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Molecular docking and admet properties of *Anacardium occidentale* methanolic nut extract against inflammatory, oxidative and apoptotic markers of diabetes

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ABSTRACT

Background. The contemporary antidiabetic drugs have side effects and adverse reactions. This demand to search for less toxic and effective treatments for diabetes from medicinal plants using computational methods. The present research investigated the molecular docking of *Anacardium occidentale* nut methanolic extract compounds with selected proteins related to diabetes and the compounds' AMDET properties.
Material and Methods. The compounds were identified using Gas chromatography-mass spectrometry analysis. The compounds'2-dimensional structure was retrieved from the PubChem compound database. Three-dimensional crystallographic structure of selected proteins; B-cell-lymphoma-2 (Bcl-2), caspase-3, glucocorticoids, interleukin-1β, myeloperoxidase and tumor necrosis factor-alpha (TNF-α) was downloaded from Protein Data Bank. Molecular docking was performed using Autodoc kvina and the active site of binding interactions was detected with the Computed Atlas of Surface Topography of proteins (CAST-P). The compounds' drug-likeness, physicochemical and ADMET were evaluated using molininspiration and admetSAR online tools.

Results. Ten compounds were identified from the *Anacardium occidentale* nut methanolic extract. All the compounds exhibited drug-likeness properties with violation of one Lipinski's rule. Two compounds, oleic acid and 3-(p-methoxyphenyl)-propionic acid exhibited the best binding energy with the active receptors site of Bcl-2, caspase-3, TNF-α and glucocorticoid. Also, tridecanoic acid exhibited good binding energy with the active site of glucocorticoid receptors. Only 3-(p-methoxyphenyl)-propionic acid exhibited moderate binding energy with the active receptors site of interleukin-1β and myeloperoxidase. All the compounds displayed excellent ADMET properties.
Conclusions. Antidiabetic drugs with the least side effects could be explored from these compounds.

Introduction

Diabetes mellitus is currently recognized as one of global epidemic diseases, affecting approximately 382 million people worldwide. The International Diabetes Federation (IDF) estimation of individuals who die from diabetes yearly is roughly 1.3 million and the global number of diabetes patients is expected to reach 629 million by 2045 [1].

Diabetes is a metabolic disorder characterized by chronic hyperglycemia accompanied by alteration in carbohydrates, fats, and proteins resulting from scanty insulin secretion or insulin action. Alterations in the metabolism of carbohydrates, fats, and proteins are associated with micro-vascular and macro-vascular diabetes complications [2].

Oxidative stress and inflammation critically contribute to the pathogenesis of diabetes mellitus-related complications. Oxidative stress and inflammation mediated by diabetes mellitus are considered to trigger apoptotic process leading to cellular injury and multiple organ damage [3].

Currently, the available antidiabetic drugs are not permanently curing diabetes and are associated with adverse effects. Therefore, much interest has been shifted towards the use and alternative medicine or derivative from food products with rich antidiabetic phytoconstituents. The presence of bioactive compounds and plant-derived products such as alkaloids, flavonoids, glycosides, gum, carbohydrates, and triterpenes with some short-peptides in medicinal plants play a major role in their therapeutic efficacy [4]. Specifically, plants nuts with rich phenolic compounds are known for their numerous biological activities including antioxidant, anti-inflammatory and antidiabetic properties [5]

Anacardium occidentale L. globally known as cashew belongs to the family *Anacardiaceae*. Several parts of the *Anacardium occidentale* tree including the leaf, stem bark and nut are often used ethno-pharmacological and investigated experimentally for their antidiabetic therapeutic efficacies [6]. Research showed that the presence of phenolic compounds, carotenoids, flavonoids, anthocyanins, tannins, and other minerals components in *Anarcadium occidentale* may be responsible for its antidiabetic properties [7]. *Anacardium occidentale* nut is well rich in unsaturated fatty acids such as oleic (ω-9) and linoleic (ω-6) acids, flavonoids, anthocyanins and tannins, fiber, folate and tocopherols [8–12].Previously, the nuts have been reported in metabolic syndrome risk modulation [13].

However, an extensive scientific investigation is necessary on valuable traditional medicinal plants to investigate their antidiabetic efficiency using modern experimental equipment and methods. Computational molecular modeling has been identified as an important sector in the natural drug product development process. *In-silico* drug design tools improve the detection of novel drugs from natural products. Computational modeling provides much detail on the molecular recognition processes underlying the interaction between disease-related target macromolecules with naturally occurring drug-like substances [14].

The physicochemical and drug-likeness properties of bioactive compounds in *Anacardium occidentale* nut for their antidiabetic therapeutic efficacies have not been elucidated. The molecular docking, drug-likeness and ADMET of *Anacardium occidentale* nut methanolic extract compounds were investigated for further identification of major compound with potent antidiabetic therapy.

Material and Methods

Anacardium occidentale Nut Collection

The nuts were freshly harvested from the *Anacardium occidentale* plant at the Agricultural

Research Farm located at Ladoke Akintola University of Technology in Ogbomosho, Oyo State, Nigeria. The nut underwent identification, and validation, and was given the voucher specimen number LH0533 by Dr. A. T. J. Ogunkunle, a faculty member of the Biology Department at Ladoke Akintola University of Technology in Ogbomoso, Oyo State, Nigeria.

