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Attenuation of hyperglycemia-associated dyslipidemic, oxidative, cognitive, and inflammatory crises via modulation of neuronal ChEs/ NF-κB/COX-2/NOx, and hepatorenal functional deficits by the *Tridax procumbens* extract

Itam Ako Hogan [a,1], Yu-Cheng Kuo [b,c,1], Asmau N. Abubakar [d], Bashir Lawal [e,f], Abdulhakeem R. Agboola [g], Halimat Yusuf Lukman [h], Sunday Amos Onikanni [i,j], Femi Olawale [k], Adewale Oluwaseun Fadaka [l], Yunusa O. Ibrahim [d], Shukurat B. Babalola [m], Gaber El-Saber Batiha [n], Sarah M. Albogami [o], Mohammed Alorabi [o], Michel De Waard [p,q,r], Hsu-Shan Huang [e,f,s,t,u,*]

[a] *Department of Internal Medicine, University of Calabar, Calabar, Cross River State, Nigeria*
[b] *Department of Pharmacology, School of Medicine, College of Medicine, Taipei Medical University, Taipei 11031, Taiwan*
[c] *School of Post-baccalaureate Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung 40402, Taiwan*
[d] *Department of Biochemistry, Federal University of Technology, Minna, Nigeria*
[e] *PhD Program for Cancer Molecular Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University and Academia Sinica, Taipei 11031, Taiwan*
[f] *Graduate Institute for Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei 11031, Taiwan*
[g] *Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Nigeria*
[h] *Department of Chemical Sciences, Biochemistry Unit, College of Natural and Applied Sciences, Summit University, Offa, PMB 4412, Nigeria*
[i] *Department of Chemical Sciences, Biochemistry Unit, Afe-Babalola University, Ado-Ekiti, Ekiti State, Nigeria*
[j] *College of Medicine, Graduate Institute of Biomedical Sciences, China Medical University, Taiwan*
[k] *Nano Gene and Drug Delivery Group, University of Kwazulu Natal, South Africa*
[l] *University of the Western Cape, Department of Biotechnology, Belleville, South Africa*
[m] *Department of Biochemistry, Federal University of Technology, Minna, Nigeria*
[n] *Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, AlBeheira, Egypt*
[o] *Department of Biotechnology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia*
[p] *Smartox Biotechnology, 6 rue des Platanes, 38120 Saint-Egrève, France*
[q] *L'institut du thorax, INSERM, CNRS, UNIV NANTES, F-44007 Nantes, France*
[r] *Université de Nice Sophia-Antipolis, LabEx "Ion Channels, Science & Therapeutics", F-06560 Valbonne, France*
[s] *Graduate Institute of Medical Sciences, National Defense Medical Center, Taipei 11490, Taiwan*
[t] *School of Pharmacy, National Defense Medical Center, Taipei 11490, Taiwan*
[u] *PhD Program in Drug Discovery and Development Industry, College of Pharmacy, Taipei Medical University, Taipei 11031, Taiwan*

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ABSTRACT

Tridax procumbens (cotton buttons) is a flowering plant with a medicinal reputation for treating infections, wounds, diabetes, and liver and kidney diseases. The present research was conducted to evaluate the possible protective effects of the *T. procumbens* methanolic extract (TPME) on an experimentally induced type 2 diabetes rat model. Wistar rats with streptozotocin (STZ)-induced diabetes were randomly allocated into five groups of five animals each, viz., a normal glycemic group (I), diabetic rats receiving distilled water group (II), diabetic rats with 150 (III) and 300 mg/kg of TPME (IV) groups, and diabetic rats with 100 mg/kg metformin group (V). All treatments were administered for 21 consecutive days through oral gavage. Results: Administration of the

Abbreviations: ABTS, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid; CAT, catalase; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GSH, reduced glutathione; HPLC, high-performance liquid chromatography; LPO, lipid peroxidation; MDA, malonaldehyde; SOD, superoxide dismutase; STZ, streptozotocin; TBA, 2-thiobarbituric acid.

* Corresponding author at: PhD Program for Cancer Molecular Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University and Academia Sinica, Taipei 11031, Taiwan.
E-mail addresses: bashirlawal12@gmail.com (B. Lawal), huanghs99@tmu.edu.tw (H.-S. Huang).
[1] These authors contributed equally to this work.

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T. procumbens extract to diabetic rats significantly restored alterations in levels of fasting blood glucose (FBG), body weight loss, serum and pancreatic insulin levels, and pancreatic histology. Furthermore, *T. procumbens* significantly attenuated the dyslipidemia (increased cholesterol, low-density lipoprotein-cholesterol (LDL-C), triglycerides, and high-density lipoprotein (HDL) in diabetic rats), serum biochemical alterations (alanine transaminase (ALT), aspartate transaminase (AST), alanine phosphatase (ALP), blood urea nitrogen (BUN), creatinine, uric acid, and urea) and full blood count distortion in rats with STZ-induced diabetes. The TPME also improved the antioxidant status as evidenced by increased superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and decreased malondialdehyde (MDA); and decreased levels of cholinesterases (acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)), and proinflammatory mediators including nuclear factor (NF)-κB, cyclooxygenase (COX)− 2, and nitrogen oxide (NOx) in the brain of rats with STZ-induced diabetes compared to rats with STZ-induced diabetes that received distilled water. However, TPME treatment failed to attenuate the elevated monoamine oxidases and decreased dopamine levels in the brain of rats with STZ-induced diabetes. Extract characterization by liquid chromatography mass spectrometry (LC-MS) identified isorhamnetin (retention time (RT)= 3.69 min, 8.8%), bixin (RT: 25.06 min, 4.72%), and lupeol (RT: 25.25 min, 2.88%) as the three most abundant bioactive compounds that could be responsible for the bioactivity of the plant. In conclusion, the TPME can be considered a promising alternative therapeutic option for managing diabetic complications owing to its antidiabetic, antihyperlipidemic, antioxidant, and anti-inflammatory effects in rats with STZ-prompted diabetes.

1. Introduction

Diabetes mellitus (DM), a metabolic disease, is associated with a defect in the pancreatic production of insulin or lack of insulin sensitivity/activity that respectively leads to type 1 or type 2 DM (T2DM) [1]. T2DM was reported to be the most common type affecting a very large percentage of the population [2]. It is symptomized by elevated blood and urine glucose levels, excessive urination, sweating, dehydration, and several tissue complications [3,4]. According to IDF, 6.7 million deaths were recorded in 2021 as a result of diabetes [5]. On average, 1 in 10 adults has diabetes totaling about 537 million cases globally. The number is projected to increase to 643 million by 2030 and 783 million by 2045 [5]. In Africa, the rate of an undiagnosed diabetes condition is about 54% with 416,000 deaths recorded [5]. Undiagnosed diabetes or improper management can lead to chronic diabetic conditions which are accompanied by several tissue/organ complications such as retinopathies, hepato-nephropathies, and encephalopathies [6]. The liver as the central metabolic organ becomes functionally impaired due to any metabolic disorder [7], while the kidneys become thick, scarred, and leaky in diabetic nephropathy [8].

Oxidative stress plays important implicative roles in the development of vascular complications in T2DM [9]. Increased free radical formation in diabetes has been attributed to non-enzymatic glycation of proteins, increased lipid peroxidation (LPO), and glucose oxidation [10–12]. During oxidative metabolism by the mitochondrion electron transport chain, a component of the utilized oxygen is reduced to water, and a free radical, known as the superoxide anion (O), which can also be converted into other reactive oxygen species (ROS) such as $ONOO^-$, OH, and H_2O_2 [13]. These free radicals can further modulate insulin signaling and induce insensitivity/resistance, a major risk factor for T2DM [14]. The build-up of free radicals in turn transfers their free unpaired electrons and causes oxidation of cellular macromolecules, including lipids, proteins, and nucleic acids [11]. It also modulates several intracellular signaling pathways [12] to promote insulin resistance (IR), pancreatic β cell damage, and the development of DM complications including coronary artery disease, neuropathies, nephropathies, retinopathies, and stroke.

Inflammation is an important pathophysiological mechanism of DM and its complications, including diabetic nephropathy (DN) [15,16]. Chronic inflammation can cause cellular injury, and impair antioxidant defense systems, resulting in provoking oxidative stress [17]. Besides the damaging effect on cellular macromolecules, excessive ROS can activate several redox-sensitive molecules such as nuclear factor (NF)-κB, resulting in the release of proinflammatory mediators, cellular dysfunction, and injury [18]. Thus, oxidative stress, activation of NF-κB, and upregulation of proinflammatory mediators were observed in experimental diabetes and DN [19,20]. This interplay between oxidative and inflammatory responses plays a pivotal role in DN [21], and constitutes therapeutic strategies for preventing disease progression.

Conventional drugs that are used to treat and manage diabetes are costly, not readily available, and not completely effective [22]. Therefore, due to the ease of accessibility and high therapeutic values in the clinics with little or no side effects [23,24], medicinal plants have been in use since time immemorial for preventing, treating, and managing different types of human diseases including parasites, infections, inflammation, oxidative stress, diabetes, and associated complications [25–30].

Tridax procumbens is a flowering plant that belongs to the Asteraceae family. It is popularly known as cotton buttons, and originates from the US but is now distributed in different parts of the world [31]. It was traditionally used for wound healing, infections, malaria, diabetes, and other metabolic disorders [32]. *Tridax procumbens* is rich in flavonoids, saponins, alkyl esters, sterols, triterpenes, fatty acids, and polysaccharides [33]. *Tridax procumbens* was scientifically reported to possess various activities including antimicrobial, antihyperuricemic, antioxidant, and antipurgative effects and for treating wounds and liver diseases, among others [34–36]. Previous studies reported amylase inhibition [37,38], blood glucose reduction [39–41], and hypolipidemic effects [41] of the crude extract of *T. procumbens* in diabetic rats. However, there is a paucity of information on evaluating its effectiveness in attenuating chronic diabetes-associated complications. Consequently, in the present study, an extract of *T. procumbens* demonstrated in vitro therapeutic efficacy, and attenuated hyperglycemia, insulin deficiency, oxidative stress, inflammation, and cognitive deficiency in an in vivo model of rats with streptozotocin (STZ)-induced diabetes.

2. Materials and methods

2.1. Chemicals and reagents

The chemicals and reagents used in this study were of analytic grade. Assay kits used for liver and kidney function analyses were products of Randox Laboratories (Antrum, UK), neurotransmitter enzyme-linked immunosorbent assay (ELISA) kits were product of Elabscience, and chemicals used for the antioxidant analysis were a product of Sigma Aldrich (St. Louis, MO, USA). The insulin ELISA kit was a product of Calbiotech (El Cajon, CA, USA), and the ELISA reader was from Sunrise (Tecan, Austria).

2.2. Collection and preparation of the extract

The methanolic extract of *T. procumbens* (TPME) was produced

according to a method described by Alozieuwa et al. [42]. Briefly, freshly picked *T. procumbens* plants were washed under running water to remove dust and air-dried for 2 weeks at room temperature until a constant dry weight was obtained. The dried plant was then pulverized using a blender into fine powder, and 300 g of powder was soaked in 1500 mL methanol for 72 h with intermittent shaking on a shaker for thorough and complete extraction. The mixture was filtered, and the filtrate concentrated under reduced pressure, to obtain a yield of 11.28 g (3.76%) and properly stored for further analysis.

2.3. Analysis of total phenolic and flavonoid contents

Total phenolic and total flavonoid contents of the TPME were determined according to a method described by Singleton et al. [43] and Chang et al. [44] using standard gallic acid and quercetin to prepare respective calibration curves.

2.4. In vitro antioxidant assay

2.4.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay

The ability of the extract to stabilize DPPH free radicals was determined using standard protocols [45] as described by Tsado et al. [46]. One hundred microliters of a DPPH solution in methanol (50 mg/mL) was incubated with 100 μL of the extract (50, 100, 200, 300, and 400 μg/mL) for 45 min in the dark. The decrease in absorbance was measured at 517 nm against a blank, and the percentage of DPPH inhibition was calculated.

2.4.2. Ferric-reducing antioxidant power (FRAP) assay

FRAP activity of the extract was assayed according to a method described by Oyaizu [45]. One milliliter of a sample containing various concentrations (50, 100, 200, 300, and 400 μg/mL) of METP was incubated at 50 ºC for 20 min in 0.2 M sodium phosphate buffer containing 1% potassium hexacyanoferrate (III) followed by the addition of 10% trichloroacetic acid (TCA). The mixture was centrifuged, and the supernatant was mixed 0.1% ferric chloride for color development. The absorbance was read at 700 nm, and the percentage FRAP activity was computed.

2.4.3. Lipid peroxidation (LPO) assays

The thiobarbituric acid-reactive substance (TBARS) protocol described by Panjamurthy et al. [47] was employed for the LPO analysis.

2.5. In vitro anti-diabetes analysis

2.5.1. Alpha-amylase inhibition assay

A method described by Worthington [48] was used to assay the α-amylase inhibitory effect of the extract. Different concentrations of the acarbose standard (12.5–100 μg/mL) and extract were prepared and incubated in a solution of porcine pancreatic enzyme at 37 °C for 10 min. A starch solution was added to initiate the reaction, the mixture was incubated for 30 min at 37 °C, and the reaction was terminated with 10 μL of HCl (1 M). Iodine was added for color development, and the absorbance was measured at 580 nm.

2.5.2. Glucose uptake by yeast cells

Various concentrations of the standard drug or extract were incubated with a glucose solution at 37 °C for 10 min. A yeast suspension was introduced and incubated at 37 °C for 60 min. The mixture was centrifuged and the percent increase in glucose uptake was measured in the supernatant.

2.6. In vitro anti-inflammatory analysis

2.6.1. Human red blood cell (RBC; HRBC) membrane stabilization test

An HRBC membrane stabilization assay was carried out according to established protocols [49]. To a 10% RBC suspension, different concentrations of the extract or standard aspirin drug were added to make 2-mL reaction mixtures. These were incubated at 56 °C for 30 min and centrifuged at 2500 rpm for 5 min, and the absorbance of the supernatants was measured at 560 nm.

2.6.2. Inhibition of protein denaturation

An assay of the inhibition of protein denaturation was carried out according to a method described by Mizushima and Kobayashi [50]. Various concentrations of the extract or standard aspirin drug were mixed and 1% bovine serum albumin in an aqueous solution). Samples were heated to 55 °C for 30 min and allowed to cool. At 660 nm, sample turbidities were read, and the percentage inhibition of protein denaturation was calculated.

2.6.3. Proteinase inhibitory assay

A proteinase inhibitory assay was performed as described by Oyedepo and Femurewa [51]. The reaction mixture (2 mL; 0.06 mg trypsin and 1 mL Tris-HCl buffer) was incubated with 1 mL of the extract at 37 °C for 5 min. The reaction was followed by the addition of 0.8% (w/v) casein, and incubation for 20 min. The reaction was terminated by the addition of 2 mL of 70% perchloric acid, and the absorbance of the supernatant obtained after centrifugation was measured at 210 nm.

