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Original Article

Dialium guineense improves Lipid Profile and Oxidative Stress in Streptozotocin-induced Glycemic-impaired Diabetic Rats

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Abstract

In the present study, *in-vivo* and *in-vitro* approaches were utilised to investigate the antioxidant, anti-hyperglycemic and anti-hyperlipidemic activities of Ethanolic (EthEx) and Ethyl Acetate extracts (EthAcEx) of *Dialium guineense* (DG). *In-vitro* analysis of the plant extracts revealed the presence of Phenolics (43.89±0.65 and 12.16±0.27 µg/100 mg GAE for EthEx and EthAcEx respectively) and Flavonoids (3.59±0.09 and 2.56±0.39 µg/100 mg QE for EthEx and EthAcEx respectively). The *in vitro* antioxidant analysis revealed a DPPH IC_{50} of 43.45±1.14 and 108.45±0.89 µg/mL for EthEx and EthAcEx respectively. Similarly, FRAP values were EthEx 3.02±0.01 and EthAcEx 0.96±0.05 respectively. *In-vitro* hypoglycemic (IC_{50}) activities were alpha-amylase: EthEx 17.15 ± 0.03 and EthAcEx 6.04±0.03 µg/mL; alpha-glucosidase EthEx 70.21±1.55 and EthAcEx 28.81±0.22 µg/mL. *In-vivo* analysis revealed that the plant extracts improved weight gain and alleviated glycemic impairment in Streptozotocin induced diabetic rats. In addition, the solvent extracts caused an improvement of dyslipidemia and increased the plasma activities of Superoxide Dismutase, Catalase and concentration of Glutathione. Malondialdehyde concentration was suppressed by administration of the plant extracts. Put together, the present study provides evidence for the antioxidant, anti-hyperglycemic, anti-hyperlipidemic and antidiabetic activities of DG. This suggests the potential of the plant as alternative therapy for the management of diabetes and its complications.

Keywords: Diabetes; *Dialium guineense*; Dyslipidemia; Hyperglycemia; Oxidative Stress; SDG3.

1. Introduction

Diabetes mellitus is one of the major endocrine disorders characterised by lack of insulin, insulin resistance and derangement of carbohydrate, protein and lipid metabolism [1]. According to the International Diabetes Federation (IDF) 537 million suffered diabetes in 2021 and it is projected that in 2045, 783 million persons will be living with the disease [2]. This continued increase in the epidemiology of the disease is attributable to key contributors which include urbanization, an

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ageing population, decreasing levels of physical activity and increasing prevalence of overweight and obesity. Diabetes mellitus is classified into type I characterised by lack of insulin, type II characterised by insulin resistance and gestational diabetes common to pregnant women [3]. Hallmarks of the disease include complications such as hyperglycemia, oxidative stress, hyperlipidemia, nephropathy, retinopathy, neuropathy and cardiovascular disease [4, 5]. Currently, exercise, diet, synthetic drugs and herbal remedies are used singly or combined for managing diabetes and its complications [6- 9]. Similarly, a combination of two or more synthetic drugs with varied mechanism of action is used to effectively tackle diabetes. Acarbose and miglitol for instance, inhibits the hydrolysis of complex carbohydrates, glibenclamide enhances the release of insulin, metformin functions by mobilizing glucose into tissues to mention but a few. However, some of these drugs are known to have side effects which undermine their pharmacological benefits [10]. Hence, the urgent need to develop new drugs.

Plants are known to be rich reservoir of phytochemicals such as polyphenol, flavonoid, alkaloids which confers pharmacological activities such as antidiabetic, anticancer, antiinflammatory benefits [11, 12]. The use of medicinal plants for the treatment of human diseases is common in many developing countries of the world. In fact, some of the drugs currently marketed in both developed and developing economies are derived from plant origin.

Dialium guineense is a legume which belongs to the family, Fabaceae and a sub-family of Caesalpinioideae. It is commonly known as velvet tamarind, also known as black velvet, it is called 'Aigele' by the Igala tribe of Nigeria, West Africa. Leaves and fruit of the plant are consumed as food while other parts of the plant are used for medicinal purposes. Reports from the literature suggest that there is growing experimental support for the use of *Dialium guineense* in herbal medicine. For instance, Ezeja et al. [13] demonstrated that methanolic stem bark extract of *Dialium guineense* had analgesic activity while anti-microbial activity of the stem bark was revealed by the study of Olajubu and co-workers [14]. Mannose/glucose-specific lectin purified from the seeds of *Dialium guineense* exhibited an intermediary toxic effect on *Artemia sp*. Nauplii [15]. In a study, the phenolic extract of the seed pup enhanced reactive oxygen species detoxification of aflatoxin B1 hepatocarconogenesis [16]. The plant has also been shown to possess activities such as wound healing [17], protects against aspirin-induced gastric injury [18], antidiarrheal activity [19] and improvement of growth performance in broiler chickens [20]. However, the antidiabetic effects of *Dialium guineense* leaf are yet to be explored. Hence, this study was designed to study the effects of ethanol and ethyl acetate extracts of *Dialium guineense* leaf on lipid profile, antioxidant activities and oxidative stress indices of Streptozotocin induced glycemic-impaired diabetic rats.

