Hypoglycemic Activity of Different Leaf Extracts of *Crateva adansonii* DC in Streptozotocin-Induced Diabetic Rats

Running title: Effect of *Crateva adansonii* DC in diabetic rats

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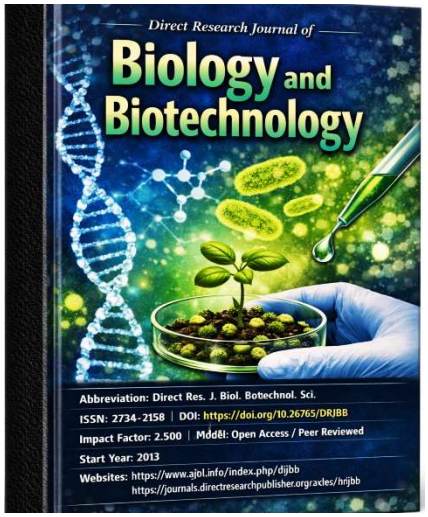
ABSTRACT

This study aimed to evaluate the antihyperglycemic activity of four leaf extracts of Cretiva adansonii DC (hexane, dichloromethane, ethyl acetate, and methanol), assess their effects on liver and kidney function indices, and identify bioactive compounds present in the most active extract. Exactly 50 mg/kg body weight of streptozotocin was used to induce diabetes in Wistar rat model and the antihyperglycemic activity was determined in-vivo. A single dose of 350 mg/kg body weight of the extracts which was establish to be an effective dose in pilot studies, was administered daily by intragastric tube for 28 days to four groups containing 5 rats each, and compared to a standard (metformin) for the different extracts. Fasting blood glucose was recorded at day 0 and day 28. Liver and kidney function test was determined in-vivo using commercial diagnostic kits and chemical compounds were determined using GC-MS. Among the tested extracts, the ethyl acetate extract demonstrated the most pronounced antihyperglycemic activity. Significant reductions ($p < 0.05$) were observed in liver enzyme markers including Aspartate Transaminase (AST) (70.33 ± 1.34 U/L), Alanine Transaminase (ALT) (60.67 ± 1.50 U/L), Alkaline Phosphatase (ALP) (82.56 ± 0.21 U/L), and Gamma-Glutamyl Transferase (GGT) (36.33 ± 1.00 U/L). Renal biomarkers were also significantly reduced, with urea and creatinine levels decreasing to 4.40 ± 0.27 mmol/L and 98.33 ± 1.37 µmol/L, respectively. In contrast, albumin levels significantly increased to 37.00 ± 1.92 g/L compared with the diabetic control group. GC–MS analysis of the ethyl acetate extract identified 22 phytochemical compounds, among which octadecanoic acid, n-hexadecanoic acid, and hexadecane have documented biological activities that may contribute to the observed antihyperglycemic and organ-protective effects. The findings of this study demonstrate that the ethyl acetate leaf extract of C. adansonii possesses significant antihyperglycemic potential and may ameliorate hepatic and renal biochemical abnormalities associated with diabetes mellitus. These results support its potential application as a source of phytotherapeutic agents for the management of hyperglycemia and related complications.



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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both, leading to disturbances in carbohydrate, lipid, and protein metabolism (Ayele *et al*., 2021). The disease has emerged as one of the most serious global public health challenges due to its rapidly increasing prevalence and associated complications. Chronic hyperglycemia predisposes affected individuals to severe microvascular complications such as nephropathy, neuropathy, and retinopathy, as well as macrovascular complications including hypertension, atherosclerosis, myocardial infarction, and stroke (Ohiagu *et al*., 2021). Despite advances in therapeutic management, DM continues to contribute significantly to morbidity, mortality, and reduced quality of life worldwide. In sub-Saharan Africa, particularly Nigeria, the burden of diabetes has increased markedly over recent decades, with Nigeria currently recording one of the highest prevalence rates in the region (Muhammad, 2020). This increasing incidence, coupled with poor access to healthcare facilities and high treatment costs, underscores the urgent need for alternative and affordable therapeutic interventions.

