AJPAM Reports
African Journal of Pharmacy and Alternative Medicine

https://doi.org/10.58460/ajpam.v5i01.196

RESEARCH ARTICLE

Gas Chromatography-Mass Spectrometry Analysis and Antidiabetic Effect of Fractionated Extract of *Strophanthus hispidus*

Muyiwa SAMUEL FAGEYINBO[1], Temitayo ESTHER ADEYEOLUWA[*1], Omotayo AKINPELUMI AYEDOGBA[1], Abidemi JAMES AKINDELE[2] & Esther OLUWATOYIN AGBAJE[2]

Author Affiliations

1 Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, University of Medical Sciences, Ondo, Nigeria.

2 Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria.

*Corresponding Author: tadeyeoluwa@unimed.edu.ng

Submitted: 8th January 2026 | Accepted: 23rd April 2026 | Published Online: 26th August 2026

ABSTRACT

Strophanthus hispidus DC (Apocynaceae) is a valued African plant that has been used as a remedy of various disease conditions. This study identified compounds present using Gas Chromatography Mass Spectrometry (GC-MS) as well as the potential antidiabetic effects of the n-butanol, ethyl acetate, and aqueous fractions. GC-MS was carried out using the Agilent 7890 A GC with a J&W HP-5MS column (30 m x 0.320 mm i.d. x 0.25 µm). The effects of the fractions (100 mg/kg each) on blood glucose levels, body weight, biochemical parameters, lipid profile, serum insulin, hepatic glycogen, and haemoglobin levels were evaluated in alloxan-induced hyperglycaemic rats. The GC-MS analysis revealed the presence of Decanoic acid methyl ester; methyl 15-acetoxy hexadecanoate; Hydrazine propyl; and Cyclobut-1-enyl methanol. A significant ($p<0.05$) reduction in blood glucose, creatinine, urea, bilirubin, liver enzymes, and glycosylated haemoglobin levels was observed with all fractions of *S. hispidus* administered compared to the untreated control. Also, an improvement in body weight, lipid profile, serum insulin, hepatic glycogen, and haemoglobin levels with *S. hispidus* fractions compared to the untreated control was observed. The study identifies the above-named compounds as potential antidiabetic agents in *S. hispidus*, with activity observed in both polar and non-polar media, warranting further investigation for compound isolation and characterization.

Keywords: *GC-MS; Strophanthus hispidus fractions; alloxan; antidiabetic; hepatic glycogen*

How to Cite this Article: FAGEYINBO, M. S., ADEYEOLUWA, T. E., AKINDELE, A. J., & AGBAJE, E. O. (2026). Gas Chromatography-Mass Spectrometry Analysis and Antidiabetic Effect of Fractionated Extract of *Strophanthus hispidus*. *African Journal of Pharmacy and Alternative Medicine*, 5(02). https://doi.org/10.58460/ajpam.v5i02.196

INTRODUCTION

Strophanthus hispidus DC (Apocynaceae) is a phytotherapeutic plant that has gained widespread recognition as a remedy in Africa, for numerous inflammatory diseases and infections (Burkill, 2000; Odugbemi, 2008; Agbaje and Fageyinbo, 2012).*S. hispidus* known as Sagere or Isagere in Yoruba language, has been used in Africa as a cure for skin diseases, diabetes (Fageyinbo et al., 2019), leprosy, ulcers, gonorrhoea, dysentery, and malaria (Burkill, 2000). It is used in traditional medicine for various purposes. Its bark or leaf sap decoction treats snakebites, while the sap is applied topically for head lice and parasites (Burkill, 2000; Odugbemi, 2008). The bark decoction is used for conjunctivitis (Burkill, 2000). The leaf and stem decoctions treat sores, fever, and serve as a laxative (Agyare et al., 2013). The root reduces inflammation and treats rheumatism (Agbaje and Fageyinbo, 2012). However, despite its extensive ethnomedicinal use, there remains limited mechanistic and compound-level evidence supporting its antidiabetic efficacy.

Diabetes mellitus is a complex, long-term non-communicable disorder defined by sustained elevation blood glucose resulting from inadequate insulin secretion, impaired insulin action, or both, and it is commonly associated with widespread metabolic disturbances that disrupt carbohydrate, lipid, and protein homeostasis while progressively affecting critical organs such as the liver, kidneys, and cardiovascular system (Defronzo, 2009, Galicia-Garcia et al., 2021). Its underlying pathogenesis extends beyond hyperglyceamia to include β-cell dysfunction, insulin resistance in peripheral tissues, enhanced hepatic gluconeogenesis, and chronic oxidative and inflammatory stress, all of which interact to drive disease progression and complications (American Diabetes Association, 2023). In response to this multifaceted pathophysiology, current therapeutic approaches increasingly adopt a holistic, multi-target framework that not only regulates blood glucose levels but also improves insulin sensitivity, corrects dyslipidemia, reduces oxidative damage, and promotes effective glycogen synthesis and storage (Rines et al., 2016; Zheng et al., 2018). This broadened strategy is critical as metabolic abnormalities, including lipid imbalance and oxidative stress, exacerbate insulin signaling impairment and pancreatic dysfunction, increasing disease severity and risk of cardiovascular and renal complications (Forbes and Cooper, 2013). Consequently, both conventional drugs and emerging bioactive compounds are now evaluated based on their capacity to simultaneously modulate these interconnected metabolic pathways, offering a more comprehensive and sustainable approach to diabetes management.

In many developing countries, medicinal plants are the preferred means of managing diabetes, while several pharmaceuticals are in fact made from these natural products (Yedjou et al., 2023). Several studies have demonstrated that medicinal plants exert antidiabetic effects through diverse mechanisms. Ahmad et al., (2021) reported enhanced insulin secretion and sensitivity with Phyllanthus emblica. Similarly, sulfur-containing compounds such as S-methylcysteinesulfoxide (SMCS) and S-allylcysteinesulfoxide (SACS) from Allium cepa (Onion) and Allium sativum (gallic) stimulate insulin release, improve hepatic glucose metabolism, and reduce oxidative stress via inhibition of lipid peroxidation (Augusti and Sheela, 1996; Rajasekaran et al., 2024; Eidi et al., 2026). In addition, phytosterols (phytosterols-lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartanol, and 24-methylene-cycloartanol) from Aloe vera significantly reduce glycated haemoglobin (HbA1c), reflecting improved long-term glyceamic control (Marles and Farnsworth, 1995; Tanaka et al., 2006). Collectively, these studies highlight key antidiabetic mechanisms including insulin secretion, insulin sensitivity, enhanced glucose uptake, oxidative stress modulation, and glycogen metabolism. Hepatic glucose uptake and glycogen storage are particularly important, as increased hepatic glycogen reflects improved insulin action and glucose utilization (Singh et al., 2001). Oxidative stress modulation is also critical, given its role in β-cell dysfunction and insulin resistance.