Anacardium occidentale Nuts Methanolic Extraction

The *Anacardium occidentale* nuts finely powered weighing 500 g was placed in a Soxhlet apparatus for extraction using 95% methanol as the solvent for a duration of 48 hours, the temperature was carefully maintained below the boiling point of the solvent. The extracts were filtered using a white muslin cloth and subsequently subjected to a double filtration process using white Whatman filter paper. The filtrate was then concentrated in a rotary evaporator at a controlled temperature of 35°C and reduced pressure until the extract had fully dried, yielding a concentrated methanolic extract. The obtained extract was then stored in a refrigerator at a constant temperature of 4°C for utilization at a later time.

Identification of *Anacardium occidentale* Nut Compounds

The bioactive compounds of the *Anacardium occidentale* nut methanolic extract were identified by gas chromatography-mass spectrometry (GC-MS) technique. GC-MS analysis of the methanolic extract was performed using on Ralte et al. [15] adopted method.

Target Proteins

Five target proteins, B-cell lymphoma-2 (Bcl-2) for anti-apoptotic, caspase-3 for apoptotic, interleukin-1β (IL-1β) for anti-inflammatory, tumor necrosis factor-alpha (TNF-α) for inflammatory, myeloperoxidase for insulin resistance and glucocorticoids receptor for insulin antagonist related to diabetes mellitus progression were selected for their interactions with the bioactive compounds from *Anacardium occidentale* nuts.

Preparation of Target Proteins

The 3-dimensional (3D) X-ray crystallographic structuzres of target proteins; B-cell lymphoma-2(Bcl-2) (PDB ID: 2YV6), caspase-3 (PDB ID: 1QX3), glucocorticoids receptor (PDB ID: 1GDC), interleukin-1β (IL-1β) (PDB ID: 9ILB), myeloperoxidase (MPO) (PDB ID: 3F9P) and tumor necrosis factor-alpha (TNF-α) (PDB ID: 1TNF) were retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (The proteins were prepared by(a) removal of hetero-atoms (water, ions), (b) addition of polar hydrogens, and (c) assignment of Kollman charges using "Clean Protein" and "Prepare Protein" Discovery Studio software (version 19.1) and the proteins were converted from PDB format into pdbqt file format via Auto-dock Tools 4.2 software.

Ligand Molecules Preparation

After the GC-MS analysis, The ligand molecules were downloaded from the pubchem database (https://pubchem.ncbi.nlm.nih.gov) in a structure database file (SDF) and converted to pdb format using Pymol [16], then to pdbqt format via Autodock tool (version 4.2) for molecular docking.

The ligand molecules were docked with all target proteins using Auto-dock 4.0 software by setting up 4 energy range and exhaustiveness value of eight 8 as default to obtain 10 different poses of ligand molecules [17]. The 2D binding interactions was detected via LigPlot+ v.2.2 (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/). After the docking process, ligands with the lowest binding energy were selected to visualize the ligand-protein interaction in Pymol.

Physicochemical and ADMET Determination

Drug-likeness physicochemical and ADMET properties of *Anacardium occidentale* nut methanolic extract compounds were determined using the molinspiration web tool and ADMETsar online server [18].

Results

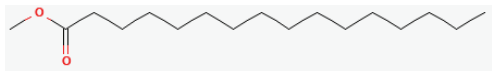
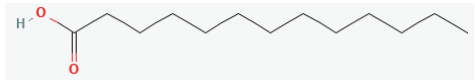
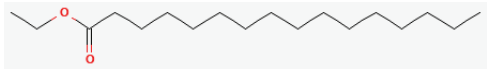
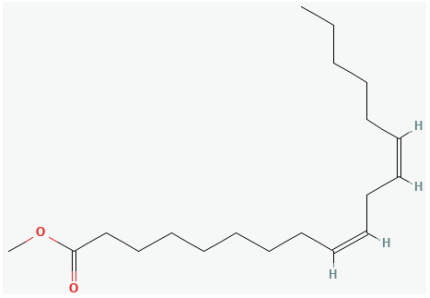
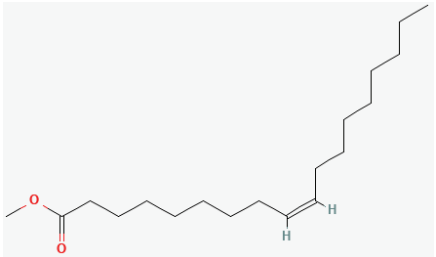
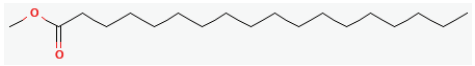
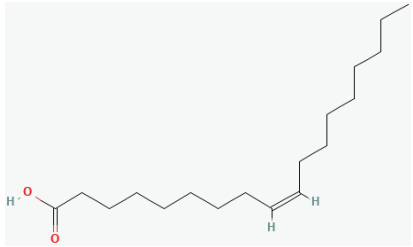
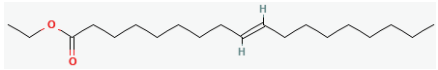
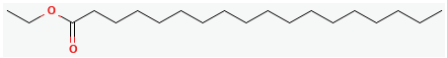
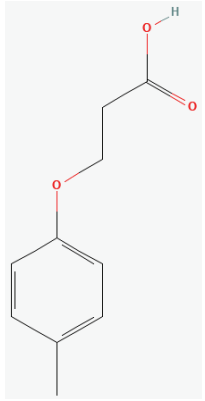
Anacardium occidentale Nut Compounds.

