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Anti-Diabetic Properties of the Root Extracts of *Salacia nitida* Benth on Alloxan Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author CIZ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CIZ and HDK managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This study was carried out to evaluate the anti-diabetic properties of root extracts of *Salacia nitida* in alloxan-induced diabetic rats.
Study Design: Experimental Animal Study.
Place and Duration of Study: Department of Pharmacology, University of Port Harcourt,Rivers State from July 2016 to February 2017.
Methodology: The study investigated the dose-dependent changes in the blood glucose levels, body weight, serum lipid profile (Total Cholesterol (TC), Total glycerides (TG), Low Density Lipoproteins (LDL) and High Density Lipoproteins (HDL), liver function (serum; Alanine Amino Transferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Total Bilirubin, Conjugated Bilirubin, Total Proteins and Albumin) and renal function (serum; creatinine and urea). The method of successive extraction was used, making use of the solvents n-hexane,

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dichloromethane, ethyl acetate, methanol and water in order of polarity to extract the root fractions to be utilised for the research. A qualitative phytochemical analysis making use of standardized methods was performed. Acute toxicity was evaluated using the Lorke's method. The anti-diabetic study was evaluated in twenty-one days, comprising two phases: induction phase and treatment phase. Fifty- five rats for the experiment were divided into eleven sets of five rats each.

Results: These results indicate the presence of chemical constituents; alkaloids, saponins, flavonoids, tannins and carbohydrates in the extracts. LD50 value was determined to be more than 5000 mg/kg, which indicates that it is safe. The extracts showed hypoglycemic activity which was evidenced by improving the body imbalance in lipid metabolism experienced during diabetes, restoring body weight to near normal, lowering blood glucose levels, restoring protein levels to near normal, decreased liver glycogen levels, and decreasing albumin, bilirubin, urea and creatinine levels.

Conclusion: This study indicated that the root extracts of *Salacia nitida* showed anti-diabetic properties in alloxan-induced diabetic rats. Thus, the extracts of *S. nitida* will be an inordinate addition to ethnomedicine in the management of diabetes and its complications.

Keywords: Alloxan; Salacia nitida; diabetes; blood glucose; lipid profile.

1. INTRODUCTION

The World Health Organization [1] has defined diabetes mellitus as a chronic hyper-glycaemic condition with unsettling disturbances in the metabolism of carbohydrate, fat and protein accompanied by elevated glucose production. It can be regarded as a disorder of metabolism arising from different causes associated with different insulin secretion abnormalities, and/or action[1].Mention must be made of diabetes as part of the killer diseases that are waging war against the survival, growth, and advancement of human beings globally, because it is a pandemic malady, which can prompt serious complications [2].

Type 2 diabetes is progressively turning into a noteworthy ceaseless disease health burden in Africa. In 2011, about 14 million individuals were evaluated to have diabetes in Africa, and this is projected to ascend to 28 million by 2030[3]. Whether it is T1 or T2 diabetes, those affected are at greater risk of developing complications, thus the need to shield patients from the principal underlying feature, which is persistent hyperglycaemia. Microvascular complications can be defined as retinopathy, which requires photocoagulation, vitreous haemorrhage, lethal or non-lethal renal failure[4]. Apart from fasting hyper-glycaemia, a few organs develop complications in diabetes. The conditions and main organs affected by diabetes are; glycogenosis and dextrinosis and steatohepatitis (liver), atherosclerosis and microangiopathy (blood vessels), retinopathy (eyes), nephropathy (kidneys), neuropathy (cerebrum and peripheral nerves) [5-7]. These harmful effects are isolated into microvascular complications like in the kidneys, nerves and eye [8] and macrovascular in the blood vessels.

Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 5, 6-deoxy uracil) an acceptable diabetogenic agent for inducing type 2 diabetes in animals [9]. A urea derivative which affects the pancreatic islet beta cells by causing a selective necrosis of it [10].

