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# Antihyperglycaemic and haematological effects of ethanol extract of *Salacia lehmbachii* leaf in alloxan-induced diabetic Wistar rats

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**ABSTRACT**

**Introduction:** *Salacia lehmbachii* is used traditionally for the treatment of diabetes mellitus. This study investigated the hypoglycaemic potentials of ethanol leaf extract of S. lehmbachii and its effects on alloxan-induced diabetic rats' haematological parameters.

**Methods:** A total of 36 male Wistar rats including normal, diabetic untreated and diabetic treated ones were used in this study. Diabetes was induced by a single intraperitoneal injection of 150 mg/kg of alloxan. When confirmed diabetes, the rats were orally administered 100 mg/kg, 200 mg/kg, and 400 mg/kg daily of the leaf extract for 21 days. The blood glucose levels and haematological parameters were determined in diabetic treated rats compared with the controls.

**Results:** The ethanol leaf extract of S. lehmbachii significantly reduced the blood glucose levels of alloxan induced diabetic rats when compared with the diabetic control ($P<0.01$). The extract also significantly ($P<0.01$) improved the haematological parameters of treated rats at three doses employed.

**Conclusion:** It can be concluded that the ethanol leaf extract of *S. lehmbachii* possesses antihyperglycaemic properties and could be considered a potential candidate for the development of new drugs in the treatment of anaemic conditions.

*Implication for health policy/practice/research/medical education:*
In this study, *Salacia lehmbachii* ethanol leaf extract significantly demonstrated antihyperglycaemic activity and improved haematological parameters. Hence, this plant can be considered as a candidate for bioassay-guided and isolation of compounds, which could possibly be developed into new lead structures for drug development programs against diseases.

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## Introduction

Diabetes mellitus has been considered as one of the present major health concerns globally. The alarming rate at which diabetes is increasing daily with a rapid increase in mortality burden shows that this disease is a leading cause of death worldwide (1). It is the most common endocrine disorder, and has been affirmed that over 300 million people are likely to be diabetic by 2030 (2). This is because managing the disease with synthetic antidiabetic agents without side effects is still a medical challenge (3,4). Despite numerous available antihyperglycaemic agents, herbal remedies are used with success to treat this disease (5). However, attention is presently being focused on herbal drugs for the treatment of diabetes throughout the world. Most of these medicinal plants are used in the treatment of diabetes and its associated complications. Furthermore, based on the WHO recommendations, investigations on antihyperglycemic agents from medicinal

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plants have become more important (6,7). More so, these plants' antihyperglycemic properties are probably due to their ability to restore the function of pancreatic tissues resulting from an increase in insulin output or decreased intestinal absorption of glucose (8). Thus, these herbal agents have β-cells protecting activities and smoothing out fluctuation in plasma glucose levels (9,10).

*Salacia lehmbachii* Loes is a shrub-like to small tree of about three meters high belonging to the family Celastraceae. The leaves are seasonally evergreen, firm, and difficult to slice. The plant is richly found in the tropical rain forest of Central, west, and East Africa (11). There are different therapeutic applications of *S. lehmbachii,* which actually justify its folkloric background. Previous studies have reported the antipyretic, anti-diarrhoea, antimotility, and anti-ulcer of this plant leaf extract (12,13). The root bark extract has been shown to possess analgesic, anti-inflammatory, anticholinergic, anti-infertility activities (14-16). The mixture of the root has also been used traditionally for the treatment of hemorrhoids (17). In addition, the stem bark extract has also been reported to possess antidiabetic and antihyperlipidemic activity (1). The present study aimed at evaluating the antihyperglycemic and haematological effects of the ethanol extracts of *S. lehmbachii* leaves in alloxan-induced diabetic Wistar rats.