2.7. Maximum tolerated dose (MTD) analysis of TPME

Preliminary MTD of TPME was determined in rats by oral administration of TPME at various concentrations of 0, 10, 100, 1000, 1600, 2800, and 500 mg/kg BW in a 7-day toxicity study as described by Lorkes [52]. The MTD was defined as the maximum dose that causes no $>$ 10% decrement in body weight and produces no mortality or external signs of toxicity that would be predicted to shorten the natural lifespan of the animal [53–55]. The animals were thereafter monitored for mortality and adverse effect over a period of 2 weeks.

2.8. In vivo antidiabetic study

In total, 30 male Wistar rats (115.78 ± 4.89 g in body weight (BW)) were procured from the animal facility of FUT Minna (Minna, Nigeria). Animals were kept under standard laboratory conditions (with a 12-h dark/light cycle) and fed a pelleted diet and H_2O ad libitum. Animal experiments were conducted according to regulations of the Ethics Committee on Animal Use of FUT Minna. Diabetes was induced by an intraperitoneal injection of 40 mg/kg BW of streptozotocin (STZ), and a 5% glucose solution 24 h after the STZ injection. Animals with fasting blood sugar (FBS) of 250 mg/dL [56] were considered to be diabetic and were divided into four groups ($n = 5$) consisting of rats receiving normal saline (group 1), 100 mg/kg BW metformin (group 2), 150 mg/kg BW (group 3), and 300 mg/kg BW (group 4) of the extract. A fifth group was given metformin as a control. All treatments were administered daily for 21 days via oral gavage.

2.9. Collection and preparation of blood, serum, and tissue homogenates

At the end of treatment, animals were anesthetized with diethyl ether and sacrificed. Blood was collected via jugular vein/cardiac puncture and allowed to clot. Clotted blood was then centrifuged at 3000 rpm for 15 min [57,58]. The serum was decanted and properly preserved in a refrigerator for the biochemical analyses. Whole blood was also collected in EDTA-coated bottles for a hematological analysis. Excised organs (brain, pancreas, and liver) were blotted to remove blood stains, weighed, and homogenized in a cold 0.25 M sucrose solution [59,60]. The mixture was centrifuged at 4000 rpm for 10 min, and the supernatant was decanted and properly preserved in a refrigerator for the biochemical analyses.

2.10. Analysis of full blood counts

Levels of hematological indices. erythrocyte indices (hemoglobin [HGB], packed cell volume [PCV], RBCs, mean-corpuscular hemoglobin [MCH], mean cell volume [MCV], and mean corpuscular hemoglobin concentration [MCHC]). leukocyte indices (white blood cells [WBCs], and their differentials), and thrombocytic indices (platelets [PLTs]) were estimated using an automated hematologic analyzer (Sysmex, KX-21, Japan) as described by Dacie and Lewis [61].

2.11. Analysis of serum biochemical parameters

Serum biochemical analysis kits were a product of Randox Laboratories (UK) or were commercial biochemical kits (Olympus, Hamburg, Germany) on an automated analyzer (Olympus AU800). Standard experimental protocols were used to analyze serum biochemical parameters; alanine transaminase (ALT) [62], aspartate transaminase (AST) [63], alkaline phosphatase (ALP) [64], total protein [65], bilirubin [66], albumin [67], creatinine [68], and urea [69]. Serum levels of the lipid profile including total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C) [70], and triglycerides (TGs) [71], were measured by colorimetric methods while the low-density lipoprotein-cholesterol (LDL-C) (mg/dl) was computed as [TC − (HDL + very LDL (VLDL))] [72]. The serum electrolyte concentration was determined according to a method described by Tietz [73].

2.12. Analysis of serum and pancreatic insulin levels

Serum and pancreatic insulin levels were assayed using insulin ELISA kits (catalog no.: IN374S; Calbiotech, El Cajon, CA, USA) and an ELISA reader (Sunrise, Tecan, Austria) following the manual's instructions. Insulin concentrations are expressed as μIU/mL.

2.13. Analysis of serum and pancreatic monoamine oxidase (MAO) activities

MAO activity was analyzed using MAO assay kits. The reaction is based on the ability of MAO to catalyze the transformation of 4-dimethylambenzylamine to p-dimethylaminobenzaldehyde. The reaction was monitored at a 355-nm wavelength.

2.14. Analysis of antioxidant parameters

Activities of superoxide dismutase (SOD) were estimated as described by Misra [74]. Briefly, to 0.2 mL of a sample, 2.5 mL of 0.05 mol/L of carbonate buffer (pH 10.2) was added. The reaction was initiated by the addition of freshly prepared 0.3 mmol/L epinephrine. The absorbance was read at 480 nm, and changes in the absorbance were recorded every 30 s for 150 s to estimate SOD activity as described by Misra [74]. Activities of catalase (CAT) were estimated as described by Sinha [75]. To 0.1 mL of the serum or tissue supernatant, 1 mL of 0.01 M phosphate buffer (pH 7.0) and 0.4 mL of 0.2 M H_2O_2 solution were added. The resulting solution was gently mixed, and the reaction was terminated by adding 2 mL dichromate acetic acid reagent. Reduced glutathione (GSH) levels were determined by a modified colorimetric protocol [76], while LPO was assayed by a thiobarbituric acid-reactive substance (TBARS) estimation [77].

2.15. Analysis of inflammatory biomarkers [cyclooxygenase (COX)-2/nitric oxide (NOx)/NF-κB]

COX-2 activity of the brain homogenate was estimated using a Rat PTGS2 (prostaglandin endoperoxide synthase 2)/COX-2 ELISA Kit (catalog no.: E-EL-R0792). NF-κB activities were analyzed using a research purpose Rat NFKB-p105 (p105 subunit) ELISA Kit (catalog no.: E-EL-R0673) based on the color development when rat NFKB-p105 was conjugated with a rat NFKB-p105-specific biotinylated detection antibody and avidin-horseradish peroxidase (HRP) conjugate. The NOx level was determined based on the reduction of nitrate to nitrite according to a procedure reported by Miranda et al. [78].

2.16. Analysis of cholinesterases (ChEs) and neurotransmitters [serotonin and dopamine]

Activities of ChEs, including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), were determined according to the method of Ellman et al. [79]. A reaction mixture containing phosphate buffer (0.1 M, pH 8.0), DTNB (10 mM), 50 μL cytosol, and 150 mM of acetylthiocholine iodide (for the AChE assay) or 150 mM of butyrylthiocholine iodide (for the BChE assay) was incubated, and changes in the absorbance were monitored at 412 nm for 3 min. The serotonin assay was conducted using an ST/5-HT (serotonin/5-hydroxytryptamine) ELISA Kit (catalog no.: E-EL-0033, Elabscience, USA), while the dopamine assay was conducted using a dopamine ELISA kit (catalog no.: E-EL-0046) according to the manufacturer's protocols.

2.17. Molecular docking analysis

The three (3D) Dimensional structure of the receptors including AChE, BChE, COX2, and NOx were obtained from the Protein Data Bank (PDB) (https://www.rcsb.org/). The mol2 file of the ligand candidates were built using the Avogadro molecular builder and visualization tool vers. 1. XX (http://avogadro.cc/) [80]. The mol2 file was transformed to PDB files with the aid of PyMOL Molecular Graphics System, vers. 1.2r3pre (Schrödinger; https://pymol.org/edu/?q=educational/). All PDB files were subsequently converted to PDBQT files using AutoDock Vina (vers. 0.8, Scripps Research Institute, La Jolla, CA, USA) [81]. Docking preparation of ligand and receptors were conducted as described in previous studies [82–85]. Docking was conducted using AutoDock Vina according to standard protocols while the docked complexes were visualized and analyzed using Discovery studio visualizer vers. 19.1.0.18287 (BIOVIA, San Diego, CA, USA) [86].

2.18. Molecular dynamic (MD) simulations

The molecular dynamic (MD) simulations were carried out using the Schrodinger suite (2020–2). The docked complexes were prepared for simulation using the system builder module in Maestro v12.4. MD simulations were carried out using the Desmond software. The orthorhombic water box was used to create a 10 Å buffer region between the atoms on the receptors and box sides [87]. The volume of the box was minimized, and Na^+ was used to neutralize the system charges. The system pressure and temperature were kept constant at 1.01325 bar and 300 Kelvin using Nose–Hoover thermostat [88] and Martyna–Tobias–Klein barostat methods. The simulations were performed using NPT ensemble by considering atoms number, pressure and timescale and the simulation time at 50 ns. The simulation study was conducted to analyze the root-mean-square fluctuation (RMSF), root-mean-square deviation (RMSD), radius of gyration (Rg), solvent-accessible surface area (SASA), secondary structure, and the number of hydrogen bonds [89]. The molecular mechanics Poisson–Boltzmann surface area (MM-PBSA) method was applied to calculate the binding free energy. The 1000 trajectory files were considered for MM-PBSA calculation.

2.19. Maximum tolerated dose (MTD) analysis of TPME

Preliminary MTD of TPME was determined in rats by oral administration of TPME at various concentrations of 0, 10, 100, 1000, 1600, 2800, and 500 mg/kg BW in a 7-day toxicity study as described by Lorkes [52]. The MTD was defined as the maximum dose that causes no $>$ 10% decrement in body weight and produces no mortality or external

signs of toxicity that would be predicted to shorten the natural lifespan of the animal [53–55]. The animals were thereafter monitored for mortality and adverse effect over a period of 2 weeks.

2.20. Data analysis

Data were analyzed with GraphPad Prism software, (GraphPad Software, La Jolla, CA, USA). Differences between the experimental groups were evaluated by an analysis of variance (ANOVA) followed by Tukey's post hoc test and Student's *t*-test.

3. Results

3.1. In vitro antioxidant, hypoglycemic and anti-inflammatory activities of the TPME

Preliminary secondary metabolite profiling revealed that the TPME contained total phenolic and flavonoid contents of 198.97 ± 1.21 mg/100 g and 95.56 ± 1.12 mg/100 g respectively. The in vitro antioxidant analysis revealed increased DPPH inhibition, FRAP activities, and inhibition of lipid peroxide in dose-dependent manners; 50% inhibitory concentration (IC_{50}) values were 186.73, 160.56, and 200.72 μg/mL, respectively, while ascorbic acid (the standard) yielded IC_{50} values of 20.90, 15.32, and 21.87 μg/mL, respectively (Fig. 1A). A hypoglycemic analysis of the extract showed that the uptake of glucose (5, 10, and 25 mM) by yeast cells proportionally increased with an increase in the extract concentration; an increase in a dose-dependent manner of α-amylase inhibition (IC_{50} of 271.60 μg/mL) was also seen (Fig. 1C). The in vitro anti-inflammatory study revealed increases in dose-dependent manners of the inhibition of protein denaturation (IC_{50} of 268.45 μg/mL), proteinase inhibition (IC_{50} of 219.00 μg/mL), and membrane stabilization (IC_{50} of 231.69 μg/mL) by the extract, while aspirin (standard control) gave maximum anti-inflammatory activities of 96.86%, 99.80%, and 97.76%, respectively, at 250 μg/mL (Fig. 1C).

3.2. maximum tolerated dose (MTD) analysis revealed the safe dose of TPME for oral remedy

We evaluated the maximum tolerated dose of TPME after oral administration to rats. The MTD was estimated based on the threshold at which all animals survived with no more than a 10% BW loss. We found that all animals treated with 10, 100, 1000, and 1600 mg/kg BW tolerated these doses, and no death or deterioration in health were recorded throughout the study period. Furthermore, none of the animals in these groups exhibited weight loss. However, rats dosed with 2800 and 5000 mg/kg bw of TPME were restless for few minutes after which they exhibited moderate writhing, abdominal tone, and profuse breathing which lasted for 1 h (Table 1). Furthermore, mortalities and severe weight loss were observed at 2800 and 5000 mg/kg dose.

3.3. TPME improved the glycemic status and insulin level in rats with STZ-induced diabetes

The TPME produced a significant ($p < 0.001$) decrease in the fasting glucose level and an improvement in BW of rats with STZ-induced diabetes compared to untreated rats (Fig. 2A, B). The standard drug, however, showed a higher percentage of glucose reduction and BW improvement than the extract-treated groups (Tables 2 and 3). Furthermore, serum and pancreatic insulin levels decreased in untreated diabetic rats compared to normal control rats. Interestingly, treatment with the extract (at 150 and 300 mg/kg BW) and the standard drug significantly attenuated the decreases in serum insulin and pancreatic insulin levels compared to untreated rats (Fig. 2C). Similarly, the decreases in serum and pancreatic monoamine oxidase in untreated diabetic rats did not significantly differ ($p > 0.05$) compared to extract-receiving groups (Fig. 2D).

3.4. Tridax procumbens demonstrated in vivo antioxidant activities in rats with STZ-induced diabetes

Our analysis of the serum and tissue antioxidant statuses following STZ intoxication and treatment with the TPME revealed that untreated diabetic rats exhibited significant decreases in serum and tissue (liver, brain, and pancreas) levels of SOD and CAT, while demonstrating elevated levels of tissue MDA compared to normal control rats. With the exception of pancreatic SOD, we found that treatment of diabetic rats with the TPME significantly attenuated the decreased activities of serum and tissue levels of SOD and CAT, and the increase in MDA concentrations compared to the untreated diabetic controls. Activities of the extract were more pronounced at 300 mg/kg BW. Similarly, serum, liver, and pancreatic GSH levels significantly increased in extract-receiving animals compared to untreated rats (Fig. 3).

3.5. Effect of the TPME on cholinesterase and neurotransmitter activities in the brains of rats with STZ-induced diabetes

Activities of ChEs (AChE and BChE) and dopamine were significantly ($p < 0.001$) elevated in the brains of rats with STZ-induced diabetes compared to activities in the brains of control rats. Interestingly, treatment with TPME induced significant ($p < 0.001$) ameliorative effects on the elevated activities of ChEs. However, no treatment-related changes in brain levels of serotonin or dopamine were observed compared to untreated diabetic rats (Fig. 4).

3.6. TPME modulates inflammatory activities in the brain of rats with STZ-induced diabetes

The brains of rats with STZ-induced diabetes exhibited a significantly ($p < 0.001$) elevated nitric oxide concentration and increased COX-2 and NF-κB activities ($p < 0.001$) compared to activities of those inflammatory markers in normal control rats. Treatment with the TPME significantly ($p < 0.001$) restored the activities of these inflammatory markers to their basal levels. Interestingly, levels of NOx and NF-κB in TPME-treated rats were significantly ($p < 0.05$) lowered compared to levels found in normal control rats (Fig. 5).