2. Materials and Methods

2.1. Chemicals

Ethanol and Ethyl acetate were products of BDH, England. 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Sodium carbonate, Methanol, Sodium hydroxide, Aluminum trichloride, Tripyridyl triazone (TPTZ), Hydrochloric acid, Quercetin,

Gallic acid, 5,5'-dithio-bis-(2-nitrobenzoic acid), Trichloroacetic acid, Thiobarbituric acid, Epinephrine, p-nitrophenyl glucopyranoside (pNPG), Potassium phosphate, Acetone, alpha amylase, Starch and Acarbose were purchased from Sigma Aldrich Co. LLC, USA. Diagnostic kits for High Density Lipoprotein, Triglycerides and Total Cholesterol were purchased from Randox Diagnostic, USA.

2.2. Collection and Extraction of Plant Material

The leaves of *Dialium guineense* were collected in Okabo, Ofu Local Government Area, Kogi State, Nigeria and were identified in the Department of Plant Science and Biotechnology of Prince Abubakar Audu University, Anyigba. The leaves were washed to remove dirt and then air dried to constant weight, after which it was blended into powder using an electric blender. Two portions of 700 g each of the air dried samples was weighed into beakers and 1500 mL of either ethanol or ethyl acetate was added separately. The mixtures were stirred and left for 72 hours to extract exhaustively. The mixture was filtered and the filtrate was placed in Water Bath at 60˚C to evaporate the solvent. The extracts obtained were designated as crude ethanolic (EthEx) and ethyl acetate extracts (EthAcEx).

2.3. Determination of Total Phenolic and Flavonoid contents

The Total phenolic content of the ethanolic and ethyl acetate extracts of *Dialium guineense* leaf was estimated using the Folin Ciocalteu method as reported by Maurício Duarte-Almeida et al. [21]. While the Flavonoid content was determined using the Aluminum trichloride method according to Dewanto and colleagues [22]. This method was based on flavonoid-aluminum complex formation. The total phenolics and flavonoids were extrapolated from the standard curves of Gallic acid and Quercetin respectively.

2.4. Gas-Chromatography Mass Spectrometry Analysis

Gas Chromatography Mass Spectrometry (GC-MS) analysis was carried out on a GC (Agilent 6890N) and MS (5973 MSD). The GC-MS was equipped with a DB-5ms capillary column (30 m× 0.25 mm: film thickness 0.25 µm). The crude solvent extract of *Dialium guineense* (2 µL) was injected and separated in the GC column using Helium as the carrier gas at a flow rate of 1mL/min. The temperature was set at 40˚C and thereafter increased to 150˚C at the rate of 10˚C/min. The temperature was again increased to 230˚C by increments of 5˚C/min. The final temperature of 310˚C was reached by increments of 20˚C/min. Other parameters for the analysis include: injector port temperature = 280˚C, detector temperature =250˚C, split ratio = 110:1 and ionization voltage = 70 eV. The outcome of the MS was compared with spectra available in the NIST database.

2.5. In vitro Antioxidant Assays

2.5.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging activity of the ethanolic and ethyl acetate extracts of *Dialium guineense* leaf was estimated using the method reported by Atanu et al. [23]. Samples (2 ml) of each extract at concentrations range 25-800 µg/ml were added to 2 ml of DPPH radical

solution in methanol for 30 minutes. Absorbance of the solution was taken at 512 nm using a spectrophotometer. Vitamin C was used as the standard. The DPPH radical scavenging effect was calculated using the following equation:

% inhibitory activity = $(A_c - A_s) / A_c \times 100$

A_s = Absorbance of sample

A_c = Absorbance of Standard (Vit C)

2.5.2. Ferric Reducing Antioxidant Power

The Ferric Reducing Antioxidant Power **(**FRAP) assay was performed according to the method of Benzie and Strain [24]. Its principle is based on the sample's ability to oxidize ferrous tripyridyltriazine (Fe (II) TPTZ) to ferric tripyridyltriazine (Fe (III) TPTZ) complex. It produces a deep blue colour at low pH. Briefly, 30 mM CH_3COONa buffer, 10 mM TPTZ, 40 mM HCl. The FRAP reagent was incubated 37˚C and mixed with the sample. Absorbance was read at 765 nm immediately. Ascorbic acid was used as the control. The FRAP value was calculated using the following equation:

FRAP value (%) = $[(A_s - A_b) / (A_c - A_b)] \times 2$

A_c = Absorbance of the positive control (Ascorbic acid + FRAP reagent)

A_s = Absorbance of sample

A_b = Absorbance of blank (H_20 + FRAP reagent).

2.6. In-vitro hypoglycemic assays

In vitro hypoglycemic assays were performed based on the ability of the extracts to inhibit carbohydrate hydrolyzing enzymes namely: alpha-amylase and alpha-glucosidase. The assays were conducted according to a previously published protocol of Atanu et al. [23]. Acarbose was used as positive control. Results were expressed as Inhibitory Concentration of the samples at 50% (IC_{50}).