## Materials and Methods

### Plant collection

Fresh leaves of *S. lehmbachii* were collected from farmland in Ukanafun, Nigeria. The plant was identified and authenticated by a taxonomist in the Herbarium Unit of Department of Botany, University of Calabar, Calabar, Nigeria. A voucher sample (herbarium No 688) was prepared and deposited in the Herbarium of Botany Department for reference. The international plant number index is Bot. Jahrb. Syst. 44(2-3):173 (1910).

### Leaf extract preparation

The leaf material of *S. lehmbachii* was air-dried to constant weight in the laboratory, powdered, and stored in an airtight container for further use. The dried material was then pulverized using a wooden mortar and pestle. Three hundred and fifty grams (350 g) of the leaf powdered plant material was extracted in 1.5 L of ethanol (75%) with constant shaking for 24 hours. The extract was filtered using Whatman No.1 filter paper. The filtrate was dried on a water bath at a reduced temperature of 40°C and a yield of 18.5 g dry extract with a greenish colour. The extract was later reconstituted in distilled water to give desired doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight.

### Phytochemical analysis

The phytochemical screening of ethanol leaf extract of *S. lehmbachii* was carried out for various secondary metabolites such as tannins (ferric chloride test), alkaloids (Mayer's and Dragendorff reagent test), saponins (Froth test), steroids (Liebermann-Burchard test), terpenoids (Salkowski test), flavonoids (ammonia and sulphuric acid test), glycosides (Keller-Kiliani test), reducing sugars (Fehling's test), phenol (ferric chloride test) and anthraquinones (Borntrager's test) (18,19).

### Animals

Male Wistar rats weighing between 170 and 190 g were obtained from the animal house of the University of Nigeria, Nsukka. Animals were maintained under standard environmental conditions at 45%–55% relative humidity for 12 hours, each of dark and light cycle and fed with a standard pellet rat diet obtained from Oladokun feed, Ibadan, Nigeria, and water was supplied *ad libitum*.

### Acute toxicity test

The $LD_{50}$ of the plant extract was tested to determine the safety of the agents using OECD (20) method. The study was carried out in two phases, on male Wistar rats. The animals were grouped into three in the first phase, each group having three rats. The rats were administered *S. lehmbachii* leaf extract at doses of 100, 600, and 1000 mg/kg using an orogastric cannula and observed for signs of toxicity and mortality for 24 hours. In the second phase of the study, rats were designated into three groups, with each having one rat and orally administered 2000 mg/kg, 3000 mg/kg, and 5000 mg/kg of the leaf extract. These animals were first observed for 4 hours and later 24 hours. Toxicity signs and mortality were recorded for each group after the observation period. The experimental rats were monitored further for 72 hours.

### Induction of diabetes

Overnight fasted rats were made diabetic by a single intraperitoneal injection of freshly prepared alloxan of 150 mg/kg in sterile saline. Seventy-two hours after alloxan injection, animals having blood glucose level >200 mg/dL were considered as being diabetic and recruited for the study (21).

### Pharmacological intervention

Rats used for this study were randomly divided into 6 groups with 6 rats each: group 1 rats (normal control) were administered distilled water (10 mL/kg), group 2 rats served as diabetic control, and were also administered distilled water (10 mL/kg). Groups 3, 4, and 5 were diabetic rats treated with *S. lehmbachii* leaf extract at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively. However, group 6 diabetic rats (positive control) were treated with glibenclamide (10 mg/kg). All treatments were carried out through oral cannula for a period of 21 days. Blood glucose levels were measured using Accu-Check glucometer test strips (Roche, Germany) at random on days 0, 7, 14, and 21 of the experiment. Blood samples from the tail vein

puncture for measurement of glucose levels were collected 24 hours after under halothane anaesthesia, while samples for determination of haematological parameters were collected by cardiac puncture from rats and dispensed into EDTA containers and analyzed within 2 hours of collection.

### Determination of haematological parameters

Mythic 18 haematological analyzer (Orphee, Switzerland) was used to determine the haematological components, including haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBCs), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBCs), platelet count (PLC), differential count (neutrophils, lymphocytes, monocytes, eosinophil and basophils), and red cell distribution width (RCDW).

