BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 2, October 2025 | Pages: 1403-1411 | DOI: 10.14421/biomedich.2025.142.1403-1411 ISSN 2540-9328 (online)

Antidiabetic, Antioxidant, and Gut Microbiota-Modulating Effects of *Cymbopogon citratus* Leaf Extract in Nicotinamide-Streptozotocin Induced Diabetic Rats

Omolola Soji-Omoniwa*, Oluwadamilola Elizabeth Britto, Precious Comfort Omogoye, Oluwafemi Obed Oluwadare, Darasimi Deborah Adeosun, Mariam Oyeladun Alimi, Ridwan Tobiloba Bolajoko, Celestina Omowunmi Amupitan, Emmanuel Ayokanmi Olufemi, Esther Afifoluwake Orekunrin
Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria.

Corresponding author*
sojiomoniwa.o@unilorin.edu.ng

Manuscript received: 13 October, 2025. Revision accepted: 29 November, 2025. Published: 24 December, 2025.

Abstract

Cymbopogon citratus Leaf Extract (CCE) is used traditionally to manage diabetes mellitus. The study aimed to to evaluate the effects of its oral administration on gut bacteria composition and antidiabetic effect in nicotinamide and streptozotocin-induced diabetic rats. Thirty-five male Wistar rats were divided into five groups comprising 7 rats each. They were; non-diabetic rats given distilled water (PC), untreated diabetic rats (NC), diabetic rats treated with 2.5 mg/kg glibenclamide (RDC), diabetic rats treated with 200 mg/kg extract (CCE1) and diabetic rats treated with 400 mg/kg extract (CCE2). Fasting blood glucose (FBG), insulin, glycogen, lipid profile, catalase, superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA), serum electrolytes, urea, uric acid, creatinine, bilirubin, albumin, alanine and aspartate amino transferases (ALT, AST), alkaline phosphatase (ALP), gut bacterial count were analysed and bacteria identified. Secondary metabolites in extract were also quantified. Results showed significant reductions ($p<0.05$) in FBG, low density lipoprotein, triglycerides, cholesterol, urea, sodium, catalase, SOD, GSH, and MDA levels in the CCE1 and CCE2 groups compared to the NC. Glycogen, ALT, AST, ALP, and HDL increased significantly. CCE1 outperformed CCE2 in most biochemical parameters. The total bacterial count increased significantly in the treatment groups and the identified species were *L. plantarum, L. lactis, C. leptum and, L. mesenteroides*. Key secondary metabolites in CCE were catechin, dihydrocytisine, steroid, aphyllidine, Narigenin, proanthocyanidine, oxalate and phytate. In conclusion, CCE exhibited glucose-lowering, antihyperlipidemic, and antioxidant effects, reversed dysfunction in organ function markers and promoted occurrence of beneficial bacterial. Further research into its nutraceutical potential is recommended.

Keywords: *Cymbopogon*; bacterial count; catechin; nutraceutical; glucose.

INTRODUCTION

Diabetes Mellitus has shown a tremendous increase in prevalence with a demographic transition in its epidemiology in recent years (Hossain et al., 2024). This metabolic disorder is characterized by hyperglycaemia, altered metabolism of lipids, carbohydrates, and proteins (Banday et al., 2020). Diabetes affects individuals of all ages, genders, and geographic locations, making it one of the most prevalent global causes of mortality and morbidity (Hossain et al., 2024). Type 2 diabetes mellitus (T2DM) is the most prevalent form, accounting for approximately 90% of all diabetes cases globally (Hossain et al., 2024). Other types of diabetes mellitus include gestational diabetes, neonatal diabetes (Banday et al., 2020). It is estimated worldwide that 240 million individuals live with undiagnosed diabetes, with nearly half of all adults with diabetes unaware of their illness (Hossain et al., 2024). In 2021, the International Diabetes Federation (IDF) estimated that there were 537 million individuals living with diabetes, accounting for 10.5% of the global population, resulting in global healthcare expenses of $966 billion. This healthcare cost is predicted to rise to more than $1054 billion by 2045 (GBDCN,2021; Hossain et al., 2024).

Virtually all parts of the human digestive system, including the gastrointestinal tract, pancreas, and liver, are affected by diabetes (Daryabor et al., 2020). The gastrointestinal tract (GIT) is populated with a myriad of microorganisms, including primarily bacteria but also archaea, viruses, fungi, and protozoan that dynamically influence the health status and homeostasis of the host (Pickard, 2017). Several observational studies have reported associations between the gut microbiota and type 2 diabetes. Consistent features of altered gut

microbiota composition in type 2 diabetes and impaired glucose tolerance/fasting glucose, found in epidemiological studies worldwide and also occurring in the metabolic syndrome, are reduced diversity and decreased abundance of bacteria that produce the short-chain fatty acid (SCFA) butyrate (Byndloss, 2024).

Diabetes mellitus (DM) constitutes a significant global health concern, with rising prevalence and associated complications such as cardiovascular disease, kidney damage, and oxidative stress. Current therapeutic options, while effective, often come with undesirable side effects, necessitating the search for alternative or complementary therapies. Medicinal plants have long been explored as potential sources of bioactive compounds with antidiabetic properties, offering a promising and less invasive approach to managing DM (Banday et al., 2020). *Cymbopogon citratus* , belonging to the Poaceae family, is a tropical grass that thrives in hot and humid regions, including Malaysia, India, America, and many African countries (Shah et al., 2011). In Nigeria, Egypt, South Africa, and Tanzania, lemongrass tea is consumed for the treatment of DM and other related disorders such as hypertension and obesity (Garba et al., 2020). Preliminary evidence suggests its efficacy in regulating blood glucose levels and improving lipid profiles, but the mechanisms underlying these benefits, including its impact on gut microbiota, a crucial player in metabolic health, remain underexplored (Júnior et al., 2024).