Currently available antidiabetic therapies, including insulin and oral hypoglycemic agents such as sulfonylureas, biguanides, and thiazolidinediones, have demonstrated effectiveness in glycemic control; however, their long-term use is often associated with several limitations. These include adverse effects such as hypoglycemia, gastrointestinal disturbances, hepatotoxicity, weight gain, and reduced therapeutic efficacy over time due to progressive β-cell dysfunction (Ayele *et al*., 2021). Furthermore, many synthetic drugs are expensive and inaccessible to individuals in low-resource settings, particularly in developing countries. These challenges have stimulated increasing scientific interest in medicinal plants as potential sources of safer, cost-effective, and multi-targeted antidiabetic agents.

The pathophysiology of DM is highly complex and involves multiple interconnected biochemical and molecular mechanisms. In type 1 diabetes mellitus, autoimmune-mediated destruction of pancreatic β-cells results in absolute insulin deficiency, whereas type 2 diabetes mellitus is primarily characterized by insulin resistance accompanied by impaired insulin secretion (American Diabetes Association, 2014). Hyperglycemia-induced oxidative stress plays a central role in disease progression through excessive generation of reactive oxygen species (ROS), which damage cellular proteins, lipids, and nucleic acids. Additionally, chronic hyperglycemia promotes glucose autoxidation, non-enzymatic glycation of proteins, activation of the polyol pathway, and increased formation of advanced glycation end products (AGEs), all of which contribute to pancreatic β-cell dysfunction and diabetic complications (Ayele *et al*., 2021). Postprandial hyperglycemia further exacerbates oxidative stress and inflammatory responses, thereby accelerating vascular and hepatic damage associated with diabetes.

Medicinal plants have historically served as important sources of therapeutic agents and continue to provide bioactive compounds for the development of modern drugs. Their widespread use in traditional medicine is largely attributed to accessibility, affordability, and perceived safety. Numerous plant species have been investigated for antidiabetic activity, with many reports demonstrating their ability to reduce hyperglycemia through antioxidant activity, enhancement of insulin secretion, improvement of insulin sensitivity, inhibition of carbohydrate hydrolyzing enzymes, and protection of pancreatic β-cells. However, many previous studies on medicinal plants have focused mainly on crude extracts without critically examining the influence of solvent polarity on extraction efficiency and biological activity. Consequently, the specific phytochemical constituents responsible for antihyperglycemic effects often remain poorly characterized. This represents a significant scientific gap that limits the standardization and therapeutic application of plant-derived antidiabetic agents.

C. adansonii, commonly known as garlic pear or sacred garlic pear, belongs to the family *Capparaceae* according to the updated Angiosperm Phylogeny Group (APG) classification system, although it was previously classified under *Capparidaceae*. The plant is widely distributed across tropical Africa and is known locally in Nigeria as Eegun orun or Ajanaka among the Yoruba, Ungududu among the Hausa, and Aamakarode among the Igbo (Tsado *et al*., 2016). Different parts of the plant have been traditionally used for the treatment of several ailments. Pharmacological investigations have demonstrated that *C. adansonii* possesses antimicrobial, anti-inflammatory, anti-gout, antioxidant, and anti-trypanosomal activities (Agboke *et al*., 2011; Abdullahi *et al*., 2012). Previous studies by Atchou *et al*. (2020) and Okoyomoh *et al*. (2023) reported the antihyperglycemic potential of hydroethanolic and ethanolic leaf extracts of *C. adansonii*, respectively. Although these findings support the ethnomedicinal relevance of the plant, the studies were limited to single-solvent extraction approaches, thereby restricting comprehensive evaluation of the diverse phytochemical constituents present in the plant.

The extraction solvent plays a crucial role in determining the type and quantity of phytochemicals isolated from plant materials because bioactive compounds differ considerably in polarity and solubility. Non-polar solvents such as hexane preferentially extract lipophilic compounds including terpenoids and fatty acids, whereas moderately polar solvents such as dichloromethane and ethyl acetate extract compounds like alkaloids, flavonoids, and certain phenolics.

