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RESEARCH

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Evaluation of the therapeutic potentials of extract fractions of *Vernonia calvoana* on streptozotocin-induced diabetic rats: approach through in silico, in vitro and in vivo studies

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Abstract

Background Diabetes is a serious metabolic disorder and many medicinal plants are used in traditional medicine to manage it. This study aimed to evaluate the therapeutic effects of *Vernonia calvoana* (*V. calvoana*) extract fractions on streptozotocin-induced diabetic rat models. In this study, we first investigated the binding affinity of ligands from extracts of *V. calvoana* crystal structure proteins using a molecular docking approach. Furthermore, the *in silico* predictions were validated by in vitro and in vivo biochemical evaluations to ascertain the efficacy of these extract fractions. The in vitro antioxidant activity of the fractions was evaluated using DPPH, FRAP, SOD, and LPx scavenging. For biological activity, extract fractions of *V. calvoana* and metformin (400 mg and 500 mg/kg body weight, respectively) were administered to diabetic rats for 21 days after induction and confirmation of diabetes.

Results The radical scavenger activities of the fractions showed a good dose-dependent reaction activity. A significant reduction in hyperglycemia, hyperlipidemia, nephrotoxicity, and hepatotoxicity was observed in all experimental treated groups. Improved hematological and histopathological changes were also observed.

Conclusion The In silico analyses revealed that all the compounds from extract fractions of *V. calvoana* have varying binding affinity for PFK and lipoprotein lipase, with some showing higher affinity than the standard drug, further validating the biological activity of the plant. The results of this study indicated that *V. calvoana* extracts might have potential value in treating complications arising from diabetes mellitus.

Keywords Diabetes mellitus, Molecular docking, *Vernonia calvoana*, Antioxidants, Bioactive compound

1 Background

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia arising from disorders in the production/secretion and action of insulin, or both. The disease is mainly influenced by various genetic factors, and people with these diseases are predisposed to developing diabetes-related complications [1]. According to a study by the WHO, approximately 422 million people have diabetes mellitus. Most people with diabetes

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mellitus fall into two broad categories: type 1 and type 2. Type 1 diabetes is characterized by a lack of insulin production in the body. Type 2 diabetes is becoming more common in lower and middle-class countries. It is estimated to be considered the leading cause of liver disease and liver disease is a primary reason of death in type 2 diabetes [2]. The link between diabetes and atherosclerosis has been extensively studied to explain the risk of cardiovascular disease in diabetes. It is associated with the hyperglycemia-induced formation of advanced glycation end products (AGEs) and/or reactive glycation oxygen species that impair the stability of the diabetic apo-lipoprotein complex and lead to an atherogenic state [3]. Access to affordable treatment is crucial for people with diabetes. Currently, drugs used to treat type 2 diabetes have high side effects and are expensive.

Vernonia calvoana (V. calvoana) is sometimes referred to as the sweet and bitter leaf. The plant is grown in swampy or wet soil and is more likely to grow in a wet environment (personal interaction with natives). Despite their extensive use of plants of the genus Vernonia in food and medicine, and a review of the genus Vernonia in humans and animals by [4], *V. calvoana* Hook. F. was one of the least studied species of the genus until recently. During the screening of antidiabetic natural products in our laboratory, it was found that *V. calvoana* leaf extract possesses phytochemical compounds with hypoglycemic activity, antioxidant activity, and hepatoprotective activity [5, 6]. A report by [7] documented the potent antioxidant potential of the inflorescences part of this plant. [8] reported on the lipid-lowering and cardio-protective effects of *V. calvoana* ethanol extract on paracetamol-induced liver toxicity in experimental rats. Authors [9] also demonstrated the enhancing potential of this plant in acetaminophen-treated rats. Reports from our laboratory, which served as a pilot study for this research, showed the presence of bioactive compounds with in vitro antioxidant activity [5, 6]. Given the reports of this plant as highlighted above and concerns about the side effects of available treatment options, the search for viable alternative treatment options that are freely available and affordable is imminent. Therefore, these studies examine the therapeutic effects of extracts of *V. calvoana* on streptozotocin-induced diabetics.

2 Method

2.1 Plant collection and preparation

Garden-fresh leaves of *V. calvoana* were bought very early in the day in Ugep, in Yakurr L.G.A., Cross River State. The leaves were identified and confirmed by Dr. Michael Eko in the University of Calabar Department of Botany, and a specimen (BCH/VC/01) was deposited in the Department's herbarium. The leaves were cleaned and left to dry for a week before being ground into powder.

2.2 Extraction

The pulverized leaves weighing 5 kg were extracted using 8000 ml (8.0 L) of 80% ethanol for 48 h. The extract was filtered twice, first with a chess cloth and then with Whatman 1 filter paper. The filtrate was concentrated to 10% by volume at 45 °C. in a rotary evaporator and then left to dry completely in a water bath, with a black paste (crude extract) obtained and refrigerated at − 4 °C.

2.3 Fractionation

The crude extract was chromatographically fractionated using separate solvents (methanol and *n*-hexane) in a column filled with 60–120 mesh silica gel.

The fractions were combined, rotary evaporated at 60 °C to 10% of their volume, and then placed in a water bath for complete dryness. The dried extract fractions were labeled and kept refrigerated at 4 °C until needed for use.

2.4 In vitro evaluation of the antioxidant activity of *n*-hexane and methanol fractions

2.4.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) method

Evaluation of the antioxidant activity of fractions of *V. calvoana* was assessed using the DPPH scavenger assay as described by [10]. Two mils of graded concentrations of the fractions (10–400 g/mL each) were dissolved with 1 mL of 0.5 mM DPPH solution. The mixture was thoroughly mixed and allowed to stand at room temperature for 30 min. The color change of DPPH was estimated by measuring absorbance at 517 nm with ascorbic acid as a reference standard.

Percent antioxidant activity was calculated as:

$$\%\text{Antioxidant Activity} = 100 - \frac{(\text{Abs sample} - \text{Abs blank})}{\text{Abs control}} \times 100$$

where Ab is absorbance.

2.4.2 Ferric reducing antioxidant power (FRAP) method

The scavenging activity of *V. calvoana* extracts was estimated using the FRAP test method as described by [11]. Freshly prepared working solution (2 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml FeCl 3 6H2O at a temperature of 37 °C) and 0.1 ml fractions in methanol were mixed together and the absorbance at 593 nm after 30 min measured. Using known concentrations (100 to 1000 mol/L) of FeSO4×7H2O, a standard plot was drawn and the antioxidant activity was estimated from the known concentrations of the Fe2+solution. Ascorbic acid was used as a standard.

The FRAP value was estimated using the formula

$$\text{FRAP value}(\mu M) = \frac{\text{change in absorbance of room } 0-4 \text{ min}}{\text{Change in absorbance of STD } 0-4 \text{ min}} \times \text{FRAP value of STD}$$

$$\text{FRAP value of STD} = 1000\ \mu M$$

2.4.3 Superoxide anion assay

Riboflavin light nitrogen blue tetrazolium (NBT) system assay was performed for superoxide scavenging activity as described by [12]. The assay mixture contained 0.5 mL phosphate buffer (50 mM, pH 7.6), 0.3 mL riboflavin (50 mM), 0.25 mL PMS (20 mM), and 0.1 mL NBT (0.5 mM) with the addition of 1 ml fraction in methanol. The test mixture was illuminated with a fluorescent lamp to initiate the reaction and the absorbance was measured at 560 nm against a control sample. Percent inhibition of superoxide anion generation was calculated using the following formula:

$$\%\ \text{scavenging activity} = \left(1 - \frac{\text{Absorbance of fraction}}{\text{Absorbance of control}}\right) \times 100$$

2.4.4 Anti-lipid peroxidation assay

The anti-lipid peroxidation assay was performed according to a modified procedure from [13]. An aliquot of egg York homogenate (0.5 ml of 10% v/v) made up of KCl (1.15%, w/v) and 0.1 ml extracts were mixed in a test tube and made up with distilled water to 1 ml, then 1.5 ml 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml 0.8% (w/v) TBA solution in 1.1% sodium dodecyl sulfate and 0.5 ml 20% TCA was added, the resulting mixture was vortex and then heated to 95 °C for 60 min. After cooling, 5.0 mL of butanol was added to all tubes and centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was observed at 532 nm. The absorbance of the supernatant at 532 nm was recorded. Percentage anti-lipid peroxidation was measured by the formula:

$$\%\ \text{anti - lipid peroxidation} = \left(1 - \frac{S}{C}\right) \times 100$$

where *C* represents the absorbance of the control, *and S* represents the absorbance of the test sample.

