

Hypoglycemic and *in vitro* antioxidant activities of *Stereospermum kunthianum* stem bark hydromethanol extract

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Abstract

This study investigated *Stereospermum kunthianum* stem bark hydromethanol extract for hypoglycemic and *in vitro* antioxidant activities. Phytochemical analyses were performed to determine the components of the extract. *In vitro* antioxidant activities of the extract were assessed with 1, 1-diphenyl-2-picrylhydrazyl radical scavenging and ferric reducing /antioxidant power assays. An oral acute toxicity study to determine the safe dose of the extract was conducted in albino Wistar rats. Evaluation of the extract's hypoglycemic activities on blood glucose levels of normal, glucose loaded and streptozotocin-induced diabetic rats were performed. The extract contained various constituents, including saponins, terpenes, tannins, flavonoids, carbohydrates, alkaloids, and steroids. 1, 1-diphenyl-2-picrylhydrazyl radical scavenging assay proved more sensitive, showing a concentration-dependent increase in antioxidant capacity of the extract, which peaked at 71.8% relative to ascorbic acid (92.9%) at the highest test concentration (400 µg mL⁻¹). The rats tolerated *Stereospermum kunthianum* extracts with the oral LD₅₀ of more than 5000 mg kg⁻¹. Interestingly, various doses (100-400 mg kg⁻¹) of the extract were effective in causing significant (p≤0.05) reductions in the blood glucose levels of normoglycemic, glucose challenged and streptozotocin-induced diabetic rats when compared to their controls. The results suggest that the stem bark extract of *S. kunthianum* was a relatively safe herbal extract, rich in phytochemicals, possessed good antioxidant activity, with potential anti-diabetic effects.

Keywords: diabetic indices; extraction; glibenclamide; hyperglycemia; oxygen radicals

Abbreviations: Abs: Absorbance; ANOVA: One-way analysis of variance; AVMA: American Veterinary Medical Association; DPPH: 1, 1-diphenyl-2-picrylhydrazyl radical; Fe(III)-TPTZ: Ferric tripyridyltriazine complex; (Fe (II)-TPTZ): Ferrous tripyridyltriazine; FRAP: Ferric Reducing/Antioxidant Power; h: hour; NIPRID: National Institute for Pharmaceutical Research and Development; SKSB: *Stereospermum kunthianum* stem bark; STZ: Streptozotocin; TETFUND: Tertiary Education Trust Fund; WHO: World Health Organization.

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Introduction

Diabetes mellitus is a metabolic disease of public health concern, contributing to severe health complications and premature deaths in many developing countries (WHO, 1985). The disease is not only a leading cause of mortality and reduced life expectancy but is considered to be one of the largest global public health concerns, imposing a heavy global burden on public health as well as socio-economic development (Lin *et al.*, 2020). In 2017, global incidence, prevalence, death, and disability-adjusted life -years associated with diabetes were reported to be 22.9 million, 476.0 million, 1.37 million, and 67.9 million, with a projection of 26.6 million, 570.9 million, 1.59 million, and 79.3 million in 2025, respectively (GBD 2015 Risk Factors Collaborators, 2016). The disorder is multifactorial, and reactive oxygen species (ROS) are implicated (Alberti and Zimmet 1998; Pizzino *et al.*, 2017). Conventional hypoglycemic therapy is expensive and requires a prolonged period of medication but generally ineffective due to the inability to restore metabolic homeostasis resulting in severe complications (Chaudhury *et al.*, 2017; Papatheodorou *et al.*, 2018). Also, the current oral anti-diabetic agents represented by insulin secretors, sensitizers and glucosidase inhibitors have modest action with limited effectiveness (Yu *et al.*, 1999). *Stereospermum kunthianum* (Bignoniaceae), commonly known as *Stereospermum kunthianum* Cham., “Sandrine Petit” or “pink jacaranda”, is often grown for ornamental purposes (Keay, 1989). The plant is a deciduous shrub or small tree (3-15 meters tall) found in tropical countries of Africa and some in Asian (Keay, 1989). Many authors have documented the tree as an effective remedy for diabetes, wounds, cough and diverse diseases in African traditional medicine (Gill, 1992; Igoli *et al.*, 2005; Tropical Plants Database, 2014). The aqueous stem bark extract displayed anti-inflammatory activity against generalized seizures in pentylenetetrazole and electro-convulsive models in rodents (Ching *et al.*, 2009). The plant extract also showed antidiarrheal activities in rodents (Chin *et al.*, 2008). Despite its acclaimed high folkloric efficacy against notable diseases, there is no information about its anti-diabetic potential. This study is therefore designed to evaluate the stem bark methanol extract of *S. kunthianum* for hypoglycemic and *in vitro* antioxidant properties using standard experimental models.

