



Cheminformatics identification of modulators of key carbohydrate-metabolizing enzymes from *C. cujete* for type-2 diabetes mellitus intervention

Fatai Oladunni Balogun¹ · Karishma Singh^{1,2} · Athika Rampadarath¹ · Ayesha Akoonjee¹ · Kayleen Naidoo¹ · Saheed Sabiu¹

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Abstract

Purpose The therapeutic use of oral hypoglycaemic agents in the management of type-2 diabetes mellitus (T2DM) is without adverse effects; thus, calls for alternative and novel candidates from natural products in medicinal plants.

Method The study explored molecular docking and molecular dynamics (MD) simulation approaches to identify key anti-diabetic metabolites from *Crescentia cujete*.

Results Molecular docking results identified four and/or five best compounds against each target enzyme (alpha-glucosidase, dipeptidyl peptidase-IV, aldose reductase, and protein tyrosine phosphatase-1B (PTP-1B)) implicated in diabetes. The resulting complexes (except against PTP-1B) had higher docking scores above respective standards (acarbose, Diprotin A, ranirestat). The MD simulation results revealed compounds such as benzoic acid (-48.414 kcal/mol) and phytol (-45.112 kcal/mol) as well as chlorogenic acid (-42.978 kcal/mol) and naringenin (-31.292 kcal/mol) had higher binding affinities than the standards [acarbose (-28.248 kcal/mol), ranirestat (-21.042 kcal/mol)] against alpha-glucosidase and aldose reductase, respectively while Diprotin A (-45.112 kcal/mol) and ursolic acid (-18.740 kcal/mol) presented superior binding affinities than the compounds [luteolin (-41.957 kcal/mol and naringenin (-16.518 kcal/mol)] against DPP-IV and PTP-1B respectively.

Conclusion While isoflavone (alpha-glucosidase), xylocaine (DPP-IV), luteolin (aldose reductase,) and chlorogenic acid (PTP-1B) were affirmed as the best inhibitors of respective enzyme targets, luteolin, and chlorogenic acid may be suggested and proposed as probable candidates against T2DM and related retinopathy complication based on their structural stability, compactness and affinity for three (DPP-IV, aldose reductase, and PTP-1B) of the four targets investigated. Further studies are warranted in vitro and in vivo on the antihyperglycaemic effects of these drug candidates.

Keywords Chlorogenic acid · *Crescentia cujete* · Isoflavone · Luteolin · Molecular dynamics simulation · Type-2 diabetes mellitus · Xylocaine

Introduction

Diabetes mellitus (DM) is an endocrine illness with increasing prevalence globally, thus, constituting a continuous health challenge with resulting huge financial implications. Diabetes mellitus is majorly classified into type-1 DM (i.e., insulin-dependent) and type-2 DM (non-insulin-dependent) with the latter accounting for 90–95% of DM incidence [1, 2]. The disease (type-2 diabetes mellitus) is reported to be attributed to a hyped concentration of glucose (hyperglycaemia) and insulin (hyperinsulinemia) within the systemic circulation [3] arising from the inability of beta cells of the islets of Langerhans within the pancreas to secrete insulin or

✉ Saheed Sabiu
sabiuss@dut.ac.za

¹ Department of Biotechnology and Food Science, Faculty of Applied Sciences, Durban University of Technology, P.O. Box 1334, Durban 4000, South Africa

² Department of Nature Conservation, Mangosuthu University of Technology, Mangosuthu, South Africa

the body tissues not responding to the available insulin [4]. The prevalence of the menace on a yearly basis has continued to remain an issue of concern for researchers (particularly diabetic), healthcare practitioners, etc., globally. In fact, according to the International Diabetes Foundation, 425 million patients are affected globally by T2DM in 2017 [5] with a further increase to 463 million in 2019 [2] and an anticipated increase to 764 million by the year 2030 [6], if no suitable solution is found.

In the past, various efforts in the management of the T2DM are tailored toward maintaining the glucose at a normal level via non-pharmacological interventions such as diets and exercise and pharmacologically using oral hypoglycaemic agents (OHAs) including the likes of sulphonylureas, biguanides, meglitinides, thiazolidinediones and alpha-glucosidase inhibitors. Thus, preventing its likely complications including retinopathy, neuropathy, and nephropathy among others [7]. The individual members or classes of these drugs (OHAs) are sometimes able to regulate the elevated glucose levels to optimal level via various mechanisms and/ or involving the inhibition of specific protein targets such as alpha-glucosidase, dipeptidyl peptidase-IV (DPP-IV), protein tyrosine phosphatase-1 B (PTP-1B) implicated in the emergence of T2DM and aldose reductase as a consequent of diabetes retinopathy.

Alpha-glucosidase is a carbohydrate-hydrolyzing enzyme of the intestines involved in the continuous breakdown of disaccharides (cleaving α -1,4-glycopyranosidic linkage) to produce simple sugars such as glucose [8, 9] during the postprandial hyperglycaemic state. While dipeptidyl peptidase IV (DPP-IV) is a surface antigen protein involved in the cleavage of the glucagon peptide-like 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) involved in the potentiation of pancreatic cells to secrete insulin [10, 11], PTP-1B catalyzes the dephosphorylation of insulin receptors (IR) and insulin receptor substrates (IRS) concerned with type-2 diabetes mellitus (T2DM) pathogenesis and obesity inducing insulin and leptin resistance, respectively [12].

Diabetes retinopathy (DR) is one of the prominent microvascular complications of diabetes [13] and a major cause of blindness in working-class individuals globally [14]. While more than 60% of T2DM cases have been reported to progress into DR after few years [15], in terms of prevalence, more than 100 million individuals suffer from this ever-increasing health challenge worldwide [14]. Aldose reductase is a key enzyme involved in polyol pathway and it is regarded as a clinical therapeutic target for complications arising from DR [16].

The inhibition of these carbohydrate enzymes by various classes of drugs (acarbose, Diprotin A, ursolic acid, ranirestat, etc.) is considered a good therapeutic approach

toward diabetes control though these OHAs are recognized with side effects including flatulence, stomachache, and diarrhoea [9]. Due to the prevalence and severity of T2DM including its complications and coupled with emanating side effects from available synthetic inhibitors, the search for novel and natural compounds with antidiabetic activity is seen as a viable method or intervention if it is to be well managed [17–19].

Medicinal plants use for the treatment of numerous diseases have been an age-long exploration or tradition [20, 21]. In fact, at a time when there has been non-existence in the use of chemical or synthetic moieties, humankind have continued to relied on these natural products or medicinal plant formulations to take care of illnesses [22] that may compromise the state of their health or threatens their survival as a race. While these medicinal plants, not limited to *Crescentia cujete* and others are (naturally) available, relatively cheap and with adjudged little or no side effects [23], they are established with one or more biological and/ or pharmacological attributes owing to the inherent compounds, many of which have in recent times been developed into drug candidates against a number of ailments [18, 19, 24].

Crescentia cujete L., commonly known as calabash tree is an important indigenous tree with wide medicinal uses including diabetes, hypertension, cancer, infectious illnesses, infertility problems attributed to a number of verified pharmacological and biological activities such as antidiabetic, antibacterial, anti-inflammatory, anthelmintic, antioxidant, antivenom, cytotoxic, neuroprotective etc., [24]. The plant belonging to the family Bignoniaceae [25] is also endowed with good nutritional potential and economic benefit. The recently published critical review of the plant provided insights into several classes of phytochemicals including flavonoids, phenolic acids, alkaloids, saponins, tannins etc. from different parts of the plant responsible for various established pharmacological potentials [24]. Inclusively, the antidiabetic property of the various parts (leaves, stem bark and roots) of the plant in various extracts (aqueous, hydroethanol, ethanol) have been investigated in both in vitro and in vivo models [26–30]. Ethanol extract and fractions (hexane, diethyl ether and ethyl acetate) in a study by Adeyemi et al. [29] showed comparative inhibition with acarbose against alpha-amylase with a superior effect over the standard against alpha-glucosidase. Additionally, aqueous root extract at 250 mg/kg body weight (b.w.) caused a reduction in the increased glucose level (brought about by alloxan) of diabetic Sprague-Dawley rats from 302.75 to 119.00 mg/dl, an effect comparable with glibenclamide (5 mg/kg b.w.) [26]. Similarly, aqueous leaves extract at concentrations of 200 and 400 mg/kg b.w. was reported to reverse elevated glucose level of streptozotocin -induced

hyperglycaemic Wistar rats [28]. Despite these reports, the molecular mechanism of antidiabetic action of the plant remains elusive. The present study is aimed at studying the interaction of natural products (secondary metabolites) in *Crescentia cujete* L with key targets implicated in T2DM through computational studies as a way of discovering novel therapeutic agents with antidiabetic properties.

Materials and methods

Data mining for *C. cujete* metabolites

The identification of the 56 phytoconstituents (Supplementary Table S1) of *C. cujete* provided in the work of Balogun and Sabiu [24] was confirmed using various databases [NPASS (<http://bidd.group/NPASS/>) and Dr. Duke's Phytochemical and Ethnobotanical Database (<https://phytochem.nal.usda.gov/phytochem/search>)].

Targets, molecular docking, and molecular dynamic simulation

The crystal structure for the target enzymes; PDB: 3W37 (alpha-glucosidase); PDB: 1IEI (aldose reductase); PDB: 1WCY (dipeptidyl-peptidase 4) and PDB: 2HNQ (protein tyrosine phosphatase 1B)] was obtained from the RSCB Protein Data Bank (<https://www.rcsb.org/>) and downloaded in PDB format. They are prepared on UCFS chimera software v. 1.14 and saved in PDB format based on [31] method. The 3D structures of the compounds and reference standards (acarbose, ranirestat, Diprotin A, ursolic acid) were collected from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) in sdf format, thereafter, prepared and optimized using Avogadro software and then saved as mol2 according to Sabiu et al. [19].

The molecular docking of the optimized ligands (*C. cujete* metabolites and reference standards) and proteins (alpha-glucosidase, aldose reductase, DPP-IV and PTP-1B) was performed using Python Prescription (PyRx) version 0.9.6. as described by Ambrose et al. [32]. Briefly, following the description in the work of Mubarak et al. [33], the x-y-z coordinates of the active sites of the proteins were identified where the docking of the ligands occurred, thereafter, the grid box was adjusted to fit perfectly within the well-defined x-y-z coordinates. AutoDock Vina (version 4.2)-coupled PyRx tools was employed to implement the molecular docking of the optimized ligands and prepared proteins. The ligands were then ranked based on the best affinities for the studied proteins (alpha-glucosidase, aldose reductase, dipeptidyl-peptidase IV, and protein tyrosine phosphatase-1B) and the highest affinity of complexes with

the best pose formed. Consequently, the top compounds with the most negative binding scores were selected as the best compounds against each enzyme and the inhibition constant (K_i) of their resulting complexes was obtained from their binding energy (ΔG) [34], before further evaluation through MD simulation.

