Phytomedicine Plus 2 (2022) 100182

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus

Phytochemical characterization, antihyperglycaemic and antihyperlipidemic activities of *Setaria megaphylla* in alloxan-induced diabetic rats

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ARTICLE INFO

Keywords:
Diabetes mellitus
Medicinal plants
Hypoglycaemic agents
Insulin
1-Triacontanol
Stigmasterol

ABSTRACT

Background: Setaria megaphylla (Steud) Dur & Schinz (Poaceae), a grass used ethnomedically by herbalists in Nigeria for the treatment of diabetes was evaluated for antidiabetic, hypolipidemic and pancreas protective potentials in rats.

Methods: Solvents fractions (hexane, dichloromethane (DCM), ethyl acetate (EA) and methanol) were investigated for antidiabetic, hypolipidemic and pancreas protective potentials in alloxan-induced diabetic rats. Glibenclamide was used as positive control. The fasting blood glucose (FBG) level, serum insulin, glycosylated hemoglobin (Hb1Ac), oral glucose tolerance test (OGTT) and lipids levels were determined. Histopathological study of the pancreas was done. Isolation of phytochemicals and their subsequent identification using Fourier transform infrared spectroscopy, gas chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy were carried out.

Results: Treatment of alloxan-induced diabetic rats with the leaf fractions caused significant ($p < 0.05$–0.001) reduction in FBG of treated diabetic rats in acute and prolonged studies as well as OGTT with DCM, EA and hexane fractions having pronounced activities. The leaf fractions also caused significant ($p < 0.01$) decreases in Hb1Ac levels and increases in serum insulin levels. The leaf fractions further caused lowering of serum total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL) with increased high density lipoprotein (HDL) level in the treated diabetic rats. Histopathological study of pancreas revealed protective effect by the leaf fractions. 1-Triacontanal, 1-triacontanol, 1-dotriacontanol, 1-triacontyl cerotate, and stigmasterol were isolated and identified from the active DCM and EA fractions of this plant for the first time .

Conclusion: The leaf fractions of *S.megaphylla* possess hypoglycemic, insulin secretion stimulatory, hypolipidemic, and pancreas protective potentials which may be due to the activities of the phytochemical constituents.

List of abbreviations

DCM- Dichloromethane
EA- Ethyl acetate
FBG- Fasting blood glucose
OGTT- Oral glucose tolerance test

; ATR, Attenuated total reflection; FT-IR, Fourier transform infrared spectroscopy; ROS, Reactive oxygen species.

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[1] Jude E. Okokon designed and supervised the work
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[5] Li Wen Wu did the GC–MS and NMR analysis and interpretation of the spectra and also edited the work.

https://doi.org/10.1016/j.phyplu.2021.100182
Received 19 October 2021; Received in revised form 15 November 2021; Accepted 27 November 2021
Available online 30 November 2021

Hb1Ac-	Glycosylated hemoglobin
GC–MS-	Gas chromatography-mass spectrometry
NMR-	Nuclear magnetic resonance spectroscopy
LDL-	Low density lipoprotein
VLDL-	Very low density lipoprotein
HDL-	High density lipoprotein
WHO-	World Health Organisation
H & E-	Heamatoxylin and eosin
T C-	Total cholesterol

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease affecting economic and social activities of all countries of the world with estimated millions of deaths worldwide due to high blood glucose and risks of associated complications, which often result in premature death (WHO, 2017). There are three major types of DM: type 1 diabetes (T1DM, insulin dependent), type 2 diabetes mellitus (T2DM), and gestational diabetes mellitus. T2DM contributes to more than 90% of all DM cases. According to International Diabetes Federation in 2019, the total adult population stands at 463 million with diabetes, which may increase to 578 million and 700 million by 2030 and 2045, respectively (Saeedi et al., 2019). The increasing prevalence of diabetes is worrisome and calls for an urgent remedial steps as the orthodox medicines used in the management of DM seem to be inadequate and challenging (Saeedi et al., 2019). The current use of orthodox drugs and insulin in the management of diabetes does not offer satisfactory treatment outcome as the drugs are associated with unpleasant side effects such as hypoglycemia, hypersensitivity, gastrointestinal disturbances, hepatotoxicity, renotoxicity and heart failure ((Basu et al., 2015); Sudasinghe and Peiris,2018). Herbal preparations are highly patronized for the management of diabetes due to their low cost, accessibility, relative safety and many biological activities compared to conventional anti-diabetic drugs (Badri et al., 2006; Khan et al., 2012; Peiris et al., 2015). Medicinal plants have been extensively investigated for the potential treatment of DM (Marles and Farnsworth, 1995; Ezuruike and Prieto, 2014; Tran et al., 2020). Various phytochemicals such as ginsenosides in *Panax ginseng*, cucurbitane type terpene glycosides in *Momordica charantia*, glycyrrhizin in *Glycyrrhiza glabra* and berberines in *Coptis chinensis* have been found to possess antidiabetic activity (Park and Jang, 2017). In particular, metformin, one of the most common drugs for DM, is derived from guanidine compounds in *Galega officinalis* (Bailey, 2004). Hence there is the need to search for new antidiabetic compounds from medicinal plants with potentials to circumvent the present challenges faced in DM management.

Setaria megaphylla (Steud) Dur & Schinz (Poaceae) is a perennial pasture grass distributed widely in tropical and subtropical countries of the world (Van Oudtshoorn, 1999). It is called 'nkwongo' in ibibio or broad leafed brittle grass. Although no detailed ethnopharmacological survey has been carried out on medicinal plants used by the Ibibios in Akwa Ibom State, reports of ethnomedicinal use of the plant (leaves and roots) by herbalists from Ibibio tribe in Niger Delta region of Nigeria for the treatment of diabetes have been published (Okokon et al., 2006; Okokon and Antia, 2007, 2007a). Preliminary reports indicated antidiabetic and hypoglycaemic activities of the leaves and roots (Okokon and Antia, 2007; Okokon et al., 2007a). Other activities of the leaves include; *in vitro* and *in vivo* antimalarial (Clarkson et al., 2004; Okokon et al., 2007b, 2017), anti-inflammatory, analgesic (Okokon et al., 2006), cytotoxic, immunomodulatory and antileishmanial (Okokon et al., 2013) and antidepressant (Okokon et al., 2016). Phytochemical constituents of the leaf extract identified through various screening and analytical methods have been reported (Okokon et al., 2006; Okokon et al., 2013). Here, we report the antihyperglycaemic and hypolipidemic potentials of the solvent leaf fractions of *Setaria megaphylla* in alloxan-induced diabetic rats, and further isolation and identification of phytochemicals in these fractions.

Materials and methods

Plant material and extraction

Fresh leaves of the plant were collected from Akwa Anwa forest, Uruan in Akwa Ibom State, Nigeria in August 2020 and identified by a taxonomist in the Department of Botany, University of Uyo, Uyo. A voucher specimen (UUPH. 221 d) of the plant was deposited at herbarium of Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo. The leaves were washed, chopped into pieces, dried and powdered. The powder (1.5 kg) was successively and gradiently macerated in 7.5 L of each of these solvents at room temperature (28 ± 2 °C) for 72 h; *n*-hexane, dichloromethane (DCM), ethyl acetate (EA) and methanol to give corresponding their fractions. The liquid filtrates obtained were concentrated to dryness at 40 °C using rotary evaporator and these were preserved in a desicator until used for the proposed experiments.