Materials and Methods

Plant identification/collection

Fresh stem bark of *S. kunthianum* was collected from Suleja in Niger State, Nigeria in December 2017. Malam I. Muazzam, a plant taxonomist with the Ethnobotany and Herbarium section of the National Institute for Pharmaceutical Research and Development (NIPRID) Abuja, Nigeria identified the plant material. A deposit of a voucher specimen (NIPRID.H.7072) was made in the herbarium of NIPRID for reference.

Extract preparation

The leaves were air-dried to constant weight and pulverised into a coarse powder using an electric hammer mill (Jiangxi, Chin) and 1,600 g macerated in 80% methanol (JHD, China) for 48 h. The filtrate was concentrated *in vacuo* using a rotary evaporator (Rotavapor R 210, Buchi, Switzerland). The extract of *Stereospermum kunthianum* stem bark (SKSB) obtained was preserved in the refrigerator at 4 °C until when used.

Phytochemical screening

Phytochemical tests for the presence of saponins, terpenes, tannins, steroids, flavonoids, anthraquinones, carbohydrates and alkaloids in SKSB extract were carried out using the methods described by Evans (2005).

*Antioxidant activity*1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay

The antioxidant activity of the SKSB extract was evaluated with a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay as described by Mensor *et al.* (2001). One millilitre of 0.3 mM DPPH methanol solution was added to different concentrations of 2.5 ml solution of the extract (25, 50, 100, 200, 400 $\mu\text{g ml}^{-1}$) and allowed to react at room temperature for 30 min and the control similarly prepared but without the extract. Methanol was used for the baseline correction. The absorbance of the resulting mixture was measured at 517 nm and calculated as percentage antioxidant activity, using the formula:

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of control} - (\text{Absorbance of sample} - \text{Absorbance of blank})}{\text{Absorbance of control}} \times \frac{100}{1} \quad (1)$$

A mixture of 1.0 ml of methanol plus 2.0 ml of the extract was used as the blank, while 1.0 ml of the 0.5 mM DPPH solution plus 2.0 ml of methanol served as a negative control. Ascorbic acid is used as a standard reference.

Ferric reducing/antioxidant power (FRAP)

The total antioxidant potential of the extract was determined using a ferric reducing ability of plasma assay of Benzie and Strain (1996) as a measure of 'antioxidant power'. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue coloured ferrous tripyridyltriazine (Fe (II)-TPTZ) compound from colourless ferric tripyridyltriazine (Fe (III)-TPTZ) complex by the action of electron-donating antioxidants, a reduction at low pH. The mixture of Sample- FRAP reagent 2 ml and extract 100 μl monitored for 5 min at 593 nm, 1 cm light path and 25 °C. The antioxidant activity of the extract (25-400 $\mu\text{g ml}^{-1}$) was compared with that of ascorbic acid as a standard, calculated as FRAP value of sample (μM):

$$\frac{\text{Change in absorbance of standard (0-5 min)} \times \text{FRAP value of the standard (1000 } \mu\text{M)}}{\text{Change in absorbance in absorbance of standard (0-5 min)}} \quad (2)$$

Experimental animal

Matured albino Wistar rats (150-200 g) of both sexes obtained from NIPRID were used for the study. Housing the animals was in wire-mesh cages; they were fed *ad libitum* with pelleted feed (Vital feed, Nigeria) and water except when fasting was required. Approval for the experimental protocol was from the Institutional Animal Ethics Committee (UAECAU/2018/003). The animal handling was in line with the Guide for the Care and Use of Laboratory Animals of the National Research Council (National Research Council, 2010).