The MD simulation was executed using the AMBER 18 software (Centre for High-Performance Computing) as earlier described [19], where the FF18SB variant of the amber force field was employed to provide the operating system functions. Using the ANTECHAMBER, the atomic partial charges of the ligand via general amber force fields (GAFF) and restrained electrostatic potentials (RESP) were created. The TIP3P potential represented water molecules, Na^+ and Cl^- counter ions were added to neutralize the system and the cut-off value of the non-bond interactions was set to 8 Å. The total simulation was carried out for 100 ns, with the Leap module SHAKE algorithm adopted to limit the expansion of chemical bonds involving hydrogen atoms. For each individual simulation was a 2 fs step size corresponding to an isobaric-isothermal ensemble (NPT) containing randomized seeding, 300 K temperature, 1 bar constant pressure, Langevin thermostat (1.0 ps collision frequency), and 2 ps pressure-coupling constant. Examined MD simulation results were presented as post-dynamic data.

The post-dynamic simulation was achieved as previously described [19]. The AMBER 14 PTRAJ program was used to combine and analyze the coordinates of the systems, followed by CPPTRAJ program used for the analyses of parameters such as root mean square fluctuation (RMSF), root mean square deviation (RMSD), radius of gyration (R_g), and solvent accessible surface area (SASA). Molecular Mechanics/GB Surface Area method (MM/GBSA) was employed to calculate the average binding free energy for each ligand-protein complex over 100 000 snapshots from the 100 ns simulation. Discovery Studio version 21.1.0 was used to analyze and visualize the interaction of the complexes for each interaction. All the data plots were generated using Origin data analysis software V18 [35], and where applicable, statistical significance between calculated means was considered at $p < 0.05$ level.

Pharmacokinetics evaluation

The pharmacokinetic profiles including absorption, distribution, metabolism and excretion (ADME) and drug-likeness properties of the compounds with the most promising complex were predicted with the SWISS ADME (Swiss Institute of Bioinformatics, Lausanne, Switzerland) server (<http://swissadme.ch/index.php>).

Results and discussions

The global scientific databases are filled with large repertoire of probable compounds identified from medicinal plants or natural products that are continuously being submitted or reported from published studies on timely basis. It is of interest to note that researchers are often times comfortable to pick alien or new medicinal plant species to study in an effort to identify their compounds but many a time they lacked the tenacity to further explore or screen these (literature available) avalanches of compounds for likely drug development. It is on this aforementioned background that prompted the development of this theoretical approach (through computational tool) with a view at making or identifying possible candidates of pharmacological importance (against a number of diseases) from the plant. Computational analyses are extensively considered during drug discovery and development to screen libraries of compounds with the potential to elicit the desired therapeutic activity [36]. Computational methods allow for the elimination of compounds without therapeutic benefit prior to

further experimental and/or clinical studies such as in vitro and in vivo testing.

Molecular docking

The docking scores obtained from the interaction of the 56 *C. cujete* compounds with the respective active sites of the four investigated targets are presented in Supplementary Table S1, where most of the compounds that exhibited, lower negative scores were compared to the reference standards. However, the findings of the selected top compounds (based on best binding scores) against each target are presented in Table 1. Compounds such as isoflavone (-7.7 kcal/mol), luteolin (-6.7 kcal/mol), and naringenin (-9.9 kcal/mol) with specific targets (alpha-glucosidase, DPP-IV, aldose reductase, respectively) revealed the most negative binding scores when compared with complexes for the respective standards [acarbose (-5.0 kcal/mol), Diprotin A (-5.5 kcal/mol), ranirestat (-9.0 kcal/mol). Chlorogenic acid with the best binding score (-6.2 kcal/mol) against PTP-1B had a lower binding score compared to the standard, ursolic acid (-7.4 kcal/mol) (Table 1). Molecular docking analysis measures the binding mode of an inhibitor or compound as it binds to a protein [37]. Molecular docking uses a scoring function to determine the inhibitor with the most appropriate (binding) orientation at the active site of an enzyme. The most negative binding score depicts the better pose and the best modulator of the enzyme [19]. The highest negative binding scores of isoflavone, luteolin, naringenin, and chlorogenic acid observed in this study were an indication of their superior affinities for the respective (alpha-glucosidase, DPP-IV, aldose reductase) targets above other compounds and standards except for ursolic acids against PTP-1B. The superior binding mode of phytochemicals over their synthetic counterpart against these targets has been reported [19]. Typically, compounds such as luteolin 7-O-beta-D-glucoside (-8.6 and -9.4 kcal/mol), 1,3-dicaffeoylquinic acid (-8.1, -9.7 kcal/mol), epicatechin (-7.7, -9.6 kcal/mol) etc. from *Carpobrotus edulis* had enhanced binding scores superior to acarbose and ranirestat (-7.5 and -8.4 kcal/mol respectively) against alpha-glucosidase (PDB: 3W37) and aldose reductase (PDB: 3RX3) [19]. Additionally, 3-cafeoylquinic acid (-7.8 kcal/mol), engeletin (-8.4 kcal/mol, and sinocrassosideA1 (-9.0 kcal/mol) from *Helichrysum petiolar* had higher binding affinity values than acarbose (-6.3 kcal/mol) against alpha-glucosidase (PDB: 3WEL) [38]. Thus, our findings in this study corroborated earlier studies [19, 39], on the best pose of some phytoconstituents as good modulators of the studied enzymes.

Inhibition constant (Ki) aimed to measure the strength of binding occurring between the inhibitor and the enzyme [40, 41]. A lower Ki value is reported as an indication of a

Table 1 Docking scores of the best five compounds against each of the diabetic target enzymes

Targets	Compound/standards	Binding energy (kcal/mol)	Ki (μM)
Alpha-glucosidase [#]	Isoflavone	-7.7	0.05
	Phytol	-7.4	0.06
	Apigenin	-7.3	0.06 [^]
	Benzoic acid	-7.2	0.06 [^]
	Acarbose*	-5.0	0.14
Dipeptidyl peptidase-IV	Luteolin	-6.7	0.07
	Xylocaine	-5.7	0.11
	Apigenin	-5.7	0.11
	Pinocembrin	-5.7	0.11
	Cistanoside D	-5.7	0.11
	Diprotin A*	-5.5	0.12
	Chlorogenic acid	-6.2	0.10
Protein Tyrosine Phosphatase-1B	1,2,4,5-tetrazine-3,6-diamine	-5.9	0.10
	Pinocembrin	-5.9	0.10
	Naringenin	-5.7	0.11
	Trans-cinnamic	-5.7	0.11
	Ursolic acid*	-7.4	0.06
	Naringenin	-9.9	0.02
	Luteolin	-9.7	0.02 [^]
Aldose reductase [#]	Isoflavone	-9.6	0.02 [^]
	Chlorogenic acid	-9.4	0.03
	Ranirestat*	-9.0	0.03 [^]

*Reference standards; [#]Four compounds selected against alpha-glucosidase and aldose reductase because many compounds have same binding energy scores (-7.1 and -8.7 kcal/mol respectively) for the next lower docking scores; [^]Values after rounding up to 2 decimal places

stronger affinity between the inhibitor and the enzyme [36]. As observed from the study, the K_i of these compounds, i.e., isoflavone, luteolin and naringenin complexed with respective enzymes (alpha-glucosidase, DPP-IV and aldose reductase) were the lowest (0.05, 0.07 and 0.02 μM , respectively) compared to the standards [acarbose (0.14 μM), Diprotin A (0.12 μM) and ranirestat (0.03 μM)]. However, the complex formed between ursolic acid (standard) and PTP-1B revealed a lower K_i (0.06 μM) compared with complexes of the phytocompounds with the enzyme (Table 1). The lower K_i values of isoflavone, luteolin and naringenin compared to the standards signify their stronger and superior inhibitory effects on the respective enzymes (alpha-glucosidase, DPP-IV, aldose reductase). In fact, a report of phytocompounds such as hypericin (9.4 mg/L) having a stronger K_i than acarbose (40.6 mg/L) had been reported [40].

Molecular dynamics simulation

The binding energy determinations (measured by thermodynamics calculations) is another parameter used as a

measure of the fitness of the ligands at the protein's binding pocket [42]. Higher binding energy depicted by the most negative values is suggestive of a stronger affinity of a ligand for an enzyme, thus, affording better complex stability [43]. The result of the thermodynamic profiles of top compounds of *C. cufete* against each of the studied enzymes is shown in Table 2. A look at these results revealed compounds such as benzoic acid (-48.415 kcal/mol) and phytol (-45.112 kcal/mol) having the most negative binding free energies against alpha-glucosidase which were better than acarbose with -28.248 kcal/mol. The superiority of some plants' compounds in showing the best complex affinities with their respective enzymes have been seen in some reports [19, 38, 44, 45]. Interestingly too, Adinortey et al. [46] also buttressed the superiority of various compounds such as taraxasterol, voruscharin, alpha-amyrin, beta-sitosterol, apigenin-7-0-glucoside, ursolic acid, quercetin-3-rutinoside, and isorhamnetin-3-Orutinoside, from *Calotropis procera* showing better affinity than acarbose (-34.3 kcal/mol) against alpha-glucosidase as depicted by their higher binding energies (ranging from -34.7 kcal/mol

