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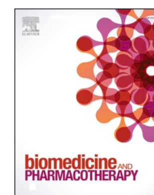

In vivo antihyperglycaemic and antihyperlipidemic activities and chemical constituents of *Solanum anomalum*

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A R T I C L E I N F O

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A B S T R A C T

Solanum anomalum is a plant used ethnomedically for the treatment of diabetes. The study was aimed to validate ethnomedical claims in rat model and identify the likely antidiabetic compounds. Leaf extract (70–210 mg/kg/day) and fractions (140 mg/kg/day) of *S. anomalum* were evaluated in hyperglycaemic rats induced using alloxan for effects on blood glucose, lipids and pancreas histology. Phytochemical characterisation of isolated compounds and their identification were performed using mass spectrometry and NMR spectroscopy. Bioinformatics tool was used to predict the possible protein targets of the identified bioactive compounds. The leaf extract/fractions on administration to diabetic rats caused significant lowering of fasting blood glucose of the diabetic rats during single dose study and on repeated administration of the extract. The hydroethanolic leaf extracts also enhanced glucose utilization capacity of the diabetic rats and caused significant lowering of glycosylated hemoglobin levels and elevation of insulin levels in the serum. Furthermore, triglycerides, LDL-cholesterol, and VLDL-cholesterol levels were lowered significantly, while HDL–cholesterol levels were also elevated in the treated diabetic rats. There was absence or few pathological signs in the treated hyperglycaemic rat pancreas compared to that present in the pancreas of control group. Diosgenin, 25(*R*)-diosgenin-3-O-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranoside, uracil, thymine, 1-octacosanol, and octacosane were isolated and identified. Protein phosphatases along with secreted proteins are predicted to be the major targets of diosgenin and the diosgenin glycoside. These results suggest that the leaf extract/fractions of *S. anomalum* possess antidiabetic and antihyperlipidemic properties, offer protection to the pancreas and stimulate insulin secretion, which can be attributable to the activities of its phytochemical constituents.

1. Introduction

Diabetes mellitus (DM), a chronic and metabolic disease, constitutes a serious health challenge threatening all countries world over economically and socially. The disease has contributed to governance problems and ailing economy of most countries. The World Health Organisation (WHO) reported that elevated blood glucose level and associated complications leading to estimated millions of deaths world over are recorded yearly ranking diabetes 8th among the deadly diseases killing people worldwide [1]. The increasing number of people with diabetes, which is projected to escalate to 700 million by 2045 [2], portrays a serious threat to human existence and therefore requires that positive steps be taken urgently to address the situation. The management of DM, which is largely by the use of insulin and a wide range of blood glucose lowering (hypoglycaemic) agents such as biguanides (metformin), sulfonylureas, meglitinides, thiazolidinediones,

Abbreviations: DCM, Dichloromethane; EA, Ethyl acetate; FBG, Fasting blood glucose; GC-MS, Gas chromatography-mass spectrometry; H & E, Heamatoxylin and eosin; Hb1Ac, Glycosylated haemoglobin; HDL, High density lipoprotein; LCMS, Liquid chromatography-mass spectrometry; LDL, Low density lipoprotein; NMR, Nuclear magnetic resonance; OGTT, Oral glucose tolerance test; PTPN1, Protein-tyrosine phosphatase 1B; VLDL, Very low density lipoprotein; WHO, World Health Organisation.

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α-glucosidase inhibitors, dipeptylpeptidase-4 (DPP-4) inhibitors, sodium-glucose cotransporter-2 (SGLT2) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and amylin mimetics [3]. These therapies seem to pose financial challenges to the patients and their families in terms of cost coupled with the associated side effects. Therefore, the search for affordable and safe alternative drugs is inevitable.

Plant based herbal preparations and isolated natural products [3,4] are used and/or investigated for DM management. A large number of phytochemicals such as flavonoids, polyphenols, terpenoids, alkaloids (e.g. berberine, tetrandrine), saponins (steroid or triterpenoid glycosides, e.g. ginsenosides), and quinones showed antidiabetic activity through various mechanisms of action [4]. Previously, we have demonstrated the in vivo efficacy of several Nigerian plant extracts such as *Anthocleista djalonensis* [5], cornhusk of *Zea mays* [6], and *Setaria megaphylla* [7] in animal models.

The *Solanum* is the largest genus in the family Solanaceae, which has about 2000 species widely distributed in the subtropical and tropical regions of Africa, Asia and Australia. Some *Solanum* species such as *S, tuberosum* (potato), *S. lycopersicum* (tomato), and *S. melongena* (eggplant or aubergine) are vegetables and are economically significant, while others possess a plethora of pharmacological activities such as antidiabetic activity [8]. Extracts of *S. indicum* [9,10], *S. nigrum* [11–14], *S. melongena* [15], *S. trilobatum* [16], *S. macrocarpon* [17], *S. lycocarpum* [18], and *S. anguivi* [19] were shown to have antidiabetic activities. Phytochemicals such as steroidal saponins, steroidal alkaloids, flavonoids, lignans, and terpenes were identified from various *Solanum* species [8].

Solanum anomalum Thonn. ex Schumach, a plant whose fruits and leaves are used medicinally and nutritionally is commonly found growing in West and East Africa sub-regions. Its parts are utilised locally to treat diabetes, gastrointestinal disorders infections, inflammation and pains [20]. The antidiabetic property of the fruits [20], anti-inflammatory [21], antioxidant and antiulcer [22] properties of the leaf extracts have been reported. Its fruit extracts have also been reported to alleviate lead acetate toxicity on male reproductive system and sperm quality of rats [23]. This investigation reports the blood glucose and lipids lowering effects of *S. anomalum* leaves in rat model as well as isolation and characterization of its potential bioactive compounds.

2. Materials and methods

2.1. Plants collection

Fresh leaves of *S. anomalum* were collected from gardens in rural areas of Uruan, Akwa Ibom State, Nigeria in August, 2020. Prof. Margaret Bassey, a taxonomist in University of Uyo, Uyo, Nigeria identified and authenticated the leaves. Leaf sample specimen (UUH.75a) was deposited at the University's Herbarium.

2.2. Extraction

Fresh leaves of *S. anomalum* were washed, sliced and left to dry for two weeks on the laboratory table. An electric blender was used in powdering the dried leaves. A portion of the leaf powder (1.5 kg) was soaked in 50% ethanol (7.5 L) for 3 days at room temperature. While another portion (1.5 kg) was soaked successively for 3 days in these solvents (2 x 5 L) following their polarity gradient, *n*-hexane, dichloromethane (DCM), ethyl acetate and methanol to obtain the solvent fractions which were concentrated and stored in a refrigerator. The yields of crude extract of *n*-hexane, DCM, ethyl acetate and methanol were 8.4%, 0.2%, 0.2%, 0.1%, and 0.3%, respectively.

