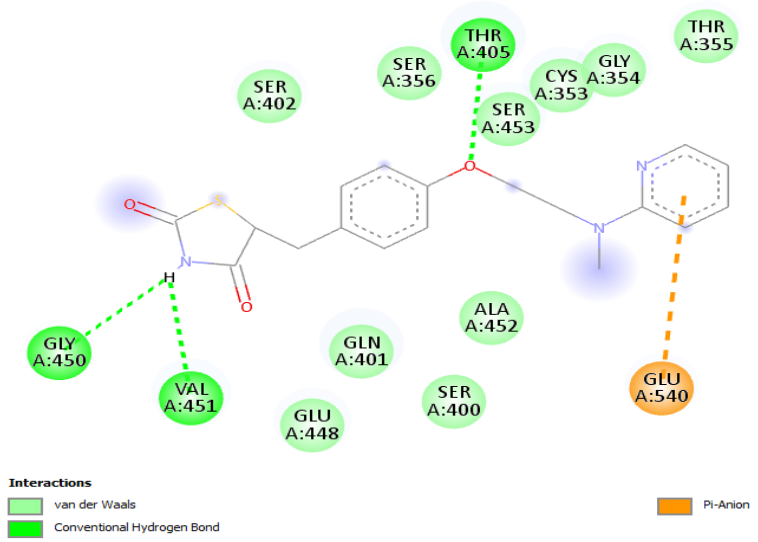
Figure 5. 3-D Binding interactions of isorhamnetin with α-glucosidase showing the bond distances (A), and bond types (B) of the receptor's active site amino acid residues



A



B



C

Figure 6. 2-D Interaction of the isorhamnetin (A), metformin (B) and acarbose (C) with GFAT
GFAT= glutamine fructose-6-phosphate amidotransferase

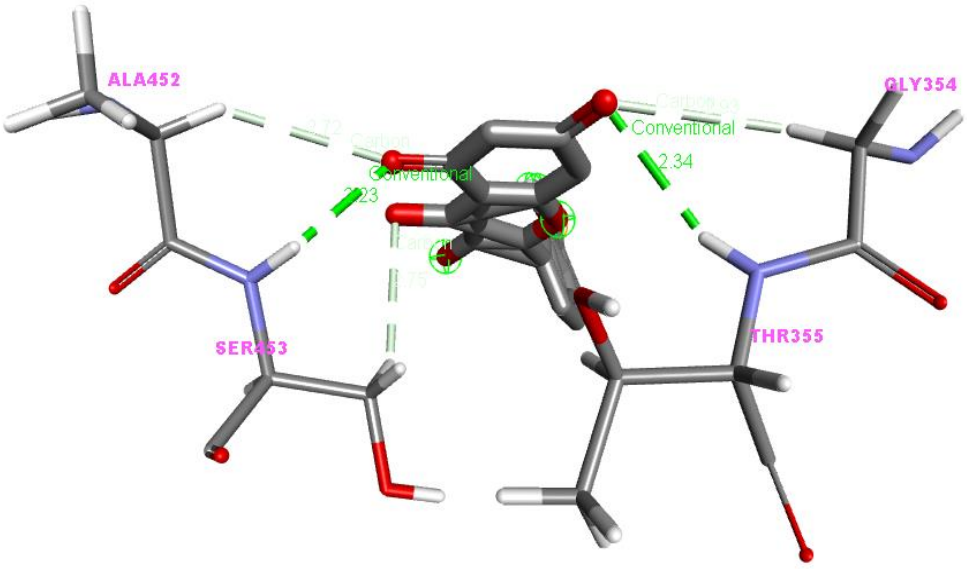


Figure 7. 3-D Isorhamnetin-GFAT complex interactions showing the bond distances and types of the receptor's active site amino acids
GFAT= glutamine fructose-6-phosphate amidotransferase

3.3 *In-Silico* Validation of the Docked Results

Prior to experimental validation, results from docking analyses were *in-silico* validated by comparing the active sites coordinates of our result and that of the original targets downloaded from PDB (Table 5) using Discovery Studio visualizer. Each pair of the targets-ligand complex has approximately equal values of XYZ Coordinate except for that of Serial no 3 as indicated in Table 5.

Table 5. The active sites XYZ coordinates of the protein-ligand complexes

S/No	Targets-Ligands Complex	XYZ Coordinates		
1.	AR-Isorhamnetin	2.01	5.92	19.72
	3S3G-Native Ligand	3.10	6.28	22.30
2.	DPP-4-Isorhamnetin	38.50	50.20	36.61
	6B1E-Native Ligand	38.89	50.98	36.62
3.	α-Glucosidase-Acarbose	-14.20	-37.92	97.40
	5NN3-Native Ligand	-17.75	-39.09	93.61
4.	GFAT-Isorhamnetin	-3.80	50.74	-47.11
	6R4F-Native Ligand	-3.61	50.01	-46.05
5.	SGLT2-Acarbose	38.26	49.50	46.94
	7VSI-NativeLigand	38.30	50.25	46.38
6.	SUR1-Glibenclamide	161.82	101.84	149.80
	6JB3-Native Ligand	161.03	98.36	156.21

AR= aldose reductase; DPP-4= dipeptidyl peptidase-4; GFAT= glutamine fructose-6-phosphate amidotransferase, SGLT2= sodium glucose co-transporter 2; SUR1= sulfonylurea receptor 1.

In-silico validation was also done by co-docking our ligands together with the native or co-crystalized compounds on the protein targets (Figure 8). A good overlapping of our ligand (pink) and the native ligand (blue) signifies a very good docking result as shown in Figure 8.