Table 2 Thermodynamic profiles of the top compounds with the target enzymes

Energy components (kcal/mol)					
Complexes	ΔE_{vdW}	ΔE_{elec}	ΔG_{gas}	ΔG_{solv}	ΔG_{bind}
Alpha-glucosidase					
AG + Acarbose	-37.431 $\pm$ 5.05	-39.417 $\pm$ 12.88	-66.466 $\pm$ 12.09	26.613 $\pm$ 6.42	-28.248 $\pm$ 3.35
AG + Apigenin	-39.997 $\pm$ 2.49	-6.959 $\pm$ 7.80	-46.956 $\pm$ 6.89	20.807 $\pm$ 4.91	-26.150 $\pm$ 3.82
AG + Benzoic acid	-49.945 $\pm$ 3.47	-56.287 $\pm$ 9.72	-106.232 $\pm$ 9.38	57.818 $\pm$ 7.27	-48.415 $\pm$ 4.44
AG + Isoflavone	-33.307 $\pm$ 2.16	-4.994 $\pm$ 3.49	-38.300 $\pm$ 4.40	11.227 $\pm$ 2.60	-27.073 $\pm$ 3.18
AG + Phytol	-48.510 $\pm$ 3.91	-8.244 $\pm$ 4.84	-56.754 $\pm$ 6.49	11.643 $\pm$ 2.25	-45.112 $\pm$ 6.10
DPP-IV					
DPP-IV + Diprotin A	-48.509 $\pm$ 3.91	-8.244 $\pm$ 4.84	-56.754 $\pm$ 6.49	11.642 $\pm$ 2.24	-45.112 $\pm$ 6.10
DPP-IV + Xylocaine	-45.001 $\pm$ 4.50	-30.372 $\pm$ 14.24	-75.373 $\pm$ 14.09	49.707 $\pm$ 9.61	-25.666 $\pm$ 6.29
DPP-IV + Pinocembrin	-20.909 $\pm$ 3.00	-22.914 $\pm$ 8.61	-43.824 $\pm$ 9.16	25.344 $\pm$ 7.01	-18.480 $\pm$ 3.74
DPP-IV + Luteolin	-34.908 $\pm$ 3.77	-54.367 $\pm$ 15.81	-89.274 $\pm$ 16.55	47.317 $\pm$ 11.98	-41.957 $\pm$ 6.49
DPP-IV + Cistanoside D	-41.517 $\pm$ 5.55	-24.131 $\pm$ 17.09	-65.648 $\pm$ 19.14	44.739 $\pm$ 14.41	-20.909 $\pm$ 7.29
DPP-IV + Apigenin	-25.667 $\pm$ 3.91	-32.933 $\pm$ 7.53	-58.600 $\pm$ 7.76	34.741 $\pm$ 4.70	-23.859 $\pm$ 5.15
PTP-1B					
PTP-1B + Ursolic acid	-30.044 $\pm$ 4.12	-8.593 $\pm$ 6.10	-31.584 $\pm$ 7.53	12.844 $\pm$ 4.36	-18.740 $\pm$ 5.25
PTP-1B + Chlorogenic acid	-24.702 $\pm$ 4.05	-22.648 $\pm$ 12.89	-47.349 $\pm$ 14.51	31.421 $\pm$ 10.80	-15.928 $\pm$ 5.11
PTP-1B + TZD	-12.423 $\pm$ 4.67	-18.396 $\pm$ 9.01	-30.820 $\pm$ 12.41	20.161 $\pm$ 8.24	-10.658 $\pm$ 4.88
PTP-1B + Naringenin	-22.280 $\pm$ 6.51	-12.275 $\pm$ 8.66	-34.555 $\pm$ 12.69	18.037 $\pm$ 6.86	-16.518 $\pm$ 7.03
PTP-1B + Pinocembrin	-22.116 $\pm$ 5.21	-7.880 $\pm$ 6.84	-30.001 $\pm$ 9.93	14.628 $\pm$ 5.27	-15.3721 $\pm$ 5.69
PTP-1B + TCA	-9.1595 $\pm$ 8.50	-7.275 $\pm$ 9.05	-16.434 $\pm$ 16.23	8.403 $\pm$ 8.11	-8.031 $\pm$ 8.47
Aldose reductase					
AR + Ranirestat	-30.729 $\pm$ 4.81	-10.134 $\pm$ 6.41	-40.863 $\pm$ 7.04	19.821 $\pm$ 4.56	-21.042 $\pm$ 3.83
AR + Chlorogenic acid	-41.940 $\pm$ 3.72	-48.666 $\pm$ 16.38	-90.606 $\pm$ 16.62	47.628 $\pm$ 7.83	-42.978 $\pm$ 9.97
AR + Isoflavone	-30.129 $\pm$ 7.32	-6.039 $\pm$ 4.66	-36.168 $\pm$ 9.46	13.280 $\pm$ 4.19	-22.887 $\pm$ 6.20
AR + Luteolin	-54.803 $\pm$ 3.88	-6.784 $\pm$ 14.19	-26.587 $\pm$ 13.13	16.378 $\pm$ 7.60	-30.209 $\pm$ 6.94
AR + Narengenin	-39.275 $\pm$ 2.38	-10.957 $\pm$ 7.90	-50.232 $\pm$ 7.40	18.940 $\pm$ 4.73	-31.292 $\pm$ 3.94

AG-Alpha-glucosidase; AR: Aldose reductase; PTP-1B: protein tyrosinase phosphatase; DPP-IV: dipeptidyl peptidase-IV; TZD: 1,2,4,5-tetrazine-3,6-diamine; TCA: trans cinnamic acid; ΔE_{vdW} =van der Waals; ΔE_{elec} =electrostatic energy; ΔG_{gas} =gas phase free energy; ΔG_{solv} =solvation free energy; ΔG_{bind} =total binding energy

to -40.2 kcal/mol). However, with respect to DPP-IV in this study, Diprotin A (standard) had the most negative binding energy value (-45.112 kcal/mol) compared to *C. cujete* compounds, although luteolin (-41.957 kcal/mol), had the most promising affinity out of the five compounds evaluated (Table 2). A similar trend in the superiority of the reference standard (ursolic acid: -18.740 kcal/mol) was also observed against PTP-1B in comparison with *C. cujete* compounds such as naringenin (-16.518 kcal/mol) being the most negative and trans-cinnamic acid (-8.031 kcal/mol), the least negative studied compound. The binding affinity values of the reference standards (Diprotin A and ursolic acid) against DPP-IV and PTP-1B were stronger or the strongest as observed in this study. The observation corroborated the finding from Bower et al. [47] for plant compounds such as hispidulin (-9.4 kcal/mol), naringenin (-8.6 kcal/mol), cirsimaritin (-8.4 kcal/mol), eriodictyol (8.9 kcal/mol) isolated from culinary herbs (*Origanum vulgare*, *Origanum majorana*, *Rosmarinus officinalis*) lower than sitagliptin (-9.6 kcal/mol) used as standard in the study. However, a report of higher negative binding free energy values of some flavonoid c-glycosides such as orientin, vitexin and apigenin above ursolic acid (standard) against PTP1B have also been reported [39]. Against aldose reductase, all the top 5 compounds had higher negative binding free energies compared to ranirestat (-21.042 kcal/mol), with chlorogenic acid (-42.978 kcal/mol) being the most promising compound (Table 2). The observation in this study regarding the affinity of the test compounds for aldose reductase is consistent with a previous study [19], where chlorogenic acid (-41.43 kcal/mol) had a better affinity for the binding pocket of aldose reductase than ranirestat (-38.51 kcal/mol).

Following the establishment of various binding affinities of the compounds and standards with respective targets, there is a need to probe further the extent of stability, flexibility, and compactness of the complex due to the likelihood of a conformational change being imminent as a result of the binding of the ligand which consequently would have an effect on the biological activity of the enzyme [44]. In post-MD simulation, important parameters such as RMSD, RMSF, Rg, and SASA are employed to study the degree of stability or equilibration of the ligand-protein complex [19]. The RMSD measures the change in the dynamics of the protein and the stability (conformation) of the ligand-protein complex [48]. A reduction in the average RMSD value of the complex relative to the apo enzyme during a simulation period is an indication of its stability [49]. Looking at the results of this investigation, the average RMSD of the apo enzyme (alpha-glucosidase) over 100 ns was 1.475 Å which was higher than those of the complexes formed with isoflavone (1.212 Å) and acarbose (1.318 Å) indicating the better stability of these compounds (Table 3). The complex formed

with phytol (1.462 Å) showed a marginal decrease ($p > 0.05$) relative to apo-enzyme (1.475 Å) which is still an indication of the stability of the complex. Isoflavone showing superior stability with its lowest average RMSD value compared to acarbose and other compounds was observed to corroborate the results of thermodynamic profiles. The superior stability of isoflavone-alpha-glucosidase complex over acarbose-alpha-glucosidase was in line with previous studies where natural (phenolic) compounds such as procyanidin, rutin, astilbin depicted lower RMSD values compared to acarbose against alpha-glucosidase [19, 50]. Figure 1a saw the convergence of all the systems at 10 and 20 ns which was maintained over 100 ns except the benzoic acid's complex which diverged from the alpha-backbone carbon of the enzyme at 20 ns. The average RMSD value of the unbound DPP-IV (1.418 Å) was lower compared with the bound complexes including the standard, DPP-IV-Diprotin A (1.756 Å) and *C. cujete* compounds DPP-IV-xylocaine (1.891 Å), DPP-IV-apigenin (1.905) and DPP-IV-luteolin (2.093 Å) (Table 3). While the superiority of Diprotin A (with marginal decrease compared with cistanoside D, 1.787 Å) in terms of stability above these compounds, as well as their high RMSD values including Diprotin A over the apo enzyme (DPP-IV), was noted, however, such increase has been reported not to be an indication of the complexes' instability [51, 52]. The finding observed in this study was in tandem with reports from Nath et al. [53] and Arif et al. [54] where the RMSD of hit compounds were < 2.5 Å and a convergence of all the systems at 15 ns was also observed before equilibration throughout the simulation period (Fig. 1b). 1,2,4,5-tetrazine-3,6-diamine and chlorogenic acid complexes (with PTP-1B) showed the lowest average RMSD values (1.162 Å, 1.242 Å, respectively) followed by PTP-1B-naringenin complex (1.291 Å) compared to the unbound system (1.681 Å). All the bound systems for the compounds revealed a significant ($p < 0.05$) reduction in average RMSD values (except pinocembrin) compared to the standard, ursolic acid-PTP-1B (1.498 Å) highlighting the significance of their superior structural stability above the standard (Table 3; Fig. 1c) and is consistent with a previous observation on RMSD values [55]. A striking fluctuation around 35–40 ns was observed for chlorogenic acid and ursolic acid (Fig. 1c). However, this finding differs from the result of the thermodynamic analyses as ursolic acid appeared to show an increased negative score relative to chlorogenic acid, naringenin, and pinocembrin. Although, the variation is noted, however, since MD simulations are used to refine the results emanating from docking scores and are an appropriate tool for providing insight into the evolutionary dynamics of the binding site interaction [56], hence, information on ligand-enzyme complex exhibiting reduced RMSD values relative to the apo enzyme is worth being accorded much importance in terms of consideration

Table 3 Average post-dynamic simulation parameters of the top compounds with the carbohydrate metabolizing enzymes