A total of 10 compounds were identified from *Anacardium occidentale* nut methanolic extract via the GC-MS analysis. The details of the compounds are given in **Table 1**.

Physicochemical Properties Analysis

The physicochemical properties and drug-likeness of the selected *Anacardium occidentale* nut

Table 1. Identified compounds from *Anacardium occidentale* nut methanolic extract.

No	Compounds structures, molecular names and formulas	Molecular weights (g/mol)	Retention time
1	 Hexadecanoic acid-methyl ester Molecular formula $C_{17}H_{34}O$	271	37.008
2	 Tridecanoic acid Molecular formula $C_{16}H_{32}O_2$	256	37.420
3	 Hexadecanoic acid-ethyl ester Molecular formula $C_{18}H_{36}O_2$	285	37.589
4	 9,12-Octadecadienoic acid (Z,Z)-methyl ester Molecular formula $C_{19}H_{34}O$	295	38.265
5	 9-Octadecenoic acid (Z)-methyl ester Molecular formula $C_{19}H_{36}O$	296	38.302
6	 Methyl stearate Molecular formula $C_{19}H_{38}O_2$	299	38.465
7	 Oleic acid Molecular formula $C_{18}H_{32}O_2$	283	38.609
8	 (E)-9-Octadecenoic acid-ethyl ester Molecular formula $C_{20}H_{38}O_2$	310	38.697
9	 Octadecanoic acid-ethyl ester Molecular formula $C_{20}H_{40}O_2$	313	38.847
10	 3-(4-Methoxyphenyl)-propionic acid Molecular formula $C_{16}H_{12}O_3$	180	40.342

methanolic extract compounds were determined using Lipinski's rule of five (RO5) [19], which stated that, a compound is considered a drug-like molecule if no more than one of the following criteria is violated: molecular weight < 500KDa, LogP < 5, hydrogen bond donor ≤ 5, hydrogen bond acceptor ≤ 10. Nine compounds of *Anacardium occidentale* nut methanolic extract violated one Lipinski's rule as their lipophilicity expressed as logP (octanol-water partition coefficient) greater than the acceptable range and one compound 3-(p-methoxyphenyl)-propionic acid violated none of the rules (**Table 2**).

Compounds ADMET Profile Analysis

The canonical SMILES of the compounds were saved and uploaded to the admetSAR web server or admet properties analysis. The compounds are screening for ADMET properties including acute oral toxicity, blood-brain barrier, carcinogenicity, cytochrome P450 inhibitors isoforms (CYP inhibitors)1, hepato-toxicity, human ether-a-go-go-related gene inhibition (hERG), human Intestinal Absorption, human oral bioavailability and P-glycoprotein inhibitor (P-gpi) (**Table 3**).

The selected compounds of *Anarcardium occidentale* nut methanolic extract have no acute oral toxicity as the majority of the compounds displayed Class III category. However, compound II (tridecanoic acid) and compound VII (oleic acid) fall into the Class IV category.

In addition, the compounds have high human intestinal absorption, good blood-brain barrier

Table 2. Physicochemical properties of Anacardium occidentale nut methanolic extract compounds.

No	Compounds	Lipinski rule violation	Molecular weight (g/mol)	nHD	nHA	Log P
1	Hexadecanoic acid	1	270.45	0	2	7.37
2	Tridecanoic acid	1	214.33	1	1	5.54
3	Hexadecanoic acid-ethyl ester	1	284.27	0	2	7.448
4	9,12-octadecadienoic acid (Z,Z)-methyl ester	1	294.26	0	2	6.992
5	9-octadecenoic acid-methyl ester	1	296.49	0	2	7.746
6	Methyl stearate	1	298.29	0	2	8.049
7	Oleic acid	1	282.26	1	2	7.131
8	(E)-9-octadecenoic acid-ethyl ester	1	310.52	0	2	7.531
9	Octadecanoic acid-ethyl ester	1	312.3	0	2	8.286
10	3-(p-methoxyphenyl)-propionic acid	0	180.08	1	3	2.148

Table 3. ADMET properties of Anacardium occidentale nut methanolic extract compounds.