2.3. Purification and isolation of compounds

The active antidiabetic leaf fractions: *n*-hexane, ethyl acetate and methanol (each 15 g), which showed greater glucose lowering potential were purified using silica gel column chromatography (Merck, 60–120 mesh). *n*-Hexane fraction was eluded alone, while a combination of ethyl acetate and methanol fractions was eluded differently. *n*-Hexane fraction was eluted with *n*-hexane, dichloromethane and ethyl acetate in different proportions and adjusting the polarity. Monitoring of purity of the pooled fractions from successive columns on silica TLC plates (Merck, Germany) using spray agents (vanillin-sulphuric acid) led to isolation **K1** (9 mg), **PP4** (6 mg) and **R10** (10 mg). Similarly, ethyl acetate and methanol combined fractions were eluted with *n*-hexane, adjusting the polarity by varying the volumes of dichloromethane, ethyl acetate and methanol successively according to their polarity. This resulted in a fraction which was further purified on Sephadex LH-20 using methanol to obtain two sub-fractions **S9** (10.5 mg) and **S10** (5.2 mg). **S9** was further purified by Agilent 1220 semipreparative high performance liquid chromatography (HPLC) using a reversed phase C-18 column and eluting with 2% B (A + B) for 5 min, an increase to 100% B over 25 min, and 100% B for 5 min and a UV detection at 215 nm at a flow rate of 4 mL/min. Solvents A and B are distilled water and methanol, respectively. The fractions eluted from HPLC at 4–5 min and 9–10 min were collected and freeze-dried to give **S9–1** and **S9–2** as white powder, respectively. **S10** was further purified by semi-preparative HPLC eluting with 20% B (A + B) and increase to 100% B over 25 min, and 100% B for 6 min with a UV detection at 203 nm to give **S235** (1.0 mg) at Rt30-30.5 min.

2.4. Gas chromatography-mass spectrometry analysis

GC-MS analysis of the *n*-hexane and DCM fractions or isolated compounds was performed using an Agilent 7890 A gas chromatograph connected with an 5975 C MSD detector (Agilent Technologies, USA). 1–2 μL of fractions or isolated compounds (with or without TMSi derivation by treatment of *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA)) [24] in hexane or DCM were injected to a HP5-MS column (5% phenyl-methylpolysiloxane, 30 m × 0.25 mm × 0.25 μm) and eluted with helium gas under a pressure of 10 psi. The oven temperature gradient was: starting at 150 °C for 3 min, increasing to 300 °C at 10 °C/min, and keeping at 300 °C for 5 min. Electron ionization mode of GC-MS at 70 eV was applied. The metabolites in the *n*-hexane and DCM fractions and isolated compounds were determined by comparison of spectral data in the NIST 2011 database.

2.5. Liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS)

1–2 μL of compounds **S9–1**, **S9–2** and **S235** dissolved in methanol were injected into a Raptor C18 column (100 ×2.1 mm, particle size 2.7 μm, Restek, UK) coupled with an Agilent Infinity 1260 series system comprising a G1316A TCC Thermostated column compartment, G1312B Binary Pump, G4225A Hip Degasser, G1329B ALS Auto sampler and 6530 Accurate Mass Q-TOF as the detector as described previously [24]. A detector with mass range of 100–1700 *m/z* and Qualitative analysis Mass Hunter workstation software were used. A flow rate of solvent was set at 0.3 mL/min by keeping 5% B over 2 min and increasing from 5% to 98% (from 2 to 21 min) and keeping it at 98% B for another 3 min. Solvent A and B are 0.1% formic acid in water and acetonitrile, respectively.

2.6. NMR spectroscopy

^{1}H and ^{13}C NMR spectra for the isolated compounds (**K1**, **PP4**, **R10**, **S9–1**, and **S9–2**) in $CDCl_3$ or DMSO-d_6 were obtained on a Bruker 400 MHz instrument. ^{1}H NMR, 2D NMR (HSQC, HMBC, TOCSY and ROESY) and 1D-TOCSY spectra for the isolated compound (**S235**) in CD_3OD were obtained on Bruker AVIII 700 MHz instrument equipped a TCI helium cryoprobe. ^{13}C NMR of **S235** was recorded on a Bruker AVIIIHD 600 MHz NMR instrument equipped with a Prodigy-N_2 broadband

cryoprobe.

2.7. Experimental animals

Albino Swiss mice (19 – 28 g) and Wistar rats (138–150 g), male and female, use for this study were gotten from Animal house of University of Uyo. Plastic cages were used to keep them, while being allowed free access to standard feed and water. Animal handling protocols of NIH (1996) were followed, and the work was approved by University of Uyo's Animal Ethics Committee (UU/CHS/AE/20/016).

2.8. Acute toxicity testing in mice

A modified method of Lorke [25] was used for acute toxicity testing of the leaf extract (100–1000 mg/kg) intraperitoneally to determine the median lethal dose (LD_{50}) in mice. Manifested toxicity signs as well as mortality were recorded in the respective groups within 24 h.

2.9. Induction of experimental diabetes in rats using alloxan monohydrate

Alloxan monohydrate (150 mg/kg) freshly prepared in saline was used to intraperitoneally induced diabetes in 60 overnight fasted healthy male and female rats of known weights. The initial post-induction hypoglycaemic state was subdued in the animals by 2 mL of 5% dextrose solution orally administered soon after induction. The animals were given 3 days rest period to allow the diabetic condition to fully developed, during which they had free access to food and water. For the study, rats attaining hyperglycaemic levels of 200 mg/dL and above with persistent glycosuria, and hyperglycaemia were selected for the experiments.

The selected diabetic rats were randomly allocated to 9 different treatment groups (n = 6). The dosing regimens were determined from the determined LD_{50} value, and the diabetic rats were treated as follows. Group 1 was the negative control group was administered 10 mL/kg/day of normal saline for 14 days, orally. Group 2 was the positive control group administered 5 mg/kg/day of glibenclamide for 14 days orally. Groups 3–5 were respectively given 70, 140 and 210 mg/kg/day of *S. anomalum* leaf extract for 14 days orally while groups 6 − 9 were respectively administered with 140 mg/kg/day of various fractions of *S. anomalum* leaves: *n*-hexane, dichloromethane, ethyl acetate and methanol fractions, orally for 14 days.

2.10. Evaluation of antihyperglycaemic potentials of leaf extract and fractions of S. anomalum in alloxan-induced diabetic rats.

Fasting blood glucose (FBG) levels of respectively treated groups of diabetic rats were monitored and recorded throughout the study at predetermined intervals. For acute or single dose study, monitoring and measurement of FBG levels of rats were done at 1, 2, 3, 5 and 7 h interval, post after receiving one dose of the extract/fractions, while on repeated administration (prolonged study) of the extract/fractions for 14 days measurement was done daily on days 1, 2, 3, 5, 7 and 14. Glucometer was used to monitor blood glucose level from tail blood of rats collected on the respective days. The diabetic rats were treated in the morning hours (7.00–8.00 am) everyday throughout the duration of the study and they were fasted of food overnight prior to the measurement of their fasting blood glucose concentrations.

2.11. Effect of S. anomalum leaf extract/fractions on diabetic rat body weights

The effect of the extract/fractions on body weight of the treated diabetic rats was determined by taking their weights at the beginning of experiment, shortly before commencement of treatments and after the treatment period.