Complexes	RMSD (Å)	Rg (Å)	RMSF (Å)	SASA (Å)
AG	1.475 ± 0.26 ^a	23.531 ± 0.12 ^a	1.096 ± 0.47 ^a	20486.80 ± 7.15 ^a
AG + Acarbose	1.318 ± 0.17 ^b	23.486 ± 0.09 ^b	0.987 ± 0.44 ^b	20389.38 ± 5.79 ^b
AG + Isoflavone	1.212 ± 0.10 ^c	23.399 ± 0.11 ^c	0.932 ± 0.39 ^b	19832.15 ± 4.98 ^c
AG + Phytol	1.462 ± 0.15 ^a	23.459 ± 0.12 ^d	1.039 ± 0.51 ^c	20160.05 ± 5.63 ^d
AG + Benzoic acid	1.921 ± 0.34 ^d	23.591 ± 0.15 ^e	1.098 ± 0.51 ^a	20512.63 ± 6.61 ^a
AG + Apigenin	1.497 ± 0.16 ^e	23.491 ± 0.07 ^b	0.956 ± 0.43 ^b	19751.53 ± 3.93 ^c
DPP-IV	1.418 ± 0.19 ^a	26.985 ± 0.12 ^a	1.211 ± 0.77 ^a	25134.84 ± 451.90 ^a
DPP-IV + Diprotin A	1.756 ± 0.23 ^b	27.118 ± 0.11 ^b	1.252 ± 0.78 ^a	24891.80 ± 378.62 ^b
DPP-IV + Luteolin	2.093 ± 0.29 ^c	27.101 ± 0.09 ^b	1.283 ± 0.76 ^c	24757.82 ± 359.15 ^b
DPP-IV + Xylocaine	1.891 ± 0.17 ^d	27.327 ± 0.13 ^c	1.164 ± 0.58 ^b	24637.47 ± 373.40 ^b
DPP-IV + Apigenin	1.905 ± 0.28 ^d	27.297 ± 0.10 ^c	1.182 ± 0.58 ^b	25601.66 ± 395.67 ^c
DPPIV + Pinocembrin	1.982 ± 0.180 ^d	27.327 ± 0.11 ^c	1.257 ± 0.77 ^a	25185.67 ± 488.93 ^d
DPPIV + Cistanoside D	1.787 ± 0.17 ^b	27.133 ± 0.09 ^b	1.187 ± 0.58 ^b	25157.60 ± 474.17 ^a
PTP-1B	1.681 ± 0.24 ^a	18.921 ± 0.07 ^a	1.277 ± 0.87 ^a	13194.41 ± 312.84 ^a
PTP-1B + Ursolic acid	1.498 ± 0.16 ^b	18.864 ± 0.07 ^b	1.109 ± 0.57 ^b	12749.91 ± 332.30 ^b
PTP-1B + Chlorogenic acid	1.242 ± 0.11 ^c	18.844 ± 0.05 ^b	0.987 ± 0.48 ^c	12804.77 ± 267.42 ^c
PTP-1B + Naringenin	1.291 ± 0.12 ^c	18.911 ± 0.10 ^a	1.076 ± 0.48 ^b	12714.82 ± 400.36 ^b
PTP-1B + Pinocembrin	1.427 ± 0.21 ^b	18.869 ± 0.07 ^b	1.182 ± 0.65 ^c	12560.01 ± 318.85 ^d
PTB1B + TZD	1.162 ± 0.08 ^d	18.871 ± 0.07 ^b	0.959 ± 0.47 ^d	12574.774 ± 321.39 ^d
PTB1B + TCA	1.319 ± 0.15 ^e	19.043 ± 0.09 ^c	1.116 ± 0.54 ^c	13074.964 ± 304.36 ^c
AR	2.126 ± 0.216 ^a	19.342 ± 0.08 ^a	1.089 ± 0.52 ^a	13783.47 ± 357.35 ^a
AR + Ranirestat	2.217 ± 0.30 ^b	19.357 ± 0.09 ^a	1.051 ± 0.58 ^b	13503.46 ± 291.10 ^b
AR + Chlorogenic acid	1.754 ± 0.22 ^c	19.215 ± 0.07 ^b	1.022 ± 0.56 ^c	13132.35 ± 272.52 ^c
AR + Naringenin	1.876 ± 0.27 ^d	19.327 ± 0.07 ^a	1.070 ± 0.67 ^a	13343.60 ± 277.74 ^d
AR + Luteolin	1.491 ± 0.18 ^e	19.165 ± 0.09 ^c	1.024 ± 0.63 ^c	13116.07 ± 294.48 ^e
AR + Isoflavone	1.735 ± 0.25 ^c	19.314 ± 0.09 ^a	1.045 ± 0.79 ^b	13316.77 ± 255.46 ^c

AG: Alpha-glucosidase; AR: Aldose reductase; DPP-IV: dipeptidyl peptidase-IV; PTP-1B: protein tyrosine phosphatase-1B; TZD: 1,2,4,5-tetrazine-3,6-diamine; TCA: trans cinnamic acid; RMSD: Root mean square deviation; RMSF: Root mean square fluctuation; Rg: Radius of gyration; SASA: Solvent accessible surface area

a,b,c,d,e Values with different superscript letters down the column for each parameter are significantly different from each other ($p < 0.05$)

or determination of better stability of the complex. Contrary to the RMSD results on alpha-glucosidase and DPP-IV, the *C. cujete* compounds complexation with aldose reductase revealed significant ($p < 0.05$) lower average RMSD values compared to apo enzyme, aldose reductase (2.126 Å) (Table 3). Interestingly, aldose reductase-ranirestat complex (2.217 Å) was observed to be significantly ($p < 0.05$) higher compared to the unbound system. While the RMSD of the former is within the acceptable range of 2.5 Å and may not be an indication of instability [51, 52], the result was found to be consistent with the binding affinity findings as chlorogenic acid and naringenin with the most negative binding energies were also among the four compounds that formed the best complexes stability with aldose-reductase having a lower average RMSD (1.754, 1.876 Å, respectively), though luteolin and isoflavone were the lowest (1.491 Å, 1.735 Å, respectively) as can also be seen in Fig. 1d. While it was evident that these compounds promoted good structural stabilities better than the standard (ranirestat) and an indication of their inhibitory role against aldose reductase or

being possible drug candidates, the best effect depicted by luteolin in this study was contrary to a previous report [19] with a related compound (luteolin-7-O-beta-D-glucoside) having enhanced negative binding energy (and increased RMSD value) compared to other compounds from *C. edulis* which might be connected to the sugar attachment. Notwithstanding the aforementioned, a study has established good structural stability of naringenin with aldose reductase [57]. Summarily, isoflavone, cistanoside, chlorogenic acid and luteolin were found in this study to reveal the lowest RMSD values often better than respective standards against each of the targets. Since RMSD is a measure of stability [58], hence, the reflection of lower RMSD values of these compounds compared to co-compounds and standard could only be suggested as an indication of better stabilities [19].

In addition to stability, the Rg determines the compactness of the complex [58] and a stable Rg is an indication of stably folded protein [59]. An increased Rg value signifies reduced compactness and vice-versa [60]. The average Rg of apo enzyme (23.531 Å) was significantly ($p < 0.05$)

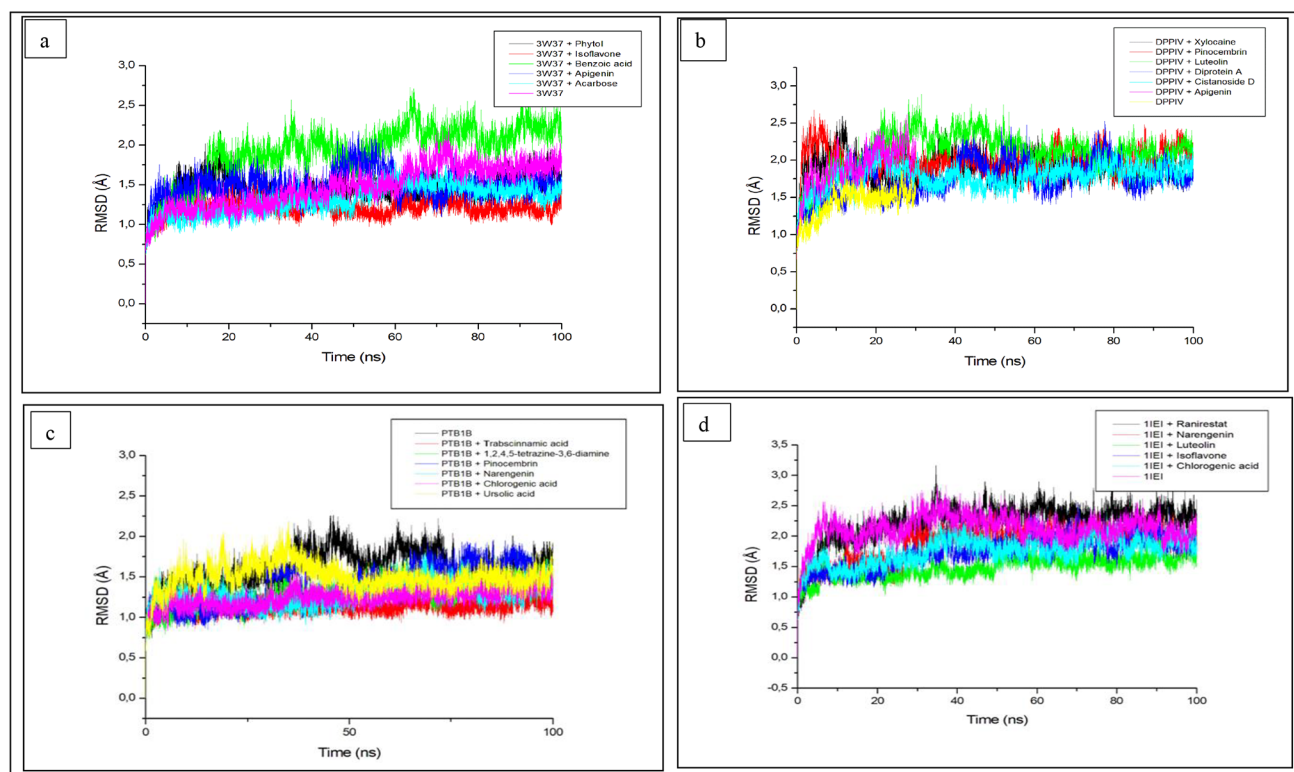


Fig. 1 Root Mean Square Deviation (RMSD) plots of comparison between the studied enzymes **a** (alpha-glucosidase), **b** (Dipeptidyl peptidase-IV), **c** (Protein tyrosine phosphatase-1B) and **d** (aldose reductase) *C. kujete* compounds and standard molecules (acarbose,

Diprotin A, ursolic acid and ranirestat)] determined over 100 ns molecular dynamics simulations. DPP-IV; dipeptidyl peptidase-IV; PTP-1B; protein tyrosine phosphatase-1B. CODES: 3W37- alpha-glucosidase; 1IEI- aldose reductase

higher than that of alpha-glucosidase-isoflavone (23.399 Å), alpha-glucosidase-phytol (23.459 Å), and alpha-glucosidase-acarbose (23.486 Å) complexes (Table 3). While the trend of the result is similar to RMSD, the lower Rg values of the complexes with isoflavone and phytol (Fig. 2a) relative to acarbose suggest their better compactness and stability since reduced Rg is synonymous to better protein stability [19, 61]. The result of the finding was similar to Eawsakul et al. [50] where astilbin was reported to be more stable with alpha-glucosidase (PDB 5ZCC) based lower Rg value compared to alpha-glucosidase-acarbose. All the complex systems were observed to converge at 10 ns though the higher inconsistency and instability of alpha-glucosidase-benzoic acid complex could be attributed to its higher average Rg value (Fig. 2a). However, against DPP-IV, all the bound systems showed a higher average Rg values compared to the unbound system (26.985 Å) with DPP-IV-luteolin complex (27.101 Å) exhibiting the lowest ($p < 0.05$) increase. The ability of luteolin to compare favourably with Diprotin A (27.118 Å) is a pointer to the superiority of the former above the latter, hence, better compactness (with DPP-IV) and stability. This observation regarding RMSD aligns with thermodynamic energy value results where luteolin had the most negative binding energy compared to Diprotin A.