The gut microbiota plays a pivotal role in modulating host metabolism, immunity, and inflammation, which are critical in the pathogenesis of diabetes. Dysbiosis, or an imbalance in gut bacterial composition, is commonly observed in individuals with diabetes and is closely linked to impaired glucose metabolism and systemic inflammation. Therefore, evaluating the influence of *C. citratus* on gut microbiota, along with its biochemical and systemic effects will provide a holistic understanding of its potential as an antidiabetic agent.

MATERIALS AND METHODS

Animals

A total of 35 (thirty-five) albino rats (Rattus norvegicus) with an average body weight of 110 g were obtained from the animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were housed in well-ventilated plastic cages with access to rat chow and water throughout the 7 days of acclimatization.

Chemicals, Apparatus and Assay Kits

Streptozotocin and Nicotinamide are product of Sigma-Aldrich (a part of Merck Group), Glibenclamide (Sanofi Adventis, Nigeria), methanol, sodium chloride, citric acid, sodium citrate. All chemicals used were of analytical grade and were prepared in distilled water. The assay kit for insulin determination was a product of RayBioteh Life Inc 3607 Parkway Lane Suite 200 Peachtree Corner, GA 30092, United States. All other assay kits used during the experiment were products of Fortress Diagnostics, United Kingdom.

Plant Preparation

Fresh leaves of *Cymbopogon citratus* were obtained from Tanke Bubu, Ilorin, Kwara State, Nigeria with a GPS coordinates: N8º28’ 13.24884’’, E4^{0}37’4.7254’’ in February, 2024. The plant was appropriately identified at the herbarium unit of the Department of Plant biology, University of Ilorin, Ilorin, Nigeria, with a voucher specimen number UILH|001|1315|2024. The leaves were collected, thoroughly rinsed, and dried at room temperature to a constant weight.

Dried leaves were pulverized using a commercial grinder and stored in air-tight containers prior to analysis. The pulverized sample (1607g) was macerated in a 15 L mixture of water and methanol in the ratio 70:30 for 72 hours with continuous stirring at intervals. It was filtered using 1.0 Whatman filter paper. The resulting filtrate was concentrated in a water bath at 40 ℃ to a constant weight.

High-performance Liquid chromatography Analysis of *Cymbopogon citratus* Leaf

High performance liquid chromatography (HPLC) analysis was performed using Shimadzu LC-10AD dual binary pumps, Shimadzu CTO-10AS column oven, and Shimadzu Prominence SPD-20A UV/Vis detector. The analysis was performed using a C-12 normal phase column (Phenomenex, Gemini 5 µ, 200 mm length × 4.8 mm internal diameter). The mobile phase consisted of acetic acid-acidified deionized water (pH 2.8) as solvent A and acetonitrile as solvent B at a flow rate of 0.8 mL/min. The column was equilibrated with 5% solvent B for 20 minutes after each sample injection. The column temperature was set to 38°C and the injection volume was 20 µL. The wavelengths were set to 280 nm for the detection of phenolics, Phenolic compound identification and quantification were performed by comparing respective retention times and peak areas with pure standard compounds utilizing the method of external standards to construct calibration curve. Gradient elution was executed as follows: 0-5 min, 5-9% solvent B; 5-15 min, 9% solvent B; 15-22 min, 9-11% solvent B; 22-38 min, 11-18% solvent B; 38-43 min, 18-23% solvent B; 43-44 min 23-90% solvent B; 44-45 min, 90-80%, solvent B; 45-55 min.

Induction and Confirmation of Diabetes Mellitus

The fasting blood glucose level of all experimental rats was determined after 8 hours fast prior to induction using Accu – check glucometer and compatible strips. Afterwards, diabetes was induced in experimental rats except those in the positive control group by

administering a single dose of 120 mg/kg body weight Nicotinamide in normal saline solution intraperitoneally to the rats. Fifteen minutes later, streptozotocin (freshly dissolved in ice- cold 0.1M sodium citrate buffer, pH 4.5) was administered to all rats except the positive control at a dose of 65mg/kg body weight.

7 days post nicotinamide and streptozotocin administration in the rats, induction was confirmed by checking their FBG concentration. Rats with FBG concentrations of 200 mg/dl were considered to be successfully induced with diabetes.

Animal Grouping and Treatments

Thirty-five rats were randomly assigned to five groups with seven rats each. They were grouped as follows:

- Positive control (PC): Non-diabetic rats fed with water and feed
- Negative control (NC): Rats induced with diabetes but not treated
- Reference drug control (RDC): Diabetic rats treated with 2.5 mg/kg body weight Glibenclamide
- CCE 1: Diabetic rats treated with 200 mg/kg body weight of *Cymbopogon citratus* leaf extract
- CCE 2: Diabetic rats treated with 400 mg/kg body weight of *Cymbopogon citratus* leaf extract

Cymbopogon citratus leaf extract was administered orally daily for 21 days. After 21 days of administration, the feed and water were withdraw from the rats 24 hours before sacrifice.

Animal Sacrifice and Tissue Collection

Twenty-four hours after the last day of treatment, rats were anaesthetised using dichloromethane and sacrificed by jugular puncture. Blood was collected into plain sample bottles. Serum was obtained after centrifugation at 3000 rpm for 10 minutes and aspirated using a Pasteur pipette into clean sample bottles. Kidney, liver, and heart were isolated and stored in phosphate buffered saline before being homogenizing and stored at -4 ^{o}C for further analysis

Bacterial isolation

The small intestine of the albino rats was aseptically dissected out into a sterile specimen bottle containing 9 ml sterile distilled water. It was vigorously shaken and one ml of it was taken for bacterial load analysis and bacterial type evaluation respectively using the method of Omoya and Momoh (Omoya & Momoh, 2019). Each analysis was done in triplicate. The stepwise isolation of the microflora from the intestine of the experimental rats was done using prepared differential and enrichment media as described previously (Cheesebrough, 2014).

Biochemical assays

Using the ACCU CHEK active glucometer and compatible strips, the FBG concentration of all experimental groups was measured prior to the induction of diabetes in the rats (day 0). The experimental rats' caudal tails were slightly cut in order to obtain the minimal amount of blood needed for this test.

