European Journal of Medicinal Plants

25(4): 1-9, 2018; Article no.EJMP.45129
ISSN: 2231-0894, NLM ID: 101583475

Anti-diabetic Activity of Convallatoxin Isolated from the Root Bark of *Parquetina nigrescens* (Afzel.) Bullock (Asclepiadaceae)

Kolade Olatubosun Faloye[1], Marcus Durojaye Ayoola[2], Bamidele Martin Amos-Tautua[3] and Samson Oluwaseyi Famuyiwa[1*]

[1]Department of Chemistry, Faculty of Science, Obafemi Awolowo University, Nigeria.
[2]Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Nigeria.
[3]Department of Pure and Applied Chemistry, Faculty of Science, Niger Delta University, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author SOF designed the study and supervised the research work, carried out structural elucidation of the compound, process the manuscript to submission. Author MDA supervised the biological part of the research work, performed the statistical analysis. Author KOF carried out the research work under the supervision of authors SOF and MDA, generated the data, wrote the first draft of the manuscript and author BMAT took the sample to South Africa for NMR analysis on his own expenses. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/45129
Editor(s):
(1) Dr. Shanfa Lu, Professor, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, China.
(2) Dr. Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Emmanuel Ifeanyi Obeagu, University Health Services, Michael Okpara University of Agriculture, Nigeria.
(2) Muruganandan Shanmugam, School of Medicine, Wayne State University, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/27100

Original Research Article

Received 07 August 2018
Accepted 31 October 2018
Published 08 November 2018

ABSTRACT

This study investigated the anti-diabetic activity of the root bark extract of *Parquetina nigrescens* and the isolated compound, convallatoxin, from the root bark. A powdered sample of the plant was extracted with methanol, and the extract (**A**) was tested in glucose-loaded normal rats at 100, 200 and 400 mg/kg for the determination of the most active dose. The anti-diabetic activity of **A** at 200 mg/kg was carried out on streptozotocin-induced diabetic rats. **A** was further partitioned to obtain its *n*-hexane ($\mathbf{B_1}$), dichloromethane ($\mathbf{B_2}$), ethyl acetate ($\mathbf{B_3}$) and mother liquor ($\mathbf{B_4}$) fractions that were tested for blood glucose lowering activity using glucose-loaded normal rats model. The anti-

**Corresponding author: E-mail: oluwaseyi_f@yahoo.com;*

diabetic activity of the isolated compound from **B_3** was carried out on streptozotocin-induced diabetic rats. The results were subjected to one way ANOVA followed by Bonferroni post hoc tests and $p< 0.05$ was considered significant. **A** showed dose-independent and time dependent blood glucose level reduction activity at 200 mg/kg with the highest percentage of 30% at 4 h that was comparable to the standard, glibenclamide at 5 mg/kg. **A** at 200 mg/kg showed a blood glucose level reduction of 49, 68 and 70% by Day 4, 7 and 10 respectively as against glibenclamide at 5 mg/kg of 18, 33 and 39% by Day 4, 7 and 10 respectively on streptozotocin-induced diabetic rats. **B_3** showed a time-dependent blood glucose level reduction activity up to the fourth hour similar to glibenclamide with 35% as against 38% of glibenclamide. **B_1**, **B_2** and **B_4** were devoid of blood glucose level reduction activity. Convallatoxin isolated from **B_3** gave 83 % blood glucose levels reduction at day 10 as against 38% of glibenclamide. The anti-diabetic activity of convallatoxin was significantly ($p<0.05$) more than glibenclamide at all-time point. The structure of convallatoxin was determined using IR, 1D and 2D NMR spectroscopy and the spectroscopic data compared well with published data in the literature.

Keywords: Parquetina nigrescens; root bark; anti-diabetes; chromatography; convallatoxin.

1. INTRODUCTION

Diabetes mellitus is a multifarious disorder commonly presented with episodes of hyperglycaemia and glucose intolerance, due to absolute lack of insulin, defective insulin action, or both [1]. Globally, it affected about 171 million people in 2003; 173 in 2004, 285 in 2009, 371 in 2012 and 422 in 2016 [2-4] and expected to increase to about 552 million people by 2030 [5]. African and Asian continents have been projected to have the highest number of people with this disease by 2030 [6-8]. The complications of the disease and the existing drugs necessitated the continuous search for new potential drugs from anti-diabetic ethno-medicinal plants [7,8,9,10].

