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# Antidiabetic potentials of *Syzygium guineense* methanol leaf extract

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## ABSTRACT

This study examines the effects of a methanol extract of *Syzygium guineense* leaves in streptozotocin (STZ) - induced diabetes, evaluates its effect on alpha glucosidase and 2, 2-diphenyl-1-picrylhydrazyl radical. Diabetes was induced in rats by a single intraperitoneal injection of streptozotocin (60 mg/kg). An oral glucose tolerance test was performed after diabetes induction and repeated after 14 days of treatment with the extract. The extract elicited antihyperglycemic action in diabetic rats evidenced by an improved oral glucose tolerance. A dose of 250 mg/kg of extract significantly ($P<0.01$, 0.001) enhanced glucose clearance at the end of treatment period and was comparable with metformin, the group also showed increase in hepatic glycogen content by 33.9% relative to the diabetic control. Serum biochemical analysis showed that the extract improved indices of renal and hepatic function by reduction in serum albumin, creatinine, liver enzymes, total and direct bilirubin. Similarly, the extract reduced serum cholesterol, triglycerides and high density lipoprotein (HDL) in a non-dose dependent manner; treatment with 250 mg/kg extract caused significant ($P<0.05$) reduction of HDL. Groups which received 250 and 500 mg/kg of extract showed reversal of glomerular damage compared with the diabetic untreated group. The extract also exhibited concentration-dependent antioxidant activity ($EC_{50}$= 0.2 mg/ml) and statistically significant ($P<0.01$, 0.001) alpha glucosidase inhibitory effect ($IC_{50}$= 6.15 mg/ml). These findings show the antidiabetic potential of *S. guineense* leaf extract, likely mediated through its ability to inhibit alpha glucosidase, scavenge free radicals and increase intrahepatic glucose uptake and storage.

**Keywords:** Alpha glucosidase, Antioxidant, *Syzygium guineense*.

## INTRODUCTION

Diabetes mellitus is a major metabolic disorder and a global threat to health owing to its high prevalence, morbidity and mortality. Diabetes is currently prevalent in 9% of adults aged 18 years and older and 80% of diabetes-related deaths occur in low and middle-income countries [1,2]. According to the World Health Organization, diabetes will be ranked the seventh leading cause of death by 2030 [3]. Non insulin-dependent diabetes mellitus or type II diabetes mellitus is a multifactorial disorder caused by either deficient insulin secretion from pancreatic beta cells or failure in insulin action and is characterized by hyperglycemia, impaired lipid metabolism, defects in redox balance, altered metabolism of major food substances [4]. Long term health complications of untreated or poorly managed diabetes include diabetic nephropathy, retinopathy, neuropathy, hypertension, and cardiovascular disease. Among other factors, free radicals have been recognized to play an important role in the development of diabetic complications [5]. Oxidative stress caused by persistent hyperglycemia leads to chronic cellular redox imbalance, with negative effects on key cellular metabolic processes and organelles; necessitating adequate control of hyperglycemia to mitigate cellular damage [6]. Oral antidiabetics commonly used to manage type II diabetes include drug classes such as sulfonylureas, thiazolidinediones, biguanides and the newer incretin mimetics/enhancers. These groups of drugs stimulate insulin secretion, sensitize tissues to insulin action or inhibit key carbohydrate metabolizing enzymes among other mechanisms [7]. Although oral antidiabetics are frequently employed to manage type II diabetes, they are occasioned with unwanted and sometimes life-threatening side effects like hypoglycemia, leading to a search for new and safer alternatives especially from natural sources. Herbal remedies have been used in traditional practice for the treatment of different diseases. For example, *Syzygium guineense* (Myrtaceae) has been used to alleviate symptoms of different diseases in some parts of Africa. It is a flowering plant native to the wooded savannah and tropical forests of Africa and bears edible fruits. Also known as 'water berry', the fruits and other parts of the plant are used locally as charcoal, timber, food, medicine, fodder, bee forage and dyes [8]. *S. guineense* leaves are also used as remedy for diarrhoea and dysentery [9]. Scientific investigation of extracts of the plant reveal its antibacterial and antihypertensive effects [10,11]. In view of these, the present study was carried out to evaluate the antidiabetic potentials of *S. guineense* methanol leaf extract in streptozotocin – induced diabetic rats.