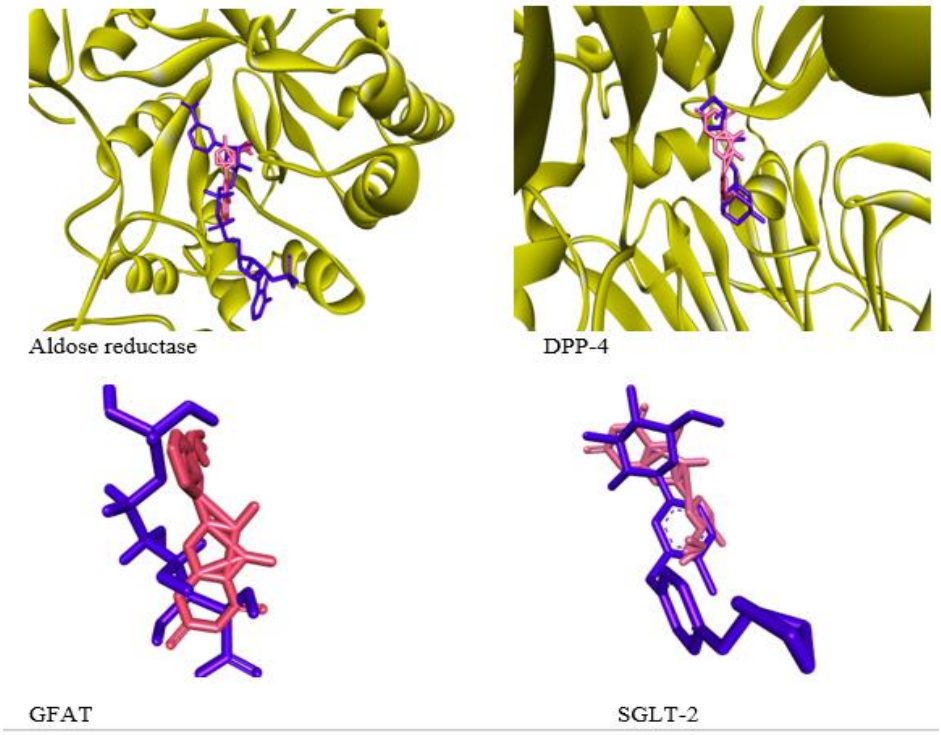


Figure 8. Isorhamnetin co-docked with the native ligands at different receptors active sites
The blue compounds were the native or co-crystalized ligands.

Experimental validation of the docking result

Isorhamnetin was the only compound among the phenolics that was able to demonstrate good binding interactions with target receptors, and thus was used for experimental validation. Table 6 presented the FBG lowering effect of isorhamnetin-containing sample on alloxan-induced diabetic rats. As indicated in this result (Table 6), both acarbose and isorhamnetin treated groups clearly had significant glucose lowering effect on 6th and last day of the treatment unlike the diabetic control. On day 7, the FBG levels of the diabetic control (untreated) group, acarbose and isorhamnetin treated groups were 421.00±9.10, 232.40±6.15 and 239.60±8.56mg/dL.

Table 6. Effect of isorhamnetin-containing sample on i*n-vivo* fasting blood glucose level of alloxan-induced diabetic rats

Groups	FBG Level (mg/dL)								
	Day0	**Day1**	**Day2**	**Day3**	**Day4**	**Day5**	**Day6**	**Day7**	**% Decrease in FBG**
NC	133.00±6.67	132.40±5.32	131.40±2.88	133.80±2.17	136.20±5.31	137.80±2.17	136.20±3.56	136.80±3.83	
DC	454.60±26.08	450.20±20.35	448.60±18.12	436.80±28.86	423.40±20.38	418.40±18.54	411.80±16.25^{a}	421.00±9.10^{b}	**10.65±3.20**
DAT	485.20±16.62	482.00±17.54	448.40±15.90	417.00±13.08	369.20±8.61	323.40±4.78	266.00±6.78^{a}	232.40±6.15^{b}	**52.07±1.78**
DIT	481.00±19.57	481.20±16.93	451.20±7.54	421.20±27.74	388.40±5.77	337.60±13.74	269.20±13.44^{a}	239.60±8.56^{b}	**50.13±2.60**

n=5, Values are Mean ± SD. Rats with FBG >250mg/dl was selected after induction. One-way ANOVA showed a statistically significant difference between mean values of the Treated groups and the Control groups with P= 0.001 at P<0.05 confidence. Also, superscripts '**a**' and '**b**' indicated that there is a statistically significant difference between DC and DAT or DIT on Days 6 and 7 respectively. But there was no statistically significant difference between mean values of acarbose and isorhamnetin treated groups following Post Hoc Turkey comparison test (P= 0.320).
NC= Normal control (given 0.9% normal saline), DC= Diabetic control (given 0.9% normal saline), DAT= Diabetic acarbose treated (given 5mg/Kg b.w), DIT= Diabetic isorhamnetin-containing sample treated (given 5mg/Kg b.w).

Figure 9 presented the graphical presentation of the percentage decrease in FBG level in other to illustrate the daily trend on blood glucose reduction upon oral adminitration of the standard drug (acarbose) and the isorhamnetin-containing sample.

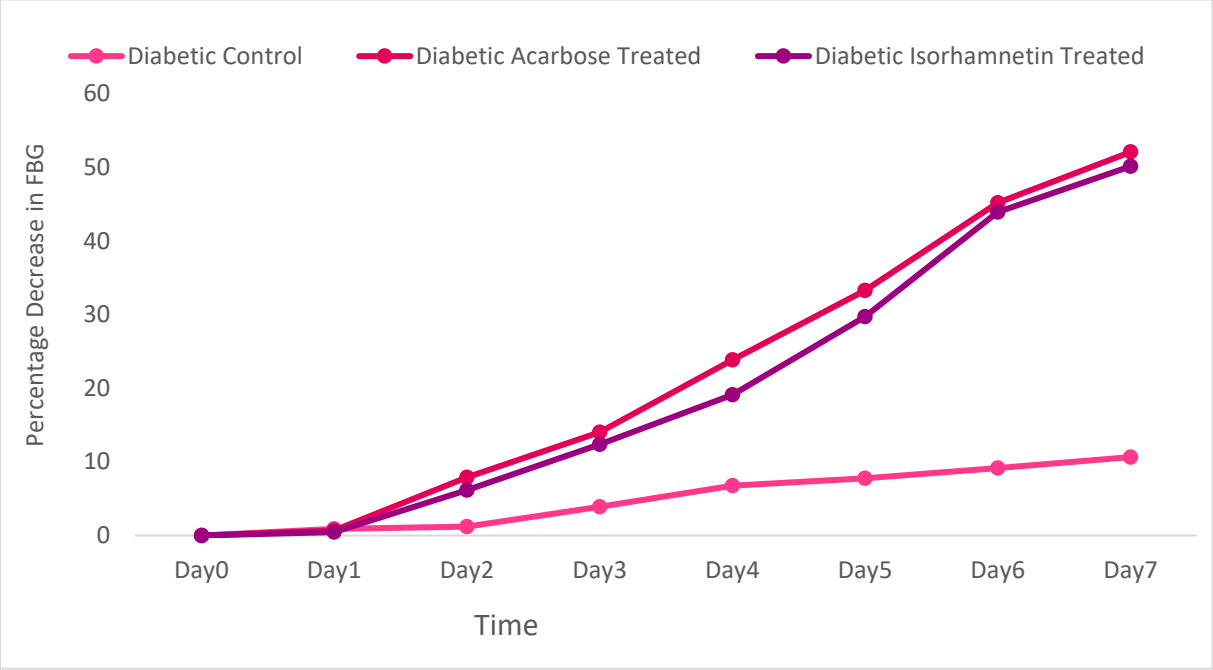


Figure 9. Daily Percentage decrease in *in-vivo* FBG level on alloxan-induced diabetic rats

