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HEPATIC FUNCTION, ANTIOXIDANT DEFENSE, LIPID PROFILE, AND OXIDATIVE-STRESS LEVELS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS TREATED WITH *TERMINALIA CATAPPA* AQUEOUS NUT EXTRACT

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ABSTRACT

Diabetes mellitus (DM) is still without cure and there's a need to manage and prevent its related complications. *Terminalia catappa* is a locally consumed, nutritive fruit whose nut is considered waste. Hence, this study evaluated the effects of *Terminalia catappa* aqueous nut extract (TCANE) on hepatic antioxidant enzymes, liver function, lipid profiles, and oxidative stress levels in streptozotocin (STZ)-induced diabetic rats. Twenty-four (24) Wistar rats were divided into four groups (n=6): control (group 1), diabetic untreated (group 2), and diabetic groups treated with TCANE (300 mg/ kg) and glibenclamide (groups 3 and 4, respectively). PyRx and Biovia Discovery Studio were used for molecular docking studies while swissADME and ProTox were used to predict the ADME/T properties. The results from this study showed that the TCANE-treated group had a significant ($p<0.05$) increase and decrease in hepatic antioxidant enzymes [superoxide dismutase (SOD) and catalase (CAT)] and liver function [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] activities compared to the untreated diabetic rats, respectively. Additionally, a significant ($p<0.05$) decrease in total cholesterol, triglycerides, low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL), and an increase in high-density lipoprotein (HDL) were observed. The *in-silico* studies showed favorable 2D and 3D interactions between TCANE bioactive compounds (epicatechin, sapogenin, and flavone) and the selected key diabetes-related proteins. The ADME/T analysis of these compounds showed good pharmacokinetic properties, with acceptable absorption, metabolism, and non-toxicity. These findings suggest that *Terminalia catappa* could be a potential therapeutic agent for managing diabetes and its related complications rather than being discarded after consuming the fruit.

Keywords: Diabetes mellitus, Waste-to-wealth, *Terminalia catappa* nut, Phytochemistry, Antioxidant enzymes, Hepatic enzymes, Oxidative stress, Lipid profile.

1.0 INTRODUCTION

The most common type of diabetes mellitus (DM) is type-2 DM and it accounts for approximately 90% of DM cases worldwide. The International Diabetes Federation (IDF) has projected the prevalence of diabetes to reach an alarming 700 million by the year 2045. DM remains a macromolecular-metabolic disorder without yet a cure. It is marked by a decreased body's capacity to respond to insulin for appropriate blood sugar (glucose) level maintenance. It is a chronic, non-communicable hyperglycemia syndrome caused by exposure to both environmental and hereditary factors [1]. It can result in various

forms of life-threatening conditions including the risk of coma, cardiovascular diseases (heart disease, stroke), blindness, and chronic kidney and liver damage amongst others [2, 3]. Streptozotocin (STZ) is a naturally occurring chemical compound derived from the bacterium *Streptomyces achromogenes* commonly used in research to induce diabetes in animal models, particularly rats and mice [4]. Due to structural similarities with glucose, STZ possesses both diabetogenic and antibacterial effects [5]. It is selectively absorbed by pancreatic β-cells via the glucose transporter-2 (GLUT-2) [6]. The insulin-producing beta cells of the pancreas are destroyed due to DNA fragmentation and alkylation that STZ causes once inside the β-cells. This causes a significant decrease in insulin secretion, resembling the symptoms of diabetes, especially Type 1 diabetes mellitus (T1DM), in which there is a complete lack of insulin secretion because the β-cells are being destroyed [7].

Diabetes affects all major organs of the body although the severity may differ. For example, the liver which plays a significant role in the metabolism of fats and carbohydrates (glucose) by regulating blood sugar levels, and managing fat storage and breakdown may be affected, leading to hepatic damage [8]. The regulations of these macromolecule mechanisms are usually compromised in the diabetic state, which may cause the liver to produce excess glucose due to insulin resistance or deficiency, contributing to hyperglycemia [9]. It is also known that the liver is essential in inflammatory cytokines (tumor necrosis factor-α, interleukins -1, 6, 8, and 12) production [10]. Common liver enzymes such as alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) are involved in these processes. They are implicated in liver damage caused by diabetic conditions [11]. Increased serum levels and activity of these enzymes indicate inflammation, which can disrupt insulin signaling, leading to hepatic injury [12].

Diabetic conditions also often result in oxidative stress, an imbalance between free-radical generation and antioxidants. This redox imbalance has been linked to insulin resistance, β-cell dysfunction, and impaired glucose intolerance, which in turn lead to type 2 diabetes (T2DM) [13]. The excessive accumulation of free radicals (e.g. reactive oxygen species) can damage various cellular components, including proteins, lipids, and DNA, resulting in cellular dysfunction and disruption of normal physiological processes. To protect the body from oxidative stress, antioxidant enzymes including catalase (CAT), and superoxide dismutase (SOD) play a vital role [14], such that SOD converts dangerous superoxide radicals (O_2^-) into less toxic molecules like oxygen (O_2) and hydrogen peroxide (H_2O_2). CAT further catalyzes hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2), lessening its toxicity and safeguarding cellular components [15]. Additionally, DM, particularly type-2 diabetes, is closely linked with altered lipid profiles, a condition known as diabetic dyslipidemia. This metabolic disturbance is characterized by changes and fluctuation in lipid profile levels which contribute significantly to the elevated cardiovascular risk in diabetic patients [16]. The relationship between diabetes and lipid abnormalities is primarily driven by insulin resistance and hyperglycemia [17]. In diabetes, insulin resistance reduces the body's ability to regulate lipid metabolism effectively. As a result, the liver increases the production of very low-density lipoprotein (VLDL) and triglycerides. At the same time, the breakdown of triglycerides in fat tissue is increased, leading to a higher release of free fatty acids into the bloodstream. These fatty acids are then taken up by the liver, further enhancing the production of triglycerides and VLDL [18].

Conventional antidiabetic agents have been associated with various side effects and researchers worldwide, including those in developed countries, are now exploring locally available natural products and their wastes, which have negligible side effects and traditional antidiabetic claims. *Terminalia catappa* belongs to *Combretaceae* family and is commonly called Almonds, Igi-furutu (Yoruba), and Baushe (Hausa) [19]. Almond nuts are reported to be significantly high in proteins, fats, carbohydrates, vitamins, and minerals [20]. *Terminalia Cattapa* bark, leaves, and fruit have been used for a variety of medicinal purposes including the treatment of dermatitis, hemostatic effects, and fever [21]. Additionally, almond nuts have been reported for heart health issues due to their high fiber, phytosterols, vitamins, minerals, antioxidants, and unsaturated fatty acid contents [22]. Most of the biological activities reported for almond nuts have been associated with its phyto-components including saponins, alkaloids,

glycosides, terpenes, volatile oils, steroids, and phenols [23]. Inhibitors of diabetes-related protein targets act to postpone the breakdown of carbohydrates in the small intestine and hence lower the postprandial blood glucose levels [24]. These protein targets are essential for managing diabetic conditions and their complications. For instance, α-amylase is found in saliva and pancreatic juice, and it helps to break down large polysaccharides like starch into molecules that can easily be absorbed. Conversely, α-glucosidase located in the mucosal brush border of the small intestine breaks down carbohydrates and catalyzes the final stage of starch and disaccharide digestion. Glucose is converted to sorbitol by aldose reductase (AR), and then into fructose by sorbitol dehydrogenase (SDH) in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) in the polyol pathway [25, 26]. Consequently, each of these proteins can be targeted to manage, stop, or minimize the consequences of diabetes and its complications. Our ethnobotanical survey on the consumption of almond fruits and/ or nuts showed they possess a hypoglycemic activity. Hence, this study aimed to investigate the *in-silico* inhibitory potentials of the secondary metabolites of *Terminalia catappa* aqueous nut extract on selected protein targets and its effects on biochemical parameters.