## MATERIALS AND METHODS

### Drugs and chemicals

Methanol, dimethylsulfoxide, 2,2-diphenyl-1-picrylhydazyl (DPPH), alpha glucosidase, p-nitrophenyl-α-D-glucopyranoside, sodium carbonate (Sigma Aldrich, Germany), metformin hydrochloride (Glucophage®, Merck, France), citric acid (BDH, England), streptozotocin and concentrated citrate solution (Santa Cruz Biotech., Germany). Other reagents used were of analytical grade.

### Animals

Adult Wistar rats of either sex were used for the study. The rats were housed in steel cages and acclimatized for two weeks to laboratory conditions before the study. They were maintained on standard rodent feed and allowed unrestricted access to potable drinking water. All applicable institutional and international guidelines for the care and use of animals were adhered to in all procedures [12].

### Plant material

Fresh leaves of *S. guineense* were collected in October from Suleja, Niger state, Nigeria and identified by a plant taxonomist. A voucher specimen was prepared (voucher number: NIPRD/H/6644) and deposited in the herbarium of the National Institute of Pharmaceutical Research and Development (NIPRD). The leaves were air-dried under shade for two weeks then milled mechanically to coarse powder.

### Extract preparation and phytochemical screening

A 200 g quantity of *S. guineense* leaf powder was extracted by maceration in 80 %v/v methanol (1:8) at room temperature. The mixture was filtered after 48 h with Whatman filter paper then the filtrate concentrated under vacuum. The concentrate obtained was dried on a hot water bath maintained at 50$^{o}$C. The dry extract (SG) was stored in an air tight container in a refrigerator at 4$^{o}$C until required. Phytochemical screening of the extract for tentative identification of the presence of free anthraquinone glycosides, combined anthraquinone glycosides, saponins, terpenes, flavonoids and alkaloids was carried out in accordance with standard rest procedures [13].

### High performance liquid chromatography fingerprinting of extract

Chromatographic separation was performed using a $C_{18}$ column (25 cm x 4.6 mm, 5 µm i.d. Phenomenex Luna®) with a compatible guard column maintained at 45°C. The mobile phase, consisting of: A, 0.01% formic acid/acetonitrile; B, 0.01% formic acid/water, was filtered through a 0.45 µm filter and degassed prior to use. A 20µL volume of extract was filtered through a 0.22 mm filter disk and injected into the column. Flow rate of the mobile phase was maintained at 0.8 mL/min, and peaks were separated according to the following linear gradient elution: 0 - 1 min, 82% A/18% B; 1 - 15 min, 82% A /18% B to 75% A/25% B; 15 - 20 min, 75% A /25% B to 65% A /35% B; 20 - 25 min, 65% A/35% B to 40% A/60% B; 25 - 26 min, 40% A/60% B to 82% A/18% B; followed by an equilibration with 82% A /18% B for 10 min. Wavelength for ultraviolet detection was 370 nm.

### Acute toxicity

Acute toxicity test in rats was done using a modification of Lorke's method, in two phases [14]. In the first phase, two groups of three rats each were given orally, 300 and 1000 mg/ kg of body weight of the methanol extract respectively and monitored for 24 h for physical signs of toxicity and mortality. The rats were subsequently observed for two weeks for delayed signs of toxicity and/or mortality. In the second phase, three groups of three rats each were orally administered 1250, 2500 and 5000 mg/kg respectively and monitored likewise. The median lethal dose in mice ($LD_{50}$) was calculated as the geometric means of the maximum dose producing 0% mortality and the minimum dose that produced 100% mortality.

### ANTIHYPERGLYCEMIC SCREENING

### Induction of experimental diabetes

Fifty rats were fasted overnight on day zero (0) during which they were granted unrestricted access to potable drinking water. Fresh streptozotocin solution in ice cold citrate buffer (0.1 M, pH 4.5) was prepared in aliquots and protected from light. The solution was immediately injected intraperitoneally to rats on day 1 at a dose of 60 mg/kg of body weight. Thereafter, the rats were granted access to food and 10%w/v sucrose solution for 48 h. After an overnight fast, blood glucose was taken at 72 h using an Accu-Chek glucometer (Roche, Mannheim, Germany) with its corresponding strips. Only rats with fasting blood glucose concentration (BGC) above 200 mg/dL were considered diabetic and used for the 2 week study.