Discussion

This study was aimed at *in-silico* evaluation of antidiabetic potentials of phenolic compounds identified from ethyl acetate fraction of *A. conyzoides* methanol leaf extract, and experimental validation through *in-vivo* FBG evaluation of the most relatively best docked compound. The phenolic compounds identified by Ozioko et al. (2024) from ethyl acetate fraction of *A. conyzoides* methanol leaf extract were *in-silico* analyzed to ascertain their drug-likeness in comparison with some representative antidiabetic oral drugs in use (Table 1). Drug-likeness evaluates a ligand's potential to become an oral medication in terms of bioavailability qualitatively. Five distinct rule-based filters (Lipinski, Amgen, GSK, Pharmacia, and Bayer) with varying ranges of attributes within which a molecule is regarded as drug-like are accessible through the SwissADME tool. The guidelines or procedures used by Amgen, GSK, Pharmacia, and Bayer were from (Ghose et al., 1999; Veber et al., 2002; Egan et al., 2000; Muegge et al., 2001), in that order respectively. Any ligand that violates any rule is considered less druggable. From the result obtained (Table 2), acarbose violated three rules (Lipinski, Amgen, and Bayer), while metformin violated two rules (Amgen and Bayer). However, other ligands had zero or one violation, including all four phenolics investigated in this research. Besides this five-rule-based filter, SwissADME also employed another model, the Abbot Bioavailability (BA) Score (Martin, 2005). The BA Score aims to forecast the likelihood of a chemical having at least 10% oral bioavailability in rats or significant Caco-2 (colon cancer cell line) permeability. From the result, all the ligands have a BA score above 10%, with acarbose having the least oral bioavailability (17%) (Table 2).

Similarly, the ADMETLab 2.0 tool used somewhat four different rule-based filter models to define the druggable status of a candidate drug. These rules were the Lipinski (Lipinski et al., 2001) (MW≤500; logP≤5; NHBA≤10; NHBD≤5), Pfizer (Hughes et al., 2008) (logP > 3; TPSA <75), GSK (Gleeson, 2008) (MW ≤ 400; logP ≤4), and Golden Triangle (GT) (Johnson et al., 2009) (200 ≤MW ≤500; -2 ≤ logD ≤5). According to the Pfizer rule, compounds with a high log P (>3) and low TPSA (<75) are likely to be toxic. Also, compounds satisfying the GSK and GT rules may have a more favourable ADMET profile. From the result presented, acarbose violated three rules (Lipinski., GSK, and GT) (Table 2), which is similar to the result output using the SwissADME tool. Furocoumarinic acid, isorhamnetin, syringin, and rosiglitazone obeyed all the rules, while glibenclamide, metformin, sitagliptin, repaglinide, and liquiritin had one violation each (Table 2).

Molecular docking is a fresh approach for developing new therapeutic candidates to generate anti-diabetic medications against diabetes provided by several common protein targets engaged in certain types of DM (Bhowmick & Banu, 2017; Ngoc & Ly, 2012). The analyses of docked ligand-protein interactions are very important in understanding structure-based drug design (SBDD). The primary goal of SBDD has reportedly been stated to be an *in-silico* prediction of the free energy of ligand-protein binding (Cournia et al., 2017). According to the results presented in Table 3, isorhamnetin, metformin, and acarbose have respective BEs of -12.75, -5.65, and 36.08 Kcal/mol when docked with AR, and isorhamnetin had the highest inhibitory effect on the target protein with a very low Ki (0.05nM) value. This was followed by metformin.

Acarbose seems to have a stimulatory effect on the target with a very high Ki (30.02 x 10^{35}nM) value. This could stem from the unfavourable bumps and acceptor-acceptor amino acids (from Pro270, Gly513, Gly544, Val545, and Leu547) interactions as indicated in the 2D acarbose-AR complex (Figure 1). This was unlike that of the isorhamnetin-AR complex, which had favourable hydrogen bond interactions and salt bridge formation, which helped stabilize the ligand at its binding site. Weak, noncovalent intermolecular interactions play an important role in stabilizing a ligand energetically at the interface of a protein. (Patil et al., 2010) There are common interacting amino acid residues (D44, K78, Q184, and Y210) at both the binding sites of isorhamnetin- and metformin-AR complexes, unlike that of acarbose (Table 3). Looking into the 3-D isorhamnetin-AR complex structure (Figure 2), the primary active site-interacting amino acids were Trp21, Lys22, Asp44, Pro212, Ser215, Ile261, Pro262, and Lys263. There is a total number of 8 H-bonding intermolecular interactions. The H-bond distances between some atoms of the ligand and active site amino acid residues- Lys22, Ser215, Ile261, Ile261, Lys263 and Lys263 were 2.38Å, 2.68Å, 2.70Å, 3.07Å, 2.29Å, and 2.60Å, respectively (Figure 2). Similarly, Ser215, Pro212, and Pro262 form C-H bonds with the ligand with different bond lengths, while Asp44 and Trp21, respectively, form charge-charge and donor-donor bonds with the ligand. These myriads of noncovalent interactions of the isorhamnetin-AR complex contributed immensely to the high affinity and stability of the complex, thus the good inhibitory action of the ligand.