2.0 MATERIALS AND METHODS

Plant Collection and Extraction Process

Ripe *Terminalia catappa* fruits were harvested in March 2024 within the Faculty of Science, Lagos State University, Southwestern Nigeria (Latitude: 6°28′1.20′′N and 3°10′58.80′′E). It was brought into the Laboratory of the Department of Biochemistry, rinsed under running water, the fruits consumed and the seeds dehulled to obtain the kernel which was then rinsed, crushed, and ground using an electric blender (model No. KW-505) until smoothly moldy. The blended mixture was sieved using a fine mesh. Fifty (50) g of the nut powder was then dissolved in 500 mL of distilled water and left to macerate for 48 hours before filtering with Whatman filter paper grade 1. The resulting filtrate was the *Terminalia catappa* aqueous nut extract (TCANE) later stored in the refrigerator until when needed.

Phytochemical Screening

The embedded phytochemical constituents of *Terminalia catappa* aqueous nut extract were screened, identified, and quantified following the procedures described by [27, 28].

High-Performance Liquid Chromatography (HPLC) Analysis of TCANE

High-performance liquid chromatography (HPLC) analyses for the quantification of flavonoid compounds in the extracts were done on an HPLC-Agilent Technologies 1200 series liquid chromatograph with a UV detector. The chromatography was performed on a reversed-phase prepacked Hypersil BDS C18 column (150 × 4.6 mm, 5 µm particle size) at 25^0C. The mobile phase is made of A (0.1% formic acid in water) and B (HPLC grade Acetonitrile) with a constant flow rate of 0.75 mL/min. The linear gradient solvent system started at 0 min, 94% A; 14 min, 83.5% A; 16 min, 83% A; 18 min, 82.5% A; 20 min, 82.5%; 22-24 min, 81.5%; and 27-40 min, 80% A. The detection wavelength was 280nm.

Ligand Modeling

The 3D crystal structures of the ligands were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih. gov/) in .sdf format. Biovia Discovery studios 2021 was used to convert them to .pdb format. The canonical SMILES of the ligands were retrieved from the PubChem database, while open babel (https://openbabel.readthedocs.io/en/ latest/Forcefields/mmff94.html) was employed to optimize the energy of the ligands after which the ligands were converted into AutoDock ligand pdbqt format.

Protein Preparation and Molecular Docking

The protein targets in this study includes α-amylase (x= 8.6407, y= -27.8388, and z=15.7172), α-glucosidase (x= -12.175, y= -35.415, and z=88.753), sorbitol dehydrogenase (x= 80.0875, y= 62.3478, and z= 3.4355), and aldose reductase (x= -0.3071, y= -0.6795, and z=15.0975) were obtained from the

Protein Data Bank (https://www.rcsb.org). The 3D structures of these proteins were modeled using a deposited *Homo sapiens* crystal structure as a reference. Biovia Discovery Studio 2021 was employed to remove water molecules and heteroatoms to prepare (purify) the proteins. Subsequently, the purified protein structures were saved in .pdb format for subsequent use.
The PyRx software was employed for the site-specific molecular docking studies and the co-crystallized ligands for the target proteins as well as acarbose and tolrestat (standard medications for diabetes) were used. The study was based on binding free energy values, and the ligand molecules were then sorted in the order of increasing binding energies.

Absorption, Distribution, Metabolism, Elimination and Toxicity (ADME/T) Prediction
The Lipinski rule of five (RO5), swissADME, admetSAR, and ProTox were employed to predict several drug-likeness parameters, physicochemical properties, pharmacokinetics, lipophilicity, water solubility, medicinal chemistry, and toxicity of the top ten ligands based on their binding energies. These predictive tools were utilized to assess the overall suitability of the ligands as potential drug candidates and provide insights into their potential therapeutic efficacy, safety, and pharmaceutical properties.

Experimental Animals
Twenty-four (24) male Wistar rats aged 8 weeks (average weight = 103 g and range = 100–105 g) were sourced from the Animal house of the Department of Biochemistry, Faculty of Science, Lagos State University, Nigeria, under normal day/night cycles. They were given access to clean drinking water and standard commercial pelleted feed (Top feed® Nigeria). The Wistar rats were maintained and used for the study in compliance with existing local and international guidelines. The study protocol was approved by the Lagos State University Research Ethics Committee with LASU/23/REC/055 as the ethical approval number.

Induction of Diabetes
Type-2 Diabetes mellitus (T2DM) was induced via intraperitoneal injection of 55 mg/kg body weight of Streptozotocin (STZ) (Sigma-Aldrich Chemicals Company, St. Louis, MO., U.S.A) dissolved in freshly prepared 0.1 M of iced-cold citrate buffer (pH 4.5) and administered into overnight fasted rats. The rats were later sustained with 20% glucose (Unique Pharmaceuticals, Sango Otta, Ogun State, Nigeria) for 6 hours after the induction and 5% glucose for the next 24 hours to prevent hypoglycemia. Gradual onset of hyperglycemia was confirmed on the 3rd day (72 hours) post-induction but all the rats became consistently hyperglycemic and stable on the 7th day post-induction. By the 7th day, rats with a fasting blood glucose of equal or greater than 200 mg/dL were considered diabetic and incorporated in this study. Treatments commenced on the 4th day post-STZ-induction and continued for 28 days. The diabetic state was assessed in the rats by measuring blood glucose concentrations using an Accu check glucometer after the collection of blood from the rats' tails. The induction success rate was above 80%.

Experimental Design
The twenty-four (24) Wistar rats used for this study were randomly divided into four groups (A–D) of 6 rats each but the mean weights of the groups were later made equal.

Group A = Normal non-diabetic rats (control) received 2 mL/kg/day of distilled water orally for 28 days.
Group B = Diabetic rats orally administered 2 mL/kg body weight/day distilled water for 28 days.
Group C = Diabetic rats orally administered 2mL/kg body weight/day of *T. catappa* aqueous nut extract (TCANE) for 28 days.
Group D = Diabetic rats orally administered 5 mg/kg body weight/day of glibenclamide (®Daonil, Hoechst Marion Roussel Limited, Mumbai, India) for 28 days.

All the animals were allowed free access to feed and clean water throughout the experimental period. The initial weights of the animals were recorded and subsequently, every week. Whole blood was collected by tail tipping method between 8:00 and 9:00 hours and, was evaluated for fasting blood glucose (FBG) at

days 1, 7, 14, 21, and 28, using the glucose oxidase method of Trinder (1969) via AccuChek Active Glucose monitor.

Anesthetization and blood sample collection

At the end of the 28th day of treatment, each rat was anesthetized with 0.2 mL ketamine injection and dissected. Anesthesia was confirmed by loss of pedal and corneal reflexes. Fresh blood samples were then collected from the heart chamber through cardiac puncture using a sterile 21 G needle mounted on a 5 mL plunger syringe (®Cliniject Hypodermic Syringe, Albert David Limited, Mandideep-462046, Raisen District, India) and kept in plain red top bottles. The blood samples were immediately centrifuged at 2500 rpm for 15 minutes, and the supernatant (serum) was collected and kept in the freezer until needed for biochemical analyses.