### Oral glucose tolerance test

The experimental rats were fasted overnight but allowed access to drinking water. After the fast, the rats were divided into 5 groups (n=5) and individual and individual pre-treatment BGC values were recorded. Group 1 was the diabetic control and received distilled water (1 ml/kg). Other treatment groups comprised diabetic rats receiving the extract (250, 500 and 1000 mg/kg) and metformin hydrochloride (100 mg/kg). Thirty minutes after treatment of all the groups, each rat was administered an oral glucose load (1 g/kg) and BGCs recorded at 0, 30, 60, 90, 120, 180 and 240 min.

### Study design

Four experimental groups were used for the study and treated once daily for 14 days as follows: Group 1 served as the diabetic control and received the aqueous vehicle alone (1ml/kg). Groups 2 and 3 were treated with 250 and 500 mg/kg of extract prepared in aqueous vehicle while group 4 was treated with metformin hydrochloride (100 mg/kg). Oral glucose tolerance test was repeated again for all the groups at the end of the study period, as described previously.

### Serum biochemical analysis

At the end of the treatment period, the rats were euthanized by chloroform inhalation. Blood samples collected from each rat by cardiac puncture were dispensed into plain tubes, allowed to clot and centrifuged at 3500 rpm for 10 min. The serum was stored at -4°C and used for evaluation of biochemical parameters including electrolytes, creatinine, urea, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lipids, total and conjugated bilirubin, using commercial kits (Randox Laboratories, Antrim, UK).

### Effect of extract administration on kidney, liver weight and liver glycogen

The kidneys and liver of each rat was excised carefully and their weights were calculated relative to body weight of the rat on the same day. Approximately 1 g of tissue was cut from each liver for estimation of glycogen content expressed as gram per gram (g/g) of liver tissue. [15].

### Renal and pancreatic morphology assessment

Kidneys and pancreas obtained from the rats were fixed in 10% formal saline for at least 48 h. These were then processed routinely

and the tissues were embedded in paraffin wax. Histological sections were cut at 5 - 6 µm and stained with haematoxylin and eosin (HE). Sample slides bearing codes were examined by a pathologist blinded to the study design and treatment groups to identify histological changes.

**Alpha glucosidase inhibitory test**

A chromogenic method described previously was used [16]. Briefly, 20 µL of extract solution (0.625 – 10 mg/mL) was incubated for 5 min with 80 µL of 100 mM phosphate buffer solution (pH 6.8) and 40 µL of enzyme solution (0.76 unit/mL). After addition of 40 µL of *p*-nitrophenyl-α-D-glucopyranoside (5 mM), the mixture was further incubated for 15 min. The reaction was stopped by addition of 20 µL of 200 mM sodium carbonate and *p*-nitrophenol generated was measured at 405 nm using a microplate reader (GF-M3000, England). Test incubations were carried out in triplicate. Blank and control incubations were prepared by replacing enzyme and extract solution with 80 µL buffer solution and 20 µL DMSO in blank and control incubations respectively. Enzyme inhibition (%) was calculated using the relation:

$$\left[1-\frac{B}{A}\right]\times 100$$

Where A = absorbance of the control without test samples, and B = net absorbance of test sample (Test minus blank absorbance). The concentration of extract necessary to inhibit enzyme activity by 50% ($IC_{50}$) was calculated by linear regression where the abscissa (x) represents extract concentrations and the ordinate (y) represents the average inhibition (%) of enzyme activity.