While it was observed that all the systems converge at 30 ns, the high divergence of xylocaine at 10 and 27 ns before stability as a probable reason for the observed increase (including pinocembrin) in average Rg values (Fig. 2b). The fluctuation of all the systems against PTP-1B ranges between 18.5 and 19.4 Å and there seems to be a convergence of the systems at around 40 ns (Fig. 2c). The average Rg of the unbound PTP-1B was the highest (18.921 Å) compared to the bound systems except trans cinnamic acid (19.043 Å) (Table 3). The marginal decrease in the average Rg value of chlorogenic (18.844 Å) and insignificant increase in the complexes with pinocembrin and 1,2,4,5-tetrazine-3,6-diamine (18.869 Å, 18.871 Å) compared to that of ursolic acid (18.864 Å) may indicate the better stability (with PTP-1B) of the compounds and standard, besides, the ursolic acid may be suggested to be superior going by its highest negative binding energy. The submission in this study contradicts the findings of Rampadarath et al. [39] on the better compactness of flavonoids c-glycosides such as orientin, vitexin, apigenin above ursolic acid. Luteolin-aldose reductase and chlorogenic acid-aldose reductase complexes revealed the lowest average Rg values of 19.165 Å and 19.215 Å, respectively compared to the unbound system, aldose reductase (19.342 Å) and other bound systems

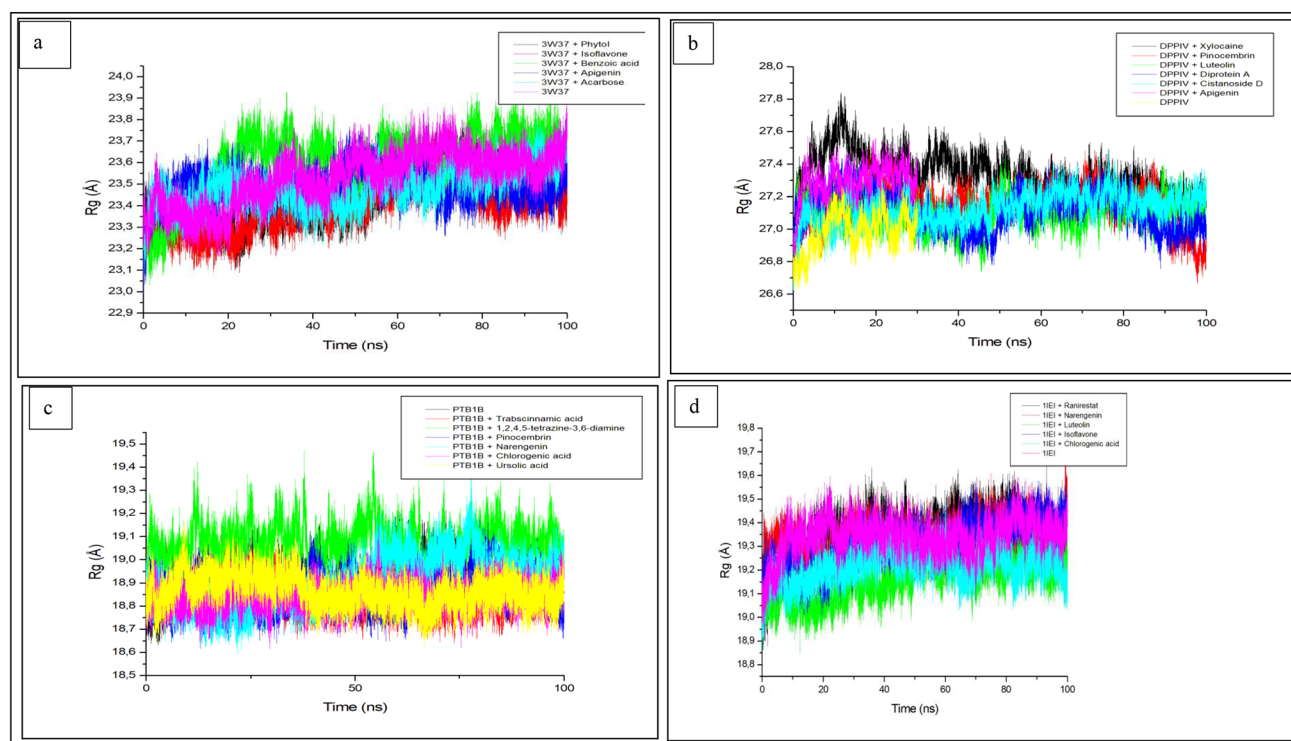


Fig. 2 Radius of Gyration (R_g) plots of comparison between the studied enzymes [a (alpha-glucosidase), b (Dipeptidyl peptidase-IV), c (Protein tyrosine phosphatase-1B) and d (aldose reductase) and *C. cujete* compounds and standard molecules (acarbose, Diprotin A, urso-

lic acid and ranirestat)] determined over 100 ns molecular dynamics simulations. DPP-IV; dipeptidyl peptidase-IV; PTP-1B; protein tyrosine phosphatase 1B

CODES: 3W37- alpha-glucosidase; 1IEI- aldose reductase

[isoflavone-aldose reductase (19.314 Å) aldose reductase-naringenin (19.327 Å)] (Table 3; Fig. 2d). The superiority of chlorogenic acid and luteolin in the compactness of the complexes was in line with the results from the thermodynamic energy profiles. The R_g value of the aldose reductase-ranirestat complex (19.357 Å) was the highest among the bound systems, signifying the less stable or instability of its complex relative to the significant and stable compactness of the compounds. While the result follows the same pattern as RMSD, however, the findings buttress the previous submission with *C. edulis* [19] where the R_g of the standard reflected less stability compared to the compounds. Above all, R_g is a function of compactness which partly is also attributed to the stability of the complex; the finding of this study summarily as it concerns R_g depicted isoflavone, luteolin and chlorogenic acid as candidates with better compactness with the four respective targets owing to their lowest R_g values and thus poses better stabilities.

Root mean square fluctuation provides insight to the structural flexibility of the ligand-protein complex [46]. It is a measure of the average displacement of a position during a trajectory period relative to a reference point [62]. An enhanced flexibility movement is concomitant to increased fluctuations existing between the ligands and the amino acids residues of the receptor and vice versa [58]. In this

study, the average RMSF value observed with the unbound alpha-glucosidase was higher (1.096 Å) compared to the acarbose-alpha-glucosidase (0.987 Å) complex (Table 3), indicating a lower fluctuation of the complex. Moreover, the average RMSF values of isoflavone-alpha-glucosidase (0.932 Å) and apigenin-alpha-glucosidase (0.956 Å) complexes were lower ($p > 0.05$) compared to acarbose-alpha-glucosidase system. However, since lower RMSF values indicates better bonding and lesser flexibility of amino acid residues at the binding domain, it could be implicative of little structural distortion [63] and better structural stability [64] of these compounds (isoflavone and apigenin) with alpha-glucosidase and as such, suggest them as better inhibitors of the enzyme than acarbose [50]. While the average RMSF of other compounds such as phytol (1.039 Å) and benzoic acid (1.098 Å) increased the flexibility of the complex, the consistent fluctuations of all the systems were observed at 50, 150, 300 and around 220–240 amino residues with isoflavone showing the lessened fluctuations (Fig. 3a) indicating its potential superior possibility of sitting well in the binding pocket of the protein. Against DPP-IV, the average RMSF value of Diprotin A-DPP-IV complex (1.252 Å) was marginally above ($p > 0.05$) that of the apo enzyme (1.211 Å) suggestive of a possible heightened instability of the complex, however, xylocaine-DPP-IV

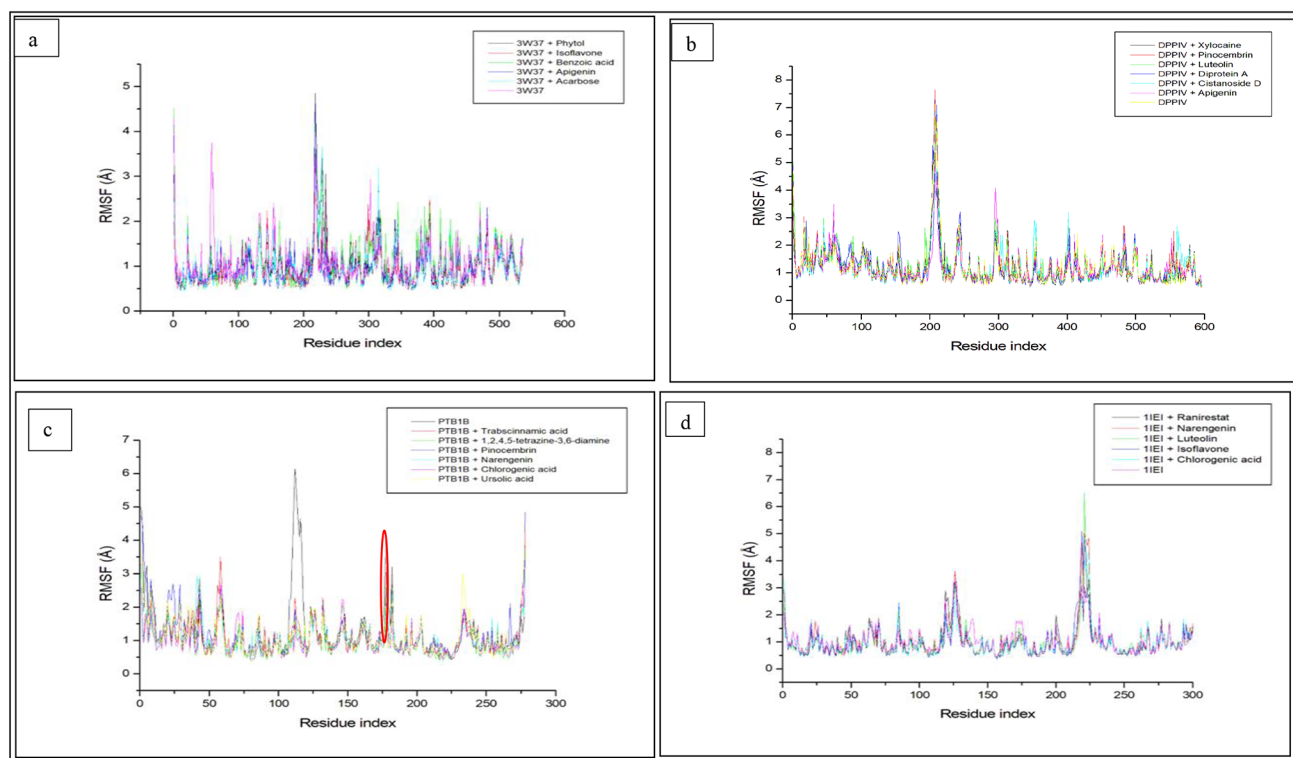


Fig. 3 Root Mean Square Fluctuation (RMSF) plots of comparison between the studied enzymes [a (alpha-glucosidase), b (Dipeptidyl peptidase-IV), c (Protein tyrosine phosphatase-1B) and d (aldose reductase)] *C. cujete* compounds and standard molecules (acarbose,