Parquetina nigrescens (Afzel.) Bullock (Asclepiadaceae) is a herbaceous perennial twine found in most part of Africa. It is known as "Ogbo" in South Western part of Nigeria and it is reported to be used ethno-medicinally for anti-diabetic, skin diseases, menstrual disorders and gonorrhoea [11-14]. The root bark is used in the management of diabetes mellitus in Akure, Ondo State of Nigeria (Personal Communication). Its analgesic, anti-inflammatory, antipyretic, anti-ulcerogenic, antioxidant, anti-sickling, antimicrobial, sympathomimetic, haematological, uterotonic and cytotoxic activities have been reported [15-22]. The aqueous leaf extract has been reported for anti-diabetic effect in alloxan-induced diabetic rats and it was also reported for its improvement in sexual activity in rats induced with sexual dysfunction [23,24]. Some of its isolated chemical constituents reported include strophanthidin, strophanthidin glycoside, cymarin and Isorhoifolin [15,25]. The chloroform soluble extract of the leaves was reported to ameliorate dichlorvos-induced cardio- and nephrotoxicity, neurotoxicity, oxidative stress and apoptosis in rats [26,27].

2. MATERIALS AND METHODS

2.1 General

NMR data was obtained from Bruker DPX Avance 400 instrument using methanol-d_4 as a solvent and internal standard. Analytical TLC plates, $60F_{254}$, were used. UV lamp (254) was used to view compounds on the TLC plates after which the plates were sprayed with vanillin-sulphuric acid solution. Accu-Chek Glucometer with Accu-Chek test strips. Streptozotocin (Sigma-Aldrich Germany).

2.2 Plant Material

The plant was collected in Akure, Ondo State, Nigeria. It was identified and authenticated at the herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife. A voucher specimen (IFE 17513) was prepared and deposited at the same herbarium.

2.3 Extraction and Partitioning

The roots of the plants were washed, peeled and air dried for 30 days. 2.5 kg of the root material was pulverised. The pulverised sample was exhaustively extracted with methanol and the solution was concentrated *in vacuo* to obtain a crude extract (155 g). The crude extract was dissolved in aqueous methanol (1:1, 500 mL). The solution was partitioned with *n*-hexane (6×300 mL), DCM (6×300 mL) and ethyl acetate (7×300 mL) to obtain their respective *n*-hexane

(28.13 g), DCM (47.75 g), ethyl acetate (19.65 g) fractions and the mother liquor (58.57 g).

2.4 Isolation of the Compound and Its Spectroscopic Data

The ethyl acetate fraction (19.65 g) was adsorbed on 20.12 g of silica gel and it was left to dry overnight to avoid solvent interference. The adsorbed silica gel was loaded into glass column dry and was gradiently eluted using n-hexane, ethyl acetate and methanol. 30 mL of the eluant was collected and 172 column fractions were obtained and these fractions were bulked into ten fractions: PE1 (1.01 g), PE2 (1.43 g), PE3 (0.79 g), PE4 (1.17 g), PE5 (3.32 g), PE6 (0.71 g), PE7 (3.97 g), PE8 (2.02 g), PE9 (0.94 g) and PE10 (1.58 g), according to their TLC profile. Bulked fraction PE7 (3.97 g), a brownish solid obtained at EtOAc/MeOH (60:40) with one prominent spot among several spots, was further subjected to column chromatography for purification and a brown solid (single spot) was obtained at EtOAc/MeOH (80:20) which yielded convallatoxin (172.00 mg). The same spot that yielded convallatoxin was found in the other bulked fractions from PE3 to PE9 in reasonable quantity by comparing their Rf values on TLC plate. The approximate quantification was carried out by spraying the TLC plate with a sulphuric acid solution and the plate was left in the oven at 105 °C to char. This showed convallatoxin to be the major component of the ethyl acetate fraction.

