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Antihyperglycaemic, Antihyperlipidemic and Antioxidant Activities of Root Extract and Fractions af *Hippocratea africana*

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ABSTRACT

Hippocratea africana is a medicinal plant whose roots are employed for the treatment of diabetes mellitus traditionally. The root extract (200-600 mg/kg) and fractions; n-hexane, dichloromethane (DCM), ethyl acetate and methanol (400 mg/kg) of *H. africana* were assessed for blood glucose and lipids lowering activities, and pancreas protective potentials in alloxan-induced diabetic rats. The fasting Blood Glucose (FBG) level was monitored using glucometer, determination of serum insulin and lipids levels and histopathological evaluation of the pancreas were carried out using standard methods. Evaluation of *in vitro* antioxidant activity of root extract and fractions was carried out using ferric reducing power assay (FRAP), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and nitric oxide (NO) scavenging assay. Extract/fraction treatment of diabetic rats lead to significant ($p<0.05$) lowering in FBG of treated diabetic rats in single and repeated dosing studies with *n*-hexane fraction exhibiting the highest effect. The extract/fractions also exhibited significant ($p<0.01$) elevation of serum insulin levels accompanied with decreased glycosylated hemoglobin (HbA1c) levels. The extract and fractions further lowered serum total cholesterol, triglycerides, low density lipoprotein (LDL)-cholesterol, very low density lipoprotein (VLDL)-cholesterol, and increased high density lipoprotein (HDL)–cholesterol level in the diabetic rats. Pancreas histology depicted fewer pathological signs in the treated diabetic rats relative to untreated diabetic rats. Root extract and fractions exhibited antioxidant activity in all assays with ethyl acetate fraction exhibiting the highest antioxidant activity. The findings suggest that the root extract and fractions of *H. africana* possess anti-hyperglycemic, insulin secretion stimulatory, antihyperlipidemic, pancreas protective and antioxidant properties.

Keywords: Antidiabetic, Hypolipidemic, Hypoglycaemic, Diabetes, Insulin

Introduction

Diabetes mellitus (DM) remains a life-threatening metabolic disease prevalent worldwide with social and economic impact.[1] World Health Organization (WHO) reported that abnormal high blood glucose level and associated complications were responsible for millions of deaths yearly ranking diabetes 8th among the killer diseases worldwide.[1] The situation is worrisome as projected prevalence of diabetes by 2045 will rise to 700 million worldwide,[2] therefore necessitating an urgent step to remedy the situation. Depending solely on conventional drug for the management of DM seems challenging to the patients economically with associated side effects, therefore the search for affordable and safe alternative drugs becomes inevitable. Medicinal plants or herbal products are used across most countries of the world in the management of diabetes.

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In most countries, herbal preparations are preferable as remedies for diabetes management because of many biological activities, relative safety, low cost and accessibility compared to conventional anti-diabetic drugs.[3] The antidiabetic effect of some of these plants has not been ascertained and also their mechanism of action. This was the focus of this research on *Hippocratea africana*, one of such plants used traditionally in diabetes management.

Hippocratea africana (Willd.) Loes. ex Engl. (Celastraceae) known in English as African paddle-pod and Eba enang enang' in Ibibio language in Nigeria, is a climber perennial plant distributed widely in tropical Africa.[4] Traditionally, the plant root has been variously utilized in herbal preparations to treat diseases like malaria and diabetes as well as liver diseases[5]. Preliminary report by Okokon *et al.*,[6] showed that the root extract possess antidiabetic and hypolipidemic activities. Previous works on the plant include; antimalarial,[5] antioedema and antinociceptive,[7] antidiarrhoeal and antiulcer,[8] hepatoprotective,[9] antileishmanial, cytotoxicity and cellular antioxidant,[10] antibacterial, anticonvulsant and depressant.[11] Also, earlier studies had reported the presence of $^{\delta}$-3-Carene and α-terpineol,[12] isolation of 1,3,7-trihydroxy-6-methoxyxanthone [isoathyrio] and 1,3,6,7-tetrahydroxyxanthone [norathyriol][13] from ethyl acetate fraction. Monoterpenes and sesquiterpenes have been identified in the *n*-hexane fraction.[9] Antioxidant as well as glucose and lipid lowering effects of the root extract and fractions of *H. africana* in alloxan-induced diabetic rats are reported in this study.

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Materials and Methods

Plants collection

The plant material *Hippocratea africana* (roots) was collected in August 2020 at Nyaan forest in Uruan area, Akwa Ibom State, Nigeria. The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Hebarium specimen UUPHB 30(i) was deposited at Department of Pharmacognosy and Natural Medicine Herbarium.

Preparation of extract and fractions

The shade dried and pulverised root of *H. africana* (HAE) (1.5 kg) was macerated in 7.0 L of ethanol (50%) for 72 h. The filtrate obtained was concentrated in a rotary evaporator at 40˚C. The crude extract (10 g) was successively partitioned further into each of n-hexane, dichloromethane, ethyl acetate and *n*-butanol to obtain their respective fractions. The extract and fractions were stored in a refrigerator until used for the experiment.