Even though orthodox drugs which have been accepted worldwide for the management of diabetes are readily accessible, statistics show that a high percentage of these patients' glycemic level have not been maintained in line with the treatment goals as endorsed by international diabetic associations [11-12]. These agents such as sulphonylurea and biguanide are related with extreme adverse effects; lactic acidosis, hypoglycaemia [13]. Therefore, the use of trado-medicine as an adjunct is fast gaining significance in the treatment of the disease. Nevertheless, alternatives to the already existing synthesized drugs are now sought for worldwide, as a result of the perceived cost-effectiveness, assumed lesser toxicity, and minimal or no reported side effects unlike the clinically approved drugs which are used for diabetes mellitus treatment [14]. Reports have shown that most plants used for diabetes management contains alkaloids, carotenoids, flavonoids, glycosides, terpenoids, and many others, that are as often as possible thought to have anti-diabetic impact [15] and free from adverse reactions than the manufactured medicines[14]. Therapeutic plants have been accounted to have hypoglycemic actions, which enhance insulin secretion, glucose uptake by adipose tissues. They also enhance the production of glucose in the liver and its absorption from the intestine [16].

An example of such plants is *Sarcopoterium spinosum* [17]. Another is *Salacia nitida* Benth.

Salacia nitida is a genus of plants in the Celastraceae family. It is a woody climbing shrub distributed in Sri- Lanka, South-West India, Thailand, Philippines, Java [18] South Africa and Southeastern part of Nigeria. In the southern part of Nigeria amongst the Ogonis, a decoction of roots *of Salacia nitida* serve as useful antimalarial agent [19]. The present study investigated the dose-dependent changes in the blood glucose levels, body weight, serum lipid profile (TC, TG, LDL and HDL), liver function (serum; ALT, AST, ALP, Total Bilirubin, Conjugated Bilirubin, Total Proteins and Albumin) and renal function (serum; creatinine and urea) in normal and alloxan-induced diabetic rats for a period of 3 weeks of the root extracts of *Salacia nitida*.

2. MATERIALS AND METHODS

2.1 Plant Material

The fresh root of *Salacia nitida* plant used in this study was obtained from a local area in Omuokiri of Aluu, Rivers, Nigeria. The plant was identified and validated. The herbarium number is UPHCO288. The roots were washed and reduced to smaller sizes and air–dried to a constant weight and then ground into powder using a grinder machine (manufactured from Indore).

2.2 Preparation of Extracts

About 1850 g of the powdered root was sequentially extracted using extraction solvent by maceration for three (3) days. The extraction was done with five (5) different solvents in their increasing order of polarity. The solvents used include n-hexane, dichloromethane, ethyl-acetate, methanol and water with regular shaking at room temperature (28°C). Each time, the plant material was filtered through Whatmann filter paper number: 45 then dried and later extracted with each of the five solvents. Each of the extracts was then carefully evaporated to dryness over a water bath under controlled temperature, afterwards was stored in the refrigerator and then used for further experimental study.

2.3 Animals

Fifty five (55) Adult Wistar rats males and females, were weighed (100-250 g). They were obtained from the Faculty of Pharmaceutical Sciences, animal house of the University of Port Harcourt and were kept in the animal house of the Faculty at room temperature and relative humidity and a 12:12 h light/ dark cycle for one week. The wood shavings (sawdust) were changed daily and the cages washed and cleaned each other day. They were allowed to become familiar with laboratory conditions for 7 days before the start of the study. The endorsement of the Animal Ethical Committee of University of Port Harcourt was sought before the start of the analyses. Every one of the protocols and investigations were led in consistency with the ethical standards and rules.

2.4 Phytochemical Evaluation

Standard method for phytochemical screening were carried out to determine the presence of Alkaloids, saponins, flavonoids, triterpenoids, tannins and reducing sugars [20,21,22,23].

2.5 Acute Toxicity Study

Acute toxicity was carried out on the experimental animals making use of method of Lorke [24]. It was carried out to determine the safety profile of the plant.

2.6 Anti-diabetic Activity

2.6.1 Experimental Induction of diabetes

Induction of diabetes in the animals was carried out after an overnight fast (12 hours) by an intraperitoneal injection of alloxan at 150 mg kg^{-1}b.wt. Calculations were done and then it was solubilized using 100ml of saline solution before injection [25]. After an hour of the administration, the animals were fed *ad libitum*and 5% dextrose solution was given to them in feeding bottles for 24 hours to overcome the early hypoglycaemic stage. The animals were then watched for 72 hours after which blood samples were collected by the clipping of the tail and blood glucose levels then evaluated. A group was chosen randomly to represent the control group, which received saline alone. The rats whose glucose level >150 mg/dl were separated into groups of 5 rats each for further experimental study [26,27].