Biochemical assays

Biochemical parameters were assessed to evaluate the effects of *T. catappa* aqueous nut extracts (TCANE) on STZ-induced diabetic Wistar rats:

Estimation of Protein Concentration

The protein concentration of the various samples was determined using the Lowry method (Lowry, 1951) with few modifications. Briefly, 0.5 mg/mL bovine serum albumin was mixed with 25 µL of each group serum, 400 µL of solution C, and 40 µL of Folin C. The resulting solution was mixed well and allowed to stand. The absorbance was measured at 650 nm against a blank that consisted of 60 µL of phosphate buffer (Ph 7.4) or water, solution C and Folin C. The protein concentration of each group was extrapolated from the Lowry standard curve and expressed as mg/mL. Solution C was made up of 9.8 mL solution A (0.1 M sodium hydroxide and 2% sodium bicarbonate) and 200 µL solution B (1% copper sulfate ($CuSO_4.5H_2O$) and 2% sodium potassium tartrate).

Lipid Profile Estimation

The serum sample of the treated Wistar rats was used to analyze lipid profile [triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-c)] using standard diagnostic test kits (Randox Laboratories, Crumlin, U.K.). Plasma low-density lipoproteins cholesterol (LDL-c) and very-low-density lipoproteins cholesterol (VLDL-c) were estimated using the Friedewald formula: LDL-c = [TC- (HDL-c + TG/5)], and VLDL-c = TAG/5, respectively (Latha*et al*., 2023).

Determination of Catalase (CAT) Activity

CAT activity was evaluated using the procedure of Claiborne (1985) with slight modification. Briefly, hydrogen peroxide (1180 µL of 19 mM solution) was mixed with 20 µL of each serum sample and then invertedly mixed. The absorbance of each sample was determined using a UV-spectrophotometer at 240 nm. The change in absorbance was noted every 10 seconds for 2 mins. Catalase activity was calculated and expressed as µmol/mg protein using the extinction coefficient of hydrogen peroxide.

Determination of Superoxide Dismutase (SOD) Activity

SOD activity was evaluated using the Epinephrine method of Misra and Fridovich (1972). Briefly, 0.1 mL of the serum was added to 2.5 mL of 0.05 M of phosphate buffer (pH 7.8). Finally, 0.3 mL of adrenaline solution (0.059%) was added at the point of absorbance measurement, and absorbance read at 750 nm every 15 seconds for 1 minute 30 seconds. SOD activity was calculated and expressed as µmol/ mg protein.

Determination of Glutathione (GSH) Levels

Glutathione (non-protein thiol) level was evaluated using Ellman's reagent (DTNB) method described by Jollow *et al*. (1974). The serum was precipitated with sulpha-salicylic (4%) in 1:1 and kept at room temperature (26 °C) for 1 hour before being subjected to centrifugation at 5000 rpm for 10 minutes at 4

°C. The supernatant (100 µL) was then mixed with 550 µL of 0.1 M phosphate buffer and 100 µL of DTNB. The absorbance was read at 412 nm and the results were expressed as µmol of GSH/mg protein.

Determination of Hydrogen Peroxide (H_2O_2) Levels

Hydrogen peroxide level was determined according to the method of Wolff (1994). Briefly, 10 mL of xylenol was mixed with 10 mL sorbitol, 50 mL ammonium ferrous sulfate, and 30 mL distilled water to form the FOX solution. The resulting FOX solution (290 µL) was later mixed with 10 µL of serum before being vortexed till it foamed. A pale pink color complex was generated after incubation for 30 minutes at room temperature (26 °C) and the absorbance was read against a blank (distilled water) at 560 nm. The concentration of the hydrogen peroxide generated was evaluated and expressed as mmol/ mL.

Determination of Nitrite/ Nitric oxide (NO) Levels

The amount of nitrite/ nitric oxide in the serum samples was evaluated using the Griess reaction method by incubating 250 µL of each sample with 250 µL of Griess reagent at room temperature for 20 minutes. The absorbance of the resulting solution was read at 550 nm with a Visible spectrophotometer. Nitrite/ nitric oxide concentration was calculated by comparison with the absorbance of the standard solution of a known sodium nitrite.

Determination of Enzymatic Activities

Activities of alanine transaminase (ALT, EC 2.6.1.2) and aspartate transaminase (AST, EC 2.6.1.1) were determined using the commercial diagnostic kits (Randox, U.K) according to the manufacturer's descriptions. The activity of each enzyme was measured spectrophotometrically at 340 nm and the results were expressed as U/I.

Statistical Analysis

The results were presented as mean ± standard error of means (SEM). GraphPad Prism (version 5.0) software (Graphpad Prism Software Inc., San Diego, CA, USA) was used to analyze the data generated. Treated and control groups were compared using one-way ANOVA while the means were separated using Bonferroni as a post-hoc test. A 95% confidence interval was used to determine statistically significant differences between the control and treated groups, with p values less than 0.05 ($p< 0.05$).

3.0 RESULTS

Tables 1 – 2 show the phytochemical analysis of the aqueous extract from *T. catappa* nut. The extract contains alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, and phenolics (Table 1). Additionally, the screening of the polyphenolic content showed that tannins were the most abundant, followed by phenolics and flavonoids. Table 2 shows comprehensive polyphenolic bioactive compounds embedded in *Terminalia Catappa* aqueous nut extract revealed by HPLC analysis. Table 3 shows the top ten (10) ligands based on their binding affinities. Epicatechin showed the overall best ligand with a binding energy of -9.8 kcal/mol.

Table 1: Preliminary phytochemical screening of *Terminalia catappa* aqueous nut extract (TCANE).

S.No.	Phytochemicals	Qualitative	Polyphenolic content (mg/100g)
1	Alkaloids	+	
2	Flavonoids	+	21.54 ± 0.11^{b}
3	Saponins	+	
4	Tannins	+	60.03 ± 0.02^{a}
5	Steroids	+	
6	Phenolics	+	43.70 ± 0.06^{a}
7	Terpenoids	+	

KEY: + = Present, - = Absent

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Table 2: HPLC Analysis of Bioactive compounds in *Terminalia catappa* Aqueous Nut Extract.

Peak No	Compounds	Molecular weight (g/mol)	Molecular formula	Conc (mg/g)	Class of compound
1	Proanthocyanin	592.50	$C_{31}H_{28}O_{12}$	10.46	Flavonoid
2	Gallic acid	170.12	$C_7H_6O_5$	623	Phenolic acid
3	Naringin	580.54	$C_{27}H_{32}O_{14}$	12.71	Flavonoid
4	Catechin	290.27	$C_{15}H_{14}O_6$	25.18	Flavonoid
5	Quinine	3242	$C_{20}H_{24}N_2O_2$	7.18	Alkaloid
6	Chlorogenic acid	3531	$C_{16}H_{18}O_9$	53.47	Phenolic acid
7	Flavan-3-ol	456.39	$C_{15}H_{14}O_2$	42.276	Flavonoid
8	Caffeic acid	180.16	$C_9H_8O_4$	46.15	Phenolic acid
9	Anthocyanin	207.30	$C_{15}H_{11}O$	6.016	Flavonoid
10	Ellagic acid	302.19	$C_{14}H_6O_8$	98.82	Polyphenol
11	Ribalinidine	275.30	$C_{31}H_{28}O_{12}$	10.366	Alkaloid
12	Naringenin	272.26	$C_{15}H_{12}O_5$	12.970	Flavonoid
13	Rutin	610.52	$C2_7H_{30}O_{16}$	45.06	Flavonoid glycoside
14	Spartein	2338	$C_{15}H_{26}N_2$	15.460	Alkaloid
15	Quercitrin	448.38	$C_{20}H_{21}O_{11}$	79.65	Flavonoid glycoside
16	Sapogenin	486.68	$C_{30}H_{46}O_5$	17.963	Saponin
17	Isoquercitrin	4638	$C_{21}H_{20}O_{12}$	52.03	Flavonoid glycoside
18	Phenol	911	C_6H_6O	20.316	Phenolics
19	Quercetin	302.24	$C_{15}H_{10}O_7$	26.71	Flavonoid
20	Flavonones	5950	$C_{29}H_{27}O_{11}$	22.730	Flavonoid
21	Steroids	288.40	$C_{19}H_{28}O_2$	25.650	Steroids
22	Epicatechin	290.26	$C_{15}H_{14}O_6$	27.536	Flavonoid
23	Kaempferol	286.23	$C_{15}H_{10}O_6$	29.860	Flavonoid
24	Phytate	660.04	$C_6H_{18}O_{24}P_6$	32.993	Anti-nutrient
25	Flavone	222.24	$C_{31}H_{28}O_{12}$	3600	Flavonoid
26	Oxalate	128.09	$C_2O_{4(2-)}$	36.876	Anti-nutrient
27	Resveratol	228.25	$C_{14}H_{12}O_3$	120	Polyphenol

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Table 3: Binding Energies (kcal/ mol) of the top ten (10) ligands with each Protein target.

