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## Research Article

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## Phytochemical composition and antidiabetic activity of ethanol root extract of *Nauclea latifolia*

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### Abstract

Diabetes is a metabolic disease that is affecting major population of developing countries causing deaths. **Objectives:** The ethanolic root extract of *Nauclea latifolia* use traditionally for the treatment of diabetes was evaluated for antidiabetic activity in alloxan-induced diabetic rats after a single dose (acute study) and prolonged treatment (chronic study). **Materials and Methods:** Diabetes was induced in rats using alloxan monohydrate (150 mg/kg). The diabetic rats were treated with the root extract (150 – 450 mg/kg). The blood glucose level (BGL) was measured by using a glucometer. **Results:** Treatment of alloxan diabetic rats with the root extract (150 – 450 mg/kg, p.o) caused a significant ( $P < 0.05 - 0.001$ ) reduction in fasting Blood Glucose levels (BGL) of the diabetic rats both in acute study and prolonged treatment (2 weeks) in a manner comparable to that of the reference drug, glibenclamide (10 mg/kg bw.p.o). **Conclusion:** This finding suggests that the root extract of *N. latifolia* posses antidiabetic effect on alloxan-induced diabetic rats which can be exploited in the management of diabetes.

**Keywords:** *Nauclea latifolia*, Antidiabetic, Alloxan, Phytoconstituents, Herbal medicine.

### Introduction

*Nauclea latifolia* Smith (Rubiaceae) is a multi-stemmed shrub or small tree which is widespread in tropical Africa and Asia. The plant is used traditionally in the treatment of various diseases. The leaves are employed traditionally for the treatment of malaria in East Africa<sup>1</sup> and in Nigeria<sup>2</sup>. In part of Nigeria, the roots of *Nauclea latifolia* are used by some traditional medicine practitioners to treat hypertension.<sup>2,3</sup> In Ghana and Ivory Coast, the roots are also used in the treatment of malaria.<sup>4,5</sup> The roots are reported to be used to induce abortion and as a purgative.<sup>6</sup> The bark is used in the treatment of wounds, coughs and gonorrhoea in Nigeria.<sup>7</sup> Biological activities reported on the roots include antibacterial and antifungal<sup>8</sup>, hepatoprotective and trypanocidal<sup>7</sup>, antimalarial<sup>4</sup> and antihypertensive<sup>2</sup>. The indole alkaloid strictosamine has been found in the root.<sup>9</sup> Antidiabetic activity has been reported on the leaves.<sup>10,11</sup> However, different parts of the plant are usually used to treat diabetes. In this study we report the antidiabetic activity of the roots as there is no previous report of the activity in the root.

### Materials and Methods

#### Plant material

The plant part (root) was identified by Dr. Margaret Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo. The roots were collected from compounds in Uyo metropolis and were authenticated. A voucher specimen of the plant was deposited in the herbarium of Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo.

### Extraction

The roots were shade dried for 2 weeks. The dried roots were further chopped into small pieces and reduced to powder using pestle and mortar. The powdered root was macerated in 50% ethanol for 72 hours to give the crude ethanolic extract. The liquid filtrates were concentrated and evaporated to dryness in vacuum at 40°C using rotary evaporator. The yield of the extract was calculated. The dry extracts were stored in a refrigerator at - 4°C until when used.

### Phytochemical Screening

Phytochemical screening of the crude root extract was carried out employing standard procedures and tests<sup>12, 13</sup> to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides among others.

### Animals

The animals (Swiss albino mice and rats) of both sexes were used for these experiments. They were obtained from University of Uyo animal house. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea Feed) and water ad libitum.

### Determination of median lethal dose (LD<sub>50</sub>)

The median lethal dose (LD<sub>50</sub>) of the extract was estimated using albino mice by intraperitoneal (i.p) route using the method of Lorke.<sup>14</sup> This involved the administration of different doses of the extract to groups of three mice each. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 hours was recorded. The LD<sub>50</sub> was calculated as

geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b).

$$LD_{50} = \sqrt{ab}$$

### Evaluation of antidiabetic and hypolipidemic activities of the Extract and fractions

Induction of diabetes and animal treatment: The animals (male rats) were fasted for 24 h and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (150 mg/kg) in ice cold 0.9% saline (NaCl) solution. The animals were given 2 ml of 5% dextrose solution using orogastric tube immediately after induction to overcome the drug induced hypoglycaemia. 72 hours later, rats with blood glucose level (BGL) above 200 mg/dl were considered diabetic and selected for the experiment.