Concentrations of serum triglycerides, high density lipoprotein (HDL), total cholesterol, albumin, total and direct bilirubin, urea, creatinine, creatine Kinase, sodium, potassium, chloride, bicarbonate ions and activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) were estimated using Fortress Diagnostic kits by following the manufacturer's instructions in the kit.

Homeostasis model assessment for insulin resistance (HOMA-IR) and homeostasis model assessment for pancreatic beta scores (HOMA–β scores) were determined by using the formular described by Matthews *et al*.[13]

$$HOMA - IR = \left[Insulin\left(\frac{\mu U}{l}\right) \times Bloodglucose\left(\frac{mmol}{l}\right)\right] \div 22.5$$

$$HOMA - \beta = [20 \times insulin\ (\mu U/l)] \div [Blood\ glucose(mmol/l) - 3.5]$$

Conversion factor: Insulin (1 U/l = 7.174 pmol/l) and blood glucose (1 mmol/l = 18 mg/dl).

Statistical Analysis

Statistical analysis was done using the IBM® statistical package for social sciences (SPSS) software version 20. The level of significant difference was determined using one-way analysis of variance (ANOVA), while post hoc multiple comparisons were done using Duncan's multiple range test. The level of significance was set at $p < 0.05$ (confidence level = 95%). All data were expressed as the mean of 7 determinations ± SEM.

RESULTS AND DISCUSSION

High Performance Liquid Chromatography Analysis of the Chemical Constituents of *Cymbopogon citratus*

Table 1 shows the constituents of the hydromethanolic leaf extract of *C. citratus.* The predominant constituents were catechin, dihydrocytisine, steroid, aphyllidine, Narigenin, proanthocyanidine, oxalate and phytate.

Table 1. High Performance Liquid Chromatography Constituents of *Cymbopogon citratus* Leaf Extract.

Component	Retention	Area	Height	External	Units
Kaempferol	0.210	5426.6760	354.141	3.1514	ug/ml
Steroid	2.390	12419.2739	701.553	7.7090	ug/ml
Epihedrine	4.120	6543.2930	371.126	3.8009	ug/ml
Catechin	6.016	18234.4938	1020.754	24.5087	ug/ml
Anthocyanin	7.470	8483.3000	480.619	3.6277 /	ug/ml
Dihydrocytisine	10.366	19624.7599	1096.770	25.1170	ug/ml
Aphyllidine	12.970	6252.9846	354.931	7.0442	ug/ml
Cyanogenic glycoside	15.460	4978.2300	282.655	5.0535	ug/ml
Aphyllidine	17.963	11350.9880	641.436	14.5837	ug/ml
Narigenin	20.313	12765.7255	680.809	7.6373	ug/ml
Tannin	22.730	9582.8779	539.389	25.4129	ug/ml
Flavonones	25.650	10089.8716	570.416	12.9801	ug/ml
Ammodendrine	27.536	11537.6368	649.849	1.5007	ug/ml
Flavone	29.860	5484.5430	311.583	7.0526	ug/ml
Proanthocyanidine	32.993	14337.0304	803.989	8.5773	ug/ml
Ribalinidine	34.600	6059.2135	344.107	4.0895	ug/ml
Spartein	36.876	6995.9865	394.000	8.9961	ug/ml
Oxalate	39.200	10236.8397	576.255	22.8264	ug/ml
Sapogenin	42.276	3509.5826	199.546	4.7151	ug/ml
Phytate	44.170	10547.4808	596.526	13.5630	ug/ml
		194460.7875		211.9471	

Fasting Blood Glucose Concentration of Nicotinamide and Streptozotocin-induced Diabetic Rats Treated with *Cymbopogon citratus* Leaf Extract

Table 2 shows the results of the FBG concentration of experimental rats pre and post diabetes induction. The FBG of all experimental groups pre induction (day 0) ranges between 75.75 to 86.50 mg/dl. By day 1 (post induction), FBG concentration of all diabetes induced group increased significantly ($p<0.05$) compared to PC, ranging between 256.50 ± 4.91 to 378.00 ± 3.54 mg/dl. Upon commencement of treatment with CCE, there was a significant reduction ($p<0.05$) in the FBG levels of RDC, CCE1 and CCE2 in a dose-dependent manner from day 4 to 20 of the experiment.

Table 2. Fasting Blood Glucose Concentration of Nicotinamide and Streptozotocin- induced diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus.*

Groups	FBG (mg/dl)						
	Day 0	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20
PC	75.75±2.84^{a}	108.7 5±4.59^{a}	108.00±3.46^{a}	87.75±4.01^{a}	62.00±1.73^{a}	60.00±3.81^{a}	97.00±2.86^{a}
NC	75.50±3.48^{a}	256.50±4.91^{b}	228.00±2.80^{b}	314.50±7.50^{c}	314.50±7.50^{d}	278.50±5.50^{c}	300.00±4.04^{e}
RDC	79.25±2.53^{a}	281.00±5.90^{c}	236.50±6.50^{b}	184.50±2.87^{b}	203.00±1.73^{c}	159.50±7.79^{c}	129.00±0.00^{b}
CCE1	72.25±5.90^{a}	383.00±6.00^{d}	266.50±4.91^{c}	291.00±2.36^{d}	205.00±5.58^{d}	201.00±4.73^{d}	252.00±0.00^{d}
CCE2	86.50±1.85^{a}	378.00±3.54^{d}	351.50±2.60^{d}	308.00±4.04^{c}	144.50±0.29^{b}	147.50±1.44^{b}	147.50±1.44^{c}

Values are mean of 7 determinations ± SEM. Values carrying different alphabets are statistically significant at $p < 0.05$ down the column. PC (non-diabetic rats given distilled water); NC (untreated diabetic rats); RDC (diabetic rats treated with 2.5 mg/kg glibenclamide); CCE1 (diabetic rats treated with 200 mg/kg leaf extract of *Cympobogon citratus*); CCE2 (diabetic rats treated with 400 mg/kg leaf extract of *Cympobogon citratus*); FBG – fasting blood glucose; Day 0 – fasting blood glucose before induction.