2.7 Experimental Design

Eleven groups of animals containing five rats each was divided as follows;

Group I: Saline (untreated/ normal control)
Group II: Hyperglycaemic rats- Diabetic control (Normal saline)
Group III: Hyperglycaemic rats + Glibenclamide (10 mg/kg/day p.o. once daily)
Group IV: Hyperglycaemic rats + dichloromethane extract of *Salacia nitida* root ;(250 mg/kg/b.wtp.o once daily)
Group V: Hyperglycaemic rats + dichloromethane e extract of *Salacia nitida* root; (500 mg/kg/b.wtp.o once daily)
Group VI: Hyperglycemic rats + ethyl acetate extract of *Salacia nitida* root (250 mg/kg/b.wtp.o once daily)
Group VII: Hyperglycemic rats + ethyl acetate extract of *Salacia nitida* root (500 mg/kg/b.wtp.o once daily)
Group VIII: Hyperglycemic rats + methanol extract of *Salacia nitida* root (250 mg/kg/b.wtp.o once daily)
Group IX: Hyperglycemic rats + methanol extract of *Salacia nitida* root (500 mg/kg/b.at p.o once daily
Group X: Hyperglycemic rats + aqueous extract of *Salacia nitida* root (250 mg/kg/b.wtp.o once daily)
Group XI: Hyperglycemic rats + aqueous extract of *Salacia nitida* root (500 mg/kg/b.wtp.o once daily)

The diabetic rats' BGLs were monitored by making small incisions on their tail and collecting blood into the available test-kit (Accu-Check Active Test Meter, Roche Diagnostics). The animals were weighed on day 0, 3, 7, 14 and 21 after 1h of treatment with the extracts and the standard anti-diabetic drug (Glibenclamide); making use of the weighing balance (GmbH, Mannheim, Germany). The drug and extracts administration was carried out for a period of 21 days using orogastric tube.

2.8 Determination of Biochemical Parameters

On day 21 of treatment with *S. nitida* extracts, the animals were sacrificed under ether anaesthesia, blood samples were then collected. The Biochemical Parameters Evaluated were Low Density Lipoproteins (LDL), High Density Lipoproteins (HDL), Triglycerides, Urea, Creatinine, Aspartate Aminotransferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Albumin, Bilirubin and total proteins.Serum urea was determined using the procedure of [28] and creatinine determined using the procedure of [29] Serum aspartate and alanine aminotransferase (AST and ALT) were evaluated making use of the method of [30]. [31] method was used to evaluate alkaline phosphate (ALP) level, while albumin level was evaluated using the BCG Assay kit [32]. The TC, TG, HDL-C and LDL-C levels were evaluated in the serum of the animals using standard procedures [33].

2.9 Statistical Analysis

Statistical analysis of results was done using SPSS version 20.0. Results were presented as Mean±Standard Error of Mean. ANOVA followed by the LSD (Least Significant Difference), were used to determine significant differences across the groups. The differences in the mean values of the groups were the criteria for the pharmacological activities. The difference in means was considered significant at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

Preliminary phytochemical screening indicated the presence of alkaloids (except in dichloromethane and ethyl acetate extracts), saponins, flavonoids and tannins.

3.2 Acute Toxicity Studies

The acute toxicity study in rats produced no death or signs of toxicity even at high dose (5000 mg/kg body weight). Thus, the LD_{50} value was found to be 5000 mg/kg.

3.3 Antidiabetic Activity

Table 3 and Table 4 shows the effect of oral administration of the root extracts of *S.nitida* and glibenclamide on body weight, Table 5 and 6 on blood glucose level respectively of alloxan-induced diabetic rats for the period of the study. There was a significant ($p<0.05$) decrease in the body weight and also hyperglycaemia on day 3 in the alloxan-induced diabetic rats when compared to the normal rats. On administration of the extracts and glibenclamide, there was a significant ($p<0.05$) increase in the body weight from day 7 with a maximal increase achieved on day 21 by the 500 mg dose of ethyl acetate. Similarly, the 500 mg dose of aqueous extract caused a maximal reduction in blood glucose level by day 21.

Table 1. Phytochemical screening results

Crude extract test	Dichloromethane	Ethyl acetate	Methanol	Aqueous
Alkaloids	—	—	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Carbohydrates	+	+	+	+
Reducing sugars	—	+	+	+
Anthraquinones	+	—	—	—

+ = present; — = absent

3.4 Effect of the Root Extracts on the Body Weight

Table 3 and Table 4 shows the effect of oral administration of the root extracts of *S.nitida* and glibenclamide on body weight.

3.5 Change in Body Weight

Table 4 shows that at the end of the study period, administration of the methanol 250 mg and ethyl acetate 500 mg extracts had the most significant increase in body weight.