Acute toxicity test

The acute toxicity study of SKSB extract was conducted using the method described by Lorke (1983). The experiment's first phase engaged nine rats of both sexes (6 males; 3 females), separated into three groups of 3 rats each (2 males and a female) treated orally with 10, 100 and 1000 mg kg^{-1} of the extract respectively. The animals were monitored every two hours for three days, and the toxic effects were noted. In the absence of mortality, new sets of rats were treated in the same manner at 2000, 3000 and 5000 mg kg^{-1} , respectively. Animals that survived were monitored for 14 days. The oral median lethal dose of extract was calculated using the formula:

$$\text{LD}_{50} = \sqrt{(\text{Minimum toxic dose} \times \text{maximum toxic dose})} \quad (\text{Abdu } et al., 2018). \quad (3)$$

Normoglycemic study

Normal rats were used to determine the anti-hyperglycemic prophylactic activity of SKSB extract. In this study, there were five groups (A, B, C, D and E). Group A consisted of normal rats treated with distilled water (10 ml kg^{-1}) to serve as the negative control. The rats in group B were treated with glibenclamide (0.2 mg

kg⁻¹ p.o) to serve as the reference hypoglycemic drug, while the rats in groups C, D, and E received the extract at doses of 100, 200 and 400 mg kg⁻¹ *per os* respectively. The blood glucose levels were measured before the half, one, two, and four h after administration and compared with those of the control group. Blood glucose levels were measured in mg dl⁻¹ with a glucometer, using the tail tipping blood sample technique (Cunha *et al.*, 2008).

Glucose challenge test

In this study, the glucose challenge test (Oral Glucose Tolerance Test, OGTT) as a standard experimental procedure was followed (Cunha *et al.*, 2008). The experiment consisted of 5 groups (A, B, C, D and E). Group A had normal rats that were given distilled water (10 ml kg⁻¹) to serve as the negative control. In contrast, the rats in group B were treated with glibenclamide (0.2 mg kg⁻¹ *p.o*), a reference hypoglycemic drug. The rats in groups C, D and E received the extract at oral doses of 100, 200 and 400 mg/kg, respectively. Before experimentation, at zero hour, the blood glucose level for each rat was measured. Hyperglycemia was induced by the oral glucose administration (10 g kg⁻¹) to the five rats in each group. Thirty minutes before the administration of glucose, the animals (5 per group) were treated with a single oral administration of the extract (100, 200, and 400 mg kg⁻¹) (extract treatment groups), distilled water (negative control group) and glibenclamide (0.2 mg kg⁻¹) (positive control group). Blood glucose levels were determined three hours, at one h intervals, and 30 min after glucose administration. A comparison of the blood glucose levels of the negative control group and the treatment groups was made. The blood glucose was measured with the glucometer (mg dl⁻¹) using the tail tipping blood sample technique (Diehl *et al.*, 2001).

Streptozotocin (STZ)-induced diabetic test

The experiment was conducted in two phases; phase 1: Induction of diabetes and phase 2: treatments/hypoglycemic evaluation over four h. Phase 1 commenced with 35 albino Wistar rats of both sexes (25 males and ten females) marked with 10% picric acid, weighed and fasted overnight for 16 h. Induction of diabetes was by a single intraperitoneal administration of a freshly prepared stz (STZ) 50 mg kg⁻¹ dissolved in normal saline. Post STZ administration, 25 rats were harvested for phase II experiment (on the basis of a range of elevated blood glucose levels of 150 mg dl⁻¹ and above but < 560 mg dl⁻¹).

Phase II: The twenty-five (25) STZ-induced diabetic rats of both sexes (20 males and five females) were randomly allocated to five experimental groups (A, B, C, D, and E), consisting of five rats per group. Group A (negative control) had STZ-induced diabetic rats to which normal saline (10 mg kg⁻¹) was administered. Group B (positive control) consisted of STZ-induced diabetic rats to which glibenclamide (0.2 mg kg⁻¹) was administered. Groups C, D, and E had STZ-induced diabetic rats, given a single oral administration of SKSB extract at 100, 200 and 400 mg kg⁻¹, respectively. The glucose levels produced were measured just prior to and at half, one, two, three and four h after extract/ drug administration (Cunha *et al.*, 2008).

Statistical analysis

Data obtained was presented as mean ± SEM and analysed using one-way analysis of variance (ANOVA). Posthoc comparisons using the Duncan test on SPSS version 23 (IBM corporation, Armonk, USA) were conducted. A value of P < 0.05 was considered significant in the study.

Results

Phytochemical analyses

Phytochemical screening revealed the presence of saponins, terpenes, tannins, steroids, flavonoids, carbohydrates and alkaloids in SKSB extract.

DPPH assay

The antioxidant activities of SKSB extract were significantly ($p < 0.05$) lower compared to ascorbic acid at all concentrations (25-400 $\mu\text{g ml}^{-1}$) tested. The extract produced $36.2 \pm 3.1\%$, $57.9 \pm 0.6\%$, $66.1 \pm 0.8\%$, $66.3 \pm 2.2\%$ and $71.8 \pm 0.2\%$ antioxidant activity at 25, 50, 100, 200 and 400 $\mu\text{g ml}^{-1}$ compared to ascorbic acid which produced antioxidant activity of $70.8 \pm 0.3\%$, $83.7 \pm 0.8\%$, $89.8 \pm 1.6\%$, $93.3 \pm 0.3\%$ and 92.9% at the same concentrations, respectively (Figure 1).