2.7. In-vivo anti-diabetic studies

2.7.1. Animal husbandry, grouping and induction of diabetes

Thirty (30) albino rats weighing 160 - 180 g were purchased from the Animal Care Facility of the Department of Biochemistry, Prince Abubakar Audu University, Anyigba, Nigeria. All animal experiments were approved by the Directorate of Research and Innovation of Prince Abubakar Audu University, Anyigba, Nigeria in accordance with the Helsinki Protocol as well as the World Medical Association (WMA) statement on animal use in Biomedical Research (PAAUDRI02.06.2022). The animals were housed in animal cages under conditions of 28±2°C and 12-hr light/ark cycle. All animals received standard pellet diet and water ad libitum. The animals were acclimatized for 2 weeks prior to commencement of treatments. Twenty-four (24) of the rats were fasted overnight before induction of diabetes by single intraperitoneal injection of freshly prepared Streptozotocin (55 mg/kg prepared 0.1M citrate buffer; pH=4.5) [25]. After 30 minutes, the rats were allowed free access to feed and water. Fasting blood glucose was measured using AccuCheck Advantage II glucometer (Roche Diagnostic Co, USA) after 72 hours of streptozotocin injection. The rats with glucose values higher or equal to 250 mg/dL were considered diabetic. The rats were allocated to five experimental groups of six rats per group as shown below:

Group 1: Normal control, **Group 2**: Diabetic control, **Group 3**: Diabetic rats administered 5 mg/kg body weight Glibenclamide. **Group 4**: Diabetic rats administered 200 mg/kg body weight of ethanolic extract of *Dialium guineense*. **Group 5**: Diabetic rats administered 200 mg/kg body weight of ethyl acetate extract of *Dialium guineense*.

The administration of the treatment was once per day and for 28 days. The Fasting blood sugar and body weight of the animals were measured every 7 days until the 28th day.

2.7.2. Animal sacrifice, sample collection and processing

On the 29th day, the experimental animals were sacrificed by cervical dislocation and whole blood was collected from the heart by cardiac puncture. Serum was separated by centrifugation at 5, 000 rpm for 5 min. Whole organs (liver, heart, kidney and pancreas) were excised, trimmed of extra tissues and weighed.

2.7.3. Determination of biochemical parameters

Total Cholesterol (TC), High Density Lipoprotein (HDL) and Triglycerides (TG) were determined using Randox kits based on manufacturer's instructions. Low Density Lipoprotein (LDL) was calculated using the formula below:

LDL = TC – HDL – [TG/5]

Glycemic variability, Atherogenic Index and Coronary Artery Index were calculated according to the formulas [26]:

Glycemic variability (%) = $[G_{28} - G_0]/G_0$ X 100

Where G_{28} and Go are Fasting Blood Glucose on the days 28 and day 0 respectively.

Atherogenic Index (AI) = [TC – HDL] /HDL

Coronary Artery Index (CAI) = LDL/HDL

Superoxide dismutase was assayed according to the method of Misra and Fridovich [27], Catalase activity was assayed based on the protocol of Beers and Sizer [28], while Glutathione concentration was determined by the method of Ellman [29]. Concentration of Malondialdehyde was determined according to the method of Ohkawa et al. [30].

2.8. Statistical analysis

Results were expressed as Mean±SEM of replicates. Data was compared using the one-way analysis of variance (ANOVA) on the GraphPad Instat statistical software. Statistical significance was determined at $p<0.05$, $p<0.01$ and $p<0.001$.

3. Results and Discussion

Diabetes has become a major cause of death hence the corresponding quest for novel antidiabetic drugs. Over the last century, attention has been drawn to medicinal plants as a reservoir for novel bioactive phytochemicals that could serve as drug leads [12]. Different research groups across the globe have adopted *in-vitro*, *in-silico* and *in-vivo* models toward drug discovery aimed at diabetes. In the present study, we demonstrated using *in-vitro* and *in-vivo* models that solvent extracts of *Dialium guineense* possess antioxidant, hypoglycemic, anti-hyperglycemic and anti-hyperlipidemic properties in Streptozotocin-induced rat model of diabetes.

3.1. Chemical composition, in vitro antioxidant and inhibition of carbohydrate hydrolyzing enzymes by extracts of Dialium guineense

Table 1 shows the results for Total Phenolic and Flavonoid contents of Ethanolic (EthEx) and Ethyl Acetate extracts (EthAcEx) of *Dialium guineense* leaf. The results revealed that EthEx (43.89 ± 0.65 µg/100 mg extract GAE) contain significantly higher concentrations of phenolic compounds than the EthAcEx (12.16 ± 0.27 µg/100 mg extract GAE; $p < 0.05$), while the flavonoid content is not significantly different in both extracts ($p>0.05$).

In vitro antioxidant activity was evaluated based on DPPH radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP) of the crude extracts. Results of the DPPH radical scavenging activity presented as IC50 values suggest that the EthEx (43.45 ± 1.14 µg/mL) had higher free radical scavenging activity than the EthAcEx (108.45 ± 0.89 µg/mL) and Vitamin C (68.42 ± 0.51 µg/mL). The reference compound Vitamin C however, had significantly higher DPPH radical scavenging activity than the EthAcEx ($p<0.05$; Table 1). Similarly, FRAP values of all three samples were significantly different ($p<0.05$). The order of activity is as follows: EthEx 3.01 ± 0.01>Vitamin C 2.00 ± 0.00> EthAcEx 0.96 ± 0.05.