3.7. TPME attenuated the hyperglycemia-related dyslipidemia in STZ-intoxicated rats

STZ intoxication caused significant ($p < 0.05$) increases in serum levels of cholesterol (CHOL) ($p < 0.001$), TGs ($p < 0.01$), and LDL-C ($p < 0.05$) and decreased levels of HDL-C ($p < 0.05$) compared to levels in normal glycemic rats. Administration of the TPME at 300 mg/kg BW significantly decreased elevated levels of CHOL, TGs, and LDL-C while increasing the level of HDL-C compared to STZ-intoxicated rats that received distilled water only. However, no treatment-associated changes in CHOL or LDL-C were seen in the group of rats treated with 150 mg/kg TPME compared to STZ-intoxicated rats that received distilled water only (Fig. 6).

3.8. TPME reversed the serum biochemical alterations in liver and kidney functional indices of STZ-intoxicated rats

Administration of STZ significantly ($p < 0.05$) increased levels of total bilirubin, direct bilirubin, bicarbonate, urea, creatinine, and uric acid, and serum activities of AST, ALP, and ALT (Table 4), while levels of total proteins, albumin, and potassium significantly ($p < 0.05$) decreased. However, treatment with the TPME at 150 or 300 mg/kg BW significantly ($p < 0.05$) reduced the STZ treatment-associated alterations in levels of serum total bilirubin, direct bilirubin, urea, creatinine, uric acid, AST, ALP, ALT, total proteins, albumin, and potassium compared to levels of STZ-treated rats that received distilled water only. Levels of sodium in all experimental groups compared favorably

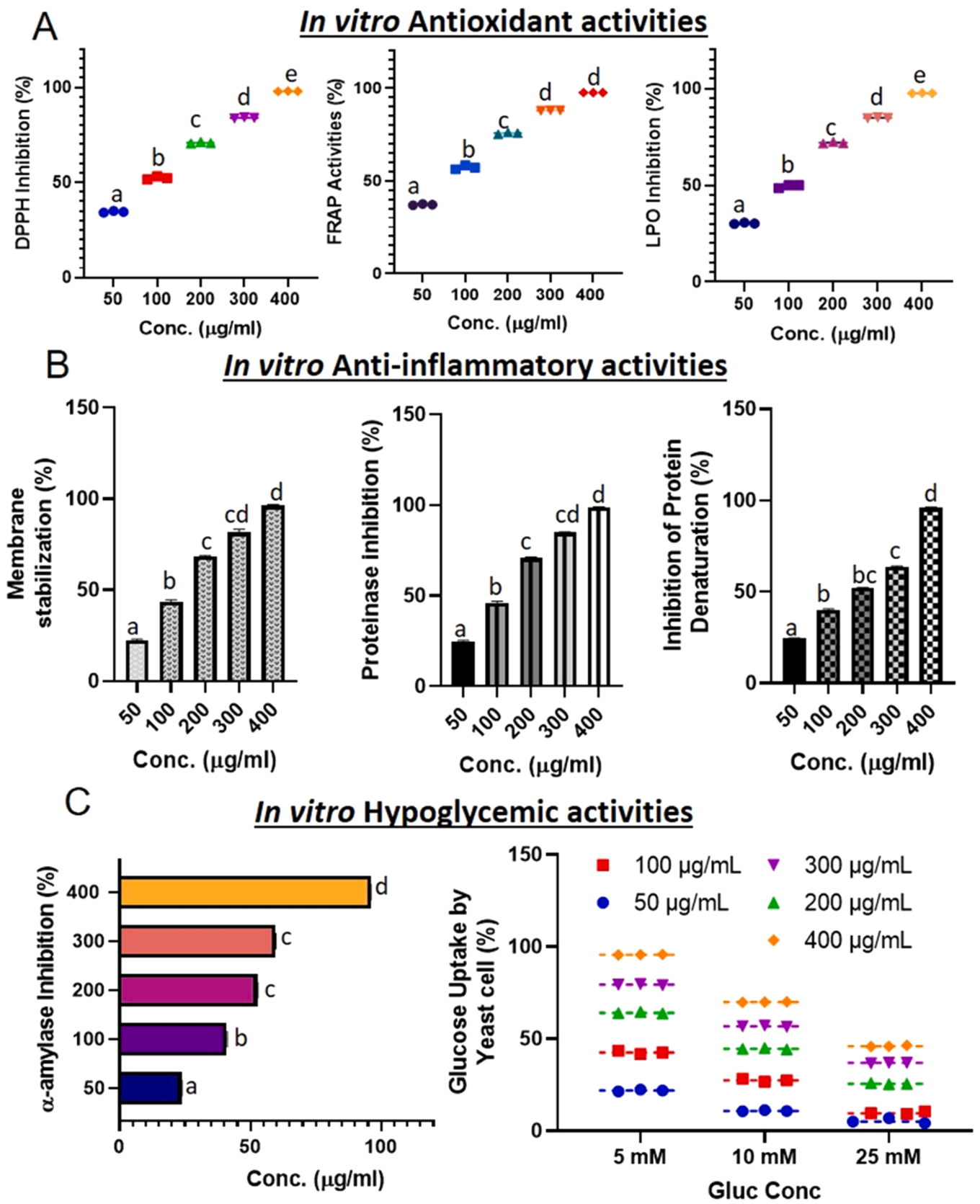


Fig. 1. *In vitro* antioxidant, hypoglycemic and anti-inflammatory activities of the *Tridax procumbens* methanolic extract. Data are the mean±standard error of the mean (SEM) of three replicate determinations. Values followed by different superscript letters significantly differ across treatment doses.

Table 1
Maximum tolerated dose (MTD) profile of TPME in rats.

Dose (mg/kg BW)	Initial BW (g)	Final BW (g)	BW gain (%)	Mortality	Physiological observation for sign of adverse effect
10	123.82 ± 1.30	129.90 ± 0.28	6.08	0/3	None
100	121.68 ± 1.70	125.95 ± 4.72	4.35	0/3	None
1000	121.66 ± 2.27	126.90 ± 1.50	5.24	0/3	None
1600	124.07 ± 3.99	125.31 ± 9.30	1.24	0/3	restlessness, profuse breathing, hyper activeness
2800	119.33 ± 5.04	112.89 ± 5.46	-6.44	1/3	moderate writhing, abdominal tone, and profuse breathing
5000	116.35 ± 3.54	109.79 ± 2.18	-6.56	2/3	Severe writhing, moderate writhing, abdominal tone, and profuse breathing

BW, body weight, MTD: maximum tolerated dose

($p > 0.05$) with the control group, and no extract treatment-related modulation of bicarbonate levels was seen compared to STZ-treated rats that received distilled water only (Table 4).

3.9. TPME ameliorated the hematological alterations in STZ-intoxicated rats

STZ administration significantly ($p < 0.05$) reduced the blood count of hemoglobin (HGB), packed cell volume (PCV), mean cell hemoglobin (MCH), RBCs, lymphocytes (Ls), and monocytes (Ms), while significantly ($p < 0.05$) increasing levels of mean corpuscular volume (MCV), platelet (PLC), WBCs, and neutrophils (Table 5). On the other hand, treatment with the TPME at 150 and 300 mg/kg BW significantly ($p < 0.05$) increased levels of HGB, PCV, MCH, RBCs, Ls, and Ms, while decreasing levels of PLC, MCV, WBCs, and neutrophils compared to those of the distilled water-treated control group (Table 5). However, the group of rats treated with 150 mg/kg BW did not significantly differ ($p > 0.05$) in TWBC compared to counts in STZ-treated rats that received distilled water. There was no extract treatment-related significant ($p > 0.05$) modulation of MCHC counts in STZ-treated rats that received distilled water.

3.10. Characterization of the T. procumbens extract

Extract characterization by LC-MS identified isorhamnetin (retention time (RT): 3.69 min, 8.8%), bixin (RT: 25.06 min, 4.72%), and lupeol (RT: 25.25, 2.88%) as the three most abundant bioactive compounds that could be responsible for the bioactivity of the plant. Other compounds identified included biochanin A, dicumarol, cucurbitacin E, myricetin, silymarin, quercetin, apigenin, echinone, akuammidine, catechin, and sitosterol. The compounds' identity profiles and chromatogram are respectively displayed in Table 6 and Fig. 7.

3.11. Molecular dynamic simulations revealed potential interaction of isorhamnetin with AChE/BChE/COX2/NOx

Molecular docking profile revealed that isorhamnetin demonstrated best interactions with stronger binding energy to AChE/BChE/COX2/NOx when compared with interaction of bixin and lupeol to the proteins (Fig. 8). Only isorhamnetin and lupeol interacted with AChE (Fig. 9) while only isorhamnetin bind with the COX2 and NOx (Fig. 10). The

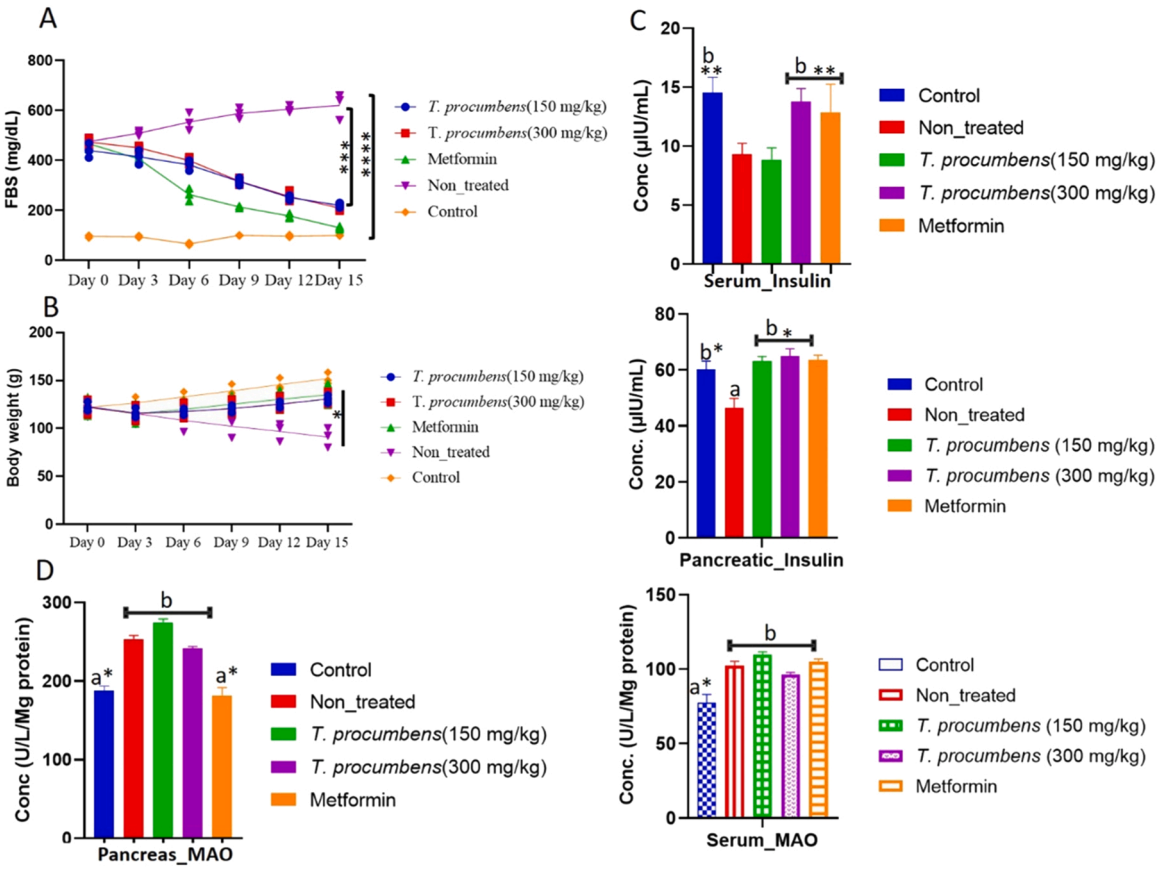


Fig. 2. *Tridax procumbens* methanolic extract (TPME) improved the glycemic status and insulin levels in rats with STZ-induced diabetes. Monoamine oxidase activities of TPME-treated rats with STZ-induced diabetes.

Table 2
Glucose concentration (mg/dL) of rats with STZ-induced diabetes treated with the methanolic extract of *Tridax procumbens* (TPME).

Group	0	3	6	9	12	15	% Change in FBS
Control	96.07 ± 2.95	94.57 ± 2.37	95.50 ± 2.69	100.10 ± 0.96	99.87 ± 1.68	99.63 ± 0.90	3.71↑
Untreated	475.33 ± 12.00	509.13 ± 5.80	553.87 ± 20.40	601.53 ± 9.60	623.50 ± 9.22	648.33 ± 9.08	36.40↑
Metformin	466.83 ± 11.08	405.90 ± 6.98	283.43 ± 9.86	213.37 ± 2.62	177.87 ± 5.83	130.53 ± 3.64	72.04↓
150 mg/kg TPME	439.50 ± 16.93	414.03 ± 16.37	382.23 ± 12.18	315.57 ± 9.03	251.50 ± 6.03	224.17 ± 3.12	48.99↓
300 mg/kg TPME	473.53 ± 9.49	450.23 ± 4.83	399.40 ± 8.10	317.27 ± 8.23	255.93 ± 12.94	218.00 ± 4.11	53.96↓

Values are presented as mean±standard error of mean of replicate determinations.
↓ = Decrease, ↑= increase.
FBS, fasting blood sugar.

Table 3
Effect of the methanolic extract of *Tridax procumbens* (TPME) on the body weight of rats with STZ-induced diabetes.