Although previous studies have reported the antidiabetic activity of *Strophanthus hispidus* (Fageyinbo et al., 2019; 2022), significant gaps remain. Notably, prior investigations relied on crude extracts without identifying bioactive compounds, comparing solvent fractions, or linking observed effects to specific metabolic mechanisms. Furthermore, the comparative efficacy of solvent-partitioned fractions (e.g., aqueous, ethyl acetate, and n-butanol) remains unknown, despite the critical role of solvent polarity in concentrating distinct phytochemical classes. There is also a lack of integrated evaluation of metabolic outcomes, including insulin levels, glycogen storage, lipid profile, and organ function parameters. Addressing these gaps is essential for advancing from ethnomedicinal claims to evidence-based pharmacological validation and eventual drug discovery.

The present study offers a novel and systematic evaluation of the antidiabetic potential of *S. hispidus* through an integrated, multi-level approach. Specifically, the phytochemical constituents of root fractions were characterized using CG-MS to provide compound-level evidence, while the antidiabetic efficacy of the aqueous, ethyl acetate, and n-butanol fractions was comparatively assessed.

Pharmacological effects were mechanistically linked to key pathways, including insulin secretion and sensitivity (via serum insulin levels), glucose homeostasis (fasting blood glucose and HbA1c), hepatic glucose utilization (glycogen content), and lipid metabolism (lipid profile), alongside evaluation of systemic metabolic integrity through liver enzymes, renal biomarkers, hematological indices, and body weight changes. This comprehensive framework distinguishes the study from prior reports by integrating biochemical, physiological, and mechanistic endpoints, thereby bridging the gap between crude extract pharmacology and compound-oriented investigation. Collectively, the findings will substantiate the ethnomedicinal relevance of *S. hispidus* and provide a robust foundation for future bioassay-guided isolation, mechanistic elucidation, and antidiabetic drug development. Accordingly, this study evaluates the phytochemical composition and antidiabetic effects of solvent fractions of *S. hispidus* at a standardized dose of 100 mg/kg (Fageyinbo et al., 2019), building on prior efficacy data while introducing fraction-specific and mechanism-oriented insights.

METHODOLOGY

Plant Material and Extraction

The root of *S. hispidus* was obtained from a village called Ile-Oluji at Oke-Igbo Local Government Area, Ondo State, Nigeria. The plant specimen (LUH 2618) authenticated by Prof J.D Olowokudejo was kept at the University of Lagos herbarium, Nigeria. Distilled water (400 mL) was added to 50.8 g of water extract of *S. hispidus* root in the beaker and dissolved properly. The resulting mixture was extracted using n-butanol and ethyl acetate. A rotary evaporator was used to evaporate the fractions. The yields of the fractions produced were 6.6% for n-butanol (n-BF) fraction, 4.62% for ethyl acetate fraction (EAF) and 7.56% for aqueous fraction (AF)

Chemical and Reagents

Alloxan monohydrate, Glibenclamide (Diatab®, May and Baker Nigeria PLC), trichloroacetic acid (TCA), Ellman's reagent, and Drabkin reagent (Sigma-Aldrich, Schnelldorf, Germany), butanol, ethyl acetate (BDH, Frankfurter, Germany).

Chemical Composition Analysis

GC-MS Analysis.

The method of Sofidiya et al., 2015 was employed using Agilent 7890 A GC with J&W HP-5MS column (30 m x 0.320 mm i.d. x 0.25 µm) for the investigation. The setting used comprise flow rate; 1.0 ml/min; spitless injection: 2 µl of the plant material, oven temperature: 70 ◦ C; injector temperature: 250 ◦ C and detector temperature: 280 ◦ C Mass spectra scan was taken at 70 eV from 40 to 500 m/z. NIST database (NIST Mass Spectral Database, 2014) was used to identify the components.

Experimental Animal

The study was carried out on male Albino rats (about 3 months old; 150 ± 2.2 g). Rats had unrestricted access to water and rodent food, and the average temperature was 26.7°C. Approval for the study protocol (CMUL/HREC/11/17/283) was obtained from the Ethics Committee, College of Medicine, University of Lagos, Lagos, Nigeria.

Acute Toxicity

The acute toxicity study of *S. hispidus* has been carried out previously (Agbaje and Fageyinbo, 2012) and the safety also established (Fageyinbo et al., 2021). The dosage (100 mg/kg) used in this study was the effective dose earlier reported (Fageyinbo et al., 2019).

Induction of Hyperglyceamia

Hyperglyceamia was evoked by a singular intraperitoneal injectant of recently liquified (in saline) alloxan monohydrate 120 mg/kg body weight (El-Demerdash et al., 2005; Fageyinbo et al., 2019), to 12 h fasted (overnight) Albino rats. After 72 h of receiving the alloxan injection, fasting blood glucose (FBG) levels were estimated. For this study, only rats with blood glucose level ≥200 mg/dL were deemed diabetic (Tzeng et al., 2014; Fageyinbo et al., 2019).

Treatment Protocol and Analysis

Forty (40) hyperglyceamia rats were randomly selected into 5 groups of 8 rats apiece namely SHP-n-butanol fraction (100 mg/kg, p.o.), SHP-ethyl acetate fraction (100 mg/kg, p.o.), SHP-aqueous fraction (100 mg/kg, p.o.), glibenclamide (5 mg/kg, p.o.), non-treated control (0.9% normal saline; 10 mL/kg, p.o). Additionally, 8 healthy rats were placed in another group and given distilled water (normal control; 10 mL/kg p.o.). All treatments were administered once a day for four weeks (Fageyinbo et al., 2019). Two droplets of venous blood were obtained from each rat's tail and placed on a glucose strip that was placed inside the Glucometer (Accu-chek®) to measure the FBG. The results were then read on the digital display. Each rat's body weight and fasting blood glucose level were recorded once a week. Each rat had its blood drawn through the retroorbital sinus on day 29. The blood was then placed in an EDTA bottle for haematological tests (glycated haemoglobin, or HbA1c) and a lithium heparinized bottle for biochemical, antioxidant,and insulin assessments.

After that, the sacrificing of the rats was done by quick cervical dislocation, and the liver of each harvested for the liver glycogen test.

Biochemical Assay

The blood samples collected were centrifugated for 10 min at 300 rpm with the assays carried out namely; renal function tests [creatinine, urea]; liver function tests [total protein, albumin, globulin, total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), as well as alanine aminotransferase (ALT), using an auto-analyser (Vitalab Flevor E, Diefen, Netherlands). Lipids (triglycerides (TG), high-density lipoprotein (HDL-c) total cholesterol (TC)] and total protein (TP).