2.5 Animal handling/design

The experimental design was approved by the Animal Ethics Committee of the Faculty of Basic Medical Sciences with approval number 149BCM3021. In the present study, 36 albino rats of both sexes weighing 100–150 g separated into 6 wooden cages of 6 animals each were used for this study. The rats were fed pellets and had access to water ad libitum at a control room temperature of 25 °C. The rats were acclimatized in the animal facility of the Department of Biochemistry for three weeks. The extracts were administered twice daily. The rats were divided into six groups of six animals each as follows:

- Group 1: Normal control (NC) treated with 0.2 mL of dimethylsulfoxide
- Group 2: Diabetic control (DC) treated with 0.2 mL of dimethylsulfoxide.
- Group 3: Diabetic treated with 500 mg/kg b.w of metformin.
- Group 4: Diabetic treated with 400 mg/kg b.w of methanol fraction of *V. calvoana*.
- Group 5: Diabetic treated with 400 mg/kg b.w of *n*-hexane fraction of *V. calvoana.*
- Group 6: Diabetic treated with 400 mg/kg b.w of crude extract of *V. calvoana*.

2.6 Induction of diabetes

Diabetes was induced in the test animal by injection of 45 mg/kg b.w of streptozotocin (intraperitoneally) in 0.1 M sodium citrate buffer (pH 4.5), obtained from Sigma (Steinheim, Switzerland) (14) with slight modifications. Fasting blood glucose (FBG) was recorded using an Accu-check glucometer. Animals with a fasting blood sugar of more than 7.8 mmol/l or 200 mg/dl were employed for the study [14]. Before the injection of streptozotocin, animals were fasted overnight but had access to water.

2.7 Experimental protocol

The grouping and treatment of experimental animals were as described above. The doses administered were as previously reported [15] and administration was twice daily (10:00 am: 4:00 pm) via oral gastric intubation. The treatment lasted 21 days experimental period.

2.8 Collection of samples for analysis

After the 21-day experimental period, the animals were fasted overnight by removing food with constant access to water. Before sacrifice, the test animal was anesthetized with chloroform vapor. Whole blood was collected by cardiac puncture using sterilized syringes and needles. The sample was poured into a test tube containing EDTA for hematological analysis, while the remaining samples were allowed to stand in an EDTA bottle at 4 °C for 2 h. Centrifugation of the sample was carried out at 3000 rpm for 10 min to obtain plasma from the cells. The plasma

was separated into plain test tubes and kept refrigerated until when needed for analysis. The liver tissue was removed and blotted with filter paper to remove blood. Thereafter, a portion of the tissue was sliced into 10% fixative (formal saline) for histological analysis.

2.9 Biochemical analysis

Serum lipid parameters: TG, T CHOL, HDL-C, VLDL and LDL, enzyme activity (ALT and AST), serum blood glucose, electrolyte profiles, namely K^+, CL^-, Na^+, HCO^{-3}, urea, and creatinine levels were determined using assay kit by AGAPPE Diagnostic (Switzerland) as described by the manufacturing method. The free fatty acid concentration was determined using BioVision's Free Fatty Acid Quantification Kit and Lipase activity by Fotress diagnostic kit method.

Estimation of Percentage Change in Fasting Blood Glucose

$$\% \text{ change in FBG} = \frac{\text{Final FBG} - \text{Initial FBG}}{\text{Final FBG}} \times 100$$

2.10 Histopathology

Histological observation of the liver of the test animals was evaluated using the differential staining method described by [16].

2.11 Statistical analysis

Results were analyzed for statistical significance by one-way ANOVA with post hoc Tukey's test at ($p < 0.05$) using the Prism GraphPad 8 (GraphPad Software, La Jolla, USA). All data were expressed as mean ± SEM ($n = 6$ replicates).

2.12 Preparation of compounds and molecular docking analysis using PyRx

Compounds reported in our earlier study [6] were employed for the *in silico* analysis. The chemical structures of these compounds and that of the co-crystallized (standard ligand) to the protein were retrieved from PubChem's database (https://pubchem.ncbi.nlm.nih.gov). The 3D structures of the protein molecules (PKF and lipoprotein lipase) were taken from the Protein Data Bank (www.rcsb.org). The ligand structural data files (SDF) were downloaded and subjected to molecular docking with PKF and lipoprotein lipase protein targets. The protein was prepared using Chimera 1.14 by removing non-essential water molecules, and non-standard proteins, and adding hydrogen and charges. The (SDF) formats of the ligands were minimized and converted into a Protein Database, Partial Charge (Q), and, Atom Type (T) (PDBQT) file using the PyRx tool. Autodock Vina from PyRX [17] was used for the docking of the ligand to the protein of the target and their binding affinities were estimated. Chimera 1.14 and Discovery Studio were used for the visualization of the interactions between the ligands and the protein.

3 Results

3.1 Molecular docking analysis

The 3D and 2D structures, docking results of the PKF and lipoprotein lipase co-crystallized compound, and some compounds with the highest affinity from extract fractions of *V. calvoana* are shown in Figs. 1a–c, 2a–c, 3a–c, 4a–c, and Table 1. Twenty-two compounds from *V. calvoana* were docked against PFK and lipoprotein lipase proteins. The results showed compounds from extract fractions of *V. calvoana* had different degrees of binding affinities for PKF and lipoprotein lipase depending on the change in Gibbs free energy. In the binding of compounds from the *n*-hexane fraction with PFK, it was observed that 8,9,13-trihydroxydocosanoic acid had the highest binding energy (6.0 kcal/mol), followed by 9-methyl-z,z-10,12-hexadecadien-1-ol acetate (-5.8) and gamma-homolinolenic acid (-5.3 kcal/mol). Also, the binding of compounds from the methanol fraction with PFK showed that 72–9 (e)-11-tetradecen-1-ol acetate had the highest binding energy (-5.6 kcal/mol), followed by sterculic acid (-5.5 kcal/mol), and then *n*-oleoylethanolamine (-5.4). The interaction of metformin with PFK was observed to have a binding energy of − 5.0 kcal/mol. The compounds (ligands) were also docked to the lipoprotein lipase protein. From the result obtained, it became apparent that in compounds from the *n*-hexane fraction, it was observed that compounds (phytol, gamma-hemolinolenic acid, and 8,9,13-trihydroxydocosanoic acid) had different binding values ranging from − 5.0 to 5.8 kcal/mol, which were either higher than metformin or − 5.0. Compounds from the methanol fraction (sterculic acid, *n*-oleoylethanolamine, and limonene oxide) also had varying binding energies ranging from − 5.0 to 5.9 kcal/mol, either higher than metformin's − 5.0 kcal/mol. However, other compounds from the fractions also showed varying degrees of binding affinities.

3.2 Effect of fractions of *V. calvoana* leaves on free radical scavenging activity using DPPH, FRAP, SOD, and anti-lipid peroxidation methods

The findings of measuring the scavenging activity utilizing DPPH, FRAP, SOD, and anti-lipid peroxidation techniques are shown in Fig. 5a–e. Figure 5a shows a comparison between the antioxidants found in *V. calvoana* and ascorbic acid in terms of their ability to scavenge free radicals. In comparison to the *n*-hexane fraction, it was found that the methanol fraction had the strongest radical scavenging activity and was most similar to