### Statistical analysis

Results were expressed as means ± SEM and analyzed with statistical package for social sciences (SPSS version 20) by using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. A difference in the mean $P < 0.05$ was considered significant.

## Results

### Phytochemical analysis

The phytochemical screening of *S. lehmbachii* ethanol leaf extract contained secondary metabolites such as alkaloid, saponins, tannins, flavonoids, terpenoids, steroids, glycosides, phenol, reducing sugar, while anthraquinone was not detected.

### Acute toxicity test

The ethanol leaf extract of *S. lehmbachii* did not produce any lethality or significant toxicity signs in rats up to 5000 mg/kg bodyweight for 24 and 72 hours post-treatment.

### Effect of the ethanol leaf extract of *Salacia lehmbachii* on blood glucose levels in Wistar rats

Antihyperglycaemic impact of the leaf extract on blood glucose levels of the experimental rats is shown in Table 1. Intraperitoneal alloxan administration into the rats significantly elevated blood glucose levels in Wistar rats compared with control (normal) rats. The blood glucose levels increased from 88.52 to 240.58 g/dL. Treatment with the ethanol leaf extract of *S. lehmbachii* at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg significantly ($P < 0.01$) reduced the blood glucose levels in dose dependent fashion compared with diabetic untreated rats. However, 400 mg/kg of the extract with the highest activity in the extract-treated groups compared favourably well with the glibenclamide (10 mg/kg) treated group.

### Effect of the leaf extract on haematological indices in rats

Table 2 shows a significant ($P < 0.05$) decrease in the levels of RBC, Hb, PCV, MCH, MCV, MCHC, RCDW as well as glibenclamide, after intraperitoneal administration of alloxan when compared with the normal control group. However, following intervention with the leaf extract and glibenclamide at different doses applied, these haematological indices significantly ($P < 0.01$) increased compared with diabetic untreated group.

### Effect of *Salacia lehmbachii* ethanol leaf extract on WBC differential in Wistar rats

There was significant ($P < 0.05$) observed reduction in serum WBC, neutrophils, lymphocyte, monocytes, eosinophils, basophils, and platelets following induction of hyperglycemia in rats with alloxan compared with normal control. The leaf extract significantly ($P < 0.01$) increased the levels of these parameters at both doses used when compared with the diabetic control group (Table 3). The best results were observed at 400 mg/kg of the extract and the standard drug (glibenclamide 10 mg/kg).

### Effect of ethanol extract of *Salacia lehmbachii* leaf on body weight of Wistar rats

There was a significant ($P < 0.05$) weight increase in all the rats given various doses of the extract and those treated with glibenclamide. However, there was a considerable weight loss in diabetic control rats (Table 4).

**Table 1.** Effect of ethanol extract of *Salacia lehmbachii* leaf on blood glucose level of alloxan-induced diabetic rats

| Treatment | Dose (mg/kg) | Blood glucose level (mg/dL) | | | |
|---|---|---|---|---|---|
| | | Day 0 | Day 7 | Day 14 | Day 21 |
| Normal control | 10 mL/kg | 88.52 ± 2.22 | 91.45 ± 2.40 | 93.48 ± 2.52 | 74.52 ± 2.34 |
| Diabetic control | 10 mL/kg | 230.40 ± 2.42 | 240.22 ± 3.25 | 269.68 ± 3.88 | 298.44 ± 4.20 |
| *S. lehmbachii* | 100 | 238. 39 ± 2.25 | 220.52 ± 2.20* | 175.49 ± 2.10** | 135.45 ± 2.02** |
| | 200 | 240.58 ± 2.35 | 190.28 ± 2.30** | 152.77 ± 2.20** | 128.10 ± 2.08** |
| | 400 | 235.52 ± 2.32 | 182.55 ± 2.23** | 142.85 ± 2.17** | 118.18 ± 2.02** |
| Glibenclamide | 10 | 230.49 ± 2.20 | 173.14 ± 2.19** | 135.59 ± 2.14** | 110.06 ± 2.06** |

Values are mean ± SEM (n = 6); * $P<0.05$ when compared with control group; ** $P<0.01$ when compared with diabetic control group.

