

HEPATIC AND ANTIOXIDANT ENZYME ACTIVITIES, 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 a cure, and there is 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 and antioxidant enzymes, 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). We used PyRx and Biovia Discovery Studio for molecular docking studies, and SwissADME and ProTox to predict ADME/T properties. The results from this study showed that the TCANE-treated group had antioxidant enzyme [superoxide dismutase (SOD) and catalase (CAT)] activities that were significantly ($p<0.05$) higher, and hepatic enzyme [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] activities that were significantly ($p<0.05$) lower, than in the untreated diabetic rats. Additionally, we observed 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). The *in silico* studies showed strong 2D and 3D interactions between TCANE bioactive compounds (epicatechin, sapogenin, and flavone) and the selected key diabetes-related proteins. ADME/T analysis 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, *Terminalia catappa* nut, Phytochemistry, Antioxidant enzymes, Hepatic enzymes, Oxidative stress.

INTRODUCTION

Type-2 DM is the most prevalent form of diabetes mellitus (DM), accounting for roughly 90% of all cases worldwide. The International Diabetes Federation (IDF) projects that the number of people living with diabetes will climb to nearly 700 million by 2045. DM remains an incurable disorder of macromolecular metabolism, characterized by the body's diminished capacity to respond to insulin and maintain normal blood glucose levels. As a chronic,

non-communicable hyperglycemic condition, it arises from a combination of environmental and hereditary factors (Dilmurodovna, 2023), and if left unchecked, can progress to life-threatening outcomes such as coma, cardiovascular disease (including heart attack and stroke), blindness, and chronic kidney or liver damage, among other complications (Yedjou et al., 2023; Wei et al., 2022). Streptozotocin (STZ), a naturally occurring compound derived from the bacterium *Streptomyces achromogenes*, is commonly used to induce diabetes in experimental animal models such as rats and mice (Ghasemi & Jeddi, 2023). Its structural resemblance to glucose gives STZ both diabetogenic and antibacterial properties (Wan Chik et al., 2023), and it is selectively taken up by pancreatic β -cells through glucose transporter-2 (GLUT-2) (Moheb-Alian et al., 2024). Once inside these cells, STZ causes DNA fragmentation and alkylation that destroys the insulin-producing β -cells, sharply lowering insulin secretion in a pattern resembling Type 1 diabetes mellitus (T1DM), where β -cell destruction leaves insulin secretion almost completely absent (Sharma et al., 2023).

Diabetes affects nearly every major organ, though the extent of damage varies. The liver, for instance, plays a central role in regulating carbohydrate and lipid metabolism, and disruption of this role in diabetes can lead to hepatic injury (Holesh et al., 2023). In diabetes, these regulatory mechanisms are often compromised, allowing the liver to overproduce glucose because of insulin resistance or deficiency, worsening hyperglycemia (Mondal et al., 2024). The liver also produces inflammatory cytokines such as tumor necrosis factor- α and interleukins-1, 6, 8, and 12 (Das & Medhi, 2023). Enzymes including alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) participate in these hepatic processes and serve as markers of diabetes-related liver damage (Rafaqat et al., 2023). When their serum levels and activity rise, this typically signals inflammation that can interfere with insulin signaling and ultimately cause hepatic injury (Ghalandari et al., 2023).

Oxidative stress, an imbalance between free-radical generation and the body's antioxidant defenses, is another common consequence of diabetes. This redox disturbance is associated with insulin resistance, β -cell dysfunction, and impaired glucose tolerance, all of which contribute to type 2 diabetes (T2DM) (Black,

2022). When free radicals such as reactive oxygen species accumulate excessively, they can damage proteins, lipids, and DNA, disrupting normal cellular function. Antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) counter this damage (Bizoni et al., 2023): SOD converts the harmful superoxide radical (O_2^-) into the less reactive oxygen (O_2) and hydrogen peroxide (H_2O_2), and CAT subsequently breaks the resulting H_2O_2 down into water (H_2O) and oxygen (O_2), reducing its toxicity and protecting cellular components (Jomova et al., 2024). Diabetes, especially the type-2 form, is also closely tied to disturbed lipid profiles, a condition termed diabetic dyslipidemia, which is a major driver of the elevated cardiovascular risk seen in people with diabetes (Lee et al., 2023) and stems largely from insulin resistance and hyperglycemia (Piccirillo et al., 2023). Because insulin resistance impairs the body's regulation of lipid metabolism, the liver ramps up production of very low-density lipoprotein (VLDL) and triglycerides, while fat tissue simultaneously releases more free fatty acids into circulation; the liver then takes up these fatty acids and converts them into still more triglycerides and VLDL (Sedgi et al., 2024).