	Body weight (g)							
Group	0	3	6	9	12	15	18	% Change
Control	121.93 ± 2.82	126.56 ± 3.36	132.88 ± 2.73	139.15 ± 3.59	145.79 ± 3.56	151.90 ± 3.41	157.63 ± 2.95	29.28
Untreated	122.86 ± 5.49	115.66 ± 5.73	108.24 ± 6.09	102.31 ± 6.18	96.87 ± 5.47	90.89 ± 5.84	83.84 ± 5.60	-31.76
Metformin	122.05 ± 5.87	115.41 ± 6.22	119.77 ± 5.44	125.26 ± 5.71	130.22 ± 6.05	135.11 ± 6.53	139.89 ± 6.04	14.62
150 mg/kg TPME	122.62 ± 2.80	116.03 ± 2.95	117.68 ± 2.13	120.67 ± 2.33	125.46 ± 2.09	130.58 ± 2.41	138.32 ± 4.26	12.80
300 mg/kg TPME	121.13 ± 4.20	116.83 ± 4.81	120.25 ± 5.30	126.70 ± 5.07	132.11 ± 5.31	136.74 ± 5.08	142.81 ± 4.90	17.90

Values are presented as the mean ± standard error of mean of replicate determinations.

higher molecular docking score of isorhamnetin-BChE complex is an indication of compact interactions. Furthermore, more hydrogen bonds indicate an increasingly stable nature for the complex. In this simulation study, the RMSD from Fig. 8 illustrated that the bixin–BChE complex had a higher RMSD trend compared with those of the other two complexes. however, bixin-BChE complexes displayed large fluctuations in RMSD trends, which indicated complex flexibility. Interesting, isorhamnetin-BChE complex demonstrated most desirably high and remained in a steady state and demonstrating a rigid profile. The degree of mobility in a biological system can be indicated by the Rg profile. Our simulation revealed that isorhamnetin-BChE complex had a lower Rg profile than other complex, indicating the compacted nature of the protein complex, whereas higher Rg values, which correlate with the repeated folding and unfolding protein behavior, were observed for the protein complexes containing both bixin and lupeol. Furthermore, the bixin-BChE complex shows fluctuated Rg value, suggesting a loose packaging of the system [90]. The surface area of the biological systems and their corresponding binding patterns with ligand molecules can be assessed through SASA analysis. The SASA analysis revealed that isorhamnetin-BChE demonstrated a stable SASA profiles with no significant deviation in the surface area and formed more rigid and stable profiles. However, the increasing and fluctuating trend in SASA observed in bixin-BChE complex represents protein expansion and comparatively loose binding of bixin to BChE.

4. Discussion

The α-Amylase inhibitory effect of TPME clearly suggest it potential to reduce postprandial glucose levels. Our findings validate the study of Sonawane [37] who worked on α-amylase inhibitory activities of several extracts of *T. procumbens*. Although inhibition of α-amylase by the extract would not cause any net nutritional caloric losses, they slow down carbohydrate digestion and glucose absorption, thus attenuating postprandial blood glucose transport and hyperglycemia. Results also indicated that the TPME had high efficiency in increasing glucose uptake by yeast cells.

The DPPH radical-scavenging activity of the TPME demonstrated its ability to serve as an electron donor, thereby attenuating the DPPH radical and preventing oxidative damage [91]. The FRAP activities also indicated that the TPME contains some bioactive compounds that react with free radicals and donate electrons to terminate the cascade of the free radical chain reactions [92]. Inflammation triggers secondary damage via free radical-induced LPO [93]. The dose-dependent increases in the inhibition of LPO by the TPME further confirm its potential antioxidant effect. HRBC membranes are analogous to lysosomal membranes, and their stabilization by TPME indicated the ability of the extract to stabilize lysosomal membranes and prevent tissue inflammation [51]. The proteinase inhibitory and protein inhibition activities of the extract further strengthens its potential for preventing tissue inflammation [42].

The loss of BW in untreated rats with STZ-induced diabetes may have been due to mobilization of stored fat and protein from muscles as sources of energy [94]. The reversal of hyperglycemia and loss of BW in diabetic rats treated with the TPME agreed with a study by Bhagwat et al. [39], who reported a significant reduction in blood glucose following oral administration of leaf extract of *T. procumbens* at 200 mg/kg BW to rats with alloxan-induced diabetes. The reduction in FBS levels by treatment with the TPME provides scientific support for the use of the plant in traditional management of DM [95]. The hypoglycemic effect of the extract could be attributed to its high phenol and flavonoid contents which were reported to exhibit antioxidant activity [96], enhance glucose uptake [97], and stimulate insulin secretion from pancreatic β cells [98,99].

The ability of the extract to increase insulin levels of the diabetic rats could be ascribed to its effect in preventing loss of β-cells or the ability to enhance insulin secretion by residual β-cells. It is worth noting that the improvement in serum insulin levels produced by the extract at 150 and 300 mg/kg was comparable to that of the reference compound, metformin, which is a known stimulator of insulin secretion by β-cells [100].

The increased MAO activities in diabetic non treated rats are associated with increase production of H_2O_2, antioxidant exhaustion, and subsequent oxidative stress [101,102]. Consistent with our findings, previous studies also reported increased MAO expressions in rats with STZ-induced diabetes treated with MAO inhibitors which reduced levels of free radical generation and oxidative stress by 50% [103,104]. However, treatment with the TPME produced no significant attenuating effects on elevated MOA activities in STZ-intoxicated rats. In line with the abovementioned results, the present study acknowledged the contributing role of MAO in oxidative status in the serum and pancreas of diabetic rats. Our data however, further revealed that the improvement in the antioxidant status of diabetic rats following treatment with the TPME was not associated with inhibition of MOA.

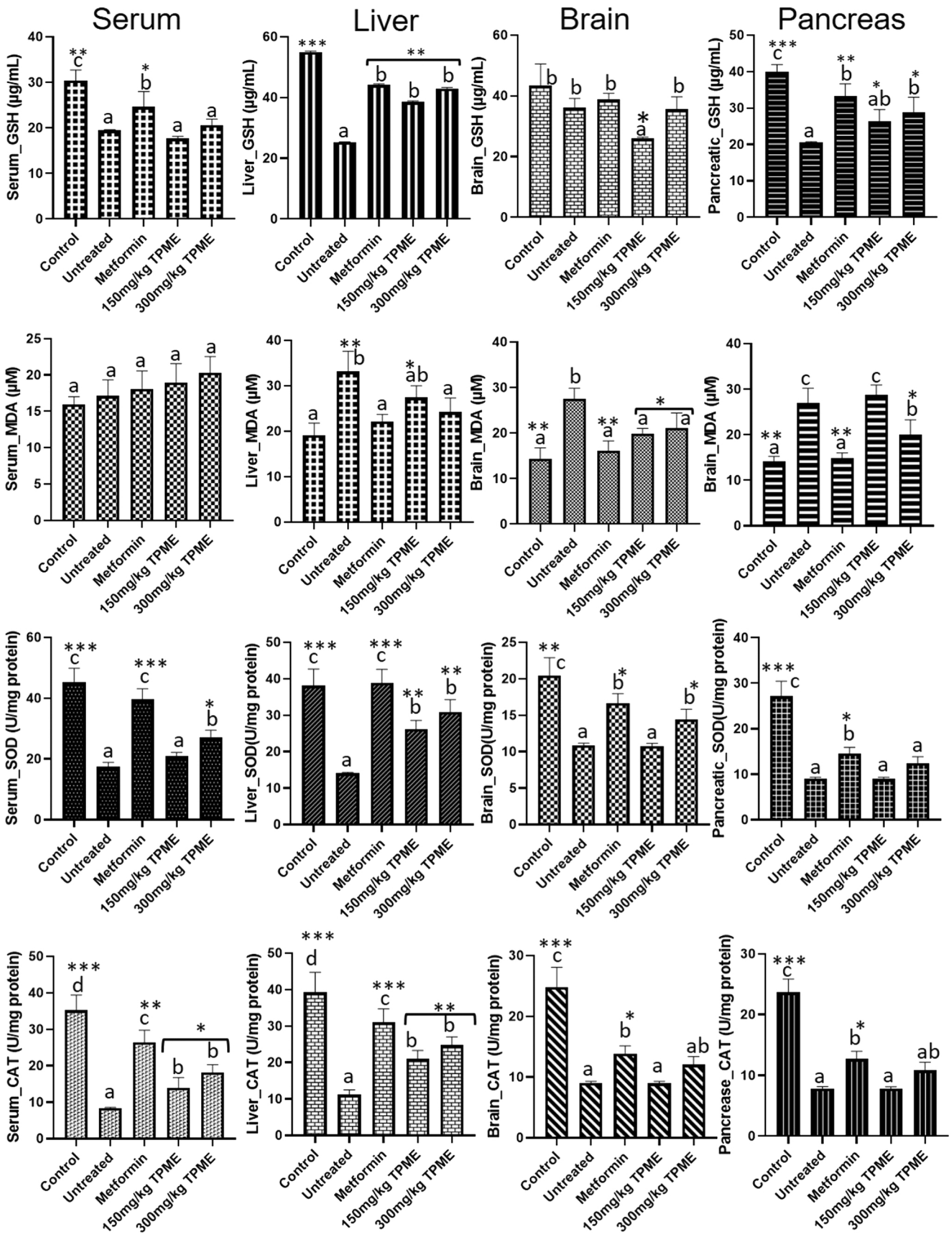


Fig. 3. Antioxidant activities in the serum and tissues of rats with STZ-induced diabetes treated with the methanolic extract of *Tridax procumbens* (TPME). Data are the mean±SEM of replicate determinations. Values followed by different superscript letters significantly differ across the groups. * Significantly differs from STZ-treated rats that received distilled water. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns, non-significant.

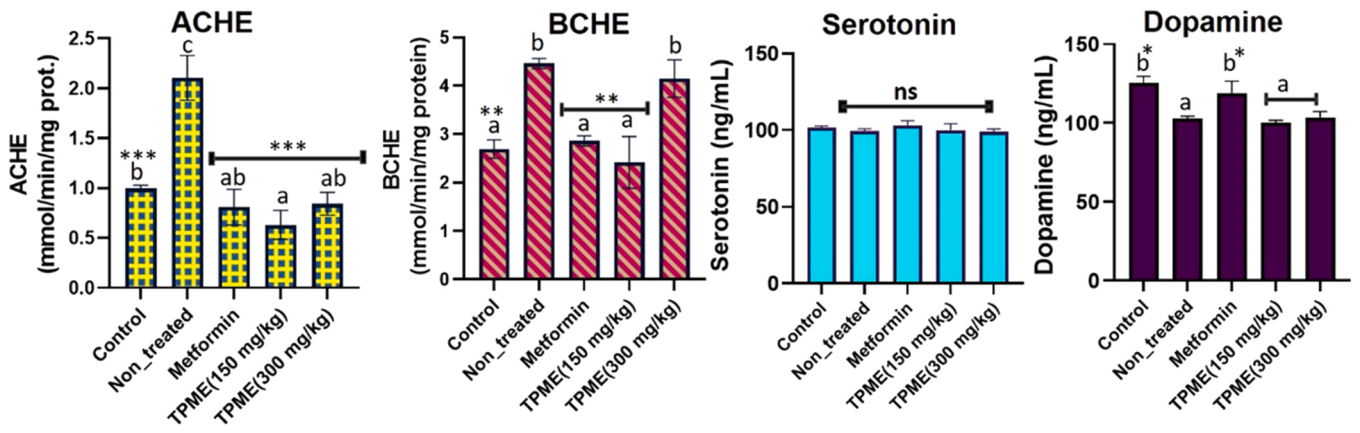


Fig. 4. Cholinesterase and neurotransmitter activities in the brain of rats with STZ-induced diabetes treated with the methanolic extract of *Tridax procumbens* (TPME). Data are the mean±SEM of replicate determinations. $^{**}p < 0.01$, $^{***}p < 0.001$, ns, non-significant.

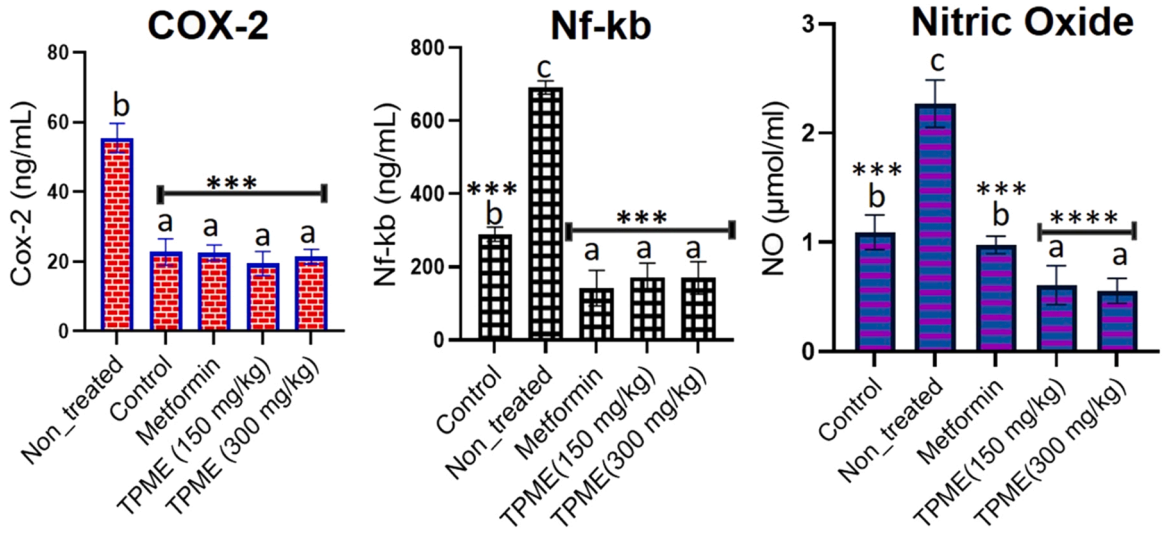


Fig. 5. Activities of inflammatory markers in the brains of rats with STZ-induced diabetes after treatment with *Tridax procumbens* extract**.** Data are the mean±SEM of replicate determinations. $^{***}p < 0.001$, $^{****}p < 0.0001$.

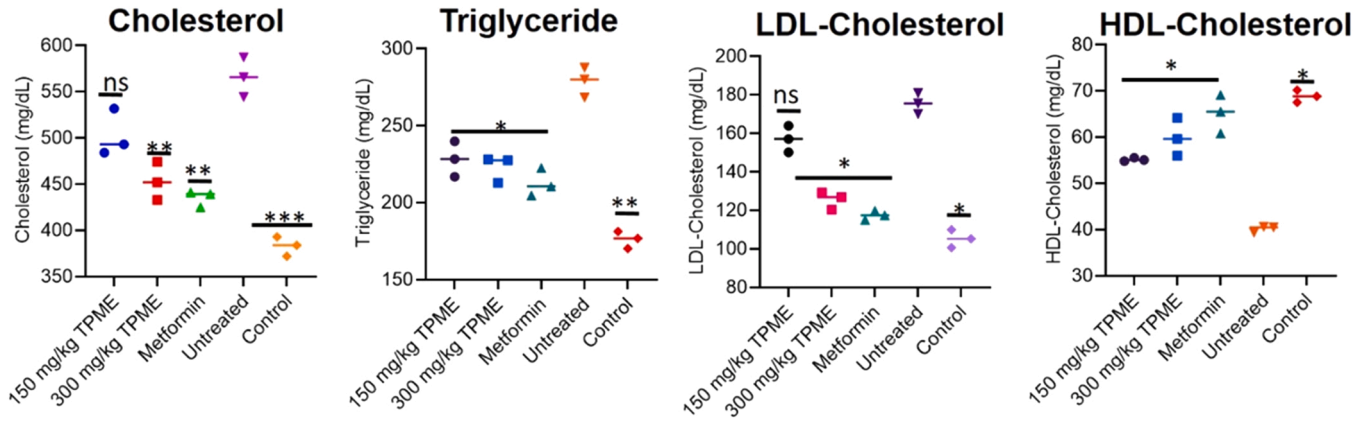


Fig. 6. Serum lipid profiles of rats with STZ-induced diabetes treated with the methanolic extract of *Tridax procumbens* (TPME). Data are the mean±SEM of five replicate determinations. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns, non-significant.