3.6 Effect of the Root extracts on Blood Glucose Level

Table 5 and 6 showed that at the end of the study period, the Aqueous extract 500mg and Methanol 250mg extracts caused a decrease in blood glucose levels which can compared to the standard drug (glibenclamide).

3.7 Effects of the Root Extracts on the Lipid Function Tests

Lipid profile: the analysis of various lipid parameters are seen in Table 7, it shows the effect of oral administration of the root extracts of *S.nitida* and glibenclamide on the lipid function tests [total cholesterol (TC), total glycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL)] of alloxan-induced diabetic rats for the period of the study. The TC, TG, and LDL-C levels significantly decreased ($p<0.05$) while there was a significant ($p<0.05$) increase in the HDL-C levels following the treatment of the rats with the extracts. The administration of the aqueous extracts produced significant effect relative to the untreated group for the serum TG and TC levels

3.8 Effects of the Root Extracts on Liver Function Tests

There was an observable elevation of the liver enzymes AST, ALT and ALP, which was markedly noticed in the untreated rats and diabetic rats before the extracts administration; however, following administration of the aqueous extracts, the liver enzymes level significantly ($p<0.05$) decreased. On administration of the extracts aqueous (500 mg & 250 mg), methanol (500 mg & 250 mg) and dichloromethane (250 mg), there was a significant ($p<0:05$) decrease in the Total bilirubin (T.B) levels when compared to the groups that were untreated with the most significant effect seen with the aqueous extracts. There was also a significant ($p<0:05$) decrease in the Conjugated bilirubin (CB) levels on the administration of the dichloromethane 500 mg. The Total protein (TP) levels on the administration of the extract ethyl acetate 500mg showed a significant ($p<0:05$) increase compared to the groups that were untreated. There was no significant change seen on the administration of the extracts on Albumin levels relative to the untreated group.

Table 2. Response and effect on the rats treated with various doses of the extract of the root of *Salacia nitida*

Dose (mg/kg)	No of rats	Survival	Death
10	3	3	0
100	3	3	0
1000	3	3	0
1600	3	3	0
2900	3	3	0
5000	3	3	0

Table 3. Effect of the root extracts on the body weight

Treatment groups (/kg daily)	Day 0	Day 3	Day7	Day 14	Day 21
Normal	227.30±1.22	226.63±1.84	240.50±3.18	249.57±1.44	251.33±3.38
Diabetic Control	116.10±1.19	111.75±1.03	110.75±1.49	108.75±1.93	107.18±2.82
Glibenclamide10 mg	169.10±12.79	124.14±6.58[a]	141.30±12.00[a]	156.86±15.48[a]	157.40±17.10[a]
Aqueous extract 500 mg	100.00±0.00	83.33±8.82[a]	103.33±3.33[a]	113.33±8.82[a]	116.67±6.67[a]
Aqueous extract 250 mg	146.67±13.33	110.00±20.82[a]	116.67±20.28[a]	120.00±17.32[a]	120.00±17.32[a]
Methanol extract 500 mg	150.00±0.00	133.33±8.82[a]	143.33±6.67[a]	146.66±13.33[a]	146.67±13.33[a]
Methanol extract 250 mg	107.50±4.79	107.50±7.50[a]	117.50±2.50[a]	127.50±2.50[a]	140.00±9.13[a]
Dichloromethane extract 500 mg	134.87±6.69	135.47±13.83[a]	136.70±5.59[a]	150.93±8.48[a]	145.53±2.21[a]
Dichloromethane extract 250 mg	163.45±6.47	143.98±12.18[a]	148.78±8.71[a]	164.78±10.04[a]	156.78±3.44[a]
Ethyl acetate extract 500 mg	131.40±9.90	130.05±5.35[a]	122.15±2.05[a]	164.40±14.70[a]	184.95±5.05[a]
Ethyl acetate extract 250 mg	170.35±20.25	140.50±0.50[a]	146.60±1.50[a]	128.85±8.55[a]	129.15±8.45[a]

Data represented as Mean± SEM (Standard error of mean) (n=5)[a] Significant values when compared with the diabetic control p<0.05(ANOVA)

Table 4. Effect of oral administration of the root extracts *S. nitida* and glibenclamide on body weight