Analyzing docking interactions with α-glucosidase, acarbose, isorhamnetin, and metformin have respective BEs of -9.86, -9.49, and -4.87 Kcal/mol, with acarbose having the best inhibition effect (Ki of 58.10 nM). This could stem from the pi-alkyl bonding interactions of the cyclic rings of acarbose with leucine hydrophobic residues (Leu556 and Leu484), and pi-cation and pi-pi T-shaped bonds with Asp552 and Trp387 respectively, at the binding site of the target (α-glucosidase) (Figure 3). The weak van der Waal forces and the conventional H-bonds equally played significant roles in the ligand-receptor complex stability. Figure 4 equally indicated the bond types and distances of the interacting active sites' amino acid residues with acarbose. Besides the conventional H-bond interactions, salt bridge formations and charge-charge bonds (from Asp310, Asp424, Asp551, Asp552, and Phe555) have also contributed immensely to the formation of a stable isorhamnetin-α-glucosidase complex alongside other bonds (Figures 5), which made isorhamnetin have close binding affinity with acarbose, the known inhibitor of α-glucosidase. For instance, Met425 formed an H-bond with a bond distance of 3.49Å, while Arg506 formed two H-bonds with different atoms of the ligand with respective bond distances of 1.99Å and 2.64Å (Figure 5). Other bond interaction types were salt bridges (Asp310 4.21Å, Asp424 4.47Å, and Asp522 2.84Å), charge-charge (Asp551 5.38Å), C-H bond (Asp551 2.19Å), and pi-cation (F555 4.07Å) formations. It was also found that isorhamnetin, acarbose, and metformin have BEs of -7.83, -6.67, and -5.11 Kcal/mol, respectively, after being docked with DPP-4 to form their respective complexes (Table 4). Isorhamnetin had the best inhibitory effect on DPP-4 with a Ki of 1790.40nM. The binding site of the enzyme (DPP-4) has two common interacting amino acids, Glu167 and Tyr509, in the three complexes. Considering the GFAT receptor, docking of the ligands yielded a BE of -7.79, -6.00, and -5.10 kcal/mol for isorhamnetin, acarbose, and metformin, respectively (Table 4), in which T405 was the only common interacting amino acid at their active site. Here, isorhamnetin exhibited the highest inhibitory effect on GFAT with a Ki of 1919.90nM. The isorhamnetin-GFAT complex 2-D interaction of the binding site residues was through the conventional H-bond, C-H bonds, and van der Waal forces (Figure 6). The complex 3-D interaction analysis showed that Thr355 and Ser453 formed H-bonds (with respective bond distances of 2.34Å and 2.23Å) with the ligand (Figure 7). Also, Gly345 (2.93Å), Ala452 (2.72Å), and Ser453 (2.75Å) formed C-H bonds with the ligand. Besides bond types, the analysis of the bond distances of ligand-receptor interacting amino acid residues helps in predicting the strength of each contributing bonding force and thus the stability of the ligand at the inner cleft of the active site of the protein receptor. In general, the smaller the bond distances, the stronger the ligand-receptor complex interaction and hence the low binding energy (i.e., high binding affinity) (Chen et al., 2016). More so, docking of the ligands with the SGLT2 protein yielded a BE of -9.57, -9.25, and -5.39 kcal/mol for acarbose, isorhamnetin, and metformin, respectively, in which N55 was the only common interacting amino acid at the active site (Table 3), with acarbose having the best inhibitory action with a Ki of 94.85 nM.

Similarly, analyses of the docked ligands on receptors or targets (i.e., GCK, FFAR-1, GPR119, PPAR-γ, and SUR1) with stimulatory mechanisms of action showed that isorhamnetin and acarbose exhibited stimulatory effects only in glucokinase with a BE of 19.43 and 11.37 kcal/mol, respectively, while glibenclamide had stimulatory action on SUR1 with a BE of 13.58 kcal/mol (Table 5). The few ligands (acarbose, isorhamnetin, and metformin) that were able to dock with some of the other targets had inhibitory effects on them with negative BEs.

Thus, among the four identified phenolics that were docked, only isorhamnetin was able to dock successfully on some of the selected targets. Surprisingly, it exhibited an outstanding inhibitory effect on AR, DPP-4, and GFAT, even better than the US FDA-approved oral antidiabetic drugs such as acarbose, glibenclamide, and metformin (Table 3). It also presented a good inhibitory effect on SGLT2, albeit lower than acarbose. It equally had the best stimulatory effect on GCK (Table 4), which serves as the cell glucose sensor. This excellent inhibitory and stimulatory effect of isorhamnetin on the target proteins could be attributed to its structure, which forms stable (ligand-target) complexes resulting from the non-covalent

bonding forces of conventional H-bonds, C-H bonds, van der Waal forces, and salt bridges without any interfering unfavourable bumps or acceptor-acceptor atoms steric hindrance. As opined by Bhinge et al. (2004) and Valdar & Thornton (2001), numerous biophysical factors, including size, shape, molecular weight, hydrogen bonds, hydrophobic interactions, van der Waals forces, and salt bridges, stabilize the positions of a small number of atoms. As a result, internal freedom as well as translational and rotational entropy are lost by each tiny molecule that binds to a target (Mobley & Dill, 2009). Thus, hydrogen bonding interactions and other noncovalent weak intermolecular forces are essential for proteins, as they provide the interface for unique folding and the affinity that supports molecular recognition in the ligand-target interactions.

Presenting a very high inhibition on AR (0.05nM), DPP-4 (1709.40nM), SGLT2 (152.23nM), and GFAT (1919.90nM), isorhamnetin from *A. conyzoides* methanol leaf extract could be the potential oral hypoglycemic molecular candidate for the treatment of DM through a multifaceted mechanisms of action. For instance, inhibition of AR and GFAT may reduce or eliminate diabetic complications in diabetic individuals (Kalai et al., 2022). The first and rate-limiting enzyme in the polyol pathway that converts glucose to sorbitol in diabetics is aldose reductase (AR) (Anand et al., 2016). In individuals without diabetes, its affinity for glucose is poor (high Km) at a normal glucose concentration of 5.5 mM. The polyol or aldose reductase pathway increases in diabetic states in tissues like the retina, kidney, peripheral nerves, and blood vessels that do not require insulin for cellular glucose uptake (Stephen et al., 2003). This results in diabetic micro-vascular complications like retinopathy, neuropathy, and nephropathy as well as macro-vascular complications like cardiovascular diseases. The cumulative effects of the AR pathway in uncontrolled diabetes include: sorbitol-stimulated osmotic stress; reduced Na^+/K^+-ATPase action; upsurge in cytosolic NADH/NAD^+ generation, which provides substrate for NADH oxidase to produce more ROS (reactive oxygen species); and a decrease in cytosolic NADPH and thus reduced glutathione, which then accelerates oxidative stress (Darenskaya et al., 2021). Interestingly, isorhamnetin can alleviate these complications and their attendant propagation effects via inhibition of AR activity. Similarly, sustained hyperglycemia in diabetics can also lead to increased glucose metabolism via the hexosamine pathway (HAP) (Buse, 2006). Being the first and rate-limiting enzyme of HAP, GFAT converts fructose-6-phosphate to glucosamine-6-phosphate, resulting in the formation of uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc), which provides substrates for the creation of O-linked glycoproteins as the primary end-product (Gaderer et al., 2017). The HAP stimulation of O-GlcNAcylation-mediated suppression of eNOS (endothelia nitric oxide synthase) activity in arterial endothelial cells is particularly significant for diabetic vascular problems. Therefore, isorhamnetin inhibition of GFAT halts this post-translational modification of tissue proteins in hyperglycemic circumstances and other propagation effects. More so, the inhibition of DPP-4 by isorhamnetin can lead to increased endogenous GLP-1(glucagon-like peptide) and GIP (glucose-dependent insulinotropic polypeptide) levels. Glucagon-like peptide 1 and GIP are entero-endocrine hormones (Chee et al., 2009) that promote pancreatic beta-cell expansion or preservation of beta-cell mass, insulin gene expression, and insulin secretion stimulation. These endogenous hormones are quickly broken down and rendered inactive in-vivo by DPP-4 by cleaving their N-terminal two amino acids. Thus, inhibition of this enzyme will increase the half-lives of GLP-1 and GIP as did isorhamnetin. This ligand was also able to inhibit the SGLT2 protein. Inhibiting SGLT2 may promote urine glucose excretion, which would lower DM patients' plasma glucose levels without the use of insulin (Morita et al., 2010).