2.12. Organs and blood samples collection

On the 15th day of the study (24 h post treatment with extract/fraction) the rats were sacrificed under anaesthesia with diethyl ether vapour after their weights were taken. Sera were separated by centrifugation from blood samples collected from the respective rats for biochemical analysis. Surgical removal of the pancreas from diabetic rats was carried out and the weights of all isolated pancreas were taken before fixing them in 10% buffered formalin for histological study.

2.13. Effect of leaf extract/fraction on Insulin and glycosylated hemoglobin levels of the diabetic rats

This was assessed by measuring serum levels of insulin [26] and glycosylated haemoglobin [27] levels of treated hyperglycaemic rats using standard kits according to previously described methods.

2.14. Evaluation of the effect of the leaf extract and fractions on the lipid profile of the treated diabetic rats

Lipid profile parameters were enzymatically determined using standard diagnostic kits, while the values of low and very low-density lipoprotein (LDL and VLDL) from determined values of basic lipid parameters such as high density lipoprotein (HDL), triglyceride and total cholesterol were determined according to the reported method [28].

2.15. Prediction of protein targets of selected isolated compounds

The protein targets of two steroidal compounds, diosgenin (**R10**) and diosgenin glycoside (**S235**) were predicted using freely available SwissTargetPredict web tool [29,30].

2.16. Statistical analysis

Values are represented as mean ± SEM and significance relative to control were considered at $p<0.05$. Data collected in this study were analyzed using one-way ANOVA followed by Tukey Kramer post-hoc test using GraphPad Prism software Inc. (La Jolla, CA, USA).

3. Results

3.1. Determination of median lethal dose (LD_{50})

The calculated LD_{50} value of the extract is 725 mg/kg. Restlessness, increased respiratory rate, reduced motor activity, gasping and coma were manifested by the mice before they died.

3.2. Effect of leaf extract and fractions on body weights of rats

Considerable differences in body weights of both the extract/fractions-treated diabetic rats and negative control were observed after the treatment period (Table 1). Administration of extract and fractions of *S. anomalum* to diabetic rats produced significant ($p < 0.05$–0.001) but non–dose-dependent increases in body weights of treated diabetic rats with the middle dose (140 mg/kg) having the highest weight increase (10.0%) followed by methanol fraction treated group (8.8%) (Table 1).

3.3. Effect of extract and fractions on pancreas weights of diabetic rats

Administration of leaf extract and fractions of *S. anomalum* to diabetic rats reduced significantly ($p < 0.001$) the pancreas weights of treated hyperglycaemic rats when compared to that of control group and DCM and methanol groups had the lowest weights (Table 1). The standard drug, glibenclamide, also exhibited significant ($p < 0.001$) lowering of pancreas weights of the treated hyperglycaemic rats when

Table 1
Effect of leaf extract and fractions of *S. anomalum* on body and pancreas weights of diabetic rats.

Treatment	Dose	Body weight (g)			Weights of pancreas (g)
	mg/kg	Day 0	Day 15	% Change	
Control normal saline	Saline	132.6 ± 18.4	129.3 ± 20.4	-1.9	1.0 ± 0.1
Glibenclamide	10	132.0 ± 9.5	141.3 ± 12.3	7.0	0.7 ± 0.1[a]
Crude extract	70	143.6 ± 12.6	158.0 ± 3.2	10.0	0.7 ± 0.2[a]
	140	152.8 ± 15.6	163.0 ± 9.3	6.7	0.7 ± 0.2[a]
	210	140.8 ± 13.3	152.6 ± 7.2	8.4	0.7 ± 0.2[a]
n-Hexane fraction	140	142.9 ± 8.5	153.6 ± 6.5	7.5	0.7 ± 0.1[a]
DCM fraction	140	138.4 ± 6.3	144.6 ± 13.7	4.7	0.6 ± 0.2[a]
Ethyl acetate fraction	140	144.3 ± 8.5	156.6 ± 9.9	8.5	0.8 ± 0.1[a]
Methanol fraction	140	145.8 ± 7.5	158.6 ± 10.6	8.8	0.6 ± 0.03[a]

Values are expressed as Mean ± SEM, Significant at $^{a}p < 0.001$, when compared to control. (n = 6).

compared to that of negative control group (Table 1).

3.4. Antihyperglycaemic activity of S. anomalum leaf extract and fractions during acute study

The administration of *S. anomalum* leaf extract and fractions to hyperglycaemic rats, non-dose-dependently reduced the FBG levels of the treated rats 2 h after treatment. However, statistically significant low values ($p < 0.05$–0.01), compared to control, were recorded in groups treated with *n*-hexane and ethyl acetate fractions 5–7 h post treatment. The reductions exhibited by *n*-hexane (40%) and ethyl acetate (30%) fractions 7 h post treatment were higher compared to that of glibenclamide (12%) (Table 2).

3.5. Antihyperglycemic activity of S. anomalum leaf extract and fractions during repeated treatment

Repeated treatment of hyperglycaemic rats with *S. anomalum* leaf extract and fractions demonstrated significant ($p < 0.05$–0.001) reductions in FBG levels of the rats following 14 days daily treatment. These reductions which compared well with that exhibited by glibenclamide, were sustained throughout the experimental period and non-dose-dependent. On day 14, the effects were 64.1%, 63.4%, 65.4% and 64.5% for 70, 140, 210 mg/kg of the crude extract and glibenclamide respectively (Table 3). The leaf fractions also exerted sustained significant ($p < 0.05$–0.001) reductions of hyperglycemia in the treated diabetic rats. Percentage reductions of FBG exerted by the different fractions on day 14 were 68.0%, 54.7%, 66.1% and 66.8% respectively for *n*-hexane, dichloromethane, ethyl acetate and methanol fractions. The antidiabetic effects exhibited by *n*-hexane, ethyl acetate and methanol fractions were comparable to effect exerted by glibenclamide, the standard drug (Table 3).

3.6. Effect of S. anomalum leaf extract and fractions on insulin level

Treatment of hyperglycaemic rats with leaf extract and fractions of *S. anomalum* caused dose-dependent elevations of insulin levels of the treated rat groups. The levels of serum insulin recorded in hydroethanolic extract, *n*-hexane, DCM and ethyl acetate fractions-treated groups were higher compared to megative control group. The extract (210 mg/kg) and ethyl acetate fraction-treated groups exhibited the highest insulin level ($p < 0.001$) relative to that of glibenclamide (postive control) (Fig. 1).

3.7. Effect of S. anomalum leaf extract and fractions on glycosylated hemoglobin level

Administration of leaf extract and fractions of *S. anomalum* caused non-dose-dependent and statistically significant ($p < 0.05$–0.001) reductions in glycosylated hemoglobin levels of the treated hyperglycaemic rat groups. The middle dose of extract (140 mg/kg) and DCM fraction demonstrated stronger reduction potential than glibenclamide (Fig. 2).