S. No.	Ligands	PubChem ID	Diabetes Implicated Protein Targets			
			Aldolase reductase	α-Amylase	α-Glucosidase	Sorbitol dehydrogenase
1	Epicatechin	72276	**-9.8**	-	**-6.7**	-6.7
2	Kaempferol	5280863	-9.5	-7.5	-6.2	-
3	Flavone	10680	-9.4	-7.2	-6.3	-
4	Anthocyanin	145858	-9.4	-7.2	-6.4	-
5	Chlorogenic acid	1794427	-9.4	-	-6.2	-6.5
6	Catechin	9064	-9.3	-7.1	-6.5	-6.9
7	Sapogenin	99474	-8.7	**-8.7**	-6.2	**-8.7**
8	Resveratol	445154	-8.6	-	-6.2	-
9	Steroids	9904	-8.5	-8.2	-6.7	-6.5
10	Caffeic acid	689043	-8.1	-	-	-

Figure 1a shows the 3D (left) and 2D (right) structures and interaction of sapogenin with various amino acids at the active site of α-Amylase. Ile235, Ala307, His305, and Leu165 formed Alkyl and Pi-Alkyl interaction while Tyr151, Lys200, Leu162, Trp58, Tyr62, Gln63, Trp59, Asp300, and Gly306 formed van der Waals interaction with sapogenin. Figure 1b illustrates the 3D (left) and 2D (right) structures and interaction of Phe649 and Try481 with α-glucosidase forming a Pi-Pi T-shaped bond, Leu678 formed a Pi-Alkyl while Asp616 formed a Pi-Anion interaction. Additionally, Met519 formed a Pi-sulfur interaction and Leu650, Arg600, Asp518, Trp376, Leu677, and Ser676 formed a van der Waal interaction with flavone. Figure 1c shows the complex interaction of sapogenin with the human sorbitol dehydrogenase. The 2D structure showed the formation of Pro98, Cys105, Phe138, and Pro304 formed an Alkyl and Pi-Alkyl interaction with flavone. Asn301 formed a carbon-hydrogen bond and an unfavorable acceptor-acceptor interaction. Tyr110, Asn101, Glu100, Arg99, Ala137 and Tyr140 formed van der Waals interaction with sapogenin, the ligand with the best binding affinity. Figure 1d illustrates the complex interaction of epicatechin, with aldose reductase. The 2D structure showed the formation of Tyr48, Trp20 and Tyr209 formed a Pi-Pi Stacked and Pi-Pi T-shaped interaction, Pro211 formed a Pi-Alkyl interaction and Cys298 formed Pi-Sulfur. Lys262, Lys21, Ile260, Ser214 formed a carbon hydrogen bond and Pi-donor hydrogen bond interaction. Additionally, Trp20, Gln183, Lys77, Ser210, Asp216 conventional hydrogen bond, while Pro215, Leu212, Pro261, Asp43, Trp111, Lys21 formed van der Waals interaction with epicatechin, the ligand with the best binding

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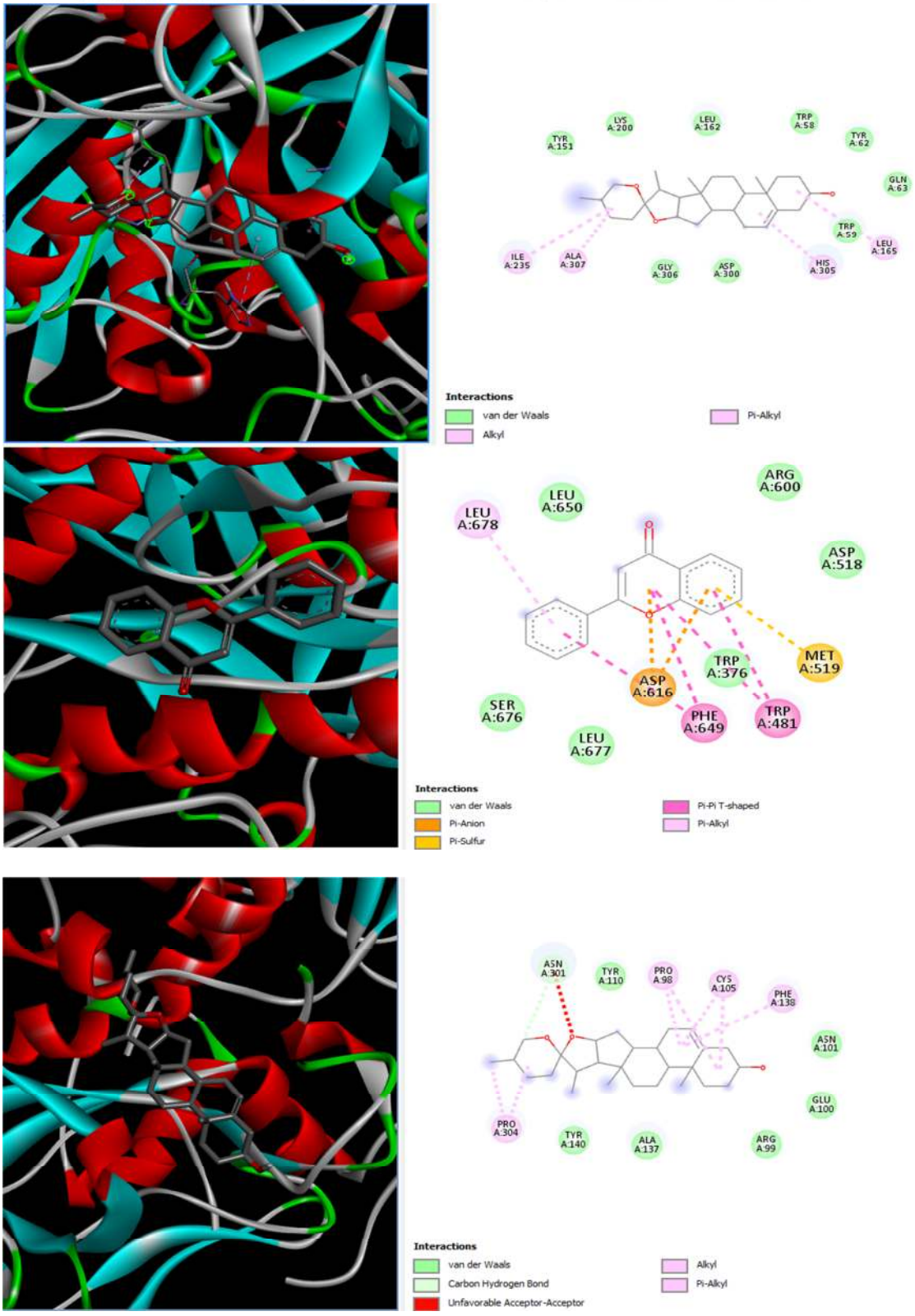