**Antioxidant effect of extract**

The test was performed as described previously with slight modification [17]. Here, 0.01% w/v solution of DPPH was freshly prepared in methanol and kept away from light. A 150 µL volume of this solution was added to 50 µl of various concentrations (0.1565 - 5 mg/mL) of extract prepared in methanol. After 30 min of incubation in the dark, absorbance was taken at 492 nm. Free radical scavenging activity was calculated using:

$$100-\left[\left\{\frac{As-Ab}{Ac}\right\}\text{ x }100\right]$$

Where $A_s$ - $A_b$ = Net absorbance of sample, $A_c$ = Absorbance of control. The effective concentration of extract necessary to decrease the initial DPPH absorbance by 50% ($EC_{50}$) was calculated by linear regression where the abscissa (x) represents extract concentrations and the ordinate (y) represents the average percentage (%) scavenging capacity.

**Data analysis**

Results were expressed as mean ± SEM. Statistical analysis was carried out using one-way analysis of variance (ANOVA) using Graph Pad Prism 5.0 software. The data obtained was further subjected to dunnet's post *hoc* test; differences between treated groups and the untreated control were accepted as significant at P<0.05.

## RESULTS

The extract did not cause any obvious toxicity at all the doses used in both phases of the acute toxicity test. No mortality was recorded within 2 weeks following administration of extract doses up to 5000 mg/kg. Orally administered extract produced dose-dependent decrease in blood sugar in diabetic rats in an oral glucose tolerance test after diabetes injection. All the diabetic groups showed impaired tolerance to oral glucose seen as elevated blood sugar concentration for over 240 min following glucose administration (Table 1). The extract produced dose-dependent decrease in blood glucose concentration with time and a maximum dose of 1000 mg/kg of extract produced 31.35% reduction at 240 min relative to the control. This effect was observed to be higher than the effect produced by metformin. In a repeated glucose tolerance test after 14-day treatment, the extract (250 mg/kg) significantly (P<0.05, 0.01, 0.001) decreased the hyperglycemic peak in diabetic rats, as seen in the rapid drop in postprandial blood glucose concentration at a significantly faster rate compared to the diabetic control (Table 2). A similar effect was also produced by metformin (100 mg/kg). The onset of action was half an hour after extract administration and lasted for over 2 h in a time dependent manner, as blood glucose concentration decreased to normoglycemic levels.

**Table 1:** Effect of extract on oral glucose tolerance test following diabetes induction

| Treatment | Dose mg/kg | BGC (mg/dl) 0 min | 30 min | 60 min | 90 min | 120 min | 180 min | 240 min |
|---|---|---|---|---|---|---|---|---|
| Diabetic control | - | 541±35.03 | 551±23.53 | 536±36.0 | 477±30.04 | 465±20.20 | 436±20.95 | 366.5±38.3 |
| SG | 250 | 541.4±32.96 | 490.4±40.01 | 477.2±38.02 | 436.8±33.49 | 450.6±45.46 | 415±31.4 | 413.4±41.6 |
| | 500 | 528.6±40.21 | 491.6±60.73 | 462±57.44 | 423.6±51.31 | 417.4±52.85 | 368.6±55.01 | 344±60.97 |
| | 1000 | 518.2±43.55 | 443.2±58.67 | 415±46.50 | 383.2±46.85 | 383.4±50.96 | 338±50.93 | 251.6±59.3 |
| Metformin | 100 | 398.8±29.45 | 446.6±27.20 | 425.8±17.2* | 411±23.91 | 344.6±15.25** | 300.6±19.98** | 311.6±22.8 |
| NDNT | - | 71.8±2.60 | 97.2±7.11 | 80.8±4.20 | 80.8±4.2 | 96.3±6.40 | 85.8±4.81 | 80.3±2.11 |

NDNT: Non diabetic non treated group; *P<0.05; **P<0.01(Data analysis and post hoc test)

Serum biochemical analysis revealed that the extract did not significantly affect electrolyte and urea levels but elicited dose-dependent reduction of serum creatinine compared to the diabetic control (Table 3). Also, the extract caused a reduction in serum levels of liver marker enzymes, total and direct bilirubin and albumin; most of these reductions were observed to be dose-dependent (Table 4). Lipid profile results show that the 250 mg/kg extract decreased cholesterol, triglycerides and significantly (*P*<0.05) reduced serum HDL (Table 5). These changes were however observed to be non dose-dependent.