Alpha-amylase and alpha-glucosidase were selected for *in vitro* hypoglycemic studies. Results presented in **Table 1** show that both extracts possess inhibitory activity against the hydrolytic activity of these enzymes. The inhibition of the extracts was compared against a reference compound Acarbose. The EthAcEx was more potent at inhibiting both enzymes than the EthEx however, Acarbose had a superior inhibition compared to both extracts ($p<0.05$).

Table 1. Chemical composition, antioxidant and *in vitro* inhibitory activity of carbohydrate hydrolyzing enzymes by *Dialium guineense*'.

	Ethanolic extract	Ethyl Acetate extract	Reference
Total Phenolics (µg/100 mg extract GAE)	43.89 ± 0.65^{a}	12.16 ± 0.27^{b}	--
Total Flavonoids (µg/100 mg extract QE)	3.59 ± 0.09^{a}	2.56 ± 0.39^{a}	--
DPPH Scavenging activity (IC_{50} µg/mL)	43.45 ± 1.14^{a}	108.45 ± 0.89^{b}	**Vit C:** 68.42 ± 0.51^{c}
FRAP value	3.01 ± 0.01^{a}	0.96 ± 0.05^{b}	**Vit C:** 2.00 ± 0.00^{c}
Alpha-Amylase inhibition (IC_{50} µg/mL)	17.15 ± 0.03^{a}	6.04 ± 0.03^{b}	**Acarbose:** 5.11 ± 0.06^{b}
Alpha-Glucosidase inhibition (IC_{50} µg/mL)	70.21 ± 1.55^{a}	28.81 ± 0.22^{b}	**Acarbose:** 5.26 ± 0.18^{c}

Note: Vit C= Vitamin C. Values with different superscript and in the same row are significantly different $p<0.05$. Values are presented as Mean ± SEM of three determinations.

Oxidative stress is implicated in several diseases; a condition characterised by increased levels of free radicals without a commensurate increase in endogenous antioxidants. It is known that oxidative stress is associated with most of the complications of diabetes. In diabetic individuals free radicals damage proteins, lipids and nucleic acids [31,32]. There is therefore the need for anti-diabetic medications to possess antioxidant capacity in addition to the other pharmacological benefits. In the present work, the antioxidant properties of the plant extracts of *Dialium guineense* leaf were evaluated in-vitro using the DPPH radical scavenging activity and FRAP assays. The antioxidant activity of the extracts in both assays is attributed to the donation of hydrogen or electrons to both DPPH radical and Fe^{3+} to attain a more stable electronic configuration. Interestingly, EthEx had significantly in-vitro antioxidant activity than EthAcEx and Vitamin C. This variation could be explained by the relative solubility/extraction of different phytochemicals by solvent of varying polarity [33]. Our results further reveal that the plant extracts are rich in phytochemicals such as phenols and flavonoids. Surprisingly, the EthEx had higher concentration of phenols and flavonoids than EthAcEx.

3.2. Gas Chromatography Mass Spectrometry analysis of leaf extracts of Dialium guineense

GC-MS fingerprinting of EthEx of *Dialium guineense* was performed in order to identify some of the phytoconstitutents that may be responsible for the activities of the plant extracts. The Gas chromatogram shown in **Figure 1** reveals twenty-nine peaks of bioactive compounds. See **Table 2** for details of the compounds identified by GC-MS. A more critical observation of the result shows that Pentadecane 2,6,10,14-tetramethyl, Octadecane, Eicosane, Heneicosane and Squalene were most prominent having an area greater than 3%.

Furthermore, the GC-MS analysis revealed the presence of bioactive compounds which are known to possess both nutritional and pharmacological potentials. These phytochemicals have been shown in previous studies to possess functional groups that confer antioxidant properties and protective effects against many human diseases. Put together, there is a positive correlation between *in-vitro* antioxidant activities of the plant extracts and its phytochemical composition.

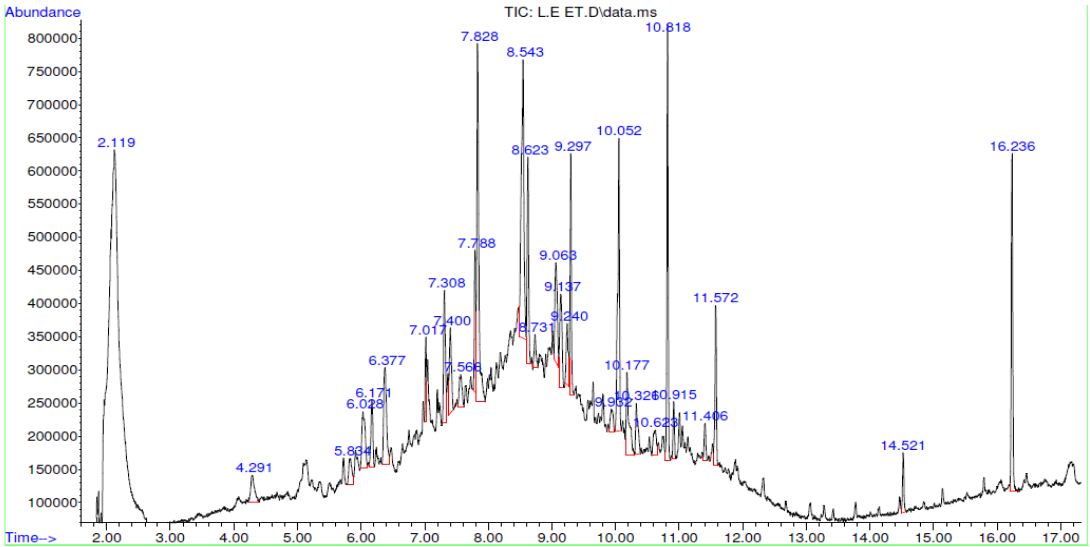

Figure 1. Gas chromatogram of *Dialium guineense* leaf extract.