	Body Weight (gm)	
Treatment groups (/kg daily)	**Initial**	**Final**
Normal	227.30±1.22	242.01.±2.46
Diabetic Control	116.10±1.19	109.60±1.86
Glibenclamide10mg	169.10±12.79	144.93±12.79[a]
Aqueous extract 500 mg	100.00±0.00	104.17±6.91[a]
Aqueous extract 250 mg	146.67±13.33	116.67±18.94[a]
Methanol extract 500 mg	150.00±0.00	142.50±10.54[a]
Methanol extract 250 mg	107.50±4.79	123.13±5.41[a]
Dichloromethane extract 500 mg	134.87±6.69	142.16±7.53[a]
Dichloromethane extract 250 mg	163.45±6.47	153.58±8.59[a]
Ethyl acetate extract 500 mg	131.40±9.90	150.39±6.79[a]
Ethyl acetate extract 250 mg	170.35±20.25	136.28±4.75[a]

Data represented as Mean± SEM (Standard error of mean) (n=5)[a] Significant values when compared with the diabetic control p<0.05(ANOVA)

Table 5. Effect of oral administration of the root extracts of *S. nitida* and glibenclamide on Blood Glucose Level (BGL)

Treatment groups(/kg body weight daily)	Day 0)(mg/dl)	Day3(mg/dl)	Day7(mg/dl)	Day 14(mg/dl)	Day 21(mg/dl)
Normal	97.00±2.08	90.00±34.12	87.67±2.03	79.33±1.45	103.33±2.40
Diabetic Control	66.25±8.05	299.50±23.67	350.00±16.63	363.75±14.14	480.25±39.06
Glibenclamide10 mg	84.60±9.14	768.40±111.60[a]	709.80±109.34[a]	452.40±54.81[a]	348.40±68.95[a]
Aqueous extract 500 mg	54.00±4.51	627.00±131.87[a]	509.00±38.89[a]	457.33±72.22[a]	317.00±16.09[a]
Aqueous extract 250 mg	71.67±4.06	481.33±24.90[a]	467.67±19.10[a]	500.67±16.91[a]	441.33±35.47[a]
Methanol extract 500 mg	57.67±4.48	497.00±205.58[a]	280.67±115.57[a]	303.33±99.35[a]	300.33±103.64[a]
Methanol extract 250 mg	60.25±8.72	655.25±132.91[a]	348.75±81.92[a]	455.50±155.32[a]	349.00±78.04[a]
Dichloromethane extract 500 mg	76.33±9.13	414.67±17.90[a]	337.67±14.05[a]	516.33±41.25[a]	328.00±44.41[a]
Dichloromethane extract 250 mg	74.50±6.36	594.25±98.89[a]	574.25±108.92[a]	549.00±110.36[a]	460.00±47.21[a]
Ethyl acetate extract500 mg	99.00±1.00	401.50±53.50[a]	655.00±225.00[a]	306.00±171.00[a]	277.50±157.50[a]
Ethyl acetate extract 250 mg	99.00±1.00	653.00±227.00[a]	404.00±73.00[a]	418.50±33.50[a]	388.50±58.50[a]

Data represented as Mean± SEM (Standard Error of Mean) (n=5) significant values when compared with diabetic control p<0.05(ANOVA)

Table 6. Change in Blood Glucose Level (BGL)

Treatment groups(/kg body weight daily)	Day3	Day21	%MAX.RED
Diabetic Control	299.50±23.67	480.25±39.06	-1.81
Glibenclamide10 mg	768.40±111.60[a]	348.40±68.95[a]	4.20
Aqueous extract 500 mg	627.00±131.87[a]	317.00±16.09[a]	3.10
Aqueous extract 250 mg	481.33±24.90[a]	441.33±35.47[a]	0.40
Methanol extract 500 mg	497.00±205.58[a]	300.33±103.64[a]	1.97
Methanol extract 250 mg	655.25±132.91[a]	349.00±78.04[a]	3.06
Dichloromethane extract 500 mg	414.67±17.90[a]	328.00±44.41[a]	0.86
Dichloromethane extract 250 mg	594.25±98.89[a]	460.00±47.21[a]	1.34
Ethyl acetate extract 500 mg	401.50±53.50[a]	277.50±157.50[a]	1.24
Ethyl acetate extract 250 mg	653.00±227.00[a]	388.50±58.50[a]	2.65

Data represented as Mean± SEM (Standard Error of Mean) (n=5) significant values when compared with diabetic control p<0.05(ANOVA)

Table 7. Effect of oral administration of the root extracts of *S. nitida* and glibenclamide on the lipid function tests