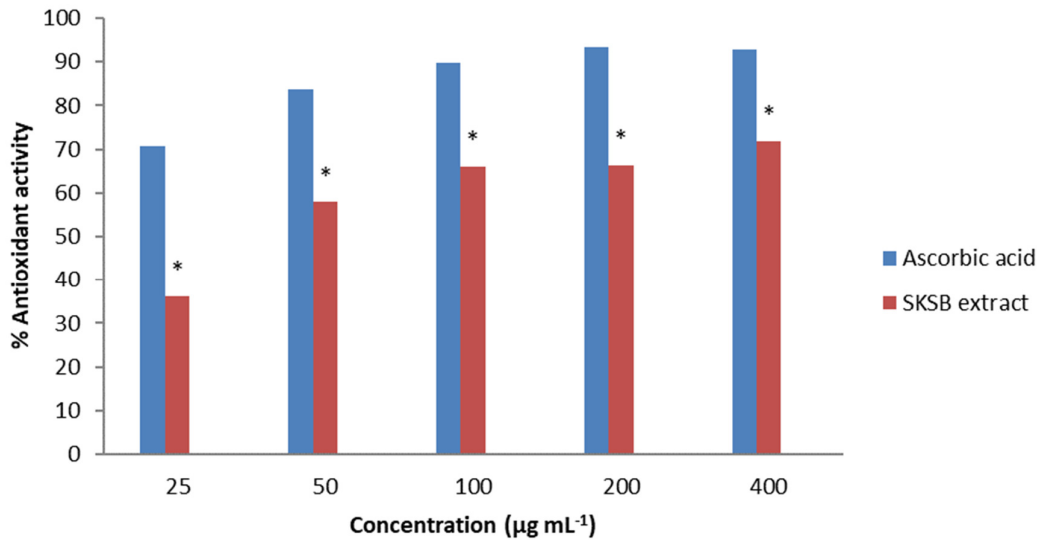


Figure 1. DPP assay of *S. kunthianum* stem bark extract

*Significantly ($P < 0.05$) reduced compared to ascorbic acid

FRAP test

The FRAP values produced by the extract were significantly ($P < 0.05$) reduced at each concentration compared to those of ascorbic acid. SKSB extract had FRAP values of 0 , 0.02 ± 0.02 , 0.05 ± 0.01 , 0.16 ± 0.0 and $0.29 \pm 0.02 \mu\text{M}$ at 25, 50, 100, 200 and 400 $\mu\text{g ml}^{-1}$ respectively while ascorbic acid produced 0.21 ± 0.00 , 0.33 ± 0.01 , 0.68 ± 0.01 , 1.27 ± 0.05 and $1.48 \pm 0.02 \mu\text{M}$ at the same concentration (Figure 2).

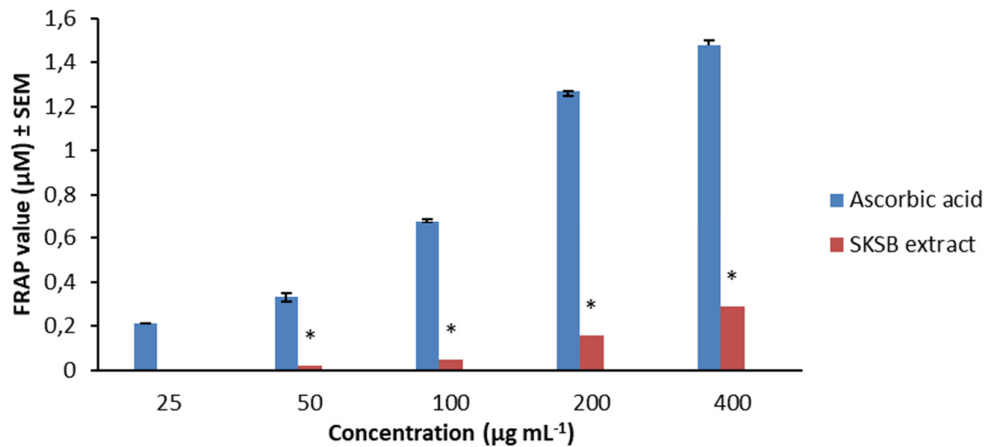


Figure 2. FRAP test with *S. kunthianum* extract

*Significantly ($P < 0.01$) reduced value compared to ascorbic acid

Acute toxicity test

No signs of toxicity and death occurred in the treated rats at the maximal test dose (mg kg^{-1}). Therefore, the LD_{50} of the extract was greater than 5000 mg kg^{-1} .