Serum Insulin Estimation

This was estimated by means of an enzyme-linked immunosorbent assay (ELISA) protocol using rat insulin ELISA kit (Mercodia Ultrasensitive Rat Insulin ELISA, Winston Salem, North Carolina, USA) (Oyetunji and Musbau, 2014, Fageyinbo et al., 2019).

Hepatic Glycogen Estimation

This was measured according to the method earlier described (Oyetunji and Musbau, 2014; Fageyinbo et al., 2019).

Haemoglobin and Glycated Haemoglobin Estimation

The haemoglobin concentration in the blood samples collected was estimated using a method earlier described (Drabkin and Austin, 1932; Fageyinbo et al., 2019). Glycated haemoglobin concentration was also measured by the method of Nayak and Pattabiraman (1981); Fageyinbo et al., (2019).

Haemoglobin and Glycated Haemoglobin Estimation

The data collected were analysed using two-way ANOVA then by Tukey's multiple comparison post hoc test (Graph-Pad Prism 6; Graph-Pad Software Inc., CA, USA). The results were expressed as Mean ± standard error of mean (S.E.M) and considered significant when $p<0.05$.

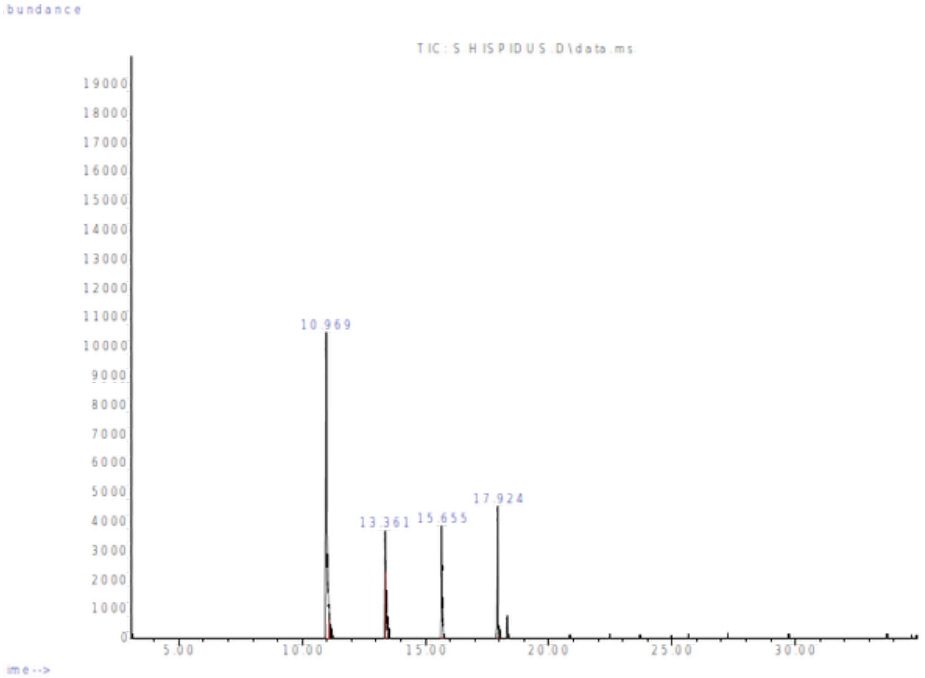
RESULTS

GC-MS Analysis of Bioactive Compounds in S. hispidus

The main compounds found in the GC-MS study were decanoic acid methyl ester (58.568%); methyl 15- acetoxy hexadecanoate (8.489%); hydrazine propyl (16.033%) and cyclobut-1-enylmethanol (16.910%) (Fig 1a, 1b and Table 1).

Hypoglycaemic Activity

The n-BF, EAF, AF and glibenclamide recorded a marked significant ($p<0.05$) reduction in the levels of blood glucose from day 7 till day 28 comparable with the diabetic control. The n-BF revealed a potent glucose reduction outcome by reducing the level of glucose to baseline after 28 days. The percentage reduction in glucose level for n-BF, EAF, AF and glibenclamide were 68.07 %; 62.50 %; 61.43 % and 61.63 % respectively after day 28 (Table 2).