No	Acute oral toxicity	Blood-Brain Barrier	Carcinogenicity	CYP1A2 inhibitor	CYP2C19inhibitior	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Hepatotoxicity	hERG inhibitor	Human Intestinal Absorption	Human Oral Bioavailability	p-glycoprotein inhibitor
1	Class III	+	-	-	-	-	-	-	-	-	+	-	-
2	Class IV	+	-	+	-	-	-	-	-	-	+	-	-
3	Class III	+	-	-	-	-	-	-	-	-	+	-	-
4	Class III	+	-	+	-	-	-	-	-	+	+	-	-
5	Class III	+	-	+	-	-	-	-	-	+	+	-	-
6	Class III	+	-	-	-	-	-	-	-	-	+	-	-
7	Class IV	+	-	+	-	-	-	-	-	-	+	-	-
8	Class III	+	-	+	-	-	-	-	-	+	+	-	-
9	Class III	+	-	-	-	-	-	-	-	-	+	-	-
10	Class III	+	-	-	-	-	-	-	-	-	+	+	-

permeation and well human oral bioavailability and compound X exhibited poor human oral bioavailability. Furthermore, the compounds are non-carcinogenic, non-hepato-toxic and non-inhibitors of permeability glycoprotein (P-gp).

Furthermore, the majority of the compounds are non-inhibitors of CYP450 isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4) and compounds II, IV, V, VII and VIII exhibited CYP1A2 inhibitors.

Also, compounds I, II, III, VI, VII, IX and X are non-inhibitor of the human ether-a-go-go related gene (hERG) and compounds IV, V and VIII exhibited hERG inhibitors.

Ligands Molecular Docking Interaction Analysis

The ten identified compounds were successfully docked with the target protein receptors and compounds with notable significant docking binding energy were selected.

Ligands Molecular Interaction with B-cell lymphoma-2 (BCL-2)

Oleic acid and 3-(p-methoxyphenyl)-propionic acid were recognized as the best-docked compounds to active receptors site of target protein B-cell lymphoma-2 (Bcl-2) (PDB ID: 2YV6) with a binding energy of -6.1 and -5.8 Kcal/mol respectively. Oleic acid established binding interaction with active receptors site of Bcl-2 via one hydrogen bond forming residue ASP 103 significant than the standard drug (metformin) and eight residues with GLY 145, ARG 107, ASP 111, PHE 153, GLU, 152, LEU 137, VAL 148 and ALA 100 via hydrophobic interactions and six forming residues with MET 115, PHE 112, VAL, 156, ALA 149, PHE, 104 and TYR 108 through alkyl-pi-alkyl interactions. The ligand 3-(p-methoxyphenyl)-propionic acid had binding interaction with active receptors site of Bcl-2 via one hydrogen bond forming residue ARG 127 and 6 residues with HIS 184, PHE 138, TYR 180, GLU 135, VAL 134 and ALA 131 through hydrophobic interactions (**Figure 1: a** & **b**).

Ligands Molecular Interaction with Caspase-3

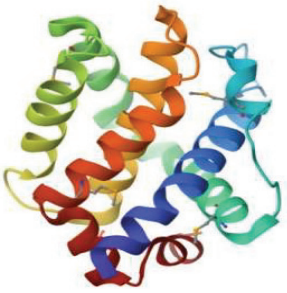
Oleic acid and 3-(p-methoxyphenyl)-propionic acid were identified as the most docked compounds with target protein caspase-3 (PDB ID: 1QX3) active receptors site and displayed lower binding energy of -3.4 and -4.0 Kcal/mol respectively in comparison with reference drug metformin (-4.3 Kcal/mol). Oleic acid had interactions with caspase-3 through one hydrogen bond forming residue TYR 195 and six residues with MET 268, ARG 164, TYR 197, GLY 125, LEU 136 and ASP 135 via hydrophobic interactions. Also, 3-(p-methoxyphenyl)-propionic acid had interactions with caspase-3 via one hydrogen bond forming residue LYS 105 and three residues via hydrophobic interactions with ARG 147, SER 150, and CYS 148 (**Figure 2: c** & **d**).

Ligands Molecular Interaction with Glucocorticoids

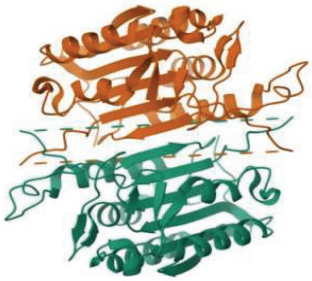
Three compounds' tridecanoic acid, oleic acid and 3-(p-methoxyphenyl)-propionic acid were discovered as excellent docked compounds with target protein glucocorticoids (PDB ID: 1GDC) active receptors site with binding energy of -4.0, -4.1 and -5.0Kcal/mol respectively. Tridecanoic acid had interactions with glucocorticoids active receptors site through one hydrogen bond forming residue SER 440 and two forming residues with TYR 455 and ARG 477 through hydrophobic interactions. Also, Oleic acid had interactions with active receptors site of glucocorticoid via one hydrogen bond forming residue ARG 477 and four residues with SER 440, TYR 455, ARG 470 and PRO 474 via hydrophobic interactions. Further, 3-(p-methoxyphenyl)-propionic acid had interactions with glucocorticoids active receptors site via two hydrogen bonds forming residues SER 440 and ARG 477 and three forming residues with TYR 455, PHE 444 and VAL 443 via hydrophobic interactions (**Figure 2: e**, **f** & **g**).