**Table 2.** Effect of ethanol extract of *Salacia lehmbachii* leaf on red blood cell indices of alloxan-induced diabetic rats

| Parameters | Normal | Hyperglycemic | 100 | 200 | 400 | 10 |
|---|---|---|---|---|---|---|
| | Control | Control | SL | SL | SL | Glibenclamide |
| RBC(x 10 $^{12}$/L) | 9.10 ± 0.06 | 6.95 ± 0.05* | 8.57 ± 0.38** | 8.70 ± 0.54** | 8.78 ± 0.68** | 8.77 ± 0.62** |
| Hb (g/dL) | 15.88 ± 0.79 | 12.89 ± 1.05* | 15.24 ± 1.00** | 15.87 ± 1.20** | 16.10 ± 1.78** | 15.92 ± 1.26** |
| PCV (%) | 37.55 ± 1.33 | 35.51 ± 1.28* | 41.48 ± 1.30** | 41.54 ± 1.29** | 41.67 ± 1.34** | 41.50 ± 1.31** |
| MCV (fl) | 60.53 ± 1.66 | 55.39 ± 1.48* | 69.44 ± 2.09** | 70.64 ± 2.11** | 72.89 ± 2.15** | 71.44 ± 2.10** |
| MCH (pg) | 17.42 ± 0.55 | 14.63 ± 0.66* | 17.97 ± 0.59** | 19.33 ± 1.20** | 19.86 ± 1.17** | 17.51 ± 0.56** |
| MCHC (g/dL) | 34.58 ± 0.66 | 20.14 ± 0.88* | 26.65 ± 0.78** | 28.50 ± 1.18** | 28.88 ± 1.15** | 28.66 ± 1.11** |
| RCDW | 14.20 ± 0.77 | 12.78 ± 0.64* | 14.58 ± 2.33** | 14.97 ± 2.28** | 15.10 ± 1.52** | 15.48 ± 1.50** |

Haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), platelet count (PLC). SL: 100, 200 and 400 = *S. lehmbachii* in mg/kg, respectively.
Values are mean ± SEM (n=6); * $P$<0.05 when compared with control group; ** $P$<0.01 when compared with diabetic control group.

**Table 3.** Effect of ethanol extract of *Salacia lehmbachii* leaf on WBC differential count of alloxan-induced diabetic Wistar rats

| Parameters | Normal | Hyperglycemic | 100 | 200 | 400 | 10 |
|---|---|---|---|---|---|---|
| | Control | Control | SL | SL | SL | Glibenclamide |
| WBC (×10$^9$/L) | 15.51 ± 2.31 | 3.12 ± 0.77* | 7.07 ± 3.07** | 7.45 ± 2.65** | 6.98 ± 2.55** | 6.50 ± 3.23** |
| Neutrophils (%) | 27.34 ± 1.66 | 3.05 ± 0.17* | 20.27 ± 0.44** | 28.33 ± 0.45** | 24.52 ± 0.67** | 23.68 ± 0.22** |
| Lymphocytes (%) | 75.48 ± 5.66 | 4.89 ± 1.23* | 68.77 ± 3.05** | 61.38 ± 2.56** | 65.87 ± 3.45** | 71.88 ± 5.16** |
| Monocytes (%) | 19.29 ± 4.30 | 4.22 ± 1.33* | 15.79 ± 0.48** | 13.48 ± 0.12** | 11.54 ± 0.52** | 17.22 ± 0.58** |
| Eosinophil (%) | 6.47 ± 1.65 | 0.85 ± 0.64* | 5.43 ± 0.19** | 5.39 ± 0.50** | 4.22 ± 0.50** | 3.18 ± 0.22** |
| Basophils (%) | 0.44 ± 0.10 | 0.03 ± 0.03* | 0.09 ± 0.03** | 0.10 ± 0.02** | 0.10 ± 0.03** | 0.30 ± 0.18** |
| Platelets (×10$^9$/L) | 460.00 ± 58.44 | 47.01 ± 36.22* | 256.02 ± 57.04** | 167.00 ± 34.00** | 189.00 ± 42.00** | 210.00 ± 57.00** |