Animals

In this study, male Wistar rats were used. The animals were sourced from University of Uyo Animal house and housed in plastic cages. The rats were fed with pelleted standard Feed (Guinea feed), with free access to drinking water. The study was approved by College of Health Sciences Animal Ethics Committee, University of Uyo (UU/CHS/AE/20/103).

Induction of diabetes using alloxan monohydrate

Fifty-six male Wistar rats were fasted for 24 h, weighed and injected with 150 mg/kg of alloxan monohydrate freshly prepared in normal saline (150 mg/kg,i.p).[14] The hypoglycaemia initially induced by alloxan was subdued by oral administration of 5% dextrose solution (2 mL). Diabetes was allowed to develop in the animals after induction with alloxan for 3 days during which the animals had free access to food and water. Rats with FBG of 200 mg/dL and above after 72 hours were selected and included in the experiments.[15]

The rats considered diabetic and selected for the study were assigned to different groups (n=6) before treatment commenced on the first day (day 0). Suitable dose regimens were determined using previously determined median lethal dose (LD_{50}) value of 2450 mg/kg,[5] and the rats were treated as follows; 10 mL/kg/day of normal saline was given to animals in group 1, while glibenclamide 10 mg/kg/day was administered to group 2 rats, *H. africana* root extract at doses of 200, 400 and 600 mg/kg/day were respectively administered to rats in groups 3-5, while animals in groups 6 - 9 were given n-hexane, dichloromethane, ethyl acetate and n-butanol fractions respectively at a dose of 400 mg/kg/day. All treatments were done orally daily for 14 days.

Effect of root extract and fractions of H. africana on FBG of alloxan-induced diabetic rat

On day 1 of treatment, monitoring of FBG of the treated diabetic rats was done at 1, 2, 3, 5- and 7 hours interval for single dose study and subsequently at 1, 2, 3, 5, 7- and 14-days interval on repeated root extract and fractions administration for 14 days in rat's tail blood using glucometer.[14] All the treatments were carried out daily before 8.00 am for 14 days and FBG was measured after an overnight fasting with food withdrawal the previous evening.

Effect of H. africana extract/fractions on body weights of the diabetic rats

The diabetic animals were weighed before and after diabetes induction and at the end of the study (14 days) to determine the effect of the treatment on their body weight.

Blood samples and organs collection

After the treatment period of 14 days, the animals were anaesthetized using light diethyl ether vapor and sacrificed. Collection of blood samples from each rat was done by cardiac puncture into plain bottles which were centrifuged at room temperature to separate the serum. Biochemical assays (lipid profile, insulin and glycosylated hemoglobin levels) were carried out on these sera samples. Pancreas of each diabetic rat was surgically removed, weighed and processed for histological evaluation.

Effect of root extract/fractions on insulin and glycosylated hemoglobin levels in the diabetic rats

The ELISA kit (Alpco Diagnostics) was used to determine serum insulin levels of the diabetic rats.[16] Briefly, different wells were prepared for standards in a Microelisa stripplate using standard dilution buffer to obtain different concentrations; 60, 40, 20, 10 and 5 ng/mL, respectively. Sample dilution buffer and samples were added (dilution factor is 5) and loaded onto the well bottom, mixed, shaken gently and incubated for 30 min at 37℃. After incubation, plates were washed gently, aspirated and refilled with the wash solution. HRP-Conjugate reagent was added to each well except the blank control well and incubated at room temperature. 50 µL Chromogen Solution A and 50 µL Chromogen Solution B were added to each well, mixed with shaken gently and incubated at 37℃ for 15 minutes. 50 µL stop solution was added to each well to terminate the reaction. The colour in the well changed from blue to yellow. Absorbance was read at 450 nm using a Microtiter Plate Reader.[16]

The method of Nathan *et al.*[17] was employed in the determination of glycosylated hemoglobin levels of the rats. Briefly, a 1.5 µL of blood sample was collected with the integrated sampling device by touching the surface of the blood drop until it filled the capillary end. Later, the sampling device was inserted into the Affinion Analyser system. The sample was automatically diluted and mixed with a buffer that lyses the erythrocytes and precipitates the haemoglobin. The analyser evaluated the precipitation on the membrane. The ratio between the glycated haemoglobin and the total haemoglobin intensities was proportional to the percentage HbA1c displayed on the Analyzer screen.[17]

Effect of the root extract and fractions on the lipid profile of the diabetic rats

Triglyceride (TG), total cholesterol (TC), and high-density lipoprotein (HDL) were determined using Randox diagnostics kits, United Kingdom, according to the manufacturer's protocol.[18] The formula of Friedwald *et al*.,[19] was used to estimate the levels of low and very low-density lipoprotein (LDL and VLDL).