MAO is responsible for metabolizing dopamine and serotonin, and increased MAO activity causes a dopamine deficiency in the brain [105]. This is consistent with the decrease levels of dopamine and serotonin that we observed in the diabetic untreated rats. Consequently, the fact that no TPME treatment-related changes in levels of serotonin or dopamine were observed, could be attributed to a failure of the extract to attenuate elevated MAO activities in rats with STZ-induced diabetes as we discussed earlier. Our finding also concurred with a study of

Table 4
Functional indices of the liver and kidney of rats with STZ-induced diabetes after oral administration of the methanolic extract of *Tridax procumbens* (TPME).

Sample	TP150	TP300	Positive	Normal	Negative
TB	1.62 ± 0.04^{b*}	1.55 ± 0.48^{b*}	1.42 ± 0.05^{ab*}	1.24 ± 0.04^{a*}	2.33 ± 0.07^{c}
DB	1.44 ± 0.35^{b}	1.18 ± 0.02^{a*}	1.86 ± 0.02^{b}	1.67 ± 0.17^{b}	1.51 ± 0.40^{b}
AST	45.35 ± 0.32^{c*}	45.77 ± 1.36^{c*}	21.87 ± 1.62^{a*}	25.60 ± 2.66^{b*}	65.75 ± 0.18^{d}
ALT	20.32 ± 0.42^{b*}	26.87 ± 0.39^{c*}	14.69 ± 2.53^{a*}	18.43 ± 0.45^{a*}	32.88 ± 0.79^{d}
ALP	166.81 ± 0.91^{b*}	238.44 ± 2.27^{d*}	177.38 ± 0.86^{c*}	67.57 ± 1.02^{a*}	298.51 ± 1.50^{e}
TP	8.93 ± 0.20^{b*}	9.63 ± 0.14^{c*}	10.60 ± 0.16^{d*}	10.33 ± 0.31^{d*}	6.49 ± 0.51^{a}
ALB	3.97 ± 0.18^{b*}	4.91 ± 0.25^{c*}	5.32 ± 0.19^{d*}	6.08 ± 0.10^{e*}	3.33 ± 0.21^{a}
Na	199.23 ± 1.43^{a}	202.11 ± 1.07^{a}	196.43 ± 0.79^{a}	195.70 ± 4.47^{a}	195.42 ± 6.48^{a}
K	10.81 ± 0.07^{b*}	13.42 ± 0.13^{d*}	14.16 ± 0.07^{e*}	12.71 ± 0.09^{c*}	7.01 ± 0.15^{a}
CO3	21.38 ± 0.12^{b}	20.35 ± 0.38^{b}	21.92 ± 0.16^{b}	18.70 ± 0.24^{a*}	22.45 ± 1.08^{b}
Urea	40.29 ± 0.69^{c*}	39.05 ± 0.38^{c*}	29.05 ± 0.14^{b*}	19.35 ± 0.99^{a*}	47.96 ± 0.68^{d}
Creatinine	8.21 ± 0.58^{b*}	5.09 ± 0.04^{a*}	5.54 ± 0.04^{a*}	4.86 ± 0.08^{a*}	9.91 ± 0.05^{c}
Uric acid	31.47 ± 0.36^{b*}	30.20 ± 1.99^{b*}	32.96 ± 0.54^{b*}	26.94 ± 0.11^{a*}	45.64 ± 0.29^{c}

Data are the mean±SEM of five replicate determinations. Values followed by different superscript letters significantly differ across the groups. * Significantly differs from the STZ-treated rats that received distilled water. TP150, TPME at 150 mg/kg of body weight; TP300, TPME at 300 mg/kg of body weight; TB, total bilirubin; DB, direct bilirubin.

Emory [106], who reported that MAO inhibition in diabetic patients attenuated a dopamine deficiency.

The higher expression levels of COX-2, NOx, and NF-κB in STZ-induced diabetes is consistent with a previous study which reported that diabetes-associated inflammation is mediated by the release of several endogenous inflammatory mediators including NOx, glutamate, COX-2, NF-κB, serotonin, and histamine [107–109]. Our results demonstrated for the first time that the TPME inhibited the releases of major inflammatory mediators including COX-2/NOx/NF-κB in diabetic rats, hence suggesting its potential for preventing diabetes-associated inflammation.

The increased levels of MDA in the pancreas and brain of untreated rats with STZ-induced diabetes indicated oxidative damage to the cellular and neuronal membranes. Conversely, decreased levels of MDA in the liver, pancreas, and brain of TPME-treated diabetic rats indicated anti-LPO activity of the extract and suggested the preservation of the integrity of neuronal and cellular membranes. Furthermore, the significant increased levels of SOD, CAT, and GSH, and improved antioxidant status of the diabetic rats, suggesting that the TPME possesses antioxidative properties capable of protecting tissues of rats with STZ-induced diabetes from oxidative stress.

Therefore, an ideal antidiabetic agent should not only regulate the blood glucose level, but also attenuate cognitive deficits and other complications associated with diabetes [110]. Hence the increased AChE activity in untreated rats with STZ-induced diabetes could result in decreased ACh (acetylcholine) levels, which may in turn disrupt nerve impulse transmissions and eventually induce memory dysfunction [111]. The inhibitory activities of TPME on AChE and BChE could prevent rapid degradation of ACh, thus ensuring proper nerve impulse transmission among neurons. This study agreed with the findings of Ramrao et al. [112], who reported that the aqueous extract of *T. procumbens* modulated cognitive functions with a nootropic effect and also reversed scopolamine-induced amnesia. Hence, significant decreases in blood glucose levels produced by the extract revealed a possible mechanism by which the TPME can prevent cognitive and learning deficits in diabetic conditions.

The elevated activities/levels of AST, ALP, ALT, total bilirubin, direct bilirubin, bicarbonate, urea, creatinine, and uric acid in diabetic untreated rats is an indication that the functional integrity of the liver and kidney have been compromised [113–115]. However, the attenuation of these parameters by TPME treatment suggests the alleviation of the STZ-induced organs injury.

Diabetic dyslipidemia, characterized by high levels of CHOL, TGs, and LDL, contributes to the increased production of ROS, activates cascades of inflammatory events, induces insulin resistance, and accelerates vascular diseases in diabetic patients [116]. Thus, regulating plasma levels of lipids is essential for patients with T2DM. Interesting, the abnormal lipid profile (elevated CHOL, TRIG, and LDL-C and decreased HDL-C) was significantly reversed in TPME-treated rats. Altogether, our data suggested that the TPME can attenuate diabetic dyslipidemia and subsequent IR, oxidative stress, and inflammation associated with T2DM.

Extract characterization by LC-MS identified isorhamnetin (RT: 3.69 min, 8.8%), bixin (RT: 25.06 min, 4.72%), and lupeol (RT: 25.25 min, 2.88%) as the three most abundant bioactive compounds that could be responsible for the bioactivity of the plant. Other compounds identified included biochanin A, dicumarol, cucurbitacin E, myricetin, silymarin, quercetin, apigenin, echinone, akuammidine, catechin, and sitosterol. The compounds' identity profiles and chromatograms are respectively displayed in Table 5 and Fig. 7. Consequently, the most abundant compounds were subjected to in silico molecular and dynamic modeling revealing that isorhamnetin demonstrated best interactions with stronger binding energy to AChE/BChE/COX2/NOx when compared with interaction of bixin and lupeol to the

Table 5
Hematological parameters in rats with STZ-induced diabetes after oral administration of the methanolic extract of *Tridax procumbens* (TPME).

Sample	TP150	TP300	Positive	Normal	Negative
Hb (g/dL)	13.25 ± 0.35^{b*}	13.40 ± 0.40^{b*}	15.20 ± 0.40^{c*}	16.10 ± 0.30^{c*}	8.00 ± 0.20^{a}
PCV (%)	37.00 ± 1.00^{b*}	39.00 ± 1.00^{b*}	39.50 ± 0.50^{b*}	39.50 ± 0.50^{b*}	29.00 ± 1.00^{a}
MCV (fi)	81.00 ± 1.00^{a*}	77.00 ± 1.00^{a*}	75.00 ± 1.00^{a*}	79.00 ± 1.00^{a*}	89.50 ± 0.50^{b}
MCH (pg)	23.50 ± 0.50^{b*}	25.50 ± 0.50^{b*}	27.00 ± 1.00^{b*}	26.00 ± 1.00^{b*}	21.00 ± 1.00^{a}
MCHC (g/dL)	25.50 ± 0.50^{a}	25.00 ± 1.00^{a}	27.00 ± 1.00^{b*}	29.00 ± 1.00^{b*}	23.00 ± 1.00^{a}
RBC ($X10^{12}$/L)	4.00 ± 0.20^{b*}	4.40 ± 0.40^{b*}	4.30 ± 0.30^{b*}	6.00 ± 0.40 ^{C*}	3.50 ± 0.30^{a}
PLC ($X10^{9}$/L)	171.50 ± 1.50^{b*}	163.50 ± 1.50^{a*}	168.00 ± 1.00^{a*}	171.00 ± 1.00^{b*}	194.00 ± 1.00^{c}
TWBC ($X10^{9}$/L)	114.00 ± 1.00^{b}	99.00 ± 1.00^{a*}	95.00 ± 1.00^{a*}	95.00 ± 1.00^{a*}	117.00 ± 1.00^{b}
N (%)	50.50 ± 1.50^{c*}	47.00 ± 1.00^{b*}	41.00 ± 1.00^{a*}	49.00 ± 1.00^{c*}	58.00 ± 1.00^{d}
L (%)	47.00 ± 1.00^{b*}	50.50 ± 0.50^{b*}	50.00 ± 1.00^{b*}	51.00 ± 1.00^{b*}	33.00 ± 1.00^{a}
M (%)	7.00 ± 1.00^{c*}	5.00 ± 1.00^{b*}	5.00 ± 1.00^{b*}	4.50 ± 0.50^{b*}	2.50 ± 0.50^{a}
E (%)	2.50 ± 0.50^{a*}	4.00 ± 1.00^{d*}	3.50 ± 0.50^{c*}	3.50 ± 0.50^{c*}	3.00 ± 1.00^{b}

Data are the mean±SEM of five replicate determinations. Values followed by different superscript letters significantly different across the groups. * Significantly differs from the STZ-treated rats that received distilled water. TP150, TPME at 150 mg/kg body weight; TP300, TPME at 300 mg/kg body weight; Hb, hemoglobin; PCV, packed cell volume.

Table 6
LC-MS characteristic of the compounds identified in the *Tridax procumbens* extract.

ID	RT (min)	Height	Height %	Area	Area %	M/Z	Identity	Fragments
1	0.475	1772	1.30057	1997	1.023139	284.2	Biochanin A	213, 284
2	5.691667	12,088	8.872057	8292.75	4.248692	317	Isorhamnetin	317
3	8.325	450	0.33028	1090.25	0.558577	337.05	Dicumarol	212, 300, 427
4	8.866667	846	0.620927	1126	0.576893	557.3	Cucurbitacin E	224, 317, 448, 529
5	9.425	740	0.543127	1039	0.532319	318.9	Myricetin	288, 318
6	11.8	967	0.709735	617.75	0.316497	482.9	Silymarin	483
7	16.7	1907	1.399654	2841.5	1.455809	302.2	Quercetin	107,302, 225
8	18.16667	1924	1.412131	2757	1.412516	271.2	Apigenin	271
9	20.33333	3131	2.298015	2981.1	1.527331	345.15	Echinone	111, 171, 225, 345
10	22.6	3116	2.287006	7007	3.589953	353.2	Akuammidine	353
11	25.06667	6444	4.729611	7860.75	4.027362	395.25	Bixin	304, 353, 395
12	25.25833	3937	2.889584	5457	2.795829	427.4	Lupeol	113,427
13	29.61667	811	0.595238	1204.25	0.616983	291.1	Catechin	291
14	36.08333	757	0.555604	1105.5	0.56639	415.05	Sitosterol	105, 415

RT, retention time; M/Z, molecular weight.