Besides exerting excellent inhibitory effects on AR, DPP-4, SGLT2, and GFAT, isorhamnetin also had the best activation effect on GCK, as presented in Table 5. Glucokinase (hexokinase IV) functions as a glucose sensor in the liver, pancreatic cells, and other peripheral tissues by causing changes in metabolism or cell function in response to rising or falling glucose levels, such as those experienced after meals or during fasting (Magnuson & Matschinsky, 2004). Unlike other hexokinases, it has a lesser affinity for glucose, and its byproduct, glucose-6-phosphate, does not inhibit it. So it is able to control a "supply-driven" metabolic pathway, and the supply of glucose determines the rate of reaction rather than the demand for the final products. Type 2 DM has been linked to lower GCK activity. Thus, isorhamnetin can enhance type 2 DM treatment by activating or stimulating GCK activity, which in turn stimulates the activation of the ATP-sensitive potassium channel, hence leading to glucose-stimulated insulin release from the pancreatic β-cells (Kalai et al., 2022; Jiang et al., 2019).

Prior to experimental validation, *in-silico* validation was done by comparing the active sites coordinates of docked result and that of the targets downloaded from PDB as well as juxtaposing or co-docking the isorhamnetin together with the respective native ligands of the protein targets. From the results presented in Table 5, the XYZ coordinates of the respective best docked ligands and the native ligands were approximately the same except for α-glucosidase. In the same vain, co-docking them and isorhamnetin on their respective protein targets presented a good overlap (Figure 8). These were indications that our docking result was good and reliable. Results were equally validated experimentally via *in-vivo* FBG level evaluations using the isorhamnetin-containing fraction that gave the best docking interactions among the four identified phenolics from the ethyl acetate fraction of *A. conyzoides* methanol leaf extract. The *in-vivo* FBG-lowering effect of isorhamnetin-containing sample in alloxan-induced diabetic rats for 7 days with acarbose as the standard drug was

carried out. Alloxan, a toxic glucose analogue, enters the pancreatic β-cell via the GLUT2 glucose transporter. This chemical plays an important role in the hyperglycemic animal model through its specific inhibition of GCK (glucokinase) and stimulation of ROS production, consequently causing necrosis and destruction of β-cells (Correia-Santos et al., 2012). Acarbose, a well-known α-glucosidase inhibitor currently used for the treatment of diabetic patients, was used as a positive control because it has been shown to additionally inhibit the absorption of D-glucose from the intestinal lumen into the blood stream (Widyawati et al., 2015; Hirsh et al., 1997). As reported from this current study, both acarbose and isorhamnetin-treated groups clearly had a significant glucose-lowering effect on the 6th and last (7th) day of the treatment (Table 6), contrary to the diabetic control. On day 7, the FBG levels of the diabetic control (untreated) group, acarbose- and isorhamnetin-treated groups were 421.00±9.10, 232.40±6.15, and 239.60±8.56 mg/dL, respectively, with a corresponding % decrease in the FBG level of 10.65±3.20, 52.07±1.78, and 50.13±2.60. A one-way ANOVA showed a statistically significant difference between the mean values of the treated groups and the control groups, with P = 0.001 at 95% confidence. However, there was no statistically significant difference between the mean values of the acarbose and isorhamnetin-treated groups following the post-hoc Turkey comparison test. Similarly, analysis of the percentage decrease in FBG level (Figure 9) indicated that both the acarbose and isorhamnetin-treated groups had a gradual and appreciable decrease in FBG level, which reached above 50% on the last day of treatment, unlike the diabetic control (~10%). The daily estimation of the percentage decrease in FBG level of a potential candidate drug molecule helps in determining the dosing formulation frequency of the drug. This similar glucose-lowering capacity of both acarbose and isorhamnetin could result from their structural and functional group resemblance, especially the presence of hydroxyl groups.

Since alloxan has been established to predominantly induce type 1 DM in rat models (Lenzen, 2008), the mechanism of reduction of FBG level by isorhamnetin-containing fraction could be through restoration of pancreatic β-cell integrity and prevention of further cell death by scavenging the ROS produced by alloxan-induced diabetes. It could also have achieved a glucose reduction effect via the activation of GCK activity, which subsequently induces glucose-stimulated insulin secretion by peripheral cells or tissues.

By and large, isorhamnetin (3'-methoxy-3,4',5,7-tetrahydroxyflavone) is an o-methylated flavonol belonging to the flavonoid class, and is highly present fruits (Holland et al., 2020). However, to our utmost knowledge, it has not been documented to be present in A. conyzoides despite myriad of research reports about its biological and pharmacological activities (Liqing et al., 2016; Jin-Jing et al., 2016; Yeon et al., 2005) of this “miracle king” grass. Isorhamnetin is utilized both as is and in a number of derivatized forms that can be made into pharmaceuticals to treat illnesses brought on by oxidative stress and cancer-causing viruses because of its purported antioxidant and antiviral qualities (Kandakumar & Manju, 2017). Numerous biological properties of this chemical have been identified, such as hepatoprotective activity (Guang-Zhi et al., 2014), cardiovascular protection (Yun et al., 2015; Liqing et al., 2016), anti-inflammatory activity (Tae et al., 2013; Marilena et al., 2015), anticancer effects (Tae et al., 2013; Jin-Jing et al., 2016), and antidiabetic effect (Yeon et al., 2005). Thus, the reason for isorhamnetins' vast biological functions could be attributed to their capacity to bind metal ions, give hydrogen atoms or electrons, and scavenge free radicals. Its ability to inhibit free radicals and protect cells and organs from degenerative diseases like cancer, diabetes, and cardiovascular diseases may also be attributed to structural features, specifically the number and positions of -OH groups and the types of substitutions on phenyl aromatic rings.