Purification of compounds from dichloromethane and ethyl acetate fractions of Setaria megaphylla

Dichloromethane and ethyl acetate fractions (15 g) found to be active during the experiment were bulked and subjected to silica gel column chromatography (Merck, 60–120 mesh) and gradient-eluted with *n*-hexane containing increasing quantity of dichloromethane, followed by increasing quantity of ethyl acetate and methanol. Eluates (20 mL each) were collected, monitored on silica thin layer chromatography (TLC) plates (Merck, Germany) in hexane: DCM: EA (2:1:1) using vanillin-sulphuric acid as spray reagent. Fifty-one fractions were obtained and bulked together based on their similar TLC characteristics (Rf values, color reaction with spray reagents) to give four semi-pure residues coded F1–F4. F2 was further purified using preparative TLC; carefully dissolved in dichloromethane and applied across the coated silica gel plate (20 × 20 cm, 0.25 mm) using a micro-Pasteur pipette (Simax, India) 1 cm above the bottom edge of the plate. The plates were developed using *n*-hexane: dichloromethane (4:1) solvent system in a Chromatank (USA). The chromatogram obtained showed two distinctly resolved layers which were carefully scrapped, separated, filtered and concentrated *in vacuo*. Further TLC evaluations indicated a single spot in each layer which were denoted **X1 (10 mg)** and **X16/17 (8 mg).** F3 on further purification with preparative TLC using hexane and dichloromethane (1:4) yielded one pure compound **N15 (10 mg).** Purification of F4 with preparative TLC using hexane and DCM (4:1) produced a pure compound **PJ3 (8 mg).** This was filtered and concentrated to give a white crystalline compound. The chemical structures of isolated pure compounds were elucidated using spectroscopic analyses.

Animals

Wistar rats (males and females) used for these experiments were obtained from Animal house in University of Uyo. The animals were accommodated in standard plastic cages and maintained on pelleted Feed (Guinea Feed) and water *ad libitum*. Permission and approval for animal studies were obtained from College of Health Sciences Animal Ethics committee, University of Uyo (UU/CHS/AE/21/024).

Induction of experimental diabetes using alloxan monohydrate

Intraperitoneal injection of freshly prepared alloxan monohydrate solution (150 mg/kg i.p) in ice cold 0.9% saline (NaCl solution), was used to induce diabetes in overnight fasted forty (40) Wistar rats (males and females) weighing (140–160 g). Immediately after the induction, 2 mL of 5% dextrose solution was orally administered to the animals to reduce the effect of initial hypoglycaemia (Okokon and Nyong, 2018). Rats with moderate diabetes, (i.e. with blood glucose levels of 200 mg/dL and above), after a post-induction rest period of 72 h, which was

allowed for the diabetes to be fully developed (Lenzen, 2008) were considered diabetic and selected for the experiments.

The sample size for the study was calculated using resource equation method of Festing (2006) and the diabetic animals were divided into 6 (six) treatment groups of 6 rats each. Based on determined median lethal dose (LD_{50}) of 2.4 ± 0.5 g/kg, (Okokon et al., al.,2006), the rats were treated with 200 mg/kg/day of respective solvent fractions of *S. megaphylla* leaf orally for 14 days as follows; group 1 was given 10 mL/kg/day of normal saline, group 2, 5 mg/kg/day of glibenclamide, group 3 – 6 were respectively administered 200 mg/kg/day of *n*-hexane, dichloromethane, ethyl acetate and methanol fractions of *S. megaphylla* leaf.

Assessment of oral glucose tolerance in treated diabetic rats

Oral glucose tolerance test (OGTT) was evaluated in a group of 36 diabetic rats induced with diabetes using alloxan as stated above. After confirmation and selection of the diabetic rats on the 3rd day, the rats were fasted for 24 h but allowed free access to water. After which the animals were weighed and randomised into 6 groups of 6 rats each. The basal Blood glucose levels (BGL) of the animals were determined by tail-tipping method using digital Glucometer. Thereafter, the diabetic rats were orally administered with 200 mg/kg of the respective solvent fractions (*n*-hexane, dichloromethane, ethyl acetate and methanol). Thirty (30) min post-administration of fractions the animals were given oral glucose load (2 g/kg). BGL were determined at 30, 60, 90, 120 and 180 min post glucose administration (Rabintossaporn et al., 2009; Okokon and Mandu, 2018).

Determination of hypoglycemic potential of the solvent fractions of S. megaphylla in alloxan-induced diabetic rats

FBG of diabetic rats were determined at hourly intervals during the periods of acute study, i.e. 1–3, 5 and 7 h interval, after a single dose of the fractions and at intervals of 1–3, 5, 7 and 14 days during prolonged study by "the tail-tipping method" using microprocessor digital blood glucometer (WHO, 1980).

Administration of fractions and drugs were carried out at a scheduled time daily and the animals were fasted every evening prior to days for measurement of the FBG concentrations.

Determination of the body weights changes of the treated diabetic rats

The body weights of the experimental animals were monitored before and after induction of diabetes and at the end of the study (Day15).

Collection of blood samples and organs

Twenty-four (24 h) after 14 days of treatment with fractions/drugs, the rats were weighed and sacrificed under light diethyl ether vapor. Blood samples were collected by cardiac puncture into plain centrifuge tubes and centrifuged immediately at 1500 rpm for 15 min to separate the serum at room temperature to avoid haemolysis. These blood samples were used for biochemical assays. The pancreas of the diabetic rats used in the study were surgically removed, weighed, fixed in 10% formaldehyde, processed, stained using Heamatoxylin and eosin (H & E) method and microscopically examined under the microscope at magnification (X400)

Determination of insulin and glycosylated hemoglobin levels in the diabetic rats

Serum insulin levels were measured with an ultra-sensitive rat insulin ELISA kit (Alpco Diagnostics) (Finlay and Dillard, 2007), while glycosylated hemoglobin was measured by method of Nathan et al. (1984).

Determination of the effect of the solvent fractions on the lipid profile (Serum TG, TC, HDL, LDL, VLDL levels) of the treated diabetic rats

Serum total cholesterol (TC), triglyceride and high density lipoprotein (HDL) levels of the diabetic rats were measured using Randox diagnostic kits, low and very low-density lipoprotein (LDL and VLDL) were calculated from the formula of Friedwald et al. (1972).

Gas chromatography-Mass spectrometry (GC–MS) analysis

GC–MS was carried out on an Agilent 7890A gas chromatograph, coupled with an Agilent MS model 5975C MSD with triple axis detector (Agilent Technologies, USA). The system was equipped with a HP5-MS column 5% phenyl-methylpolysiloxane, 30 $m \times 0.25$ mm $\times$ 0.25 μm (Agilent Technologies, USA). The carrier gas was helium with a gas flow under a constant pressure of 10 psi. The injector temperature was set at 280 °C. The initial oven temperature was 160 °C and increased to 320 °C at 10 °C/min, and the final temperature was held for 6 min at 320 °C. The mass spectrometer was operated in the electron ionization mode at 70 eV. The 0.2–0.4 mg of each compounds were dissolved in $CHCl_3$ (5 mg/mL) (X16/17 and PJ3) or treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (N15) for 20 min at room temperature before injection of 1 μL to GC–MS system (Aldulaimi et al., 2017). The compounds were identified by comparison of spectral data and fragmentation patter with reference compounds in the NIST 2011 database.