3.8. Antihyperlipidemic effect of S. anomalum leaf extract and fractions

Administration of *S. anomalum* leaf extract and fractions to diabetic rats did not affect the total cholesterol level of the rats significantly ($p > 0.05$) except in the groups treated with middle dose of the extract (140 mg/kg) and ethyl acetate fraction where significant ($p < 0.05$–0.01) reductions were observed. However, the leaf extract and fractions exhibited statistically significant ($p < 0.05$–0.001) reductions of TG, LDL and VLDL levels in the treated hyperglycaemic rats relative to the control. However, the HDL levels of the groups treated with standard drug, glibenclamide, extract (70–210 mg/kg) and leaf fractions were significantly ($p < 0.05$–0.001) increased relative to the control (Table 4).

3.9. Effect of leaf extract and fractions on pancreas of diabetic rats

Pathological findings on the histological sections of untreated diabetic rat pancreas in negative control group, depicted areas of gross degeneration and degranulation of various cells, while pancreas of hyperglycaemic rats administered with glibenclamide, leaf extract/fractions had normal features without any pathological lesion (Fig. 3).

Table 2
Antihyperglycaemic activity of ethanol leaf extract and fractions of *S. anomalum* on blood glucose level of alloxan-induced diabetic rats during acute study.

Treatment	Dose	Blood glucose level (mg/dL) in hours					
	mg/kg	0 HR	1 HR	2 HR	3 HR	5 HR	7 HR
Control normal saline	Saline	266.0 ± 17.2	274.3 ± 16.0	281.0 ± 35.6	260.0 ± 16.4	269.3 ± 19.8	253.8 ± 17.4
Glibenclamide	10	233.0 ± 12.0	223.6 ± 20.2	222.0 ± 28.5	213.6 ± 21.9	216.0 ± 14.0	205.2 ± 17.8
Crude extract	70	279.3 ± 14.7	256.3 ± 41.7	258.6 ± 20.2	266.6 ± 12.6	255.3 ± 8.0	249.0 ± 5.5
	140	260.6 ± 8.3	251.6 ± 6.9	237.6 ± 27.2	230.6 ± 9.8	232.3 ± 7.1	218.6 ± 8.0
	210	257.0 ± 3.7	252.6 ± 23.7	236.3 ± 13.4	238.6 ± 14.4	224.0 ± 9.1	220.0 ± 16.7
n-Hexane fraction	140	264.3 ± 14.5	223.1 ± 12.4	283.6 ± 18.9	266.1 ± 40.1	169.6 ± 10.4[b]	158.6 ± 36.7[b]
DCM fraction	140	236.0 ± 37.6	227.6 ± 16.7	230.6 ± 19.8	236.3 ± 60.3	215.3 ± 17.6	206.6 ± 9.3
Ethyl acetate fraction	140	271.3 ± 13.0	241.6 ± 15.8	235.6 ± 13.8	232.0 ± 17.8	214.0 ± 32.2	189.3 ± 21.4[a]
Methanol fraction	140	245.6 ± 12.3	234.0 ± 14.5	252.6 ± 17.0	212.3 ± 21.4	221.3 ± 29.1	206.6 ± 26.2

values 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 ethanol leaf extract and fractions of *Solanum anomalum* on blood glucose level of alloxan- induced diabetic rats during chronic study.

Treatment	Dose	blood glucose level mg/dL in hours					
	mg/kg	0 HR	1st DAY	3RD DAY	5TH DAY	7TH DAY	14TH DAY
Control normal saline	saline	266.0 ± 17.2	243.6 ± 53.2	231.6 ± 44.2	225.3 ± 18.4	324.6 ± 23.1	249.0 ± 14.0
Glibenclamide	10	233.0 ± 12.0	208.0 ± 18.6	143.0 ± 18.6	113.3 ± 14.5^{a}	94.6 ± 16.2^{a}	82.6 ± 8.2^{b}
Crude extract	70	279.3 ± 14.7	195.0 ± 14.7	196.6 ± 14.6	165.0 ± 18.2	128.0 ± 17.0^{b}	100.3 ± 16.7^{b}
	140	260.6 ± 8.3	177.6 ± 18.5	151.0 ± 14.9	120.3 ± 19.4^{a}	101.6 ± 11.3	95.3 ± 11.3^{a}
	210	257.0 ± 3.7	167.5 ± 16.3^{a}	169.3 ± 23.4	108.8 ± 9.6^{c}	96.0 ± 14.2^{a}	88.3 ± 8.8^{b}
n-Hexane fraction	140	264.3 ± 14.5	158.6 ± 36.7^{c}	142.8 ± 8.5	116.0 ± 18.8^{a}	100.8 ± 11.1^{b}	84.6 ± 40.1^{c}
DCM fraction	140	236.0 ± 37.6	206.6 ± 9.3^{c}	148.3 ± 9.4	158.3 ± 20.7	120.6 ± 17.6^{b}	107.0 ± 14.5^{b}
Ethyl acetate fraction	140	271.3 ± 13.0	189.3 ± 21.4	169.6 ± 29.4	131.0 ± 13.0	115.2 ± 22.3	92.0 ± 14.3^{a}
Methanol fraction	140	245.6 ± 12.3	208.5 ± 18.6	143.0 ± 17.6	123.3 ± 11.5	94.6 ± 16.4^{a}	81.6 ± 18.5^{b}

Values 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).

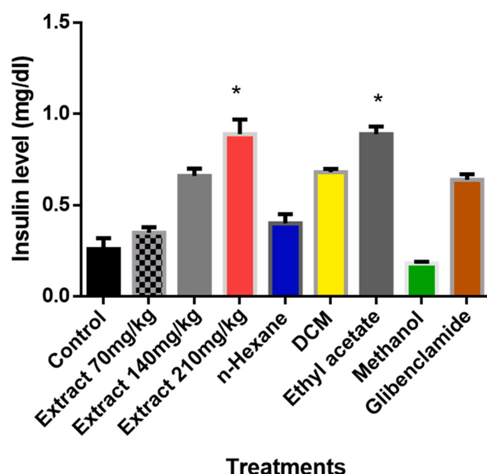

Fig. 1. Effect of *S. anomalum* leaf extract and fractions on insulin levels of diabetic rats. Significant at $*p < 0.01$ relative to control (n = 6).

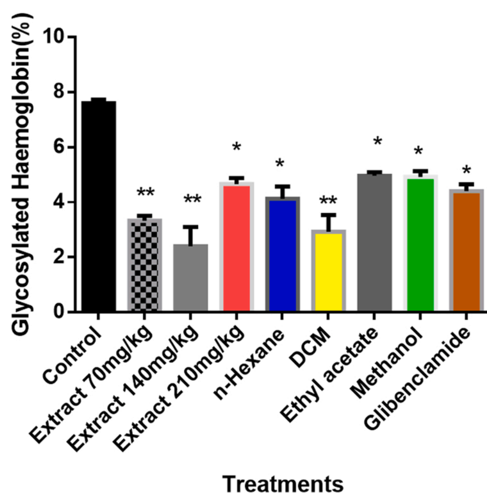

Fig. 2. Effect of *S. anomalum* leaf extract and fractions on glycosylated haemoglobin levels of diabetic rats. Significant at $*p < 0.05$, $**< 0.001$ relative to control (n = 6).