Table 2. Phytocompounds in extracts of *Dialium guineense* identified by Gas Chromatography-Mass Spectrometry Analysis.

S.No.	Retention time (min)	Area (%)	Compound	Molecular weight (g/mol)	Molecular formula
1.	5.834	0.73	Cis-9-Tetradecenoic acid, isobutyl ester	282.5	$C_{18}H_{34}O_2$
2.	5.834	0.73	11,13-Dimethyl-12-tetradecen-1-ol acetate	282.5	$C_{18}H_{34}O_2$
3.	6.028	1.63	2-Hydroxy-3-methoxybenzaldehyde, 2 - methylpropyl ether	243.2	$C_{13}H_{13}N_3O_2$
4.	6.171	1.16	Carbonic acid, undecyl vinyl ester	242.3	$C_{14}H_{26}O_3$
5.	6.377	2.55	Hexa-2, 4-diyn-1-yl benzene	154,2	$C_{12}H_{10}$
6.	7.017	1.01	Hexadecane	226.44	$C_{16}H_{34}$
7.	7.400	1.55	Oxalic acid, 3,5-difluorophenyl tetradecyl ester	398.5	$C_{22}H_{32}F_2O_4$
8.	7.566	0.81	Pentadecafluorooctanoic acid, octadecyl ester	666.5	$C_{26}H_{37}F_{15}O_2$
9.	7.566	0.81	17-Pentatriacontene	490.9	$C_{35}H_{70}$
10.	7.566	0.81	2-Octadecyl-propane-1,3-diol	328.6	$C_{21}H_{44}O_2$
11.	7.788	1.76	Heptadecane	240.5	$C_{17}H_{36}$
12.	7.828	6.36	Pentadecane 2,6,10,14-tetramethyl	268.5	$C_{19}H_{40}$
13.	8.543	6.48	Octadecane	254	$C_{18}H_{38}$
14.	8.731	0.55	2-Hexenoic acid, methyl Ester	128.7	$C_7H_{12}O_2$
15.	8.731	0.55	5-Undecanol	172.3	$C_{11}H_{24}O$
16.	8.731	0.55	Oxirane-2-carboxylic acid, 2-amino carbonyl-3-methyl-3-(1-methylethyl)-ethyl ester	201.22	$C_9H_{15}NO_4$
17.	9.297	2.67	Nonadecane	278.5	$C_{19}H_{40}$
18.	9.932	0.58	9-Undecenal, 2,6,10-trimethyl	210.3	$C_{14}H_{26}O$
19.	10.052	4.69	Eicosane		$C_{20}H_{42}$
20.	10.177	2.04	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	324.8	$C_{16}H_{22}O_4$
21.	10.326	1.04	Phthalic acid, butyl undecyl ester	376.5	$C_{23}H_{36}O_4$
22.	10.623	0.73	2-methyl nanodecane	282.5	$C_{20}H_{42}$
23.	10.623	0.73	E-8-Methyl-9-tetradecen-1-ol acetate	268.2	$C_{17}H_{32}O_2$
24.	10.623	0.73	Octyldodecanol	298.5	$C_{20}H_{42}O$
25.	10.818	4.80	Heneicosane	296.6	$C_{21}H_{44}$
26.	11.572	2.11	Docosane	310.6	$C_{22}H_{46}$
27.	14.521	0.72	Di(2-ethylhexyl) phthalate	390.6	$C_{24}H_{38}O_4$
28.	14.521	0.72	Diisooctyl phthalate	390.6	$(C_8H_{17}COO)_2C_6H_4$
29.	16.236	4.34	Squalene	234.6	$(C_5H_8)_6$

3.3. Anti-hyperglycemic effect of leaf extracts of Dialium guineense in diabetic rats

Diabetes was induced by single intraperitoneal injection of Streptozotocin. Rats induced with diabetes had significantly higher Fasting Blood Glucose (FBG) by day 1 post induction compared to rats in the normal control group ($p<0.001$). However, treatment of the diabetic rats with either Glibenclamide or plant extracts for 28th caused a significantly decreased FBG compared to the negative control (diabetic control) ($p<0.001$); however, FBG of treatment groups were not significantly different from the normal control group (**Figure 2a**). Similarly, the glycemic variation which is a measure of the glucose surge over the 28-day period was significantly lower in the treatment groups

compared to the control as shown in **Figure 2b** ($p<0.001$). It appeared that the EthEx was more effective at managing hyperglycemia in the diabetic rats than the EthAcEx.