Nuclear magnetic resonance (NMR) spectroscopy

^{1}H and ^{13}C NMR spectra for the isolated compounds were obtained on a Bruker 400 and 100 MHz instrument, respectively. Chemical shifts were reported in δ (ppm) using the solvent $CDCl_3$ or $CDCl_3 + CD_3OD$ (9:1) as standard and coupling constants (J) were measured in Hertz.

Fourier transform infrared (FT-IR) spectroscopy

Fourier transform infrared spectrometer (Nicolet AVATAR360, Thermo Fisher Scientific) fitted with the attenuated total reflection (ATR) accessory was used to record FTIR spectra with a resolution of 4.0 cm^{-1} from 4000 to 800 $cm^{-1.}$

Statistical analysis

Data obtained from this work are expressed as MEAN ± SEM and were analysed statistically using one way ANOVA followed by Tukey-Kramer multiple comparison test using Instat Graphpad software, San Diego, USA. Differences between means were considered significant at 5% level of significance ie $p \leq 0.05$.

Results

Effect of solvent fractions on body and pancreas weights of rats

There were considerable changes in the body weights of the treated and untreated alloxan-induced diabetic rats (Table 1). Treatment of the diabetic rats with the leaf fractions caused significant ($p < 0.05$–0.001) increases in the body weight of the diabetic rats when compared to control. The highest increases were recorded in EA fraction-treated group (9.64%), followed by hexane group (8.30%) (Table 1). Significant ($p < 0.001$) decreases in the pancreas weights of the diabetic rats compared to control was observed following treatment of alloxan –induced diabetic rats with leaf fractions of *S. megaphylla* with the hexane fraction exerting the highest reduction (Table 1).

Table 1
Effect of leaf fractions of *Setaria megaphylla* on body and pancreas weights of diabetic rats.

Treatment	Dose mg/kg	Weight (g) Day 0	Day 15	% Increase	Weights of pancreas (g)
Control normal saline	–	154.6 ± 11.18	148.3 ± 14.60	−4.07	0.71 ± 0.03
Glibenclamide	10	147.3 ± 0.85	157.68 ± 11.20	7.04	0.44 ± 0.01[a]
***n*-hexane fraction**	200	144.96 ± 1.81	157.0 ± 24.61	8.30	0.50 ± 0.02 [a]
Dichloromethane fraction	200	145.26 ± 4.67	151.25 ± 10.84	4.12	0.53 ± 0.01 [a]
Ethyl acetate fraction	200	144.4 ± 8.50	158.33 ± 16.02	9.64	0.54 ± 0.04 [a]
Methanol fraction	200	152.46 ± 12.10	159.33 ± 15.16	4.50	0.58 ± 0.03 [a]

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

Effects on the FBG levels of alloxan-induced diabetic rats during acute treatment

The leaf fractions, 2 h post treatment, exerted considerable reductions in FBG of the diabetic rats which were significant at the 7 h ($p < 0.05$–0.001) with the dichloromethane and EA fractions-treated groups having the most significant ($p < 0.01$) reductions of FBG. The effect of the dichloromethane fraction was stronger than that of standard drug, glibenclamide (7 h) (Table 2).

Antidiabetic activities of the leaf fractions during prolonged treatment

The leaf fractions caused significant ($p < 0.05$–0.001) and sustained lowering of FBG levels of the diabetic rats throughout the duration of the study. The effect of the standard drug, glibenclamide was higher than that of the solvents fractions. On the last day of the study (day 14), the FBG levels of all the groups treated with the fractions were reduced significantly ($p < 0.001$) with EA fraction exerting the highest effect followed by *n*-hexane and dichloromethane fractions which exert comparable effects (Table 3).

Effect of leaf fractions on glycosylated hemoglobin level

There was significant ($p < 0.001$) reduction of Hb1Ac levels in fractions-treated groups with the hexane and dichloromethane groups exerting the highest reductions. The effect of the hexane group was more than that of the standard drug, glibenclamide. The hexane group was followed by DCM and EA groups (Fig. 1).

Effect of leaf fractions on insulin level

The solvent fractions treatment significantly ($p < 0.05$–0.01) increased the serum insulin levels of the respective treated groups relative to control. These increases were highest in the glibenclamide and hexane fraction treated groups. This was followed by DCM and EA groups (Table 2). The serum insulin level of hexane group was comparable to that of the standard drug, glibenclamide (Fig. 2).

Effect of solvents fractions on oral glucose tolerance test (OGTT)

Table 2 shows the effect of different solvent fractions on oral glucose tolerance test of alloxan-induced diabetic rats. The leaf fractions caused lowering of BGL of the diabetic rats. These reductions were significant ($p < 0.01$–0.05) when compared with control and peaked effects were recorded at 180 min with the various fractions producing (34.07–50.29%) inhibition of elevation of BGL, while the methanol fraction had the highest percentage inhibition of 50.98% followed by hexane (50.29%) and dichloromethane (43.84%) (Table 4).

Hypolipidemic effect of leaf fractions

The leaf fractions of *S. megaphylla* did not cause any significant effect ($p > 0.05$) on the levels of total cholesterol, triglyceride, HDL, LDL and VLDL. Although there were prominent decreases in LDL and VLDL levels, as well as increases in HDL levels of the fractions-treated groups, these changes were not significant ($p > 0.05$) when compared to control group. The standard drug, glibenclamide, caused significant ($p < 0.05$) reductions in LDL and VLDL levels (Table 5).

Histological studies of the pancreas

Histopathological study of pancreas of untreated diabetic rats treated with normal saline only (10 mL/kg) at X400 revealed distorted pancreas with areas of islets cells degeneration with very few cells, degranulated islet cells and degranulated β-cells and α-cells as well as areas of reduced islet cells. Pancreas of diabetic rats treated with glibenclamide and leaf fractions revealed some normal areas though with few areas of reduced islet cells of Langerhans than normal, degranulated or degenerated cell, thereby showing a significant protective effect (Fig. 3).

Identification of compounds

Five compounds were isolated and identified using FT-IR, ^{1}H and ^{13}C NMR (Supplementary materials) and GC-MS along with comparison with literature data as 1-triacontanal, 1-triacontanol, 1-dotriacontanol, 1-triacontyl cerotate, and stigmasterol (Fig. 4). These compounds are reported for the first time from *S. megaphylla*.