Highly polar solvents such as methanol are particularly effective for extracting polyphenols, glycosides, tannins, and saponins. Therefore, the use of multiple solvents with increasing polarity enables broader recovery of phytochemicals and facilitates identification of the fractions with the greatest biological activity. Such an approach provides a more systematic understanding of the relationship between phytochemical composition and pharmacological effects. Several phytochemicals reported in medicinal plants, including flavonoids, alkaloids, phenolic compounds, tannins, saponins, and terpenoids, possess significant antidiabetic potential through different mechanisms of action. Flavonoids and phenolic compounds exhibit potent antioxidant activities capable of scavenging free radicals and reducing oxidative stress-mediated β-cell damage. Alkaloids and saponins may improve insulin secretion and glucose uptake, while tannins have been shown to inhibit α-amylase and α-glucosidase enzymes, thereby reducing postprandial hyperglycemia. Terpenoids may also enhance peripheral glucose utilization and improve insulin sensitivity. The presence of these phytochemicals in *C. adansonii* may therefore contribute synergistically to its antihyperglycemic activity.

In view of the increasing burden of diabetes and the limitations associated with existing therapies, there is a need for further investigation of medicinal plants with potential antidiabetic properties. However, there remains limited information regarding the comparative antihyperglycemic efficacy of different solvent extracts of *C. adansonii* and the phytochemical constituents responsible for such activity. Therefore, this study was designed to evaluate the antihyperglycemic effects of hexane, dichloromethane, ethyl acetate, and methanol extracts of *C. adansonii* in streptozotocin-induced diabetic rats, assess the liver function status of treated animals, and identify the bioactive compounds present in the extract exhibiting the highest antihyperglycemic activity.

MATERIALS AND METHODS

Plant Collection

Fresh leaf sample of *C. adansonii* was collected from an uncultivated farm land in Fufore Local Government Area of Adamawa State, Nigeria. It was identified and authenticated in the Department of Plant Science, Modibbo Adama University Yola, Adamawa State.

Plant Extract Preparation

The fresh leaves of *C. adansonii* were washed thoroughly under running tap water to remove dirt and other contaminants, and subsequently shade-dried at room temperature to prevent degradation of heat-labile phytoconstituents. The dried leaves were pulverized into fine powder using a mechanical grinder and stored in airtight containers until extraction. Exactly 200 g of the powdered leaf material was successively extracted using solvents of increasing polarity, namely n-hexane, dichloromethane, ethyl acetate, and methanol, in order to fractionate phytochemicals based on their polarity and solubility characteristics. This sequential extraction approach was employed to enhance the recovery of a broad spectrum of bioactive compounds, ranging from non-polar lipophilic constituents to highly polar phenolic compounds.

For each extraction step, the powdered material was soaked in solvent at a plant-to-solvent ratio of 1:3 (w/v) for 5 days with intermittent agitation to facilitate solvent penetration and phytochemical dissolution. The mixture was filtered using Whatman No. 1 filter paper, after which the solvent was removed under reduced pressure using a rotary evaporator at a temperature below 50°C to preserve thermolabile constituents. The marc obtained after each extraction was air-dried before subsequent extraction with the next solvent in increasing order of polarity. The resulting concentrated extracts were transferred into sterile sample bottles and stored at 4°C until further analysis (Fasihuddin *et al*., 2010).

Experimental Animals

A total of thirty-five (35) adult Wistar rats weighing 120 ± 20 g were used for this study. The animals were obtained from the Animal Breeding Unit of the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The rats were housed in clean polypropylene cages under standard laboratory conditions with adequate ventilation, controlled temperature, and a 12 h light/dark cycle. They were allowed free access to standard rat chow (Vital Feeds®, Jos, Nigeria) and clean drinking water ad libitum. The animals were acclimatized for two weeks prior to the commencement of the experiment. All experimental procedures involving animals were conducted in accordance with standard guidelines for the care and use of laboratory animals.