In vitro antioxidant activity of the root extract and fractions

Nitric oxide (NO) scavenging assay

Nitric oxide generated from sodium nitroprusside (SNP) was measured according to the modified method of Awah and Verla.[20] Three milliters (3 mL) of SNP in phosphate buffered saline (pH 7.4) was added to 2 mL of different concentrations of the root fractions and ascorbic acid (20, 40, 60, 80, and 100 µg/mL) the resulting solutions was then incubated at 25^{o}c for 60 minutes. A similar procedure was repeated with methanol as blank, which served as control. To 3 mL of the incubated sample, 5 mL of Griess reagent (1% sulphonamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride) was added. The absorbance of chromophore (purple azo dye) that were formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with naphthyl ethylenediamine dihydrochloride was measured at 540 nm. The assays were carried out in triplicates and the results were expressed as mean values ± standard deviations,

Ferric Reducing Power assay (FRAP)

This was determined using the method of Oyaizu.[21] Various concentrations 20, 40, 60, 80, and 100 µg/mL of the *H. africana* root fractions and ascorbic acid (1 mL) was mixed with 1 mL of 1% potassium ferricyanide. The mixture was incubated at 50^{o}c for 20 minutes. After which 1 mL of 10% trichloroacetic acid was added and the resulting mixture centrifuged at 650 rpm for 10 minutes. The mixture (4 mL) was then mixed with 4 mL of de-ionised water and 1 mL of 0.1% ferric chloride and the absorbance was measured at 700 nm. A similar procedure was repeated with methanol as blank, which served as control. Higher absorbance indicates higher reducing power. The assays were carried out in triplicates and the results were expressed as mean values ± standard deviations.

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Determination of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH free radical scavenging potential of the root fractions of *Hippocratea africana* and ascorbic acid prepared in methanol at concentrations of 20, 40, 60, 80 and 100 µg/mL were evaluated according to the method of Nabavi et al.[22] 1mL of DPPH was added to 3 mL of the solutions prepared with the root fractions (ethyl acetate, n-hexane, n-butanol, and dichloromethane) and ascorbic acid and stirred for 1 minute. Each mixture was kept in the dark for 30 minutes and the absorbance (A_s) was measured at 517 nm. The assays were carried out in triplicates and the results expressed as mean values ± standard deviations. Lower absorbance of the reaction mixture indicated higher free radical activity. A similar procedure was repeated with methanol as blank, which served as control. The percent DPPH scavenging effect was calculated using the following equations:

DPPH scavenging effect (%) or Percent inhibition= $[(A_0 – A_s) / A_0] \times 100$.

Where A_0 is the absorbance of control reaction and A_s is the absorbance in the presence of test or standard sample (ascorbic acid).

Statistical analysis

Results from this study were statistically analyzed using Graph pad prism software Inc. La Jolla, CA, USA. One - way ANOVA followed by Tukey-Kramer multiple comparison test were used to establish the significant difference in the various groups. Values were presented as mean ± SEM and significance difference relative to control were considered at $p< 0.05$.

Results and Discussion

Changes in body weights of diabetic rats

The root extract and fractions affected the body weights of the diabetic animals as was seen on the weights gain of the treated and untreated diabetic rats (Table 1). The body weights of the diabetic rats were dose-dependently and significantly ($p<0.05$-0.001) improved due to treatment with the root extract and fractions when compared to control. Percentage increases of 6.74 and 9.64 were recorded for highest dose (600 mg/kg) and ethyl acetate fraction respectively (Table 1). The treatments further produced significant ($p<0.001$) decreases in pancreas weights of the diabetic rats compared to control with dichloromethane fraction exerting the highest reduction (Table 1).

Effect of single dose of H. africana root extract/fractions on FBG of diabetic rats

Pronounced lowering of FBG of diabetic rats was observed 1hour post treatment with the root extract and fractions. At 5 h and 7h, the reductions were non-dose dependent and significant ($p<0.05$-0.001). At 7 h all the treated groups had their FBG lowered significantly ($p<0.001$) and the groups treated with *n*-hexane and dichloromethane fractions had the most significant ($p<0.001$) reduced FBG levels. The activity exhibited by *n*-hexane fraction was significant ($p<0.001$) relative to control and better compared to that of glibenclamide (7 h) (Table 2).

Table 1: Effect of ethanol root extract and fractions of *H. africana* on body and pancreas weights of alloxan- induced diabetic rats.