Ligands Molecular Interaction with Interleukin-1beta (IL-1β)

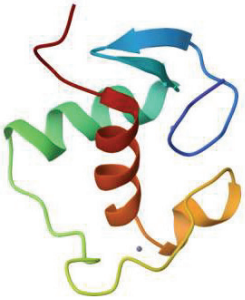
The compound's 3-(p-methoxyphenyl)-propionic acid exhibited a docking pattern of the binding energy of – 5.2 Kcal/mol with the protein interleukin-1β (PDB: 9ILB) active receptors site of all the 10 compounds. 3-(p-methoxyphenyl)-propionic acid had no hydrogen bonding forming residue interactions with IL-1β. The stabilization of interaction of 3-(p-methoxyphenyl)-propionic acid with the active receptors site was mediated via hydrophobic interactions forming residues with LYS 63, TRY 68, TRY 90, ASN 66, SER 5, GLU



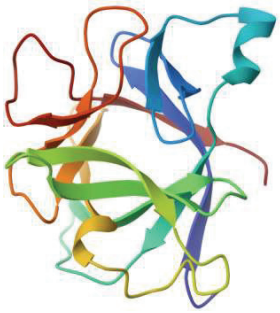
a: B-cell lymphoma-2 (Bcl-2)



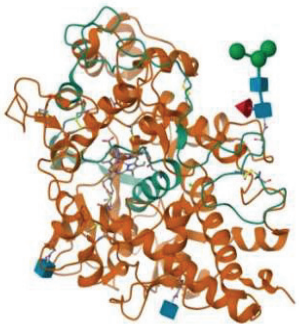
b: Caspase-3



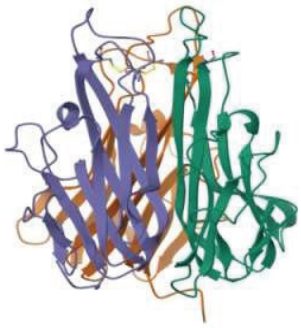
c: Glucocorticoid receptors



d: Interleukin-1β (IL-1β)



e: Myeloperoxidase



f: Tumor necrosis factor-alpha (TNF-α)

Figure 1. Depict 3-D crystallographic structure of the target proteins related to diabetes for Molecular docking.

64 and SER 43 and alkyl-pi-alkyl interaction residue with PRO 87 (**Figure: 2h**).

Ligands Molecular Interaction with Myeloperoxidase (MPO)

The docking analysis revealed only compound 3-(p-methoxyphenyl)-propionic acid showed binding energy of –5.3Kcal/mol with protein myeloperoxidase (PDB: 3F9P) and had interactions with through one hydrogen bond forming residue ARG 507 and six forming residues with TRY 273, TRY 313, LEU 310, THR 312, TRP 513 and PRO 311 via hydrophobic interactions and four alkyl-pi-alkyl interactions forming residues with ALA 529, TRP 514, ILE 290 and ARG 294 (**Figure: 2i**).

Ligands Molecular Interaction with Tumor Necrosis Factor-Alpha (TNF-α)

The compounds' oleic acid and 3-(p-methoxyphenyl)-propionic acid exhibited a significant docked pattern of the binding energy of -4.1 and -4.1Kcal/mol respectively with target protein tumor necrosis factor- alpha (PDB ID: 1TNF) active receptors site. Oleic acid