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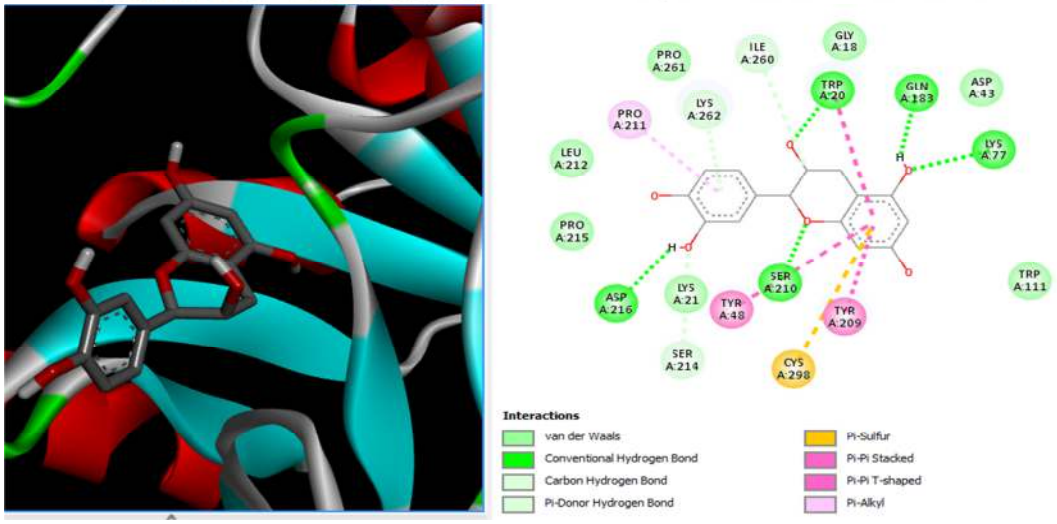

Figure 1: 3D (left) and 2D (right) structures and interactions of the amino acids of (a) α-amylase docked with sapogenin, (b) α-glucosidase docked with flavone, (c) sorbitol dehydrogenase docked with sapogenin, and (d) aldose reductase docked with epicatechin.

Table 4.1-4.6 illustrates the absorption, distribution, metabolism, elimination and toxicity (ADMET) properties of the overall, best ten (10) Ligands according to their binding affinity (kcal/ mol). Table 4.1 shows the physiochemical properties of the best 10 ligands embedded in *Terminalia catappa* nut extract. From the table, the compounds embedded in the nut obeyed the Lipinski rule of five (RO5) which states that a molecule is more likely to be druggable if it has no more than five violations of the following criteria: molecular weight less than 500, logP less than 5, hydrogen bond donors less than 5, hydrogen bond acceptors less than 10, and 10 or fewer rotatable bond. Table 4.2 reveals the assessment of lipophilicity properties and water solubility of the top ten ligands. Sapogenin showed the best logarithm of the partition coefficient (Log P) with a value of 5.02. Meanwhile, other ligands are also within the ranges away from zero (0) indicating their good lipophilicity properties in drug discovery except chlorogenic acid with Log P of -0.39. Table 4.3 shows the pharmacokinetic properties of the best ten ligands, it shows high gastro- intestinal absorption for nine of the ligands except catechin which has a low gastro-intestinal absorption. Table 4.4 shows that the top ten ligands have a good bio-availability score since a minimum of 0.10 bioavailability score is required of a compound to be considered as a drug candidate. Table 4.5 shows the medicinal chemistry of the top ten ligands, from this result, it can be deduced that 4 of the ligands epicatechin, anthocyanin, chlorogenic acid and catechin showed a positive pan-assay interference compounds (PAINS) while epicatechin, kaempferol, flavone and catechin showed a positive lead-likeness. Table 4.6 shows the toxicity of the best ten ligands.

Table 4.1: Physiochemical properties prediction of the top ten (10) active compounds in TCANE.

Ligands	Formula	MW (g/mol)	NRB	NHBA	NHBD
Epicatechin	$C_{15}H_{14}O_6$	290.27	1	6	5
Kaempferol	$C_{15}H_{10}O_6$	286.24	1	6	4
Flavone	$C_{15}H_{10}O_2$	2224	1	2	0
Anthocyanin	$C_{15}H_{11}O+$	207.25	1	1	0
Chlorogenic acid	$C_{16}H_{18}O_9$	3531	5	9	6
Catechin	$C_{15}H_{14}O_6$	290.27	1	6	5
Sapogenin	$C_{30}H_{46}O_5$	486.68	2	5	3
Resveratol	$C_{14}H_{12}O_3$	228.24	2	2	3
Steroids	$C_{18}H_{26}O_2$	2740	0	2	1

Caffeic acid	$C_9H_8O_4$	180.16	2	4	3
*Acarbose	$C_{25}H_{43}NO_{18}$	645.60	9	19	14
*Tolrestat	$C_{16}H_{14}F_3NO_3S$	357.35	6	6	1

*denotes standard drugs incorporated in this study, MW denoted molecular weight, NRB denotes number of rotatable bonds, NHBA denotes number of rotatable hydrogen bond acceptor, NHBD denotes number of hydrogen bond donor.

Table 4.2: Water solubility and Lipophilicity prediction of top ten (10) active compounds in TCANE.

Ligands	**Log S (ESOL)**	**Class**	**Log $P_{o/w}$ (iLOGP)**
Epicatechin	-2.22	Soluble	1.47
Kaempferol	-3.31	Soluble	1.70
Flavone	-4.09	Soluble	2.55
Anthocyanin	-4.01	Soluble	-0.76
Chlorogenic acid	-1.62	Soluble	0.87
Catechin	-2.22	Soluble	1.33
Sapogenin	-5.98	Poorly soluble	4.66
Resveratol	-3.62	Moderately soluble	1.71
Steroids	-3.19	Soluble	2.77
Caffeic acid	-1.89	Soluble	0.97
*Acarbose	2.13	Highly soluble	1.43
*Tolrestat	-29	Moderately soluble	2.42

*denotes standard drugs incorporated in this study

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Table 4.3: Pharmacokinetics properties prediction of the top ten (10) active compounds in TCANE.

Ligands	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Epicatechin	High	No	Yes	No	No	No	No	No
Kaempferol	High	No	No	Yes	No	No	Yes	Yes
Flavone	High	Yes	No	Yes	Yes	No	No	No
Anthocyanin	High	Yes	Yes	Yes	No	No	Yes	No
Chlorogenic acid	High	No	No	No	No	No	No	No
Catechin	Low	No	Yes	No	No	No	No	No
Sapogenin	High	Yes	No	No	No	No	No	No
Resveratol	High	Yes	No	Yes	No	Yes	No	Yes
Steroids	High	Yes	No	No	No	No	No	No
Caffeic acid	High	No	No	No	No	No	No	No
*Acarbose	Low	No	Yes	No	No	No	No	No
*Tolrestat	High	No	No	Yes	Yes	No	No	No

*denotes standard drugs incorporated in this study

Table 4.4: Drug likeness properties prediction of top ten (10) active compounds in TCANE.

Ligands	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
Epicatechin	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
Kaempferol	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
Flavone	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
Anthocyanin	Yes; 0 violation	Yes	Yes	Yes	No; 1 violation	0.55
Chlorogenic acid	Yes; 1 violation	No; 1 violation	No; 1 violation	No; 1 violation	No; 1 violation	0.11
Catechin	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
Sapogenin	Yes; 1 violation	No; 3 violations	Yes	Yes	No; 1 violation	0.55
Resveratol	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
Steroids	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
Caffeic acid	Yes; 0 violation	Yes	Yes	Yes	Yes	0.56
*Acarbose	No; 3 violations	No; 4 violations	No; 1 violation	No; 1 violation	No; 5 violations	0.17
*Tolrestat	Yes; 0 violation	Yes	Yes	Yes	Yes	0.56

*denotes standard drugs incorporated in this study.