Treatment	Dose (mg/kg)	Weight (g)			Weights of pancreas (g)
		Day 0	Day 15	% Increase	
Normal saline	10mL/kg	154.60 ± 11.18	148.30 ± 14.60	-4.07	0.71 ± 0.03
Glibenclamide	10	147.30 ± 0.85	157.68 ±11.20	7.04	0.44 ± 0.01[a]
Crude extract	200	150.60 ±19.78	159.20 ± 22.10	5.71	0.51 ± 0.10[a]
	400	159.00 ± 4.41	168.5 ± 31.20	5.97	0.52 ± 0.05[a]
	600	159.03 ± 4.32	169.75 ± 31.20	6.74	0.50 ± 0.01[a]
n- hexane fraction	400	144.96 ± 1.81	157.00 ± 24.61	8.30	0.50 ± 0.02[a]
Dichloromethane fraction	400	145.26 ± 4.67	151.25 ±10.84	4.12	0.53 ± 0.01[a]
Ethyl acetate fraction	400	144.40 ± 8.50	158.33 ±16.02	9.64	0.54 ± 0.04[a]
n-butanol fraction	400	152.46 ± 12.10	159.33 ±15.16	4.50	0.58 ± 0.03[a]

Data are expressed as MEAN ± SEM, Significant at [a]$p< 0.001$, when compared to control. (n = 6).

Table 2: Antidiabetic effect of ethanol root extract and fractions of *H. africana* on blood glucose level of alloxan- induced diabetic rats during acute study

Treatment	Dose	Blood glucose level mg/dL in hours					
	(mg/kg)	0 HR	1 HR	2 HR	3 HR	5 HR	7 HR
Normal saline	10 mL/kg	341.7 ± 13.87	397.0 ± 16.01	387.0 ± 5.50	345.3 ± 21.16	338.7 ± 55.48	336.2 ± 10.68
Glibenclamide	10	360.3 ± 10.33	373.3 ± 12.01	261.7 ± 30.68	198.6 ± 31.89	182.0 ± 40.15 [a]	182.5 ± 14.64[c]
Crude extract	200	344.0 ± 23.72	328.3 ± 11.28	273.0 ± 52.04	227.3 ± 17.90	201.6 ± 38.26 [a]	205.0 ± 12.68 [c]
	400	350.0 ± 66.10	353.0 ± 28.36	307.6 ± 49.49	263.3 ± 33.22	204.0 ± 19.75	196.0 ± 15.19 [c]
	600	369.3 ± 86.24	369.3 ± 86.24	280.3 ± 88.46	276.0 ± 87.06	225.0 ± 18.68	214.7 ± 15.61[c]
n-hexane fraction	400	347.0 ± 59.18	287.0 ± 59.18	239.3 ± 42.83	154.0 ± 25.63	168.0 ± 12.85[b]	173.5 ± 14.23[c]
DCM fraction	400	350.7 ± 58.04	284.3 ± 82.56	298.3 ± 81.94	229.6 ± 28.35	197.3 ± 9.77[a]	196.7 ± 13.40[c]
Ethylacetate fraction	400	343.3 ± 58.33	207.0 ± 64.45	228.0 ± 31.89	207.6 ± 17.14	210.6 ± 20.20	205.2 ± 28.20 [c]
n-butanol fraction	400	302.5 ± 12.10	306.3 ± 67.83	306.3 ± 67.83	210.7 ± 20.34	204.3 ± 18.12	206.7 ± 24.74 [c]

Data are expressed as MEAN ± SEM, Significant at [a]$p<0.05$, [b]$p < 0.01$, [c]$p< 0.001$, when compared to control. (n = 6).

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Effect of repeated administration of root extract and fractions of H. africana on FBG of diabetic rats

Repeated administration of root extract and fractions of *H. africana* to diabetic rats lowered FBG levels significantly ($p < 0.05$-0.001) in the diabetic rats from day 3 to day 14, which was comparable to the activity of glibenclamide. On day 14, all the treated groups had their FBG reduced significantly ($p<0.001$) with the high dose (600 mg/kg), n-hexane and ethyl acetate fractions exhibiting highest effects (Table 3).

Antiglycation and effect of H. africana root extract and fractions on serum insulin level of diabetic rats

The root extract and fractions significantly ($p<0.05$-0.01) elevated insulin levels in serum of treated diabetic rats when compared to control. These dose-dependent increases were higher in high dose (600 mg/kg) and n-hexane fraction-treated groups (Figure 1). The level of serum insulin in high extract dose (600 mg/kg) treated group was higher than that of glibenclamide. Significant ($p<0.001$) lowering of HbA1c levels in root extract/fractions-treated groups compared to untreated group was observed. The root extract exerted a non-dose-dependent effect with the highest lowering effect recorded in *n*-butanol fraction-treated group followed by ethyl acetate fraction group (Figure 2).

Effect of H. africana root extract and fractions on lipid profile parameters of diabetic rats

Treatment of diabetic rats with *H. africana* root extract and fractions did not affect the total cholesterol levels of treated and untreated rats significantly ($p>0.05$). However, statistically significant ($p<0.05$-0.001) lowering of serum triglyceride, LDL and VLDL levels of treated rats relative to the negative control was observed. The HDL level was significantly ($p<0.05$-0.001) elevated in glibenclamide, extract (200-600 mg/kg) and all the groups treated with fractions (Table 4).