3.10. Chemical characterization of purified compounds from Solanum anomalum

Compound **R10** was isolated from the bioactive *n*-hexane fraction and identified as (25 *R*)-spirost-5-en-3β-ol, diosgenin (a steroid sapogenin) by GC-MS, ^{1}H NMR and ^{13}C NMR (Supplementary material) and comparing with the data reported [31].

Compound **S235** was isolated as white powder from a combination of ethyl acetate and methanol fractions. HR-ESI (negative)-MS gave an ion of 767.4247, corresponding to molecular formula $C_{40}H_{64}O_{14}$. However, ^{13}C NMR only showed the presence of only 39 carbon peaks rather than 40. Further LC-(negative ion) ESI-MS/MS analysis of the molecular ion of **S235** indicated a base mass of 721.4197 due to loss of mass of 46.00, corresponding to the mass of formic acid, which was used in the LC-MS experiment. The observed molecular mass of 767.4247 is the deprotonated formic acid adduct of **S235** rather than the usually expected deprotonated mass $[M-H]^{-}$, thus its molecular formula is determined to be $C_{39}H_{62}O_{12}$. Comparing ^{13}C NMR spectrum of **S235** to that of diosgenin (**R10**), they are very similar except the presence of additional 11 peaks distributed between 60 and 102 ppm and a peak at 16.43 ppm in the spectrum of **S235**, which indicated the presence of two pyranose sugars. 1D TOCSY spectra of the two sugar moieties indicated they are *β*-D-Glucopyranose (Glc) and *α*-Rhamnose (Rha) due to their characteristic coupling patterns (Table 5, supplementary materials). HSQC, TOCSY and ROESY analysis further allowed to assign all the ^{1}H and ^{13}C peaks. On the HMBC spectrum (Supplementary materials)., the presence of crossing peaks between 78.28 ppm (C3 of aglycone) and 4.42 ppm (H1-Glc) suggested that *β*-D-glucose is attached to the C-3 of aglycone diosgenin, while rhamnose is further attached to the glucose. The observations of the crossing peaks between 101.48 ppm (C1-Rha) and 3.54 ppm (H4-Glc); 78.50 ppm (C4-Glc) and 4.87 (H1-Rha) on HMBC indicated that the linkage between Rha and Glc is the 1→4 linkage (Supplementary materials). The observation of a fragmentation ion at 575.3571 ($C_{33}H_{51}O_8$, calcd. 575.3589) due to the loss of mass of Rha (146) together with mass of formic acid (46) on the LC-MS/MS of the molecular ion of *m/z* at 767.4259 supported this structure. Thus, **S235** is identified as 25(*R*)-diosgenin-3-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside, a steroidal glycoside or saponin type compound (Fig. 4).

Compounds **S9-1** and **S9-2** were identified as uracil and thymine (5-methyluracil) by 1D and 2D NMR spectroscopy and LC-TOF-MS, respectively. These nucleobases were previously found in other plants such as the roots of *Xanthium sibiricum* [32]. **PP4** was identified as 1-octacosanol by GC-MS after TMSi derivation, ^{1}H NMR, ^{13}C NMR and HSQC spectra. **K1** was identified as octacosane by GC-MS.

Diosgenin (R10). White powder, FT-IR (ν_{max}, cm^{-1}): 3298, 3017, 2848, 1654, 1456, 1377, 1063, 973. GC-MS (Rt, 18.293 min): *m/z* (Relative %): 396.4 $[M-18]^{+}$(5), 342.1 (7), 282.1 (35), 239.2 (5), 139.8 (100), 91.0 (62), 55.0 (70), 43.0 (50). ^{1}H NMR (400 MHz, $CDCl_3$), δ_H: 5.37 (d, $J = 5.1$ Hz, 1 H); 4.43 (dd, 1 H, $J = 15.0, 7.0$ Hz), 3.51 (1 H, m), 3.39 (1 H, t, $J = 10.8$ Hz), 2.30 (2 H, m), 2.00 (2 H, m), 1.87 (3 H, m), 1.05 (3 H, s), 0.99 (3 H, d, $J = 6.9$ Hz), 0.81 (3 H, t, $J = 3$ Hz), ^{13}C NMR (100 MHz, $CDCl_3$), $\delta_{c,}$ 140.91, 121.45, 109.25, 80.63, 71.74, 66.85, 62.12, 56.53, 50.08, 42.29, 41.62, 40.28, 39.80, 37.23, 36.65, 32.06, 31.86, 31.64, 31.45,41.40, 30.31, 29.70, 28.81, 20.88, 19.42, 17.13, 16.28, 14.52. These data are in agreement with those reported [31].

25(*R*)-diosgenin-3-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside (**S235**), white powder. LC-HR-(negative)-ESI-MS, *m/z*:

Table 4
Effect of leaf extract and fractions of *Solanum anomalum* 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	saline	4.2 ± 0.2	3.33 ± 0.84	1.43 ± 0.12	0.99 ± 0.01	0.66 ± 0.05
Glibenclamide	10	2.2 ± 0.5^{c}	1.2 ± 0.1^{c}	2.4 ± 0.2^{b}	0.4 ± 0.2^{a}	0.27 ± 0.07^{c}
Crude extract	70	3.1 ± 0.3	1.1 ± 0.1^{c}	2.4 ± 0.3^{a}	0.7 ± 0.1	0.29 ± 0.05^{c}
	140	2.3 ± 0.5^{b}	1.2 ± 0.3^{c}	2.4 ± 0.3^{b}	0.4 ± 0.1^{a}	0.27 ± 0.02^{c}
	210	2.7 ± 0.1	1.4 ± 0.1^{c}	2.7 ± 0.1^{c}	0.3 ± 0.1^{b}	0.32 ± 0.02^{c}
n-Hexane fraction	140	2.9 ± 0.1	1.3 ± 0.04^{c}	2.6 ± 0.2^{b}	0.4 ± 0.1^{c}	0.29 ± 0.04^{c}
DCM fraction	140	2.8 ± 0.2	1.1 ± 0.04^{c}	2.6 ± 0.3^{b}	0.4 ± 0.3^{a}	0.26 ± 0.04^{c}
Ethyl acetate fraction	140	2.6 ± 0.1^{a}	1.1 ± 0.1^{c}	2.7 ± 0.2^{c}	0.4 ± 0.2^{a}	0.25 ± 0.02^{c}
Methanol fraction	140	2.8 ± 0.2	1.2 ± 01^{c}	2.6 ± 0.3^{c}	0.6 ± 0.3	0.28 ± 0.01^{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).