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Table 4.5: Medicinal chemistry prediction of top ten (10) active compounds in TCANE.

Ligands	PAINS	Brenk	Leadlikeness	Synthetic Accessibility
Epicatechin	1 alert	1 alert	Yes	3.50
Kaempferol	0 alert	0 alert	Yes	3.14
Flavone	0 alert	0 alert	Yes	2.88
Anthocyanin	1 alert	1 alert	No; 2 violations	2.78
Chlorogenic acid	1 alert	2 alerts	No; 1 violation	16
Catechin	1 alert	1 alert	Yes	3.50
Sapogenin	0 alert	1 alert	No; 2 violations	6.94
Resveratol	0 alert	1 alert	No; 1 violation	2.02
Steroids	0 alert	0 alert	Yes	51
Caffeic acid	0 alert	2 alerts	No; 1 violation	1.81
*Acarbose	0 alert	1 alert	No; 2 violations	7.34
*Tolrestat	0 alert	1 alert	No; 2 violations	2.34

*denotes standard drugs incorporated in this study.

Table 4.6: Toxicity properties prediction of the top ten (10) active compounds in TCANE.

Ligands	Carcinogenicity (Binary)	Hepa-toxicity	Respiratory toxicity	Reproductive toxicity	Mitochondrial toxicity	Nephro-toxicity	Acute oral toxicity (Class)
Epicatechin	-	-	+	+	+	-	IV
Kaempferol	-	+	+	+	+	-	II
Flavone	-	+	+	+	-	-	III
Anthocyanin	-	+	-	-	-	+	III
Chlorogenic acid	-	+	+	+	+	-	III
Catechin	-	-	+	+	+	-	IV
Sapogenin	+	-	+	+	+	-	IV
Resveratol	-	-	-	-	-	+	III
Steroids	-	+	+	+	+	-	III
Caffeic acid	-	-	+	+	+	-	III
*Acarbose	-	+	+	+	+	-	IV
*Tolrestat	-	+	+	+	-	-	III

*denotes standard drugs incorporated in this study
+ denotes PRESENT
- denotes ABSENT

Toxicity category I is highly toxic and severely irritating, Toxicity category II is moderately toxic and moderately irritating, Toxicity category III is slightly toxic and slightly irritating, Toxicity category IV is practically non-toxic and not an irritant.

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Table 5 shows the effect of TCANE on glucose level and protein concentration. The administration of TCANE significantly ($p<0.05$) decreased and increased high levels of glucose and protein, respectively, compared to the STZ-untreated group.

Table 5: Effect of TCANE on Glucose and Protein concentration.

S/N	Groups	Glucose Conc. [mg/dL]	Protein Conc. [mg/ mL]
1	Control	90.38 ± 1.93^{a}	0.31 ± 0.06^{a}
2	STZ	201.2 ± 23.77^{b}	0.15 ± 0.03^{b}
3	STZ + TCANE 300mg	101.8 ± 3.21^{c}	0.22 ± 0.15^{c}
4	STZ + Glb	71.74 ± 1.15^{a}	0.29 ± 0.06^{c}

Data shows mean ± SEM (n=5) and values with different alphabets in the same column are statistically significant at $p<0.05$. STZ = Streptozotocin, TCANE= *Terminalia catappa* aqueous nut extract, Glb= Glibenclamide.

Figure 2 shows the effect of TCANE on antioxidant activities. It was observed that the administration of TCANE significantly ($p<0.05$) increased superoxide dismutase (SOD) activity and catalase (CAT) activities compared to the STZ-untreated group. However, the standard, glibenclamide, significantly ($p<0.05$) increased both the SOD and CAT activities.

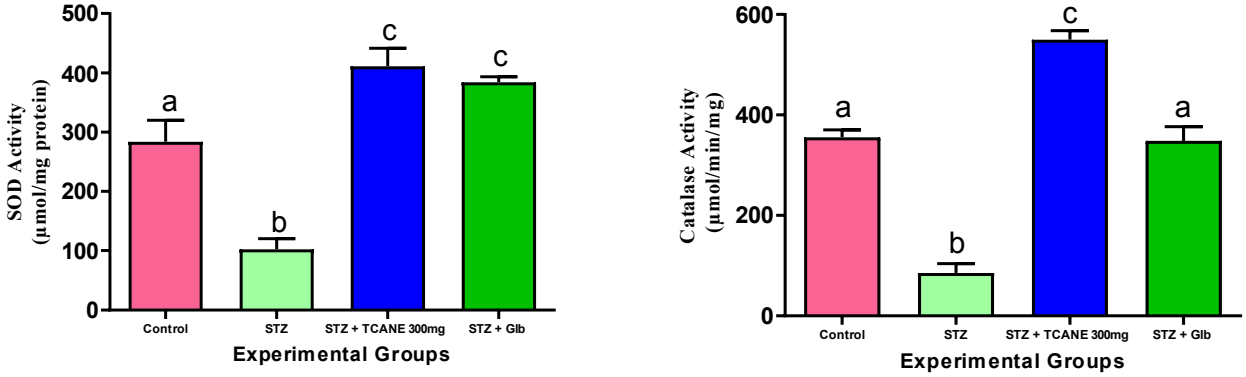

Fig. 2: Effect of *Terminalia catappa* aqueous nut extract (TCANE) on Antioxidant Activities in STZ-Induced Diabetic Wistar rats. Each bar represents mean ± SEM (n=5) and bars with different alphabets are statistically significantly significant at $p < 0.05$. STZ = Streptozotocin, TCANE= *Terminalia catappa* aqueous nut extract, Glb= Glibenclamide.

Figure 3 shows the effect of TCANE on liver enzyme activities. It was observed that the administration of TCANE significantly ($p<0.05$) decreased alanine transferase (ALT) activity and aspartate transferase (AST) compared to STZ-untreated diabetic and glibenclamide-treated rats.

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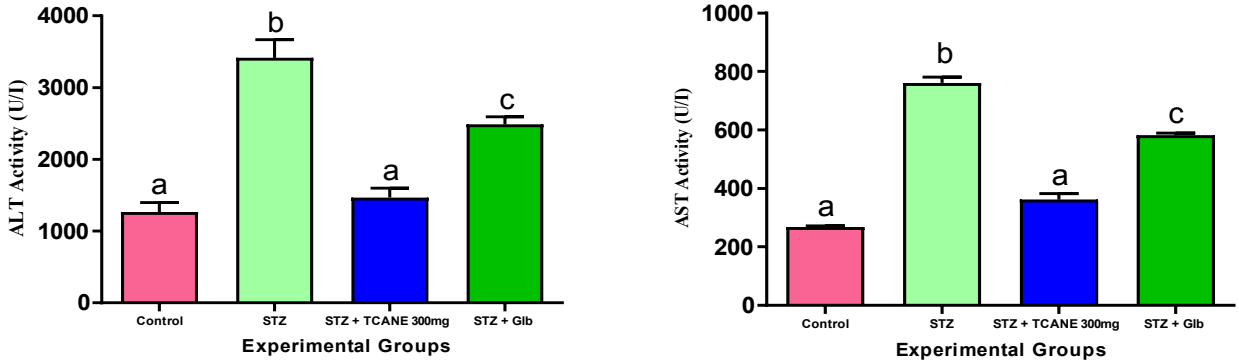

Figure 3: Effect of *Terminalia catappa* aqueous nut extract (TCANE) on Liver enzyme activities in STZ-Induced diabetic Wistar rats. Each bar represents mean ± SEM (n=5) and bars with different alphabets are statistically significantly significant at $p < 0.05$. STZ = Streptozotocin, TCANE= *Terminalia catappa* aqueous nut extract, Glb= Glibenclamide.