Induction of Diabetes

Experimental diabetes was induced following overnight fasting of the animals using a single intraperitoneal injection of freshly prepared streptozotocin (STZ) at a dose of 50 mg/kg body weight dissolved in citrate buffer (Al-Hariri, 2012). Streptozotocin is selectively toxic to pancreatic β-cells and is widely used for the induction of experimental diabetes mellitus. Following STZ administration, the rats were provided with 5% glucose solution overnight to prevent sudden hypoglycemic shock. Forty-eight hours after STZ administration, fasting blood glucose levels were measured using a glucometer. Rats with fasting blood glucose levels greater than >200

Table 1: Experimental design.

Group description	Treatment
Sham control (control group)	Normal food and water
STZ-induced diabetic rats (diabetic control)	STZ + normal food and water
STZ-induced diabetic rats (standard control)	50 mg/kg body weight Metformin
STZ-induced diabetic rats	350 mg/kg bwt of hexane leaf extract of *C. adansonii*.
STZ-induced diabetic rats	350mg/kg bwt of Dichloromethane leaf extract of *C. adansonii*
STZ-induced diabetic rats	350mg/kg bwt of Ethyl acetate leaf extract of *C. adansonii*.
STZ-induced diabetic rats	350mg/kg bwt of Methanol leaf extract of *C. adansonii.*

mg/dL were considered diabetic and included in the study.

Experimental Design

Thirty-five (35) Wistar rats were randomly assigned into seven groups containing five rats each. The grouping and treatment regimen are presented in (Table 1). Treatments were administered according to the experimental protocol for 28 consecutive days.

Measurement of blood glucose level (BGL)

Blood samples were collected from the tail vein of the rats by carefully cutting the tip of the tail using sterile lancet. After sample collection, the tail tip was disinfected using 70% ethanol to prevent infection. Fasting blood glucose levels were measured daily using a glucometer and compatible glucose test strips (SD CodeFree™ Blood Glucose Monitoring System, SD Biosensor Inc., Korea). Blood glucose levels were recorded before treatment initiation (Day 0) and on Day 28 following an overnight fast of 14 h (Kifle *et al*., 2021).

Collection and Preparation of Blood Sample

At the end of the experimental period, blood samples were collected from overnight-fasted rats through cardiac puncture and retro-orbital plexus puncture techniques under appropriate handling conditions. The blood samples were transferred into sterile plain tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3,000 rpm for 10 min and subsequently used for biochemical analyses (Li *et al*., 2008).

Biochemical assay

The separated serum samples were analyzed for liver and kidney function biomarkers including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), albumin, urea, and creatinine using commercially available diagnostic kits according to the manufacturers' instructions. ALT and AST activities were determined using the method of Reitman and Frankel (1957), while urea, albumin, GGT, and creatinine estimations were performed according to standard established protocols (Bowers, 1980; Vassault *et al*., 1986).

Gas Chromatography – Mass Spectrometry (GC-MS)

The extract exhibiting the highest antihyperglycemic activity was subjected to Gas Chromatography–Mass Spectrometry (GC–MS) analysis for the identification of bioactive phytochemical constituents. GC–MS analysis was performed according to the method described by Kalaiselvan *et al*. (2012) with slight modifications. The analysis was carried out using a GC–MS system equipped with an electron ionization (EI) detector operating at an ionization energy of 70 eV. Helium gas (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. Exactly 1 µL of the purified extract was injected into the system using split injection mode at a split ratio of 25:1. The injector temperature was maintained at 260°C. The oven temperature program was initially set at 60°C and increased at a rate of 10°C/min to 280°C, followed by an isothermal hold at 280°C for 10 min. Mass spectra were recorded at a scan interval of 0.5 s within a mass range of 45–450 m/z. Identification of phytochemical constituents was achieved by comparing the obtained mass spectra and retention times with those available in standard spectral libraries. The relative abundance of each detected compound was calculated from the corresponding peak area relative to the total chromatographic peak area.