2.4.1 3β-O-(α-L-rhamnopyranosyl)-5β,14β-dihydroxy-19-oxo-17β-card-20(22)-enolide

Brown powder; IR (KBr): υ_{max} (cm^{-1}) 3427 (OH), 1735 (C=O), 1730 (C=O), 1620 (C=C), 1074 (C-O-C). ^{1}H and ^{13}C NMR, Table 1.

2.5 Bioassay

2.5.1 Animals

Healthy albino rats (120–160 g) of both sexes bred under standard conditions (temperature 25 ± 3 °C) at the animal house, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria were used for the experiment. They were fed on a standard pellet diet (Vital Feeds, Nigeria) and water was given *ad libitum*. All animal experiments conformed to the *Guide for the Care and Use of Laboratory Animals* [28].

2.5.2 Glucose lowering activity of extract and fractions

A glucose tolerant test was carried out by giving glucose (10 g/kg, *p.o.*) to rats that were fasted for 24 h. Those with high blood glucose level (blood glucose level ≥ 7 mmol/L (126 mg/dL) after 0.5 h (time point 0 h, T_o) were divided into groups of five and administered (*p.o.*) with 1% tween 80 in normal saline (negative control), extract (100, 200 and 400 mg/kg), or partitioned fraction (200 mg/kg) or glibenclamide (5 mg/kg) to determine their blood glucose level reduction activity. A drop of blood, taken from the tip of the tail of each rat, was dropped onto a glucometer strip and the blood glucose (bg) level read off directly. The blood glucose levels at 0 h (T_o) were taken as 100% while those at other times were percentages of these values. Their blood glucose (bg) levels were determined and recorded at 0, 0.5, 1, 2 and 4 h after administration of the normal saline/extract/fraction/drug [29].

2.5.3 Anti-diabetic activity of extract and isolate on streptozotocin-induced diabetic rats

Diabetic rats were obtained by intraperitonial injection of streptozotocin (65 mg/kg) that was dissolved in freshly prepared 0.1M sodium citrate buffer with a pH of 4.5 on pH meter. Rats with fasting blood glucose levels ≥ 14 mmol/L after 72 h of injection of streptozotocin were taken to be diabetic. The diabetic rats were divided into three groups of 10 rats each. They were given 1 % tween 80 in normal saline, extract (200 mg/kg) or isolate or glibenclamide (5 mg/kg) for 10 days. Their fasting blood glucose levels were determined on days 1, 4, 7 and 10 respectively [30].

2.6 Statistical Analysis

Data obtained from this study were expressed as the mean ± SEM for the number (N) of animals in the group. One way Analysis of variance (ANOVA) was first used followed by Bonferroni post hoc test to determine the source of significant differences for all determinations and $p < 0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Glucose Lowering Activity of the Extract

The glucose-loaded normal rats that were given normal saline (negative control) showed time-

dependent blood glucose reduction up to the fourth hour. This indicated that the homeostatic regulatory mechanism and the pancreases of the rats were functioning well [31]. The glucose lowering activity of the extract at 100 and 400 mg/kg was not time-dependent and it was insignificant with 8 and 7% blood glucose reduction at 4 h respectively (Table 1). The blood glucose reduction of the extract at 200 mg/kg was time-dependent up to the fourth hour and it was comparable ($p > 0.05$) to that of glibenclamide (5 mg/kg) between 0.5-4 h indicating similar minor extra-pancreatic and major insulin stimulating effects of glibenclamide at this dose (Table 1) [32].

3.2 Anti-diabetic Activity of the Extract on Streptozotocin-induced Diabetic Rats

The most active dose of the extract (200 mg/kg) in glucose lowering assay (Table 1) was used in the study of the anti-diabetic activity of the extract in streptozotocin-induced diabetic rats. The negative control group diabetic rats were consistently hyperglycaemic throughout the period of the experiment (Table 2). The extract showed 49, 68 and 70% blood glucose level reduction on days 4, 7 and 10 respectively that were significantly higher ($p<0.05$) than the 18, 33 and 39% given by glibenclamide (5 mg/kg) (Table 2). This result indicated that the root bark extract of *P. nigrescens* was more active than its aqueous leaf extract that was reported to show 68% activity at 1000 mg/kg on the fourth week [23].