**Table 2:** Effect of extract on oral glucose tolerance test after 14-day treatment

| Treatment | Dose (mg/kg) | BGC (mg/dl) 0 min | 30 min | 60 min | 90 min | 120 min | 180 min | 240 min |
|---|---|---|---|---|---|---|---|---|
| Diabetic control | - | 404±98.61 | 365±54.85 | 370.3±43.9 | 357.3±40.11 | 321.3±35.26 | 280.3±62.41 | 235.3±80.68 |
| SG | 250 | 108±22.73 | 221.8±14.12* | 167.8±12.8** | 96±10.55** | 91±10.46*** | 84.75±19.3** | 66.25±30.96 |
| | 500 | 362±145.5 | 371±61.25 | 360.3±45.54 | 344±39.46 | 326.3±36.4 | 357.7±41.09 | 247.7±55.48 |
| Metformin | 100 | 141.8±29.3 | 178±12.76* | 122.3±21.1*** | 80.3±15*** | 69.25±8.3*** | 71.25±3.82** | 67±4.42 |
| NDNT | - | 64.4±3.42 | 92.8±4.51 | 86.8±5.77 | 73.6±4.12 | 73.6±3.28 | 69.0±3.29 | 56.0±2.83 |

*P<0.05, **P<0.01, ***P<0.001(Data analysis and post hoc test)

**Table 3:** Effect of extract on serum electrolytes and creatinine

| Treatment | Dose (mg/kg) | $Na^+$ | $K^+$ | $Cl^-$ | $HCO^-$ | Urea | Creatinine |
|---|---|---|---|---|---|---|---|
| Diabetic | - | 136.7±2.4 | 6.67±0.87 | 102.3±1.76 | 23±0.58 | 11.77±0.96 | 40.33±12.41 |
| SG | 250 | 137±2.89 | 7.70±0.35 | 105±3.46 | 22.5±0.29 | 12.63±1.76 | 37±8.66 |
| | 500 | 137.7±1.2 | 6.0±0.29 | 103.7±2.67 | 23±0.58 | 13.4±1.25 | 28.67±1.20 |
| Metformin | 100 | 147.2±1.07** | 18.34±5.96 | 117.6±2.71** | 25.2±1.16 | 20.16±6.35 | 35.20±6.32 |

**P<0.01(Data analysis and post hoc test)

**Table 4:** Effect of extract on liver enzymes, serum bilirubin and protein

| Treatment | Dose (mg/kg) | AST | ALT | ALP | TBIL | DBIL | TP | ALB |
|---|---|---|---|---|---|---|---|---|
| Diabetic | - | 386±184.6 | 154±24.58 | 362.7±110.9 | 2.83±0.37 | 1.53±0.43 | 60.67±7.62 | 24.0±4.36 |
| SG | 250 | 270.5±15.88 | 154.5±40.13 | 337.5±97.28 | 2.8±0.51 | 1.0±0.12 | 63.5±0.87 | 23.0±0.58 |
| | 500 | 257±74.45 | 131±28.69 | 430.7±51.34 | 2.4±0.45 | 0.87±0.13 | 56.67±4.91 | 20.0±2.89 |
| Metformin | 100 | 245±103.7 | 301±134.6 | 72.80±21.85* | 9.34±1.5** | 3.22±0.56 | 46.80±4.65 | 21.6±4.47 |

*P<0.05, **P<0.01 (Data analysis and post hoc test)

**Table 5:** Effect of extract on serum lipid profile

| Treatment | Dose(mg/kg) | CHOL | HDL | LDL | TGLY |
|---|---|---|---|---|---|
| Diabetic | - | 47.33±4.33 | 37.33±2.96 | 1.0±0.58 | 54.33±7.42 |
| SG | 250 | 25.0±6.93 | 20.50±6.06* | 1.0±0.00 | 30.83±6.42 |
| | 500 | 32.67±6.74 | 26.0±6.11 | 1.33±0.67 | 39.33±9.6 |
| Metformin | 100 | 44.8±8.81 | 2.40±0.25*** | 3.60±1.08 | 60.80±13.44 |

*P<0.05, ***P<0.001(Data analysis and post hoc test)

**Table 6:** Antioxidant activity of *S. guineense* leaf extract

| Concentration (mg/ml) | Antioxidant activity |
|---|---|
| 0.1565 | 32.79 |
| 0.3125 | 77.28 |
| 0.6250 | 81.23 |
| 1.2500 | 82.41 |
| 2.5000 | 79.87 |
| 5.0000 | 73.91 |

$EC_{50}$= 0.2 mg/ml.