Effect of root extract and fractions of H. africana on pancreas histology of diabetic rats

Pancreas histologic sections of rats in control group treated with normal saline (10 mL/kg) only at magnification (X400) depicted areas of severe degeneration and degranulation of islets cells, β- and α- cells as well as reduced pleomorphic prominent nuclei areas. Animals groups treated with glibenclamide (10 mg/kg), root extract (200-600 mg/kg) and fractions had pancreas with visible absence or reductions of these abnormal features (Figure 3).

Table 3: Antidiabetic effect of ethanol root extract and fractions of *H. africana* on blood glucose level of alloxan-induced diabetic rats during prolonged study

Treatment	Dose (mg/kg)	Blood glucose level mg/dL in days					
		0 DAY	1st DAY	3RD DAY	5TH DAY	7TH DAY	14TH DAY
Normal saline	10mL/kg	341.7±13.87	300.0±21.05	298.3±5.17	206.3±27.84	196.7±14.78	132.0±4.33
Glibenclamide	10	360.3±10.33	308.0±12.14	187.3±16.25^{c}	102.6±5.89^{b}	90.7±9.87^{c}	80.7±2.02^{c}
Crude extract	200	344.0±23.72	350.0±22.93	204.3±10.58^{c}	94.3±12.83^{c}	89.3±6.17^{c}	91.3±2.96^{c}
	400	350.0±66.10	220.7±23.33	201.7±17.05^{c}	125.0±12.66^{a}	83.7±9.52^{c}	92.0±1.52^{c}
	600	369.3±86.24	290.2±20.19	199.7±6.56^{c}	141.0±3.50^{a}	96.0±7.00^{c}	84.3±2.96^{c}
n -hexane fraction	400	347.0±59.18	308.0±18.41	168.0±1.56^{c}	68.3±2.33^{c}	97.3±7.96^{c}	85.7±4.70^{c}
DCM fraction	400	350.7±58.04	310.5±25.94	105.0±2.51^{c}	62.0±4.35^{c}	137.3±8.16^{a}	88.0±2.08^{c}
Ethylacetate fraction	400	343.3±58.33	260.0±23.33	96.0±1.00^{c}	78.7±6.88^{c}	87.7±6.48^{c}	84.7±3.66^{c}
n-butanol fraction	400	302.5±12.10	350.0±19.56	178.0±15.53^{c}	168.3±7.91^{a}	132.7±6.34^{a}	99.0±2.08^{b}

Data is expressed as MEAN ± SEM, Significant at $^{a}p<0.05$, $^{b}p< 0.01$, $^{c}p< 0.001$, when compared to control. (n=6).

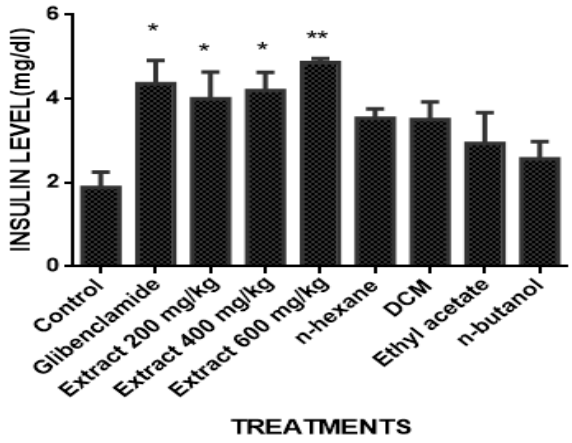

Figure 1: Effect of *H. africana* root extract and fractions on insulin levels of diabetic rats. Data is expressed as MEAN ± SEM. Significant at $^{*}p<0.05$, $^{**}p< 0.01$, $^{***}p< 0.001$, when compared to control. (n = 6).

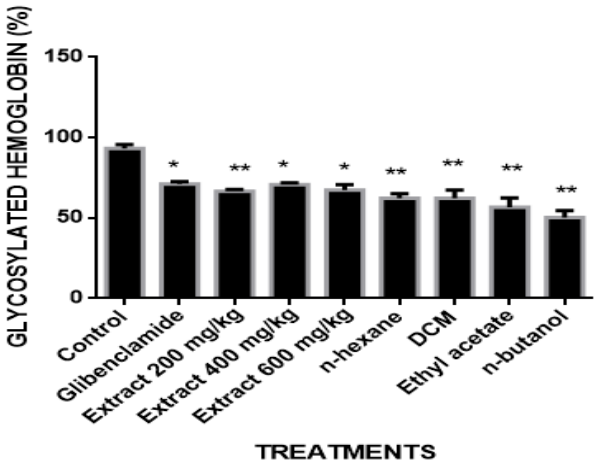

Figure 2: Effect of *H. africana* root extract and fractions on glycosylated haemoglobin concentration of diabetic rats. Data is expressed as MEAN ± SEM. Significant at $^{*}p<0.05$, $^{**}p< 0.01$, $^{***}p< 0.001$, when compared to control. (n = 6).