3.3 Glucose Lowering Activity of the Partitioned Fractions

The result of the glucose lowering effect of the partition fractions of *P. nigrescens* showed that its dichloromethane fraction lacked appreciable activity between 0.5-4 h while *n*-hexane and aqueous fractions gave similar activity at all-time points that were significantly lower than that of glibenclamide (Table 3). The ethyl acetate fraction with comparable activity to glibenclamide (5 mg/kg) and the extract at 0.5-4 h, however, showed the highest activity among the partition fractions, indicating that the glucose lowering constituents of the extract are mostly concentrated in this fraction and may be insulinnotropic in action (Table 3).

3.4 Anti-diabetic Activity of the Isolate on Streptozotocin-induced Diabetic Rats

The isolated compound at 20 mg/kg, gave a time dependent blood glucose level reduction activity of 43, 56 and 83% that was significantly higher than 18, 33 and 39% showed by glibenclamide (5 mg/kg) on days 4, 7 and 10 respectively (Table 4). The activity of the isolated compound was comparable to the extract (200 mg/kg) on days 4 and 7 but significantly higher on day 10 (Table 4), indicating that the isolate was 10 times more active than the extract.

Table 1. Dose related glucose lowering effect of *P. nigrescens* root bark methanolic extract

Dose of extract (mg/kg)	Blood glucose level as percentage of To (reduction in blood glucose relative to negative control at Tt)				
	0.0 h	**0.5 h**	**1 h**	**2 h**	**4 h**
GLU(10 g/kg)	100	83.79±3.81^{a}	85.89±0.50^{b}	76.45±1.71^{b}	74.18±1.97^{b}
A (100)	100	89.40±3.16^{a} (-6.70%)	84.43±3.51^{a,b} (1.70%)	77.43±5.72^{a,b} (-1.28%)	67.95±7.53^{a,b} (8.40%)
A (200)	100	78.52±8.92^{a} (6.29%)	71.59±7.24^{a,b} (16.65%)	59.74±7.76^{a,b} (21.86%)	51.68±6.50^{a} (30.33%)
A (400)	100	81.64±5.85^{a} (2.57%)	77.94±6.93^{a,b} (9.26%)	73.53±7.81^{a,b} (3.82%)	69.19±8.13^{a,b} (6.73%)
GLI (5)	100	75.64±6.73^{a} (9.73%)	70.68±6.86^{a,b} (17.71%)	58.32±6.44^{a} (23.72%)	45.27±6.88^{a} (38.97%)

*Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at 0h (To), n=5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Bonferroni t-test). GLU (negative control); **A**: Extract of P. nigrescens root bark; GLI: Glibenclamide (positive control)*

Table 2. Anti-diabetic activity of the extract of *P. nigrescens* root bark in streptozotocin-induced rats

Dose of extract (mg/kg)	Blood glucose level as percentage of To (reduction in blood glucose relative to negative control at Tt)			
	Day 1	Day 4	Day 7	Day 10
Negative control	100.00	106.83 ± 2.45[c]	101.04 ± 3.26[c]	102.76 ± 1.83[c]
A (200 mg/kg)	100.00	54.97 ± 5.76[a] (48.54%)	32.76 ± 4.80[a] (67.58%)	30.79 ± 3.0[a] (69.86%)
GLI (5 mg/kg)	100.00	87.30 ± 2.20[b] (18.28%)	67.52 ± 2.55[b] (33.18%)	62.54 ± 4.41[b] (39.14%)

*Data show the mean ± SEM blood glucose levels at the different time interval expressed as percentages of levels at 0 h (T_o), n=5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$, one way analysis of variance followed by Bonferroni t test). GLI: (positive control), **A**: Extract of Parquetina nigrescens*

Table 3. Glucose lowering effect of the partition fractions of *P. nigrescens* root bark