Statistical analysis

Data obtained from the study were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 26.0. Differences among experimental groups were analyzed using one-way analysis of variance (ANOVA), followed by appropriate post hoc multiple comparison tests where applicable. Values were considered statistically significant at $p < 0.05$. All analyses were conducted in triplicate where applicable.

Table 2: Solvent Leaf Extracts of *C. adansonii* on Blood Glucose Level of STZ-induced Hyperglycemic Rats.

Groups (Treatment per kg/bwt)	Blood glucose concentration	
	Initial	Final
Sham control	37.00 ± 1.92	106.20±4.44
Diabetic control	37.00 ± 1.92	293.56 ± 0.76
Standard control (met 5mg/kg bwt)	37.00 ± 1.92	110.52 ± 1.02
Diabetic + 350mg/kg bwt HEX	37.00 ± 1.92	153.72 ± 1.07
Diabetic + 350mg/kg bwt MET	37.00 ± 1.92	250.80 ± 0.63
Diabetic + 350mg/kg bwt DCM	37.00 ± 1.92	269.80 ± 0.70
Diabetic + 350mg/kg bwt EA	338.76 ± 0.76	159.84 ± 0.81

Values are Mean ± SEM; (n=5); p<0.05; HEX=Hexane; MET=Methanol; CM=Dichloromethane; EA=Ethyl acetate; met=Metformin

Table 3. Serum levels of Liver indices in STZ-induced hyperglycemia.

Group- Treatment (kg/bwt)	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (g/L)	Albumin (g/L)
Sham control	65.33 ± 1.20	57.67 ± 1.35	76.00 ± 0.34	37.66 ± 1.23	36.33 ± 0.73
Diabetic control	109.00 ± 1.15	107.00±1.16	137.66±1.31	54.66 ± 1.04	20.33 ± 1.23
Standard control (met 5mg/kg bwt)	66.00 ± 1.52	57.00 ± 0.99	76.03 ± 0.56	35.00 ± 0.34	36.00 ± 0.54
Diabetic + 350 mg/kg bwt HEX	85.67 ± 1.56	75.00 ± 1.34	103.33±1.02	45.66 ± 1.56	27.00 ± 1.31
Diabetic + 350 mg/kg bwt DCM	73.00 ± 2.10	76.66 ± 1.38	96.33 ± 1.17	47.00 ± 0.48	28.33 ± 1.23
Diabetic + 350 mg/kg bwt EA	70.33 ± 1.34	60.667 ± 1.5	82.56 ± 0.21	36.33 ± 1.0	37.00 ± 1.92
Diabetic + 350 mg/kg bwt MET	77.33 ± 2.01	68.33 ± 0.9	83.33 ± 0.45	37.33 ± 1.00	31.66 ± 1.11

Values are mean ± SEM of 5 replicates.
HEX=Hexane; MET=Methanol; DCM=Dichloromethane; EA=Ethyl acetate; met=Metformin.
AST= Aspartate Aminotransferase; ALT= Alanine Transaminase; ALP= Alkaline Phosphatase; GGT= Gamma Glutamyl Transpeptidase.

RESULTS

Antihyperglycemic Activity

The effect of leaf extracts of *C. adansonii* on blood glucose level (BGL) of STZ-induced hyperglycemic rats is shown in (Table 2). The administration of STZ caused a significant increase ($p < 0.05$) in the BGL of all the groups. Treatment with the different solvent leaf extracts of *C. adansonii* resulted in a significant ($p < 0.05$) decrease in the BGL after 28 days, when compared to the untreated (diabetic control) hyperglycemic rats with the ethyl acetate extract having the highest activity.

Analysis of serum levels of ALT, AST, ALP, GGT and Albumin in STZ-induced hyperglycemia.