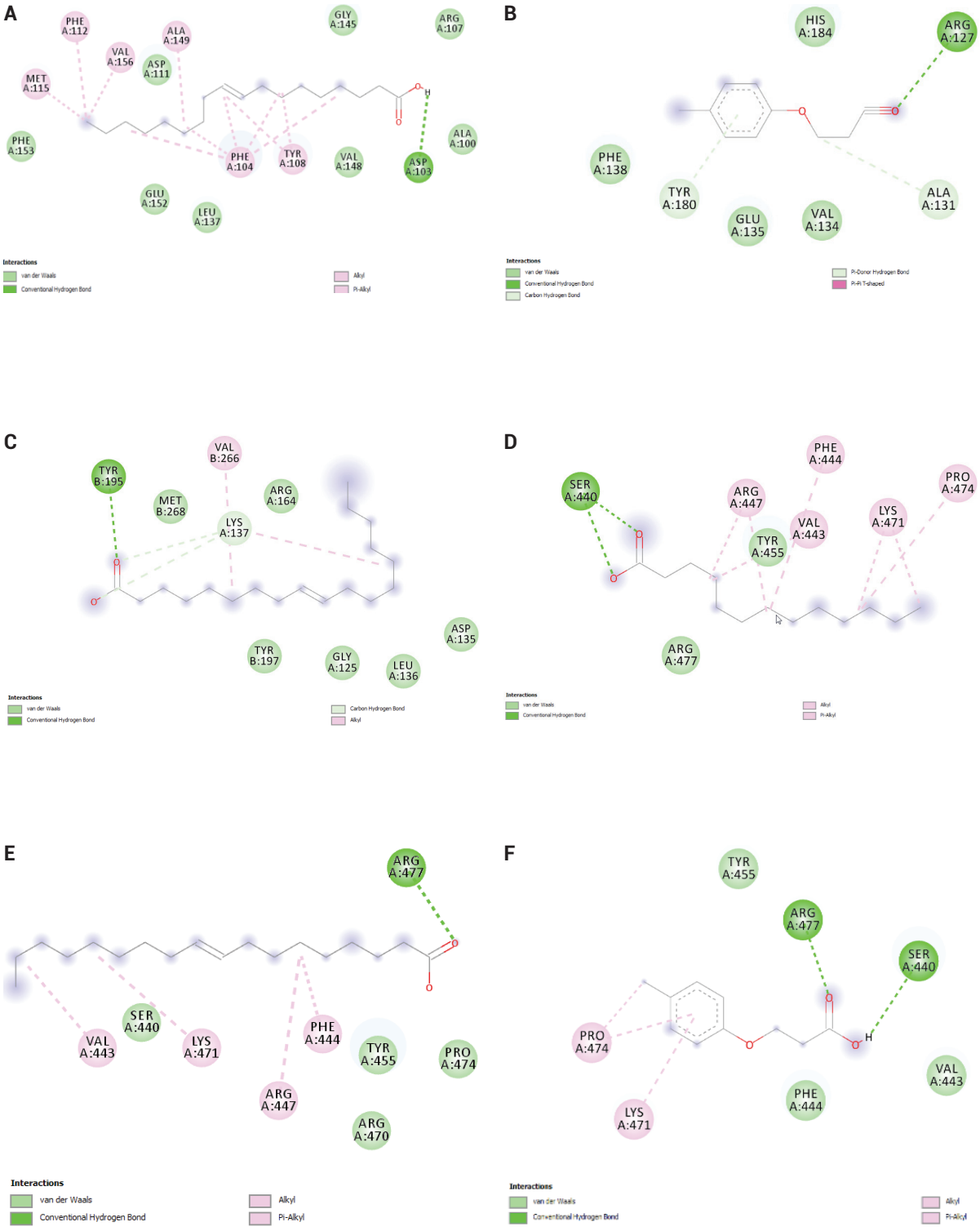
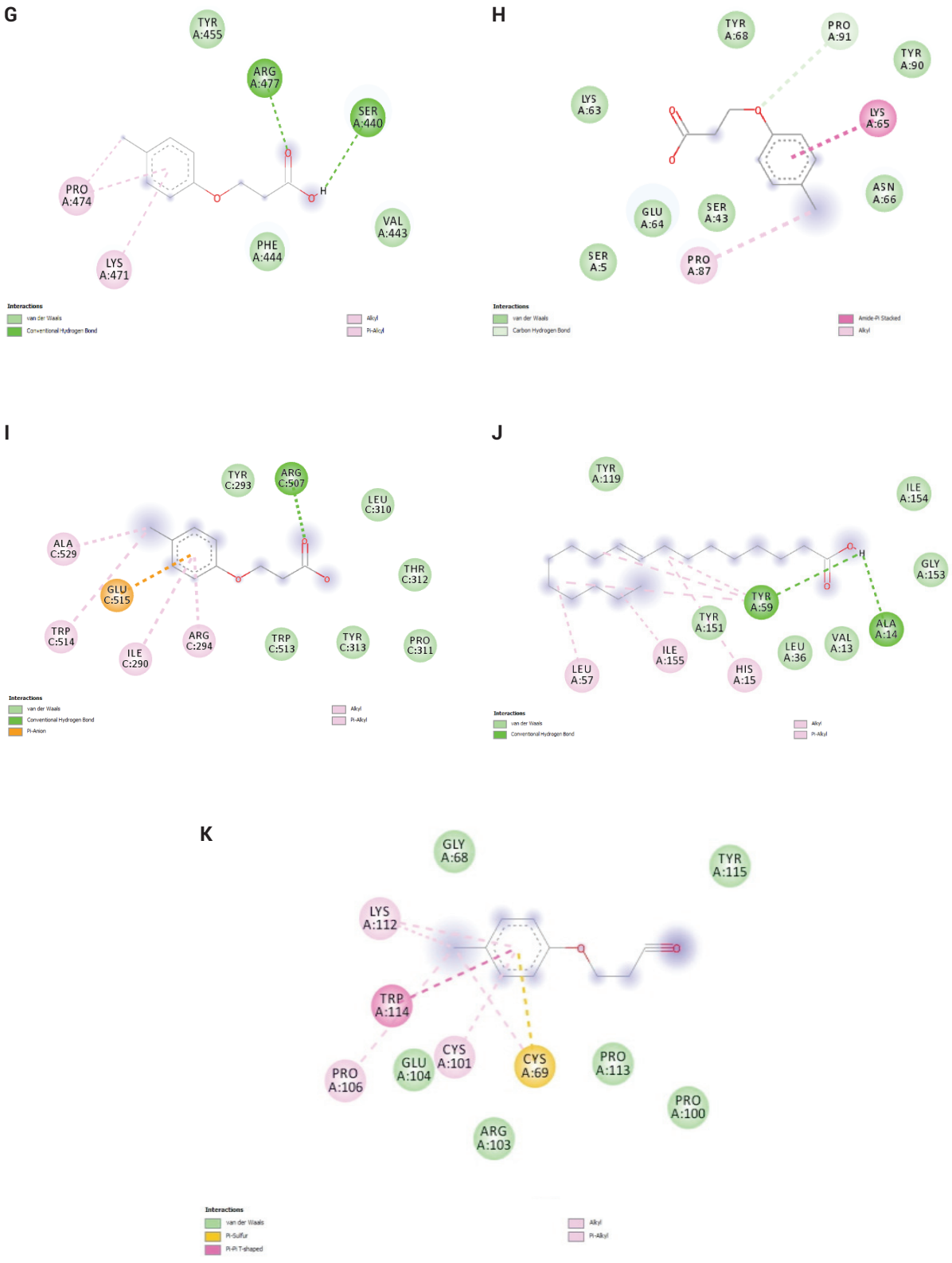