Normoglycemic test

The normoglycemic test results are presented in Tables 1a and 1b. There were no significant ($P > 0.05$) differences between the blood glucose levels of treated rats and the negative control at 0 h. The blood glucose levels of all the experimental rats increased significantly ($P < 0.05$) at 1/2 h and 1 h compared to their respective normal blood glucose levels at 0 h (baseline) showing hyperglycemia immediately following treatments (Tables 1a). However, at 2, 3 and 4 h, there were significant ($P < 0.05$) reductions in the blood glucose values of treated rats compared to the corresponding levels at half and one h post-treatment. The onset of blood glucose reductions (hypoglycemic activity) was observed to commence in both glibenclamide and extract ($100\text{-}400 \text{ mg kg}^{-1}$) treated groups at two hours and progressed to the end of the study (4 h) (Table 1a). The differences in the blood glucose produced by the various doses of extract and glibenclamide was significantly ($P < 0.05$) higher at two h post-treatment than at three or four h post-treatment. At two h, glibenclamide (0.2 mg kg^{-1}) achieved percentage glucose reduction of 26.8% while SKSB extract at 100, 200 and 400 mg kg^{-1} produced 24.5%, 31.1% and 20.9% blood glucose reductions, respectively (Table 1b).

Table 1a. Hypoglycemic activity of SKSB extract in normal rats

Group	Time post-treatment (hours)					
	0 h	½ h	1 h	2 h	3 h	4 h
	Rat blood glucose mg dl^{-1}					
Control	64.20 ± 6.8	76.20 ± 6.5	72.40 ± 9.6	69.80 ± 5.5	65.80 ± 4.7	65.20 ± 8.1
Glibenclamide (0.2 mg kg^{-1})	55.20 ± 0.9	80.00 ± 4.7	79.00 ± 4.7	40.40 ± 1.8*	48.00 ± 3.1*	49.20 ± 3.1*
SKSB extract (100 mg kg^{-1})	55.40 ± 5.3	71.40 ± 5.4	69.20 ± 4.5	41.80 ± 2.9*	49.40 ± 2.7*	51.20 ± 4.6*
SKSB extract (200 mg kg^{-1})	68.20 ± 4.0	78.80 ± 7.0	73.80 ± 3.8	47.00 ± 2.6*	60.80 ± 5.9*	61.00 ± 3.9*
SKSB extract (400 mg kg^{-1})	64.00 ± 2.4	94.80 ± 5.3	73.20 ± 4.6	50.60 ± 0.5*	56.60 ± 2.1*	60.20 ± 1.6*

n=5; SKSB= *Stereospermum kunthianum* stem bark extract; *significant ($P < 0.05$) reductions compared to values at 0 h, ½ h and 1 h post treatment; h = hour.

Table 1b. Calculated blood glucose difference & Percentage reduction post-treatment of normal rats

Group	Time post-treatment (hours)					
	0 h	½ h	1 h	2 h	3 h	4 h
	blood glucose difference & %glucose reduction in parentheses					
Control	0	-12 (-18.7%)	-8.2 (-12.8%)	-5.6 (-8.7%)	-1.6 (-2.5%)	-1 (-1.6%)
Glibenclamide (0.2 mg kg^{-1})	0	-24.8 (-44.9%)	-23.8 (-43.1%)	14.8** (26.8%)	7.2* (13.0%)	6* (10.9%)
SKSB extract (100 mg kg^{-1})	0	-16 (-28.9%)	-13.8 (-24.9%)	13.6** (24.5%)	6* (10.8%)	4.2* (7.6%)
SKSB extract (200 mg kg^{-1})	0	-10.6 (-15.5%)	-5.6 (-8.2%)	21.2** (31.1%)	7.4* (10.9%)	7.2* (10.6%)
SKSB extract (400 mg kg^{-1})	0	-30.8 (-48.1%)	-9.2 (-14.4%)	13.4** (20.9%)	7.4* (11.6%)	3.8* (5.9%)

n=5; ** $P < 0.01$; * $P < 0.05$ when compared with distilled water treated control within same treatment period (2, 3 and 4 h); h = hour; SKSB = *Stereospermum kunthianum* stem bark