Serum ALT, AST, ALP, GGT and albumin levels were assessed in hyperglycemic rats in order to investigate the impact of *C. adansonii* leaf extracts on the control of biochemical markers. In comparison to the normal control values, STZ significantly increased ALT, AST, ALP, and GGT while decreasing albumin levels. The above-mentioned parameters were significantly improved by oral administration of 350 mg/kg bwt of *C. adansonii* leaf extracts and 50 mg/kg bwt of metformin (Table 3). In comparison to the other solvents, ethyl acetate extract appears to have more potential to reverse the levels of serum albumin, ALT, AST, ALP, and GGT. Additionally, there was no significant difference (p>0.05) in the effects of the extracts of ethyl acetate and methanol on GGT levels and ethyl acetate on albumin levels when compared to the effects of the drug (metformin).

Analysis of serum creatine and urea level in STZ-induced hyperglycemia in rats after 28 days of treatment

Effects of *C. adansonii* on the serum urea and creatine level were studied to access the renal function. STZ induced a significant elevation in serum urea and creatine levels of rats when compared to the normal control values. Oral administration of *C. adansonii* leaf extracts at 350 mg/kg.bw dose and metformin at 50 mg/kg.bw significantly reduced the above parameters when compared to the diabetic control rats (Table 4). A slight but no significant ($p > 0.05$) difference was observed between the urea levels of all the groups. The drug used (metformin) and ethyl acetate extract had comparable effects on creatine levels.

Identification of Compounds (GC-MS Profiling)

Ethyl acetate leaf extract was found to be more potent amongst the leaf extract; hence the ethyl acetate leaf extract was fractionated into two fractions (1 and 2) and was subjected to GC-MS analysis. The GC-MS chromatogram showed peaks of various useful chemical compounds present in ethyl acetate fractions of *C. adansonii*. Table 5 presents some of the compounds that were revealed to be present in ethyl acetate Fraction 1 of leaf extract of *C. adansonii* and Table 6 presents some of

Table 4: Serum levels of Urea and Creatine in STZ-induced hyperglycemia.

Group- Treatment (kg/bwt)	Urea (mmol/L	Creatinine (µmol/L)
Sham control	4.46 ± 0.23	96.00 ± 1.45
Diabetic control	9.90 ± 0.43	135.33 ± 1.23
Standard control (met 5mg/kg bwt)	4.43 ± 1.45	98.33 ± 1.03
Diabetic + 350mg/kg bwt HEX	5.46 ± 1.89	109.00 ± 1.20
Diabetic + 350mg/kg bwt DCM	5.06 ± 1.51	111.66 ± 1.11
Diabetic + 350mg/kg bwt EA	4.40 ± 0.27	98.33 ± 1.37
Diabetic + 350mg/kg bwt MET	4.70 ± 0.23	102.66 ± 1.05

Values are mean ± SEM of 5 replicates.
HEX=Hexane; MET=Methanol; DCM=Dichloromethane; EA=Ethyl acetate; met=Metformin.

Table 5: Chemical Compounds of Fraction 1 of Ethyl acetate Leaf Extract of *C. adansonii*

Retention time	Peak area (%)	Compound name	Molecular formular	Molecular weight (g/mol)
9.054	0.19	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34
9.609	4.82	1,2-Benzenedicarboxylic acid bis (2-methylpropyl) esters	$C_{16}H_{22}O_4$	278.34
10.584	0.78	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	234.13
10.968	26.88	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390.6
11.935	3.05	9-Oxabicyclo [6.1.0] non-4-ene	$C_8H_{12}O$	124.18
12.257	39.14	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6
14.745	4.35	10-Undecyn-1-ol	$C_{11}H_{20}O$	168.28
17.257	1.54	13-Tetradece-11-yn-1-ol	$C_{14}H_{24}O$	208.34

Table 6: Chemical Compounds of Fraction 2 of Ethyl acetate Leaf Extract of *C. adansonii*