Conclusion

This study has demonstrated that furocoumarinic acid, liquiritin, isorhamnetin, and syringin phenolic compounds derived from ethyl acetate fraction of *A. conyzoides* methanol leaf were found to exhibit relatively good drug-likeness potential according to the rule-based filter models developed by well-known pharmaceutical firms, with oral bioavailability scores of 55% better than acarbose (17%). Out of these four phenolics, isorhamnetin was able to inhibit AR, DPP-4, GFAT, and SGLT2 activities and activate GCK action through docking simulation interactions. This compound (isorhamnetin-containing sample) equally exhibited very good *in-vivo* FBG-lowering effects relative to acarbose. Thus, isorhamnetin isolated from the ethyl acetate fraction of *A. conyzoides* methanol leaf extract could be a potential antidiabetic drug candidate that need further refinement and validation experimentally.

Significance of the study:

Isorhamnetin and acarbose had a percentage decrease in *in-vivo* FBG of 50.13% and 52.07%, respectively. However, *in-silico* drug-likeness evaluations indicated isorhamnetin to have better drug-like potentials with 55% bioavailability over acarbose, which has only 17%. Isorhamnetin also inhibited AR and GFAT with a BE of -12.72 and -7.79 kcal/mol, better than acarbose with a BE of 36.08 and -6.00 kcal/mol, respectively. So, it could be potentially used for the management of diabetes microvascular complications upon further purification.

Recommendations

It is recommended that partially purified isorhamnetin sample of the ethyl acetate fraction of *A. conyzoides* methanol leaf extract be formulated and dosed into herbal supplement for the management of diabetes mellitus.

The isorhamnetin from *A. conyzoides* methanol leaf extract should be synthesized, purified, and formulated into an oral drug for the treatment of DM and its associated complications.

List of abbreviations

ADME T	Absorption, distribution, metabolism, excretion, and toxicity
AR	Aldose reductase
CADD	Computer-aided drug design
CVDs	Cardiovascular diseases
DM	Diabetes mellitus
DPP-4	Dipeptidyl peptidase-4
FFAR1	Free fatty acid receptor 1
GCK	Glucokinase
GFAT	Glutamine fructose-6-phosphate amidotransferase
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GT	Golden triangle
HDA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
LogP	Partition coefficient or lipophilicity
MD	Molecular docking
NCBI	National Center for Biotechnology Information
PK	Pharmacokinetic
PPAR- γ	Peroxisome proliferator-activated receptor-gamma
PTP1 β	Protein tyrosine phosphatase 1 beta
ROS	Reactive oxygen species
SBDD	Structure-based drug design
SGLT2	Sodium glucose co-transporter 2
SURI	Sulfonylurea receptor 1
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 Diabetes mellitus
TPSA	Topological polar surface area
UCSF	University of Califonia San-Francio
VS	virtual screening

Author contributions

PC Ozioko conceptualized and designed the research idea under the supervisions of A Ibrahim and YY Muhammad. PC Ozioko and A Ibrahim analyzed the results. The writing of the manuscript was equally scrutinized by A Ibrahim and YY Muhammed. All the authors approve the manuscript.

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Conflict of interest

The author declares no conflict of interest. The manuscript has not been submitted for publication in other journal.

Ethics approval

The need for ethical approval was waived off by the Animal Cure and Use Research Ethics Committee (ACUREC) of Directorate of Research, Innovation and Partnership (DRIP) of Bayero University Kano, Nigeria because of the research encompasses more of *in silico* study with only 20 rats used for validation of the study.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

AI usage declaration

We did not use artificial intelligence in writing this research in any way.

References

Anand, S., Arasakumari, M., Prabu, P. & Amalraj, A. R. (2016). Anti-Diabetic and Aldose Reductase Inhibitory Potential of *Psidium Guajava* by *In-Vitro* Analysis. *International Journal of Pharmacy and Pharmaceutical Sciences*, *8*(9), 271-276.

Anurak, C. & Kesara, N. B. (2018). "A systematic review: application of in silico models for antimalarial drug discovery," *African Journal of Pharmacy and Pharmacology*, *12*(13), 159-167.

Atawodi, S. E., Adepoju, O. A., & Nzelibe, H. C. (2017). Antihyperglycaemic and hypolipidemic effect of methanol extracts of *Ageratum conyzoides* L (Asteraceae) in normal and diabetic rats. *Tropical Journal of Pharmaceutical Research, 16*(5), 989–996.

Batool, M., Ahmad, B. & Choi, S. (2019). "A structure-based drug discovery paradigm," *International Journal of Molecular Sciences*, *20*(11), 2783.

Bhinge. A., Chakrabarti, P., Uthanumallian, K., Bajaj, K., Chakraborty, K., et al. (2004) Accurate detection of protein-ligand binding sites using molecular dynamics simulations. *Structure*, 12: 1989–1999.

Bhowmick, A. & Banu, S. (2017). Therapeutic targets of type 2 diabetes: an overview. *MOJ Drug Design and Development Therapy*; *1*(3), 60–64.

Biovia, D. S. (2021). Discovery Studio Visualizer. San Diego.

Burley, S. K., Bhikadiya, C., Bi, C., Bittrich, S., Chen, L., Crichlow, G., et al. (2021). RCSB Protein Data Bank: Powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering, and energy sciences. *Nucleic Acids Res*earch, *49*, D437–D451.

Buse, M.G. (2006). Hexosamines, insulin resistance, and the complications of diabetes: current status. *American Journal of Physiology Endocrinology and Metabolism*, *290*(1): 1–8.

Chee, W., Egan, C. & Egan, J. M. (2009). Incretin-Based Therapies in Type 2 Diabetes Mellitus. *Current Protein and Peptide Science*, *10*, 46-55.