Values are mean ± SEM (n=6); * $P$<0.05 when compared with control group; ** $P$<0.01 when compared with diabetic control group. SL: 100, 200 and 400 = *S. lehmbachii* in mg/kg, respectively.

**Table 4.** Effect of ethanol extract of *Salacia lehmbachii* leaf on body weight of alloxan-induced diabetic Wistar rats

| Treatment | Dose (mg/kg) | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|---|
| Control | 20 mL/kg | 185.50 ±1.44 | 198.65 ± 3.13 | 210.66 ± 3.40 | 215.69 ± 3.29 |
| Diabetic control | 20 mL/kg | 189.90 ± 2.67 | 185.30 ± 2.43 | 171.51 ± 1. 52 | 164.34 ± 1.40 |
| *S. lehmbachii* | 100 | 190.00 ± 1.42 | 197.80 ± 2.43* | 207.60 ± 2.62* | 214.11 ± 2.50* |
| | 200 | 188.75 ± 1.24 | 195.38 ± 2. 40* | 205.80 ±2.35* | 210.10 ± 2.06* |
| | 400 | 190.30 ± 1.37 | 197.30 ± 1.40* | 205.55 ± 2.36* | 211.48 ± 2.40* |
| Glibenclamide | 10 | 190. 60 ± 2.66 | 198.40 ± 2.33* | 206.78 ± 2.64* | 211.64 ± 2.13* |

Values are mean± SEM (n=6); * $P$<0.05 when compared with control group.

## Discussion

Plant parts have been used globally in traditional medicine for the treatment of different diseases and disorders. More than two thousand species of medicinal plants have been scientifically recorded as herbal medicines for diabetes. These agents have been widely acknowledged to be safe with minimum side effects, efficacious, and affordable (22-24). This study investigated antihyperglycemic and haematological activities of *S. lehmbachii* ethanol leaf extract in alloxan-induced hyperglycemic rats for 21 days. Its phytochemical analysis was equally carried out, and the result indicates the presence of a variety of phytoconstituents in the extract. Each of these constituents is known for its various protective and therapeutic effects (25).

Alloxan selectively destroys the insulin-producing beta cells found in the pancreas. Hence it affects endogenous insulin release and, as a result, increases blood glucose level (26). The repeated administration of different doses (100 mg/kg, 200 mg/kg, and 400 mg/kg) of the ethanol extract of *S. lehmbachii* leaf for 21 days resulted in a significant decrease in blood glucose levels in alloxan-

induced diabetic rats with a higher decrease observed in 400 mg/kg. Medicinal plant extracts have been reported to possess valuable antidiabetic properties and a stimulatory insulin release activity. (27). However, the reference drug (glibenclamide 10 mg/kg) demonstrated higher activity than the leaf extract to reduce the blood glucose level compared with diabetic control. It has been established that glibenclamide produces antidiabetic activity by increasing insulin secretion from the existing pancreatic β-cells (28). In the present study, the observed antihyperglycemic action of *S. lehmbachii* leaf extract may be by potentiating the insulin effect, probably by increasing the pancreatic secretion of insulin from the β-cells or by modulating the intracellular glucose utilization (29). Thus, the antihyperglycemic effect of *S. lehmbachii* leaf extract may be linked to the presence flavonoids, terpenoids and other secondary metabolites in the leaf extract. These phytoconstituents have been implicated in the antidiabetic activities of many plants (30,31).