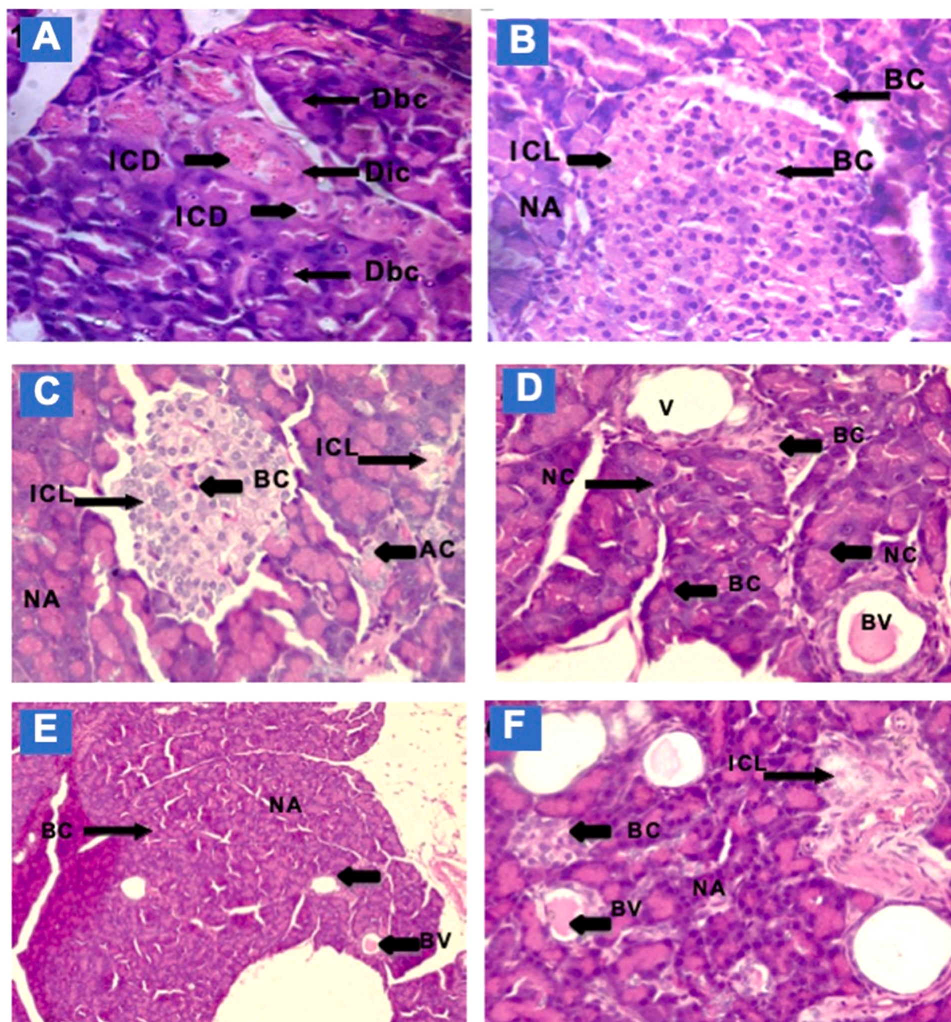

Fig. 3. Histological sections of pancreas of alloxan-induced diabetic rats treated with normal saline 10 mL/kg (A), glibenclamide 10 mg/kg bw (B), leaf extract 70 mg/kg bw (C), leaf extract 140 mg/kg bw (D), leaf extract 210 mg/kg bw (E), *n*-hexane fraction 140 mg/kg (F) at magnification (x400), stained with H&E method. Abbreviations used in the Figure: islet cells degeneration (ICD), degranulated islet cells (DIC), blood vessel (BV), degranulated Beta Cells (DBC), islet cells of langharns (ICL), beta cell (BC), blood vessels (BV), normal acini (NA).

767.4247 [M+CHOO]$^{-}$, calculated monotopic mass for $C_{40}H_{63}O_{14}$, 767.4218. The formula of **S235** is $C_{39}H_{60}O_{12}$. ^{1}H and ^{13}C NMR data of **S235** in CD_3OD are assigned and listed in Table 5, which showed expected slight difference from those data measured in a different solvent pyridine-d_5 but in a comparable pattern [33,34].

Uracil (Compound **S9-1**). White powder. $C_4H_4N_2O_2$. LC-HR-(positive(-ESI-MS, *m/z*: 113.0354 [M+H]$^{+}$; 135.0171 [M+Na]$^{+}$. LC-HR-(negative)-MS, *m/z*: 111.0192 [M-H]$^{-}$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 5.45 (1 H, d, *J* = 7.6 Hz, H-5), 7.39 (1 H, d, *J* = 7.6 Hz, H-6), 10.85 (2 H, br, NH-1, NH-3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 100.67 (C-5, d), 142.67 (C-6, d), 151.98 (C-2, s), 164.80 (C-4, s). The NMR data conformed with the previous literature[32].

Thymine (Compound **S9-2**). White powder. $C_5H_6N_2O_2$. LC-HR-(positive)-ESI-MS, *m/z*: 127.0503 [M+H]$^{+}$; 149.0320 [M+Na]$^{+}$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.72 (3 H, brd, H-7, *J*=0.86), 7.25 (1 H, brd, H-6, *J*=0.73 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.24 (C-7), 108.12 (C-5, s), 138.19 (C-6, d), 151.51 (C-2), 165.48 (C-4). These data were in accordance with those of thymine [32].

1-Octacosanol (**PP4**), white powder, FT-IR (ν_{max}, cm^{-1}): 3274, 2918, 2854, 1468, 1064.^{1}H NMR (400 MHz, $CDCl_3$), δ_H: 0.92 (t, *J* =7.0 Hz, 3 H), 1.29 (brs, 50 H), 1.60 (2 H, m), 3.66 (t, *J* = 6.6 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$), δ_c, 63.13 (C-1), 32.64 (C-2), 31.94 (C-3), 29.71 (C-4), 29.63 (C-5–23), 29.45 (C-24), 29.37 (C-25), 25.76 (C-26), 22.70 (C-27), 14.12 (C-28). GC-MS, TMSi-Octacosanol (Rt, 16.217 min): *m/z* (Relative

Table 5
^{1}H (700 MHz) and ^{13}C NMR (150 MHz) data of **S235** (CD_3OD).

No.	^{1}H (*J*, Hz)	^{13}C	No.	^{1}H (*J*, Hz)	^{13}C
1	1.90, 1.09 (m)	37.11	22	–	109.21
2	1.94, 1.32 (m)	29.30	23	2.00;1.58 (m)	31.00
3	3.54 (m)	78.28	24	1.65; 1.44 (m)	28.47
4	2.45 (dt, 13.2, 2.3); 2.28 (dt, 13.2, 0.8)	38.29	25	1.62 (m)	30.03
5	–	140.56	26	3.47 (d), 3.34 (dd)	66.45
6	5.30 (brs)	121.17	27	0.82 (s)	16.08
7	2.00, 1.31 (m)	31.32	Glc (3-O)		
8	1.68 (m)	31.39	1'	4.42 (d,7.8)	100.96
9	1.00 (m)	50.27	2'	3.20 (dd, 7.8, 9.4)	73.85
10	–	36.61	3'	3.34 (ddd, 9.4, 4.4, 2.2)	75.32
11	1.59, 1.53 (m)	20.58	4'	3.54 (t,10.5)	78.50
12	1.79, 1.21 (m)	39.51	5'	3.48 (t,10.5)	75.5
13	–	40.01	6'	3.82 (brd, 12.1) 3.68 (dd, 12.1, 4.2)	60.55
14	1.17 (m)	56.38	Rha (4'-O)		
15	2.05 (m)	31.77	1''	4.87 (d, 1.5)	101.48 (C-H, 169 Hz)
16	4.42 (m)	80.82	2''	3.86 (d,3.5)	71.04
17	1.76 (m)	62.31	3''	3.65 (dd,3.3, 9.5)	70.80
18	0.84 (s)	15.35	4''	3.40 (t,10.0)	72.4
19	1.07 (s)	18.43	5''	3.98 (dt, 6.0, 10.0)	69.25
20	1.93 (m)	41.50	6''	1.29 (d, 6.3)	16.43
21	0.98 (s)	13.46			

%): 567.5 $[M-15]^{+}$(100), 75 (35), 57.0 (30), 43.0 (30). These data are in agreement with those reported for 1-octacosanol [35].