Chen, D., Oezguen, N., Urvil, P., Ferguson, C., Dann, S. M., & Savidge, T. C. (2016). Regulation of protein-ligand binding affinity by hydrogen bond pairing. *Science advances*, *2*(3), e1501240. https://doi.org/10.1126/sciadv.1501240

Correia-Santos, A. M., Suzuki, A., Anjos, J. S., Rêgo, T. S., Almeida, K. C. L. & Boaventura, G. T. (2012). Induction of Type 2 Diabetes by low dose of streptozotocin and high-fat diet-fed in wistar rats. *Medicina (Ribeirão Preto)*, *45*(4), 436-444.

Cournia, Z., Allen, B. & Sherman, W. (2017). Relative binding free energy calculations in drug discovery: recent advances and practical considerations. *Journal of Chemical Informatic Model*, 57, 2911–2937. https://doi.org/10.1021/acs.jcim.7b00564

Daina, A., Olivier, M., & Vincent, Z. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Report*; *7*, 42717.

Darenskaya, M. A., Kolesnikova, L. I. & Kolesnikov, S. I. (2021). Oxidative Stress: Pathogenetic Role in Diabetes Mellitus and Its Complications and Therapeutic Approaches to Correction. *Bulletin of Experimental Biology and Medicine*, *171*(2),179-189. https://doi.org/10.1007/s10517-021-05191-7

de Carvalho, E. N., de Carvalho, N. A. S. & Ferreira, L. M. (2003). Experimental model of induction of diabetes mellitus in rats. *Acta Cir. Bras*; *18*: 60–64.

Egan, W. J., Merz, K. M. & Baldwin, J. J. (2000). Prediction of Drug Absorption Using Multivariate Statistics. *Journal of Medicinal Chemistry, 43*, 3867-3877.

Fatema, N. (2013). Antioxidant and cytotoxic activities of *Ageratum conyzoides* stems. *International Current Pharmaceutical Journal*, *2*, 33-37.

Gaderer, R., Seidl-Seiboth, V., de Vries, R. P., Seiboth, B. & Kappel, L. (2017). N-acetylglucosamine, the building block of chitin, inhibits growth of Neurospora crassa. *Fungal Genetics and Biology*, *107*, 1–11.

Ghose, A. K., Viswanadhan, V. N. & Wendoloski, J. J. (1999). A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery: A qualitative and quantitative characterization of known drug databases. *Journal of Combinatorial Chemistry*, 1: 55–68.

Gleeson, M. P. (2008). Generation of a set of simple, interpretable ADMET rules of thumb. *Journal of Medicinal Chemistry*, *51*(4), 817-34.

Guang-Zhi, D., Ju-Hee, L., Sung-Hwan, K., Ji-Hye, Y., Il-Je, C., Seung, H. K. et al. (2014). AMPK activation by isorhamnetin protects hepatocytes against oxidative stress and mitochondrial dysfunction. *European Journal of Pharmacology*, *740*, 634-640. https://doi.org/10.1016/j.ejphar.2014.06.017

Hirsh, A. J., Yao, S. Y., Young, J. D. & Cheeseman, C. I. (1997). Inhibition of glucose absorption in the rat jejunum: a novel action of alpha-D-glucosidase inhibitors. *Gastroenterology*, *113*, 205-211.

Holland, T. M., Agarwal, P., Wang, Y., Leurgans, S. E., Bennett, D. A., Booth, S. L. & Morris, M. C. (2020). "Dietary flavonols and risk of Alzheimer dementia". *Neurology*, *94*(16), e1749–e1756.

Hughes, J. D., Blagg, J., Price, D. A., Zhang, Y. et al. (2008). Physiochemical drug properties associated with in vivo toxicological outcomes. *Bioorganic and Medicinal Chemistry Lett*er, *18*(17), 4872-4875.

Jain, A. N. & Nicholls, A. (2008). Recommendations for evaluation of computational methods. *Journal of Computational Aided Molecular Design*, *22*(34), 133-142.

Jiang, H., Yamashita, Y., Nakamura, A., Croft K. & Ashida, H. (2019). Quercetin and its metabolite isorhamnetin promote glucose uptake through different signalling pathways in myotubes. *Scientific Repor*t, *9*, 2690. https://doi.org/10.1038/s41598-019-38711-7

Jin-Jing, Z., Jin-Qing, S., Shu-Yi, P. & Kai, W. (2016). Treatment with isorhamnetin protects the brain against ischemic injury in mice. *Neurochemistry Research*, *41*(8), 1939-1948.

Johnson, T. W., Dress, K. R. & Edwards, M. (2009). Using the Golden Triangle to optimize clearance and oral absorption. *Bioorganic and Medicinal Chemistry Lett*er, *19*(19), 5560-5564.

Kalai, F. Z., Boulaaba, M., Ferdousi, F. & Isoda, H. (2022). Effects of Isorhamnetin on Diabetes and Its Associated Complications: A Review of In Vitro and In Vivo Studies and a Post Hoc Transcriptome Analysis of Involved Molecular Pathways. *Int J Mol Sci*., *23*(2), 704. https://doi.org/10.3390/ijms23020704

Kandakumar, S. & Manju, V. (2017). Pharmacological Applications of Isorhamnetin: A Short Review. *International Journal of Trend in Scientific Research and Development*, *1*(4), 172-178. https://doi.org/10.31142/ijtsrd2202.

Kaur, R. & Dogra, N. K. (2014). A review on traditional uses, chemical constituents and pharmacology of *Ageratum conyzoides* L. (Asteraceae). *International Journal of Pharmaceutical and Biological Archive*, *5*, 33-45.

Kim, S., Thiessen, P.A., Bolton, E.E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B.A., Wang, J., Yu, B., Zhang, J., and Bryant, S.H. (2016). PubChem Substance and Compound databases. *Nucleic acids research*; *44*(D1), D1202-D1213.

Lenzen, S. (2008). The mechanisms of alloxan- and strteptozotocin-induce diabetes. *Diabetologia*, *51*(2), 216-226.

Lipinski, C. A., Lombardo, F., Dominy, B. W. & Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advance in Drug Delivery and Review, 46*, 3-26 (2001).

Liqing, H., Huan, H., Zhantu, L., Dan, L., Dong, Y. & Ming, H. (2016). Protective effects of isorhamnetin on cardiomyocytes against anoxia/reoxygenation1induced injury is mediated by SIRT1. *Journal of Cardiovascular Pharmacology*, *67*, 526-537.

Magnuson, M. A. & Matschinsky, F. M. (2004). "Glucokinase as a glucose sensor: past, present, and future". In Matschinsky, F.M. and Magnuson, M.A. (eds.). Glucokinase and Glycemic Disease: From Basics to Novel Therapeutics (Frontiers in Diabetes). Basel: S. Karger AG (Switzerland). Pp.18-30.