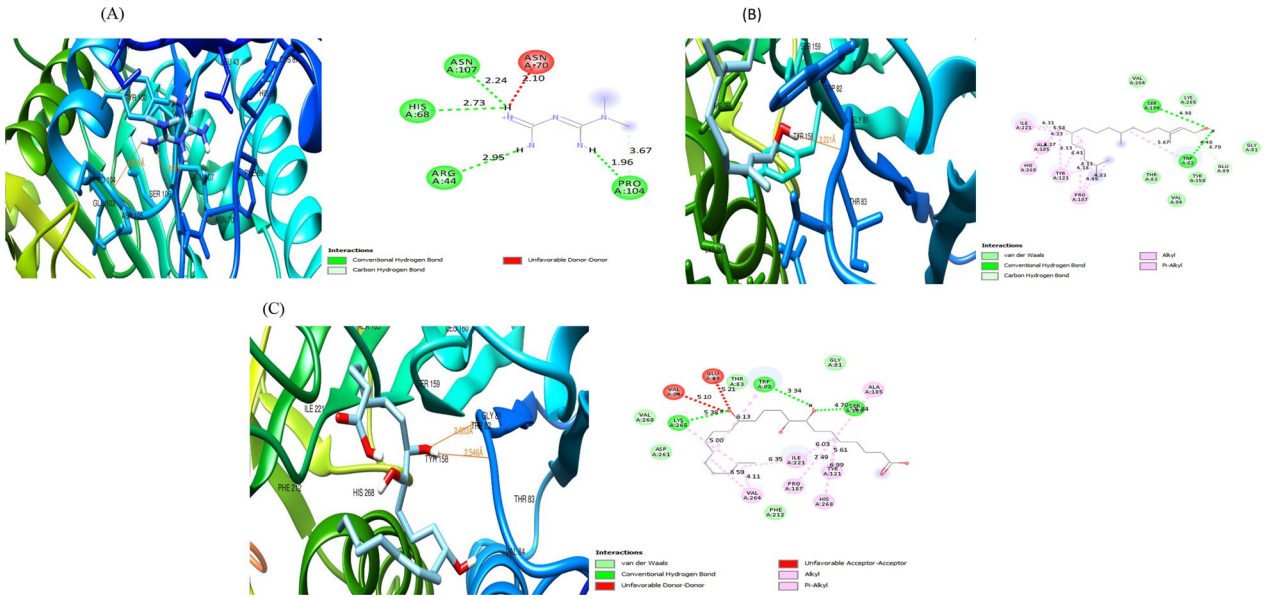

Fig. 1 3D and 2D view of the molecular interactions of amino acid residues of PFK with (**A**) Metformin (**B**) 8, 9, 13-trihydroxydocosanoic acid (**C**) 9-methyl-z,z-10,12-hexadecadien-1-ol acetat compounds of *n*-hexane fraction of *V. calvoana*

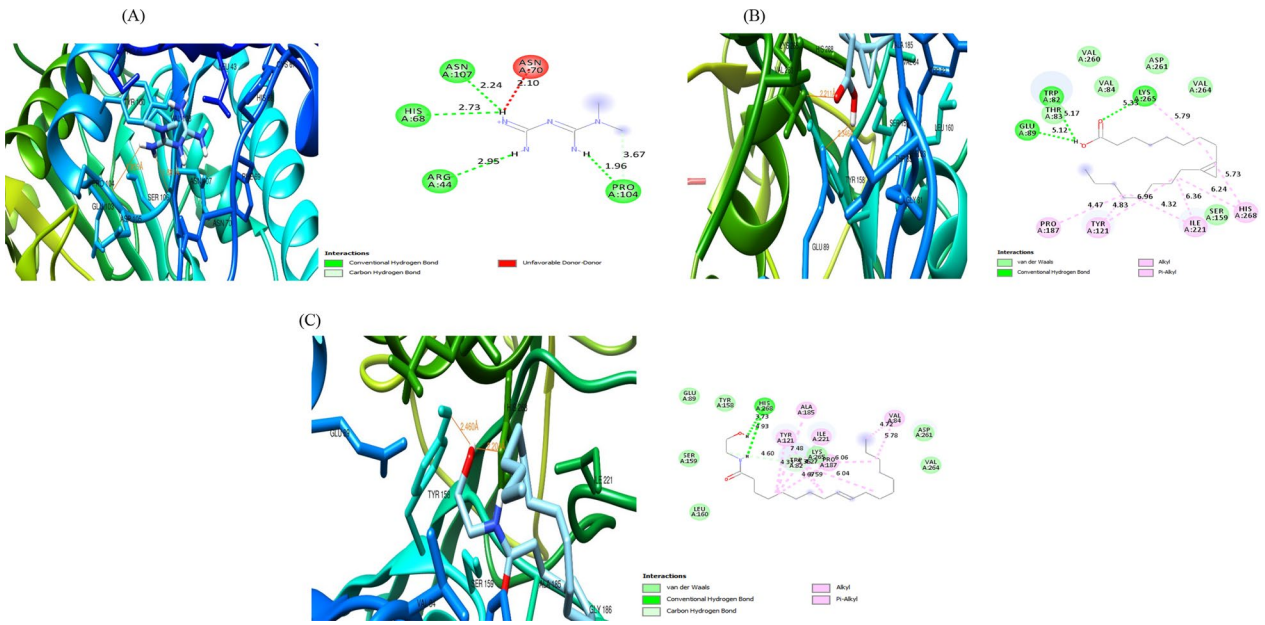

Fig. 2 3D and 2D view of the molecular interactions of amino acid residues of PFK with (**A**) Metformin (**B**) 72–9 (e)-11-tetradecen-1-ol acetate (**C**) Sterculic acid compounds of methanol fraction of *V. calvoana*

ascorbic acid. Additionally, the DPPH's ability to scavenge free radicals was dose-dependent and most effective for the methanol fraction (70.03%) at 400 g/mL, whereas *n*-hexane (48.97%) was compared to ascorbic acid (77.16%). Additionally, the *n*-hexane fraction's FRAP activity was shown to be maximum at 400 g/mL (1.919%) and strongly connected to ascorbic acid, as shown in Fig. 5c.

In the present study, both anti-lipid peroxidation and superoxide anion activities were also assessed. Based on the results obtained (Fig. 5d, e), extracts from *V. calvoana* showed impressive scavenging activities, with significant

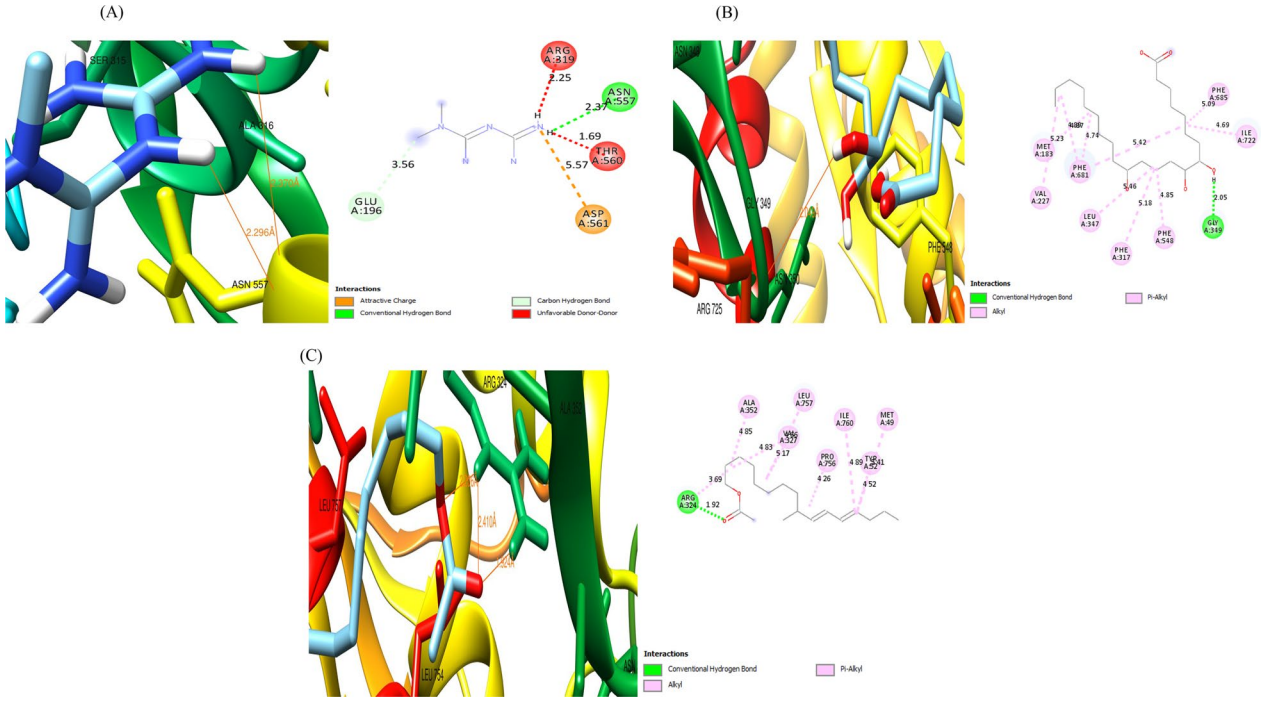

Fig. 3 3D and 2D view of the molecular interactions of amino acid residues of LPL with (**A**) Metformin (**B**) phytol (**C**) 8, 9, 13-trihydroxydocosanoic acid compounds of *n*-hexane fraction of *V. calvoana*