**Table 7:** Alpha glucosidase inhibitory activity of *S. guineense* ethanol extract

| Concentration (mg/ml) | mean absorbance due to p-nitrophenol generated | Inhibition (%) |
|---|---|---|
| Control | 3.071 ± 0.16 | - |
| 0.625 | 2.615 ± 0.04** | 14.86 |
| 1.25 | 2.367 ± 0.01*** | 22.94 |
| 2.5 | 2.168 ± 0.04*** | 29.40 |
| 5 | 1.793 ± 0.04*** | 41.63 |
| 10 | 0.649 ± 0.04*** | 79.19 |

$IC_{50}$= 6.15 mg/ml. **P<0.01; **P<0.001 (Data analysis and post hoc test)

**Table 8:** Effect of extract on absolute kidney, liver weight and liver glycogen

| Treatment | Dose (mg/kg) | Relative kidney weight (g) | Relative liver weight (g) | Liver glycogen (gram/gram of tissue) |
|---|---|---|---|---|
| Diabetic control | - | 0.66±0.03 | 3.50±0.31 | 0.59±0.03 |
| SG | 250 | 0.68±0.07 | 2.99±0.19 | 0.79±0.19 (33.9%) |
| | 500 | 0.70±0.05 | 3.26±0.23 | 0.55±0.004 |
| Metformin | 100 | 0.97±0.03** | 3.35±0.23 | 0.59±0.03 |

Value in parenthesis (%) represents percentage increase in liver glycogen content relative to the diabetic control group. **P<0.01(Data analysis and post hoc test)

Serum biochemical analysis revealed that the extract did not significantly affect electrolyte and urea levels but elicited dose-dependent reduction of serum creatinine compared to the diabetic control (Table 3). Also, the extract caused a reduction in serum levels of liver marker enzymes, total and direct bilirubin and albumin; most of these reductions were observed to be dose-dependent (Table 4). Lipid profile results show that the 250 mg/kg extract decreased cholesterol, triglycerides and significantly ($P$<0.05) reduced serum HDL (Table 5). These changes were however observed to be non dose-dependent.

An antioxidant effect was observed to be produced by the extract in a concentration-dependent manner (Table 6). A maximum radical scavenging effect was produced at a concentration of 1.25 mg/mL of extract and a concentration of 0.2 mg/ml was estimated to be the efficient concentration required to elicit 50 % radical scavenging capacity ($EC_{50}$). Similarly, the extract elicited significant (P < 0.01, 0.001), concentration-dependent inhibition of alpha glucosidase at all tested concentrations and the concentration required to inhibit enzyme activity by 50 % ($IC_{50}$) was estimated to be 6.15 mg/ml (Table 7).

Gross examination of excised liver and kidneys of treated and control groups revealed that the extract did not significantly alter the absolute and relative weights of these organs. Further studies however showed that liver glycogen content was elevated in diabetic rats treated with 250 mg/kg extract, compared to the diabetic untreated control (Table VIII). Histopathological analysis of kidneys of the diabetic untreated control revealed complete loss of nuclei within the collecting duct which appeared dense with epithelial destruction and glomerular atrophy. Diabetic groups which received metformin, 250 and 500 mg/kg of extract showed normal glomeruli although the nuclei within collecting duct appeared slightly enlarged or diffuse (Figure 1). Pancreatic tissue of the diabetic untreated group appeared to have fewer acinar cells with widened interstitial spaces compared to the normoglycemic and extract-treated groups (Figure 2).

Alkaloids, flavonoids, saponins and terpenoids were detected in the extract, whereas glycosides and anthraquinone derivatives where not detected. High performance liquid chromatoghy fingerprint of the extract revealed rutin and quercitrin as some of the polyphenolic constituents (Figure 3). Three unknown, prominent constituents with retention times of 3.538, 4.661 and 13.218 min respectively were also present in the extract.