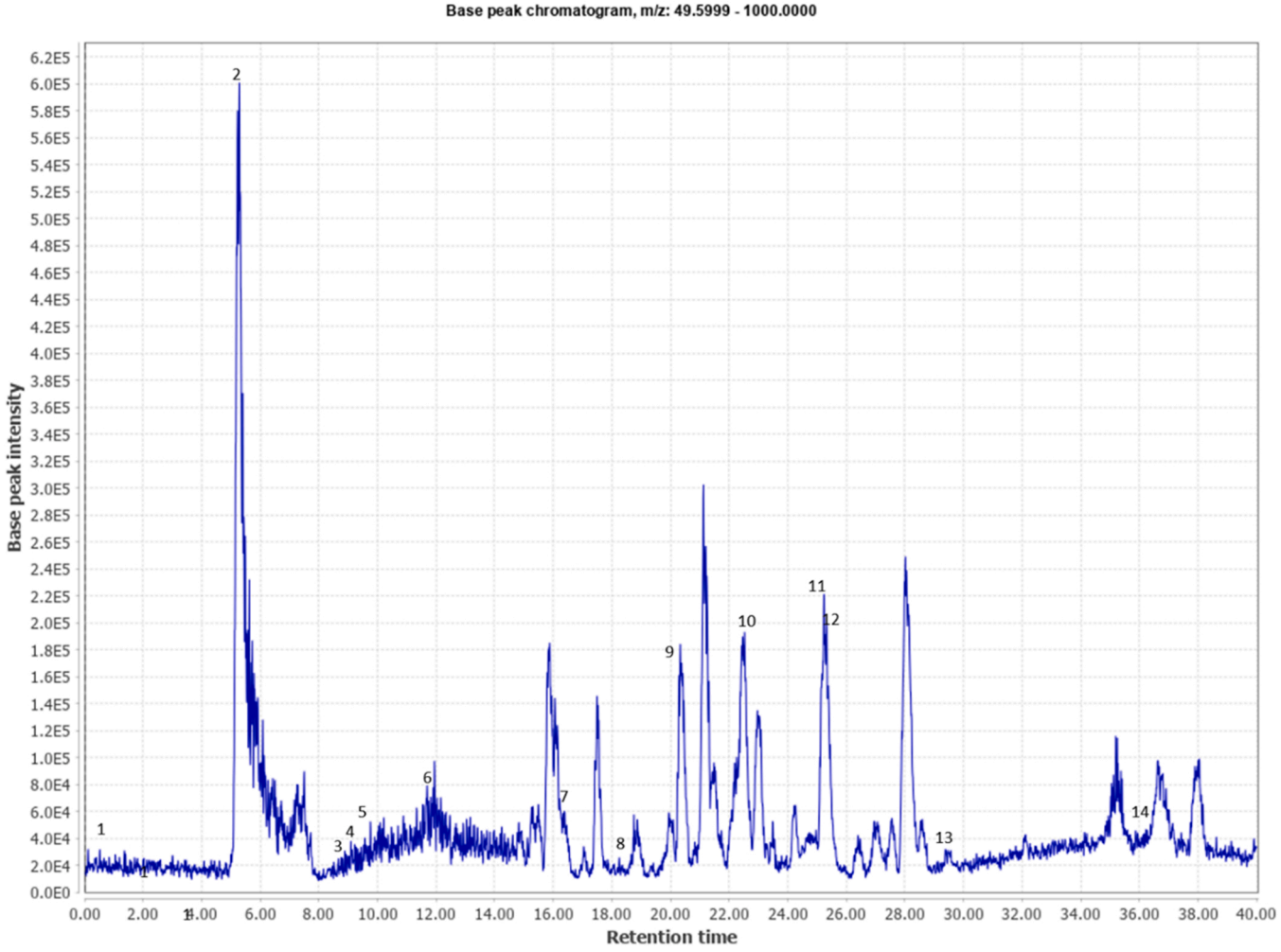


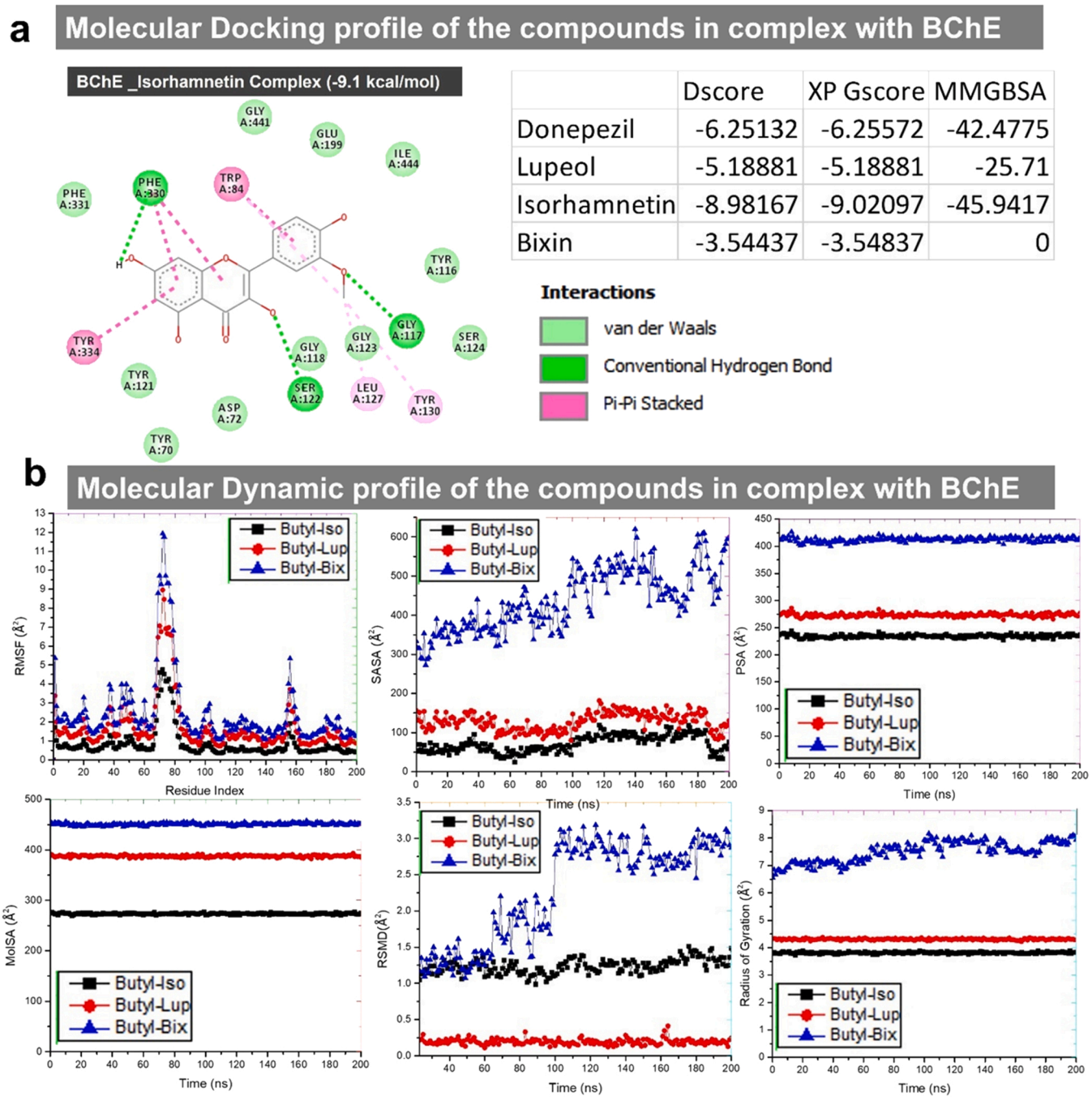
Fig. 7. LC-MS chromatogram of *Tridax procumbens*.

proteins. The high hydrogen bonds indicate an increasingly stable nature for the complex. furthermore, isorhamnetin-BChE complex demonstrated most desirably high simulation profile and remained in a steady state, and thus serve as a potential therapeutic template for future study.

However, the limitation of our study must be acknowledged. The absence of cellular experiment marked an important area that required further studies. Further experiment including molecular experiments, targets and therapeutic validation in cells are necessary for the clinical validation, and applicability of our findings. Further preclinical studies are currently in progress to examine the potential activities of these compound against diabetes and its associated complications.

5. Conclusions

Conclusively, the TPME could be considered a promising alternative therapeutic option for managing diabetic complications owing to its antidiabetic, antihyperlipidemic, antioxidant, and anti-inflammatory



	Dscore	XP Gscore	MMGBSA
Donepezil	-6.25132	-6.25572	-42.4775
Lupeol	-5.18881	-5.18881	-25.71
Isorhamnetin	-8.98167	-9.02097	-45.9417
Bixin	-3.54437	-3.54837	0

Fig. 8. The molecular docking and dynamics simulation of the TPME compounds in the docked cavity of BChE. (A) Two (2) dimensional simulation of receptor-ligand interactions between isorhamnetin and BChE (B) Present, RMSD, RMSF, Rg, SASA, PSA, and molSA.

effects in rats with STZ-induced diabetes.

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CRediT authorship contribution statement

Itam Ako Hogan: Conceptualization, Methodology, Data analysis, Software, Investigation, Visualization, Writing – original draft. **Bashir Lawal:** Conceptualization, Methodology, Data analysis, Software, Investigation, Visualization, Writing – original draft. **Yu-Cheng Kuo:** Conceptualization, Methodology, Data analysis, Software, Investigation, Writing – original draft. **Amos S. Onikanni:** Conceptualization, Methodology, Data analysis, Software, Investigation, Writing – original draft. **Abdulhakeem R. Agboola:** Conceptualization, Funding acquisition, Methodology, Data analysis, Software, Investigation, Writing – original draft. **Shukurat B. Babalola:** Data curation, Investigation, Software, Validation, Writing – review & editing. **Asmau N. Abubakar:** Data analysis, Software, Writing – review & editing, Supervision, Funding acquisition. **Yunusa O. Ibrahim:** Conceptualization, Methodology, Data analysis, Software, Investigation, Writing – original draft. **Gaber El-**

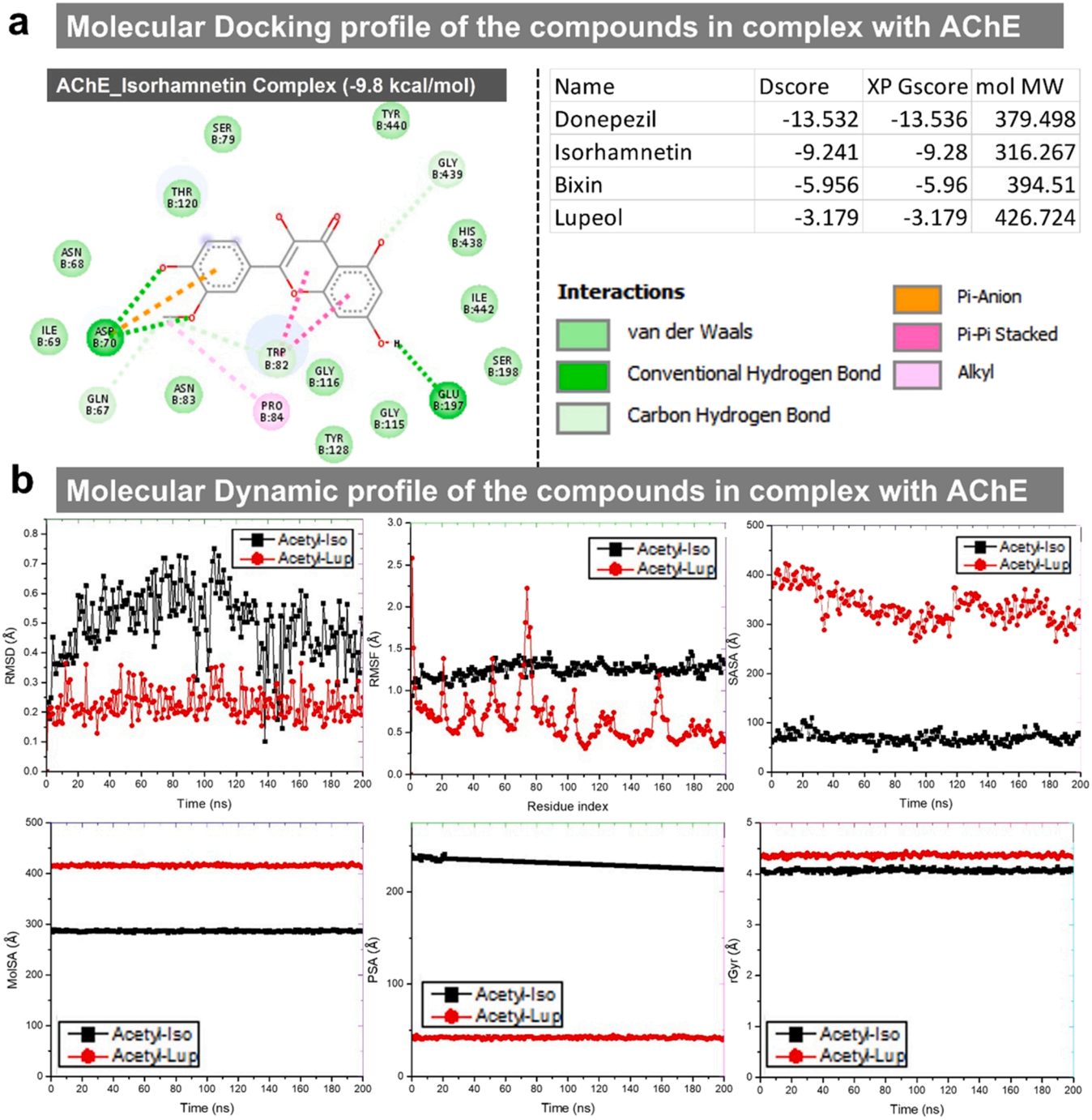

Fig. 9. The molecular docking and dynamics simulation of the TPME compounds in the docked cavity of AChE. (A) Two (2) dimensional simulation of receptor-ligand interactions between isorhamnetin and AChE (B) Present, RMSD, RMSF, Rg, SASA, PSA, and molSA.

Saber Batiha: Conceptualization, Supervision, Writing – review & editing, Software, Funding acquisition. **Hsu-Shan Huang:** Conceptualization, Methodology, Data analysis, Software, Investigation, Writing – original draft. **Halimat Yusuf Lukman:** Conceptualization, Methodology, Data analysis, Software, Investigation, Writing – original draft. **Femi Olawale:** Conceptualization, Funding acquisition, Methodology, Data analysis, Software, Investigation, Writing – original draft. **Adewale Oluwaseun Fadaka:** Data curation, Investigation, Software, Validation, Writing – review & editing. **Sarah M. Albogami:** Conceptualization, Methodology, Data analysis, Software, Investigation, Writing – original draft. **Mohammed Alorabi:** Conceptualization, Funding acquisition, Methodology, Data analysis, Software, Investigation, Writing – original draft. **Michel De Waard:** Data curation, Investigation, Software, Validation, Writing – review & editing. All authors read and approved the final version of the manuscript.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability

All replicate data can be made available upon reasonable request.

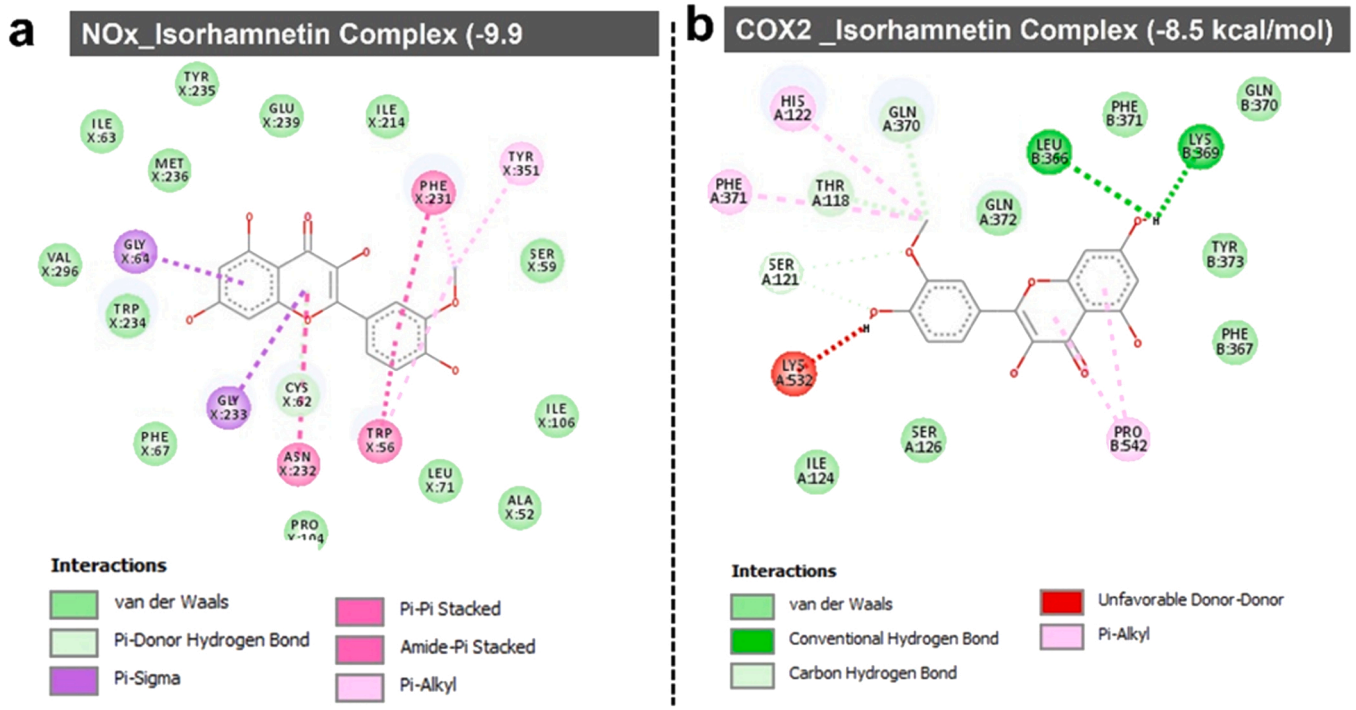

Fig. 10. The molecular docking profile of the TPME compounds in the docked cavity of (A) nitric oxide synthase, and (B) COX2.