Selected glycemic indices of Nicotinamide and Streptozotocin-induced Diabetic Rats Treated with *Cymbopogon citratus* Leaf Extract

Table 3 shows results of serum insulin, hepatic glycogen concentrations, HOMA-IR and HOMA – β scores for diabetic rats treated with *C. citratus* leaf extract. There was a significant decrease in the serum insulin concentration and HOMA-IR of CCE1 and CCE2 compared to NC, while a significant increase in hepatic glycogen concentration and HOMA– β scores of the test groups compared to NC was recorded in this study.

Table 3. Selected glycemic indices of Nicotinamide and Streptozotocin- induced diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus.*

Groups	Serum Insulin (mIU/L)	Hepatic glycogen (mmol/l)	HOMA-IR	HOMA – β scores
PC	2.48 ± 0.01^{a}	5.13 ± 0.06^{a}	0.83 ± 0.08^{a}	38.06 ± 4.18^{a}
NC	5.52 ± 0.12^{b}	3.43 ± 0.21^{b}	4.35 ± 0.58^{b}	6.22 ± 6.80^{b}
RDC	3.36 ± 0.93^{c}	4.99 ± 0.56^{a}	2.11 ± 0.35^{c}	33.15 ± 4.01^{a}
CCE1	3.62 ± 0.02^{c}	4.27 ± 0.19^{a}	2.62 ± 0.01^{c}	33.82 ± 0.16^{a}
CCE2	3.44 ± 0.78^{c}	5.43 ± 0.61^{a}	1.93 ± 0.30^{c}	34.78 ± 1.01^{a}

Values are mean of 7 determinations ± SEM. Values carrying different alphabets are statistically significant at $p < 0.05$ down the column. PC (non-diabetic rats given distilled water); NC (untreated diabetic rats); RDC (diabetic rats treated with 2.5 mg/kg glibenclamide); CCE1 (diabetic rats treated with 200 mg/kg leaf extract of *Cympobogon citratus*); CCE2 (diabetic rats treated with 400 mg/kg leaf extract of *Cympobogon citratus*); HOMA-IR - Homeostasis model assessment for insulin resistance; HOMA – β scores - Homeostasis model assessment for pancreatic β scores.

Total Lipid Profile of diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus*

The result of total lipid profile of Nicotinamide and Streptozotocin-induced diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus* is presented in Table 4. There was a significant increase ($p < 0.05$) in HDL concentrations of CCE1 and CCE2 groups compared to NC. Total cholesterol, LDL, VLDL, triglycerides and AI reduced significantly ($p < 0.05$) in *Cymbopogon citratus* treated groups compared to NC.

Table 4. Total lipid profile of Nicotinamide and Streptozotocin-induced diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus.*

Groups	HDL (mmol/l)	Triglycerides (mmol/l)	Total cholestrol (mmol/l)	VLDL (mmol/l)	LDL (mmol/l)	AI
PC	0.98 ± 0.06^{a}	0.80 ± 0.04^{a}	1.39 ± 0.02^{a}	0.12 ± 0.01^{a}	0.43 ± 0.03^{a}	0.09 ± 0.04^{a}
NC	0.63 ± 0.03^{b}	1.74 ± 0.05^{b}	2.37 ± 0.13^{b}	0.26 ± 0.01^{b}	1.99 ± 0.09^{b}	0.17 ± 0.01^{b}
RDC	1.18 ± 0.13^{a}	0.81 ± 0.07^{a}	1.03 ± 0.27^{b}	0.14 ± 0.01^{a}	0.46 ± 0.20^{b}	0.12 ± 0.03^{a}
CCE1	1.27 ± 0.17^{a}	0.85 ± 0.25^{a}	1.11 ± 0.32^{b}	0.13 ± 0.05^{a}	0.73 ± 0.04^{ab}	0.08 ± 0.05^{a}
CCE2	1.07 ± 0.09^{a}	0.82 ± 0.02^{a}	1.80 ± 0.06^{c}	0.14 ± 0.00^{a}	0.43 ± 0.04^{a}	0.10 ± 0.02^{a}

Values are mean of 7 determinations ± SEM. Values carrying different alphabets are statistically significant at $p < 0.05$ down the column. PC (non-diabetic rats given distilled water); NC (untreated diabetic rats); RDC (diabetic rats treated with 2.5 mg/kg glibenclamide); CCE1 (diabetic rats treated with 200 mg/kg leaf extract of *Cympobogon citratus*); CCE2 (diabetic rats treated with 400 mg/kg leaf extract of *Cympobogon citratus*); HDL – High density lipoprotein; VLDL - Very low density lipoprotein; LDL - Low density lipoprotein; AI-Atherogenic index.

Kidney function indices of diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus*

Table 5 shows the Kidney function indices of diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus.* There was no significant difference ($p > 0.05$) in the uric acid concentrations, serum and kidney ALP activities of the experimental rats. However, there was a significant decrease ($p < 0.05$) in serum urea, creatinine, bicarbonate ion, potassium ion, chloride ion and sodium ion concentrations of CCE1 and CCE2 compared to NC.