1-Triacontanal (X16/17), white powder. IR (ATR, ν_{max}, cm^{-1}): 2921, 2854, 1726, 1479, 1461. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm), δ_H 9.70 (1H, t, $J = 1.83$ Hz, H-1), 2.41 (2H, td, $J = 7.34$, 1.83 Hz, H-2), 1.63 (2H, m, H-3), 1.25 (16H, brs), 0.88 (3H, t, $J = 7.0$ Hz, H-30); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.11 (C-30), 22.10 (C-29), 22.69 (C-28), 29.18 (C-27), 29.36 (C-26), 29.43 (C-25), 29.59 (C-4), 29.70 (C-(5–24)), 31.93 (C-3), 43.92 (C-2), 202.95 (C-1). GC–MS (Rt, 17.918 min). *m/z* (relative%): 418.4 $[M - H_2O]^+$ (10), 268.1 (5), 147.0 (5), 96 (50), 82 (60), 71.1 (95), 57.1 (100). GC–MS was consistent with data reported by Tulloch. (1987). The NMR data of 1-triacontanal were first reported.

1-Triacontanol and 1-Dotriacontanol (ratio 1:3 by GC–MS, N15),

Table 2
Antidiabetic effect of leaf fractions of *Setaria megaphylla* on BGL of alloxan- induced diabetic rats during acute study.

Treatment	Dose mg/kg	Blood glucose level mg/dl in hours 0 hr	1 hr	2 hr	3 hr	5 hr	7 hr
Control normal saline	10 mL/kg	342.0 ± 47.60	243.0 ± 49.56	243.0 ± 2.94	248.66 ± 57.56	312.6 ± 65.59	326.22 ± 45.28
Glibenclamide	10	344.0 ± 87.55	272.6 ± 87.41	203.3 ± 75.76	192.6 ± 55.36	171.3 ± 64.86	184.3 ± 61.18 [c]
***n*-hexane fraction**	200	334.6 ± 58.85	242.0 ± 42.93	198.6 ± 40.20	234.6 ± 57.81	273.0 ± 71.93	275.5 ± 44.23[c]
Dichloromethane fraction	200	314.3 ± 77.62	202.33±68.00	182.0 ± 58.34	154.33 ± 42.55	156.6 ± 64.87[a]	113.33 ± 13.77[c]
Ethyl acetate fraction	200	301.0 ± 72.38	217.3 ± 70.33	187.6 ± 59.53	170.33 ± 57.62	188.0 ± 76.70	186.2 ± 45.23[c]
Methanol fraction	200	319.6 ± 87.18	276.0 ± 77.39	233.33 ± 77.28	214.33 ± 92.96	230.6 ± 84.76	226.7 ± 65.33[c]

Data are expressed as MEAN ± SEM, Significant at [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, when compared to control. ($n = 6$).

Table 3
Antidiabetic effect of solvent leaf fractions of *Setaria megaphylla* on blood glucose level of alloxan- induced diabetic rats during prolonged study.

Treatment	Dose mg/kg	Blood glucose level mg/dl in hours 0 hr	24 hr	3rd day	5th day	7th day	10th day	14th day
Control normal saline	10 mg/ml	342.0 ± 47.60	320.0 ± 52.15	312.6 ± 65.59	206.33±27.84	196.66±34.78	176.3 ± 10.13	145.3 ± 22.78
Glibenclamide	10	344.0 ± 87.55	325.6 ± 52.44	159.3 ± 5.34 ^{c}	85.6 ± 3.52^{b}	76.0 ± 6.92^{c}	74.0 ± 6.65^{c}	61.3 ± 6.36^{c}
***n*-hexane fraction**	200	334.6 ± 58.85	346.0 ± 86.52	148.5 ± 9.20^{c}	133.3 ± 4.91^{c}	73.0 ± 2.30^{c}	79.66 ± 7.21^{c}	78.0 ± 8.08^{c}
Dichloromethane fraction	200	314.3 ± 77.62	184.3 ± 40.53	123.0 ± 5.94^{c}	118.0 ± 4.04^{c}	94.66 ± 6.00^{c}	80.00 ± 4.93^{c}	77.66 ± 4.33^{c}
Ethyl acetate fraction	200	301.0 ± 72.38	248.3 ± 90.03	134.0 ± 4.00^{c}	123.6 ± 2.60^{c}	90.33 ± 2.02^{c}	92.33 ± 21.75^{c}	90.66 ± 2.99^{c}
Methanol fraction	200	319.6 ± 87.18	354.3 ± 74.46	143.0 ± 9.57	190.3 ± 4.57	83.66±7.42	77.0 ± 10.97^{c}	70.66 ± 8.19^{c}

Data is expressed as MEAN ± SEM, Significant at $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, when compared to control. ($n = 6$).

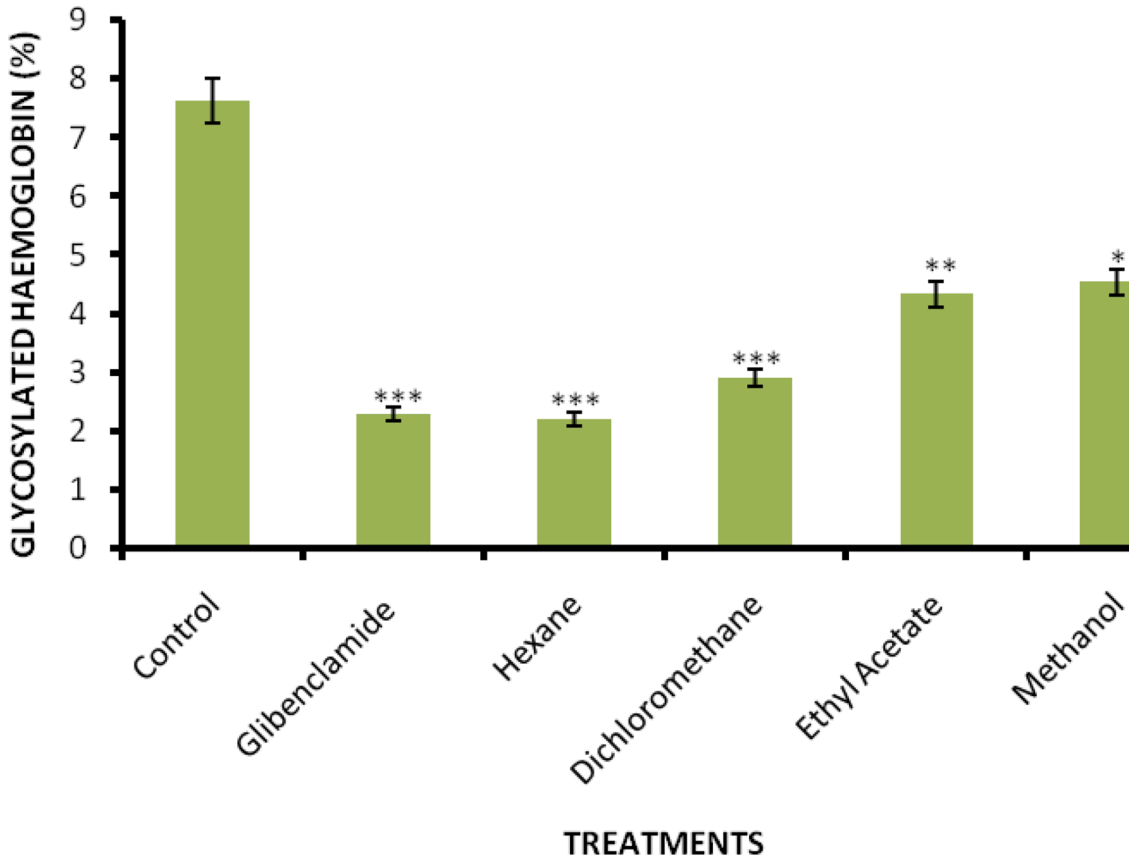

Fig. 1. Effect of solvent leaf fractions of *Setaria megaphylla* on glycosylated hemoglobin concentration of alloxan-induced diabetic rats. Data is expressed as MEAN ± SEM. Significant at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, when compared to control. ($n = 6$).