Because conventional antidiabetic drugs often carry unwanted side effects, researchers, including those in developed countries, are increasingly turning to locally available natural products and their by-products, which tend to have fewer side effects and a history of traditional antidiabetic use. *Terminalia catappa*, a member of the *Combretaceae* family, is known locally as Almond, Igi-furutu (Yoruba), and Baushe (Hausa) (Adomi, 2021), and its nuts are rich in protein, fat, carbohydrate, vitamins, and minerals (Chukwuma et al., 2024). Various parts of *Terminalia catappa* (bark, leaves, and fruit) have long been used medicinally, including for dermatitis, hemostasis, and fever (Habibullah et al., 2023). At the same time, the nuts have been linked to cardiovascular benefits because of their fiber, phytosterol, vitamin, mineral, antioxidant, and unsaturated fatty acid content (Dantas & Costa, 2022). Researchers have attributed much of this bioactivity to phytoconstituents such as saponins, alkaloids, glycosides, terpenes, volatile oils, steroids, and phenols (Iyekowa et al., 2023). Targeting the protein pathways involved in diabetes offers another route to lowering post-meal blood glucose by slowing carbohydrate breakdown in the small intestine (Dubey et al., 2020). α -amylase, present in saliva and pancreatic juice, breaks large starch polysaccharides into smaller, absorbable units. At the same time, α -glucosidase, located at the intestinal brush border, completes the final stages of starch and disaccharide digestion. Aldose reductase (AR) converts glucose to sorbitol, which sorbitol dehydrogenase (SDH) then converts to fructose using nicotinamide adenine dinucleotide phosphate (NADPH) via the polyol pathway (Oso & Olaoye, 2020; Olatunde et al., 2023). These proteins therefore represent viable targets for managing diabetes and its complications. Building on our ethnobotanical survey, which indicated that almond fruit and nut consumption has hypoglycemic effects, this study investigated the *in silico* inhibitory potential of secondary metabolites in *Terminalia catappa* aqueous nut extract against these protein targets, along with their effects on relevant biochemical parameters.

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). The fruits were brought to the Laboratory of the Department of Biochemistry, rinsed under running water and consumed. The seeds were dehulled to obtain the kernel, which was then rinsed, crushed, and ground using an electric blender (model No. KW-505) until smoothly milled. 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 macerated for 48 hours before filtering through Whatman filter paper grade 1. The resulting filtrate was the *Terminalia catappa* aqueous nut extract (TCANE), later stored in the refrigerator until needed.

Phytochemical Screening

The embedded phytochemical constituents of *Terminalia catappa* aqueous nut extract were screened, identified, and quantified following the procedures described by Awote et al. (2023) and Behlil et al. 2019).

High-Performance Liquid Chromatography (HPLC) Analysis of TCANE

We performed high-performance liquid chromatography (HPLC) analyses to quantify flavonoid compounds in the extracts on an Agilent Technologies 1200 series HPLC with a UV detector. Chromatography was performed on a reversed-phase prepacked Hypersil BDS C18 column (150 × 4.6 mm, 5 μ m particle size) at 25 °C. The mobile phase consisted of A (0.1% formic acid in water) and B (HPLC-grade acetonitrile) at 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. We used BioVIVA Discovery Studio 2021 to convert them to .pdb format. We retrieved the canonical Simplified Molecular Input Line Entry System (SMILES) notation of the ligands from the PubChem database. At the same time, Open Babel (<https://openbabel.readthedocs.io/en/latest/Forcefields/mmmff94.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>). We modeled these proteins using a deposited *Homo sapiens* crystal structure as a reference. We used BioVIVA Discovery Studio 2021 to remove water molecules and heteroatoms to prepare (purify) the proteins. We then saved the purified protein structures in PDB format for subsequent use. PyRx was used for site-specific molecular docking studies, and the co-crystallized ligands for the target proteins, along with acarbose and tolrestat (standard diabetes medications), were used. The study used binding free energy values, and the ligand molecules were then sorted in order of increasing binding energy.

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 Lagos State University Research Ethics Committee approved the study protocol under LASU/23/REC/055.

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 iced-cold citrate buffer (pH 4.5) and administered to overnight fasted rats. The rats were then fed 20% glucose (Unique Pharmaceuticals, Sango Otta, Ogun State, Nigeria) for 6 hours after induction, followed by 5% glucose for the next 24 hours to prevent hypoglycemia. Gradual hyperglycemia was confirmed on the 3rd day (72 hours) post-induction, but all rats became consistently hyperglycemic and stable on the 7th day post-induction. By day 7, rats with fasting blood glucose of >200 mg/dL were considered diabetic and included in this study. Treatments began on day 4 post-STZ induction and continued for 28 days. We assessed the diabetic state by measuring blood glucose concentrations with an Accu-Chek glucometer after collecting blood from the rats' tails. The induction success rate was above 80%.

Experimental Design

The twenty-four (24) Wistar rats used in this study were randomly divided into four groups (A–D) of 6 rats each, but the group mean weights 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 of distilled water for 28 days.

Group C = Diabetic rats orally administered 300 mg/kg body weight/day of *T. catappa* aqueous nut extract (TCANE), dissolved in distilled water to a dosing volume of 2 mL/kg body weight, 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 animals had free access to feed and clean water throughout the experimental period. We recorded the animals' initial weights and then weekly. Whole blood was collected by the tail-tipping method

between 8:00 and 9:00 hours. We measured fasting blood glucose (FBG) on days 1, 7, 14, 21, and 28 using the glucose oxidase method (Trinder, 1969) with the AccuChek Active Glucose monitor.