Diprotin A, ursolic acid and ranirestat)] determined over 100 ns molecular dynamics simulations. DPP-IV; dipeptidyl peptidase-IV; PTP-1B; protein tyrosine phosphatase 1B. CODES: 3W37- alpha-glucosidase; 1IEI- aldose reductase

(1.164 Å), apigenin-DPP-IV (1.182 Å) and cistanoside D (1.187 Å) complexes presented reduced fluctuations lower than Diprotin A indicating lesser flexibilities and as such afforded better stabilities of the complexes. The lower flexibilities observed with these compounds particularly with apigenin (phenolic compound) was in line with findings from a previous study, where chlorogenic acid had lesser flexibility compared to Diprotin A [65]. It was observed that there was a similarity in the pattern of fluctuations for the systems, though, these fluctuations were observed around 20, 50, 150, 200 and 550 amino acids residues (Fig. 3b). The importance of these identified residues is that these compounds (xylocaine, apigenin and castanoside D) might possess enhanced effects to maintain a good stability at the enzyme's receptor site. Against PTP-1B, the apo enzyme depicted a high RMSF value (1.277 Å), this fluctuation was brought down by ursolic acid (1.109 Å) to infer the stability of the complex. Interestingly, the average RMSF of 1,2,4,5-tetrazine-3,6-diamine (0.959 Å) and chlorogenic acid-PTP-1B (0.987 Å) followed by naringenin-PTP-1B (1.076 Å) suggests the superiority of these compounds to provide lesser flexibilities, reduced or no distortions and profound stability. While phenolic compounds such as vitexin and orientin have been reported in Ramparadath et al. [39]

study to reveal reduced flexibilities, the lower RMSF values of compounds including naringenin, chlorogenic acid and 1,2,4,5-tetrazine-3,6-diamine from the present work could be said to corroborate earlier study and thus, may be better candidates in the development of probable moieties for diabetes control. Additionally, fluctuations of the systems were observed at 120, 175 and 280 amino acids residues though a reduced fluctuation of the compounds was observed at catalytic region, 175 representing the WPD loop (circled in red) (Fig. 3c) as also indicated in the work of Ramparadath et al. [39]. The bound systems revealed a reduction in the fluctuation of the complex when compared to the apo enzyme, aldose reductase with an average RMSF value of 1.089 Å. The average RMSF values of chlorogenic acid-aldose reductase and luteolin-aldose reductase complexes were the lowest (1.022, 1.024 Å respectively) compared to ranirestat-aldose reductase (1.051 Å) and other systems; indicating the potential of the compounds to offer better stability of the complex and thus suggest them as probable candidates for aldose reductase inhibition. The observed effect of chlorogenic and luteolin with reduced distortion by virtue of their lower flexibilities compared to ranirestat aligns with the report of Sabiu et al. [19] where isorhamnetin-3-O-rutinoside and luteolin-7-O-beta-D-glucoside displayed similar

characteristics. While fluctuations at 125, 225 amino acids residues were noted for all the systems depicting the highest fluctuation of naringenin (Fig. 3d), the observed average higher RMSF of ranirestat relative to lower *C. kujete* phytochemicals in this study was also in tandem with a previous study [19] on *C. edulis* where a related compound, luteolin-7-O-beta-D-glucoside was similarly observed to greatly lessen the flexibility of aldose reductase. Isoflavone, xylocaine, 1,2,4,5-tetrazine-3,6-diamine and chlorogenic acid are compounds with the lowest RMSF values against the studied targets. Lower RMSF values has been reported as indication of lower flexibility and more stability [64]. Hence, with better stabilities of these compounds with respective targets could consider them as likely compound for further drug development.

Solvent accessible surface area determines the degree of hydrophobic exposures of amino acid residues in an effort to predict the stability of the complex [66]. The extent of exposure may cause a conformational change to the protein structure [67]. A decreased SASA value of the bound system relative to the unbound apo enzyme suggests enhanced exposure of the non-polar amino acids residues of the resulting complex, hence conferring better stability [68]. The results obtained with regards to SASA in this study revealed a heightened SASA value with alpha-glucosidase

(20486.80 Å), this was brought down to 20389.38 Å with the binding of acarbose indicating the stability of the complex (Table 3). However, apigenin and isoflavone presented the superior stability of the complex beyond acarbose with their lowest average SASA values (19751.53 Å, 19832.15 Å respectively) thus, buttressing their potential as a possible viable candidate in managing postprandial hyperglycaemia. A continuous fluctuation was observed with the apo enzyme over 100 ns, however, the bound systems revealed inconsistent fluctuations (Fig. 4a). In a similar trend with alpha-glucosidase, DPP-IV depicted a high SASA value (25134.84 Å) when compared with other bound systems except for apigenin-DPP-IV (25601.66 Å) (Table 3). The ability of the Diprotin A-DPP-IV complex to have a lower SASA value (24891.80 Å) against the apo enzyme was an indication of the complex's stability. A further reduction was observed with xylocaine, thus, exhibiting its profound stability with Diprotin A. While it was observed that consistency of fluctuations varies in the flexibility of the bound systems throughout the simulation period, that of the apo enzyme (and by extension pinocembrin complex) was found at 30 ns (Fig. 4b). The average SASA values for the apoenzymes, PTP-1B and aldose reductase were also hyped (13194.41 Å, 13783.49 Å, respectively) when compared with their complexation with respective standards, ursolic

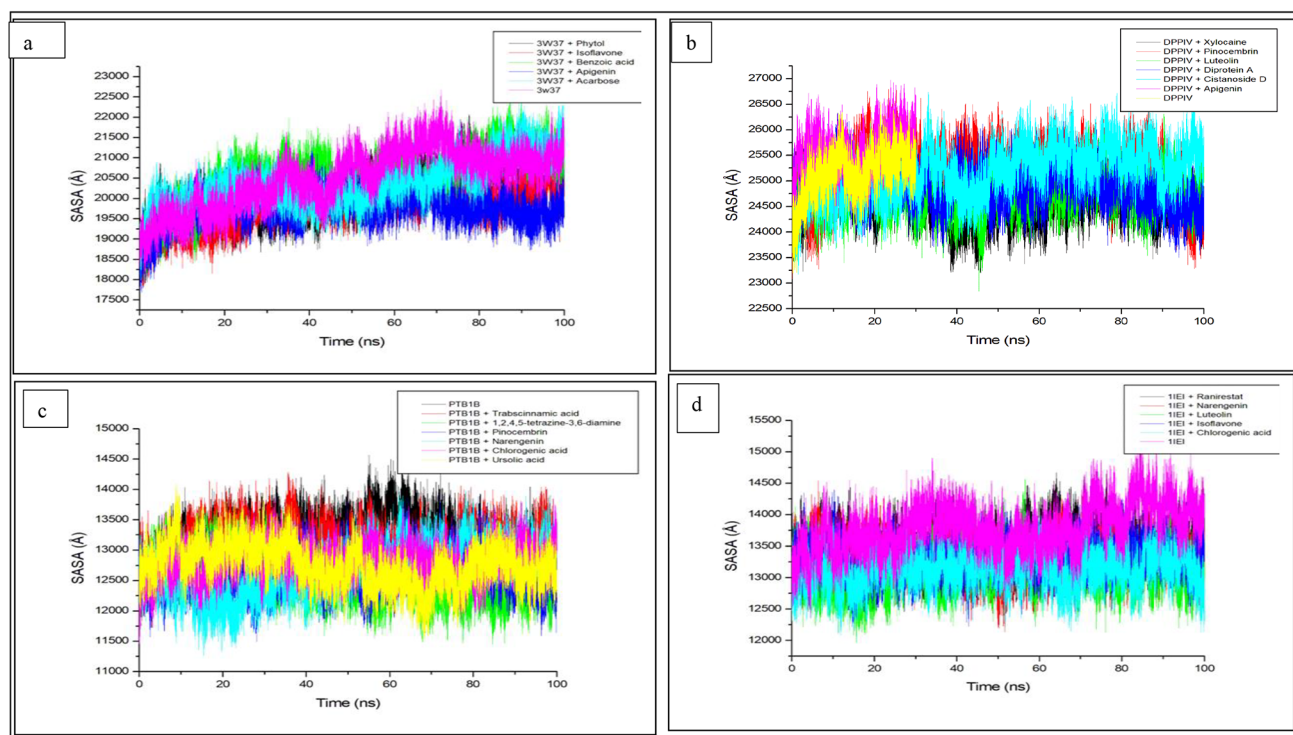


Fig. 4 Solvent Accessible Surface Area (SASA) plots of comparison between the studied enzymes [a (alpha-glucosidase), b (Dipeptidyl peptidase-IV), c (Protein tyrosine phosphatase-1B and d (aldose reductase)] *C. kujete* compounds and standard molecules (acarbose,

Diprotin A, ursolic acid and ranirestat)] determined over 100 ns molecular dynamics simulations. DPP-IV; dipeptidyl peptidase-IV; PTP-1B; protein tyrosine phosphatase 1B. CODES: 3W37- alpha-glucosidase; 1IEI- aldose reductase

acid and ranirestat (12749.91 Å, 13503.46 Å, respectively). However, the respective bound systems [pinocembrin (12560.01 Å) and luteolin (13116.07 Å)] better stabilized the complexes than the respective standards. The superiority of pinocembrin above ranirestat and luteolin over ursolic acid is an indication that they could serve as possible inhibitors in T2DM and diabetes retinopathy therapies. The fluctuations of the apo enzyme and chlorogenic acid showed consistencies throughout the simulation while other systems were not, though that of pinocembrin (Fig. 4c) and luteolin (Fig. 4d) and were less flexible. However, ursolic acid only revealed consistent fluctuations against PTP-1B (Fig. 4c). Apigenin, xylocaine, pinocembrin and luteolin presented lowest SASA values compared to apo enzymes (alpha-glucosidase, DPP-IV, PTP-1B and aldose reductase respectively) indicating increased exposure of hydrophobic non polar residues which is a consequence of complex stability [68].