Glucose challenge test

In this study, there were significant ($P < 0.05$) increases in the blood sugar levels (hyperglycemia) of all rats in the different experimental groups at one h post glucose challenge. The increased blood sugar level in the control group was not significantly ($P > 0.5$) reduced/normalised to the baseline value even at the end of the study (4 h). Glibenclamide (0.2 mg kg^{-1}) treatment significantly ($P < 0.05$) reversed the hyperglycemia observed at one h to normal/baseline value within two h and three h, and further reduced the blood glucose level to $60.8 \pm 4.7 \text{ mg dl}^{-1}$ below the baseline value ($79.2 \pm 5.6 \text{ mg dl}^{-1}$) at four h. In the same manner, at two, three and four h post-treatment, 100 and 200 mg kg^{-1} of the extract significantly ($P < 0.05$) reversed the respective hyperglycemic values of $102.8 \pm 7.3 \text{ mg dl}^{-1}$ and $89.2 \pm 4.73 \text{ mg dl}^{-1}$ to values not significantly ($P > 0.05$) different from their baseline. SKSB extract (400 mg kg^{-1}) had the most profound hypoglycemic effect in lowering the high blood sugar level of $109.2 \pm 5.7 \text{ mg dl}^{-1}$ to 75.6 ± 5.7 , 71.4 ± 3.2 and $72.6 \pm 4.1 \text{ mg dl}^{-1}$ at two, three and four h post-treatment, respectively. The blood glucose level at each of the three time points (two, three and four h) was significantly ($P < 0.05$) lowered below the baseline value of $94.0 \pm 2.8 \text{ mg dl}^{-1}$ for the group (Table 2).

Table 2. Effects of SKSB extract on Glucose challenge test (Oral glucose tolerance test, OGTT)

Group treatment	Time post-treatment (hours)				
	0 h	1 h	2 h	3 h	4 h
	Blood sugar level (mg dl^{-1})				
Negative control	64.2 ± 6.5	$121.6 \pm 2.9^*$	$98.2 \pm 2.5^*$	$91.8 \pm 4.9^*$	$93.6 \pm 6.0^*$
Glibenclamide (0.2 mg kg^{-1})	79.2 ± 5.6	$106.0 \pm 3.9^*$	86.4 ± 2.8	73.6 ± 3.5	$60.8 \pm 4.7^{**}$
SKSB extract (100 mg kg^{-1})	89.6 ± 4.7	$102.8 \pm 7.3^*$	91.4 ± 3.1	89.6 ± 4.2	88.6 ± 4.8
SKSB extract (200 mg kg^{-1})	85.4 ± 7.8	$89.2 \pm 4.7^*$	78.6 ± 1.1	73.4 ± 2.4	75.8 ± 2.2
SKSB extract (400 mg kg^{-1})	94.0 ± 2.8	$109.2 \pm 5.7^*$	$75.6 \pm 5.7^{**}$	$71.4 \pm 3.2^{**}$	$72.6 \pm 4.1^{**}$

n=5; SKSB= *Stereospermum kunthianum* stem bark; *Significant ($P \leq 0.05$) increases compared to baseline value at 0 h; **Significantly ($P \leq 0.05$) lower compared to baseline value at 0 h; h = hour

Streptozotocin (STZ)-induced diabetic test

The results of STZ-induced diabetic test presented in Tables 3 showed the blood glucose levels of STZ-induced diabetic rats at one to four hours post-treatment while Table 4 calculated blood glucose difference and the percentage reduction in blood glucose further clarified the relative handling of the blood glucose by the treatment groups compared to the controls. At 30 min post-treatment, there were no significant ($P > 0.05$) differences in the effects of glibenclamide, 100 mg kg^{-1} of extract and the negative control, but 200 and 400 mg kg^{-1} of the extract caused significant ($p < 0.05$) reductions in the blood glucose levels (Table 3). Hence, the percentage reduction in the blood glucose produced by glibenclamide, 100 mg kg^{-1} of extract and the negative control were 12.7%, 10.8% and 10.1% compared to 16.8% and 19.0% for 200 and 400 mg kg^{-1} of the extract, respectively (Table 4). However, at one, three and four hours post-treatment, glibenclamide and the various doses (100 , 200 and 400 mg kg^{-1} extract) caused significant ($p < 0.05$) reductions in the rat blood glucose levels relative to the negative control (distilled water). At 3 and 4 h, the calculated blood glucose differences of 24.1% and 28.5% in the negative control were observed to be lower compared to those of glibenclamide: 26.4% and 28.5%; 100 mg kg^{-1} extract: 31.5% and 24.8%; 200 mg kg^{-1} extract: 25.8% and 28.3% and 400 mg kg^{-1} extract: 44% and 48.2% at both periods respectively. Interestingly, 400 mg kg^{-1} of extract induced a higher hypoglycemic potency of 44% and 48.2% at 3 and 4 h post-treatment compared to glibenclamide (positive control) which produced 26.4% and 28.5% respectively at the same periods (Table 4).