Table 6 shows the effect of TCANE on oxidative stress indices. The treatment with TCANE 300mg significantly ($p<0.05$) decreased hydrogen peroxide (H_2O_2), nitric oxide (NO), and malondialdehyde (MDA) concentrations but, increased non-protein thiol (GSH) concentration compared to the STZ-untreated group.

Table 6: Effect of TCANE on Oxidative stress indices

S/N	Groups	H_2O_2 [mmol/ mL]	NO [mmol/ mL]	GSH [µmol/ mg protein]	MDA [nmol]
1	Control	0.053 ± 0.01^a	0.358 ± 0.00^a	309.8 ± 13.78^a	63.02 ± 2.745^a
2	STZ	0.192 ± 0.04^b	0.942 ± 0.01^b	171.5 ± 8.21^b	197.4 ± 0.8650^b
3	STZ + TCANE 300mg	0.054 ± 0.02^a	0.338 ± 0.00^a	392.1 ± 16.71^c	58.54 ± 3.775^a
4	STZ + Glb	0.067 ± 0.00^a	0.446 ± 0.00^c	288.5 ± 6.830^a	90.61 ± 2.120^c

Data shows mean ± SEM (n=5) and values with different alphabets in the same column are statistically significant at $p<0.05$. STZ = Streptozotocin, TCANE= *Terminalia catappa* aqueous nut extract, Glb= Glibenclamide.

Table 7 shows the effect of TCANE on Lipid profile. The administration of TCANE significantly ($p<0.05$) decreased total cholesterol, triglyceride, LDL-cholesterol level, and VLDL-cholesterol levels, and increased HDL-cholesterol levels.

Table 7: Effect of TCANE on Lipid profile.

S/N	Groups	Total-Chol [mg/dL]	TAG [mg/dL]	HDL-Chol [mg/dL]	LDL-Chol [mg/dL]	VLDL-Chol [mg/dL]
1.	Control	391.4 ± 0.08^a	196.2 ± 2.81^a	285.4 ± 9.92^a	66.75 ± 0.29^a	39.25 ± 0.56^a
2.	STZ	559.2 ± 39.62^b	492.6 ± 0.82^b	117.0 ± 1.04^b	343.68 ± 11.28^b	98.52 ± 0.16^b
3.	STZ + TCANE 300mg	295.6 ± 0.38^c	212.4 ± 0.66^a	196.5 ± 3.81^c	56.62 ± 1.03^a	59.12 ± 0.13^c
4.	STZ + Glb	389.4 ± 6.13^a	237.8 ± 14.07^a	171.8 ± 5.73^c	139.72 ± 4.64^c	77.88 ± 2.27^c

Data shows mean ± SEM (n=5) and values with different alphabets in the same column are statistically significant at $p<0.05$. STZ = Streptozotocin, TCANE= *Terminalia catappa* aqueous nut extract, Glb= Glibenclamide.

Discussion

Most recent research has shown the wide range of pharmacological uses of *Terminalia catappa* parts, particularly its antioxidant, anti-inflammatory, and antidiabetic activities [29], most of which have been attributed to the plant's phytochemical constituents capable of mitigating oxidative stress thereby improving liver function [30, 31]. Here in this study, investigation into the phytochemical composition of the aqueous nut extract of *Terminalia catappa* showed the presence of key bioactive compounds including phenols, flavonoids, tannins, alkaloids, saponins, and steroids. These phytoconstituents are known to enhance oxidative stress and metabolic conditions associated with diabetes. Phenol, the most abundant compound in the extract, has been reported to play a significant role in diabetes due to its high antioxidant capacity, which is essential for reducing oxidative stress, a major factor in the progression of diabetes [32]. Tannins are also known to reduce the levels of reactive oxygen species (ROS), and malondialdehyde (MDA), and, at the same time, increase activities of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH). It also suppresses the expressions of IL-6, IL-8, and TNF-α and enhances both the antioxidant and anti-inflammatory properties of *Terminalia catappa* nut extract [33]. Flavonoids have been reported to enhance defective insulin pathways in non-classical sites like the kidney, brain, and endothelium [34]. Diabetes-related vascular complications are closely linked to decreased nitric oxide (NO) synthesis in the endothelium during hyperglycemia conditions due to the inefficiency of the IR/AKT/endothelial nitric oxide synthase (eNOS) mechanism. This results in endothelium-dependent relaxation of the aorta [35].

α-amylase, α–glucosidase, aldose reductase (AR), and sorbitol dehydrogenase (SDH) are pharmacotherapeutic protein targets used in the treatment of diabetes complications [36]. Inhibiting these enzymes reduces the elevated blood glucose after a carbohydrate diet, thereby, reducing the rate of glucose absorption [37]. In recent years, molecular docking has emerged as a novel approach used in the pharmaceutical industries for the development of drugs [38]. The use of molecular docking techniques helps to understand and predict molecular recognition, both energetically (i.e., predicting binding affinity) and structurally (i.e., identifying potential binding modes) [39]. Twenty-eight (28) ligands (Table 2) were obtained from the HPLC analysis of the TCANE, retrieved from PubChem, and docked against the catalytic site of four (4) selected diabetic target proteins (α-amylase, α-glucosidase, AR, and SDH). As shown in Table 3, the docking result showed epicatechin as the overall lead and best-fit compound with a binding energy of -9.8 kcal/mol. This may not be surprising because studies have shown that epicatechin, a natural flavonoid, has anti-oxidant and anti-inflammatory properties [40]. Other top ligands include; kaempferol, flavone, anthocyanin, chlorogenic acid, catechin, sapogenin, resveratrol, steroids, and caffeic acid which are also natural polyphenols found in traditional medicines with reported anti-inflammatory, anti-oxidative, anti-atherosclerotic, hypoglycemic, and hypolipidemic properties [41, 42].

The water solubility and lipophilicity property predictions in Table 4.2 indicated that epicatechin is both water and lipid-soluble and thus may be used to synthesize lipid-soluble nanoparticles that can pass through the cell membrane barrier for the treatment of psychological disorders such as Alzheimer's disease, schizophrenia, dementia, etc. This may also indicate the amphipathic nature of this ligand which can enable it to travel through the blood stream and have improved binding efficiency and stability when it reaches the binding receptor. The pharmacokinetic properties shown in Table 4.3 predicted high gastro-intestinal absorption for nine of the ten best ligands except chlorogenic acid which shows a low gastro-intestinal absorption. The high gastrointestinal (GI) absorption may indicate that when the compounds are ingested, they are readily absorbed through the digestive tract into the bloodstream. This property is significant for orally administered drugs because it suggests that the ligand can effectively reach systemic circulation and exert its effect. High GI absorption generally implies good bioavailability and better pharmacokinetics. The prediction of the blood-brain barrier permeability showed a positive indication for five of the best ten ligands: flavone, anthocyanin, sapogenin, resveratrol, and steroids. As a result, these five compounds could be used to treat disorders of the central nervous system (CNS), including neurological conditions like Alzheimer's disease (AD), schizophrenia, and dementia, among others [43]. On the other hand, epicatechin, anthocyanin, and catechin were identified as a P-glycoprotein substrate. P-glycoproteins is a transmembrane efflux pump that is responsible for expelling substances from cells,

this suggests that the cellular absorption of this potential medication may be minimal. More so, only kaempferol and resveratrol of the best ten ligands were predicted to be a CYP34A4 inhibitor. Meanwhile, flavone, anthocyanin, and resveratrol were predicted to be CYP1A2 inhibitors, while only flavone was predicted to be a CYP2C19 inhibitor. Additionally, only resveratrol was predicted to be a CYP2C9 inhibitor, while kaempferol and anthocyanin were predicted to be a CYP2D6 inhibitor.