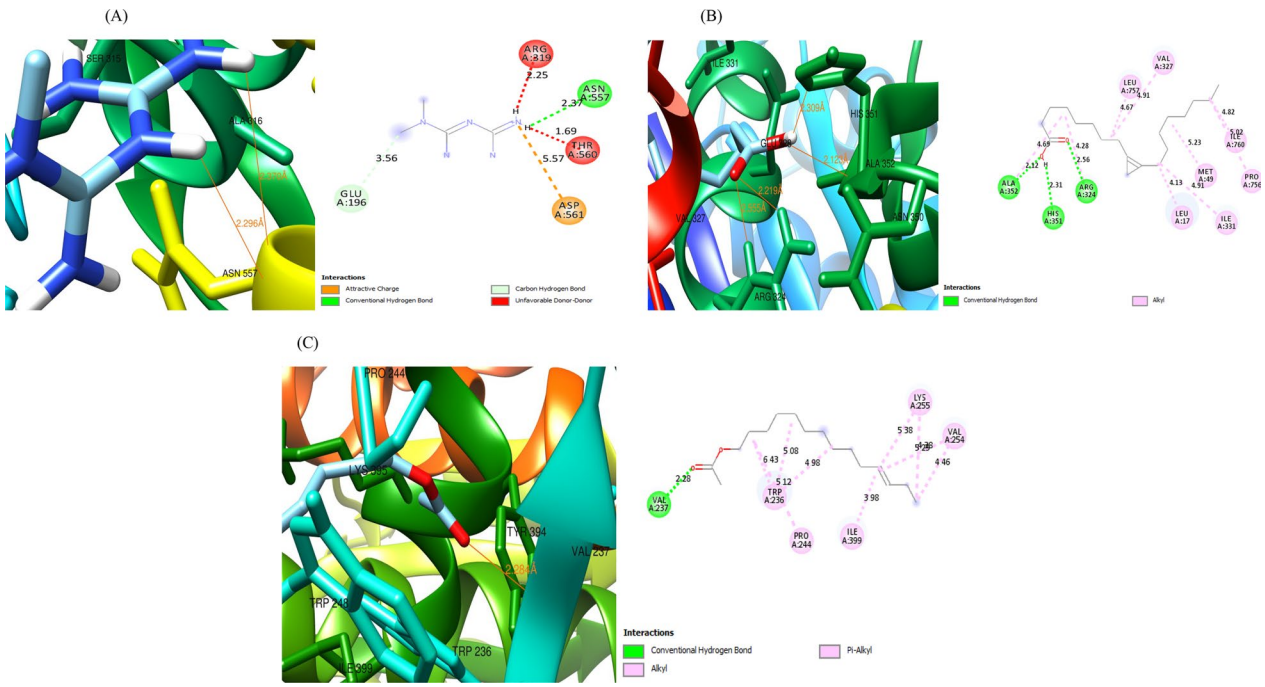

Fig. 4 3D and 2D view of the molecular interactions of amino acid residues of LPL with (**A**) Metformin (**B**) Sterculic acid (**C**) *n*-oleoylethanolamine compounds of methanol fraction of *V. calvoana*

Table 1 IC_{50} Values of different antioxidant assays (µg/ml)

Activity	*n*-hexane	Methanol	Standard
Superoxide radical scavenging activity	337.6 ± 0.3	235.3 ± 0.3	68.0 ± 0.2
Anti-lipid peroxidation	329.0 ± 0.4	158. 6 ± 0.2	37.6 ± 0.1
Ferric reducing antioxidant power	0.97 ± 0.4	0.6 ± 0.2	2.0 ± 0.0

Values are means ± SD of three independent determinations. Values with different letters in the same line are significantly different ($p < 0.05$)

activity observed in the methanol fraction. The activities that were seen also had a dosage dependency. A pattern comparable to that shown for DPPH was seen in the IC50 data (Table 2) for FRAP, SOD, and anti-lipid peroxidation.

3.3 Effect of extracts of *V. calvoana* and metformin on fasting blood glucose (FBG)

In Fig. 6a–c are the results of the effect of crude extracts of *V. calvoana* and metformin on FBG. As can be seen from the result, the serum blood a significant increase in concentration in the DC group compared to the NC group. On treatment, a significant decrease in concentration was observed in the extract and metformin-treated groups compared to the DC group (Fig. 6A). Also in Fig. 6b, the percentage change in FBG was recorded with the result showing a significant decrease in FBG of the metformin and VC nhexane group compared to the DC group. However, groups treated with VC crude and VC meth recorded a significant ($p < 0$.increaseased in FBG compared to the other experimental groups.

3.4 Effect of extracts of *V. calvoana* and metformin on liver serum enzyme and lipase activities

The results of the effects of *V. calvoana* and metformin on some liver serum enzymes (AL, AST, and AST: ALT) are shown in Fig. 7a–c. From the result, no significant ($p > 0.05$) change was observed in ALT activity in all experimental groups (Fig. 7a). Also observed was a significant ($p < 0.05$) increase in AST activity of the DC group compared to NC, which on treatment with the crude extract, methanol, and metformin, a significant ($p < 0.05$) decrease in enzyme activity was observed compared to the DC group (Fig. 7b). Moreover, the AST: ALT ratio enzyme activity was observed to increase significantly ($p < 0.05$) the DC group compared to NC. The observed activity was significantly decreased ($p < 0.05$) in groups treated with metformin, crude extract and methanol compared to the DC group (Fig. 7c). Pancreatic lipase enzyme activity were significantly ($p < 0.05$) increased in all experimental groups compared to DC group (Fig. 7d).

3.5 Effects of treatment on histology of liver tissue

In Fig. 8a–f, the effect of *V. calvoana* extracts and metformin on the cellular architecture of liver tissue is shown. In the NC group (Fig. 8a) it was observed that the liver tissue was preserved with a noticeable central vein with the hepatocytes observed to radiate outwardly and intensely stained nuclei indicating a clear cytoplasm. The portal tract was intact with dilated sinusoid spaces. The DC group (Fig. 8b) consisted of a conserved architectural layer of liver cells radiating from a central vein. Reduction in the sinusoidal spaces was noticed with intact limiting portal area and containing a bile duct, a hepatic artery and a portal vein. When treated with extracts of *V. calvoana* and metformin (Fig. 8c–f), the hepatocytes were seen as normal intact sinusoidal spaces and portal tract, with the cells having abundant cytoplasm, prominent basophilic nuclei and free space around them.

3.6 Effect of extracts of *V. calvoana* and metformin on electrolyte parameters

The levels of K^+ were observed to increase in the experimental treatment groups when compare to the DC with similar trend observed for sodium concentration. A significant ($p < 0.05$) decrease in chloride concentration in the DC group was observed compared to other experimental groups. In addition, the urea and creatinine concentrations increased significantly in the DC groups compared to the NC groups ($p < 0.05$). After treatment, their concentrations decreased significantly ($p < 0.05$) in all treated groups compared to the DC group, while the bicarbonate level decreased significantly in the *n*-hexane treated group compared to DC group($p < 0.05$). (Fig. 9a-f).

3.7 Effect of extracts of *V. calvoana* and metformin on hematological parameters

White blood cell counts in the DC, metformin, and crude extract-treated groups increased significantly ($p < 0.05$) compared to the NC group and decreased compared to the DC group. In addition, it was observed that MCV and PLT concentrations increased significantly ($p < 0.05$) in the extract-treated groups compared to the DC group (Tables 3 and 4).