Table 5. Kidney function indices of Nicotinamide and Streptozotocin-induced diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus.*

Groups	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (µmol/l)	Serum ALP (U/L)	Kidney ALP (U/L)	Bicarbonate ion (mmol/l)	Potassium ion (mmol/l)	Chloride ion (mmol/l)	Sodium ion (mmol/l)
PC	10.13 ± 1.34^{a}	10.41 ± 0.67^{a}	14.30 ± 2.98^{a}	12.62 ± 0.89^{a}	54.54 ± 1.38^{a}	48.28 ± 1.49^{a}	3.00 ± 0.59^{a}	46.99 ± 2.33^{a}	128.69 ± 2.88^{a}
NC	55.21 ± 3.80^{b}	10.11 ± 0.96^{a}	53.33 ± 2.95^{a}	12.79 ± 0.99^{a}	53.87 ± 0.05^{a}	58.05 ± 3.24^{b}	5.24 ± 0.89^{b}	76.75 ± 0.96^{b}	156.92 ± 3.89^{b}
RDC	32.29 ± 1.02^{c}	10.70 ± 0.38^{a}	17.84 ± 2.86^{ac}	12.26 ± 1.57^{a}	52.75 ± 1.44^{a}	48.63 ± 8.31^{a}	3.59 ± 0.25^{a}	41.13 ± 1.06^{a}	129.33 ± 2.21^{a}
CCE1	33.33 ± 1.60^{c}	10.59 ± 0.70^{a}	20.77 ± 1.82^{c}	13.23 ± 2.65^{a}	51.33 ± 0.81^{a}	46.25 ± 2.07^{a}	3.39 ± 0.09^{a}	48.08 ± 1.49^{a}	125.78 ± 2.70^{a}
CCE2	37.50 ± 1.63^{c}	10.22 ± 1.56^{a}	22.93 ± 0.00^{c}	11.06 ± 1.98^{a}	52.26 ± 3.35^{a}	43.60 ± 3.83^{a}	3.87 ± 0.05^{a}	49.66 ± 1.46^{a}	127.78 ± 2.36^{a}

Values are mean of 7 determinations ± SEM. Values carrying different alphabets are statistically significant at $p < 0.05$ down the column. PC (non-diabetic rats given distilled water); NC (untreated diabetic rats); RDC (diabetic rats treated with 2.5 mg/kg glibenclamide); CCE1 (diabetic rats treated with 200 mg/kg leaf extract of *Cympobogon citratus*); CCE2 (diabetic rats treated with 400 mg/kg leaf extract of *Cympobogon citratus*); ALP-Alkaline phosphatase.

Liver function indices and Oxidative stress markers of diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus*

Table 6 presents the results of selected serum liver function indices and oxidative stress markers of diabetic rats treated with a hydromethanolic leaf extract of *Cymbopogon citratus.* Albumin concentration was significantly increased ($p < 0.05$) in the CCE treated groups compared to NC, while direct bilirubin, total bilirubin, ALT and AST decreased significantly ($p < 0.05$). However, there was no significant difference ($p < 0.05$) in the results of CCE1 compared to CCE2.

The oxidative stress markers, MDA, were reduced significantly ($p < 0.05$) compared to NC and there was no significant difference ($p > 0.05$) in GSH concentrations of test groups compared to NC, PC and RDC. Catalase and SOD activities decreased significantly ($p < 0.05$) compared to NC, while the activity of catalase for CCE2 compared favorably with PC and both CCE1 and CCE compared favourably to PC in SOD activity.

Table 6. Selected serum liver function and oxidative stress markers of nicotinamide and streptozotocin-induced diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus.*

Groups	Albumin (g/dl)	Direct bilirubin (µmol/L)	Total bilirubin (µmol/L)	AST (U/L)	ALT (U/L)	MDA (µM)	Catalase (U/ml)	SOD (U/ml)	GSH (mM)
PC	3.58± 0.10^{a}	26.69 ±0.99^{a}	31.88 ±1.30^{a}	37.36 ±0.20^{a}	32.65 ±1.89^{a}	0.78±0.05^{a}	20.04±0.36^{a}	0.27±0.10^{a}	0.15±0.02^{a}
NC	2.45± 0.20^{b}	30.51 ±0.26^{b}	41.38 ±0.50^{b}	67.29 ±0.35^{b}	55.04 ±1.65^{b}	0.93±0.02^{b}	28.83±0.23^{b}	1.41±0.03^{b}	0.17±0.00^{a}
RDC	2.64 ± 0.23^{b}	26.87 ±0.08^{a}	36.75 ±1.50^{c}	41.12 ±0.28^{c}	36.56 ±0.50^{c}	0.83±0.04^{c}	23.17±0.33^{c}	0.56±0.14^{c}	0.15±0.01^{a}
CCE1	3.30± 0.02^{c}	26.75 ±4.55^{a}	33.01 ±2.00^{a}	36.30 ±0.21^{a}	34.03 ±0.00^{c}	0.85±0.08^{c}	22.21±0.17^{c}	0.28±0.03^{a}	0.18±0.02^{a}
CCE2	3.22± 0.09^{c}	26.37± 1.23^{a}	31.88±2.12^{a}	36.81 ±0.40^{a}	33.63 ±0.51^{d}	0.84±0.20^{c}	20.00±0.25^{a}	0.27±0.03^{a}	0.16±0.01^{a}

Values are mean of 7 determinations ± SEM. Values carrying different alphabets are statistically significant at $p < 0.05$ down the column. PC (non-diabetic rats given distilled water); NC (untreated diabetic rats); RDC (diabetic rats treated with 2.5 mg/kg glibenclamide); CCE1 (diabetic rats treated with 200 mg/kg leaf extract of *Cympobogon citratus*); CCE2 (diabetic rats treated with 400 mg/kg leaf extract of *Cympobogon citratus*); AST – Aspartate aminotransferase; ALT-Alanine aminotransferase; ALP-Alkaline phosphatase; MDA - Malondialdehyde; SOD-Superoxide dismutase; GSH- Reduced glutathione.

Distribution of Bacterial Species Isolated from Guts of Diabetic Rats Treated with Hydromethanolic Leaf Extract of *Cymbopogon citratus*

Figure 1 shows the distribution of bacterial species isolated from the guts of diabetic rats treated with hydromethanolic leaf extract of *C. citratus.* Six different species were isolated across all groups. *L. plantarum* was the dominant bacteria across the groups, with the highest abundance in NC. *L. lactis* was found in, NC, and CCE1 but was absent in RDC and CCE2. *L. mesenteroides* on the other hand, was found in all groups except CCE2. It was highly dominant in RDC and CCE1. Similarly, *S. aureus* was found in all groups, with two species present in PC and CCE1, and one species in other groups. *C. leptum* was found in PC, NC, and CCE1, and absent in RDC and CCE2. Lastly, *C. stratum* was present in NC, CCE1 and CCE2 but absent in PC and RDC.