The control of postprandial blood glucose concentration is cardinal to the management of diabetes [34,35]. Postprandial glucose concentration increases due to the activities of carbohydrate hydrolyzing enzymes such as pancreatic alpha-amylase and intestinal alpha-glucosidase [36]. These enzymes release monosaccharides from poly/oligosaccharides thereby increasing the sugar levels in the blood after a meal. In diabetic patients, elevated postprandial glucose and an increased gluconeogenesis activity increase the risk of hyperglycemia and diabetic complications. In the present study, extracts of *Dialium guineense* inhibited the activities of both enzymes which imply that they could be effective at regulating postprandial blood glucose. Contrary to the *in vitro* antioxidant experiments, the EthAcEx had a higher inhibition of both hydrolytic enzymes. This loss of correlation between the antioxidant activity and inhibitory activity against enzyme may suggest that different bioactive compounds may be responsible for the aforementioned activities. The link between hyperglycemia and glycation of proteins in diabetics has been established by previous researches. Such protein modification is responsible for suboptimal functionality of proteins [37,38]. Although there exist inhibitors of alpha-amylase and alpha-glucosidase currently marketed as pharmaceutical products, these drugs are known to cause extreme inhibition thereby inhibiting gastrointestinal digestion of carbohydrates hence causing gastrointestinal discomfort [36, 39].

Hence, there is a need for discovery of new hypoglycemic drugs without side effects. The *in-vitro* hypoglycemic activity of the plant extracts was further confirmed *in-vivo* using Streptozotocin-induced glycemic-impaired diabetic rats. The plant extracts significantly countered hyperglycemia caused by Streptozotocin evidenced by a regulated glycemic variation in the diabetic rats.

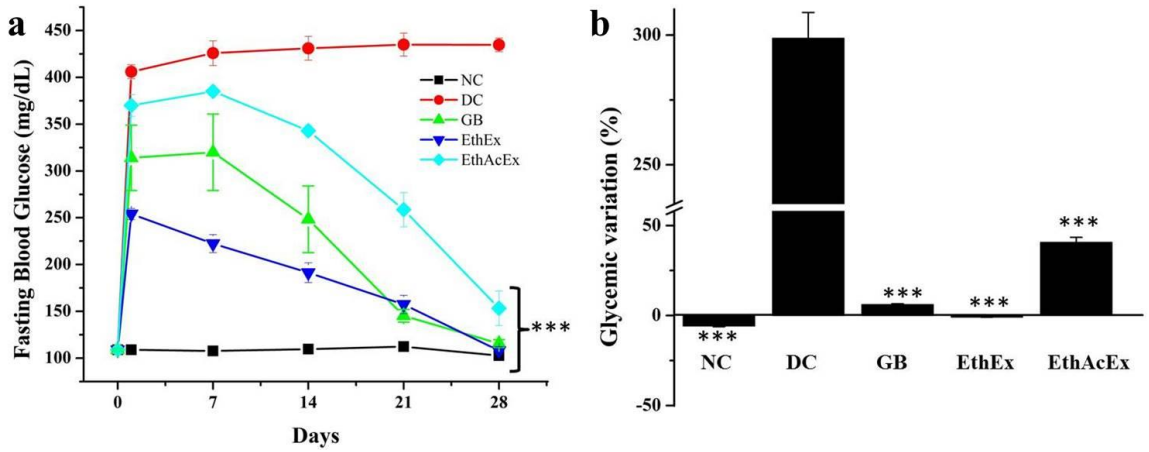

Figure 2. Anti-hyperglycemic effect of *Dialium guineense*.(a) Line graph showing the effect of *Dialium guineense* on Fasting Blood Glucose concentration and (b) Glycemic variability in diabetic rats after 28 days treatment. Values are presented as Mean±SEM (n=6). *p<0.05, **p<0.01 and ***p<0.001 significantly different compared to the Diabetic control group. NC= Normal control, DC= Diabetic control, GB= 5 mg/kg BW Glibenclamide, EthEx= Ethanol Extracts of *Dialium guineense* and EthAcEx= Ethyl Acetate Extracts of *Dialium guineense*.

3.4. Effects of leaf extracts of Dialium guineense on body weight of diabetic rats

Table 3 shows the effect *Dialium guineense* leaf extracts on weight of diabetic rats. Induction of diabetes caused a significant weight loss whereas the rats in the normal control group gained weight. The percentage weight change for each group revealed that treatment with either Glibenclamide or *Dialium guineense* leaf extracts significantly ameliorated weight loss in diabetic rats ($p<0.001$). EthAcEx was more effective at managing the weight loss than EthEx as shown in Table 3. Whereas induction of diabetes did not significantly affect the relative whole body weight to the weight of the heart and the liver, kidneys and pancreas was significantly affected. Diabetic rats had a significantly higher relative whole body weight to weight of the liver and pancreas but a significantly lower ratio for the kidneys. The extracts had no significant positive effect on the ratios for the liver, however, EthAcEx was effected on improving the ratio for the kidneys and pancreas ($p<0.001$).

Previous studies have provided evidence for weight loss as an important clinical symptom of diabetes [40,41].

Weight loss could be due to the inability of inability of insulin to drive glucose into cells hence leading to the breakdown of body fats and proteins for production of energy. In the present study, diabetic rats lost weight drastically however, administration of the plant extracts caused ameliorated the weight loss. The ameliorating effect of *Dialium guineense* extracts may be attributed to improved glucose production/utilisation and hence fat/protein sparing in the diabetic animals.