3.8 Effect of extracts of *V. calvoana* and metformin on lipid parameters and free fatty acid levels

It was found that the TC and LDL-C concentrations increased significantly in the DC group compared to the NC group ($p < 0.05$). When treated, a decrease in TC concentration was observed in all treated groups compared to the DC group (Fig. 10a, e). More so, TG, HDL-C, and VLDL-C concentrations showed a significant increase ($p < 0.05$) in all experimental treated groups compared to

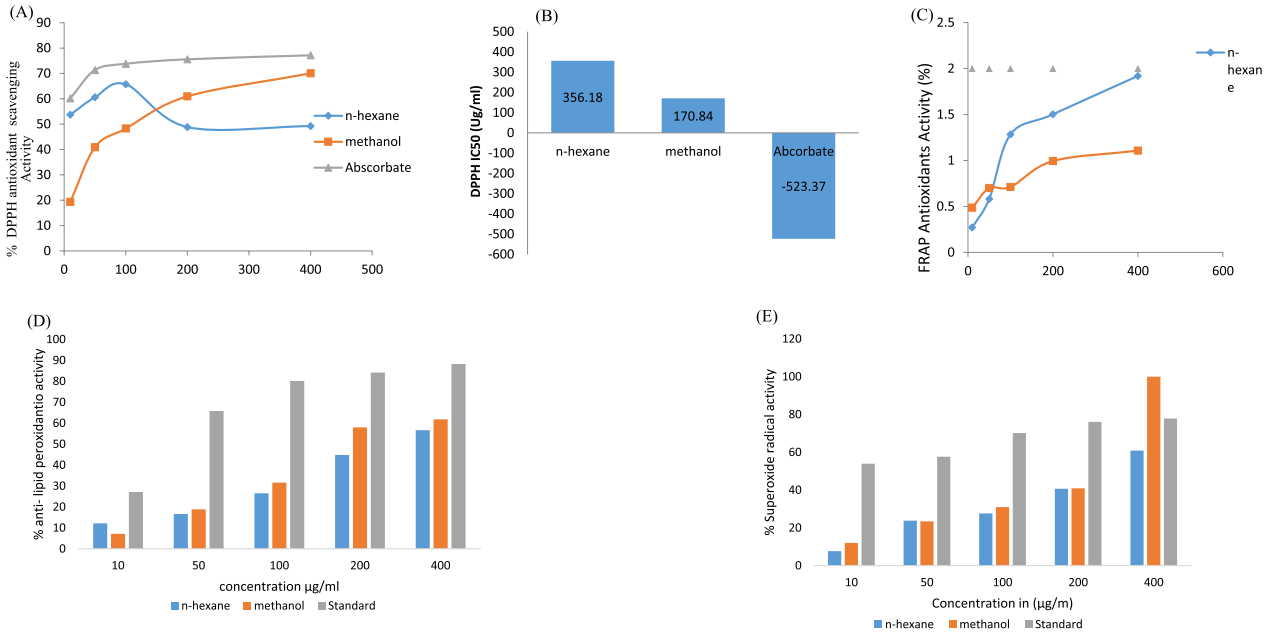

Fig. 5 Estimation of in vitro antioxidant activity of extracts of VC. **A** DPPH **B** IC50 of DPPH **C** FRAP **D** anti-lipid peroxidation **E** superoxide radical. Data are represented as mean ± SD. *N* = 3

Table 2 Binding affinities (ΔG in kcal/mol) of PFK and LPL with bioactive components of *V. calvoana* (ΔG Energy (Kcal/mol)

Compound name	CID Numbers	Binding Affinity (kcal/mol)	
		PFK	LPL
Methanol			
Valeric acid	7991	− 3.3	− 4.2
Isovaleric acid	10430	− 3.7	− 4.4
Cycloheptatriene;	11000	− 3.4	− 4.5
Sterculic acid	12921	− 5.5	− 5.9
Limonene oxide	91496	− 3.5	− 5.4
Trans-vaccenic acid	5281127	− 3.5	− 5.1
72–9 (e)-11-tetradecen-1-ol acetate	5367650	− 5.6	− 4.9
N,n-bis(3-methylbutyl)hydroxylamine	88536504	− 3.6	− 5.0
N-oleoylethanolamine	5283454	− 5.4	− 5.6
Z-4-nonadecen-1-ol acetate	5363395	− 3.6	− 5.4
1,26-hexacosanediol	16747787	− 3.3	− 4.7
***n*-hexane**			
Palmitic acid	985 − 4.8	− 5.3	
Furfuryl alcohol	7361	− 3.5	− 4.0
Phytol	5280435	− 3.7	− 5.8
Y-homolinolenic acid	5280581	− 5.3	− 5.4
9-methyl-z,z-10,12-hexadecadien-1-ol acetate	5363214	− 5.8	− 5.4
Trans-2-tridecen-1-ol	5364949	− 3.3	− 5.0
9,12,15-octadecatrien-1-ol	5367327	− 3.6	− 5.4
Stearic acid	5281	− 4.8	− 5.1
Nonadecane	12401	− 3.2	− 4.9
8,9,13-trihydroxydocosanoic acid	99112	− 6.0	− 5.6
Metformin (standard)	4091	− 5.0	− 5.0

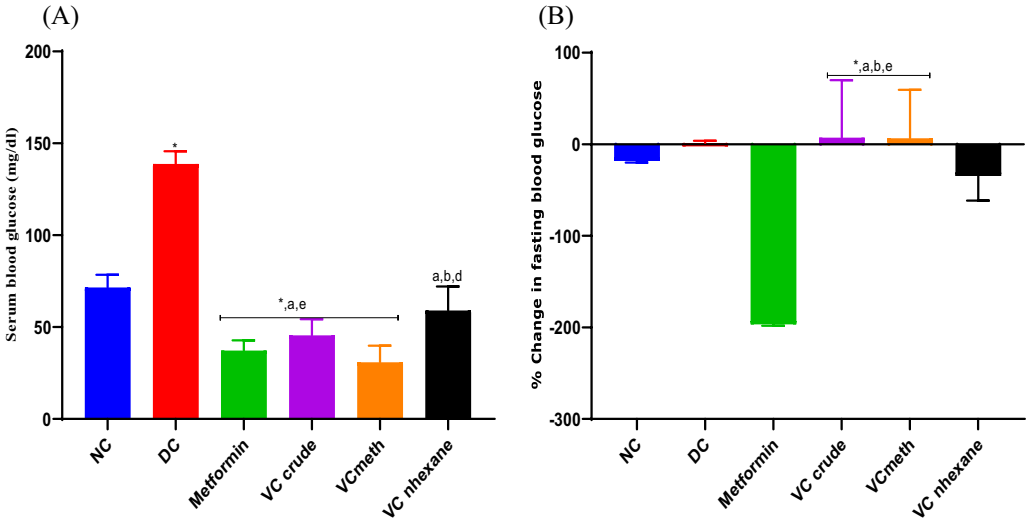

Fig. 6 **a**, **b** Serum blood glucose (**A**) and % changes in fasting blood glucose (**B**) after 21 days administration of extract of HC and metformin in diabetic rat models. NC: Normal control; DC: Diabetic control; MET: Metformin, VC crude: *V. calvoana* crude, VC meth: *V. calvoana* Methanol and VC nhexane: *V. calvoana n*-hexane. Data are represented as mean ± SEM. *$p < 0.05$ versus NC and.[a]$p < 0.05$ versus DC. $n = 6$

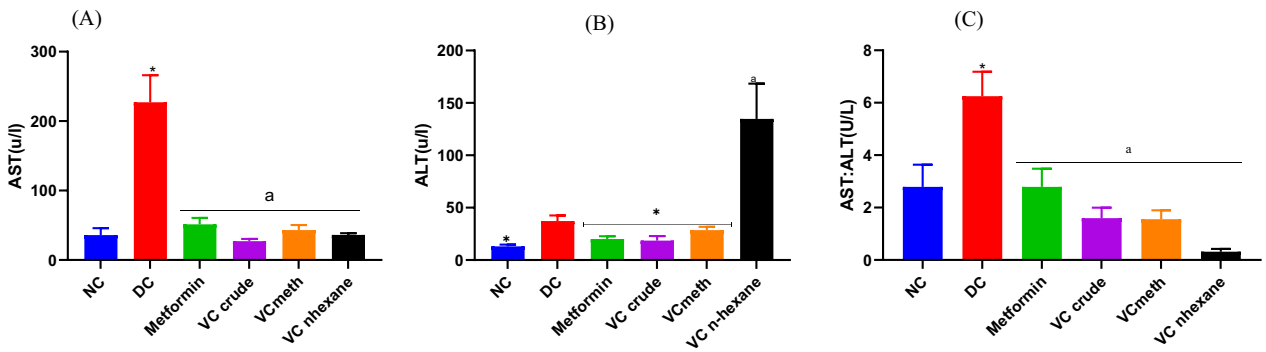

Fig. 7 Liver enzyme activity after 21 days administration of extract of HC and metformin in diabetic rat models. **A** AST **B** ALT and **C** ALT: AST. **D** Pancreatic lipase enzyme. NC: Normal control; DC, Diabetic control; MET, Metformin, VC crude: *V. calvoana* crude, VC meth: *V. calvoana* Methanol and VC nhexane: *V. calvoana n hexane*. Data are represented as mean ± SEM. *$p < 0.05$ versus NC and.[a]$p < 0.05$ versus DC. $n = 6$

the DC group (Fig. 10b–d). Free fatty acid concentration was significantly ($p < 0.05$) decreased in all experimental groups compared to the DC group (Fig. 10f).