Octacosane (**K1**), GC-MS (Rt, 17.537), *m/z* (Relative %): 337.4 (1), 282.1 (2), 207.0 (3), 141.1 (3), 99.1 (5), 87.1(40), 71.1 (60), 57.1 (100), 43.1 (80).

3.11. Gas chromatography-mass spectrometry (GC-MS) analysis of the hexane and dichloromethane fractions

GC-MS analysis of the *n*-hexane fraction of *S. anomalum* revealed the presence of six compounds (Table 6). GC-MS analysis of sub-fraction after silica gel column chromatography disclosed additional compounds such as fumaric acid, *trans*-hex-3-enyl undecyl ester, (*Z,Z,Z*)−9,12,15-octadecatrienoic acid, and docosanoic acid methyl ester (Table S2, Supplementary materials). GC-MS analysis of the dichloromethane fraction of *S. anomalum* revealed the presence of six compounds similar as the compounds in the hexane fraction (Table S3).

3.12. Prediction of protein targets of diosgenin (R10) and diosgenin glycoside (S235) using a bioinformatic tool

Using the SwissTargetPrediction web tool, the targets of diosgenin and diosgenin glycoside are predicted. The major predicted targets of diosgenin (**R10**) are phosphatase (e.g. protein-tyrosine phosphatase 1B (PTPN1)), secreted proteins (interleukin-2), nuclear receptor (e.g. bile acid receptor FXR, LXR-alpha), and kinases (e.g. ALK tyrosine kinase receptor) in mice and humans (Supplementary materials). The major predicted targets of diosgenin glycoside (**S235**) are phosphatases including protein phosphatase 2 C, serine/threonine protein phosphatase PP1 and 2 A, signal transducer and activator of transcription 3, interleukin-2 (Supplementary materials).

4. Discussion

Diabetes mellitus is a metabolic disease under stress of a high free radical level [36]. Biotransformation of alloxan, a useful tool in experimental diabetes in the laboratory, to dialuric acid induces elevation of free radical levels and thereby causing oxidative injuries to the pancreatic cells [37,38]. Consequently, blood glucose level rises due to diminished level of insulin, precipitating type 2 diabetes mellitus, leaving few pancreatic β-cells with insulin secreting ability. Alloxan monohydrate treated rats were used as the model of diabetes through this study.

Various parts of *S. anomalum,* a medicinal plant, are used locally by the Ibibios of southern Nigeria for the management of diabetic condition among others [20]. This work was aimed to evaluate antihyperglycaemic, antihyperlipidemic as well as pancreas protective potentials of *S. anomalum* leaf extract/fractions in diabetic rats and also isolation and characterisation of the phytochemicals from this plant

Table 6
GC-MS analysis of *n*-hexane fraction of *S. anomalum.*

PEAK	RT (min)	COMPOUND NAME	FORMULA	MOL. MASS
1	10.044	*cis*-Pinane	$C_{10}H_{18}$	138.25
2	12.031	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256.24
3	14.877	2,6,10,15-tetramethyl-Heptadecane	$C_{21}H_{44}$	296.34
4	18.315	Squalene	$C_{30}H_{50}$	410.39
5	20.503	Heneicosane	$C_{21}H_{44}$	296.34
6	22.847	Octacosane	$C_{28}H_{58}$	394.45

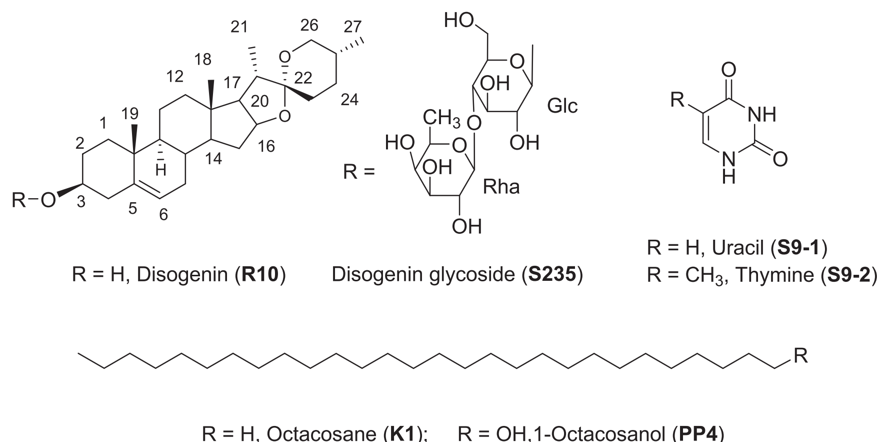

Fig. 4. Chemical structures of the isolated and identified compounds from *S. anomalum.*

which might contribute to the antidiabetic activities.

In this study, findings showed that sustained hypoglycaemic effect was achieved with *S. anomalum* leaf extract and fractions both on single dosing and repeated administration for 14 days as the *n*-hexane and methanol fractions demonstrated the most prominent effects. The findings of this study corroborate previously reported antidiabetic activities of other species of *Solanum* such as *S. indicum* [9,10], *S. melongena* [15], *S. trilobatum* [16], *S. macrocarpon* [17], *S. lycocarpum* [18], and *S. anguivi* [19], validating the local use of this plant for the management of diabetes.

Phytochemical studies of *S. anomalum* revealed the presence of diosgenin, a diosgenin glycoside, along with two nucleobases and long chain alcohol and fatty acids. Diosgenin is widely present in many herbal plants such as *Dioscorea alata*, *Trigonella foenum* graecum and *Smilax china* [39]. It has also been found in many other *Solanum* species [40–44]. Diosgenin glycoside (**S235**) was isolated from the *Solanum* genus and fully characterized by 1D and 2D NMR spectroscopic analysis for the first time, although it was recently reported to be present in the rhizomes of *Asparagus cochinchinensis* [34] and *Dioscorea composita* [31], and characterised as a microbial transformation product of polyphyllin III by *Curvularia lunata* [33]. Uracil and thymine were identified from *S. anomalum* for the first time. Further GC-MS analyses of the leaf hexane and dichloromethane fractions revealed pharmacological active compounds which include saturated and mono/polyunsaturated fatty acids (PUFAs). These mono and PUFAs have history of significant hypoglycaemic effect by stimulating beta cells to secrete insulin [45]. Also, squalene and β-sitosterol identified to be constituents of the leaf extract also exert antidiabetic activity [46,47].