Dose of extract (mg/kg)	Blood glucose level as percentage of To (reduction in blood glucose relative to negative control at Tt)				
	0.0 h	0.5 h	1 h	2 h	4 h
GLU (10)	100.00	83.79±3.81[a]	85.89±0.50[ab]	76.45±1.71[b]	74.18±1.97[b]
A (200)	100.00	78.52±8.92[a] (6.29%)	71.59±7.24[a] (16.65%)	59.74±7.76[a] (21.86%)	51.68±6.50[a] (30.33%)
B_1 (200)	100.00	86.69±5.99[a] (-3.46%)	73.11±6.40[a] (14.88%)	64.94±5.62[a,b] (15.06%)	60.01±6.18[a,b] (19.10%)
B_2 (200)	100.00	87.98±2.26[a] (-5.00%)	81.68±2.17[a] (4.90%)	75.01±5.71[b] (1.88%)	68.71±7.92[a,b] (7.37%)
B_3 (200)	100.00	81.59±1.53[a] (2.63%)	65.32±1.89[a] (23.95%)	55.58±1.45[a] (27.30%)	47.72±2.45 [a] (35.67%)
B_4 (200)	100.00	82.36±6.21[a] (1.71%)	75.62±3.94[a] (11.96%)	70.45±3.98[a,b] (7.85%)	61.21±4.65[a,b] (17.49%)
GLI (5)	100.00	75.64±6.73[a] (9.73%)	70.68±6.86[a] (17.71%)	58.32±6.44[a] (23.72%)	45.27±6.88[a] (38.97%)

*Data show the mean ± SEM blood glucose levels at the different time interval expressed as percentages of levels at 0 h (T_o), n=5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$, one way analysis of variance followed by Bonferroni t test). GLU: glucose in 1% of tween 80 normal saline (negative control), **A**: Extract of Parquetina nigrescens, **B_1**: n-Hexane fraction, **B_2**: Dichloromethane fraction, **B_3**: Ethyl acetate fraction, **B_4**: Mother liquor fraction, GLI: Glibenclamide*

Table 4. Anti-diabetic activity of the isolated compound in streptozotocin-induced rats

Dose of extract (mg/kg)	Blood glucose level as percentage of To (reduction in blood glucose relative to negative control at Tt)			
	Day 1	Day 4	Day 7	Day 10
Negative control	100.00	106.83 ± 2.45[d]	101.04 ± 3.26[d]	102.76 ± 1.83[d]
A (200 mg/kg)	100.00	54.97 ± 5.76[a] (48.54%)	32.76 ± 4.80[a] (67.58%)	30.79 ± 3.0[b] (69.86%)
Isolate (20 mg/kg)	100.00	61.32±9.76[a] (42.60 %)	44.84 ± 11.50[a] (55.62 %)	17.86 ±1.61[a] (82.62%)
GLI (5 mg/kg)	100.00	87.30 ± 2.20[c] (18.28%)	67.52 ± 2.55[c] (33.18%)	62.54 ± 4.41[c] (39.14%)

*Data show the mean ± SEM blood glucose levels at the different time interval expressed as percentages of levels at 0 h (T_o), n=5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$, one way analysis of variance followed by Bonferroni t test). **A**: Extract of Parquetina nigrescens, GLI: Glibenclamide (positive control)*

Table 5. The comparison of ^{1}H-NMR (400 MHz, MeOD) and ^{13}C-NMR (100 MHz, MeOD) of the isolated compound and convallatoxin [33]

Position	Isolated compound		Literature		Position	Isolated Compound		Literature	
	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C	δ_H	δ_C
1	-	25.2	1.66 m, 2.00 m	24.9	16	1.86 m	28.0	1.86 m, 2.10 m	27.5
2	-	26.0	1.67 m, 1.72 m	25.7	17	-	50.8	2.81 m	50.1
3	4.06 m	74.2	4.06 m	74.2	18	0.87 m	16.3	0.86 m	16.0
4	1.55 d	36.8	1.56 m, 2.15 m	36.0	19	10.07 m	210.1	10.86 m	208.3
5	-	75.4		74.3	20	-	178.3	-	178.3
6	-	37.2	1.56 m, 2.05 m	37.2	21	-	75.3	4.90 dd	73.9
7	2.16 m	18.7	2.16 m	18.7	22	5.90 brs	118.0	5.90 brs	117.8
8	-	42.6	1.92 m	42.4	23	-	177.2	-	174.3
9	-	39.6	1.72 m	39.8	1'	-	98.4	4.85 brs	100.4
10	-	56.2	-	55.6	2'	-	76.2	3.77 brs	72.2
11	-	23.3	1.45 m	22.9	3'	-	75.3	3.59 m	72.5
12	-	40.4	1.44 m	40.0	4'	-	70.1	3.39 t	73.5
13	-	51.8	-	51.3	5'	-	69.1	3.63 m	70.1
14	-	86.0	-	85.2	6'	1.16 d	18.6	1.20 d	18.1
15	-	32.5	1.12 m, 1.65 m	32.5					