Anesthetization and blood sample collection

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

Protein concentration in the various samples was determined using the Lowry method (Lowry et al., 1951) with a few modifications. Briefly, 0.5 mg/mL bovine serum albumin was mixed with 25 µL of each group's serum, 400 µL of solution C, and 40 µL of Folin C. The resulting solution was mixed well and allowed to stand. Measure absorbance at 650 nm against a blank of 60 µL 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 (CuSO₄.5H₂O) and 2% sodium potassium tartrate).

Lipid Profile Estimation

Serum samples from treated Wistar rats were used to analyze lipid profiles [triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-c)] using standard diagnostic test kits (Randox Laboratories, Crumlin, U.K.). Plasma low-density lipoprotein cholesterol (LDL-c) and very-low-density lipoprotein cholesterol (VLDL-c) were estimated using the Friedewald formula: LDL-c = [TC - (HDL-c + TG/5)], and VLDL-c = TAG/5, respectively.

Determination of Catalase (CAT) Activity

We evaluated CAT activity using the procedure of Claiborne (1985), with slight modifications. Briefly, we mixed hydrogen peroxide (1180 µL of 19 mM solution) with 20 µL of each serum sample and then inverted. We determined the absorbance of each sample using a UV spectrophotometer at 240 nm. We recorded the change in absorbance every 10 seconds for 2 min. We calculated catalase activity and expressed it 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 (Misra & Fridovich, 1972). Briefly, 0.1 mL of the serum was added to 2.5 mL of 0.05 M phosphate buffer (pH 7.8). Finally, add 0.3 mL of adrenaline solution (0.059%) at the point of absorbance measurement, and read absorbance at 750 nm every 15 seconds for 1 minute 30 seconds. SOD activity was calculated and

expressed as $\mu\text{mol}/\text{mg}$ protein.

Determination of Glutathione (GSH) Levels

Glutathione (non-protein thiol) level was evaluated using Ellman's reagent (DTNB) method (Jollow et al., 1974). Precipitate the serum with sulfo-salicylic acid (4%) at a 1:1 ratio and keep it at room temperature (26°C) for 1 hour before centrifuging at 5000 rpm for 10 minutes at 4°C . Then, mix the supernatant (100 μL) with 550 μL of 0.1 M phosphate buffer and 100 μL of DTNB. Read the absorbance at 412 nm, and express the results as μmol GSH/mg protein.

Determination of Hydrogen Peroxide (H_2O_2) Levels

Hydrogen peroxide level was determined according to the method (Wolff, 1994). Briefly, mix 10 mL xyleneol 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 mixed with 10 μL of serum and vortexed until it foamed. After incubation for 30 minutes at room temperature (26°C), a pale pink complex formed, and absorbance was read against a blank (distilled water) at 560 nm. The concentration of 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 concentration.

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 commercial diagnostic kits (Randox, U.K.) according to the manufacturer's instructions. We measured each enzyme activity spectrophotometrically at 340 nm and expressed results as U/l.

Statistical Analysis

Results were presented as mean $\pm$ standard error of the mean (SEM). We used GraphPad Prism (version 5.0) (GraphPad Prism Software Inc., San Diego, CA, USA) to analyze the data. 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 ($p < 0.05$).

RESULTS

Tables 1 and 2 show the phytochemical constituents 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 the comprehensive polyphenolic bioactive compounds in Terminalia Catappa aqueous nut extract, as 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

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	$\text{C}_{31}\text{H}_{28}\text{O}_{12}$	10.46	Flavonoid
2	Gallic acid	170.12	$\text{C}_7\text{H}_6\text{O}_5$	623	Phenolic acid
3	Naringin	580.54	$\text{C}_{27}\text{H}_{32}\text{O}_{14}$	12.71	Flavonoid
4	Catechin	290.27	$\text{C}_{15}\text{H}_{14}\text{O}_6$	25.18	Flavonoid
5	Quinine	3242	$\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2$	7.18	Alkaloid
6	Chlorogenic acid	3531	$\text{C}_{16}\text{H}_{18}\text{O}_9$	53.47	Phenolic acid
7	Flavan-3-ol	456.39	$\text{C}_{15}\text{H}_{14}\text{O}_2$	42.276	Flavonoid
8	Caffeic acid	180.16	$\text{C}_9\text{H}_8\text{O}_4$	46.15	Phenolic acid
9	Anthocyanin	207.30	$\text{C}_{15}\text{H}_{11}\text{O}$	6.016	Flavonoid
10	Ellagic acid	302.19	$\text{C}_{14}\text{H}_6\text{O}_8$	98.82	Polyphenol
11	Ribalinidine	275.30	$\text{C}_{31}\text{H}_{28}\text{O}_{12}$	10.366	Alkaloid
12	Naringenin	272.26	$\text{C}_{15}\text{H}_{12}\text{O}_5$	12.970	Flavonoid
13	Rutin	610.52	$\text{C}_{27}\text{H}_{30}\text{O}_{16}$	45.06	Flavonoid glycoside
14	Sparteine	2338	$\text{C}_{15}\text{H}_{26}\text{N}_2$	15.460	Alkaloid
15	Quercitrin	448.38	$\text{C}_{20}\text{H}_{21}\text{O}_{11}$	79.65	Flavonoid glycoside
16	Sapogenin	486.68	$\text{C}_{30}\text{H}_{46}\text{O}_5$	17.963	Saponin
17	Isoquercitrin	4638	$\text{C}_{21}\text{H}_{20}\text{O}_{12}$	52.03	Flavonoid glycoside
18	Phenol	911	$\text{C}_6\text{H}_6\text{O}$	20.316	Phenolics
19	Quercetin	302.24	$\text{C}_{15}\text{H}_{10}\text{O}_7$	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	Resveratrol	228.25	$C_{14}H_{12}O_3$	120	Polyphenol