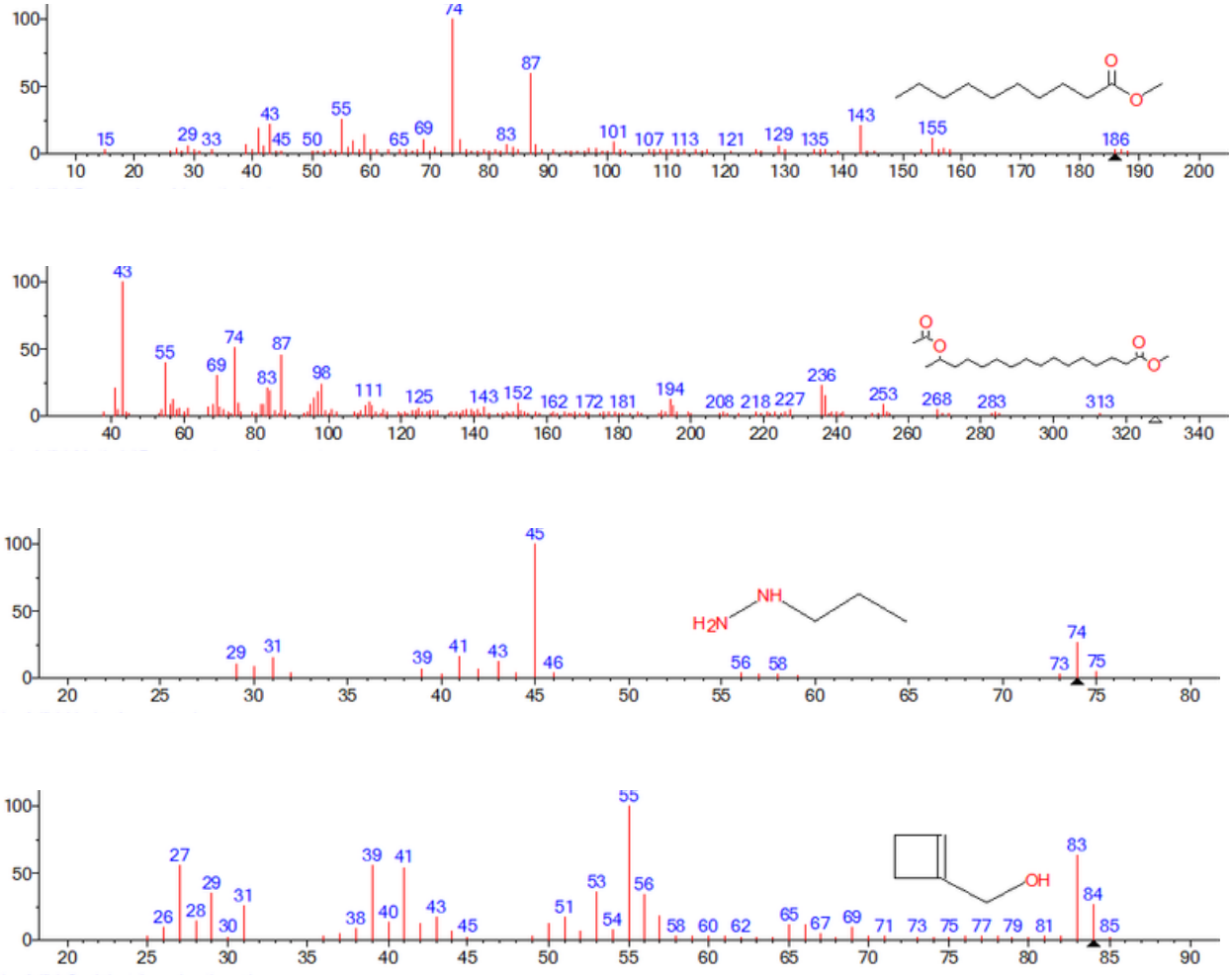


Fig 1b: Fragmentation Pattern of Compounds Presents in S. hispidus

Table 1:

GC-MS Analysis of Bioactive Compounds in S. hispidus

Compounds	Retention time (min)	Amount (%)	Molecular formula	Molecular weight(g/mol)
Decanoic acid methylester	10.989	58.568	$C_{11}H_{22}O_2$	186.295
Methyl 15-acetoxy hexadecanoate	13.361	8.489	$C_{19}H_{36}O_2$	328.493
Hydrazine propyl	15.666	16.033	$C_3H_{10}N_2$	74.127
Cyclobut-1-enyl methanol	17.924	16.910	C_5H_8O	84.118

Table 2:

Hypoglycaemic Activities of n-Butanol, Ethyl Acetate, and Aqueous Fractions of Aqueous Root Extract of S. hispidus

Glucose level (mg/dl.)								
Treatments	**Dose (mg/kg)**	**Basal**	**Day 0**	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Normal control	10 mL/kg	77.4 ± 1.32	77.0 ± 1.41	76.0 ± 0.83	78.0 ± 0.83	77.2 ± 1.65	73.8 ± 1.65	73.8 ± 0.80
n-Butanol	100	77.2 ± 1.39	269.4± 14.11	266.6±14.20 (13)	193.6 ± 2.63* (28.13)	123.2±1.28* (54.26)	97.4± 3.26* (63.84)	86.00±2.48* (68.07)
Ethyl acetate	100	81.6 ± 1.36	268.8 ± 25.30	265.0±23.95 (1.41)	203.6 ± 5.33* (24.25)	166.2±12.7* (38.16)	118.0±4.4* (56.10)	100.8±4.27* (62.50)
Aqueous	100	74.8 ± 3.35	271.2 ± 15.11	268.0 ± 13.14 (1.17)	225.0 ± 7.45* (17.03)	134.4±7.71* (50.44)	123.2±5.4* (54.57)	104.6±2.53* (61.43)
Glibenclamide	5	79.0 ± 2.07	299.2 ± 6.06	295.4±7.41 (1.50)	233.4±10.10* (21.99)	185.2±4.95* (38.10)	141.8±3.3* (52.60)	114.8±2.31* (61.63)
Diabetic control	–	80.8 ± 2.92	268.2 ± 13.4	275.0 ± 12.09	296.8 ± 4.61	291.0±3.84*	296.0± 2.96	299.8 ± 1.01

Diabetic control received normal saline 10 mL/kg; Normal control received distilled water 10 mL/kg. Results are presented as mean ± S.E.M. (n = 5).

- $p < 0.05$ statistically significant compared to diabetic non-treated control (2-way ANOVA followed by Tukey's multiple comparison).
- Values in parentheses indicate percentage reduction in blood glucose level.

Effect of n-butanol (n-BF), Ethyl acetate (EAF) and Aqueous Fractions (AF) on Body Weight

The body weight of n-BF, EAF, AF and glibenclamide treated rats decreased but not significantly ($p>0.05$) from day seven until day fourteen. However, significant ($p<0.05$) increase in the body weight of n-BF, EAF, AF and glibenclamide treated rats were observed at 28 days in comparison to diabetic untreated group. Reduction in the weights of diabetic rats was recorded and was significant ($p<0.05$) at days 21 and 28, as shown in table 3.

Biochemical Parameters and Lipid Profile

The diabetic control group produced a significant ($p<0.05$) amplification in creatinine, urea, bilirubin, ALT, AST, albumin, ALP, LDL and total cholesterol levels. Correspondingly, an increment in triglycerides (TG) levels was observed but it was not significant ($p<0.05$). Likewise, significant reduction ($p<0.05$) in HDL and total protein levels was produced by diabetic control in comparison to normal control (Tables 4 and 5). Nevertheless, n-BF, EAF, AF and glibenclamide treated rats produced significant ($p<0.05$) reduction in urea, creatinine, bilirubin, ALT, AST, albumin, ALP, LDL and total cholesterol levels. In addition, significant ($p<0.05$) amplification in HDL and total protein was recorded in these groups in comparison with the diabetic control.

Table 3: S. hispidus on Body Weight

Body weight(kg)							
Treatments	**Dose (mg/kg)**	**Basal**	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Normal control	10 mL/kg	0.114 ± 0.002	0.115 ± 0.002	0.120 ± 0.001	0.125 ± 0.001	0.130 ± 0.001	0.137 ± 0.002*
n-Butanol	100	0.121 ± 0.002	0.119 ± 0.002 (1.65 ↓)	0.114 ± 0.002 (4.20 ↓)	0.111 ± 0.003 (6.72 ↓)	0.115 ± 0.001 (3.36 ↓)	0.118 ± 0.002* (0.84 ↓)
Ethyl acetate	100	0.124 ± 0.001	0.124 ± 0.001 (0.00 ↓)	0.119 ± 0.001 (4.03 ↓)	0.114 ± 0.001 (8.06 ↓)	0.118 ± 0.002 (4.83 ↓)	0.120 ± 0.001* (3.22 ↓)
Aqueous	100	0.122 ± 0.002	0.123 ± 0.002 (0.81 ↑)	0.113 ± 0.004 (7.31 ↓)	0.114 ± 0.002 (6.55 ↓)	0.117 ± 0.002 (4.09 ↓)	0.120 ± 0.002* (1.63 ↓)

Glibenclamide	5	0.124 ± 0.002	0.124 ± 0.002 (0.00 ↓)	0.096 ± 0.021[a] (22.58 ↓)	0.095 ± 0.002 (23.38 ↓)	0.112 ± 0.005[a] (9.67 ↓)	0.117 ± 0.001* (5.64 ↓)
Diabetic control	–	0.128 ± 0.001	0.126 ± 0.002 (1.56 ↓)	0.117 ± 0.002 (8.59 ↓)	0.112 ± 0.001 (12.5 ↓)	0.101 ± 0.002[a] (21.09 ↓)	0.094 ± 0.002[a] (26.56 ↓)

Diabetic control received normal saline 10 mL/kg; Normal control received distilled water 10 mL/kg. Results are presented as mean ± S.E.M. (n = 5).