white powder, FT-IR (ATR, ν_{max}, cm^{-1}): 3275, 2918, 2854, 1467, 1064. ^{1}H NMR (400 MHz, $CDCl_3$+CH_3OD (9:1)), δ_H 0.80 (t, $J = 7.0$ Hz, 3H), 1.22 (brs, 54–58 H), 1.50 (2H, m), 3.60 (t, $J = 5.0$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$+CH_3OD (9:1)), δ: 62.62 (C-1), 32.56 (C-2), 31.86 (C-3), 29.62 (C-4), 29.56 (20C and 22C, C-5–25 and 5–27), 29.40, 29.28, 25.70, 22.61, 13.98. GC–MS, TMSi-Triacontanol: *m/z* (Relative%): 595.5 $[M\text{-}15]^{+}$ (100), 75 (35), 57.0 (40), 43.0 (35). TMSi-Dotriacontanol: *m/z* (relative %): 523.6 $[M\text{-}15]^{+}$ (100), 75 (35), 57.0 (40), 43.0 (35). These NMR data are in agreement with those reported for 1-triacontanol (Mahmuod et al., 2021), and 1-dotriacontanol (Parmer et al., 1998).

1-Triacontyl cerotate (X1). White solid, IR (ATR, ν_{max}, cm^{-1}): 2925, 2853, 1743, 1471, 1175; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.05 (*t, J* = 6.8 Hz, 2H, -$C\underline{H}_2$O), 2.30 (*t, J* = 7.0 Hz, 2H, -$C\underline{H}_2$CO), 1.61 (*quin, J* = 7.0 Hz, 4H, -$C\underline{H}_2CH_2$O and –$C\underline{H}_2CH_2$CO), 1.33–1.24 (*m*, 98 H, 49 x $C\underline{H}_2$), 0.88 (*t, J* = 7.0 Hz, 6H, 2 x $C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.12, 22.70, 25.06, 25.96, 28.68, 29.18, 29.27, 29.37, 29.49, 29.54, 29.62, 29.67, 29.71, 31.94, 34.44 ($\underline{C}H_2$CO), 64.41 (O—CH_2), 174.62 (COO). FT-IR and NMR data are consistent with reported data (Snehunsu et al., al.,2015).

Stigmasterol ((24*S*)−5,22-Stigmastadien-3*β*-ol, **PJ3);** white powder. FT-IR (ATR, ν_{max}, cm^{-1}): 2918, 2851, 1732, 1479, 1290. $C_{29}H_{48}O$, MW= 412.37. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 0.69 (3H, d, $J = 7.3$ Hz, CH_3–21), 0.81 (3H, t, $J = 7.0$, CH_3–29), 0.90 (3H, d, $J = 6.4$ Hz, CH_3–26), 1.01 (3H, d, $J = 7.5$ Hz, CH_3–27), 1.25 (3H, s, CH_3–18), 3.53 (1H, m, H-3), 5.02 (dd, 1H, $J = 8.6$, 15.0 Hz, H-22), 5.16 (1H, dd, $J = 6.6$, 15.2 Hz, H-23) and 5.34 (1H, d, $J = 4.9$ Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 140.77 (C-5), 138.32 (C-22), 129.30 (C-23), 121.72 (C-6), 71.84 (C-3), 56.89, 55.99, 50.19, 42.33, 40.49, 39.70, 37.28, 36.53, 31.92, 31.69,29.71, 28.92, 25.41, 24.38, 21.22 (C-21), 21.09 (C27), 19.41 (C-19), 18.99 (C-26), 12.25 (C-29), 12.06 (C-18). GC–MS (Rt, 17.813 min), *m/z* (%): 412.2 $[M]^{+}$(25), 351.3 (5), 300.1 ((8), 255.2 (20), 213.1 (8), 145.0 (25), 105.0 (40), 55. 0 (100). NMR data are consistent with those reported (Chen et al., 2021).

Discussion

Diabetes mellitus, a chronic metabolic disorder, is reportedly associated with increased generation of free radicals especially reactive

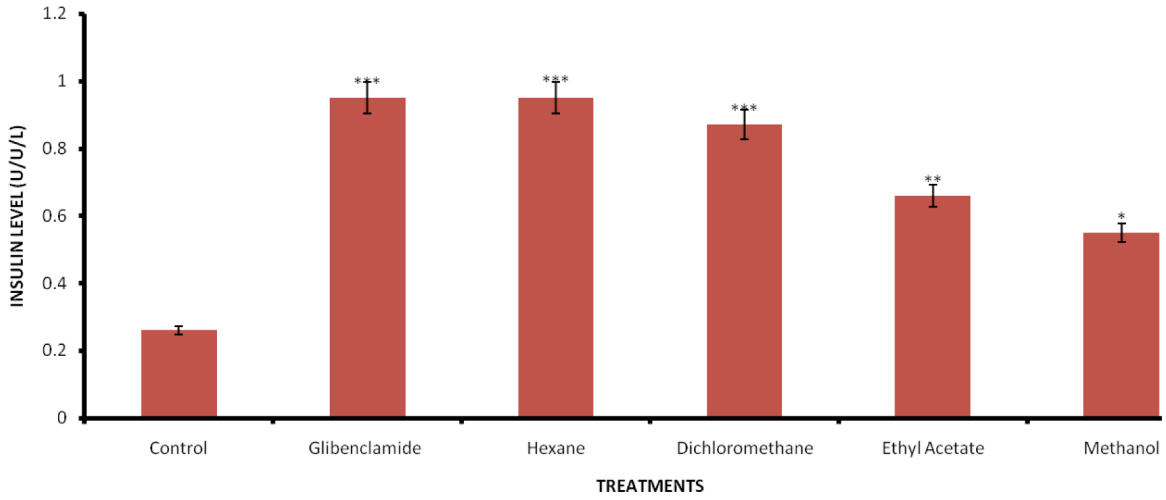

Fig. 2. Effect of solvent leaf fractions of *Setaria megaphylla* on insulin levels of alloxan-induced diabetic rats. Data is expressed as MEAN ± SEM. Significant at $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, when compared to control. ($n = 6$).

Table 4
Effect of solvent leaf fractions of *Setaria megaphylla* on Oral glucose tolerance of diabetic rats.