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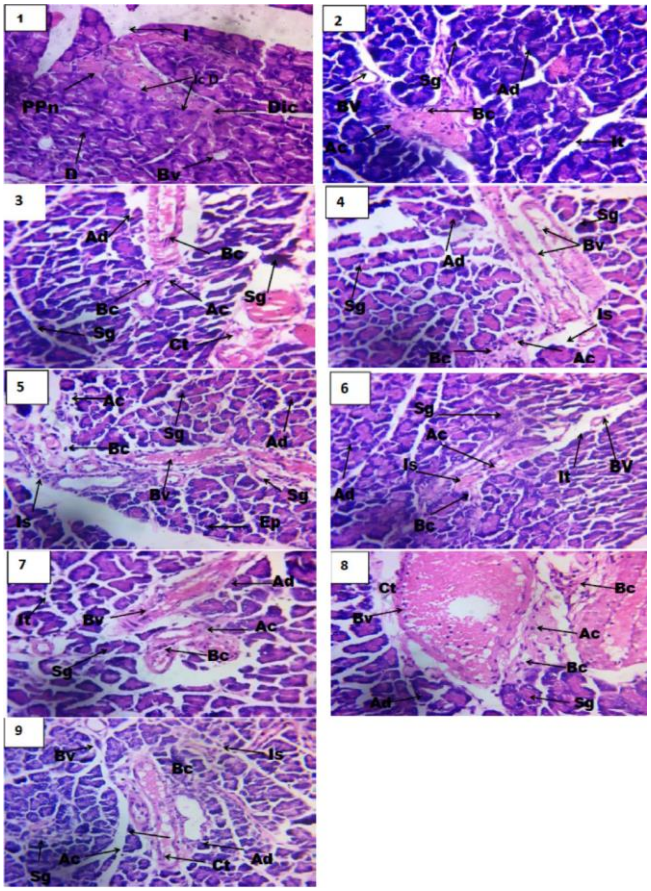
Table 4 :Effect of root extract and fractions of *H. africana* on lipid profile of alloxan-induced diabetic rats

Treatment	Dose mg/kg	Total cholesterol (mMol/L)	Triglyceride (mMol/L)	HDL-C (mMol/L)	LDL-C (mMol/L)	VLDL (mMol/L)
Control	10 mL/kg	4.25 ± 0.12	3.40 ± 0.74	1.56 ± 0.04	0.89 ± 0.01	0.68 ± 0.02
Glibenclamide	10	3.51 ± 0.33	1.28 ± 0.16[b]	2.26 ± 0.03[a]	0.81 ± 0.01[a]	0.22 ± 0.01[c]
Crude extract	200	3.45 ± 0.81	2.15 ± 0.64	2.14 ± 0.01[a]	0.72 ± 0.01[c]	0.28 ± 0.01[c]
	400	3.66 ± 0.22	2.04 ± 0.16	2.19 ± 0.02	0.64 ± 0.02[c]	0.22 ± 0.01[c]
	600	3.50 ± 0.36	1.86 ± 0.11[a]	2.35 ± 0.0 [c]	0.70 ± 0.01[c]	0.30 ± 0.01 [c]
n-hexane fraction	400	3.88 ± 0.12	1.05 ± 0.06[c]	2.30 ± 0.12[c]	0.63 ± 0.05 [c]	0.20 ± 0.01[c]
DCM fraction	400	3.90 ± 0.42	1.12 ± 0.08[b]	2.68 ± 0.20[c]	0.69 ± 0.02 [a]	0.25 ± 0.02[c]
Ethyl acetate fraction	400	3.36 ± 0.72	1.04 ± 0.10[c]	2.78 ± 0.12[c]	0.64 ± 0.02 [c]	0.21 ± 0.01[c]
n-butanol fraction	400	3.48 ± 0.38	1.28 ± 0.16[b]	2.88 ± 0.14[c]	0.79 ± 0.01	0.23 ± 0.01[c]

Data is expressed as mean ± SEM, Significant at [a]p<0.05, [b]p< 0.01, [c]p< 0.001, when compared to control, (n=6).



Figures 3: Histological sections of Pancreas of alloxan-induced diabetic rats.
Treated with Normal saline (Control) 10 mL/kg (1), Glibenclamide 10 mg/kg bw (2),extract 200 mg/kg bw (3) extract 400 mg/kg bw (4), extract 600 mg/kg bw (5), n-hexane fraction 400 mg/kg bw (6), dichloromethane fraction 400 mg/kg bw (7), ethyl acetate fraction 400 mg/kg bw (8), n-butanol fraction 400 mg/kg bw (9) at Magnification B (x400), stained with H&E Method. Keys: Endocrine portion (Ep), Ducts (D), Interstitium (It), Islet cells degeneration(Icd), Endocrine protein (Ep), Blood vessel (BV), Pleumorphic Prominent Nucleus (PPn), Ducts (D), Islet cells of langerhans (Is), Alpha cell (Ac), Beta cell (Bc), Adipocytes (Ad), Connective tissue (Ct), Acini Duct (AD), Serous gland (Sg).