The evaluation of the drug-likeness characteristics in Table 4.4 showed that the best ten ligands have good bioavailability scores within the range of 0.11 to 0.56, considered a good indicator for drug candidates [44]. Both chlorogenic acid and acarbose showed one violation for Egan and Veber which predicts that these ligands lack permeability and oral bioavailability. More so, chlorogenic acid, sapogenin, and acarbose have violations for Ghose. Ligands that pass the Ghose filter are considered likely to interact favorably with biological targets while the ligands that pass the Muegge filter indicate that the compounds have drug-likeness properties and can cross the membrane bilayer and avoid toxicity.

In Table 4.5, epicatechin, chlorogenic acid, catechin, and anthocyanin showed one alert each for positive pan-assay interference compounds (PAINS). This may suggest that the ligand contains at least one structural feature that is known to commonly cause false positives in biochemical assays. Such ligands are flagged because they may interfere non-specifically with assay readouts, often by mechanisms unrelated to genuine target binding. A single PAINS alert may mean the compound binds non-specifically, generates artifacts in assays, and may be chemically reactive. This may indicate that the compounds possess certain physicochemical properties that make them a promising starting point, or "lead," for drug development. Lead-like compounds are known to be smaller and simpler than drug-like compounds and are easier to modify chemically. In contrast, epicatechin, kaempferol, catechin, and steroids showed positive lead-likeness indications. Additionally, all the best ten ligands indicated a synthetic accessibility score of approximately 1.81 to 6.94 whereas the ligand sapogenin showed the best synthetic accessibility. Kaempferol, flavone, and steroid showed zero Brenk alerts which suggests that these compounds don't contain certain structural features, known as structural alerts, that are associated with undesirable properties, such as toxicity, metabolic instability, or poor drug-likeness. Some common structural features that trigger Brenk alert include reactive functional groups, unstable functional groups, and lipophilic or high hydrophobic groups.

SwissADME, admetSAR, and ProTox predictions in Table 4.6 showed that among the ten best-fit ligands, epicatechin, sapogenin, catechin, and caffeic acid may not induce liver and kidney toxicity, unlike anthocyanin which shows a positive indication for liver and kidney toxicity. Other compounds that also showed kidney and/or liver toxicity include; kaempferol, flavone, chlorogenic acid, resveratrol, and steroids. All the ligands showed a negative indication for carcinogenicity except sapogenin. Chlorogenic acid, sapogenin, and caffeic acid showed a negative indication of eye and skin irritation, while kaempferol, flavone, anthocyanin, and resveratrol, sapogenin showed a positive indication of eye and skin irritation. All the ligands showed a positive indication for respiratory, reproductive, and mitochondrial toxicity except anthocyanin which showed a negative indication for respiratory, reproductive, and mitochondrial toxicity. Flavone also showed a negative indication of mitochondrial toxicity. Kaempferol was predicted to belong to a toxicity class II, which indicates that kaempferol is moderately toxic and irritating. Flavone, anthocyanin, chlorogenic acid, resveratrol, steroids, and caffeic acid showed a toxicity category III, which indicates that these ligands are slightly toxic but irritating. Additionally, epicatechin, catechin, and sapogenin showed class IV toxicity, which indicates that the ligands are both non-toxic and non-irritant. Figure 1 a-d shows the interacting amino acids and bonds responsible for the stability of the protein-ligand complex. The active site of each protein contains different amino acid residues, such that α-amylase contains Ile235, Ala307, His305, Tyr151, Lys200, Leu162, Trp58, Tyr62, Gln63, Trp59, Asp300, and Gly306; α-glucosidase contains Phe649, Try481, Leu678, Asp616, Met519 Leu650, Arg600, Asp518, Trp376, Leu677, and Ser676; sorbitol dehydrogenase contains Pro98, Cys105, Phe138, Pro304, Asn301 Tyr110, Asn101, Glu100, Arg99, Ala137, and Tyr140; and aldolase reductase contains Tyr48, Trp20 and Tyr209, Pro211, Cys298, Lys262, Lys21, Ile260, Ser214, Trp20, Gln183, Lys77, Ser210, Pro215, Leu212, Pro261, Asp43, Trp111, and Lys21. Each of these amino acid residues plays different

biological roles in the protein-ligand interaction. For example, aspartate and glutamate help to coordinate the actions of metal ions such as calcium, zinc, and magnesium, while asparagine and glutamine can help to aid in substrate binding by forming hydrogen bonds with the substrate [45]. Histidine functions as a universal acid/base as it is involved in both substrate binding and catalysis [46]. Tyrosine is involved in substrate stabilization and hydrogen bonding [47]. Serine is frequently a part of catalytic processes and can attack substrates by functioning as a nucleophile [48]. Threonine is similar to serine by also acting as a nucleophile [49]. Our study showed that epicatechin has the lowest binding energy compared to other ligands and the known commercially available anti-diabetic drugs, acarbose and tolrestat, incorporated in this study. Epicatechin and sapogenin were observed to interact with some of the active site amino acid residues via specific bonds. The presence of such associated bonds may indicate a positive response to the inhibition of these enzymes. Furthermore, the plant's alleged traditional antidiabetic qualities may be attributed to the phytochemicals present in this extract.

In the i*n-vivo* studies, the treatment of the STZ-induced diabetic rats with *Terminalia catappa* aqueous nut extract (TCANE) showed significant reductions in blood glucose levels compared to the STZ-untreated rats. This may indicate that the extract has a hypoglycemic property. The extract also improved protein concentration, often diminished in diabetic conditions. The enhancement of antioxidant enzyme activities, particularly catalase and superoxide dismutase, showed the extract's potential to counteract oxidative stress, which is a major contributor to the progression of diabetes [50]. Reactive oxygen species buildup occurs in tissues when there is a reduction in the activity of SOD which leads to oxidative stress. Superoxide dismutase and catalase are known to contribute significantly to antioxidant defense systems [51], and play a vital role in cellular defense mechanisms by mitigating oxidative stress caused by reactive oxygen species (ROS) [52]. In this study, the reduction in oxidative stress markers such as malondialdehyde (MDA), hydrogen peroxide, (H_2O_2), and nitric oxide (NO), along with the increase in non-protein thiol (GSH) concentrations, demonstrated the ability of TCANE to reduce oxidative damage and promote cellular health in diabetic states [53]. Malondialdehyde (MDA) is a marker of oxidative stress, formed when free radicals cause damage to lipids in cell membranes. High MDA levels indicate increased oxidative damage, which is common in diabetic conditions due to elevated glucose levels [54]. Nitric oxide (NO), a key signaling molecule, helps regulate blood vessel function by promoting vasodilation. In diabetes, NO production can become imbalanced, leading to endothelial dysfunction [55]. Oxidative stress can also reduce NO availability, further impairing blood vessel health. This imbalance contributes to complications like impaired circulation in diabetes [56]. The decrease in hydrogen peroxide concentration showed that TCANE can minimize oxidative stress, a critical factor in the management of diabetes [57]. The activities of ALT and AST were reduced significantly by the administration of TCANE compared to the STZ-untreated group. It is well-known that diabetes causes liver dysfunction by elevating blood hepatic enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST), etc. These enzymes are typically released into the bloodstream when the liver is damaged, a complication of DM [11]. The reduction in the level of these enzymes indicates that the TCANE can help to reduce liver damage [58]. This makes *Terminalia catappa* aqueous nut extract a promising candidate for further development in the treatment and management of diabetes and its associated complications.

Conclusion

This study concludes that the nut of *Terminalia catappa* aqueous nut extract (TCANE) is useful and its embedded bioactive compounds can play a significant role in improving hepatic function, glucose and lipid metabolism, and antioxidant defenses, but also reduce oxidative stress associated with diabetes mellitus.

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