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	Resveratrol	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 interactions 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 interactions of Phe649 and Tyr481 with α -glucosidase, forming a π - π T-shaped bond; Leu678 formed a π -alkyl interaction, while Asp616 formed a π -anion interaction. Additionally, Met519 formed a Pi-sulfur interaction, and Leu650, Arg600, Asp518, Trp376, Leu677, and Ser676 formed van der Waals interactions with flavone. Figure 1c shows the complex interaction of sapogenin with the human sorbitol dehydrogenase. The 2D structure showed that Pro98, Cys105, Phe138, and Pro304

formed alkyl and pi-alkyl interactions 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 interactions with sapogenin, the ligand with the best binding affinity. Figure 1d illustrates the complex interaction of epicatechin with aldose reductase. The 2D structure showed that Tyr48, Trp20 and Tyr209 formed Pi-Pi Stacked and Pi-Pi T-shaped interactions; Pro211 formed a Pi-alkyl interaction; and Cys298 formed a Pi-sulfur interaction. Lys262, Lys21, Ile260 and Ser214 formed carbon-hydrogen and pi-donor hydrogen-bond interactions. Additionally, Trp20, Gln183, Lys77, Ser210 and Asp216 formed conventional hydrogen bonds, while Pro215, Leu212, Pro261, Asp43, Trp111 and Lys21 formed van der Waals interactions with epicatechin, the ligand with the best binding

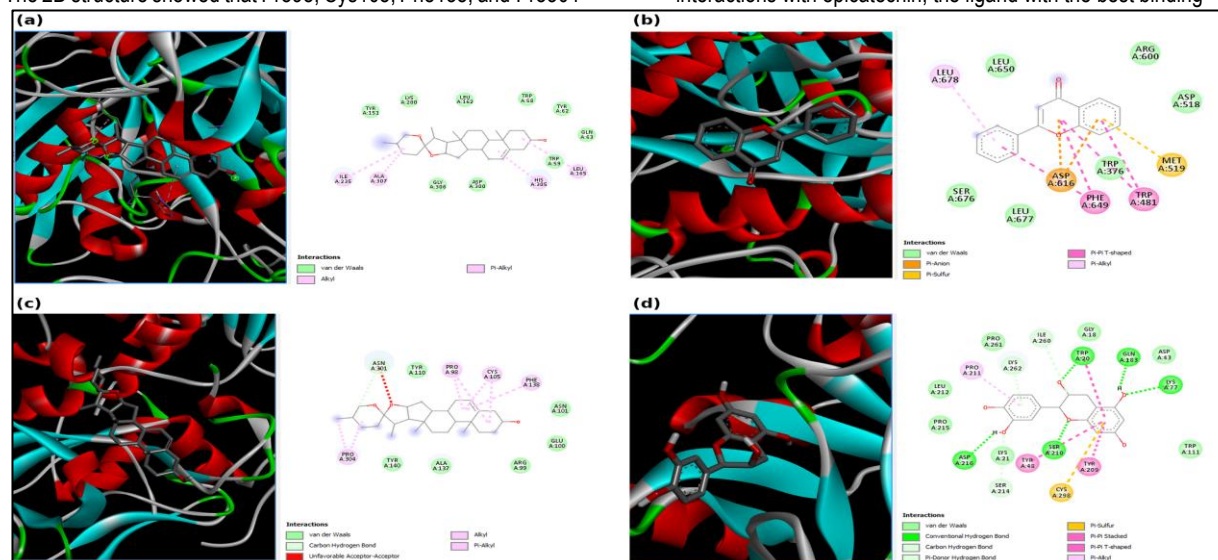


Figure 1: 3D (left) and 2D (right) structures and interactions of the amino acids of (a) α -amylase docked with sapogenin, (b) α -glucosidase docked with flavone, (c) sorbitol dehydrogenase docked with sapogenin, and (d) aldose reductase docked with epicatechin.

Tables 4.1 to 4.6 illustrate 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 physicochemical 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 bonds. Table 4.2 reveals the assessment of lipophilicity properties and water solubility of the top ten ligands. Sapogenin showed the highest log P value (5.02). Meanwhile, other ligands are also within the range away from zero (0),

indicating their good lipophilicity properties in drug discovery, except chlorogenic acid with a Log P of -0.39. Table 4.3 shows the pharmacokinetic properties of the best ten ligands; nine have high gastrointestinal absorption, except catechin, which has low gastrointestinal absorption. Table 4.4 shows that the top ten ligands have a good bioavailability score, since a minimum bioavailability score of 0.10 is required for a compound to be considered 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 positive pan-assay interference compounds (PAINS). In contrast, epicatechin, kaempferol, flavone and catechin showed positive lead-likeness. Table 4.6 shows the toxicity of the best ten ligands.