More so, the antihyperglycemic properties of *S. lehmbachii* leaf is comparable to the stem bark extract (*S. lehmbachii*), which has demonstrated significant antidiabetic and antihyperlipidemic effect on alloxan-induced diabetes in rats (1) Furthermore, oral administration of the extract significantly improved the weight of diabetic rats probably due to protective effect of the extract on tissue structural constituents (1).

Moreover, the assessment of haematological indices could be used to show the detrimental effect of most plant extracts on blood constituents of animals. These parameters are equally used to ascertain possible alterations in the levels of biomolecules like metabolic products, haematology, normal functioning, and histomorphology of organs (32,33).

The anaemic condition in diabetes mellitus has been due to the increased non-enzymatic glycosylation of red blood cell membrane proteins (32). In the present study, the red blood cell parameters, which include Hb, PCV, MCV, MCH, MCHC, and RCDW were assessed to determine the beneficial effect of *S. lehmbachii* on the levels of anaemic diabetic rats. However, the study revealed significant depletion of haematological parameters in the hyperglycemic untreated rats compared to the normal control. These abnormalities have been implicated in anaemic condition in man (34,35). Administration of the ethanol extract of *S. lehmbachii* elicited a positive change in the levels of haematological indices of diabetic rats, especially at 400 mg/kg. Hence, an increase in RBC and its related indices by the leaf extract is an indication of its ameliorative effect on anaemic condition in alloxan-induced diabetic rats.

Furthermore, there was a significant reduction in the levels of WBC, neutrophils, lymphocytes, monocytes, eosinophils, and basophils in alloxan-induced diabetic rats compared to the normal group. The extract at both doses applied markedly improved the levels of WBC, neutrophils lymphocytes, monocytes, eosinophils, and basophils compared with glibenclamide treated group. In addition, there was a decrease in the level of platelet in alloxan-induced diabetic rats compared to normal. The platelets' ability to cause aggregation in diabetic animals with poor glycaemic control probably resulted from insufficient insulin (29). Prolonged depletion of platelet could lead to internal and external bleeding and may result in death. The continuous administration of the leaf extract of *S. lehmbachii* showed improvement in the level of the platelets. However, the observed activity of the extract suggests that it can control bleeding. The acute toxicity studies showed the non-toxic nature of *S. lehmbachii* ethanol leaf extract. There was no lethality or any toxic reactions observed throughout the duration of the toxicity study.

## Conclusion

The findings from this study show that ethanol leaf extract of *S. lehmbachii* may possess antihyperglycemic potentials and improve haematological indices in anaemic conditions. Thus, the information from this study may serve as a guide for the discovery of new antihyperglycemic and antianaemic drugs. Further studies are ongoing in our laboratory to isolate and identify various active principle(s) in the leaf extract responsible for the antihyperglycemic and antianaemic activities.

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## Authors' contributions

GCA conceived the research idea and designed the work, GCA, SCO and AUM carried out the experiment, PMU wrote the first draft of the manuscript, IMO carried out the literature search, JUC carried out the statistical analysis, while KCC and FVU supervised the study. All authors read and approved the final manuscript for publication.

## Conflict of interests

The authors declare no conflict of interest.

## Ethical considerations

The study protocol was carried out as per the rules and regulations of the Institutional Animal Ethical Committee, Faculty of Medicine, Ebonyi State University, Abakaliki (EBSU/DRIC/UREC/04/054) as well as the international guidelines on the Use and Handling of Experimental Animals .