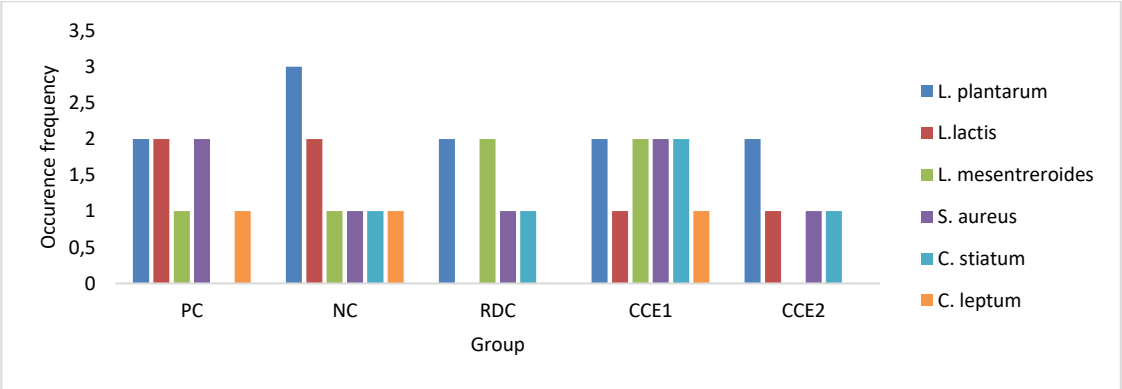

Figure 1. Distribution of Bacterial Species Isolated from Guts of Diabetic Rats Treated with Hydromethanolic Leaf Extract of *Cymbopogon citratus.*

PC (non-diabetic rats given distilled water); NC (untreated diabetic rats); RDC (diabetic rats treated with 2.5 mg/kg glibenclamide); CCE1 (diabetic rats treated with 200 mg/kg leaf extract of *Cympobogon citratus*); CCE2 (diabetic rats treated with 400 mg/kg leaf extract of *Cympobogon citratus*)

Total bacterial and Coliform count of diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus*

The Total bacterial and Coliform count of diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus* is shown in Table 7. There was a significant decrease ($p < 0.05$) in the total bacterial count of rats in the NC group compared to the PC group. Upon treatment, the bacterial count increased significantly ($p < 0.05$) compared to NC. The total coliform count for rats in RDC and CCE1 groups was zero, while there was no significant difference ($p > 0.05$) in the numbers detected for PC, NC and CCE.

Table 7. Total bacterial and Coliform count of diabetic rats treated with hydromethanolic leaf extract of *Cymbopogon citratus.*

Groups	Total Bacterial Count (CFU/ML)	Total Coliform Count (CFU/ML)
PC	12.43 ± 0.69^{a}	3.46 ± 1.93^{a}
NC	10.30 ± 1.16^{b}	3.23 ± 0.73^{a}
RDC	14.00 ± 1.07^{ac}	0.00 ± 0.00^{b}
CCE 1	11.11 ± 0.89^{b}	0.00 ± 0.00^{b}
CCE 2	15.30 ± 0.00^{c}	4.55 ± 0.22^{a}

Values are mean of 7 determinations ± SEM. Values carrying different alphabets are statistically significant at $p < 0.05$ down the column. PC (non-diabetic rats given distilled water); NC (untreated diabetic rats); RDC (diabetic rats treated with 2.5 mg/kg glibenclamide); CCE1 (diabetic rats treated with 200 mg/kg leaf extract of *Cympobogon citratus*); CCE2 (diabetic rats treated with 400 mg/kg leaf extract of *Cympobogon citratus*).

Discussion

Findings from this study showed that *Cymbopogon citratus* leaf extract elicited glucose lowering effect (Table 2). The hyperinsulinemia associated with DM was also ameliorated upon consumption of the *C. citratus* at the end of the experiment. This suggests improvement in insulin sensitivity, as further demonstrated by HOMA-IR and HOMA- β scores results (Table 3). Similarly, hepatic glycogen was also restored to normal after treatment. Blood glucose level, serum insulin, hepatic glycogen concentrations, and insulin resistance indices are markedly altered in T2DM. The improvement recorded for these parameters in this study might be due to the presence of phytochemicals in the leaf extract of *C. citratus* (Oladeji et al., 2019). Although, the secondary metabolites were not isolated and their effects studied individually on DM, result of HPLC analysis revealed the presence of secondary metabolites that have been previously reported to possess antidiabetic properties. Chief among them was catechin, a polyphenolic compound (Table 1), which was previously reported to elicit hypoglycemic effect (Addepalli & Suryavanshi, 2018). Similarly, Kaemferol and phytate had been reported to be beneficial in the management of DM (Den Hartogh et al., 2019; Yang et al., 2022). The identified secondary metabolites in *C. citratus* leaf extract might be acting in synergy with the observed antihyperglycemic effect.