Treatment	Dose mg/kg	Blood glucose level (mg/dL) in min 0 min	30 min	60 min	120 min	180 min
Control normal saline	–	154.18 ± 44.68	293.9 ± 33.24(90.62)	254.54 ± 33.68(65.09)	215.94 ± 39.36(40.05)	192.8 ± 12.96(25.04)
Glibenclamide	10	170.28 ± 15.48	135.0 ± 21.42(20.71)	97.2 ± 11.80(42.91)	67.14 ± 9.00(60.57)	69.48 ± 11.52(59.19)
***n*-hexane fraction**	200	176.0 ± 51.22	180.8 ± 55.76(2.72)	91.8 ± 13.60(47.84)	97.2 ± 12.80(44.77)	87.48 ± 14.16(50.29)
Dichloromethane fraction	200	151.7 ± 2.16	283.14 ± 24.48(86.61)	151.2 ± 8.46(0.32)	99.0 ± 9.36(34.73)	85.18 ± 5.04(43.84)
Ethyl acetate fraction	200	174.74 ± 48.78	139.68 ± 24.48(20.06)	137.88 ± 15.22(21.09)	122.9 ± 9.90(29.66)	115.2 ± 10.08(34.07)
methanol fraction	200	183.6 ± 44.10	185.4 ± 44.46(0.98)	117.54 ± 11.70(35.98)	86.94 ± 19.0 (52.64)	90.0 ± 8.10(50.98)

Data is expressed as MEAN ± SEM, Significant at $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, when compared to control. ($n = 6$). Values in parenthesis represent% inhibition of elevation of blood glucose calculated relative to 0 min.

Table 5
Effect of solvent leaf fractions of *Setaria megaphylla* on lipid profile of alloxan-induced diabetic rats.

Treatment	Dose mg/kg	Total cholesterol (mMol/L)	Triglyceride (mMol/L)	HDL-C (mMol/L)	LDL-C (mMol/L)	VLDL (mMol/L)
Control	10 mL/kg	2.70 ± 0.05	1.47 ± 0.08	1.81 ± 0.08	1.55 ± 0.09	0.67 ± 0.04
Glibenclamide	10	2.20 ± 0.11	1.02 ± 0.11^{a}	1.39 ± 0.12	1.27 ± 0.03	0.46 ± 0.04^{a}
***n*-hexane fraction**	200	2.50 ± 0.12	1.41 ± 0.03	1.70 ± 0.25	1.46 ± 0.08	0.63 ± 0.01
Dichloromethane fraction	200	2.70 ± 0.05	1.45 ± 0.05	1.87 ± 0.06	1.49 ± 0.11	0.65 ± 0.02
Ethyl acetate fraction	200	2.90 ± 0.05	1.58 ± 0.10^{c}	2.17 ± 0.01	1.47 ± 0.03	0.71 ± 0.04
Methanol fraction	200	2.66 ± 0.08	1.50 ± 0.09	1.95 ± 0.24	1.38 ± 0.15	0.67 ± 0.04

Data is expressed as MEAN ± SEM, Significant at $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, when compared to control. ($n = 6$).

oxygen species (ROS) (Okutana et al., 2005; Papachristoforou et al., 2020). Alloxan, which was used to induce diabetes in this study is reported to be biotransformed to dialuric acid with accompanying generation of free radicals (Mathews and Leiter, 1999) and subsequently, partial destruction of pancreatic β-cells of islet of Langerhans (Abdel--Barry et al., 1997). This reduces insulin level resulting in type 2 diabetes mellitus (Ighodaro et al., 2017), with some remnant pancreatic β-cells having insulin producing potentials as observed in a study with hypoglycemic agents like sulphonylureas in alloxan-induced diabetic rats (Subramoniam et al., 1996). The insulin deficiency resultantly stimulates lipolysis in adipose tissues and gives rise to hyperlipidemia (Ahmad et al., 2014).

In this study, *S. megaphylla* leaf fractions demonstrated sustained significant antidiabetic activities during acute and prolonged studies with dichloromethane, ethyl acetate and *n*-hexane fractions exerting prominent activities. The FBG levels of the treated diabetic rats were significantly reduced when compared to those of untreated diabetic rats (control). The antidiabetic results observed in this study corroborate those of previous reports (Okokon and Antia, 2007; Okokon et al., 2007a). Thus, confirming and validating the antidiabetic potentials of this plant in ethnomedicine.

Diabetes mellitus causes body glucose regulatory processes to be compromised leading to chronic hyperglycemia (Champe et al., 2005). The observed reduced FBG of diabetic rats following treatment with the leaf fractions suggests blood glucose lowering potentials probably through insulin secretion stimulation as was observed in this study.

The solvents fractions were observed to cause elevation of serum insulin levels of the diabetic rats with *n*-hexane fraction producing the highest effect followed by DCM fraction. These fractions were observed to offer considerable protection to the pancreas of the diabetic rats and may account for the high insulin levels of the groups. This result suggests insulin secretion stimulatory potential as well as recovery activities of injured β-cells in alloxan-induced diabetic rats. These effects may have resulted from the antioxidative stress activities of the fractions as reported earlier by Okokon et al., al.(2013), and 1-triacontyl cerotate (Snehunsu et al., 2015), as well as pancreatic cell stimulatory effect of stigmasterol which is reported to increase serum insulin levels in diabetic rats (Nualkaew et al., 2015), probably through regeneration of