Group(/kg body weight daily)	TC	TG	HDL	LDL
Normal	1.73±0.09	1.04±0.12	0.56±0.02	0.67±0.09
Diabetic Control	2.38±0.13	1.22±0.10	0.85±0.05	0.95±0.14
Glibenclamide10 mg	2.26±0.09	1.19±0.08	0.83±0.06	0.88±0.11
Aqueous 500 mg	1.97±0.03[a]	0.68±0.04[a]	0.64±0.04	1.03±0.03
Aqueous 250 mg	1.80±0.06[a]	0.65±0.03a	0.50±0.04	1.03±0.09
Methanol 500 mg	1.83±0.07[a]	0.90±0.17[a]	0.60±0.05	0.83±0.07[a]
Methanol 250 mg	2.10±0.07[a]	0.84±0.03[a]	0.66±0.04	1.05±0.03
Dichloromethane 500 mg	2.17±0.35[a]	1.53±0.35	0.68±0.12	0.83±0.13[a]
Dichloromethane 250 mg	2.10±0.16[a]	1.19±0.08[a]	0.51±0.08	0.98±0.13
Ethyl acetate 500 mg	2.40±0.10	1.71±0.41	0.72±0.10[a]	0.90±0.20[a]
Ethyl acetate 250 mg	3.00±0.40	1.16±0.10[a]	0.80±0.12[a]	1.50±0.10

Data represented as Mean± SEM (Standard error of the mean) (n=5)[a] Significant values when compared with diabetic control p<0.05(ANOVA).

Table 8. Effect of oral administration of the root extracts of *S. nitida* and glibenclamide on the liver function tests

Group(/kg body weight daily)	AST	ALT	ALP
Normal	195.67±1.67	45.67±0.88	234.33±3.84
Diabetic Control	238.00±16.72	59.25±6.42	780.00±159.58
Glibenclamide10 mg	220.00±13.24	59.40±5.96	594.60±84.92
Aqueous extract 500 mg	56.33±23.92[a]	49.33±23.17[a]	751.33±60.88[a]
Aqueous extract 250 mg	25.00±20.50[a]	19.00±17.50[a]	431.67±168.38[a]
Methanol extract 500 mg	74.67±41.70[a]	29.00±13.32[a]	414.67±141.24[a]
Methanol extract 250 mg	75.25±14.83[a]	50.75±15.12[a]	352.25±92.70[a]
Dichloromethane extract 500 mg	291.00±67.31	106.00±36.67	1191.33±362.21
Dichloromethane extract 250 mg	242.75±66.77	229.25±74.58	1831.75±340.97
Ethyl acetate extract 500 mg	211.50±71.50[a]	261.00±177.00	1584.50±307.50
Ethyl acetate extract 250 mg	173.50±0.50[a]	54.00±4.00[a]	490.50±117.50[a]

Data represented as Mean± SEM (Standard error of the mean)(n=5) Significant values when compared with diabetic control p<0.05(ANOVA).

Table 9. Effect of oral administration of the root extracts of *S. nitida* and glibenclamideon the liver function tests

Group(/kg body weight daily)	TB	CB	TP	ALB
Normal	4.30±0.10	0.73±0.09	71.67±2.19	35.00±3.21
Diabetic Control	4.10±0.18	0.98±0.15	77.25±1.38	34.25±1.25
Glibenclamide10 mg	4.06±0.18	0.84±0.05	74.60±2.48	32.80±0.97
Aqueous extract 500 mg	2.87±0.18[a]	1.67±0.09	70.00±4.73[a]	30.67±0.67
Aqueous extract 250 mg	2.20±0.31[a]	1.73±0.09	69.67±6.17[a]	28.67±2.33
Methanol extract 500 mg	2.97±0.22[a]	2.57±0.03	72.67±1.33[a]	28.00±0.57
Methanol extract 250 mg	3.43±0.27[a]	2.70±0.11	70.25±1.11[a]	29.25±0.48
Dichloromethane extract 500 mg	4.73±0.37	0.63±0.29[a]	74.67±3.93[a]	32.67±2.17
Dichloromethane extract 250 mg	4.08±0.19[a]	0.98±0.09	74.75±3.09[a]	34.00±2.08
Ethyl acetate extract 500 mg	4.10±0.20	0.95±0.15	82.00±1.00[a]	31.00±1.00
Ethyl acetate extract 250 mg	4.30±0.30	1.05±0.05	70.50±1.50[a]	33.00±1.00

Data represented as Mean± SEM (Standard error of the mean) (n=5)[a] Significant values when compared with diabetic control p<0.05(ANOVA).