Diosgenin is known for its prominent antidiabetic activity in many studies [48,49]. Diosgenin was shown to be involved in multiple pathways and multiple targets such as inhibiting alpha-amylase and alpha-glucosidase [50] to reduce intestinal glucose absorption, inhibiting the sodium-glucose cotransporter-1 (SGLT-1) and reducing intestinal Na^{+}-K^{+}-ATPase activity [51]. Steroidal glycosides such as **S235** and other steroidal saponins were also commonly present in the *Solanum* species [8] and reported to exert hypoglycaemic activities [52–54]. Protein tyrosine phosphatase 1B (PTP1B) and protein serine/threonine phosphatase are predicted to be the targets of diosgenin and its glycoside (**S235**), respectively. Steroid saponins from *Dioscorea* and *Solanum* species have been shown to allow the restoration of insulin response, increase plasma insulin levels, induct insulin release from the pancreas, inhibit alpha-glucose, and possess antioxidant activity [54]. PTP1B is intensively researched as a drug target of diabetes because it negatively regulates the tyrosine phosphorylation cascade in the insulin signaling pathway [55,56]. It would be interesting to test if diosgenin and diosgenin glycoside possess inhibitory activity against PTP1B in the future.

Serum insulin level was found to be significantly elevated following repeated administration of the extract and fractions especially with the extract (210 mg/kg) and ethyl acetate fraction having the highest insulin levels. It is likely that the pancreas of these groups suffered less injuries consequent of the protection offered by these treatments which can be suggested to be partly responsible for the observed elevated insulin levels, besides stimulatory effects on the beta cells. This finding corroborates previous works on *S. nigrum* which increases in insulin level/secretion and revival of damaged β-cells in hyperglycaemic rats [13]. Diosgenin [57], and β-sitosterol[58] have previously been reported to stimulate insulin secretion through regeneration of pancreas beta cells. These compounds and other phytoconstituents in the leaf extract/fractions may have protected the pancreatic cells against injurious effect of alloxan [37] which explains the observed high insulin level.

Glycosylation of body proteins including haemoglobin is common in poorly controlled diabetes. Progression of diabetes diminishes glycosylated hemoglobin affinity to carry oxygen leading to serious complications common with diabetes. The progression or degeneration of glycemic state is indicated by glycosylated haemoglobin level due to its irreversible formation and stability throughout the existence of the red blood cells. The glycosylated haemoglobin levels of the hyperglycaemic rats were observed to have been reduced following repeated administration of the leaf extract/fractions. This indicated a regulated glycaemic state especially in animals treated with dichloromethane fractions as was the case in this study. However, elevated levels of Hb1Ac with corresponding raised hyperglycemia were visible in alloxan-induced hyperglycaemic rats especially in untreated diabetic group, which corroborates the previous report [59]. These findings indicated that the *S. anomalum* leaves produced its hypoglycaemic effect primarily by stimulating the beta cells to secrete insulin, thereby causing lowering of plasma blood glucose and HbA1c values. Diosgenin [57], squalene [60] and β-sitosterol [58] have also been reported to cause similar decreases in glycosylated haemoglobin level of hyperglycaemic rats, which may likely be the case in this study. Circulatory cholesterol and other lipids levels are elevated in diabetic condition, and this leads to associated heart and liver diseases. The dyslipidemia in diabetics results from disordered lipid metabolic processes. The leaf extract and fractions were found to lower considerably the various lipid parameters of the hyperglycaemic rats such as total cholesterol, TG, LDL and VLDL-chloesterol, and also raised HDL levels in the diabetic rats. The reduction of the raised blood glucose level by treatment of hyperglycaemic rats with the leaf extract/fractions restored a near normal glucose metabolism and also that of fats. Compounds like stigmasterol present in the leaf extract/fractions also share similar mode of antidiabetic action [61]. Besides, octacosanol (policosanol) reportedly exert hypolipidemic effects through reduction of adipose tissue weight [62], inhibition of cholesterol biosynthesis [63] and reduction of LDL and VLDL as well as elevation of HDL-cholesterol [64].

Significant body weight gains by diabetic rats were observed resulting from treatment with the leaf extract /fractions. Severe body weight loss is a common feature of diabetes which results from structural proteins depletion. Treatment of hyperglycaemic rats with leaf extract /fractions of *S. anomalum* alleviated this condition probably through lowering of blood glucose level and protein synthesis stimulation.

Pancreas of the untreated diabetic control group had various pathological lesions as were observed in the histological section. However, these pathologic abnormalities were absent in the extract/fractions treated groups suggesting protective and ameliorative effect of the extract. This result corroborates previous works on *Solanum spp* which similar results were reported [8,13]. Diosgenin and β-sitosterol are also reported to cause healing and regeneration of pancreas beta cells [57, 58]. These phytoconstituents present in this extract and fractions may have been responsible for the observed regenerative effect on the pancreas. The protective effect on the pancreas could have restored the normal functions of the beta cells with resultant increased insulin levels in treated diabetic and therefore hypoglycaemic effect. The observed protective effect could have resulted from free radical scavenging activities of the phytochemical constituents (squalene and β-sitosterol) of the extract and fractions [58] as well as isolated compounds such as diosgenin, diosgenin glycoside, octacosane and 1-octacosanol, which are potent antioxidants [65] and other phenolic compounds present in the extract.

We must recognize there are limitations in this study. Only alloxan-induced rat model was used here, alternative animals models induced by other chemical agents [38] are needed to validate the effect of the plant extract for potential antidiabetic use. Due to limited quantity of isolated compounds such as diosgenin and its glycoside, testing of the toxicity and efficacy of the isolated pure compounds is not possible. The doses of plant extract/fractions used in this study were high, to achieve clinical use in humans the most potent compounds such as diosgenin (steroid) and steroidal saponins should be enriched to increase its efficacy.

In conclusion, the findings of this investigation revealed that *S. anomalum* leaves possess antihyperglycaemic, antihyperlipidemic as well as pancreas protective properties which are likely attributed to the activities of its phytochemical constituents such as diosgenin and

diosgenin glycoside.

CRediT authorship contribution statement

Jude E. Okokon (JEO) designed and supervised the work, **Idongesit Etuk** carried out the animal studies and did the statistical analysis, **Paul S. Thomas** carried out the isolation and purification of the compounds, **Wen-Wu Li (WWL)** performed the HPLC isolation of S9 and S235, GC-MS, LC-MS and NMR data analysis a well as bioinformatics analysis. **Falko P. Drijfhout** and **Tim D.W. Claridge** performed LC-MS and NMR analysis, respectively. **JEO** and **WWL** wrote the paper and all others edited the work.

Declaration of competing interest

There is no conflict of interests to be declared.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.113153.

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