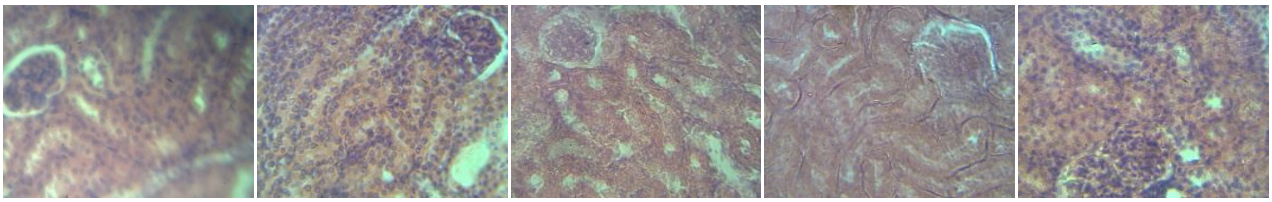


**Figure 1:** A- E: Photomicrographs (haematoxylin/eosin, ×400 magnification) of kidney tissue of A: Normoglycemic, B: Diabetic non treated, C: Diabetic + 250 mg/kg extract, D: Diabetic + 500 mg/kg extract, E: Diabetic+ metformin groups

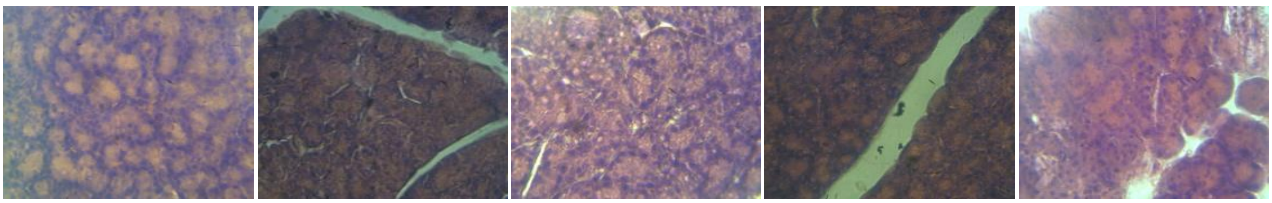


**Figure 2:** A- E: Photomicrographs (haematoxylin/eosin, ×400 magnification) of pancreatic tissue of A: Normoglycemic, B: Diabetic non treated, C: Diabetic + 250 mg/kg extract, D: Diabetic + 500 mg/kg extract, E: diabetic+ metformin groups

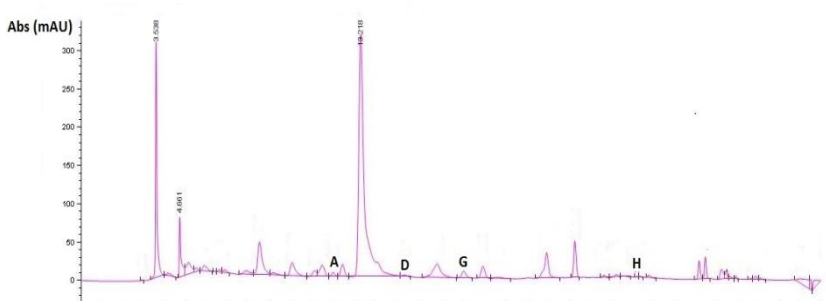


**Figure 3:** High performance liquid chromatogram of *S. guineense* methanol leaf extract showing presence of: A. rutin, D. kaempferol-3-O-rutinoside, G. quercitrin and H. quercetin