The different interactions formed in complexes arising from the binding of compounds (standard drugs and *C. cujete* compounds) to the investigated enzymes are presented in Table 4 and Supplementary Figure S1. The nature of interactions (type and/ or number) existing between the ligand and the enzyme presents insight into the degree of affinity [69–71]. Additionally, the interaction (bond) between the ligand and the respective targets has an influence on the binding free energy of the complex [72]. In this study, the interactions existing between the top compounds of *C. cujete* and respective targets are summarized in Table 4. Looking at the results, acarbose had the highest interactions (30) comprising 20 van der Waal forces, 3 H-bonds, 1 C-H bond, 1 unfavourable donor-donor bond and 1 unfavourable bump in its complexation with alpha-glucosidase (Fig. 5a) as compared to *C. cujete* compounds [phytol (23), apigenin (16), isoflavone (16) and benzoic acid (15)]-alpha-glucosidase complexes (Table 4). The number of interactions observed from each of the complexes did not translate or conform with the report of the binding affinity as benzoic acid (-48.414 Å) with the most negative binding affinity depicted the lowest number of interactions (15) (Fig. 5b) whereas, acarbose with a moderate binding affinity (-28.248 Å) in comparison with other systems showed the highest interactions (30). However, a view at the bond length revealed the bond distance between H, with O atoms and amino acid residues (Asn328, Gly225) of benzoic acid to be shorter thus, suggesting its stability; a shorter bond length has been reported to contribute to the stability of the complex than longer bond length [73]. While acarbose depicted a high number of interactions, the presence of unfavourable (bump and donor-donor) bonds might be suggested as probable a reason for its moderate binding affinity or lower binding affinity (compared to benzoic acid and phytol). Similarly, H-bonds contribute to

higher energies to the complex [46], hence, the lower binding affinity may suggest the lack of contribution of these H-bonds residues (Asn301, Asp202, Arg200, Gln170) to the complex [46]. Against DPP-IV, luteolin and xylocaine had 15 interactions each [luteolin (3 H-bonds, 11 van der Waals, and 1 π - π stacked), xylocaine (5 H-bonds, 8 van der waal, 1 unfavourable donor-donor and 1 unfavourable acceptor-acceptor bonds) which was the highest followed by pinocembrin [(12); 3 H-bonds, 8 van der Waals forces 1 π -sigma bond], Diprotin A (2 H-bonds, 3 van der Waals, 3 π -alkyl, 2 salt bridges and 1 attractive charge) and castanoside (4 H-bonds, 6 van der Waals, 1 π -alkyl) with 11 interactions (Table 4). Although, luteolin and xylocaine have an equal number of interactions but their energy profiles differ with luteolin having increased negative binding energies (Table 2) which could be attributed to the higher number of important interactions such as conventional H and carbon H-bonds (Fig. 5c) since the presence of important interactions have been reported to present higher negative binding energy [46, 69]. However, the highest binding affinity of Diprotin A (-45.112 Å) which was not justified by the fewer number of interactions (Fig. 5d) could be indicative of a variety of interaction types (H-bonds, van der Waal, π -alkyl bonds, salt bridge, unattractive charges) present. While it could be noted that the two H bonds could have been responsible for increased binding affinity recorded, at the same time electrostatic interaction such as H-N-H formed with amino acid residues (Glu168, Glu169) on the complex may also influence the binding affinity of the complex [74]. Ursolic acid (Fig. 5e) and trans-cinnamic acid (Fig. 5f) formed 13 interactions each against PTP-1B (Table 4). The increased number of interactions of ursolic acid (which was not only corroborated by the binding energy profile) made it superior to trans-cinnamic acid and could be attributed to a higher number of π bonds as the presence of π -bond or interaction between amino acids residues, especially π - π stacked interactions have been reported to influence higher bond energy [19] and involved in drug development [75]. Naringenin came next with 10 interactions which also confirms its next higher negative binding energy. For aldose reductase, the complex formed with luteolin had 25 interactions (9 H-bonds, 14 van der Waals, 1 π - π stacked, 1 π -alkyl), this was followed by chlorogenic acid-aldose reductase [20 (7 H-bonds, 11 van der Waals, 1 π - π T-shaped, 1 π -alkyl)], naringenin-aldose reductase (16) and the standard, ranirestat-aldose reductase (11) (Table 4). While the result of the interaction plots was in tandem with the finding of energy profiles between the standard and the compounds, however, the complex with chlorogenic acid with the highest binding free energy values revealed a lesser number of interactions as compared to luteolin (Fig. 5g) with a higher number of interactions and reduced negative energies. This

Table 4 Identified interactions between the top phytoconstituents and carbohydrate enzymes' amino acid residues

Phytocompounds	Number of interactions	Number of H-bonds and interaction residues	Number of van der Waal forces and interaction residues	Other important interactions and residues
AG-Acarbose	30	4 (Asp202, Arg200, Gln170, Asn301)	20 (Phe297, Gly228, Tyr389, Phe397, Glu231, Met302, Leu300, Val335, Val334, Leu227, His105, Phe206, Ile146, Phe166, Asp62, Tyr65, Arg404, His332, Phe147, Thr203)	6 (Arg400, Glu271, Asp333, Pro230, Ala229, Arg340)
AG-Apigenin	16	2 (Phe294, Asp330)	9 (Glu228, Phe394, Leu390, Arg397, Val331, Leu297, Asn298, Asp295, Met299)	5 (Leu224, Ala226, Tyr386, Pro227, Gly225)
AG-Benzoic acid	15	3 (Asn328, Gly225, Glu268)	10 (Met299, Asn298, Val331, Leu297, Gly270, Ile143, Phe163, Asp330, Arg197, Phe203)	2 (Hie329, Phe294)
AG-Isoflavone	16	-	14 (Ile143, Ala226, Gly225, Tyr232, Phe203, Leu241, Gly270, Asn202, Ile269, Glu268, Thr200, Phe294, Asp330, Arg397)	2 (Phe163, Val331)
AG-Phytol	23	1 (Asp199)	17 (Leu224, Gly225, Asn328, Glu268, Arg197, Hie102, Tyr200, Val189, Phe163, Leu164, Asp330, Trp48, Phe144, Ile143, Asn298, Met299, Arg337)	5 (Tyr62, Leu297, Tyr292, Phe294, Val331)
DPP-IV-Diprotin A	11	2 (Ser593, Tyr510)	3 (Tyr625, Asn673, Arg88)	6 (Tyr629, Glu169, Glu168, Asp626, Hid703, Trp592)
DPP-IV-Xylocaime	15	4 (Gln516, Arg392, Tyr510, Lys517)	8 (Tyr926, Cys514, Phe320, Tyr548, Glu168, Asn673, Hid703, Ser593)	3 (Asp672, Arg88, Ser515)
DPP-IV-Pinocembrin	12	3 (Trp592, Val509, Gly591)	8 (Gly595, Gly596, Ser593, Tyr594, Tyr629, Trp590, Asp508, Lys517)	1 (Tyr510)
DPP-IV-Cistanoside D	11	1 (Tyr11)	6 (Tyr715, Hid711, Trp592, Ser172, Tyr510, Tyr594)	4 (Ser593, Ala706, Gly704, Hid703)
DPP-IV-Apigenin	15	3 (Asp508, Tyr625, Ser593)	11 (Val509, Gly591, Lys517, Trp590, Asn525, Tyr715, Hid703, Tyr510, Val674, Asn673, Gly704)	1 (Trp592)
PTP-1B-Ursolic acid	13	-	7 (Asp44, Met254, Gly255, Gln258, Ile215, Ser212, Leu115)	6 (Val45, Tyr42, Lys112, Lys116, Arg41, Ala213)
AR-Naringenin	16	3 (Asp43, Trp20, Gly18)	9 (Tyr48, Gln183, Thr19, Ser263, Pro261, Ser210, Leu212, Pro211, Ser214)	4 (Lys77, Ile260, Tyr209, Lys262, 3 (Lys1, Met231, Val270)
PTP-1B-TCA	13	2 (Phe3, Glu2)	8 (Glu4, Asp241, Ile242, Val240, Cys227, Pro237, Ala274, Ile271)	2 (Hie56, Leu136)
PTP-1B-TZD	9	3 (Thr134, Asn158, Asn135)	4 (Glu97, Glu93, Trp96, Thr160)	2 (Tyr42, Ile215)
PTP-1B-Pinocembrin	9	2 (Ala213, Gln258)	5 (Arg41, Ser212, Gly255, Met254, Val45)	3 (Ile53, Gln98, Leu67)
PTP-1B-Naringenin	10	3 (Lys54, Hie56, Lys69)	4 (Glu97, Ile78, Pro206, Arg52)	1 (Ala13)
PTP-1B-Chlorogenic acid	6	2 (Ala260, Thr259)	3 (Asp261, Gln258, Tyr16)	3 (Val297, Leu300, Cys298)
AR-Ranirestat	11	2 (Try20, Ala299)	6 (Tyr209, Trp111, Lys21, Trp79, Phe122, Phe121)	4 (Trp20, Lys262, Gly18, Tyr209)
AR-Luteolin	25	7 (Asp216, Glu185, Trp111, Cys298, Lys77, Asp43, Ser210)	14 (Leu212, Pro211, Pro261, Pro215, Ser214, Val297, Arg296, Phe311, Ala299, Asn160, Hie110, Tyr48, Gln183, Ile260)	
AR-Isoflavone	4	-	4 (Thr286, Ser290, Leu289, Asn292)	
AR-Chlorogenic acid	20	7 (Asp43, Thr19, Trp20, Ser210, Pro218, Asp216, Gly18)	11 (Ile260, Gln183, Lys77, Tyr48, Hie110, Trp111, Pro215, Ser214, Arg217, Tyr209, Lys262)	
AR-Narengenin	16	2 (Asp43, Trp20)	9 (Gln183, Tyr48, Thr19, Ser263, Pro261, Ser210, Leu212, Pro211, Ser214)	5 (Lys77, Gly18, Lys262, Tyr209, Ile260)

AG: Alpha-glucosidase; AR: Aldose reductase; DPP-IV: Dipeptidyl peptidase; PTP-1B: Protein tyrosine phosphatase; TZD: 1,2,4,5-tetrazine-3,6-diamine; TCA: trans cinnamic acid;