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## References


1. Akuodor GC, Eban LK, Ajoku GA, Nwobodo NN, Akpan JL, Ezeokpo BC, et al. Antidiabetic and antihyperlipidemic potential of ethanol extract of Salacia lehmbachii stem bark in alloxan-induced diabetic rats. J Basic Clin Physiol Pharmacol. 2018;30(2):239-44. doi: 10.1515/jbcpp-2018-0059.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27(5):1047-53. doi: 10.2337/diacare.27.5.1047.
3. Mohammed RK, Ibrahim S, Atawodi SE, Eze ED, Suleiman JB. Anti-diabetic and haematological effects of n-butanol fraction of *Alchornea cordifolia* leaf extract in streptozotocin-induced diabetic Wistar rats. Glob J Med Plant Res. 2012;1(1):14-21.
4. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010;87(1):4-14. doi: 10.1016/j.diabres.2009.10.007.
5. Bhattaram VA, Graefe U, Kohlert C, Veit M, Derendorf H. Pharmacokinetics and bioavailability of herbal medicinal products. Phytomedicine. 2002;9 Suppl 3:1-33. doi: 10.1078/1433-187x-00210.
6. WHO Expert Committee on Diabetes Mellitus, Technical reports series. Geneva: WHO; 1980.
7. Kumar S, Rashmi, Kumar D. Evaluation of antidiabetic activity of *Euphorbia hirta* Linn. in streptozotocin induced diabetic mice. Indian J Nat Prod Resour. 2010;1(2):200-3.
8. Pari L, Saravanan R. Antidiabetic effect of diasulin, a herbal drug, on blood glucose, plasma insulin and hepatic enzymes of glucose metabolism in hyperglycaemic rats. Diabetes Obes Metab. 2004;6(4):286-92. doi: 10.1111/j.1462-8902.2004.0349.x.
9. Jia W, Gao WY, Xiao PG. Antidiabetic drugs of plant origin used in China: compositions, pharmacology, and hypoglycemic mechanisms. Zhongguo Zhong Yao Za Zhi. 2003;28(2):108-13.
10. Elder C. Ayurveda for diabetes mellitus: a review of the biomedical literature. Altern Ther Health Med. 2004;10(1):44-50.
11. Corstiaen PC, Sosef SM. Revision of African genus *Annickia*. Syst Geogr Plants. 2007;77:146-52.
12. Essien AD, Akuodor GC, Edidara AE, Asika EC, Chilaka KC, Nwadum SK. Evaluation of antipyretic potential of the ethanolic leaf extract of *Salacia lehmbachii* Loes. Asian J Med Sci. 2015;7(2):22-5.
13. Essien AD, Akuodor GC, Aja DO, Nwadike KI, Nwachukwu DC, Chilaka KC, et al. Studies on gastrointestinal properties of ethanolic leaf extract of *Salacia lehmbachii* in Wistar rats. Afr J Pharm Pharmacol. 2016;10(20):451-7. doi: 10.5897/ajpp2016.4536.
14. Takem LP, Lawal BA, Udia PM. Analgesic and acute anti-inflammatory activities of aqueous root extract of *Salacia lehmbachii*. Br J Pharm Res. 2014;4(18):2172-81. doi: 10.9734/bjpr/2014/9250.
15. Essien AD, Takem LP, Anele EI. In vitro cholinergic and acute toxicity evaluations *Salacia lehmbachii*. Int J Pharm Pharmacol Res. 2015;5(1):200-7.
16. Essiet GA, Essien AD, Udoh FV, Essiet A. Anti-fertility effects of ethanol extract of *Salacia lehmbachii* root bark in albino rats. J Adv Med Pharm Sci. 2016;8(4):1-8. doi: 10.9734/jamps/2016/26576.
17. Sofowora A. Medicinal plants and traditional medicine in Africa. Ibadan: Spectrum Books; 1993.
18. Aziz MA. Qualitative phytochemical screening and evaluation of anti-inflammatory, analgesic and antipyretic activities of *Microcos paniculata* barks and fruits. J Integr Med. 2015;13(3):173-84. doi: 10.1016/s2095-4964(15)60179-0.