Figure 2. Depicted the 2-D structure molecular binding interactions of (**A**) oleic acid and Bcl-2, (**B**) 3-(p-methoxyphenyl)-propionic acid and Bcl-2, (**C**) oleic acid and caspase-3, (**D**) 3-(p-methoxyphenyl)-propionic acid and caspase-3, (**E**) tridecanoic acid and glucocorticoids receptor (**F**) oleic acid and glucocorticoids receptor, (**G**) 3-(p-methoxyphenyl)-propionic acid and glucocorticoid, (**H**) 3-(p-methoxyphenyl)-propionic acid and IL-1β, (**I**) 3-(p-methoxyphenyl)-propionic acid and myeloperoxidase, (**J**) oleic acid and TNF-α, (**K**) 3-(p-methoxyphenyl-)-propionic acid and TNF-α.



had interaction with the active receptor site of TNF-α through two hydrogen bonds forming residues TYR 59 and ALA 14 and six residues with TYR 119, TRY 151, ILE 154, LEU 36, and VAL via hydrophobic interactions and three alkyl-pi-alkyl interactions residues with LUE 57, ILE 155 and HIS. The interaction of compound 3-(p-methoxyphenyl)-propionic acid with TNF-α active receptor site was mediated via hydrophobic interactions forming residues with GLY 68,

TYR 115, GLU 104, ARG 103, PRO 100 and PRO 113 and three alkyl-pi-alkyl interactions forming residues with LYS 112, PRO 106 and CYS 101 (**Figure 2: j** & **k**).

Discussion

Diabetes is emerging as the third "quiet killer" of humankind, following cancer and cardiovascular diseases owing to its escalation prevalence, morbidity and mortality [20]. Despite significant advancements in the drug discovery field, effectively managing diabetes remains challenging and poses a major problem within the medical arena [21].

Molecular docking serves a cardinal role in the development and designing of novel drugs. It precisely envisions the binding mode and affinity of natural compounds within the active binding site of the drug target [22]. Furthermore, *in-silico* techniques serve as a screening tool to acquire physicochemical, drug-likeness and ADMET information for drug designing [23]. The current study investigated drug-likeness, ADMET properties and molecular docking of compounds identified from *Anacardium occidentale* nut methanolic extract with target protein of diabetes mellitus progression using *in-silico* technique.

Hyperglycemia has been implicated in the induction of β-cell apoptosis in diabetes mellitus [24]. Anti-apoptotic B-cell lymphoma-2 (Bcl-2) is a member of the Bcl proteins family. The up-regulation of the Bcl-2 suppresses apoptosis by regulating the sensitivity of cells to apoptotic stimuli [25]. Oleic acid and 3-(p-methoxyphenyl)-propionic acid of *Anacardium occidentale* nut methanolic extract-compounds interact with active receptors site of Bcl-2 protein which are implicated in the pathogenesis of human diabetes. These novel compounds interact in the same manner as the reference drug (metformin) and efficiently fit the binding pocket of Bcl-2 protein receptors and may facilitate the up-regulation of the Bcl-2 to impede β-cell apoptosis.

Caspase-3 protein is a member of the caspase family and plays a critical role in cell apoptosis execution [26]. Inhibitors of caspase-3 peptide avert β-cell apoptosis and improve the function of islet graft [27]. Also, these two compounds oleic acid and 3-(p-methoxyphenyl)- propionic acid fit accurately into the binding pocket of the caspase-3 protein active receptors site. Therefore, could serve as an effective caspase-3 peptide inhibitors candidate to treat diabetes mellitus.

Glucocorticoids are powerful insulin action antagonists and promote hepatic gluconeogenesis thereby leading to hyperglycemia in diabetes. The determination of active glucocorticoids to their receptors at the tissue level is governed by 11beta-hydroxysteroid dehydrogenase type 1(11β-HSD1) [28]. 11β-HSD1 is an enzyme that depends on nicotinamide adenine dinucleotide phosphate (NADPH) to catalyze the inter-conversion of glucocorticoids, cortisone, and cortisol in humans. Elevated circulating levels of the active glucocorticoid cortisol can instigate insulin resistance causing hepatic gluconeogenesis eventually leading to insulin-resistant and macro-vascular diabetes complications [29]. Tridecanoic acid, oleic acid and 3-(p-methoxyphenyl) propionic acid compounds of *Anacardium occidentale* nut methanolic extract fit clearly into the binding pocket of 11β-HSD1 active receptors site and these interactions suggested that the three compounds are novel inhibitors of 11β-HSD1for diabetes therapy.