In vitro antioxidant activity
Nitric oxide (NO) scavenging assay
Generation of nitric oxide was suppressed by root extract and fractions of *H. africana* as compared to the standard (ascorbic acid), with the highest effect exhibited by ethyl acetate fraction followed by the *n*-butanol fraction and crude extract, as shown in Figure 4.

Ferric reducing power assay (FRAP)
The extract and fractions were evaluated based on their ability to reduce Fe^{3+} to Fe^{2+}. The extract and fractions showed concentration dependent and significant (p<0.05-0.001) increases in their reducing power when compared to control. These increases were lower when compared to that of ascorbic acid, which is the standard drug. (Figure 5).

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity
The extract and fractions inhibited DPPH radical activity through hydrogen donation concentration-dependently with highest activity exhibited by ethyl acetate (77.10%) when compared with the standard (ascorbic acid) as shown in Figure 6.
The study evaluated the *in vitro* antioxidant, antihyperglycemic and antihyperlipidemic potentials of root extract and fractions of *H. africana* in alloxan-induced diabetic rats and also explored their possible antidiabetic mechanism of action.
DM is a metabolic disorder with high level of free radicals produced,[23] In experimentally-induced diabetes with alloxan, dialuric acid formed from biotransformation of alloxan induces high level of free radicals leading to partial damaged of pancreatic cells, hyperglycemia and reduced insulin production.[23]
The study demonstrated that *H. africana* root extract and fractions exhibited sustained significant lowering of FBG during single dose and repeated doses study with the fractions; ethyl acetate, n-hexane and dichloromethane exerting high activities. This finding corroborates that of Okokon et al.[6], validating the local claims and confirming strongly the hypoglycemic potentials of this root as employed in ethnomedicine.
DM limits the various glucose metabolic and utilization processes thereby complicating the disease condition. The extract/fractions ability to lower FBG of diabetic rats following treatment, suggests that the root extract and fractions potentials to stimulate insulin release as seen in this study in addition to alpha amylase and alpha glucosidase inhibitory effect previously reported on the root extract and fraction.[24]
Glycosylated hemoglobin is an index of glucose level regulation and control as its formation is irreversible.[25] The significant (p<0.05) lowering of HbA1c level in this study indicates a controlled regulation of the blood glucose level especially in animals treated with the extract (600 mg/kg) and n-butanol fraction as observed in this study.

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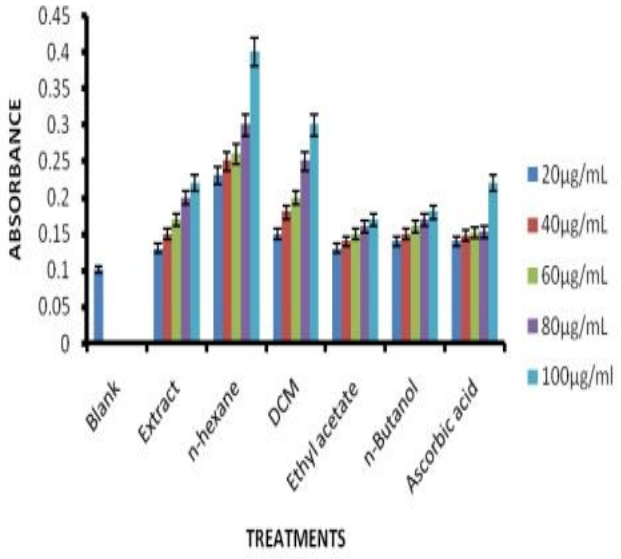