Table 4.1: Physicochemical 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	222.4	1	2	0
Anthocyanin	C ₁₅ H ₁₁ O ₊	207.25	1	1	0
Chlorogenic acid	C ₁₆ H ₁₈ O ₉	353.1	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
Resveratrol	C ₁₄ H ₁₂ O ₃	228.24	2	2	3
Steroids	C ₁₈ H ₂₆ O ₂	274.0	0	2	1
Caffeic acid	C ₉ H ₈ O ₄	180.16	2	4	3
*Acarbose	C ₂₅ H ₄₃ NO ₁₈	645.60	9	19	14
*Tolrestat	C ₁₆ H ₁₄ F ₃ NO ₃ S	357.35	6	6	1

*denotes standard drugs incorporated in this study; MW denotes molecular weight; NRB denotes the number of rotatable bonds; NHBA denotes the number of rotatable hydrogen bond acceptors; NHBD denotes the number of hydrogen bond donors.

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

Ligands	Log S (ESOL)	Class	Log P _{ow} (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
Resveratrol	-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

Table 4.3: Pharmacokinetic 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

Resveratrol	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 the 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
Resveratrol	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.

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
Resveratrol	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
Resveratrol	-	-	-	-	-	+	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.

Table 5 shows the effect of TCANE on glucose level and protein concentration. Blood glucose levels were significantly ($p < 0.05$) lower, and protein concentration significantly ($p < 0.05$) higher, in the TCANE-treated group than in 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 $\pm$ 1.93 ^a	0.31 $\pm$ 0.06 ^a
2	STZ	201.2 $\pm$ 23.77 ^b	0.15 $\pm$ 0.03 ^b
3	STZ + TCANE 300 mg	101.8 $\pm$ 3.21 ^c	0.22 $\pm$ 0.15 ^c
4	STZ + Glb	71.74 $\pm$ 1.15 ^a	0.29 $\pm$ 0.06 ^c

Data show mean $\pm$ SEM (n=5), and values with different letters 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. Superoxide dismutase (SOD) and catalase (CAT) activities were significantly ($p < 0.05$) higher in the TCANE-treated group than in

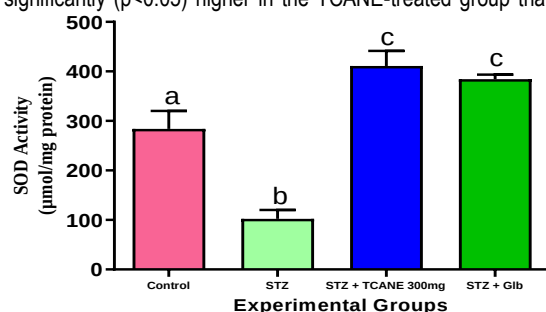


Fig. 2: Effect of *Terminalia catappa* aqueous nut extract (TCANE) on Antioxidant Activities in STZ-Induced Diabetic Wistar rats. Each bar represents the mean $\pm$ SEM (n=5), and bars with different letters are statistically significantly different at $p < 0.05$. STZ = Streptozotocin, TCANE= *Terminalia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

the STZ-untreated group. Similarly, the standard drug, glibenclamide, produced significantly ($p < 0.05$) higher SOD and CAT activities than the STZ-untreated group.

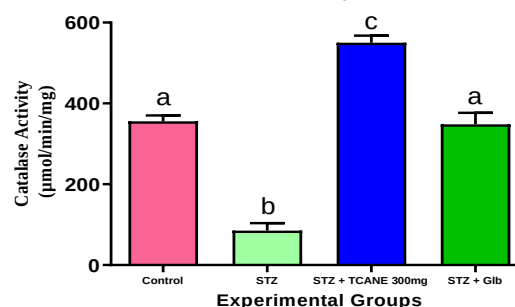


Figure 3 shows the effect of TCANE on liver enzyme activities. Alanine transferase (ALT) and aspartate transferase (AST) activities were significantly ($p < 0.05$) lower in the TCANE-treated

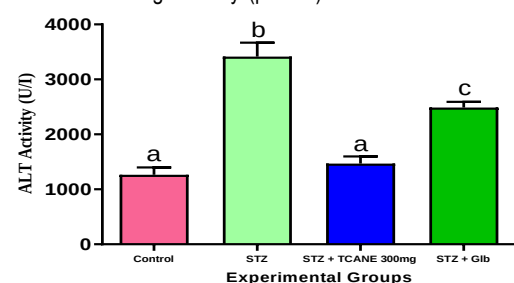


Figure 3: Effect of *Terminalia catappa* aqueous nut extract (TCANE) on Liver enzyme activities in STZ-Induced diabetic Wistar rats. Each bar represents the mean $\pm$ SEM (n=5), and bars with different letters are statistically significantly different at $p < 0.05$. STZ = Streptozotocin, TCANE= *Terminalia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

group than in the STZ-untreated diabetic and glibenclamide-treated rats.