Marilena, A., Janet, A. G., Carlos**,** M. & Sergio, O.S.S. (2015). Topical anti-inflammatory ffects of isorhamnetin glycosides isolated from Opuntia ficus-indica. *BioMed Research International*, *2015*(1), 847320. https://doi.org/10.1155/2015/847320.

Martin, Y. C. (2005). A Bioavailability Score. *Journal of Medicinal Chemistry, 48*, 3164-3170.

Meng, C., Xuan-Yu, M., Hong-Xing, Z. & Mihaly, M. (2011). Molecular Docking: A powerful approach for structure-based drug discovery. *Current Computational Aided Drug Design*, *7*(2), 146-157.

Mobley, D. L. & Dill, K. A. (2009). Binding of small-molecule ligands to proteins: ‘‘what you see’’ is not always ‘‘what you get’’. *Structure, 17*, 489–498.

Morita, H., Deguchi, J., Motegi, Y., Sato, S., Aoyama, C., Takeo, J., et al. (2014). Sodium Glucose Co-transporter 2 (SGLT2) Inhibitors: New among antidiabetic drugs, Cardiovascular Drugs. *Therapy*, *28*, 331-334.

Moura, A. C., Silva, E. L., Fraga, M. C., Wanderley, A. G., Afiatpour, P. & Maia, M. B. (2005). Anti-inflammatory and chronic toxicity study of the leaves of *Ageratum conyzoides* L. in rats. *Phytomedicine*, *12*, 138–142.

Muegge, I., Heald, S. L. & Brittelli, D. (2001). Simple selection criteria for drug-like chemical matter. *Journal of Medicinal Chemistry*, *44*, 1841-1846.

Mullard, A. (2014). 2013 FDA drug approvals. *Nature Reviews Drug Discovery, 13*, 85 –89.

Ngoc Doan, T. N., & Ly Thi, L. (2012). Targeted proteins for diabetes drug design. *Advanced Natural Science Nanotechnology*, *3*, 1-9.

Ozioko, P. C., Muhammed, Y. Y. & Ibrahim, A. (2022). *Ageratum conyzoides* Methanol Leaf Extract: Phytochemicals with Antidiabetic Potential via Antioxidant Activity. *Asian Journal of Biochemistry*, *17*(1), 15-24. https://doi.org/10.3923/ajb.2022.15.24

Ozioko, P. C., Ibrahim, A., Muhammad, Y. Y., & Bashir, M. (2024). Fractionation and Identification of Phenolic Compounds from Ethylacetate Fraction of *Ageratum Conyzoides* Methanolic Leaf Extract. *International Journal of Emerging Multidisciplinarie: Biomedical and Clinical Research*, *2*(*1*), 1-21. https://doi.org/10.54938/ijemdbmcr.2024.02.1.272

Patil, R., Das, S., Stanley, A., Yadav, L., Sudhakar, A. & Varma, A. K. (2010). Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing. *PLoS One*. *5*(8), e12029. https://doi.org/10.1371/journal.pone.0012029.

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C. & Ferrin, T. E. (2004). UCSF chimera: a visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, 25, 1605-1612. https://doi.org/10.1002/jcc.20084.

Piero, M. N., Njagi, J. M., Kibiti, C. M., Ngeranwa, J. J. N., Njagi, A. N. M., Njue, W. M., & Gathumbi, P. K. (2012). Herbal management of diabetes mellitus: a rapidly expanding research avenue. *International Journal of Current Pharmaceutical Research*, *4*(2), 1-4.

Rose, Y., Duarte, J. M., Lowe, R., Segura, J., Bi, C., Bhikadiya, C., et al. (2020). RCSB Protein Data Bank: Architectural advances towards integrated searching and efficient access to macromolecular structure data from the PDB archive. *Journal of Molecular Biology*, *16*, 6704.

Shapovalov, M. S. & Dunbrack, R. L. Jr. (2011). A smoothed backbone-dependent rotomer library for proteins derived from adaptive kernel density estimates and regressions. *Structure*, *19*, 844-858.

Stephen S. M. C., Eric, C. M. H., Karen S. L. L. & Sookja, K. C. (2003). Contribution of Polyol Pathway to Diabetes-Induced Oxidative Stress. *Journal of American Society of Nephrology, 14*, S233–S236.

Tae, H. K., Sae-Kwang, K. & Jong-Sup, B. (2013). Anti-Inflammatory activities of isorhamnetin-3-O1galactoside against HMGB1-induced inflammatory responses in both HUVECs and CLP-induced septic mice. *Journal of Cellular Biochemistry 114*, 336-345. https://doi.org/10.1002/jcb.24361

Trott, O. & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *Journal of Computational Chemistry*, *31*, 455-461.

Valdar, W. S. & Thornton, J. M. (2001). Protein-protein interfaces: analysis of amino acid conservation in homodimers. *Proteins*, 42, 108-124.

Veber, D. F. Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W. & Kopple K. D. (2002). Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry*, *45*, 2615-2623. https://doi.org/10.1021/jm020017n.

Widyawati, T, Yusoff, A. N., Asmawi, Z. M. & Ahmad, M. (2015). Antihyperglycemic effect of methanol extract of Syzygium polyanthum (Wight.) leaf in streptozotocininduced diabetic rats. *Nutrients*, *7*(9), 7764-7780.

Xiong, G. L., Wu, Z. X., Yi, J. C., Fu, L., Yang, Z. J., Hsieh, C. H., et al. (2021). ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. *Nucliec Acids Research*, *49*(W1), W5-W14. PMID:33893803.

Yeon, S. S., Hye, S. B, Kazuo, O. & Kuk, H. (2005). Inhibitory effects of Isorhamnetin-3-O-b - D-glucoside from *Salicornia herbacea* on rat lens aldose reductase and sorbitol accumulation in streptozotocin-induced diabetic rat tissues. *Biological and Pharmaceutical Bulletin*, *28*(5), 916-918. https://doi.org/10.1248/bpb.28.916.

Yun, L., Guibo, S., Xi, D., Min, W., Meng, Q., Yingli, Y. et al. (2015). Isorhamnetin attenuates atherosclerosis by inhibiting macrophage apoptosis via PI3K/ AKT activation and HO-1 induction. *PLoS ONE*, *10*(3), 0120259. https://doi.org/10.1371/journal.pone.0120259