Cytokines (small proteins produced by immune cells and other cell types) are considered in diabetes pathogenesis. Active innate immune cells assemble to cause activation of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) [30]. Elevated circulating levels of cytokine TNF-α (the protein responsible for inflammation) and diminished levels of anti-inflammatory interleukin-1β (IL-1β) protein have been proposed to induce β-cells apoptosis and insulin resistance leading to chronic hyperglycemia of diabetes mellitus [31,32]. 3-(p-methoxyphenyl)-propionic acid compound of all the compounds interact with target IL-1β protein receptors binding pocket. Furthermore, two compounds oleic acid and 3-(p-methoxyphenyl)-propionic acid compactly interact with the binding pocket of TNF-α protein active receptor site like metformin, showing the compounds possess inhibitory potential on pro-inflammatory cytokine TNF-α, and suggest it anti-inflammatory efficacy for diabetes.

Also, the heme protein myeloperoxidase (MPO) derived from leukocytes greatly contributes to the instigation and progression of diabetes. A mildly

elevated level of myeloperoxidase is associated with macro-vascular diabetes complications [33]. 3-(p-methoxyphenyl)-propionic acid compound tightly interacts with the binding pocket of myeloperoxidase protein active receptors site as the metformin which confirms the compound's myeloperoxidase inhibitory potent for therapy of macro-vascular complication of diabetes.

Efficacy and risk-free are the main goals for searching for a novel drug as every drug can assist to treat diseases as well as induce perilous effects [34]. *In-silico* analysis has served an enormous role to evaluate multiple ADMET (pharmacokinetics) properties of compounds in drug research, discovery and design [35]. The drug-likeness characteristics of the selected compounds were screened by Lipinski's rule of five (Ro5) criteria in the current study. All the compounds fulfill the drug-likeness criteria as they violated one rule of five.

The selected compounds also passed the drug-like evaluation as potential candidates via the ADMET analysis. The compounds are safe from acute oral toxicity as some of the compounds belong to Class III except for tridecanoic acid and oleic acid. High absorption from the gastrointestinal tract (GIT) and blood-brain barrier permeation connote that these compounds could be better absorbed from GIT excellently via oral administration than other routes of administration and can achieve bioavailability as well in neurological pathways, thus, serving as therapeutic for neurological degeneration.

The LogP value predicts a compound's permeability through a lipid membrane. For a potential drug, it should be ≤5. The number of hydrogen bond acceptors and donors describes its ability to bind with other compounds, which therefore describes its solubility and permeability [36]. Among the compounds, only 3-(p-methoxyphenyl)-propionic acid has better lipophilicity with LogP < 5, implying its good permeability across the lipid membrane. All the compounds exhibited an excellent number of hydrogen-bound acceptors and donors and this suggests the compounds' high solubility and affinity to bind with other compounds.

Metabolism plays a significant role in drug bioavailability as well as drug-drug interactions. Permeability glycoprotein (P-gp) belongs to the ATP-binding cassette transporters (ABC) and is essential for assessing active efflux through biological membranes (from the wall of GIT to the lumen or from the central nervous system) [37]. CYP450 enzymes with isoforms CYP450 (CYP 3A4, CYP 2D6, CYP 1A2, CYP 2C9, and CYP 2C19) facilitate drug elimination via metabolic biotransformation [38]. Both P-gp and CYP 450 have been proposed to synergistically process small molecules to improve tissue protection [39]. Inhibition of these iso-enzymes may lead to pharmacokinetics-associated drug-drug interactions that might result in detrimental effects by diminishing the solubility and the drug metabolites. Except for compounds, IV, V and VIII that demonstrated inhibitor of CYP1A2 of CYP 450 isoform, all the top hit compounds of methanolic extract of *Anacardium occidentale* nut are non-inhibitor of P-gp and CYP 450 enzymes. These compounds of non-inhibitor of P-gp and CYP 450 will be metabolized normally and safe from inducing unwanted adverse side effects.

The aptness of small molecules to be selected as candidate compounds in drug discovery depends on the compound's toxicity levels [40]. AdmetSar predicts toxicity and carcinogenic of compounds. All the selected compounds are non-carcinogenic and non-hepato-toxic and, therefore, free from inducing DNA mutation(s) and hepatic damage upon ingestion.

The human ether-a-go-go-related gene (hERG) encodes the potassium channel known for normal heart function. Research showed that many drugs have been withdrawn from use owing to their cardio-toxicity through the blockage of hERG activity [41,42]. The selected compounds are also non-inhibitory of hERG, hence safe from cardio-toxicity induction. Moreover, compounds IV, V, and VIII are hERG inhibitors and might probably induce cardiac blockage.

Conclusions

The docking analysis revealed oleic acid, 3-(p-methoxyphenyl)-propionic acid and tridecanoic acid from *Anacardium occidentale* nut methanolic extract were excellent molecules with drug-likeness owing to their inhibitory potentials on selected proteins related to diabetes mellitus pathogenesis progression. The compounds also exhibited good ADMET properties and may lead to

the design of potent novel antidiabetic drugs with minimal side effects. In *vitro and in vivo* studies of these compounds can be further investigated.

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Conflict of interest statement
The authors declare no conflict of interest.

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