- $p < 0.05$ statistically significant compared to diabetic control.
- [a] $p < 0.05$ statistically significant compared to normal control (2-way ANOVA followed by Tukey's multiple comparison test).
- Values in parentheses indicate percentage gain ↑ or loss ↓ in body weight of the rats.

Table 4:

S. hispidus on Biochemical Parameters

Treatments	Dose (mg/kg)	CREA (µmol/L)	UREA (mmol/L)	BIL (µmol/L)	ALT (IU/L)	AST (IU/L)	ALB (mg/dL)	ALP (IU/L)	TP (mg/dL)
Normal control	–	32.94 ± 1.74[a]	5.42 ± 0.26[a]	3.99 ± 0.01[a]	42.31 ± 0.85[a]	48.81 ± 0.72[a]	31.56 ± 0.29[a]	60.14 ± 1.16[a]	67.65 ± 0.51[a]
n-Butanol	100	25.42 ± 0.37[a]	6.32 ± 0.15[a]	2.87 ± 0.05[a]	33.63 ± 0.79[a]	51.21 ± 0.52[a]	28.58 ± 1.28[a]	63.61 ± 1.31[a]	65.47 ± 1.04[a]
Ethyl acetate	100	37.85 ± 0.68[a]	7.51 ± 0.24[a]	4.04 ± 0.08[a]	45.63 ± 1.71[a]	58.36 ± 1.89[a]	36.32 ± 1.56[a]	69.48 ± 1.09[a]	59.46 ± 1.97[a]

Aqueous	100	31.32 ± 0.42[a]	7.11 ± 0.26[a]	2.80 ± 0.07[a]	35.61 ± 0.49[a]	64.72 ± 1.89[a]	37.72 ± 1.95[a]	62.59 ± 1.28[a]	66.58 ± 1.19[a]
Glibenclamide	5	44.58 ± 0.59[a]	10.24 ± 0.18[a]	2.17 ± 0.07[a]	33.58 ± 1.35[a]	93.28 ± 1.93[a]	39.75 ± 0.65[a]	65.72 ± 0.44[a]	74.88 ± 2.19[a]
Diabetic control	–	95.25 ± 1.31*	27.72 ± 1.10*	17.95 ± 0.78*	123.63 ± 1.46*	132.11 ± 0.68*	45.93 ± 1.86*	177.37 ± 1.28*	35.77 ± 0.30*

Diabetic control received normal saline 10 mL/kg; Normal control received distilled water 10 mL/kg. Results are presented as mean ± S.E.M. (n = 5).

- [a] $p < 0.05$ statistically significant compared to diabetic control group.
- $p < 0.05$ statistically significant compared to normal control group (2-way ANOVA followed by Tukey's multiple comparison test).

Table 5:

S. hispidus on Lipid Profile

Treatments	Dose (mg/kg)	TC (mmol/L)	TG (mmol/L)	HDL (mg/dL)	LD (mg/dL)
Normal control	–	0.85 ± 0.06[a]	0.48 ± 0.02	62.82 ± 0.69[a]	28.50 ± 0.93[a]
n-Butanol	100	0.98 ± 0.09[a]	0.50 ± 0.04	81.57 ± 1.72[a]	19.54 ± 0.53[a]
Ethyl acetate	100	1.99 ± 0.04[a]	0.86 ± 0.06	54.63 ± 0.71[a]	29.75 ± 0.67[a]
Aqueous	100	2.24 ± 0.18[a]	1.28 ± 0.11	62.12 ± 1.61[a]	23.50 ± 1.46[a]
Glibenclamide	5	1.81 ± 0.10[a]	1.00 ± 0.01	53.76 ± 1.20[a]	30.36 ± 1.27[a]
Diabetic control	–	3.73 ± 0.19*	2.99 ± 0.03	10.02 ± 0.31*	99.79 ± 0.92*

Diabetic control received normal saline 10 mL/kg; Normal control received distilled water 10 mL/kg. Results are presented as mean ± S.E.M. (n = 5).

- [a] $p < 0.05$ statistically significant compared to diabetic control group.
- $p < 0.05$ statistically significant compared to normal control group (2-way ANOVA followed by Tukey's multiple comparison test).
- TC: Total cholesterol
- TG: Triglyceride ALP: Alkaline phosphatase
- HDL: High density lipoprotein
- LDL: Low density lipoprotein

Serum Insulin Level

The serum insulin levels in groups treated with n-BF, EAF, AF and glibenclamide were significantly ($p<0.05$) enhanced in comparison with the diabetic control. The diabetic control elicited significant ($p<0.05$) decrement in the level of serum insulin in comparison with the normal control. Nevertheless, the insulin level in the normal control group significantly ($p<0.05$) increased compared to diabetic control (Figure 2). Among the treated groups, n-BF (100 mg/kg) displayed maximum insulin level.

Hepatic Glycogen Level

The level of hepatic glycogen significantly ($p<0.05$) decreased in the diabetic control in comparison with the normal control. Conversely, n-BF, EAF, AF and glibenclamide-treated groups elicited significant ($p<0.05$) increment in hepatic glycogen level in comparison with diabetic control (Figure). This effect was more prominent with n-BF among the treated groups which was virtually the same value as the normal control group.

Haemoglobin and Glycated Haemoglobin Levels

The haemoglobin level significantly ($p<0.05$) decreased in diabetic control in comparison with the normal control group while significant ($p<0.05$) increase in haemoglobin levels were observed in groups treated with n-BF, EAF, AF and glibenclamide in comparison with the diabetic control. Significant ($p<0.05$) decrease in glycated haemoglobin level was produced by n-BF, EAF, AF and glibenclamide in comparison with diabetic control whereas significant increase ($p<0.05$) in glycated haemoglobin was displayed by diabetic control in comparison with the normal control (Figure 4).