References

[1] S.A. Mezil, B.A. Abed, Complication of diabetes mellitus, Ann. Rom. Soc. Cell Biol. (2021) 1546–1556.

[2] J.M. Forbes, M.E. Cooper, Mechanisms of diabetic complications, Physiol. Rev. 93 (1) (2013) 137–188.

[3] D. Sireesh, U. Dhamodharan, K. Ezhilarasi, V. Vijay, K.M. Ramkumar, Association of NF-E2 related factor 2 (Nrf2) and inflammatory cytokines in recent onset type 2 diabetes mellitus, Sci. Rep. 8 (1) (2018) 1–10.

[4] M. Ellenberg, Diabetic complications without manifest diabetes: complications as presenting clinical symptoms, JAMA 183 (11) (1963) 926–930.

[5] H. Sun, P. Saeedi, S. Karuranga, M. Pinkepank, K. Ogurtsova, B.B. Duncan, C. Stein, A. Basit, J.C. Chan, J.C. Mbanya, IDF diabetes atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045, Diabetes Res. Clin. Pract. 183 (2022), 109119.

[6] D.M. Nathan, Long-term complications of diabetes mellitus, N. Engl. J. Med. 328 (23) (1993) 1676–1685.

[7] P. Aja, E. Nwafor, A. Ibiam, O. Orji, N. Ezeani, B. Nwali, Evaluation of anti-diabetic and liver enzymes activity of aqueous extracts of Moringa oleifera and Bridelia ferruginea leaves in alloxan induced diabetic Albino rats, Int. J. Biochem. Res. Rev. 3 (3) (2013) 248.

[8] M. Pavan, R. Ranganath, A. Chaudhari, A. Aiyangar, K. Upadhayaya, H. Mehta, Incidence and measures to prevent intradialytic hypotension in patients on maintenance hemodialysis in a tertiary care centre in India, J. Nephrol. Ther. 1 (1) (2011) 101.

[9] K. Stadler, Oxidative stress in diabetes, in: S.I. Ahmad (Ed.), Diabetes: An Old Disease, A New Insight, Springer New York, New York, NY, 2013, pp. 272–287.

[10] L. Piconi, L. Quagliaro, A. Ceriello. Oxidative Stress in Diabetes, 2003, pp. 1144–1149.

[11] U. Asmat, K. Abad, K. Ismail, Diabetes mellitus and oxidative stress—a concise review, Saudi Pharm. J. 24 (5) (2016) 547–553.

[12] J.S. Bhatti, A. Sehrawat, J. Mishra, I.S. Sidhu, U. Navik, N. Khullar, S. Kumar, G. K. Bhatti, P.H. Reddy, Oxidative stress in the pathophysiology of type 2 diabetes and related complications: current therapeutics strategies and future perspectives, Free Radic. Biol. Med. 184 (2022) 114–134.

[13] M. Jaganjac, L. Milkovic, N. Zarkovic, K. Zarkovic, Oxidative stress and regeneration, Free Radic. Biol. Med. 181 (2022) 154–165.

[14] H. Yang, X. Jin, C.W.K. Lam, S.-K. Yan, Oxidative stress and diabetes mellitus, Clin. Chem. Lab. Med. 49 (11) (2011) 1773–1782.

[15] S.A. Antar, W. Abdo, R.S. Taha, A.E. Farage, L.E. El-Moselhy, M.E. Amer, A. S. Abdel Monsef, A.M. Abdel Hamid, E.M. Kamel, A.F. Ahmeda, A.M. Mahmoud, Telmisartan attenuates diabetic nephropathy by mitigating oxidative stress and inflammation, and upregulating Nrf2/HO-1 signaling in diabetic rats, Life Sci. 291 (2022), 120260.

[16] D. Nguyen, F. Ping, W. Mu, P. Hill, R.C. Atkins, S.J. Chadban, Macrophage accumulation in human progressive diabetic nephropathy, Nephrology 11 (3) (2006) 226–231.

[17] L. Ma, F. Wu, Q. Shao, G. Chen, L. Xu, F. Lu, Baicalin alleviates oxidative stress and inflammation in diabetic nephropathy via Nrf2 and MAPK signaling pathway, Drug Des. Dev. Ther. 15 (2021) 3207.

[18] K. Kachhawa, M. Varma, P. Kachhawa, D. Agrawal, M. Shaikh, S. Kumar, Study of dyslipidemia and antioxidant status in chronic kidney disease patients at a hospital in South East Asia, J. Health Res. Rev. 3 (1) (2016) 28.

[19] A.M. Mahmoud, A. El-Twab, M. Sanaa, E.S. Abdel-Reheim, Consumption of polyphenol-rich Morus alba leaves extract attenuates early diabetic retinopathy: the underlying mechanism, Eur. J. Nutr. 56 (4) (2017) 1671–1684.

[20] N.M. Al-Rasheed, N.M. Al-Rasheed, Y.A. Bassiouni, I.H. Hasan, M.A. Al-Amin, H. N. Al-Ajmi, A.M. Mahmoud, Simvastatin ameliorates diabetic nephropathy by attenuating oxidative stress and apoptosis in a rat model of streptozotocin-induced type 1 diabetes, Biomed. Pharmacother. 105 (2018) 290–298.

[21] A. Mima, Inflammation and oxidative stress in diabetic nephropathy: new insights on its inhibition as new therapeutic targets, J. Diabetes Res. 2013 (2013).

[22] S. Inzucchi, R. Bergenstal, J. Buse, M. Diamant, E. Ferrannini, M. Nauck, A. Peters, A. Tsapas, R. Wender, a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes, Diabetes Care 38 (2015) 140–149.

[23] T.C. Okoye, P.F. Uzor, C.A. Onyeto, E.K. Okereke, 18 – Safe African medicinal plants for clinical studies, in: V. Kuete (Ed.), Toxicological Survey of African Medicinal Plants, Elsevier, 2014, pp. 535–555.

[24] K. Biswas, I. Chattopadhyay, R.K. Banerjee, U. Bandyopadhyay, Biological activities and medicinal properties of neem (Azadirachta indica), Curr. Sci. (2002) 1336–1345.

[25] M. Diop, F. Niang-Diop, S.D. Dieng, A. Samb, G.E.D. Manga, A.P. Sané, M.B. Sène, B. Sambou, A. Goudiaby, E.A. Diatta, Ethnobotanical study of medicinal plants for treatment of diabetes and hypertension used in communities near Fathala Forest, Senegal, Ethnobot. Res. Appl. 23 (2022) 1–15.

[26] G.P. Khumalo, B.E. Van Wyk, Y. Feng, I.E. Cock, A review of the traditional use of Southern African medicinal plants for the treatment of inflammation and inflammatory pain, J. Ethnopharmacol. 283 (2022), 114436.

[27] A. Mohammed, N. Tajuddeen, Antidiabetic compounds from medicinal plants traditionally used for the treatment of diabetes in Africa: a review update (2015–2020), S. Afr. J. Bot. 146 (2022) 585–602.

[28] S. Arora, K. Kaur, S. Kaur, Indian medicinal plants as a reservoir of protective phytochemicals, Teratog. Carcinog. Mutagen. 23 (S1) (2003) 295–300.

[29] M.M. Iwu, African Medicinal Plants, CRC Press, Maryland, 1993.

[30] D.A. Adesina, S.F. Adefolalu, A.A. Jigam, B. Lawal, Antiplasmodial effect and sub-acute toxicity of alkaloid, flavonoid and phenolic extracts of Sida acuta leaf on Plasmodium berghei-infected animals, J. Taibah Univ. Sci. 14 (1) (2020) 943–953.

[31] S. Mundada, R. Shivhare, Pharmacology of Tridax procumbens a weed, Int. J. PharmTech Res. 2 (2) (2010) 1391–1394.

[32] S. Beck, H. Mathison, T. Todorov, E. Calder, O.R. Kopp, A review of medicinal uses and pharmacological activities of Tridax procumbens (L.), J. Plant Stud. 10 (2018).

[33] B. Pardeshi, V. Bhiungade, Tridax procumbens: a medicinal gift of nature for healing diabetic wound, Int. J. Chem. Phys. Sci. 5 (2016) 107–112.