4 Discussion

In our previous studies, GC–MS analysis of fractions of *V. calvoana* indicated the presence of bioactive compounds with possible antidiabetic and antioxidant activities and may be suggested to be responsible for these observed biological activities [5, 6]. In the present study, the molecular interactions of the bioactive compounds present in the fractions of *V. calvoana* were first evaluated against human phosphofructokinase (PFK) and lipoprotein lipase (LPL) crystal structure proteins using molecular docking analyses with further validation of this activity with biological analysis. The compounds from fractions of *V. calvoana* showed varying binding affinities for the target proteins, with some of the compounds exhibiting a docking score that was either equal to or higher than that of metformin. Phosphofructokinase plays a central role as it is considered to be the pacemaker enzyme of the signaling pathway [18]. It plays a rate-limiting role as it acts as an indicator of the glycolysis pathway, which is the main pathway of glucose utilization, and thus could affect glycemic control. [19, 20] reported that metformin maintains the inhibition of PFK in skeletal muscle, liver, and adipose tissue and also reverses its down-regulation and intracellular distribution in the heart of diabetic mice. Pancreatic lipoprotein lipase independently regulates islet glucose metabolism and normal insulin secretion. It catalyzes the partial hydrolysis of core triglycerides from chylomicrons and VLDL to monoglycerides and fatty

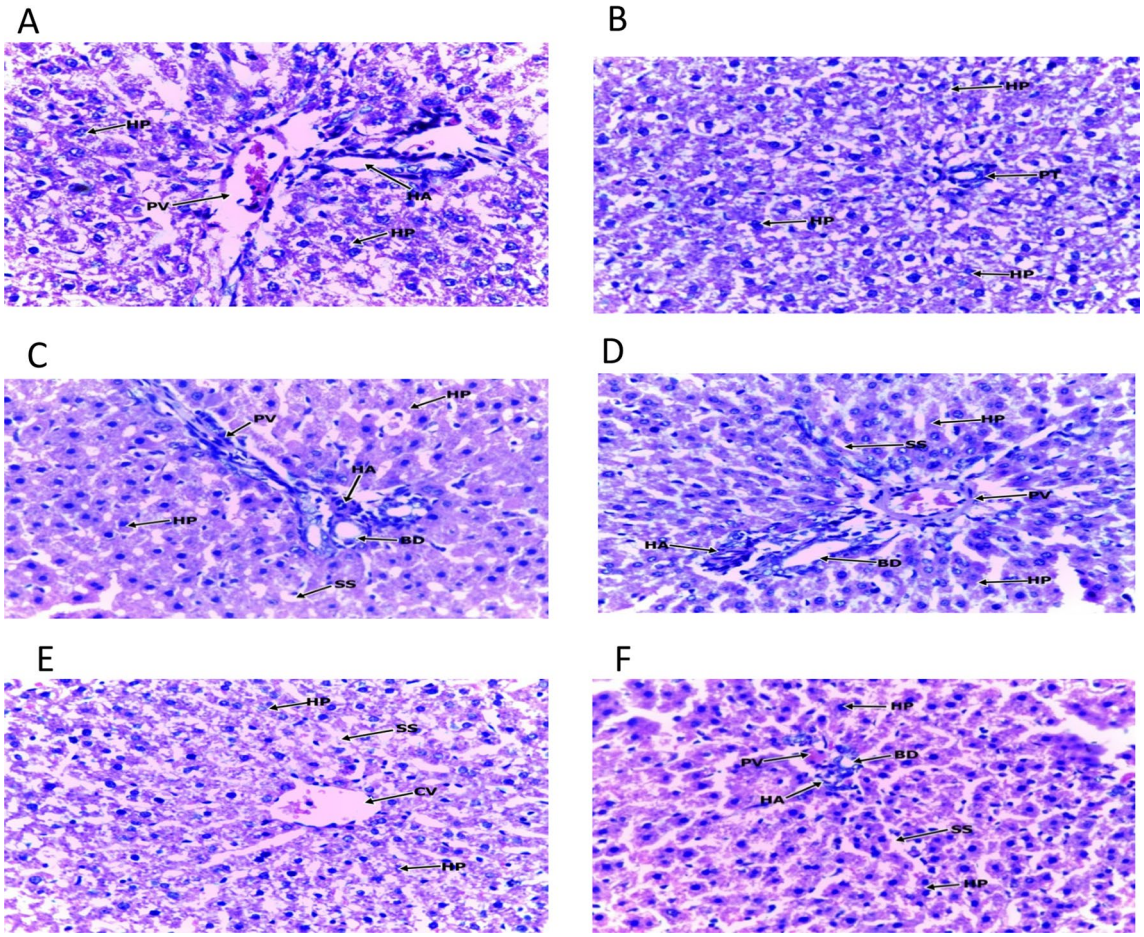

Fig. 8 Photomicrographs of liver tissue. **A** Normal control **B** diabetic control **C** metformin **D** crude extract **E** methanol group and **F** *n* hexane group. (×400)

acids [21]. Metformin is known to increase bulk serum lipoprotein lipase (LPL) levels [22]. The observed high docking score of *V. calvoana* bioactive compounds compared to metformin suggests extracts of *V. calvoana* may present an alternative therapeutic agent in the management of diabetic and associated cardiovascular disease.

Oxidation by lipid peroxidation is believed to play a crucial role in the pathogenesis of diabetes mellitus. DNA and protein damage leads to the development of diabetes and its associated complications [23]. In recent years, the search for bioactive constituents with biological activities has been on the increase due to their possible therapeutic use in the management of numerous chronic and infectious diseases. In exploring for new medicinal plant with therapeutic efficacy, within the ongoing research, we carried out investigations on the in vitro antioxidant activities of extracts of *V. calvoana* due to the diverse nature of free radicals; we tested *V. calvoana* extracts against numerous reactive oxygen species radicals to demonstrate their antioxidant activity through different mechanisms The antioxidant properties of the extracts are initially assessed based on their ability to scavenge free radicals DPPH. It is a stable free radical that easily accepts an electron and is converted into a stable molecule [24]. In this study, it was observed that extracts from methanol had a higher scavenging potential against DPPH compared to *n*-hexane. Furthermore, this observed scavenging property was dose-dependent and most effective at a higher concentration.

In addition, the reducing power of the extracts was evaluated using the FRAP test, which serves as an indicator of their antioxidant potential. The result of this study thus showed a remarkable difference in the activity of the extracts, with the *n*-hexane fraction showing greater activity against iron radicals compared to methanol. This indicates the ability of the extract to reduce Fe3 + to Fe2 + by donating electrons and this can be attributed to the bioactive components present in this plant that we previously reported, which can serve as electron donors.