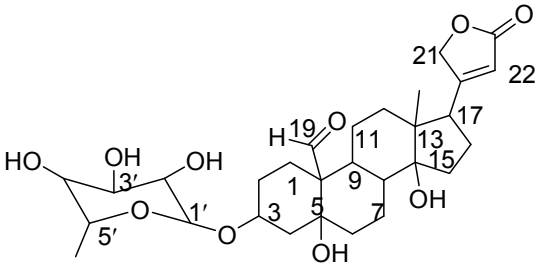

Fig. 1. Structure of 3β-O-(α-L-rhamnopyranosyl)-5β,14β-dihydroxy-19-oxo-17β-card-20(22)-enolide

3.5 Structural Elucidation of the Isolated Compound

The ^{1}H-NMR (400 MHz, MeOD) spectrum showed high field signals apart from an aldehydic proton at 10.07 (s). It also exhibited a diagnostic olefinic proton signal of α, β-unsaturated-γ-lactone ring at 5.61 (s). Two methyl groups were observed at 0.91 (s) and 1.16 (d). The analyses of ^{13}C (100 MHz, MeOD) and DEPT 135 NMR spectra showed two methyl carbons at 16.3 and 18.6, nine methylene carbons at 25.2, 26.0, 36.8, 37.2, 18.7, 23.3, 40.4, 32.5 and 28.0 with one oxygenated methylene carbon at 75.3, three methine, two oxygenated methine, three hydroxylated methine, one anomeric and one aldehydic carbon atoms at 42.6, 39.6, 50.8, 76.2, 75.3, 70.1, 69.1, 74.2, 98.4 and 210.7, and two quaternary, two hydroxylated quaternary, two vinyl and one carboxylic carbon atoms at 56.2, 51.8, 75.4, 86.0, 178.3, 118.0 and 177.2 which gave molecular formula of $C_{29}H_{42}O_{10}$. According to Vouffo *et al*. (2010) the NMR data compared with a previously isolated compound from the literature, (Table 5). Thus, the compound was identified as [3β-O-(α-L-rhamnopyranosyl)-5β,14β-dihydroxy-19-oxo-17β-card-20(22)-enolide], Fig 1.

4. CONCLUSION

The overall results of the anti-diabetic activity of *P. nigrescens* root bark both in glucose-loaded normal rats and streptozotocin-induced diabetic rats in this study have justified its ethno-medicinal anti-diabetic use. The active compounds of the extract were mostly concentrated in the ethyl acetate fraction. Isolated convallatoxin (20 mg/kg) that was significantly more active than glibenclamide (5 mg/kg) at all the tested days was confirmed as one of the active constituents of *P. nigrescens*.

Convallatoxin has been reported for its various therapeutic activities, antivirus [34], antitumor [35,36], anti-angiogenic [37]. The LD_{50} for orally delivered convallatoxin in mice was reported to be 2 g/kg [38]. Therefore, the dose reported in this study for its anti-diabetic activity falls below the toxic dose. Our studies indicate that convallatoxin from plant origin has a therapeutic

potential for the management of hyperglycemia associated with diabetes mellitus.

ACKNOWLEDGEMENTS

Authors appreciate Mr Nwamadi Mutshiyalo of the Department of Spectrau, University of Johannesburg, South Africa in the acquisition of the NMR spectra. SOF is grateful to Dr Anthony Adejuwon of the Department of English, Faculty of Arts, Obafemi Awolowo University, Ile-Ife for proofreading the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The company names used for this research are commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and companies because we do not intend to use these companies as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the companies rather it was funded by personal efforts of the authors.