The animals were randomly divided into five groups of 6 rats each and treated as follows:

Group I: Diabetic rats administered *Nauclea latifolia* extract (150 mg/kg/day) orally for 14 days.

Group II: Diabetic rats given *Nauclea latifolia* extract (300 mg/kg/day) orally for 14 days.

Group III: Diabetic rats administered orally with *Nauclea latifolia* extract (450 mg/kg/day) for 14 days.

Group IV: Diabetic rats given Glibenclamide (10mg/kg/day) for 14 days orally.

Group V: Diabetic control rats receiving normal saline (10ml/kg) for 14 days.

The change in body weight and fasting BGL of all the rats were recorded at regular intervals during the experimental period. For acute study, the BGL was monitored after 1, 3, 5 and 7 h of administration of a single dose of the extract and at the end of 1, 3, 5, 7 and 14 days for prolonged treatments. The BGL was monitored in the blood of the diabetic rats by tail tipping method. The blood was dropped on the dextrostix reagent pad. This was inserted into microprocessor digital blood glucometer and the readings were recorded.<sup>15</sup>

### Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using Students't-test and ANOVA (One- or Two - way) followed by a post test (Tukey - Kramer multiple comparison test). Differences between means will be

considered significant at 1% and 5% level of significance i.e  $P \leq 0.01$  and  $0.05$ .

## Results

### Phytochemical screening

Phytochemical screening of the ethanolic root extract of *Nauclea latifolia* revealed the presence of compounds like Tannins, saponins, alkaloids, terpenes, cardiac glycosides, flavonoids and anthraquinones.

### Acute toxicity

The extract (100 – 2000 mg/kg) produced physical signs of toxicity such as writhing, gasping, palpitation, decreased respiratory rate, and death. All the mice treated with 1600 mg/kg and above died. The i.p LD<sub>50</sub> of the extract in mice was calculated to be 1549.19 mg/kg.

### Antidiabetic activity

There were observable changes in the body weight of treated and untreated diabetic rats. Treatment of diabetic rats with ethanolic root extract of *Nauclea latifolia* or glibenclamide improved the weight gain compared to untreated diabetic rats (Table 1).

A dose-dependent reduction in BGL was observed in alloxan-induced diabetic rats treated with ethanolic root extract of *Nauclea latifolia*. After a single dose of the extract on the alloxan-induced diabetic rats, there was a significant ( $P < 0.01 - 0.001$ ) reduction in BGL of the diabetic rats within the period of acute study compared to control with the maximum effect at 7h with the highest dose of the extract (450 mg/kg). The effect of the root extract was comparable to that of the standard drug, glibenclamide (Table 2).

**Table 1:** Effect of ethanolic root extract of *Nauclea latifolia* on body weights of alloxan - induced diabetic rats

Treatment	Dose (mg/kg)	Day 0	Day 15	% increase/ decrease in body weight
Control	0.2ml	177.9±9.17	145.8 ± 15.32	-18.04
Extract	150	180.2±11.29	178.6±3.82 <sup>a</sup>	0.88
	300	186.6±8.42	188.6±2.41 <sup>a</sup>	1.07
	450	182.3±7.98	184.6±10.12 <sup>a</sup>	1.26
Glibenclamide	10	194.3±12.04	196.6±11.23 <sup>a</sup>	1.18

Data are represented as mean ±SEM. significant at  $p < 0.001$ . When compared to control (n=6)

**Table 2:** Antidiabetic effect of ethanolic root extract of *Nauclea latifolia* on blood glucose level of alloxan- induced diabetic rats during acute study

Treatment	Dose (mg/kg)	Blood Glucose Level (mg/dl) in Hours				
		0hr	1hr	3hr	5hr	7hr
Control	0.2ml	229.4± 2.82	235.0± 3.39	241.0± 4.24	248.4 ± 5.22	254.5± 2.33
Crude Extract	150	227.6 ± 6.09	227.6 ± 4.27	224.6 ± 5.30 <sup>b</sup>	200.0 ± 4.28 <sup>b</sup>	198.0 ± 4.89 <sup>b</sup>
	300	230.4 ± 6.17	226.3 ± 6.30 <sup>c</sup>	214.4 ± 3.23 <sup>b</sup>	194.0 ± 5.21 <sup>c</sup>	185.4±4.22 <sup>c</sup>
	450	231.8± 5.97	224.5 ± 2.68 <sup>a</sup>	210.0 ± 2.64 <sup>c</sup>	185.9 ± 2.43 <sup>c</sup>	175.4±2.58 <sup>c</sup>
Glibenclamide	10	232.4± 4.61	227.0 ± 2.24	215.2 ± 4.02 <sup>c</sup>	206.3 ± 6.25 <sup>c</sup>	177.0 ± 5.47 <sup>c</sup>