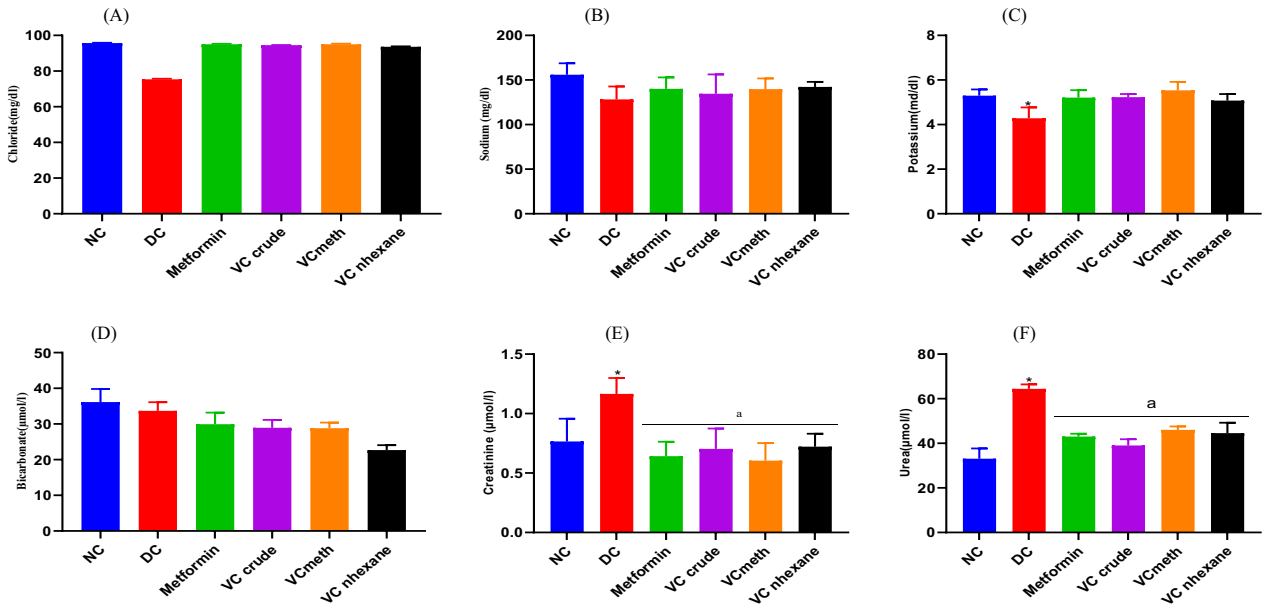

Fig. 9 Electrolytes parameters concentration after 21 days administration of extract of HC and metformin in diabetic rat models. **A** Chloride **B** Sodium **C** Potassium **D** Bicarbonate **E** Creatinine **F** Urea Normal control; DC, Diabetic control; MET, Metformin, VC crude: *V. calvoana* crude, VC meth: *V. calvoana* Methanol and VC nhexane: *V. calvoana n hexane*. Data are represented as mean ± SEM. *$p < 0.05$ versus NC and.[a]$p < 0.05$ versus DC. $n = 6$

Table 3 Effect of crude extract, methanol, *n*-hexane fractions of V.C leaves and metformin Hematological parameters in different experimental groups

Grouping	PLT	WBC (X10^3/µl)	RBC (X10^3/µl)	HGB (g/dl)	HCT (%)	MCV (fl)	MCHC (dl)	MCH (Pg)
NC	12.03 ± 1.03	7.97 ± 0.57	14.40 ± 0.55	47.93 ± 1.57	60.78 ± 4.78	30.13 ± 1.56	18.16 ± 0.63	712 ± 56.58
DC	17.63 ± 1.92*	8.05 ± 0.25	14.66 ± 0.27	48.13 ± 1.58	59.76 ± 0.40	30.50 ± 0.45	18.26 ± 0.24	737 ± 99.34
MET	15.16 ± 2.16*[a]	7.73 ± 0.21	14.93 ± 0.54	47.53 ± 1.33	61.43 ± 03.17	31.49 ± 0.51	19.36 ± 0.41	744 ± 75.80
VC crude	18.32 ± 9.46*	7.93 ± 0.98	14.13 ± 1.27	43.76 ± 4.77*[a]	55.63 ± 1.22*	32.43 ± 0.79*[a]	18.12 ± 0.83	426 ± 87.12*[a,b]
VC meth	10.91 ± 0.15[a]	7.53 ± 0.31	14.18 ± 0.50	43.53 ± 2.06*[a]	57.73 ± 1.32*	32.43 ± 0.43*[a]	18.73 ± 0.40	893 ± 38.49*[a,b]
VC nhexane	12.13 ± 1.48[a]	7.25 ± 0.39	13.06 ± 0.34	40.06 ± 2.33*[a]	55.30 ± 0.79*	32.73 ± 1.05*[a]	18.06 ± 0.61	822.33 ± 40.35*[a,b]

Values are Mean ± SEM. NC, Normal control; DC: Diabetic control; MET: Metformin, V.C crude; *Vernonia calvoana* crude, VC meth: *Vernonia calvoana* methanol and VC *n*-hexane: *Vernonia calvoana n* hexane. Data are represented as mean ± SEM. *$p < 0.05$ versus NC and [a]$p < 0.05$ versus DC. $n = 6$

Table 4 Effects of the extract of VC leaves on differential White blood cell count in the different experimental groups

Grouping	LYMP (×10^3/ul)	MXD (×10^3/ul)	NEU (×10^3/ul)
NC	9.40 ± 0.81	0.53 ± 0.14	2.10 ± 0.43
DC	13.83 ± 1.29*	1.00 ± 0.14*	2.80 ± 0.43
MET	10.96 ± 1.61*[a]	0.63 ± 0.13*	3.56 ± 0.78
VC crude	12.20 ± 5.34*[a]	0.93 ± 0.78*	5.16 ± 3.37*[a]
VC meth	8.93 ± 0.03[a]	0.26 ± 0.03[a]	1.70 ± 0.11
VC nhexane	9.23 ± 1.25[a]	0.46 ± 0.03[a]	2.43 ± 0.23

Values are Mean ± SEM. NC, Normal control; DC: Diabetic control; MET: Metformin, V.C crude; *Vernonia calvoana* crude, VC meth: *Vernonia calvoana* methanol and VC *n*-hexane: *Vernonia calvoana n* hexane. Data are represented as mean ± SEM. *$p < 0.05$ versus NC and [a]$p < 0.05$ versus DC. $n = 6$

Superoxide radicals are very damaging to biological materials and also serve as a precursor for the generation of various reactive oxygen species [25], and can also cause the generation of H_2O_2 in the biological system through a dismutation reaction. Medicinal plant extracts are composed of numerous components that scavenge free radicals and may act synergistically in scavenging free radicals in a range of oxidative stress and diseases such as diabetes [26]. The experimental finding showed that extracts from *V. calvoana* exhibited remarkable scavenging activities against superoxide anion and lipid peroxidation, with marked activities being observed in the methanol fraction. The observed activities were also

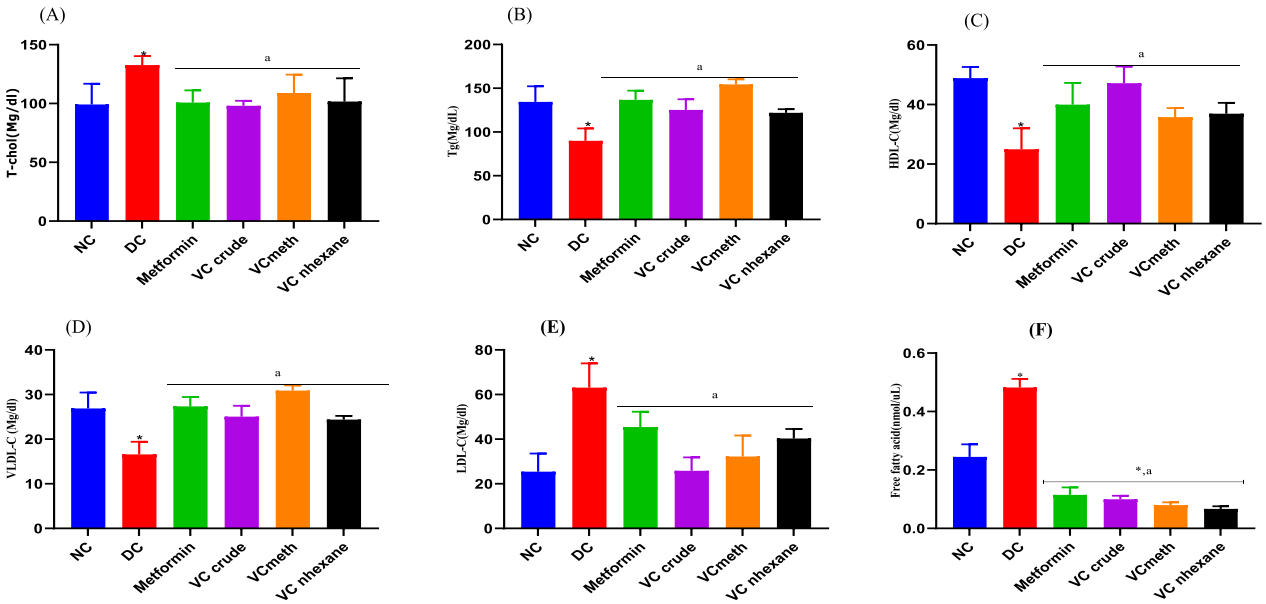

Fig. 10 Lipid parameters concentration after 21 days administration of extract of HC and metformin in diabetic rat models. **A** T-Chol **B** TG and **C** HDL-C **D** VLDL-C **E** LDL-C **F** Free fatty acid. *NC* Normal control; *DC* Diabetic control; *MET* Metformin, *VC crude V. calvoana* crude, *VC meth*: *V. calvoana* Methanol and VC nhexane: *V. calvoana n hexane*. Data are represented as mean ± SEM. *$p < 0.05$ versus NC and.[a]$p < 0.05$ versus DC. $n = 6$

dose-dependent and may be attributed to the bioactive compounds present in these plant extracts.