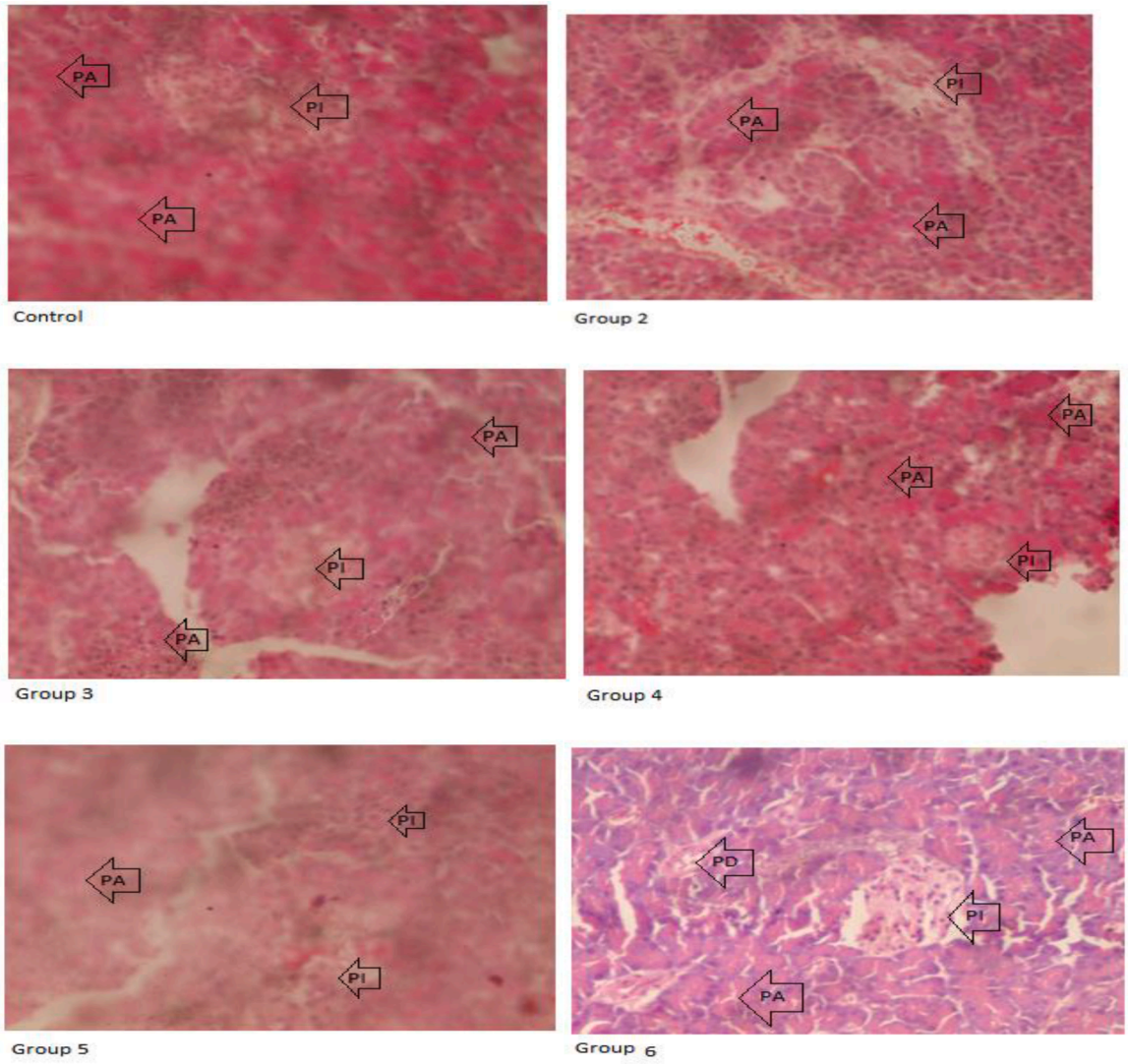

Fig. 3. Histological sections of pancreas of alloxan-induced diabetic rats treated with normal saline (control) 10 mL/kg(1), glibenclamide 10 mg/kg bw (2), *n*-hexane fraction 200 mg/kg bw (3), dichloromethane fraction 200 mg/kg bw (4), ethyl acetate fraction 200 mg/kg bw(5), methanol fraction 200 mg/kg bw(6) at magnification B(x400), stained with H&E method. Keys: Pancreastic acini (PA), Pancreatic Islets (PI), Pancreastic degeneration (PD).

pancreatic β-cells. The presence of these compounds and earlier reported compounds (Okokon et al., 2007b, 2013, 2017; Udobang et al., 2017) in the fractions must have offered protection to the pancreas and β-cells against the effect of alloxan as observed in the histopathology which could have been responsible for the high insulin level. Thus, this explains the observed pancreas protection potential.

The solvent fractions were found to considerably reduced FBG levels of treated diabetic rats during oral glucose tolerance test (OGTT). Oral glucose tolerance test measures the body's ability to use glucose, the main source of energy (Gold, 1970). Lowering of BGL effect was observed post administration of the solvent fractions with methanol and hexane fractions exerting the highest inhibition of BGL increases. This may be due to the activities of phytochemicals present in the solvent fraction as earlier reported (Okokon et al., 2006, 2013). Blood glucose level after glucose loading is dependent on insulin secretion, glucose utilization, intestinal absorption and intestinal motility (Peungvicha et al., 1996), alternative mechanism by the respective fractions could have involved insulin secretion stimulation, glucose utilization and inhibition of intestinal absorption and intestinal motility. Naveen and Baskaran (2018) had reported that the possible modes of natural products action in glycemic control include stimulation of glucose uptake by tissues, inhibition of intestinal glucose absorption, increased glycogen synthesis, inhibition of dipeptidyl peptidase-IV (DPP-IV), among others.

In this study, the fractions were observed to improved glucose tolerance which could be attributable to potentiation of the insulin effect on the plasma glucose through increased pancreatic insulin secretion from existing β-cells or its release from bound insulin as was observed in this study. This could have also resulted from the inhibitory potentials of the leaf fractions on α-amylase and α-glucosidase activities as reported earlier by Okokon et al. (2021), thereby inhibiting glucose absorption from the intestines, in addition to insulin secretion stimulation in response to glucose load and increased peripheral utilization of glucose which are the probable mechanisms of antidiabetic action (Andrikopoulos et al., 2008; Regginato et al., 2021).

In uncontrolled diabetes, high level of glycosylation of some proteins including hemoglobin occurs, contributing to long-term complications of diabetes (Latha and Pari, 2004). Hb1Ac level usually indicates glycemic control (Daisy and Rajathi, 2009) and significant lowering of Hb1Ac levels of diabetic rats by the leaf fractions indicates controlled blood glucose level especially in hexane and dichloromethane fractions-treated groups. However, high Hb1Ac levels with corresponding increased plasma glucose levels were observed in untreated diabetic rats. These results corroborate the findings of previous work (Adeneye and Adeyemi, 2009) and confirm antidiabetic activity of leaf

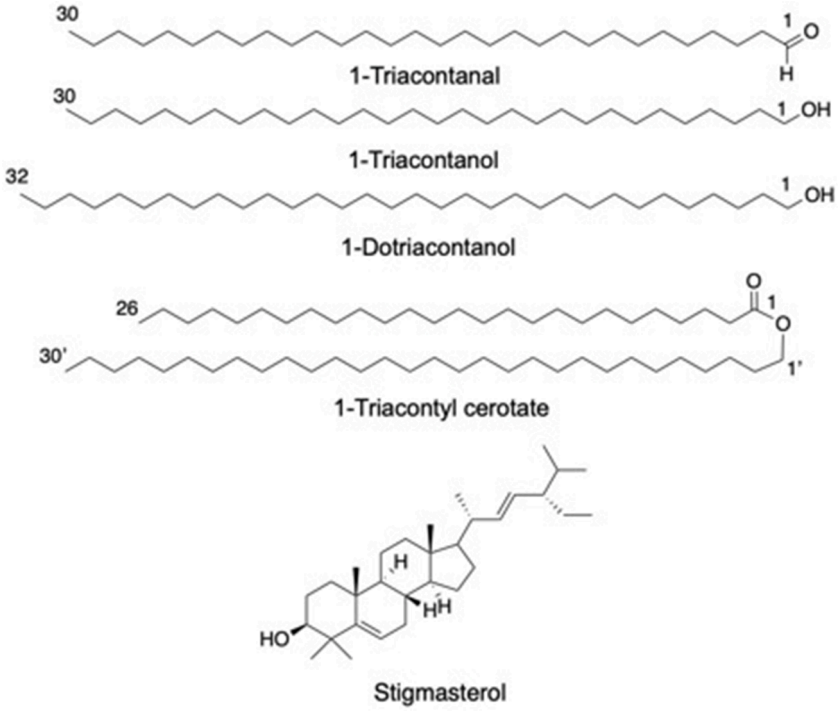

Fig. 4. Chemical structures of isolated and identified compounds from *S. megaphylla.*

fractions of *S. megaphylla* via insulin secretion stimulation, resulting in lowering of BGL and HbA1c levels.