Data are expressed as mean ±SEM. Significant at  $p < 0.05$ ,  $bp < 0.01$ ,  $cp < 0.001$ . When compared to control (n=6)

**Table 3:** Effect of ethanolic root extract of *Nauclea latifolia* on blood glucose level of alloxan- induced diabetic rats during prolonged treatment

Treatment	Dose in (mg/kg)	Blood glucose level (mg/dl) in days					
		Day 0	Day 1	Day 3	Day 5	Day 7	Day 15
Control	0.2ml	229.4± 2.82	256.4 ± 5.01	259.2 ± 4.69	252.6 ± 5.31	245.2 ± 3.56	238.2± 4.56
Crude extract	150	227.6 ± 6.09	200.3 ± 2.16 <sup>a</sup>	172.2 ± 3.32 <sup>a</sup>	142.3 ± 4.24 <sup>a</sup>	136.6 ± 3.52 <sup>a</sup>	119.8± 6.34 <sup>a</sup>
	300	230.4 ± 6.17	195.6±5.18 <sup>a</sup>	164.8 ± 5.16 <sup>a</sup>	136.2± 3.05 <sup>a</sup>	116.6 ± 2.56 <sup>a</sup>	102.7±3.69 <sup>a</sup>
	450	231.8± 5.97	193.0 ± 4.06 <sup>a</sup>	142.4± 4.54 <sup>a</sup>	128.4 ± 1.26 <sup>a</sup>	110.2 ± 1.60 <sup>a</sup>	98.3±3.22 <sup>a</sup>
Glibenclamide	10	232.4± 4.61	195.8 ± 7.30 <sup>a</sup>	140.5 ± 6.96 <sup>a</sup>	124.6 ± 3.42 <sup>a</sup>	108.8 ± 3.10 <sup>a</sup>	90.5±2.46 <sup>a</sup>

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

## Discussion

Evaluation of antidiabetic activity of ethanolic root extract of *Nauclea latifolia* was carried out in alloxan- induced diabetic rats. The extract which showed moderate toxicity was observed to demonstrate significant antidiabetic activity in alloxan-induced diabetic rats. The leaf extract of the plant has previously been reported to exhibit hypoglycaemic as well as antidiabetic activities.<sup>10, 11</sup> Some phytochemical compounds such as polysaccharides<sup>16</sup>, terpenes and tannins<sup>17</sup>, steroids<sup>18</sup>, and alkaloids<sup>19</sup> have been implicated in the antidiabetic activities of plants. Phytochemical study of the root extract revealed the presence of terpenes, saponins, tannins flavonoids and alkaloids. Shigemori<sup>20</sup> had reported the presence of indole alkaloids in the root of *N. latifolia* which have been reported to exert antidiabetic activity of plants<sup>21</sup>. These constituents may in part be responsible for the observed significant activity of this extract either singly or in synergy with one another. Sulphonylureas cause hypoglycemia by stimulating insulin secretion from the pancreas and these compounds are potent in mild alloxan induced diabetes and inactive in intense alloxan induced diabetes whereby nearly all  $\beta$ -cells have been destroyed.<sup>22</sup> The observed reduction in BGL of the diabetic rats by glibenclamide in this study portrays an in severe state of diabetes. In this study, continuous treatment with the root extract of *N. latifolia* for a period of 2 weeks caused significant decrease in BGL of treated rats compared to untreated diabetic rats. This was followed by corresponding increase in body weight of the treated rats. Diabetes is characterised by a severe loss in body weight due to loss or degradation of structural proteins.<sup>23</sup> This

condition was alleviated by the treatment of the diabetic rats with root extract of *N. latifolia*. Some plants' extracts are reported to exert hypoglycemic action by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of langerhans or its release from bound insulin.<sup>24</sup> While: others act through extra pancreatic mechanisms by inhibition of hepatic glucose production<sup>25</sup> or corrections of insulin resistance<sup>26</sup>. The root extract may have acted through one of the above mechanisms.

## Conclusion

In conclusion, the results of this study show that ethanolic root extract of *Nauclea latifolia* possessed antidiabetic properties. This confirmation justifies its use in ethnomedical medicine for the treatment of diabetes.

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## Conflict of Interest

No conflict between the authors.

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