Retention time	Peak (%)	Compound name	Molecular formular	Molecular weight (g/mol)
8.850	0.43	Hexadecane	$C_{16}H_{34}$	226.44
9.041	3.11	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester aka Di isobutyl phthalate	$C_{16}H_{22}O_4$	278.34
9.299	7.99	Phthalic acid, isobutyl octyl ester	$C_{20}H_{30}O_4$	334.4
9.604	5.23	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34
9.814	4.82	1 ,2-Benzenedicarboxylic acid	$C_{14}H_{18}O_4$	250.29
10.167	2.96	dipropyl ester aka dipropyl phthalate2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrN_O$	234.13
10.473	1.64	n-Hexadecanoic acid aka palmitic acid	$C_{16}H_{32}O_2$	256.42
10.806	8.10	Octadecanoic acid aka stearic acid	$C_{18}H_{36}O_2$	284.5
11.444	2.52	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296.5
12.245	21.78	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	390.6
13.609	3.73	N-Isovaleroylglycine	$C_7H_{13}NO_3$	159.18
14.370	5.68	Hexanoic acid, octadecyl ester	$C_{24}H_{48}O_2$	368.6
16.854	0.26	Z,E-3,13-Octadecadien-1-ol	$C_{18}H_{34}O$	266.5
18.083	0.006	Heptadecanoic acid, heptadecylic ester	$C_{34}H_{68}O_2$	508.9

the compounds that were revealed to be present in ethyl acetate Fraction 2 of leaf extract of *C. adansonii*, alongside their retention time (RT), relative percentages peak area (%), molecular formula and Molecular Weight.

DISCUSSION

The present study evaluated the antihyperglycemic potential of leaf extracts of *C. adansonii* in streptozotocin (STZ)-induced diabetic rats. Streptozotocin is known to selectively destroy pancreatic β-cells, thereby impairing insulin secretion and resulting in persistent hyperglycemia (Kifle *et al*., 2021). This mechanism explains the significant elevation in blood glucose levels observed following intraperitoneal administration of STZ in the diabetic control group. However, treatment with the ethyl acetate leaf extract of *C. adansonii* produced the most pronounced reduction ($p < 0.05$) in blood glucose levels when compared with the diabetic control group, indicating marked antihyperglycemic activity.

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by appropriate post-hoc multiple comparison tests, and differences were considered statistically significant at $p < 0.05$.

Previous phytochemical investigations of *C. adansonii* leaf extracts and fractions (ethyl acetate, methanol, hexane, and dichloromethane) revealed the presence of several bioactive secondary metabolites, including alkaloids, flavonoids, saponins, phenolics, terpenoids, and tannins (Ajanaku *et al*., 2016; Adjagba *et al*., 2017; Madaki *et al*., 2020). These phytoconstituents have been widely associated with antihyperglycemic effects through different mechanisms such as inhibition of intestinal glucose absorption, enhancement of insulin sensitivity, stimulation of peripheral glucose uptake, and promotion of glycogen storage (Atchou *et al*., 2020; Kifle *et al*., 2021). Therefore, the observed antihyperglycemic activity of the ethyl acetate extract may be attributed to the synergistic or individual actions of these bioactive compounds. The present findings are consistent with the report of Okoyomoh *et al*. (2023), who demonstrated

significant reductions in blood glucose levels following treatment with ethanol leaf extract of *C. adansonii*. In contrast, the methanol and dichloromethane extracts did not significantly reduce blood glucose levels throughout the treatment period ($p > 0.05$), suggesting that these fractions may contain lower concentrations or less active antihyperglycemic constituents.

The hepato-renal protective effects of *C. adansonii* were also investigated in the present study. Elevated serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), as well as increased urea and creatinine concentrations are established indicators of hepatic and renal dysfunction. Such elevations are indicative of cellular leakage and loss of membrane integrity resulting from oxidative stress and tissue injury (Muhammad *et al*., 2020). In this study, STZ administration significantly increased serum ALT, AST, ALP, GGT, creatinine, and urea levels, while significantly reducing albumin concentration ($p < 0.05$) compared with the normal control group. Treatment with the ethyl acetate leaf extract of *C. adansonii* significantly reversed these alterations, demonstrating considerable hepato-renal protective activity. The ameliorative effects observed may be associated with the antioxidant and free radical scavenging properties of the phytochemicals present in the extract, which may enhance endogenous antioxidant defense systems, stabilize cellular membranes, and promote regeneration of damaged hepatic tissues (Lal *et al*., 2009; Sharma *et al*., 2019; Zhang *et al*., 2020).