REFERENCES

1. Sicree R, Shaw J, Zimmet P. The global burden. Diabetes and impaired glucose tolerance. Prevalence and Projections. In: Gan, D. ed. Diabetes Atlas, 3rd edition. Brussels: International Diabetes Federation. 2006;16-103.
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; 27:1047-1053.
3. L'Heveder R, Nolan T. International diabetes federation. Diabetes Research and Clinical Practice. 2013;101: 349-351.
4. World Health Organization. Global report on diabetes. World Health Organization; 2016.
5. International Diabetes Federation (IDF). Diabetes Atlas. 5th edition. Brussels, Belgium; 2012.
6. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Research and Clinical Practice. 2011; 94:311-321.
7. Adebajo AC, Ayoola MD, Obagbemi OR, Obuotor EM, Ogunsina MO, Verspohl EJ. Antihyperglycaemic and antioxidant activities of *Eugenia uniflora* leaf: Evaluation of ethnomedical claims IV. Ife Journal of Science and Technology. 2013a;1:1-18.
8. Adebajo AC, Ayoola MD, Odediran SA, Aladesanmi AJ, Schmidt TJ, Verspohl EJ. Evaluation of ethnomedical claims III: Antihyperglycemic activities of *Gongronema latifolium* root and stem. Journal of Diabetes. 2013b;5:336-343.
9. Adebajo AC, Olawode EO, Omobuwajo OR, Adesanya SA, Begrow F, Elkhawad A. Hypoglycaemic constituents of *Stachytarpheta cayennensis* leaf. Planta Medica. 2007;73:241-250.
10. Adebajo AC, Iwalewa EO, Obuotor EM, Ibikunle GF, Omisore NO, Adewunmi CO. Pharmacological properties of the extract and some isolated compounds of *Clausena lansium* stem bark: anti-trichomonal, antidiabetic, anti-inflammatory, hepatoprotective and antioxidant effects. Journal of Ethnopharmacology. 2009;122:10-19.
11. Irvine FR. Woody plants of Ghana. Oxford University Press, London; 1961.
12. Sofowora A. Medicinal leafs and traditional medicines in Africa. Chichester John Willey & Sons New York. 1993;256.
13. Dokosi O.B. Herbs of Ghana. Ghana Universities Press; 1998.
14. Odetola AA, Oluwole FS, Adeniyi BA, Olatiregun AM, Ikupolowo OR, Labode O. Antimicrobial and gastroprotective properties of *Parquetina nigrescens* (Afzel.) Bullock. Journal of Biological Sciences. 1998;6:701-705.
15. Marks WH, Fong HHS, Tin-Wa M, Farnsworth NR. Cytotoxic principles of *Parquetina nigrescens (Afzel.)* bullock *(Asclepiadaceae)*. Journal of Pharmaceutical Sciences. 1975;64:1674-1676.
16. Datte J, Offoumou AM, Manda OM. Uterotonic effects of hydromethanolic extract of *Parquetina nigrescens* (Periplocaceae) on spontaneous contractile activity in the isolated myometrium of pregnant rats. Journal of Ethnopharmacology. 1996;53(1):15-20.
17. Datté JY, Ziegler A, Tillement JP. Sympathomimetic effects of *Parquetina nigrescens* (Periplocaceae) extract in isolated portal vein smooth muscle. General Pharmacology: The Vascular System. 1999;32(5):551-556.