Table 3. Effects of SKSB extract on blood glucose level of Streptozotocin-induced diabetic rats

Group	Time post-treatment (hours)					
	0 h	½ h	1 h	2 h	3 h	4 h
	Blood glucose mg dl ⁻¹					
Negative control	429.30 ± 6.9	386.20 ± 17.0	380.0 ± 11.5	319.8 ± 14.6	326.0 ± 0.0	332.3 ± 12.8
Glibenclamide (0.2 mg kg ⁻¹)	518.7 ± 10.8	453.0 ± 26.1	425.3 ± 23.2*	402.3 ± 22.1	382.0 ± 20.1*	371.0 ± 24.0*
SKSB extract (100 mg kg ⁻¹)	473.0 ± 18.5	422.7 ± 19.1	408.0 ± 10.4	382.0 ± 17.3	324.0 ± 2.9*	355.7 ± 17.0*
SKSB extract (200 mg kg ⁻¹)	471.0 ± 14.1	392.0 ± 12.2*	366.0 ± 13.3*	342.7 ± 8.4*	325.0 ± 9.5*	349.3 ± 19.8*
SKSB extract (400 mg kg ⁻¹)	461.0 ± 16.7	373.3 ± 5.2*	368.0 ± 17.3*	320.3 ± 7.8*	258.0 ± 21.2*	238.7 ± 20.4*

n = 5; SKSB = *Stereospermum kunthianum* stem bark; *P ≤ 0.05 compared to distilled water treated control; h = hour

Table 4. Calculated blood glucose difference and percentage reduction in Streptozotocin-induced diabetic rats post treatment with SKSB extract

Group	Time post-treatment (hours)					
	0 h	½ h	1 h	2 h	3 h	4 h
	blood glucose difference & % reduction in parentheses					
Negative Control (Distilled water)	0	43.3 (10.1%)	49.3 (11.5%)	110.3 (25.7%)	103.3 (24.1%)	97 (22.6%)
Glibenclamide (0.2 mg kg ⁻¹)	0	65.7 (12.7%)	93.7 (18.1%)*	116.4 (22.4%)	136.7* (26.4%)	147.7* (28.5%)
SKSB extract (100 mg kg ⁻¹)	0	51.0 (10.8%)	65.0 (13.7%)	91 (19.2%)	149* (31.5%)	117.3* (24.8%)
SKSB extract (200 mg kg ⁻¹)	0	79.0* (16.8%)	105.0* (22.3%)	128.3* (27.2%)	121.7* (25.8%)	121.7* (28.3%)
SKSB extract (400 mg kg ⁻¹)	0	87.7* (19.0%)	93.0* (20.2%)	140.7* (30.5%)	203.0* (44.0%)	222.3* (48.2%)

n=5; *significant (P < 0.05) increase within same treatment period compared to control; SKSB = *Stereospermum kunthianum* stem bark; h = hour

Discussion

This study evaluated the hypoglycemic and *in vitro* antioxidant activities of SKSB extract. The extract contained diverse constituents, including saponins, terpenes, tannins, steroids, flavonoids, carbohydrates and alkaloids, an indication of the richness of the plant in phytochemicals that may have bioactive properties (Bhattacharjee *et al.*, 2018). It has been reported that the common phytoconstituents found in medicinal plants with anti-diabetic potential include polyphenols, flavonoids, terpenoids, tannins, alkaloids, saponins (Mohd *et al.*, 2017). The stem bark extract of *S. kunthianum*, which contains most of these active compounds, may have anti-diabetic potential.