A major novel contribution of the present study is the GC–MS characterization of the ethyl acetate leaf extract of *C. adansonii*, which enabled the identification of bioactive compounds potentially associated with the observed antihyperglycemic activity. The GC–MS analysis revealed several compounds with varying retention times and relative abundances. Importantly, compounds such as octadecanoic acid, *n*-hexadecanoic acid, and hexadecane have previously been reported to possess antihyperglycemic and related pharmacological activities. Octadecanoic acid (stearic acid) has been associated with reductions in low-density lipoprotein (LDL) cholesterol, increased high-density lipoprotein (HDL) cholesterol, decreased visceral adiposity, and reduced blood glucose concentrations (Hunter *et al*., 2010; Shen *et al*., 2014). Reduction in visceral fat accumulation may contribute significantly to improved glucose metabolism and reduced metabolic dysfunction (Mgbeje *et al*., 2020). Similarly, *n*-hexadecanoic acid has been reported to exhibit antihyperglycemic, antioxidant, insulin-stimulatory, anti-inflammatory, and hypocholesterolemic properties (Parker *et al*., 2003; Zuraini *et al*., 2012; Tan *et al*., 2019; Olivia *et al*., 2021). Hexadecane has also been reported to possess antihyperglycemic and antimicrobial activities (Siporin and Cooney, 1976; Balamurugan *et al*., 2013). The identification of these compounds provides important scientific evidence supporting the ethnomedicinal use of *C. adansonii* in diabetes management and suggests that the antihyperglycemic activity of the ethyl acetate extract may be mediated through multiple bioactive constituents acting synergistically.

Despite these promising findings, the present study has certain limitations. The study did not isolate and characterize the individual active compounds responsible for the observed antihyperglycemic activity. Additionally, the molecular mechanisms underlying the antihyperglycemic and hepato-renal protective effects were not investigated. Histopathological examination of pancreatic, hepatic, and renal tissues was also not conducted, which could have provided further evidence of tissue protection and regeneration. Furthermore, the relatively short duration of treatment and the use of a single animal model may limit extrapolation of the findings to clinical settings.

Future studies should therefore focus on bioassay-guided isolation and purification of the active compounds identified through GC–MS analysis, followed by mechanistic studies to elucidate their modes of action. Additional investigations involving histopathological evaluation, oxidative stress biomarkers, insulin assays, gene expression analysis, and long-term toxicity studies are also recommended. Clinical studies may further validate the therapeutic potential and safety profile of *C. adansonii* as a candidate for the development of novel antihyperglycemic agents.

Ethical Approval Statement

All experimental procedures involving animals were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Ethics Committee of the Department of Biochemistry, Modibbo Adama University, Yola, Nigeria. Efforts were made to minimize animal suffering and to use the minimum number of animals required for the study.

Conclusion

The findings of the present study demonstrated that among the four solvent extracts investigated, the ethyl acetate leaf extract of *C. adansonii* exhibited the most significant antihyperglycemic activity in STZ-induced diabetic rats, as evidenced by the marked reduction in blood glucose levels ($p < 0.05$). Furthermore, treatment with the extract ameliorated altered hepatic and renal biochemical parameters, indicating hepato-renal protective effects. GC–MS analysis of the ethyl acetate extract revealed the presence of several bioactive compounds, including octadecanoic acid, *n*-hexadecanoic acid, and hexadecane, which may have contributed to the observed antihyperglycemic activity.

These findings provide scientific support for the traditional use of *C. adansonii* in the management of diabetes mellitus and suggest that the plant may serve as a potential source of novel antihyperglycemic agents.

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