Table 3. Body weight and relative whole body to organ weights of diabetic rats treated with extracts of *Dialium guineense* leaf.

Group	Change in Body Weight (%)	Relative whole body to organ weight			
		Heart (%)	Liver (%)	Kidney (%)	Pancreas (%)
Normal control	14.24 ± 0.38***	0.26 ± 0.01	3.19 ± 0.07**	0.58 ± 0.01***	0.20 ± 0.00***
Diabetic control	-19.11 ± 2.43	0.35 ± 0.01	2.19 ± 0.09	1.29 ± 0.09	0.09 ± 0.00
5 mg/kg BW GB	-3.18 ± 0.74***	0.24 ± 0.00	3.55 ± 0.28***	0.59 ± 0.01***	0.25 ± 0.16***
200 mg/kg BW EthEx	-8.45 ± 1.08***	0.32 ± 0.01	2.61 ± 0.17	0.70 ± 0.03	0.15 ± 0.01*
200 mg/kg BW EthAcEx	-3.48 ± 0.93***	0.26 ± 0.12	2.51 ± 0.07	0.69 ± 0.02***	0.21 ± 0.01***

Values are presented as Mean±SEM (n=6). *p<0.05, **p<0.01 and ***p<0.001 significantly different compared to the Diabetic control group. GB= Glibenclamide; EthEx= Ethanol Extracts, and EthAcEx= Ethyl Acetate Extracts.

3.5. Effects of leaf extracts of Dialium guineense on lipid profile of diabetic rats

Lipid profile of diabetic rats treated with extracts of *Dialium guineense* leaf is presented in **Table 4**. Total cholesterol was significantly increased in diabetic rats as evidenced in the recorded 162.13±0.77 mg/dL in the diabetic control group compared to the normal control group 128.78±0.79 mg/dL. However, treatment with the plant extracts caused a significant reduction in the plasma concentration of total cholesterol ($p<0.001$). Similar trend was observed for Low Density Lipoprotein (LDL) levels in the experimental groups. High Density Lipoprotein (HDL) on the other hand drastically decreased consequent to induction of diabetes. Treatment with extracts of *Dialium guineense* initiated slight increases of HDL in diabetic rats which were significantly different at $p<0.05$. Triglycerides increased after induction of diabetes but were significantly suppressed in all treatment groups. The best efficacy of treatment was observed in the EthEx group.

Cardiovascular risk indices namely: atherogenic index (AI) and coronary artery index (CAI) were calculated from the values of lipid profile parameters in **Table 4**. The results reveal that both indices were significantly elevated in diabetic rats. Although CAI could not be significantly mitigated ($p>0.05$) by *Dialium guineense* extracts, AI was significantly reduced in all treatment groups ($p<0.001$).

Streptozotocin induces diabetes by destruction of pancreatic β-cells consequently there is a deficit of insulin secretion. In diabetic patients the activity of lipoprotein lipase is decreased, leading to hypertriglyceridemia [26]. Previous reports have also demonstrated increased activity of HMG CoA-reductase in diabetic animals and a corresponding increase in cholesterol synthesis [42,43]. These imbalances in lipid metabolism are evidenced by high levels of triglycerides and cholesterol and low levels of Low Density Lipoprotein (LDL) in diabetics.

Table 4. Lipid profile and cardiovascular risk indicators of diabetic rats treated with *Dialium guineense* leaf extract.

Group	Lipid Profile				Cardiovascular risk Indicators	
	TC (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)	AI	CAI
Normal control	128.78±0.79***	22.34±0.53***	89.24±0.89***	107.73±0.53***	4.76±0.08***	3.68±0.23**
Diabetic control	162.13±0.77	15.64±0.96	105.4±1.52	188.56±0.56	9.43±0.26	7.09±0.34
5 mg/kg BW GB	149.32±1.58***	17.52±0.49	100.97±0.82	172.68±0.98***	7.29±0.47***	5.43±1.31
200mg/kg BW EthEx	143.73±0.84***	18.35±0.22	101.47±1.04	137.06±1.09***	6.80±0.14***	5.42±0.22
200mg/kg BW EthAcEx	144.83±1.38***	18.86±0.82*	96.69±1.31***	160.56±0.47***	6.71±0.23***	5.02±0.44

Values are presented as Mean±SEM (n=6). *p<0.05, **p<0.01 and ***p<0.001 significantly different compared to the Diabetic control group. GB= Glibenclamide; EthEx= Ethanol Extracts, EthAcEx= Ethyl Acetate Extracts, HDL= High Density Lipoprotein, LDL= Low Density Lipoprotein, TG= Triglycerides, TC= Total Cholesterol, AI= Atherogenic Index and CAI= Coronary Artery Index.

It was observed in this present experiment that the plant extracts countered hyperlipidemia induced by Streptozotocin by decreasing the plasma concentration of total cholesterol, LDL and triglycerides and concomitant increase in High Density Lipoprotein (HDL). Hyperlipidemia in diabetes is a risk factor for cardiovascular disease. LDL in diabetics is oxidized and becomes cytotoxic to arterial tissues [44,45]. The plant extracts could have recovered the lipid profile due to its high content of phenolic compounds known to have antioxidant properties.