[34] N.A. Ingle, H.V. Dubey, N. Kaur, R. Gupta, Tridax procumbens: a multiuseful weed a review, J. Adv. Oral Res. 5 (1) (2014) 14–16.
[35] S. Udupa, A. Udupa, D. Kulkarni, Influence of Tridax procumbens on lysyl oxidase activity and wound healing, Planta Med. 57 (04) (1991) 325–327.
[36] Y. Andriana, T.D. Xuan, T.N. Quy, T.N. Minh, T.M. Van, T.D. Viet, Antihyperuricemia, antioxidant, and antibacterial activities of Tridax procumbens L, Foods 8 (1) (2019) 21.
[37] A. Sonawane, R.S. Srivastava, N. Sanghavi, Y. Malode, B. Chavan, Anti-diabetic activity of Tridax procumbens, J. Sci. Innov. Res. 3 (2) (2014) 221–226.
[38] R. Kumar, P. Samuel, M. Selvakumar, K. Shalini, Anti-oxidant, anti-diabetic, antimicrobial and hemolytic activity of Tridax procumbens, J. Chem. Pharm. Res. 8 (2) (2016) 808–812.
[39] D.A. Bhagwat, S.G. Killedar, R.S. Adnaik, Anti-diabetic activity of leaf extract of Tridax procumbens, Int. J. Green Pharm. (IJGP) 2 (2) (2008).
[40] H. Pareek, S. Sharma, B.S. Khajja, K. Jain, G.C. Jain, Evaluation of hypoglycemic and anti-hyperglycemic potential of Tridax procumbens (Linn.), BMC Complement. Altern. Med. 9 (1) (2009) 48.
[41] R.R. Petchi, S. Parasuraman, C. Vijaya, Antidiabetic and antihyperlipidemic effects of an ethanolic extract of the whole plant of Tridax procumbens (Linn.) in streptozotocin-induced diabetic rats, J. Basic Clin. Pharm. 4 (4) (2013) 88–92.
[42] U.B. Alozieuwa, B. Lawal, S. Sani, A.S. Onikanni, O. Osuji, Y.O. Ibrahim, S. B. Babalola, G. Mostafa-Hedeab, A.A. Alsayegh, S. Albogami, G.E.-S. Batiha, A.T. H. Wu, H.-S. Huang, C.A. Conte-Junior, Luteolin-rich extract of Thespesia garckeana F. Hoffm. (Snot Apple) contains potential drug-like candidates and modulates glycemic and oxidoinflammatory aberrations in experimental Animals, Oxid. Med. Cell. Longev. 2022 (2022) 1215097.
[43] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology, Elsevier, 1999, pp. 152–178.
[44] C.-C. Chang, M.-H. Yang, H.-M. Wen, J.-C. Chern, Estimation of total flavonoid content in propolis by two complementary colorimetric methods, J. Food Drug Anal. 10 (3) (2002).
[45] M. Oyaizu, Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine, Jpn. J. Nutr. Diet. 44 (6) (1986) 307–315.
[46] N. Tsado, B. Lawal, P. Ossa, A. Jagaba, G.N. Kontagora, J.A. Gboke, A. Umar, J. Oladunjoye, Antioxidants and antimicrobial activities of methanol extract of Newbouldia laevis and Crateva adansonii, J. Pharm. Allied Health Sci. 6 (2016) 14–19.
[47] K. Panjamurthy, S. Manoharan, C.R. Ramachandran, Lipid peroxidation and antioxidant status in patients with periodontitis, Cell. Mol. Biol. Lett. 10 (2) (2005) 255–264.
[48] K. Worthington, Alpha Amylase Worthington Enzyme Manual, Worthington Biochemical Corporation, Lakewood, NJ, 1993, pp. 36–41 (Google Scholar).
[49] V. Thenmozhi, V. Elango, J. Sadique, Anti-inflamatory activity of some Indian medicinal plants, Anc. Sci. Life 8 (3–4) (1989) 258.
[50] Y. Mizushima, M. Kobayashi, Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins, J. Pharm. Pharmacol. 20 (3) (1968) 169–173.
[51] O. Oyedapo, A.J. Famurewa, Antiprotease and membrane stabilizing activities of extracts of Fagara zanthoxyloides, Olax subscorpioides and Tetrapleura tetraptera, Int. J. Pharmacogn. 33 (1) (1995) 65–69.
[52] D. Lorke, A new approach to practical acute toxicity testing, Arch. Toxicol. 54 (4) (1983) 275–287.
[53] B. Lawal, Y.-C. Kuo, M.R. Sumitra, A.T. Wu, H.-S. Huang, In vivo pharmacokinetic and anticancer studies of HH-N25, a selective inhibitor of Topoisomerase I, and hormonal signaling for treating breast cancer, J. Inflamm. Res. 14 (2021) 4901.
[54] S. Dhar, N. Kolishetti, S.J. Lippard, O.C. Farokhzad, Targeted delivery of a cisplatin prodrug for safer and more effective prostate cancer therapy in vivo, Proc. Natl. Acad. Sci. 108 (5) (2011) 1850–1855.
[55] C.V. Rao, B.S. Reddy, V.E. Steele, C.-X. Wang, X. Liu, N. Ouyang, J.M.R. Patlolla, B. Simi, L. Kopelovich, B. Rigas, Nitric oxide–releasing aspirin and indomethacin are potent inhibitors against colon cancer in azoxymethane-treated rats: effects on molecular targets, Mol. Cancer Ther. 5 (6) (2006) 1530–1538.
[56] E. Etuk, Animals models for studying diabetes mellitus, Agric. Biol. JN Am. 1 (2) (2010) 130–134.
[57] O. Shittu, B. Lawal, O. Oluyomi, Effects of methanol extract of Musca domestica larvae on antioxidants enzymes in T. Brucei infected rats, Niger. J. Biochem. Mol. Biol. 29 (2) (2014) 1–10.
[58] L. Bashir, O.K. Shittu, M.B. Busari, S. Sani, M.I. Aisha. Safety evaluation of giant African land snails (Archachatina maginata) haemolymph on hematological and biochemical parameters of albino rats, *J. Adv. Med. Pharm. Sci*, *3*(3) (2015)122-30.
[59] J.O. Olugbodi, K. Samaila, B. Lawal, O.O. Anunobi, R.S. Baty, O.B. Ilesanmi, G.E.-S. Batiha, Computational and preclinical evidence of anti-ischemic properties of L-carnitine-rich supplement via stimulation of anti-inflammatory and antioxidant events in testicular torsed rats, Oxid. Med. Cell. Longev. 2021 (2021) 5543340.
[60] J.O. Olugbodi, O. David, E.N. Oketa, B. Lawal, B.J. Okoli, F. Mtunzi, Silver nanoparticles stimulates spermatogenesis impairments and hematological alterations in testis and epididymis of male rats, Molecules 25 (5) (2020) 1063.
[61] J. Dacie, S. Lewis. Practical Textbook of Haematology, seventh ed., Church Livingstone, Edinburgh, 1991, pp. 54–79.
[62] F. De Ritis, M. Coltorti, G. Giusti, Serum-transaminase activities in liver disease, Lancet 1 (7752) (1972) 685–687.
[63] R. Rej, Measurement of aminotransferases: part 1. Aspartate aminotransferase, Crit. Rev. Clin. Lab Sci. 21 (2) (1984) 99–186.
[64] P.J. Wright, D.T. Plummer, The use of urinary enzyme measurements to detect renal damage caused by nephrotoxic compounds, Biochem. Pharmacol. 23 (1) (1974) 65–73.
[65] A.G. Gornall, C.J. Bardawill, M.M. David, Determination of serum proteins by means of the biuret reaction, J. Biol. Chem. 177 (2) (1949) 751–766.
[66] Y. Suzuki, Y. Sakagishi, Determination of serum bilirubin by the diazo method using the diazotized 3-nitroaniline reacting readily with the photoproducts of bilirubin, 臨床化学 23 (2) (1994) 158–163.
[67] B.T. Doumas, W.A. Watson, H.G. Biggs, Albumin standards and the measurement of serum albumin with bromcresol green, Clin. Chim. Acta 31 (1) (1971) 87–96.
[68] J.R. Delanghe, M.M. Speeckaert, Creatinine determination according to Jaffe-what does it stand for? NDT 4 (2) (2011) 83–86.
[69] P.L. Searle, The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen. A review, Analyst 109 (5) (1984) 549–568.
[70] J.J. Albers, G.R. Warnick, M.C. Chenng, Quantitation of high density lipoproteins, Lipids 13 (12) (1978) 926–932.
[71] E. Van Handel, D. Zilversmit, Micromethod for the direct determination of serum triglycerides, J. Lab. Clin. Med. 50 (1) (1957) 152–157.
[72] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (6) (1972) 499–502.
[73] P. Finley, N. Tietz, Clinical Guide to Laboratory Tests, WB Saunders Company, 1996.
[74] H.P. Misra, I. Fridovich, The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase, J. Biol. Chem. 247 (10) (1972) 3170–3175.
[75] A.K. Sinha, Colorimetric assay of catalase, Anal. Biochem. 47 (2) (1972) 389–394.
[76] L. Kum-Tatt, I.-K. Tan, A new colorimetric method for the determination of glutathione in erythrocytes, Clin. Chim. Acta 53 (2) (1974) 153–161.
[77] K. Shagirtha, N. Bashir, S. MiltonPrabu, Neuroprotective efficacy of hesperetin against cadmium induced oxidative stress in the brain of rats, Toxicol. Ind. Health 33 (5) (2017) 454–468.
[78] K.M. Miranda, M.G. Espey, D.A. Wink, A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite, Nitric Oxide 5 (1) (2001) 62–71.
[79] G.L. Ellman, K.D. Courtney, V. Andres Jr, R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 (2) (1961) 88–95.
[80] D.E.C. Marcus, D. Hanwell, David C. Lonie, Tim Vandermeersch, Eva Zurek, Geoffrey R. Hutchison, Avogadro: An advanced semantic chemical editor, visualization, and analysis platform, J. Cheminform. 4 (2012) 17.
[81] O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. 31 (2) (2010) 455–461.
[82] B. Lawal, Y.-L. Liu, N. Mokgautsi, H. Khedkar, M.R. Sumitra, A.T.H. Wu, H.-S. Huang, Pharmacoinformatics and preclinical studies of NSC765690 and NSC765599, potential STAT3/CDK2/4/6 inhibitors with antitumor activities against NCI60 human tumor cell lines, Biomedicines 9 (1) (2021) 92.
[83] B. Lawal, C.-Y. Lee, N. Mokgautsi, M.R. Sumitra, H. Khedkar, A.T.H. Wu, H.-S. Huang, mTOR/EGFR/iNOS/MAP2K1/FGFR/TGFB1 are druggable candidates for N-(2,4-difluorophenyl)-2′,4′-difluoro-4-hydroxybiphenyl-3-carboxamide (NSC765598), with consequent anticancer implications, Front. Oncol. 11 (932) (2021).
[84] S.-Y. Wu, K.-C. Lin, B. Lawal, A.T.H. Wu, C.-Z. Wu, MXD3 as an onco-immunological biomarker encompassing the tumor microenvironment, disease staging, prognoses, and therapeutic responses in multiple cancer types, Comput. Struct. Biotechnol. J. (2021).
[85] J.-H. Chen, A.T.H. Wu, B. Lawal, D.T.W. Tzeng, J.-C. Lee, C.-L. Ho, T.-Y. Chao, Identification of cancer hub gene signatures associated with immune-suppressive tumor microenvironment and ovatodiolide as a potential cancer immunotherapeutic agent, Cancers 13 (15) (2021) 3847.
[86] D.S. Visualizer, BIOVIA, Dassault Systèmes, BIOVIA Workbook, Release 2020; BIOVIA Pipeline Pilot, Release 2020, San Diego: Dassault Systèmes, 2020.
[87] A.O. Fadaka, N.R.S. Sibuyi, A.M. Madiehe, M. Meyer, Computational insight of dexamethasone against potential targets of SARS-CoV-2, J. Biomol. Struct. Dyn. 40 (2) (2022) 875–885.
[88] W.G. Hoover, Canonical dynamics: equilibrium phase-space distributions, Phys. Rev. A 31 (3) (1985) 1695.
[89] S. Mahmud, M.R. Parves, Y.M. Riza, K.M. Sujon, S. Ray, F.A. Tithi, Z.F. Zaoti, S. Alam, N. Absar, Exploring the potent inhibitors and binding modes of phospholipase A2 through in silico investigation, J. Biomol. Struct. Dyn. 38 (14) (2020) 4221–4231.
[90] M. Dutta, A.M. Tareq, A. Rakib, S. Mahmud, S.A. Sami, J. Mallick, M.N. Islam, M. Majumder, M.Z. Uddin, A. Alsubaie, A.S.A. Almalki, M.U. Khandaker, D. A. Bradley, M.S. Rana, T.B. Emran, Phytochemicals from Leucas zeylanica targeting main protease of SARS-CoV-2: chemical profiles, molecular docking, and molecular dynamics simulations, Biology 10 (8) (2021) 789.
[91] D. Villaño, M. Fernández-Pachón, M.L. Moyá, A. Troncoso, M. García-Parrilla, Radical scavenging ability of polyphenolic compounds towards DPPH free radical, Talanta 71 (1) (2007) 230–235.
[92] V.H.M. Santos, I.O. Minatel, G.P.P. Lima, R.M.G. Silva, C.-Y.O. Chen, Antioxidant capacity and phytochemical characterization of Spathodea campanulata growing in different climatic zones in Brazil, Biocatal. Agric. Biotechnol. 24 (2020), 101536.

[93] Z. Li, Y.-N. Geng, J.-D. Jiang, W.-J. Kong, Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus, Evid. Based Complement. Altern. Med. 2014 (2014), 289264.
[94] A.S. Onikanni, B. Lawal, A.O. Olusola, J.O. Olugbodi, S. Sani, B.O. Ajiboye, O. B. Ilesanmi, M. Alqarni, G. Mostafa-Hedeab, A.J. Obaidullah, G.E. Batiha, A.T. H. Wu, Sterculia tragacantha lindl leaf extract ameliorates STZ-induced diabetes, oxidative stress, inflammation and neuronal impairment, J. Inflamm. Res. 14 (2021) 6749–6764.
[95] A.B. Thalkari, P.N. Karwa, P.S. Shinde, C.S. Gawli, P.S. Chopane, Pharmacological actions of Tridax procumbens L.: a scientific review, Res. J. Pharmacogn. Phytochem. 12 (1) (2020) 27–30.
[96] D. Tungmunnithum, A. Thongboonyou, A. Pholboon, A. Yangsabai, Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview, Medicines 5 (3) (2018) 93.
[97] F. Hajiaghaalipour, M. Khalilpourfarshbafi, A. Arya, Modulation of glucose transporter protein by dietary flavonoids in type 2 diabetes mellitus, Int. J. Biol. Sci. 11 (5) (2015) 508.
[98] J.C. Ikewuchi, Alteration of plasma biochemical, haematological and ocular oxidative indices of alloxan induced diabetic rats by aqueous extract of Tridax procumbens Linn (Asteraceae), Excli J. 11 (2012) 291–308.
[99] D. Álvarez-Cilleros, M.Á. Martín, S. Ramos, (-)-Epicatechin and the colonic 2, 3–dihydroxybenzoic acid metabolite regulate glucose uptake, glucose production, and improve insulin signaling in renal NRK-52E cells, Mol. Nutr. Food Res. 62 (4) (2018) 1700470.
[100] G. Rena, D.G. Hardie, E.R. Pearson, The mechanisms of action of metformin, Diabetologia 60 (9) (2017) 1577–1585.
[101] P. Bianchi, O. Kunduzova, E. Masini, C. Cambon, D. Bani, L. Raimondi, M.-H. Seguelas, S. Nistri, W. Colucci, N. Leducq, A. Parini, Oxidative Stress by Monoamine Oxidase Mediates Receptor-Independent Cardiomyocyte Apoptosis by Serotonin and Postischemic Myocardial Injury, Circulation 112 (21) (2005) 3297–3305.
[102] N. Kaludercic, J. Mialet-Perez, N. Paolocci, A. Parini, F. Di Lisa, Monoamine oxidases as sources of oxidants in the heart, J. Mol. Cell. Cardiol. 73 (2014) 34–42.
[103] A. Sturza, L. Noveanu, O. Duicu, D.M. Muntean, P172Monoamine oxidase inhibition corrects endothelial dysfunction in experimental diabetes, Cardiovasc. Res. 103 (Suppl. 1) (2014). S30-S30.
[104] A. Sturza, O.M. Duicu, A. Vaduva, M.D. Dănilă, L. Noveanu, A. Varró, D. M. Muntean, Monoamine oxidases are novel sources of cardiovascular oxidative stress in experimental diabetes, Can. J. Physiol. Pharmacol. 93 (7) (2015) 555–561.
[105] J. Tong, G. Rathitharan, J.H. Meyer, Y. Furukawa, L.-C. Ang, I. Boileau, M. Guttman, O. Hornykiewicz, S.J. Kish, Brain monoamine oxidase B and A in human parkinsonian dopamine deficiency disorders, Brain 140 (9) (2017) 2460–2474.
[106] H. Emory, N. Mizrahi, Monoamine oxidase inhibition in a patient with type 1 diabetes and depression, J. Diabetes Sci. Technol. 10 (5) (2016) 1203–1204.
[107] M.M. Elseweidy, N. Zein, M.M. Hassan, F.Y. Mohamed, Modulation of Serum Inflammatory Pattern, Oxidative Stress, Selected Neurotransmitters in Cerebral Cortex of Alloxan Diabetic Rats: Role of Curcuminoids and Fish Oil as Therapeutic Agents.
[108] A. Rübsam, S. Parikh, P.E. Fort, Role of inflammation in diabetic retinopathy, Int. J. Mol. Sci. 19 (4) (2018) 942.
[109] M. Sobeh, M. El-Raey, S. Rezq, M.A.O. Abdelfattah, G. Petruk, S. Osman, A.M. El-Shazly, H.A. El-Beshbishy, M.F. Mahmoud, M. Wink, Chemical profiling of secondary metabolites of Eugenia uniflora and their antioxidant, anti-inflammatory, pain killing and anti-diabetic activities: a comprehensive approach, J. Ethnopharmacol. 240 (2019), 111939.
[110] S.A. Adefegha, F.A. Dada, S.I. Oyeleye, G. Oboh, Effects of berberine on cholinesterases and monoamine oxidase activities, and antioxidant status in the brain of streptozotocin (STZ)-induced diabetic rats, J. Basic Clin. Physiol. Pharmacol. (20200173) (2021).
[111] K.M. Capiotti, D.A. De Moraes, F.P. Menezes, L.W. Kist, M.R. Bogo, R.S. Da Silva, Hyperglycemia induces memory impairment linked to increased acetylcholinesterase activity in zebrafish (Danio rerio), Behav. Brain Res. 274 (2014) 319–325.
[112] M. Ramrao, S. Ladde, Evaluation of nootropic effects of aqueous extract of Tridax procumbens Linn on cognitive functions in mice, Res. J. Life Sci. Bioinform. Pharm. Chem. Sci. 4 (2018) 242.
[113] O.K. Shittu, B. Lawal, B.U. Alozieuwa, G.M. Haruna, A.N. Abubakar, E. B. Berinyuy, Alteration in biochemical indices following chronic administration of methanolic extract of Nigeria bee propolis in Wistar rats, Asian Pac. J. Trop. Dis. 5 (8) (2015) 654–657.
[114] B. Lawal, O.K. Shittu, F.I. Oibiokpa, H. Mohammed, S.I. Umar, G.M. Haruna, Antimicrobial evaluation, acute and sub-acute toxicity studies of Allium sativum, J. Acute Dis. 5 (4) (2016) 296–301.
[115] J. Ibrahim, A.Y. Kabiru, T. Abdulrasheed-Adeleke, B. Lawal, A.H. Adewuyi, Antioxidant and hepatoprotective potentials of curcuminoid isolates from turmeric (Curcuma longa) rhizome on CCl4-induced hepatic damage in Wistar rats, J. Taibah Univ. Sci. 14 (1) (2020) 908–915.
[116] B. Al-Trad, H. Alkhateeb, W. Alsmadi, M. Al-Zoubi, Eugenol ameliorates insulin resistance, oxidative stress and inflammation in high fat-diet/streptozotocin-induced diabetic rat, Life Sci. 216 (2019) 183–188.