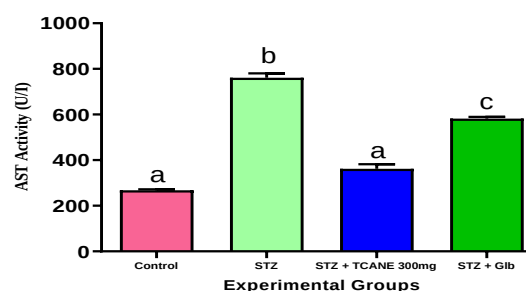


Table 6 shows the effect of TCANE on oxidative stress indices. Hydrogen peroxide (H_2O_2), nitric oxide (NO), and malondialdehyde (MDA) concentrations were significantly ($p < 0.05$) lower, and non-

protein thiol (GSH) concentration significantly ($p < 0.05$) higher, in the TCANE 300 mg/kg-treated group than in 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 $\pm$ 0.01 ^a	0.358 $\pm$ 0.00 ^a	309.8 $\pm$ 13.78 ^a	63.02 $\pm$ 2.745 ^a
2	STZ	0.192 $\pm$ 0.04 ^b	0.942 $\pm$ 0.01 ^b	171.5 $\pm$ 8.21 ^b	197.4 $\pm$ 0.8650 ^b
3	STZ + TCANE 300 mg	0.054 $\pm$ 0.02 ^a	0.338 $\pm$ 0.00 ^a	392.1 $\pm$ 16.71 ^c	58.54 $\pm$ 3.775 ^a
4	STZ + Glb	0.067 $\pm$ 0.00 ^a	0.446 $\pm$ 0.00 ^c	288.5 $\pm$ 6.830 ^a	90.61 $\pm$ 2.120 ^c

Hepatic and Antioxidant Enzyme Activities, Lipid Profile, and Oxidative Stress Levels in Streptozotocin-induced Diabetic Rats Treated with *Terminalia catappa* Aqueous Nut Extract

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

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

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 $\pm$ 0.08 ^a	196.2 $\pm$ 2.81 ^a	285.4 $\pm$ 9.92 ^a	66.75 $\pm$ 0.29 ^a	39.25 $\pm$ 0.56 ^a
2.	STZ	559.2 $\pm$ 39.62 ^b	492.6 $\pm$ 0.82 ^b	117.0 $\pm$ 1.04 ^b	343.68 $\pm$ 11.28 ^b	98.52 $\pm$ 0.16 ^b
3.	STZ + TCANE 300 mg	295.6 $\pm$ 0.38 ^c	212.4 $\pm$ 0.66 ^a	196.5 $\pm$ 3.81 ^c	56.62 $\pm$ 1.03 ^a	59.12 $\pm$ 0.13 ^c
4.	STZ + Glib	389.4 $\pm$ 6.13 ^a	237.8 $\pm$ 14.07 ^a	171.8 $\pm$ 5.73 ^c	139.72 $\pm$ 4.64 ^c	77.88 $\pm$ 2.27 ^c

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

DISCUSSION

A growing body of recent literature documents the broad pharmacological profile of *Terminalia catappa*, particularly its antioxidant, anti-inflammatory, and antidiabetic effects (Ariantari et al., 2023), largely attributed to phytochemical constituents that mitigate oxidative stress and thereby support liver function (Umoren et al., 2023; Habibullah et al., 2023). Consistent with this, our phytochemical screening of the aqueous nut extract identified phenols, flavonoids, tannins, alkaloids, saponins, and steroids, compounds known to influence the oxidative and metabolic disturbances characteristic of diabetes. Phenol, the most abundant of these constituents, is thought to contribute to diabetes management through its strong antioxidant capacity, helping curb the oxidative stress that drives disease progression (Arabshomali et al., 2023). Tannins have similarly been shown to lower reactive oxygen species (ROS) and malondialdehyde (MDA) while boosting SOD, CAT, and glutathione (GSH) activity, and to suppress IL-6, IL-8, and TNF- α expression, reinforcing the extract's combined antioxidant and anti-inflammatory action (Sahakyan et al., 2022). Flavonoids, in turn, have been reported to restore impaired insulin signaling in non-classical tissues such as the kidney, brain, and endothelium (Manavi et al., 2021). Vascular complications of diabetes are closely tied to reduced endothelial nitric oxide (NO) synthesis under hyperglycemic conditions, stemming from impaired IR/AKT/eNOS signaling and resulting in abnormal aortic relaxation (Chis et al., 2023).