19. Billmary C, Janne R, Lucero M, María C. Preliminary phytochemical screening of *Pimenta racemosa* var. *racemosa* (Myrtaceae) from Táchira—Venezuela. Pharmacologyonline. 2014;2:61-8.
20. National Institutes of Health (NIH). Guide for the Care and Use of Laboratory Animals. 8th ed. Bethesda, MD: NIH; 2011. p. 82-3.
21. Organisation for Economic Co-operation and Development (OECD). OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD; 2002. Test No. 423: Acute Oral toxicity - Acute Toxic Class Method.
22. Yakubu MT, Akanji MA, Nafiu MO. Anti-diabetic activity of aqueous extract of *Cochlospermum planchonii* root in alloxan-induced diabetic rats. Cameroon J Exp Biol. 2010;6(2):91-100. doi: 10.4314/cajeb.v6i2.68526.
23. Onoja SO, Udem SC, Anaga AO, Asuzu IU. Acute and chronic toxicity studies of hydromethanol leaf extract of *Helianthus annuus* Linn. in rats. Asian Pac J Trop Med. 2018;11(9):534-9. doi: 10.4103/1995-7645.242311.
24. Ali-Shtayeh MS, Jamous RM, Salameh NM, Jamous RM, Hamadeh AM. Complementary and alternative medicine use among cancer patients in Palestine with special reference to safety-related concerns. J Ethnopharmacol. 2016;187:104-22. doi: 10.1016/j.jep.2016.04.038.
25. Asaolu MF, Oyeyemi OA, Olanlokun JO. Chemical compositions, phytochemical constituents and in vitro biological activity of various extracts of *Cymbopogon citratus*. Pak J Nutr. 2009;8(12):1920-2. doi: 10.3923/pjn.2009.1920.1922.
26. Nastaran JS. Antihyperglycaemia and antilipidaemic effect of *Ziziphus vulgaris* L on streptozotocin induced diabetic adult male Wistar rats. Physiol Pharmacol. 2011;47(1):219-23.
27. Kumar S, Kumar V, Prakash OM. Antidiabetic and anti-lipemic effects of *Cassia siamea* leaves extract in streptozotocin induced diabetic rats. Asian Pac J Trop Med. 2010;3(11):871-3. doi: 10.1016/s1995-7645(10)60209-x.
28. Rajasekaran S, Ravi K, Sivagnanam K, Subramanian S. Beneficial effects of aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes. Clin Exp Pharmacol Physiol. 2006;33(3):232-7. doi: 10.1111/j.1440-1681.2006.04351.x.
29. Jarald E, Joshi SB, Jain D. Diabetes and herbal medicines. Iran J Pharmacol Ther. 2008;7(1):97-106.
30. Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as nutraceuticals: a review. Trop J Pharm Res. 2008:7(3):1089-99.
31. Okokon JE, Ita BN, Udokpoh AE. The in-vivo antimalarial activities of *Uvaria chamae* and *Hippocratea africana*. Ann Trop Med Parasitol. 2006;100(7):585-90. doi: 10.1179/136485906x118512.

32. Oyedemi SO, Adewusi EA, Aiyegoro OA, Akinpelu DA. Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic Wistar rats. Asian Pac J Trop Biomed. 2011;1(5):353-8. doi: 10.1016/s2221-1691(11)60079-8.
33. Magalhães PM, Appell HJ, Duarte JA. Involvement of advanced glycation end products in the pathogenesis of diabetic complications: the protective role of regular physical activity. Eur Rev Aging Phys Act. 2008;5(1):17-29. doi: 10.1007/s11556-008-0032-7.
34. Muhammad NO, Oloyede OB. Haematological parameters of broiler chicks fed *Aspergillus niger*-fermented *Terminalia catappa* seed meal-based diet. Global J Biotechnol Biochem. 2009;4(2):179-83.
35. Balasubramanian T, Lal MS, Sarkar M, Chatterjee TK. Antihyperglycemic and antioxidant activities of medicinal plant *Stereospermum suaveolens* in streptozotocin-induced diabetic rats. J Diet Suppl. 2009;6(3):227-51. doi: 10.1080/19390210903070780.