Phytochemical screening of the leaf extract revealed the presence of saponins, tannins, flavonoids, alkaloids and terpenes (Okokon et al., 2007). Also, previous GC-MS analysis of the *n*-hexane fraction of the leaf revealed the presence of (Z,Z,Z)-8,11,14-eicosatrienoic acid, phthalic acid, diisooctyl ester, vitamin E, ϒ-elemene, urs-12-ene, bicyclogermacrene, α-muurolene, germacrene-A, and guaiol among others (Okokon et al., 2013), while GC-MS analysis of ethyl acetate leaf fraction revealed the presence of (*E*)-β-ocimene, *p*-metha-1(7),8-diene, (3a)-D:A-friedooleannan-3-ol,stigmastone-3,6-dione(5a), bicyclo[2.2.1] heptan-2-ol, 4, 7,7-trimethyl, *p*-cymene (Okokon et al., 2017). Borneol, astaxanthin, α-terpineol, terpinen-4-ol, β-*cis*-bergamotene, citronellol, germacrene D among others were reported in dichloromethane fraction and 3-methyl--undecane, hexadecanoic acid, 1,2-benzene dicarboxylic acid, isodecyl octyl ester, carvacrol, linanlool, camphor, borneol, menthofuran, menthone, α-terpineol, and α-eudesmol were found in *n*-butanol fraction (Udobang et al., 2017).

In this study, additional compounds including 1-triacontanal, 1-triacontanol, 1-dotriacontanol, 1-triacontyl cerotate, and stigmasterol were isolated and identified. Some of these phytochemicals in these fractions may in part be responsible for the observed activities of these fractions either singly or in synergy. Several plants rich in terpenes, such as sesquiterpenes and monoterpenes as found in this leaf fractions have been shown to have an effect on blood glucose levels (Alam et al., 2018; Belhadj et al., 2018; Ding et al., 2018) by stimulating insulin secretion and glucose uptake, improving glycogen synthesis and inhibiting α-glucosidase (Zhao et al., 2012; Naveen and Baskaran, 2018). According to Brahmachari (2011), flavonoids also exhibit glycaemic control and also could regulate the rate-determining enzymes vital for metabolic pathways of carbohydrate. Similarly, several studies have shown that ß-sitosterol, stigmasterol, betulin, ergost-8(14)-en-3-ol, *n*-hexadecanoic acid, and palmitic acid exert hypoglycemic effects by reducing the absorptions of cholesterol from the gut ((Rajasekaran et al., 2006); Gosh et al., 2014) and their presence in these fractions could likely be responsible for the observed antidiabetic activity

Hyperglycemia and associated diabetic dyslipidemia lead to several comorbidities including macro-and microvascular damage (Naveen and Baskaran, 2018). Elevated blood glucose levels equally gives rise to low levels of HDL cholesterol and escalation of LDL cholesterol, thus increasing risk of coronary heart diseases (Sudasinghe and Peiris, 2018). Additionally, stimulation of catabolic activity and increased mobilization of free fatty acids from peripheral deposits by diabetic condition as well as lipolytic action of hormones and inhibition of hormone sensitive lipase production by insulin result in elevated lipid levels, thereby, increasing the risk of myocardial dysfunction (Rojop et al., 2012). Therefore, both diabetes and lipid levels must be managed properly to achieve a satisfactory treatment outcome. The leaf fractions were found to have insignificant lowering effect on total cholesterol, TG, VLDL and LDL levels of the diabetic rats and considerably increased the level of HDL –cholesterol in the diabetic rats. The administration of the fractions may have partly increased glucose utiization, thereby suppressing fats mobilization and lipolysis involving plasma lipoprotein lipase, though not significantly (BenKhedher et al., 2018).

Plants sterol such as stigmasterol present in the dichloromethane fraction exerts antihyperlipidemic activity through different mechanisms to inhibit the absorption of cholesterol from the gut (Batta et al., 2006). The antihyperlipidemic effects observed in this study may have resulted from the activities of this and other phytochemical compounds.

Body weight loss which is due to increased muscle wasting and loss of tissue proteins is common in diabetic rats (Shirwaikar et al., 2005). Treatment with the leaf fractions especially ethyl acetate and hexane fractions, remedied this situation perhaps due to the chemical constituents of these fractions which have the ability to reduce hyperglycaemia by increased glucose metabolism, inhibition of α-amylase and α-glucosidase enzymes, and protein synthesis stimulation thereby suppressing muscle wasting through reversal of gluconeogenesis (Mestry et al., 2017).

Alloxan monohydrate has been reported to cause various injuries to the pancreas which results in partial destruction of pancreatic β-cells of islet of Langerhans (Abdel-Barry et al., 1997). The pancreas of untreated diabetic rats were observed to have areas of islets cell degeneration, degranulated islet, degranulated β- and α- cells as well as reduced islet cells among others. These pathologic features were not prominent in the

leaf fractions treated groups, suggesting regenerative effect of the extract. The phytoconstituents in these leaf fractions such as stigmasterol, hexadecanoic acid and 1-triacontanol cerotate among others, through their antioxidative effect, could have caused the regeneration of the pancreas and increased level of insulin in treated diabetic rats in this study resulting in hypoglycaemic activity. The protection maybe due to the free radical scavenging potentials of the leaf fractions as reported by Okokon et al.,(2013) and recently by Umana (2021) which revealed strong antioxidant potentials of the hexane and ethyl acetate fractions of the leaf extract in the various *in vitro* models. This activity inherent in the leaf extract/fractions could have counteracted the vast free radicals generated by alloxan and the diabetic condition thereby leading to the observed antidiabetic and pancreas protective effects in this study.

However, this study could not determined the quantities of these identified and isolated compounds in the fractions, which is necessary for the standardization of the extract/fractions in order to be used effectively and safely nor established the antioxidant and antidiabetic activities of the isolated compounds. These were the limitations of this study which further study has been recommended to be carried out on the leaf extract.

Conclusion

The results of this study show that the leaf fractions of *S. megaphylla* possess antidiabetic, hypolipidemic and pancreas protective potentials, which may be partially attributed to the identified compounds from this plant.

Ethics approval and consent to participate

Permission and approval for animal studies were obtained from College of Health Sciences Animal Ethics committee, University of Uyo (UU/CHS/AE/21/024).

Consent for publication

All the authors read and approved the final content of this manuscript for publication.

Availability of data and material

All data generated in this project were included in this manuscript, supplementary materialsand stored in our computer hard drive and external storage drive which will be available upon request.

Authors' statements

Jude E. Okokon designed and supervised the work, Lekara John and Klinton Iwara carried the animal studies, Koofreh Davies did the statistical analysis, Paul S. Thomas carried out the isolation and purification of the compounds, Wen-Wu Li did the GC–MS and NMR analysis and interpretation of the spectra and also edited the work.

All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Competing Interest

The authors declare that there are no competing interests regarding the publication of this paper.

Acknowledgements

This research work did not receive any grant from government, profit and nonprofit organizations. Research was conducted in the Department of Pharmacology and Toxicology as well as Department of Pharmacognosy and Natural medicine, University of Uyo, Uyo, Nigeria. The authors are grateful to the management of University of Uyo for providing the enabling environment to carry out this work and Mr Nsikan Malachy of Pharmacology and Toxicology Department for providing technical assistance. We thank Dr. Sian Woodfine and Falko Drijfhout at Keele University for recording NMR and obitrap MS spectra, respectively.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2021.100182.

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