## DISCUSSION

The absence of mortality at 5000 mg/kg shows that the extract has a wide safety margin following oral acute administration. The induction of diabetes by injection of streptozotocin generates free radicals and causes breaks in DNA of pancreatic beta cells and these results in their selective destruction, producing a type II diabetes mellitus disease model [18]. This ultimately manifests as insulin deficiency and hyperglycemia and is characterized by increased glycosylation of haemoglobin, lipid peroxidation and reduced glutathione activity [18]. The blood glucose lowering effect of *S. guineense* leaf extract observed in the oral glucose test before sub acute treatment may be attributed to improved tissue uptake and storage of glucose, similar to the mechanism of action of metformin [7]. In addtion to this effect, continuous treatment with the extract may also stimulate residual beta cells to secrete insulin and improve tissue insulinotropic responses such as glycogen synthesis and storage, facilitating systemic glucose clearance. This is likely as some *Syzygium* species have also been reported to improve insulin sensitivity and promote insulin-mediated glucose uptake and storage in liver and adipose tissue [19]. The antioxidant activity exhibited by the extract may also mitigate disease progression, as reactive oxygen species are involved in the development of diabetic complications. Extracts of *S. guineense* leaves have been shown to possess strong antioxidant effects and this supports the antioxidant effect of the extract seen in our study [20]. Tissue damage in diabetes is mediated by free radicals which act on cell membranes and cause peroxidation of unsaturated fatty acids, ultimately leading to extensive membrane damage and dysfunction [21]. Also, there is an increased, uninhibited mobilization of free fatty acids from adipose tissue when insulin is deficient causing elevation in serum lipids. Hence, the lipid lowering effects and antioxidant activity of the extract potentially contributes to its antidiabetic effects observed in this study. The anti-dyslipidemic effect of the extract may be a secondary one, following the simulation of insulin release and action which increase in lipoprotein lipase activity and lowers plasma triglyceride levels [22].

The elevation of serum biomarker enzymes such as ALT, AST and ALP in untreated diabetes is an indication of impaired liver function due to hepatic damage induced by hyperglycemia [23]. The ability of the extract to reduce serum levels of these marker enzymes indicates its ability to alleviate the oxidative degenerative effects of streptozotocin on hepatocytes. This finding is supported by a previous report on the antioxidant effects of *S. guineense* on oxidative stress in the liver [20]. Likewise, the serum creatinine-lowering and albumin-reducing effect of the extract may also be attributed to its ability to ameliorate the progression of renal dysfunction in diabetes. Diabetic nephropathy is a leading cause of end stage renal failure and a relatively common complication of diabetes mellitus that ocurs when there is progessive oxidative renal injury and fibrosis, which manifests in early stage of disease as albuminuria and inscrease in serum creatinine [24]. The extract likely scavenges free radicals generated in renal tissue, reversing tubular damage. Further evidence to this is seen in the restorative effect in kidneys of extract-treated groups where glomerular damage was reversed, similar to the metformin-treated group.

The inhibition of alpha glucosidase by the extract indicates its ability to significantly prevent increase in blood glucose concentration following a meal. Notably, drugs which inhibit carbohydrate metabolizing enzymes like alpha glucosidase are commonly used in combination with regulated diet to control post prandial hyperglycemia [7]. They prevent the breakdown of carbohydrates such as dextrins, maltose, sucrose and starch to monosaccharides in intestinal brush borders and retard the release of large quantities of glucose from the intestine into bloodstream and its absorption following a meal [25]. In recent years, some plants have been known to be important inhibitors of these enzymes and have been receiving attention for their potential for development as antihyperglycemic agents [26]. The inhibitory action of the extract on alpha glucosidase elucidates it as a promising agent in this regard. This effect may be related to the phytochemicals contained within the some species of *Syzygium* as species like *S. cumini* and *S.aromaticum* reportedly show alpha glucosidase inhibitory activity [27,28]. Secondary metabolites such as flavonoids and terpenoids detected in the extract have been shown to have antihyperglycemic effect in other plant extracts. They may exert their effects by acting singly or synergistically to improve glucose homeostasis and oxidative metabolism in diabetes [29]. They have also been reported to reduce hyperglycemia through modulation of a glucose transporter protein [30]. Of the polyphenols; rutin, kaempferol-3-o-rutinoside, quercitrin and quercetin identified in the chromatogram of the extract, rutin and quercetin have been reviewed as promising oral antidiabetic agents [31,32]. These polyphenols also scavenge free radicals and may contribute to the antioxidant effects produced by the extract in this study.

## CONCLUSION

This study shows that *S. guineense* methanol leaf extract shows potential for development as an antidiabetic agent. Antioxidant, enzyme inhibitory activities and tissue glucose uptake are likely mechanisms through which its antidiabetic effects are mediated.

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