The effect of *C. citratus* was also monitored on selected lipid profiles. Findings showed that the impairment in the lipid metabolic pathway that characterized DM was ameliorated upon treatment. The result was however, dose-dependent as observed for the glycemic markers, with CCE at 400 mg/kg giving the best result. The constituents of *C. citratus* may act by promoting insulin sensitivity and glucose uptake in peripheral tissues, as well as inhibiting the glycogenolysis and lipolysis pathways. Similarly, kidney and liver function indices monitored in this study showed that the administration of *C. citratus* ameliorated kidney and liver dysfunction in the experimental rats (Tables 5 and 6). Chronic hyperglycaemia induces oxidative stress via enhanced reactive oxygen species (ROS) production and diminished antioxidant defence in diabetic kidney disease (DKD) (Jha et al., 2024). Chronic hyperglycaemia disrupts the homeostatic balance between pro-oxidant and antioxidant pathways whereby there is an upregulation of pro-oxidant enzyme-derived reactive oxygen species (ROS) formation and accompanying reduction in antioxidants, causing oxidative stress within the kidneys (DeFronzo et al., 2021; Ding et al., 2021). Recent accumulating evidence demonstrates the overproduction of intrarenal ROS in diabetes and subsequent haemodynamic alterations and metabolic changes as a key mediator and a common denominator of the pathways leading to disrupted renal function and pathological structural changes in DKD (Ding et al., 2021). Similarly, DM causes excessive accumulation of fat cells in the liver resulting in a fatty liver. Previous research has found that the liver stops oxidising fatty acids and uses them instead to synthesise triglycerides which then accumulate abnormally in the liver (Levinthal & Tavill, 1999).

At the center of organ damage in diabetic state is oxidative stress, which was observed in the NC group in this study (Table 6). However, upon treatment with *C. citratus*, the derangement was reversed. Excessive ROS production results in several deleterious events, including an irreversible oxidative modification of lipids, proteins and carbohydrates (Parveen et al., 2010; Leung and Nieto, 2013). Additionally, it will induce apoptosis in hepatocytes and the release of inflammatory cytokines,

thereby increasing the expression of adhesion molecules and facilitating the infiltration of leukocytes. A combination of all of these processes causes massive tissue destruction in the liver (Welt, 2004). Previous research has proven that a decrease in superoxide dismutase and catalase activities within a hyperglycaemic state leads to an increase in ROS, which eventually contributes to oxidation-induced liver damage (Han, 2006 and Parveen, 2010).

L. plantarum was the most dominant bacteria across all groups, with the highest abundance in Group 2 (Negative control) (Figure 1). This observation aligns with studies indicating that *L. plantarum* can proliferate in diabetic conditions due to altered gut environments (Kleerebezem et al., 2003). *L. lactis* on the other hand, thrives in both non-diabetic and untreated diabetic conditions. *L. lactis* is known for its beneficial effects on gut health and immune modulation (Gänzle, 2015). *L. mesenteroides* was found in all groups except CCE2. It was highly dominant in RDC and CCE1. This bacterium ferments dietary fibers into beneficial short-chain fatty acids, crucial for gut health (Linares et al., 2017). The dominance in Groups 3 and 4 suggests that both glibenclamide and moderate doses of *Cymbopogon citratus* extract support a favorable gut environment for *L. mesenteroides. C. leptum* found in the experimental groups is critical for maintaining gut health through its role in butyrate production, essential for colon health (Louis and Flint, 2009). Total Bacterial Count indicates the overall bacterial load in the gut, reflecting gut health and microbial balance. The high count in CCE1 and CCE 2 signifies a healthy gut microbiome. Total Coliform Count on the other hand measures coliform bacteria, which are indicators of potential contamination and gut health issues. Lower counts are generally desirable for health (Table 7).

CONCLUSION

Cymbopogon citratus leaf extract (200 and 400 mg/kg) demonstrated significant antihyperglycemic, antihyperlipidemic, and antioxidant activities, improved organ function markers, and enhanced beneficial gut bacteria. The 200 mg/kg dose showed superior efficacy, supporting its potential as a nutraceutical candidate for diabetes management.

Authors' Contributions: Omolola Soji-Omoniwa designed the study. Oluwadamilola Elizabeth Britto, Precious Comfort Omogoye, Oluwafemi Obed Oluwadare, Darasimi Deborah Adeosun, Maryam Oyeladun Alimi, Ridwan Tobiloba Bolajoko, Celestina Omowumi Amupitan, Emmanuel Ayokanmi Olufemi and Esther Afifoluwake Orekunrin, carried out the laboratory work. Maryam Oyeladun Alimi analyzed the data. Omolola Soji-Omoniwa and Oluwadamilola Elizabeth Britto wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

REFERENCES

Addepalli, V., & Suryavanshi, S. V. (2018). Catechin attenuates diabetic autonomic neuropathy in streptozotocin-induced diabetic rats. *Biomedicine & Pharmacotherapy, 108*, 1517–1523. https://doi.org/10.1016/j.biopha.2018.09.179

Banday, M. Z., Sameer, A. S., & Nissar, S. (2020). Pathophysiology of diabetes: An overview. *Avicenna Journal of Medicine, 10*(4), 174–188. https://doi.org/10.4103/ajm.ajm_53_20

Byndloss, M., Devkota, S., Duca, F., Niess, J. H., Nieuwdorp, M., Orho-Melander, M., Sanz, Y., Tremaroli, V., & Zhao, L. (2024). The gut microbiota and diabetes: Research, translation, and clinical applications—2023 Diabetes, Diabetes Care, and Diabetologia Expert Forum. *Diabetologia, 67*(9), 1760–1782. https://doi.org/10.1007/s00125-024-06198-1

Cheesebrough, M. (2014). *District laboratory practice in tropical countries* (2nd ed.). Cambridge University Press.

Daryabor, G., Atashzar, M. R., Kabelitz, D., Meri, S., & Kalantar, K. (2020). The effects of type 2 diabetes mellitus on organ metabolism and the immune system. *Frontiers in Immunology, 11*, 1582. https://doi.org/10.3389/fimmu.2020.01582

DeFronzo, R. A., Reeves, W. B., & Awad, A. S. (2021). Pathophysiology of diabetic kidney disease: Impact of SGLT2 inhibitors. *Nature Reviews Nephrology, 17*, 319–334.

Den Hartogh, D. J., Gabriel, A., & Tsiani, E. (2019). Antidiabetic properties of curcumin II: Evidence from in vivo studies. *Nutrients, 12*(1), 58. https://doi.org/10.3390/nu12010058

Ding, M., Tang, Z., Liu, W., Shao, T., Yuan, P., Chen, K., Zhou, Y., Han, J., Zhang, J., & Wang, G. (2021). Burdock fructooligosaccharide attenuates high glucose-induced apoptosis and oxidative stress injury in renal tubular epithelial cells. *Frontiers in Pharmacology, 12*, 784187.