Injection of streptozotocin caused diabetes mellitus, probably due to the destruction of cells in the islets of Langerhans in the pancreas [27]. This leads to excessive glucose production and reduction in tissue utilization which forms the basis of hyperglycemia in diabetes mellitus. In this study, induction of diabetes with streptozotocin resulted in an increase in the test animal's fasting blood glucose level compared to the control. The elevated blood glucose levels observed in the study are consistent with reports from several researchers that STZ-induced diabetes mellitus leads to elevated blood glucose levels [27]. However, the reduction in serum blood glucose observed after treatment with *V. calvoana* extracts further confirms our previous study on this plant, [5] attributed the anti-hyperglycemic activity of this plant to the presence of a bioactive component in the plant.

Diabetes is considered the commonest cause of liver disease and is believed to play a significant role in the cause of death in people with type 2 diabetes [28]. Liver tissue plays an important role in glucose metabolism, including glucose uptake, storage, and synthesis. Distributed in the liver are transaminase enzymes (including ALT, AST, and GGT) that synthesize and break down some amino acids to derive energy from these stored molecules. Changes in enzymatic activities such as AST, ALT, and ALP in diabetics are of physiological and clinical importance [29]. In this study, it was observed that induction of diabetes with streptozotocin in rats significantly increased AST and ALT activity indicating liver damage. When treated with extracts of *V. calvoana* and metformin, however, a significant decrease in the activities of these enzymes was observed indicating a possible hepato-protective effect of these plant extracts against diabetes-induced hepatotoxicity. The findings from this study corroborated with the report by [5] who documented that this plant has a protective effect against paracetamol-induced liver damage in rats. The functional integrity of liver tissue can be assessed based on the histological integrity of liver tissue [30].

Streptozotocin-induced diabetes in rats led to a disorganized liver architecture in this investigation, which is comparable with the report of [30] on the liver's integrity in diabetic rats. Therefore, the increase in serum liver enzyme concentrations that was previously noted in this investigation coincides with the observed alteration in the integrity of the liver cells. However, the disrupted architecture of hepatocytes gradually recovered after being treated with metformin and *V. calvoana* extracts.

Hypertriglyceridemia and other lipoprotein alterations that support atherosclerosis occur as a result of decreased lipoprotein lipase activity and decreased breakdown of triacylglycerol-rich lipoproteins in diabetic circumstances. Low levels of circulating LPL and low LPL protein mass have also been linked to insulin resistance [21]. The loss of the insulin-producing pancreatic beta cell caused a significant increase in the serum pancreatic

lipase enzyme activity in this study when diabetes was induced by streptozotocin. Several studies have reported elevated lipase activity in subjects with type 2 diabetes [31] All experimental groups that received *V. calvoana* extract showed an increase in pancreatic lipase enzyme activity, suggesting that this extract may have the ability to promote the circulation of these enzymes and potential repair of pancreatic cell integrity.

Diabetes leads to elevated blood sugar levels, which leads to a loss of body fluids and electrolytes. Poor retention of body fluids and electrolytes in diabetics is often compounded by the underlying use of conventional medications to treat diabetes and renal failure [32], which can lead to changes in electrolyte homeostasis. These alterations in sodium and potassium levels may be pathophysiologic and clinically characteristic of diabetes. In this present study, the levels of K^+, Na^+, and CL^- were observed to slightly increase in the experimental treatment group. The observed change in electrolyte levels in animals treated with an extract of *V. calvoana* and metformin correlates with a finding earlier reported by [33] on the effect of *Vernonia amygdalina* on diabetic animals.

High levels of urea and creatinine have been implicated in the development of insulin resistance. The impairment of creatinine and urea are the indicators of chronic kidney disease in diabetic subjects [34]. The result from this study indicated a significant reduction in the urea and creatinine concentration which may be suggestive that this plant has the ability to ameliorate chronic kidney disease which is an associated complication of diabetes mellitus. However, [35] documented that while the change in electrolyte imbalance is noticeable in diabetics, no actual cause can be given for body fluids and electrolytes.

Diabetes mellitus is one of the important secondary causes of dyslipidemia since the atherogenic combination of high triacylglycerol, high low-density lipoprotein, and reduced high-density lipoprotein concentration is known to play a role in diabetics [36]. However, from this study, it was observed that triacylglycerol and high-density lipoprotein were present in a significant concentration in all experimentally treated animals. This indicates the ability of these plant extracts to ameliorate dyslipidemia conditions associated with the diabetic condition as a result of increasing levels of high-density lipoprotein, which aids in the transport of lipids from cells.

One of the illnesses that are more frequently linked to diabetes mellitus is anemia [37]. Due to the kidneys' failure to produce enough erythropoietin, reports have shown a link between the development of anemia and a lack of renal clearance in diabetes illness. Additionally, studies have associated hyperglycemia-induced non-enzymatic glycosylation of RBC membrane proteins with an increase in anemia in diabetics [38]. In this investigation, there was no discernible difference between the experimental groups given plant extract treatment and NC in terms of RBC, HGB, HCT, MCHC, and MCH levels. The creation of diabetes was found to greatly enhance platelet count, nevertheless. [39] asserts that hypertriglyceridemia and hyperglycemia increase platelet reactivity by directly simulating the glycation of platelet proteins. And the combination of insulin resistance and insulin insufficiency makes this worse. [40] It has been proposed that giving insulin to diabetic patients may possibly limit platelet activity. In order to improve metabolic control, medications that can also improve insulin sensitivity and preserve pancreatic cell function are likely to decrease platelet reactivity and enhance the effects of antiplatelet medications. According to this study's findings, therapy with a *V. calvoana* extract dramatically decreased levels of platelet count, which is consistent with an earlier proposal made by [41]. As oxidative stress intensifies this impact by decreasing NO activity and encouraging platelet activation, this suggests that extracts from this plant may be linked to a decrease in platelet aggregation through the antioxidant function of the plant.

5 Conclusion

In this study, the therapeutic effects of *V. calvoana* extract fractions on streptozotocin-induced diabetic rats were evaluated. First, we investigated the binding affinity of metformin and compounds from extracts of *V. calvoana* to the binding domains of human phosphofructokinase and lipoprotein lipase and further validated our results with in vitro and in vivo biochemical assessments. From this, it can be concluded that extract fractions of *V. calvoana* possess the ability to prevent the occurrence of hyperglycemia and may be exploited in the management of diabetes and it associated complications.

Abbreviations

V. C	*V. calvoana*
PFK	Phosphofructokinase
LPL	Lipoprotein lipase
DPPh	2,2-Diphenyl-1-picrylhydrazyl2, 2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
SOD	Super oxide demutase
LPx	Lipid peroxidation
WHO	World Health Organization
BCH	Biochemistry
NBT	Nitrpgen blue tetraolium
TCHOL	Total cholesterol
TG	Triacylglycerol
HDL-C	High density lipoprotein cholesterol
VLDL-C	Very low density lipoproteins cholesterol
LDL-C	Low density lipoproteins
AST	Aspartate amino transferase
ALT	Alanine amino transferase

K^+	Potassium
CL^-	Chloride
Na^+	Sodium
HCO^{-3}	Hydrogen bicarbonate
ANOVA	Analysis of variance
SDF	Structural data files
PDBQT	Protein Database, Partial Charge (Q) and Atom Type (T)
FBG	Fasting blood glucose
DC	Diabetic control
NC	Normal control
MCV	Mean corpuscular volume
GGT	Gamma-glutamy transferase
MCHC	Mean corpuscular hemoglobin concentration
HCT	Hematocrit
MCH	Mean corpuscular hemoglobin
RBC	Red blood cell
HGB	Hemoglobin
PLT	Platelet

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Author contributions
IAI contributed to conceptualization and investigation; VSE contributed to conceptualization; GOI contributed to supervision, methodology, and validation. JEE contributed to methodology. EOM contributed to formal analysis and roles/writing—original draft. MOO contributed to roles/writing—original draft. FEU contributed to resources and writing—review and editing. MEU contributed to project administration, validation, and resources. OEE contributed to project administration.

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Declarations

Ethics approval and consent to participate
The experimental design was approved by the Animal Ethics Committee of the Faculty of Basic Medical Sciences with approval number 149BCM3021. Consent to participate is not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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