3.9 Effects of the root extracts on kidney function tests

Table 10 shows the effect of oral administration of the root extracts of *S.nitida* and glibenclamide on the kidney function tests (urea, creatinine) of alloxan-induced diabetic rats for the period of the study. It was observed that for the untreated diabetic rats there were elevated levels of serum urea, and creatinine but on the administration of the aqueous and methanol extracts there was significant($p<0.05$) decrease in the creatinine levels.

4. DISCUSSION

Diabetes mellitus management has globally remained despite the easy accessibility to synthetic agents and insulin. There are presently now quite some medicinal plants which are promising alternatives to conventional drugs and may be utilised in the treatment of this endless ailment because of their anti-glycaemic activity and minimal side effects. One of such plants is Salacia nitida. The preliminary phytochemical screening of the root extracts of *Salacia nitida* indicated that flavonoids, saponins, reducing sugars (except in the dichloromethane extract) and tannins were present. These compounds from studies have been reported to possess various biological actions and functions [34-36]. This finding was in agreement with studies in literature [37-39].

The World Health Organization has advocated for the determination of the safety profile of medicinal plants to standardise their use [40]. This prompted the acute toxicity study of the root extracts of *Salacia nitida, and* it was discovered to be greater than 5000 mg/kg body weight as no animal died. This finding suggests a high safety profile of *S. nitida* root extracts which makes it an excellent choice for use in the management of chronic diseases such as diabetes mellitus. This finding is similar to a study by [39].

Table 10. Effect of oral administration of the root extracts of *S. nitida* and glibenclamideon the kidney function tests

Group(/kg body weight daily)	Urea	Creatinine
Normal	7.50±0.23	220.33±0.88
Diabetic Control	5.05±1.09	235.75±109.78
Glibenclamide10 mg	6.62±0.55	144.20±19.64
Aqueous extract 500 mg	9.60±1.49	52.00±4.00
Aqueous extract 250 mg	15.17±2.10	65.33±8.35
Methanol extract 500 mg	6.53±0.59	50.33±7.69
Methanol extract 250 mg	7.95±1.66	56.00±3.94
Dichloromethane extract 500 mg	12.33±2.07	285.00±85.59[a]
Dichloromethane extract 250 mg	14.15±1.44	199.00±55.02
Ethyl acetate extract 500 mg	12.40±2.80	162.00±45.00
Ethyl acetate extract 250 mg	9.20±4.50	132.00±44.00

Data represented as Mean± SEM (Standard error of the mean)(n=5)[a] Significant values when compared with diabetic control p<0.05(ANOVA).

In the present study, on day 3, the blood sugar level in the diabetic rats after induction of diabetes by alloxan was observed to rise significantly. Alloxan brings about a diminution in insulin secretion by the destruction of ß-cells of the islets of Langerhans, and a substantial increase in the cytosolic calcium concentration which induces further destruction of pancreatic islet cells [41-43], this situation led to insulin insufficiency and caused an elevation of the blood glucose, triglyceride and total cholesterol level [44].

S. nitida extracts significantly lowered the blood glucose level of the treated rats from the 7th day of administration at different doses when compared to the untreated group. The observed changes are indications of the hypoglycaemic and anti-hyperglycaemic effects of *S. nitida*, which is comparable to that of other studies [45-47].

The maximal blood glucose lowering activity which was higher than that of glibenclamide was seen at the 500mg dose of aqueous extract. The extracts contain bioflavonoids and saponins which have been reported to have hypoglycemic effect in diabetic animal models [48-49]. So also does the presence of phenols as in V. persica phenolic-rich extracts which revealed an excellent diabetic control when compared to orthodox anti-diabetic agents [50]. The potential mechanism of action of this medicinal plant is likely to be associated with pancreatic β-cell regeneration [51-56].

One of the significant complexities of type I diabetes is weight loss which arises as a result of the reduction in insulin activity which is required for the conversion of glucose into glycogen and break down of fats; there is also the inhibition of lipolysis due to its unavailability because of destruction in beta cells[57]. Alloxan-induced weight loss mimics that of diabetes as was noticed from the results on the 3rd day. However, on commencement of treatment with *Salacia nitida* extracts, the body weight significantly improved from day 7 when compared to the diabetic rats that did not receive the extracts. The finding suggests that the extracts had blood glucose lowering ability by potentiating glucose metabolism which may be as a result of the extract action on muscle tissues, controlling their wasting and this can be achieved through reversed gluconeogenesis [58]. However, of all the treatments, the methanol 250mg and ethyl acetate 500 mg/kg dose restored the weight towards normal. Thus, *Salacia nitida* might be used to control body weight in people with diabetes. [59] noted ethanol cardamom leaves extract (ECLE) contains flavonoids, which serves to control body weight. *Salacia nitida* extracts also contain flavonoids, which may explain the extracts ability to regulate body weights. This finding is similar to results in [39].