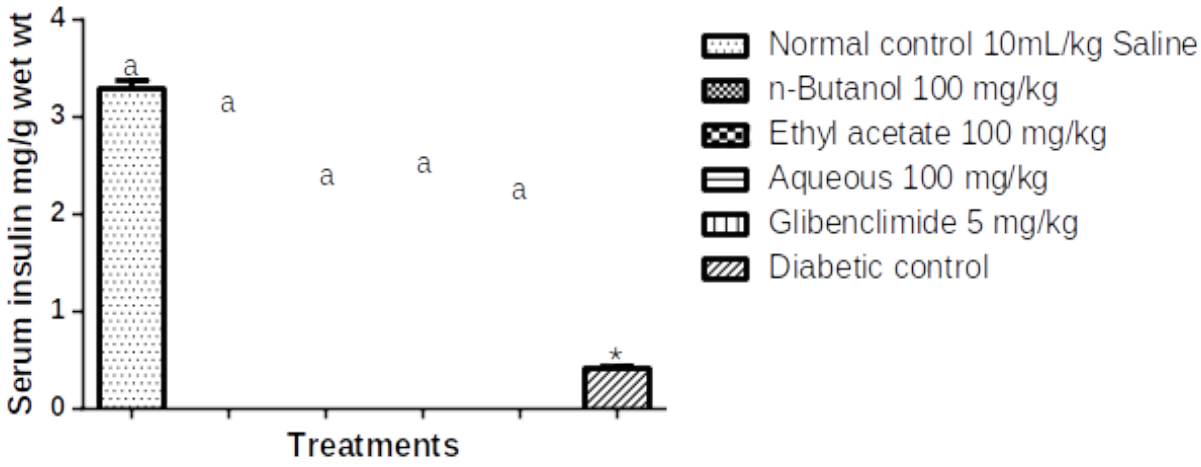


Fig: 2: Serum Insulin Level

Results are presented as Mean ±SEM (n=3), [a]$p<0.05$ statistically significant compared to diabetic control group; [*]$p<0.05$ statistically significant compared to normal control group (2-way ANOVA followed by Tukey's multiple comparison).

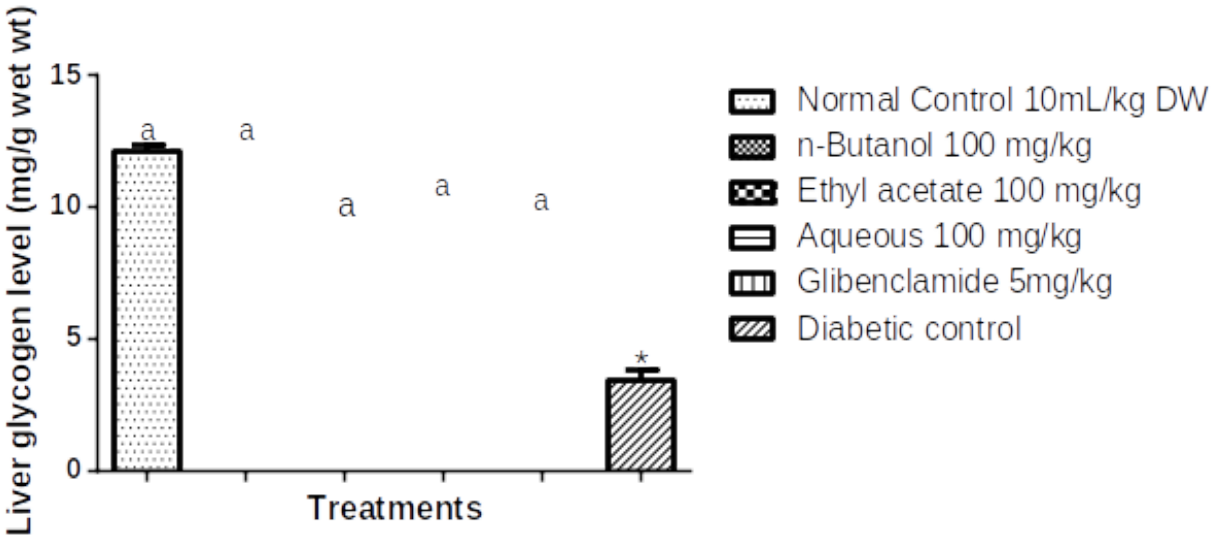


Fig 3: Hepatic Glycogen Level

Results are presented as Mean ±SEM (n=3), [a]p<0.05 statistically significant compared to diabetic control group; *p<0.05 statistically significant compared to normal control group (2-way ANOVA followed by Tukey's multiple comparison).

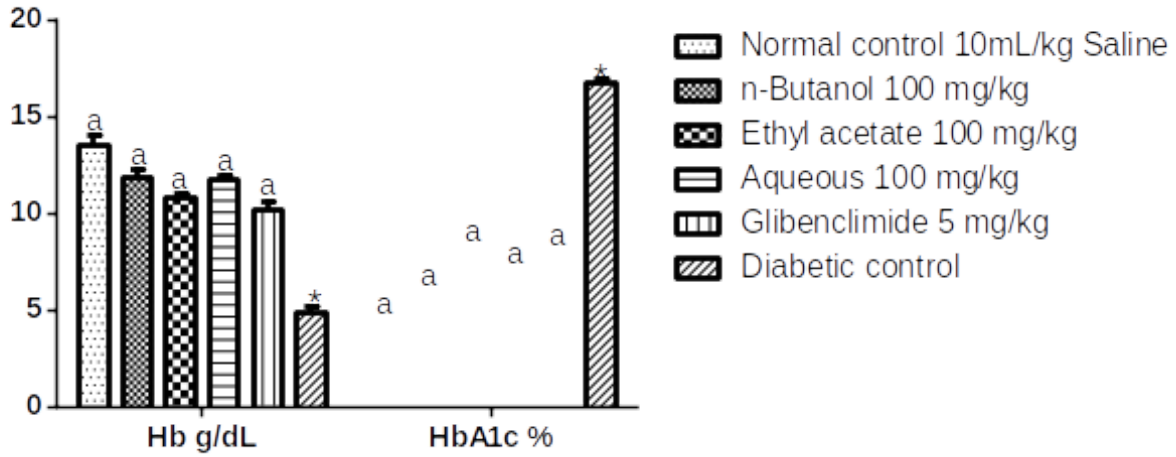


Fig 4: Haemoglobin (Hb) and Glycosylated Haemoglobin (HbA1c)

Results are presented as Mean ±SEM (n=3), [a]p<0.05 statistically significant compared to diabetic control group; *p<0.05 statistically significant compared to normal control group (2-way ANOVA followed by Tukey's multiple comparison).

DISCUSSION

Swift and acceptable blood glucose level control is imperative to improve the outcome of management and lengthen lifespan of diabetics (Weinstock et al., 2020).The current study explored the antidiabetic activity of n-Butanol (n-BF), ethyl acetate (EAF) and aqueous (AF) fractions of *S. hispidus* on alloxan-induced diabetic rats as well as identified the bioactive compounds present in the plant extract A single oral dose of 100 mg/kg was used in this antidiabetic investigation. This is because 100 mg/kg crude extract of *S. hispidus* was earlier reported to effectively lower blood glucose level (Fageyinbo et al., 2019). This study was carried out aiming to isolate the bioactive compound responsible for its observed antidiabetic activity. Hence, the use of fractionated extracts of *S. hispidus*.