Figure 4: Nitric oxide (NO) scavenging assay at 540nm

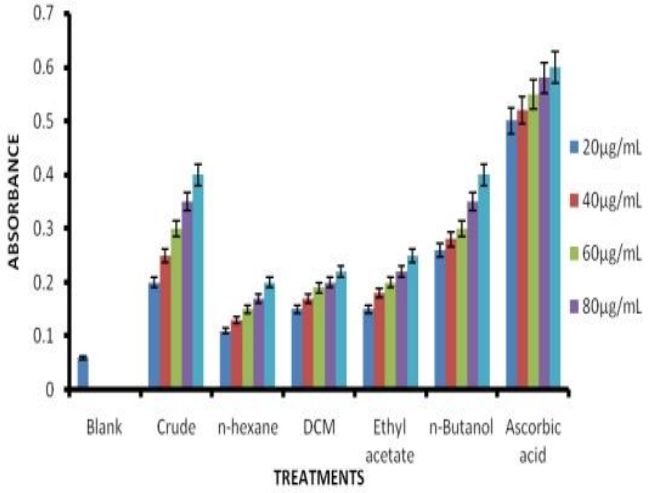

Figure 5: Reducing Power Assay at 700 nm

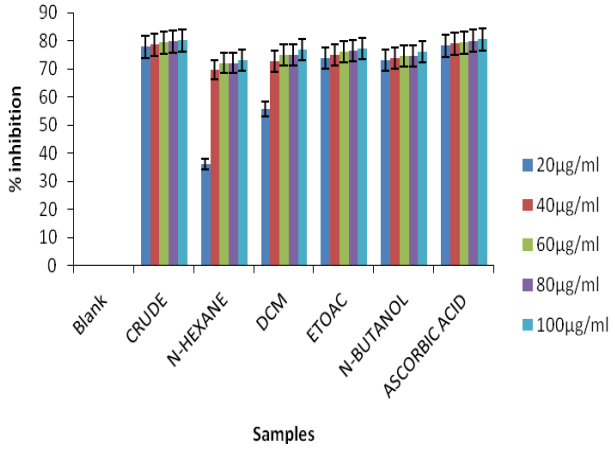

Figure 6: DPPH Radical scavenging activity of *H. africana* root extract and fractions

However, elevated Hb1Ac levels were seen in untreated diabetic rats with corresponding increased plasma glucose levels. This indicates that insulin secretion stimulation maybe one of the modes of blood glucose lowering potential of root extract and fractions resulting in reduced FBG and HbA1c values.

Phytochemical compounds have been reported in the root extract, hexane, dichloromethane and ethyl acetate fractions.[5,9,11,12] The phytoconstituents in these extract/fractions may in part be accountable for the antidiabetic activities observed in this study. Plants rich in terpenes (monoterpenes and sesquiterpenes) and xanthones as found in the root extract and fractions understudy have previously been documented to exhibit blood glucose level lowering effects[26-29] via some mechanisms such as insulin secretion stimulation and glucose uptake, and inhibition of α-glucosidase[30] and the blood glucose lowering effect observed can be attributed to their presence in these fractions.

Serum levels of insulin were found to be significantly increased in extract/fractions-treated groups especially in *n*-hexane and DCM fractions-treated groups which corroborate the pancreas protective potentials of these fractions as observed which is likely to be responsible for the elevated insulin level of these groups. This result suggests that the root extract/fractions have insulin secretion stimulatory effect and pancreas protective potentials in diabetic rats.

In diabetes, glucose underutilization and insulin inhibition of lipase cause a rise in the levels of circulatory cholesterol and other lipids, leading to cardiovascular system complications common in diabetics.[31] Lowered serum levels of TG, LDL-cholesterol and VLDL-chlolesterol as well as increased the level of HDL –cholesterol were observed in the treated diabetic rats. This is likely due to lowered blood glucose levels by the root extract which could have suppressed fats mobilization. These findings corroborate earlier report by Okokon *et al.*[6]

The root extract and fractions were found to increase body weights of diabetic rats. This corroborates the findings of Aloke et al.[32] who reported that degradation of structural proteins during diabetes often leads to reduced body weight. This was remedied by the extract/fraction treatment probably resulting from lowering of blood glucose and protein synthesis stimulation. This may also have resulted from reversal of the weight loss in extract- treated groups via cessation of proteolysis, gluconeogenesis, and glycogenolysis. [31]

The destructive effect of alloxan on pancreas was observed on the untreated diabetic rats with prominent areas of degeneration and degranulation of cells. These were, however, suppressed by the root extract/fractions treatment of the diabetic rats. Thus, suggesting protective effect of the extract leading to the observed increased insulin level and reduced glucose level, thereby implicating phytoconstituents of extract and fractions in this action. This may have been through the extract potentials to protect and cause regeneration of the *β*-cells of the pancreas or increase cellularity of the islet tissue and regeneration of the granules in the β-cells.[33]

The extract and fractions were found to exert antioxidant effect with ethyl acetate fraction exerting the highest scavenging activity. This result corroborates the findings of Okokon *et al.*[9] who reported improved activities of antioxidative stress marker enzymes in rats following treatment with the root extract and cellular antioxidant activities of the root extract and fractions.[11] Also, Umoh *et al.*,[13] had reported prominent antioxidant activity of the xanthones isolated from ethyl acetate fraction of the root, further supporting the antioxidant activity of the root extract and fractions which may be due to the presence of phytochemical compounds earlier reported on this plant. This activity explains the pancreas protective property of the root extract against generated free radicals by alloxan and consequently, its antidiabetic activity.

However, this study could not quantify, isolate nor identify compounds responsible for the observed activities in the fractions. Also, standardization of the extract/fractions was not carried out for effective and safe use of the plant. These constitute the limitations of this study which necessitate further study to be carried out on the root extract.

Conclusion

The findings of this investigation revealed that the root extract and fractions of *Hippocratea africana* possess antihyperglycemic, hypolipidemic, pancreas protective and antioxidant potentials which may be attributed to the activities of its phytochemical constituents.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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