18. Agbor AG, Odetola AA. Hematological studies of *Parquetina nigrescens* on haemorrhagic anaemic rats. African Journal of Medicine and Medical Sciences. 2001;30(1-2):105-109.
19. Owoyele BV, Nafiu AB, Oyewole IA, Oyewole LA, Soladoye AO. Studies on the analgesic, anti-inflammatory and antipyretic effects of *Parquetina nigrescens* leaf extract. Journal of Ethnopharmacology. 2009;122:86-90.
20. Imaga NOA, Gbenle GO, Okochi VI, Adenekan SO, Edeoghon SO, Kehinde MO. Antisickling and toxicological profiles of leaf and stem of *Parquetina nigrescens L.* Journal of Medicinal Plants Research. 2010;4:639-643.
21. Aderibigbe OR, Odetola AA, Oluwole FS, Farombi EO, Onabanjo OO, Jiboku OA. Antioxidant properties of methanol extract of *Parquetina nigrescens* in ulcerated rats. International Journal of Tropical Medicine. 2011;6:25-29.
22. Oyagbemi TO, Ogunleye AO, Lawal TO. Oyagbemi AA. Antimicrobial activity of *Parquetina nigrescens* on some multidrug resistant pathogens isolated from poultry and cases of otitis media in dogs from Nigeria. African Journal of Biotechnology. 2013;1559-1563.
23. Saba AB, Oyagbemi AA, Azeez OI. Antidiabetic and haematinic effects of *Parquetina nigrescens* on alloxan induced type-1 diabetes and normocytic normochromic anaemia in Wistar rats. African Health Sciences. 2010;10(3):276-282.
24. Kayode OT, Yakubu MT. *Parquetina nigrescens* leaves: Chemical profile and influence on the physical and biochemical indices of sexual activity of male Wistar rats. Journal of Integrative Medicine. 2017; 15(1):64-76.
25. Ogundaini AO, Okafor EO. Isorhoifolin, a flavonoid glycoside from *Periploca nigrescens* leaves. Planta Medica. 1987; 53:391-391.
26. Ochigbo GO, Saba AB, Oyagbemi AA, Omobowale TO, Asenuga ER. Polyphenol-rich fraction of Parquetina nigrescens mitigates dichlorvos-induced neurotoxicity and apoptosis. Journal of Ayurveda and Integrative Medicine. 2017;8:27-36.
27. Oyagbemi AA, Omobowale TO, Ochigbo GO, Asenuga ER, Ola-Davies OE, Ajibade TO. Polyphenol-rich fraction of *Parquetina nigrescens* mitigates dichlorvos-induced cardiorenal dysfunction through reduction in cardiac nitrotyrosine and renal p38 expressions in wistar rats. Journal of Dietary Supplements. 2018;15(3):269-284.
28. Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, Division on Earth and Life Studies, National Research Council of the National Academies. Guide for the Care and Use of Laboratory Animals, 8th edn. The National Academies Press, Washington, DC; 2011.
29. Ayoola MD, Balogun JO, Famuyiwa FG, Yeboah SO, Famuyiwa SO. Isolation and characterization of 2-hydroxy-3-[4-hydroxyphenyl]-2-propenoic acid and 4-bromophenol from anti-diabetic extract of the root bark of *Uvaria afzelii*. South African Journal of Botany. 2017;112:527–532.
30. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi SH, Farhangi A, Verdi AA. Induction of diabetes by Streptozotocin in rats. Indian Journal of Clinical Biochemistry. 2007;22:60-64.
31. Kar A, Choudhary BK, Bandyopadhyay NG. Preliminary studies of the inorganic constituents of some indigenous hypoglycaemic herbs on oral glucose tolerance test. Journal of Ethnopharmacology. 1999;64:179-184.
32. Luzi L, Pozza G. Glibenclamide: An old drug with a novel mechanism of action? Acta Diabetologica. 1997;34:239-244.
33. Vouffo B, Dongo E, Facey P, Thorn A, Sheldrick G, Maier A, Fiebig HH, Laatsch H. Antiarol cinnamate and africanoside, a cinnamoyl triterpene and a hydroperoxy-cardenolide from the stem bark of *Antiaris africana*. Planta Medica. 2010;76:1-7.
34. Cohen T, Williams JD, Opperman TJ, Sanchez R, Lurain NS, Tortorella D. Convallatoxin-induced reduction of methionine import effectively inhibits human cytomegalovirus infection and replication. Journal of Virology. 2016;90: 10715-10727.
35. Kaushik V, Azad N, Yakisich JS, Iyer AK. Antitumor effects of naturally occurring cardiac glycosides convallatoxin and peruvoside on human ER+ and triple-negative breast cancers. Cell Death Discovery. 2017;3:17009.
36. Anderson SE, Barton CE. The cardiac glycoside convallatoxin inhibits the growth of colorectal cancer cells in a p53-

independent manner. Molecular Genetics and Metabolism Reports. 2017;13:42-45.

37. Yang SY, Kim NH, Cho YS, Lee H, Kwon HJ. Convallatoxin, a dual inducer of autophagy and apoptosis, inhibits angiogenesis in vitro and in vivo. PloS One. 2014;9:e91094.

38. Lorenz D, Stoeckert I. The native cardiac glycosides of *Convallaria majalis*. II. Pharmacology. (Article in German). Arzneimittelforschung. 1958;8:557-564.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27100