One of the challenges discerned among diabetic patients is the presence of compound comorbidities which predisposes the patient to several incidences of drug interactions as a result of polypharmacy. Thus, herbs which possess the ability to affect several metabolic targets are being sorted after such as the herb *Brassica napus* and *Boswellia Serrata* gum resin which has shown positive effects on lipid profile as well as antihyperglycemic effects in these patients [60, 61]. S.*nitida* has also demonstrated its lipid-lowering properties in addition to its blood glucose lowering properties this may be attributed to the rich phytochemicals such as saponins and tannins; as studies have shown they exert a positive effect on dyslipidemia [62-63]. Flavonoids from plants have been implicated in the reduction of lipids by [64-66] inhibition of HMG-CoA reductase. It, therefore, follows that the underlying mechanism of fat lowering effect of the *Salacia nitida* extracts could be by inhibition of lipid absorption due to [67] the presence of saponins, flavonoids and tannins.

The liver plays a dominant role in the entire body glucose homeostasis. It is the organ responsible for facilitating de-novo glucose production [68]. Insulin secretion in normal physiological conditions activates signalling pathways which leads to the excretion of more glucose and more uptake of glucose to the required tissues, also the activation of anabolic pathways which aids glycogen synthesis in liver and muscles, and the inhibition of hepatic gluconeogenesis. Hyperglycemia results therefore from the concomitant disorders in insulin action which results from both low glucose disposal and unrepressed glucose production. Assay for liver enzymes namely Alkaline Phosphatase, Aspartate aminotransferase and Alanine transaminase is critical in evaluating ideal liver capacity amid diabetes, when the levels are elevated, it is an indication of liver dysfunction [69], there was observable elevation of the liver enzymes, which was markedly noticed in the untreated rats and diabetic rats before the extracts administration; however following

administration, the liver enzyme levels significantly reduced.

Therefore, this increased serum enzyme levels in the untreated diabetic rats confirms damage to the plasma membrane, leading to a compromise of membrane integrity but its absence in the treated is suggestive of the extracts cellular membrane and hepatocellular protective effects of the plant extract [70,71]. These seeming hepatic protective activities of the extracts are indicative that once-daily chronic application may not necessarily predispose to hepatic toxicity. The comparable observation was likewise detailed in [72,73].

An increase in tissue or serum bilirubin concentration results in jaundice, and it occurs in toxic or infectious disease of the liver, e.g. hepatitis [74]. After the 21 days of treatment, both total and conjugated bilirubin was significantly decreased, and it is an indication of protective haemoglobin metabolism and liver function of the treated rats. Studies on experimental animals have shown a significant relationship between insulin deficiency and metabolic distortion such as the excessive breakdown of proteins and amino acids leading to decreased protein content [75]. Administration of the extracts restored the protein levels to near normal levels. In this study, the albumin levels of the group treated with the extracts were not significantly different from the diabetic group that did not receive the extracts. The liver's function to synthesise albumin and globulin is decreased in the light of its dysfunction [76], and it is an indication of hepatitis and liver cirrhosis (liver damage).

Diabetic nephropathy was observed in the untreated diabetic rats with elevated levels of serum protein, urea, and creatinine. The creatinine levels were found to have significantly decreased following the administration of the aqueous and methanol extract.

5. CONCLUSION

Oral administration of the aqueous, dichloromethane, methanol and ethyl acetate extracts of *Salacia nitida* Benth showed hypoglycemic activity in alloxan-induced diabetes in experimental Wistar rats which was evidenced by improving the body imbalance in lipid metabolism experienced during diabetes, restoring body weight to near normal, decreased liver glycogen levels, and decreasing albumin, bilirubin, urea and creatinine levels. Further research to determine the particular compounds responsible for the anti-diabetic property in the extracts should be studied. A combination of the extracts (especially the ethyl acetate and aqueous extracts) may have additive effects which could likely lead to enhanced prevention and treatment of diabetes in humans.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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