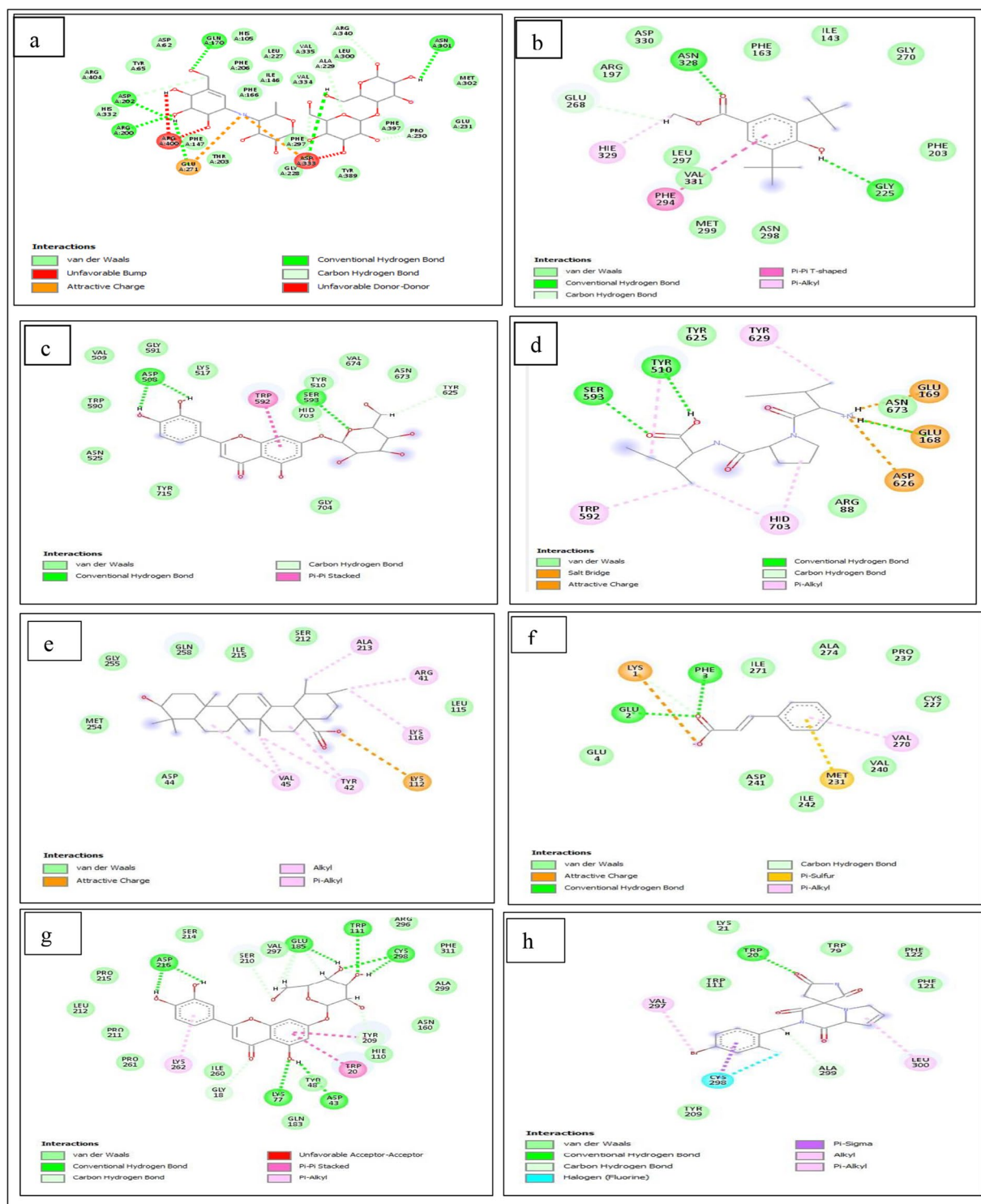


Fig. 5 Plots of interaction of (a) alpha-glucosidase-acarbose, (b) alpha-glucosidase-benzoic acid, (c) DPP-IV-luteolin, (d) DPP-IV-Diprotin A (e) PTP-1B-ursolic acid, (f) PTP-1B-trans cinnamic acid, (g) aldose reductase-luteolin, (h) aldose reductase-ranirestat

observation could be said to be attributed to several factors such as probable failure of the H-bonds responsible for contributing energies to the complex for higher binding affinity to do so [46], bond lengths, other unfavourable bonds and

lack of interaction with important catalytic amino acids residues such as Tyr48 and His110. Additionally, the increased number of interactions for chlorogenic acid-aldose reductase complex over ranirestat-aldose reductase interactions

(Fig. 5h) is consistent with a previous report [19], where chlorogenic acid had 16 interactions as compared to ranirestat with 14 interactions.

Pharmacokinetics

The ADME properties prediction of a compound gives an idea of its profiles in terms of bioavailability and toxicity when considered as a drug for possible development [76]. In determining the pharmacological profiles and coming to a decision on the prospective bioactive drug moiety, Lipinski's rule of 5 which explores the molecular weight (> 500 kilo Dalton), number of H donor (less or equal to 5), number of H acceptor (less or equal to 10) and octanol coefficient (less than 5) is usually employed. Based on the result of this investigation which looked at the overall 14 best compounds and respective standards, 12 of the compounds passed Lipinski's rule while castanoside D and catalposide failed with acarbose (which is unexpectedly being a standard drug) (Table 5). The implication is that these 12 compounds have the potentials to be able to pass through the systemic

circulation (unhindered) to elicit their pharmacological action as drug candidates [77]. Since the rate of absorption and amount of unchanged drug passing the blood is assessed by their bioavailability score (BS), this submission on Lipinski's rule was corroborated by the BS, where 11 of the compounds except chlorogenic acid (11%), castanoside D (17%) and catalposide (17%) had bioavailability scores above 20%. While benzoic acid was best bioavailable (85%) and the minimally acceptable bioavailability limit for a drug candidate is 10% [78], it then means that all the 14 compounds have worthy profiles as probable candidates in the management of T2DM though it must be noted that ursolic acid among the reference standards was poorly absorbed. In terms of aqueous solubility and gastrointestinal absorption, all the 14 compounds and standards revealed varying levels of solubility, however, chlorogenic acid, castanoside D, catalposide, glucopyranoside, phytol among the test compounds and acarbose as well as ursolic acid presented low GI absorption. This could mean that these compounds (chlorogenic acid, castanoside D, catalposide, glucopyranoside, phytol) might require further optimization or modification

Table 5 ADME properties prediction for phytocompounds and reference standards

Compounds	Properties											
	Bio-availability score	Water solubility	Lipo-philicity (ilgP)	GIT absorption	Hydro-gen bond acceptors	Hydro-gen bond donors	Lipin-ski rule	Cytochrome inhibitors				
								CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
Acarbose	0.17	Highly soluble	0.63	Low	19	14	N	N	N	N	N	N
Diprotin A	0.55	Very soluble	2.14	High	5	3	Y	N	N	N	N	N
Ursolic acid	Poorly soluble		3.95	Low	3	2	Y	N	N	N	N	N
Ranirestat	0.55	Soluble	2.15	High	5	1	Y	N	N	N	N	N
Apigenin	0.55	Moderate	1.89	High	5	3	Y	Y	N	N	Y	Y
Benzoic acid	0.85	Soluble	1.11	High	2	1	Y	N	N	N	N	N
Catalposide	0.17	Soluble	1.65	Low	12	6	N	N	N	N	N	N
Chlorogenic acid	0.11	Soluble	0.96	Low	9	6	Y	N	N	N	N	N
Cistanoside D	0.17	Soluble	2.59	Low	15	7	N	N	N	N	N	N
Glucopyranoside	0.55	Highly soluble	0.24	Low	6	5	Y	N	N	N	N	N
Isoflavone	0.55	Soluble	2.51	High	2	0	Y	Y	Y	N	N	N
Luteolin	0.55	Soluble	1.86	High	6	4	Y	Y	N	N	Y	Y
Naringenin	0.55	Soluble	1.75	High	5	3	Y	Y	N	N	N	Y
Phytol	0.55	Mod-erately soluble	4.71	Low	1	1	Y	N	N	Y	N	N
Pinocembrin	0.55	soluble	2.11	High	4	2	Y	Y	Y	N	N	N
1,2,4,5- tet-razine- 3,6 diamine	0.55	Very soluble	1.56	High	4	2	Y	N	N	N	N	N
Trans-cin-namic acid	0.85	Soluble	1.55	High	2	1	Y	N	N	N	N	N
Xylocaine	0.55	Soluble	2.86	High	2	1	Y	N	N	N	Y	N

CYP: Cytochrome; Y: Yes; N: No

for them to serve as good inhibitors to targets of T2DM, besides, none of these promising candidates against aldose reductase are poorly GI absorbed. Apigenin, isoflavone and pinocembrin among the 14 compounds inhibited at most 2 of the cytochrome isoenzymes while others were observed not to inhibit four or five of the isoenzymes (Table 5). Cytochrome isoenzymes are key in drug metabolism and the ability of seven (benzoic acid, castanoside D, catalposide, glucopyranoside, chlorogenic acid, trans cinnamic acid and 1,2,4,5- tetrazine- 3,6 diamine) of the compounds not to inhibit all the cytochromes signifies their potential safety (in causing drug-drug toxicity) and superiority over others [64].

The use of alternative therapeutic medicine with natural products is considered a laudable approach in the management of T2DM and its related complication such as diabetic retinopathy. Since alpha-glucosidase, PTP-1B and DPP-IV as well aldose reductase are prominent targets for effective treatment of T2DM and diabetic retinopathy respectively, the identification and discovery of isoflavone (based on all parameters checked in this study) observed to form or maintain good stability with alpha-glucosidase, and xylocaine as well as chlorogenic acid against DPP-IV and PTP-1B, respectively preferred them as ideal candidates from *C. cujete* towards T2DM therapy. Additionally, with luteolin replicating similar effect as isoflavone, xylocaine and chlorogenic acid, it may also be handy in the development of a good drug in the management of diabetic retinopathy.

Conclusion

The prevalence of T2DM is concerning and the hallmark in its management is towards regulating the blood glucose to normalcy which since time immemorial are managed with different classes of OHA influencing the key carbohydrate-metabolizing enzymes. While the use of synthetic drugs has largely undermined any side effects, consideration of alternative options in medicinal plants such as *C. cujete* with established antidiabetic effects is welcoming and laudable, though, the implicated phytochemicals is/are wanting or yet to be discovered. The present study explored the therapeutic action of various identified compounds from *C. cujete* as probable therapeutic drug candidates through computational studies. Based on the findings from thermodynamic profiles, MD and post-MD simulation metrics, binding interactions and pharmacokinetic profiles of each of the compounds with the respective target, the study concludes that compounds such as isoflavone (against alpha-glucosidase), xylocaine (DPP-IV), chlorogenic acid (PTP-1B) and luteolin (aldose reductase) as promising inhibitors of the studied carbohydrate-metabolizing enzymes. Hence, exploiting them as novel drug moieties in the management

of T2DM and its related retinopathy complication (luteolin) would go a long way in drastically reducing the disorder's continuous emergence.

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Declarations

Competing interests No conflicting interest among the authors.

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