3.6. Effects of leaf extracts of Dialium guineense on oxidative stress parameters of diabetic rats

The activities of superoxide dismutase, catalase and concentrations of glutathione, malondialdehyde were determined in order to understand the redox mechanisms involved in the antidiabetic properties of *Dialium guineense* leaf extracts. The results are shown in **Figure 3**. Superoxide dismutase and catalase activities were negatively affected in diabetic rats as compared to rats in the normal control group.

However, treatment with either Glibenclamide or *Dialium guineense* leaf extracts significantly improved the activities of these enzymes (**Figure 3a** and **b**). The level of glutathione was decreased in diabetic rats, treatment with plant extract did not cause an increase that is statistically significant, but glibenclamide increased plasma glutathione levels ($p<0.01$; **Figure 3c**). Malondialdehyde concentration in the plasma increased in diabetic rats ($p<0.001$) but was significantly mitigated by administration of EthEx ($p<0.01$). Glibenclamide and EthAcEx treated groups had decreased malondialdehyde concentrations though not statistically significantly different from the diabetic control group (**Figure 3d**).

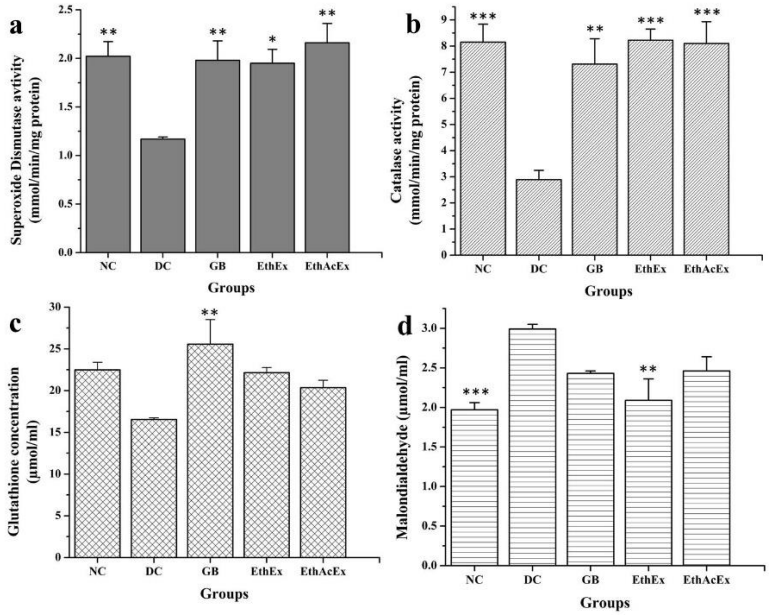

Figure 3. Effect of extracts of *Dialium guineense* on antioxidant enzymes and oxidative stress parameters in Streptozotocin induced diabetic rats. Bar graphs showing the effect of *Dialium guineense* on (a) Superoxide activity (b) Catalase activity (C) Glutathione concentration and (d) Malondialdehyde levels in Streptozotocin induced diabetic rats. Values are presented as Mean±SEM (n=6). *p<0.05, **p<0.01 and ***p<0.001 significantly different compared to the Diabetic control group. NC= Normal control, DC= Diabetic control, GB= 5 mg/kg BW Glibenclamide, EthEx= Ethanol Extracts of *Dalium guineense* and EthAcEx= Ethyl Acetate Extracts of *Dalium guineense*.

The underlying mechanism of action of Streptozotocin involves an increased generation of superoxide anion and hydroxyl radicals that induces tissue damage and lipid peroxidation [46]. In addition, hyperglycemia leads to increased mitochondrial activity and oxidative stress. Literature abounds to sufficiently demonstrate disruption of antioxidant defense mechanisms in diabetics [47-48]. Frontline antioxidant enzyme activities such as superoxide dismutase (SOD), catalase and glutathione peroxidase are decreased whereas the levels of lipid peroxides and other products of oxidative damage increases. This scenario mirrors the results in this report; however, treatment with the plant extracts reversed the narrative. The increase of SOD, Catalase and glutathione in diabetic rats treated with *Dialium guineense* extracts may reflect the antioxidant property of the plant or the ability to stimulate synthesis of these antioxidant systems. These antioxidant systems when synthesized rid the body of oxidative assaults from free radicals. Flavonoids are reported to have the ability to increase the activity of SOD, catalase and the concentration of glutathione [49]. Other phytochemicals have also been reported to reduce the levels of lipid peroxides capable of tissue damage and several pathologies.

Collectively, the present study demonstrated that *Dialium guineense* leaf extracts has hypoglycemic and anti-hyperlipidaemic effects as well as antioxidant activity *in-vitro* and *in-vivo* which could be attributed to it high phenolic and flavonoid contents. Thus, this plant could be considered for the development of a therapy for diabetes and its complications.

4. Conclusion

In conclusion, the present study utilised *in-vitro* and *in-vivo* approaches to provide evidence for the anti-diabetic potential of *Dialium guineense* extracts. It further assessed the ability of the plant extracts in ameliorating some complications of diabetes using the Streptozotocin model of diabetes. Our results reveal that *Dialium guineense* extracts are effective against diabetes and complications such as hyperlipidemia, weight loss and oxidative stress. These findings raise awareness of the possibility of developing a new therapy for diabetes from this plant.

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Conflict of interest

The authors declare to have no conflict of interest.

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