This study discovered a fundamental diminution in levels of serum glucose by n-BF, EAF and AF fractions of *S. hispidus*. However, this significant reduction in blood glucose level was more evident with the n-butanol fraction(n-BF). Significant reduction in AST, ALP, ALT levels was documented with these fractions and this further supports the hepatoprotective role of *S. hispidus* earlier postulated (Fageyinbo et al., 2019).

The protective role of *S. hispidus* on the kidney was also being confirmed owing to substantial reduction in creatinine, urea and bilirubin levels obtained with n-BF, EAF and AF of *S. hispidus* compared to the diabetic control group. Again, the n-butanol fraction produced prominent effect.

A decrease in body weight was discovered in all the SHP fraction-treated animals but it was not significant. However, significant increase was witnessed at 28 days. The non-significant reduction in body weight also suggests prevention of tissue wasting and adequate control over breakdown of tissue proteins, which confer satisfactory blood glucose control. Studies have shown that adequate control of body weight in diabetic confer proper glyceamic control (Salahuddin and Jalalpure, 2010).

From the foregoing, n-BF, EAF and AF of *S. hispidus* significantly reduced the HbA1c level and increased Hb level in contrast with elevated HbA1c level and reduced level of Hb observed in the diabetic control. It has been established that reduction in HbA1c is indicated effective glyceamic control (Selvaraj et al., 2005). The hepatic glycogen in addition to the serum insulin levels were significantly augmented in the n-BF, EAF and AF treated rats compared with the diabetic control and this further reaffirmed the insulinotropic property of *S. hispidus* (Fageyinbo et al., 2019). Potent blood glucose lowering effect was displayed by n-BF, EAF and AF of *S. hispidus*.

In this study, the prominent effect of the n-BF was observed, though the difference in its activity compared to the other fractions is not very significant, thus, suggesting that the active constituents present in *S. hispidus* responsible for the observed activities might reside partly in polar and non-polar medium.

The GC-MS analysis of *S. hispidus* revealed the existence of the following compounds: Decanoic acid methyl ester, Methyl 15- acetoxy hexadecanoate, propyl hydrazine and Cyclobut-1-enyl methanol.Decanoic acid methyl ester also referred to as methyl decanoate; methyl caprate; methyl caprinate; Capric acid methyl ester which has been reported to enhance the uptake of glucose, possibly through activating of Glut4 and control of the PI3K/AKT pathway (Lee et al., 2016). It has also been shown to possess anti-inflammatory activity (Gomathi and Elango, 2015). Methyl 15- acetoxy hexadecanoate has been reported for its anti-oxidant, hypocholesterolemia, antiandrogenic and hemolytic-5-α reductase inhibitor (Gomathi and Elango, 2015). Hydrazine derivatives reportedly possess several biological impacts such as anti-inflammatory, analgesic, cardio-protective, antiplatelet, antiprotozoan and anti-cancer actions (Verma et al., 2014). The presence of these compounds' accounts for the potent and beneficial antidiabetic activities observed in the SHP fractions.

Conclusion

This study revealed the presence of decanoic acid methyl ester, Methyl 15- acetoxy hexadecanoate, propyl hydrazine and Cyclobut-1-enyl methanol in *Strophanthus hispidus* which are responsible for the glucose lowering activity. This study also established that the observed glucose lowering activity of *S. hispidus* is partly in polar and non-polar medium. Hence, further studies are ongoing for the isolation of these compound

Recommendations

The isolation of the active constituents of *Strophanthus hispidus* DC roots and further development into supplements or drugs may be essential in the formulation of antidiabetic preparations in pharmaceutical industrie

Conflict of Interest

No funding organizations played a role in the study or the decision for submission.

Authors' Contribution

MS Fageyinbo designed, conceptualized, and wrote the manuscript. TE AdeyeOluwa edited and wrote the manuscript. OA Ayedogba edited and wrote the manuscript. AJ Akindele and EO Agbaje supervised the study and managed datasets.

Funding

The study was not funded by any funding body.

Availability of Data and Materials

All data was generated by the authors and will make data available on request

REFERENCES

Agbaje, E. O., & Fageyinbo, M. S. (2012). Anti-Inflammatory activity of aqueous root extract of *Strophanthus hispidus* DC. (Apocynaceae). International Journal of Applied Research in Natural Products 4(4), 7-14. https://www.researchgate.net/publication/279489295_Evaluating_Anti-Inflammatory_activity_of_aqueous_root_extract_of_Strophanthus_hispidus_DC_Apocynaceae

Agyare, C., Dwobeng, A. S., Agyepong, N., Boakye, D., Mensah, K. B., Ayande, P. G., & Adarkwa-Yiadom, M. (2013). Antimicrobial, antioxidant and wound healing properties of Kigeliaafricana(Lam.) Beneth. and *Strophanthus hispidus* DC. Advances in Pharmacological and Pharmaceutical Sciences. 1-10. https://doi.org/10.1155/2013/692613

Ahmad, B., Hafeez, N., Rauf, A., Bashir, S., Linfang, H., Rehman, M., Mubarak, M.S., Uddin, S., Bawazeer, S., Shariati, MA., Daglia, M., Wan, C., Rengasamy, KRR. (2021). Phyllanthus emblica: A comprehensive review of its therapeutic benefits. S Afr J Bot.; 138:278-310. doi: 10.1016/j.sajb.2020.10.003.

American Diabetes Association. (2023). Classification and diagnosis of diabetes: Standards of care in diabetes—2023. Diabetes Care, 46(Supplement_1), S19–S40. https://doi.org/10.2337/dc23-S002

Augusti, K.T., Sheela, C.G. (1996). Antiperoxide effect of S-allyl cysteine sulfoxide, an insulin secretagogue, in diabetic rats. Experientia 52: 115-120. https://doi.org/10.1007/BF01923354

Burkill, H. M. (2000). The useful plants of West Tropical Africa. 2nd ed. Volume 5, Families S-Z, Addenda. royal botanic garden, Kew, Richmond, United Kingdom, UK. https://ui.adsabs.harvard.edu/abs/1986KewBu..41..471W/abstract

Defronzo, R. A. (2009). From the triumvirate to the ominous octet: A new paradigm for the treatment of type 2 diabetes mellitus. Diabetes, 58, 773–795. https://doi.org/10.2337/db09-9028

Drabkin, D. L., & Austin, J. H. (1932). Spectrophotometric studies: Spectrophotometric constant for common hemoglobin derivatives in human, dogs and rabbit blood. Journal of Biological Chemistry, 98, 719-733. https://doi.org/10.1016/S0021-9258(18)76122-X

Eidi, A., Eidi, M., Esmaeili, E. (2006). Antidiabetic effect of garlic (Allium sativum L.) in normal and streptozotocin-induced diabetic rats. Phytomedicine 13: 624-629 https://www.sciencedirect.com/science/article/abs/pii/S0944711305002175

El-Demerdash,F. M., Yousef, M. I., & AbouEl-Naga, N. I. (2005). Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food and Chemical Toxicology, 4(1),57-63.https://doi.org/10.1016/j.fct.2004.08.012