Diabetes complications are commonly managed by targeting pharmacotherapeutic proteins such as α -amylase, α -glucosidase, aldose reductase (AR), and sorbitol dehydrogenase (SDH), whose inhibition slows postprandial glucose absorption (Özaslan et al., 2019; Demir et al., 2020). Molecular docking has become an increasingly valuable computational tool in drug development (Sahoo et al., 2022), offering insight into molecular recognition both energetically, through binding-affinity prediction, and structurally, through identification of likely binding modes (Stanzione et al., 2021). In this study, we retrieved twenty-eight (28) ligands identified by HPLC analysis of TCANE (Table 2) from PubChem. We docked them against the catalytic sites of four selected diabetes-related proteins (α -amylase, α -glucosidase, AR, and SDH). Epicatechin emerged as the top-performing ligand, with a binding energy of -9.8 kcal/mol (Table 3), a result consistent with its known antioxidant and anti-inflammatory activity as a natural flavonoid (Qu et al., 2021). The remaining top ligands (kaempferol, flavone, anthocyanin, chlorogenic acid, catechin, sapogenin,

resveratrol, steroids, and caffeic acid) are likewise natural polyphenols documented in traditional medicine for anti-inflammatory, antioxidative, anti-atherosclerotic, hypoglycemic, and hypolipidemic effects (Y. Yang et al., 2022; Reddy, 2021). Water-solubility and lipophilicity data in Table 4.2 show that epicatechin is soluble in both aqueous and lipid environments, an amphipathic property that could support its formulation into lipid-soluble nanoparticles able to cross cell membranes, a feature relevant to treating conditions such as Alzheimer's disease, schizophrenia, and dementia, and that may also aid its transport through the bloodstream and stability once it reaches its binding target. Pharmacokinetic predictions in Table 4.3 indicate high gastrointestinal (GI) absorption for nine of the ten leading ligands, with chlorogenic acid the exception. High GI absorption implies that, upon ingestion, a compound is efficiently taken up into systemic circulation, a property generally associated with favorable bioavailability and pharmacokinetics for orally administered drugs. Blood-brain barrier permeability was predicted for five of the ten ligands (flavone, anthocyanin, sapogenin, resveratrol, and steroids), suggesting potential application in central nervous system (CNS) disorders such as Alzheimer's disease (AD), schizophrenia, and dementia (Kadry et al., 2020). Epicatechin, anthocyanin, and catechin, meanwhile, were flagged as P-glycoprotein substrates; since P-glycoprotein is a transmembrane efflux pump that actively removes substances from cells, this raises the possibility of limited cellular absorption for these compounds. Among the cytochrome P450 predictions, only kaempferol and resveratrol were flagged as CYP3A4 inhibitors; flavone, anthocyanin, and resveratrol as CYP1A2 inhibitors; flavone alone as a CYP2C19 inhibitor; resveratrol alone as a CYP2C9 inhibitor; and kaempferol and anthocyanin as CYP2D6 inhibitors.

Drug-likeness evaluation in Table 4.4 shows that the ten leading ligands achieved bioavailability scores between 0.11 and 0.56, a range regarded as favorable for candidate drugs (M. Singh et al., 2023). Chlorogenic acid and acarbose each recorded a single Egan and Veber violation, pointing to reduced permeability and oral bioavailability, while chlorogenic acid, sapogenin, and acarbose also violated the Ghose criteria. Passing the Ghose filter generally indicates favorable interaction with biological targets, whereas passing the Muegge filter suggests drug-likeness and the ability to cross the membrane bilayer without incurring toxicity.

Table 4.5 shows that epicatechin, chlorogenic acid, catechin, and anthocyanin each triggered a single pan-assay interference

compound (PAINS) alert, indicating the presence of a structural feature commonly associated with false positives in biochemical assays; such compounds are flagged because they can interfere non-specifically with assay readouts through mechanisms unrelated to genuine target binding, potentially reflecting non-specific binding, assay artifacts, or chemical reactivity. This does not necessarily rule out their value; compounds with such features can still serve as promising starting points, or “lead” compounds, for drug development, since lead-like molecules tend to be smaller, simpler, and easier to modify chemically than fully drug-like compounds. Epicatechin, kaempferol, catechin, and steroids each showed positive lead-likeness in this analysis. Synthetic accessibility scores for the top ten ligands ranged from roughly 1.81 to 6.94, with sapogenin the easiest to synthesize. Kaempferol, flavone, and steroids returned zero Brenk alerts, meaning they lack the structural features, such as reactive or unstable functional groups or highly lipophilic moieties, typically linked to toxicity, metabolic instability, or poor drug-likeness.