DPPH assay revealed the moderately high antioxidant activity of the extract at high concentrations; for example, 71.8% compared to 92.9% with ascorbic acid at the highest concentration (400 µg mL⁻¹). The FRAP values produced by the extract were however found to be remarkably lower than that of ascorbic acid at each of the concentrations (25-400 µg mL⁻¹) tested. For example, the extract had 0.29 ± 0.02 µM relative to 1.48 ± 0.02 µM for ascorbic acid at 400 µg mL⁻¹. The reduced FRAP values observed may be due to the crude nature of the

extract but ascorbic acid is a reference antioxidant. In this study, the DPPH assay appeared to be more sensitive, showing appreciable antioxidant activity of SKSB extract at high concentrations. The flavonoid and phenolic content of plants are directly related to their antioxidant properties. These metabolites act as reducing agents, hydrogen donors and are capable of scavenging free radicals (Jing *et al.*, 2015). ROS is implicated in various health challenges, including diabetes, inflammatory disorders, coronary heart diseases, cancer and many more (Pizzino *et al.*, 2017). The high antioxidant activity of *S. kunthianum* extract may underlie the folkloric use of the stem bark in concoctions to treat several diseases, including diabetes mellitus.

Rats tolerated well a single dose of SKSB extract (5000 mg kg⁻¹) as no signs of morbidity or mortality were recorded. The LD₅₀ may be greater than 5000 mg kg⁻¹. This level considers a test compound practically non-toxic on an acute exposure (OECD, 2001). Okpo and Ching (2013) had previously recorded no acute adverse behavioural effects or mortality in Swiss mice given an oral dose of the extract at 8000 mg kg⁻¹.

The hypoglycemic activity of *S. kunthianum* extract in normal rats showed that various doses (100, 200 and 400 mg kg⁻¹) of extract and glibenclamide (positive control) significantly reduced normal rats' blood glucose levels from the second hour of treatment. The reason behind the observed increase in glucose levels of all the experimental rats at ½ h and two h was not clearly understood. However, it may probably be a physiological response to the treatments. The blood glucose difference and percentage reductions at two h post-treatment helped to further elucidate the significance, showing the negative control group had -5.6 (-8.7%), negative and poor glucose handling relative to glibenclamide/extract (100, 200 and 400 mg kg⁻¹) treatment groups which positively reduced their blood glucose levels to 14.8 (26.8%), 13.6 (24.5%), 21.2 (31.1%) and 13.4 (20.9%) respectively. This finding is positive, that the extract can be an oral hypoglycemic agent.

The possible hypoglycemic activity of the extract became more evident in the Glucose challenge test (Oral glucose loading animal model). Often referred to as physiological induction of diabetes mellitus, it causes the blood glucose level of the animal to transiently increase with no damage to the pancreas (Yu *et al.*, 1999). Here the extract and glibenclamide treatment groups were seen to have significantly affected the handling of the hyperglycemia caused by the high loading of the experimental animals with glucose. In contrast, the control (distilled water) treated group showed a significant poor control/handling of the induced hyperglycemia. Hyperglycemic values in rats reversed to baseline values at two and three h but further reduced below the baseline at four h following treatment with glibenclamide (0.2 mg kg⁻¹). The lower test doses (100 and 200 mg kg⁻¹) of SKSB extract were merely able to normalize the increased rat blood glucose levels at two, three and four h post-treatment. However, the most profound hypoglycemic activity was exhibited when 400 mg kg⁻¹ of the extract depressed the hyperglycemic value in glucose challenged rats below the baseline value of 94.0±2.8 mg dl⁻¹ at the interval of two, three and four h following treatment.

Treatment of STZ-induced diabetic rats with glibenclamide (0.2 mg kg⁻¹) and the different doses (100, 200 and 400 mg kg⁻¹) of SKSB extract induced higher percentage blood glucose reductions compared to distilled water treated negative control at the one, three and four h post-treatment. Again, the dose of 400 mg kg⁻¹ of extract appeared to be most effective, producing a hypoglycemic effect (glucose reduction) of 30.5%, 44.0% and 48.2% at two-, three- and four-hour post treatment respectively.

Conclusions

The findings from the study suggest that methanol extract of *Stereospermum kunthianum* stem bark is relatively safe, rich in phytochemicals, possesses good antioxidant activity, and has potent hypoglycemic effects due to its ability to lower blood glucose levels in normal rats. It suppressed a postprandial rise in blood glucose levels and also reduced blood glucose levels in streptozotocin-induced diabetic rats.

Authors' Contributions

JOO, FCN, and MOE were involved in the conceptualization, design, execution, draft, and review of the manuscript; MMO, DDA, and SOO participated in the design, sourcing of material, statistical analysis, and review of the manuscript while GMA, HGM, and MA were involved in the supervision, review, and editing of the manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

The Institutional Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Abuja approved the experimental protocol.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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