Fageyinbo, M. S., Akindele, A. J., & Agbaje, E. O. (2021).Sub-chronic toxicological evaluation of *Strophanthus hispidus* DC (Apocynaceae) aqueous root extract DC (Apocynaceae). Journal of Complementary and Integrative Medicine, 18(4), 753-760. https://doi.org/10.1515/jcim-2020-0088

Fageyinbo, M. S., Akindele, A. J., Adenekan, S. O., & Agbaje, E. O. (2019). Evaluation of in-vitro and in-vivo antidiabetic, antilipidemic and antioxidant potentials of aqueous root extract of *Strophanthus hispidus* DC (Apocynaceae). Journal of Complementary and Integrative Medicine, 16, 1–20. https://doi.org/10.1515/jcim-2018-0055

Fageyinbo, M.S., Akindele, A.J., Falade, J., Agbaje, E.O. (2022). Aqueous Root Extract of *Strophanthus hispidus* Demonstrates Antidiabetic Effect in Fructose-Streptozotocin-Induced Diabetic Rats. Pharmacol Toxicol Nat Med 2(3): 1-8DOI: 10.52406/ptnm. v2i3.40 https://doi.org/10.52406/ptnm.v2i3.40

Forbes, J. M., & Cooper, M. E. (2013). Mechanisms of diabetic complications. Physiological Reviews, 93(1), 137–188. https://doi.org/10.1152/physrev.00045.2011

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. (2020). Pathophysiology of Type 2 Diabetes Mellitus. International Journal of Molecular Sciences, 21(17),6275. https://doi.org/10.3390/ijms21176275

Gomathi, R. L., & Elango, V. (2015). Identification of bioactive components and its biological activities of Evolvulus alsinoides Linn- A GC-MS study. International journal of chemistry studies, 3(1), 41-44. https://doi.org/10.1007/s13197-013-1105-9

Lee, C. L., Lee, S. L., Chen, C. J., Chen, H. C., Kao, M. C., Liu, C. H., Chen, J. Y., Lai, Y. T., & Wu, Y. C. (2016). Characterization of secondary metabolites from purple Ipomoea batatas leaves and their effects on glucose uptake. Molecules, 21(6), 745-760. https://doi.org/10.3390/molecules21060745

Marles, R.J., Farnsworth, N.R. (1995). Antidiabetic plants and their active constituents. Phytomedicine 2: 137-189 https://www.sciencedirect.com/science/article/abs/pii/S0944711311800590

Nayak, S. S., & Pattabiraman, T. N. (1981). A new colorimetric method for the estimation of HbA1C. Clinica Chimica Acta. 109:267–274. https://doi.org/10.1016/0009-8981(81)90312-0

Nayak, S. S., & Pattabiraman, T. N. (1981). A new colorimetric method for the estimation of HbA1C. Clinica Chimica Acta. 109:267–274. https://doi.org/10.1016/0009-8981(81)90312-0

Oyetunji, T. K., & Musbau, A. A. (2014). Effect of extract of leaves of Newbouldia laevis on the activities of some enzymes of hepatic glucose metabolism in diabetic rats. African Journal of Biotechnology, 13(22), 2273-2281. https://doi.org/10.5897/AJB2014.136

Rajasekaran, S., Sivagnanam, K., Ravi, K., Subramanian, S. (2004) Hypoglycemic effect of Aloe avera gel on streptozotocin-induced diabetes in experimental rats. J Med Food 7: 61-66.Rines, A. K., Sharabi, K., Tavares, C. D. J., & Puigserver, P. (2016). Targeting hepatic glucose metabolism in the treatment of type 2 diabetes. Nature Reviews Drug Discovery,15(11),786–804. https://doi.org/10.1038/nrd.2016.151

Salahuddin, M., & Jalalpure, S. S. (2010). Antidiabetic activity of aqueous fruit extract of Cucumis trigonus Roxb. in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology,127,565–567. https://doi.org/10.1016/j.jep.2009.10.018

Selvaraj, N., Bobby, Z., Koner, B. C., & Das, A. K. (2005). Reassessing the increased glycation of hemoglobin in nondiabetic chronic renal failure patients: a hypothesis on the role of lipid peroxides.Clinica Chimica Acta,360, 108–113. https://doi.org/10.1016/j.cccn.2005.04.015

Singh, S.N., Vats, P., Suri, S., Shyam, R., Kumria, M.M., Ranganathan, S., Sridharan, K. (2001) Effect of an antidiabetic extract of Catharanthus roseus on enzymic activities in streptozotocin induced diabetic rats. J Ethnopharmacol 76: 269-277 https://doi.org/10.1016/S0378-8741(01)00254-9

Sofidiya, M. O., Orisaremi, C. O., Sansaliyu, I., & Adetunde, T. O. (2015). Gastroprotective and antioxidant potentials of ethanolic stem bark extract of Margaritaria discoidea (Euphorbiaceae) in rats. Journal of Ethnopharmacology, 171, 240–246. https://doi.org/10.1016/j.jep.2015.05.059

Tanaka, M., Misawa, E., Ito, Y., Habara, N., Nomaguchi, K., Yamada, M., Toida, T., Hayasawa, H.M., Takase, M., Higuchi, I.R. (2006). Identification of Five Phytosterols from Aloe vera Gel as Antidiabetic Compounds. Biological and Pharmaceutical Bulletin, 29, 1418-1422. http://dx.doi.org/10.1248/bpb.29.1418

Tzeng, T. F., Liou, S. S., Chang, C. J., & Liu, I. M. (2014). The ethanol extract of Lonicera japonica (Japanese Honeysuckle) attenuates diabetic nephropathy by inhibiting p-8 MAPK activity in streptozotocin-induced diabetic rats. Planta Medica, 80(3), 121–139. https://doi.org/10.1055/s-0033-1360196

Verma, G., Marella, A., Shaquiquzzaman, M., Akhtar, M., Ali, M. R., & Alam, M. M. (2014). A review exploring biological activities of hydrazones. Journal of Pharmacy And Bioallied Sciences, 6(2), 69–80. doi: 10.4103/0975-7406.129170

Weinstock, R. S., Aleppo, G., Bailey, T. S., Bergenstal, R. M., Fisher, W. A., Greenwood, D. A., Young, L. A. (2020). The Role of Blood Glucose Monitoring in Diabetes Management. Arlington (VA): American Diabetes Association. https://www.ncbi.nlm.nih.gov/books/NBK566165/doi: 10.2337/db2020-31

Zheng, Y., Ley, S. H., & Hu, F. B. (2018). Global etiology and epidemiology of type 2 diabetes mellitus and its complications. Nature Reviews Endocrinology, 14(2), 88–98. https://doi.org/10.1038/nrendo.2017.15