Toxicity predictions from SwissADME, admetSAR, and ProTox (Table 4.6) indicate that epicatechin, sapogenin, catechin, and caffeic acid are unlikely to cause liver or kidney toxicity, unlike anthocyanin, which showed a positive signal for both. Kaempferol, flavone, chlorogenic acid, resveratrol, and steroids also showed some indication of kidney and/or liver toxicity. None of the ligands were flagged as carcinogenic except sapogenin. Chlorogenic acid, sapogenin, and caffeic acid showed no indication of eye or skin irritation, whereas kaempferol, flavone, anthocyanin, resveratrol, and sapogenin did. Most ligands showed a positive signal for respiratory, reproductive, and mitochondrial toxicity, except anthocyanin, which was negative for all three, and flavone, which was also negative for mitochondrial toxicity. Kaempferol fell into toxicity class II (moderately toxic and irritating); flavone, anthocyanin, chlorogenic acid, resveratrol, steroids, and caffeic acid fell into class III (slightly toxic and irritating); and epicatechin, catechin, and sapogenin fell into class IV, indicating negligible toxicity or irritation. Figure 1a-d shows the amino acid residues and bond types stabilizing each protein-ligand complex. The α -amylase active site comprises Ile235, Ala307, His305, Tyr151, Lys200, Leu162, Trp58, Tyr62, Gln63, Trp59, Asp300, and Gly306; α -glucosidase comprises Phe649, Try481, Leu678, Asp616, Met519, Leu650, Arg600, Asp518, Trp376, Leu677, and Ser676; sorbitol dehydrogenase comprises Pro98, Cys105, Phe138, Pro304, Asn301, Tyr110, Asn101, Glu100, Arg99, Ala137, and Tyr140; and aldose reductase comprises Tyr48, Trp20, Tyr209, Pro211, Cys298, Lys262, Lys21, Ile260, Ser214, Trp20, Gln183, Lys77, Ser210, Pro215, Leu212, Pro261, Asp43, Trp111, and Lys21. Each residue type plays a distinct functional role: aspartate and glutamate coordinate metal ions such as calcium, zinc, and magnesium, while asparagine and glutamine support substrate binding via hydrogen bonding (Jindal et al., 2023). Histidine acts as a universal acid/base in both binding and catalysis (Bordeleau et al., 2024), tyrosine contributes to substrate stabilization and hydrogen bonding (C. Yang et al., 2022), and serine and threonine both function as nucleophiles during catalysis (Urlacher et al., 2024; Cuesta et al., 2020). In our study, epicatechin recorded the lowest (most favorable) binding energy of all compounds tested, including the reference antidiabetic drugs acarbose and tolrestat, and, together with sapogenin, formed specific bonds with active-site residues that may signal effective enzyme inhibition. These findings support the plant's traditional antidiabetic reputation, which

may derive from the phytochemicals identified in this extract.

Turning to the *in vivo* findings, treatment of STZ-induced diabetic rats with *Terminalia catappa* aqueous nut extract (TCANE) produced significant reductions in blood glucose relative to untreated diabetic rats, pointing to a hypoglycemic effect consistent with the broader antidiabetic profile reported for *Terminalia catappa* and other polyphenol-rich plant extracts in STZ-induced rat models (Ariantari et al., 2023; Reddy, 2021), alongside improved protein concentration, which is typically depressed in diabetic states. The extract's enhancement of antioxidant enzyme activity, particularly catalase and superoxide dismutase, reflects its capacity to counter the oxidative stress that drives diabetes progression (Akbari et al., 2022), since reduced SOD activity allows reactive oxygen species to accumulate in tissue and both enzymes are central to cellular antioxidant defense (Tekin & Seven, 2022; Anwar et al., 2024). Here, TCANE lowered oxidative stress markers, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and nitric oxide (NO), while raising non-protein thiol (GSH) levels, together indicating reduced oxidative damage and improved cellular health under diabetic conditions (Demirci-Cekic et al., 2022). MDA, formed when free radicals damage membrane lipids, is a standard oxidative-stress marker whose elevation reflects the oxidative damage common in hyperglycemic states (Azeez et al., 2023). NO, a key vasodilatory signaling molecule, tends to become dysregulated in diabetes, contributing to endothelial dysfunction (Cheng et al., 2023); oxidative stress further reduces NO availability, compounding circulatory complications (A. Singh et al., 2022). The observed drop in hydrogen peroxide concentration therefore suggests that TCANE helps limit oxidative stress, a central concern in diabetes management (Nhung, 2024). TCANE administration also significantly reduced ALT and AST activity relative to the untreated group, a hepatoprotective pattern comparable to that reported for other antioxidant-rich plant extracts, such as mulberry leaf extract, against oxidative liver injury in rat models (Yu et al., 2022). Because diabetes-related liver damage typically elevates these hepatic enzymes as they leak into the bloodstream (Rafaqat et al., 2023), their reduction here suggests that TCANE helps protect against liver injury. TCANE treatment likewise significantly lowered total cholesterol, triglycerides, LDL, and VLDL while raising HDL, mirroring the lipid-normalizing effect reported for other antioxidant-rich plant extracts in STZ-induced diabetic rats and consistent with the mechanistic link between insulin resistance, hyperglycemia, and diabetic dyslipidemia described earlier (Lee et al., 2023; Piccirillo et al., 2023; Sedgi et al., 2024); by curbing oxidative stress and improving insulin sensitivity, TCANE may reduce hepatic VLDL/triglyceride overproduction and the compensatory release of free fatty acids that drive this dyslipidemic pattern. Taken together, these findings reinforce *Terminalia catappa* aqueous nut extract's promise as a candidate for further development in the treatment and management of diabetes and its associated complications.

Conclusion

Taken together, these findings indicate that *Terminalia catappa* aqueous nut extract (TCANE) and its bioactive constituents can meaningfully support hepatic function, glucose and lipid metabolism, and antioxidant defenses, while reducing the oxidative stress associated with diabetes mellitus.

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