Garba, H. A., Mohammed, A., & Ibrahim, M. A. (2020). Effect of lemongrass (*Cymbopogon citratus* Stapf) tea in a type 2 diabetes rat model. *Clinical Phytoscience, 6*, 19. https://doi.org/10.1186/s40816-020-00167-y

Global Burden of Disease Collaborative Network. (2024). *Global Burden of Disease Study 2021 results.* Institute for Health Metrics and Evaluation. https://vizhub.healthdata.org/gbd-results/

Han, D., Hanawa, N., Saberi, B., & Kaplowitz, N. (2006). Mechanisms of liver injury: III. Role of glutathione redox status in liver injury. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 291*(1), G1–G7. https://doi.org/10.1152/ajpgi.00001.2006

Hossain, M. J., Al-Mamun, M., & Islam, M. R. (2024). Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. *Health Science Reports, 7*(3), e2004. https://doi.org/10.1002/hsr2.2004

Jha, R., Lopez-Trevino, S., Kankanamalage, H. R., & Jha, J. C. (2024). Diabetes and renal complications: An overview on pathophysiology, biomarkers, and therapeutic interventions. *Biomedicines, 12*(5), 1098. https://doi.org/10.3390/biomedicines12051098

Júnior, A. S. S., Aidar, F. J., Silva, L. A. S., de B. Silva, T., de Almeida, S. F. M., Teles, D. C. S., de L. Junior, W., Schimieguel, D. M., de Souza, D. A., Nascimento, A. C. S., Camargo, E. A., Dos Santos, J. L., de O. E. Silva, A. M., de S. Nunes, R., Borges, L. P., & Lira, A. A. M. (2024). Influence of lemongrass essential oil (*Cymbopogon flexuosus*) supplementation on diabetes in a rat model. *Life, 14*(3), 336. https://doi.org/10.3390/life14030336

Kleerebezem, M., Boekhorst, J., van Kranenburg, R., Molenaar, D., Kuipers, O. P., Leer, R., Tarchini, R., Peters, S. A., Sandbrink, H. M., Fiers, M. W., Stiekema, W., Lankhorst, R. M., Bron, P. A., Hoffer, S. M., Groot, M. N., Kerkhoven, R., de Vries, M., Ursing, B., de Vos, W. M., & Siezen, R. J. (2003). Complete genome sequence of *Lactobacillus plantarum* WCFS1. *Proceedings of the National Academy of Sciences, 100*(4), 1990–1995. https://doi.org/10.1073/pnas.0337704100

Leung, T. M., & Nieto, N. (2013). CYP2E1 and oxidant stress in alcoholic and non-alcoholic fatty liver disease. *Journal of Hepatology, 58*(2), 395–398. https://doi.org/10.1016/j.jhep.2012.08.018

Levinthal, G. N., & Tavill, A. S. (1999). Liver disease and diabetes mellitus. *Clinical Diabetes, 17*(3), 73–79.

Louis, P., & Flint, H. J. (2009). Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiology Letters, 294*(1), 1–8. https://doi.org/10.1111/j.1574-6968.2009.01514.x

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28*(7), 412–419.

Michael, G. G. (2015). Lactic metabolism revisited: Metabolism of lactic acid bacteria in food fermentations and food spoilage. *Current Opinion in Food Science, 2*, 106–117. https://doi.org/10.1016/j.cofs.2015.03.001

Mohamed, J., Nazratun Nafizah, A. H., Zariyantey, A. H., & Budin, S. B. (2016). Mechanisms of diabetes-induced liver damage: The role of oxidative stress and inflammation. *Sultan Qaboos University Medical Journal, 16*(2), e132–e141. https://doi.org/10.18295/squmj.2016.16.02.002

Omoya, F. O., & Momoh, A. O. (2019). Comparative effects of garlic, yoghurt, beniseed liquor and fresh orange juice on induced type-1 diabetes mellitus in rabbits using streptozotocin. *Journal of Food & Nutritional Disorders, 8*(4), 1–9.

Oladeji, O. S., Adelowo, F. E., Ayodele, D. T., & Odelade, K. A. (2019). Phytochemistry and pharmacological activities of *Cymbopogon citratus*: A review. *Scientific African, 6*, e00137. https://doi.org/10.1016/j.sciaf.2019.e00137

Parveen, K., Khan, M. R., Mujeeb, M., & Siddiqui, W. A. (2010). Protective effects of pycnogenol on hyperglycemia-induced oxidative damage in the liver of type 2 diabetic rats. *Chemico-Biological Interactions, 186*(2), 219–227. https://doi.org/10.1016/j.cbi.2010.04.023

Pickard, J. M., Zeng, M. Y., Caruso, R., & Núñez, G. (2017). Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. *Immunological Reviews, 279*(1), 70–89. https://doi.org/10.1111/imr.12567

Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., & Mann, A. S. (2011). Scientific basis for the therapeutic use of *Cymbopogon citratus* Stapf (lemongrass). *Journal of Advanced Pharmaceutical Technology & Research, 2*(1), 3–8. https://doi.org/10.4103/2231-4040.79796

Welt, K., Weiss, J., Martin, R., Dettmer, D., Hermsdorf, T., Asayama, K., et al. (2004). Ultrastructural, immunohistochemical and biochemical investigations of the rat liver exposed to experimental diabetes and acute hypoxia with and without application of Ginkgo extract. *Experimental and Toxicologic Pathology, 55*(5), 331–345. https://doi.org/10.1078/0940-2993-00337

Yang, Y., Chen, Z., Zhao, X., Xie, H., Du, L., Gao, H., & Xie, C. (2022). Mechanisms of kaempferol in the treatment of diabetes: A comprehensive and latest review. *Frontiers in Endocrinology, 13*, 990299.

THIS PAGE INTENTIONALLY LEFT BLANK