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GC-MS, Radicals Scavenging Capacity and Antidiabetic Effect of *Senna alata* Seed Extract in Type II-Induced Diabetes Mellitus in Rats

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ABSTRACT

Background and Objective: *Senna alata* (candle bush) is an important medicinal plant and it's also an ornamental flowering plant in the sub-family of Caesalpinioideae. This study evaluated the phytochemical, free radicals scavenging activity and antidiabetic effect of *Senna alata* seed extract in an animal model of Type II Diabetes Mellitus. **Materials and Methods:** The GC-MS profiling and DPPH radical scavenging activity for antioxidants of the extract were carried out. Animals were made diabetic by the administration of Streptozotocin (STZ). Consequently, they were treated with varying doses of the extract and specific doses of the standard drug. **Results:** The presence of flavonoids, triterpenoids, alkaloids, tannins, saponins and phenolic compounds was detected in the extract. Increased scavenging activities were observed at 40 µg mL^{-1} concentrations for hexane and methanol with 9.23 and 43.35% inhibition, respectively. The hyperglycaemic examination revealed that there was a significant reduction in the glycaemic index of the animals treated with *Senna alata* seed extract comparable with Glibenclamide. **Conclusion:** This research justifies the empirical use of *Senna alata* seed extracts in the treatment of hyperglycaemia in diabetic patients.

KEYWORDS

GC-MS, radicals, antidiabetic, *Senna alata*, type II diabetes

INTRODUCTION

Medicinal plants have been documented as an important source for discovering new pharmaceutical molecules that have been used to treat serious diseases[1-3]. Previous reports stated that natural products and their derived compounds exhibit absence or slight side effects and improved efficacy than other synthetic drugs[4]. Various plant species have been reported to have chemical constituents with pharmacological activities and this is attributable to these phytoconstituents such as glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes and accordingly[5].

Candle bush (*Senna alata*) is an important medicinal plant and it is also an ornamental flowering plant in the sub-family of Caesalpinioideae. It is called emperor's candlesticks, candle bush, candelabra bush as

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well as Christmas candles, empress candle plant. Also, it is called a ringworm shrub as well as a candle tree due to the erect flower spikes on its bud that appear like yellow candles. It has diuretic characteristics and is often used for the treatment of ringworm, constipation and inhibition of fungi growth and as well acts as an anti-inflammatory agent[6].

The plant is a remarkable species of Senna under the genus of Herpetica. The plant is native to most Neotropics from Mexico and the West Indies of Paraguay. It is an invasive species in Nigeria and it grows up to 3-4 m long. Its seeds and flowers have been reported with compounds like glycosides, serotonin, flavonoids and sterols[7].

Phytochemical studies are known assessments done to identify the active components of plant exudates that are responsible for their therapeutic potency. Generally, the screening of plants' phytochemicals with healing potency has increased among researchers to discover new therapeutic molecules from the plant that would be able to tackle some of the emerging and recalcitrant illnesses[8].

The potential of these bioactive compounds should be analysed for their capacity in the treatments of various ailments[9]. Plant-based medicines are often prepared from crude plant extracts comprising a complex mixture of different phytochemicals[10]. These phytochemicals have unique and complex structures and are used in treating prolonged as well as contagious diseases[11]. An enormous pool of bioactive secondary metabolites exists in various plant species, but merely a small proportion of them have been examined and sustained to be a significant source of therapeutic molecules. In the search for new compounds and also for quality control, the development of suitable screening methods is very important[9]. Extractions and characterizations of numerous such bioactive compounds from various medicinal plants have led to the delivery of certain medicines with high-activity profile[11].

Modern-day synthetic and chemical drugs are often explored with hesitant owing to their perceived side effects[7], hence traditional herbals are gaining huge interest as they are more natural, environment-friendly and exhibit little or no side effects[8]. The initial screening of medicinal plants by spectrometric and chromatographic methods provides basic information on chemical and pharmacological activities, which helps to select the biologically active plants[12]. In recent years, Fourier-Transform Infrared (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) has commonly been employed for the detection of functional groups and identification of various bioactive therapeutic compounds that are present in medicinal plants[13,14]. The GC-MS is one of the best, fast and most accurate techniques to detect various compounds, including alcohols, alkaloids, nitro compounds, long chain hydrocarbons, organic acids, steroids, esters and amino acids and requires a small volume of plant extracts. Hence, in the present study, the GC-MS technique was adopted for the detection and identification of phytochemical compounds present in the medicinal plant, *Senna alata* a sub-family of Caesalpinioideae.

Complementary and alternative medicines are largely found in developing countries such as Africa and Asia because the people of these countries have used traditional herbs for ages. In traditional medicine, *Senna alata* has been recognized for its beneficial medicinal properties such as antimicrobial, antifungal, purgative, anti-inflammatory, analgesic and antitumor potentials[15].

One of the most prevalent chronic diseases in the world is diabetes. The link between oxidative stress and many pathological situations, such as diabetes mellitus and other human diseases, is gaining scientific and popular attention[16]. According to earlier experimental and clinical research, oxidative stress is a significant factor in the pathophysiology and emergence of problems in both kinds of diabetes mellitus[17]. However, the precise mechanism through which oxidative stress may contribute to and hasten the onset of problems in diabetes mellitus is yet unclear[18].

Side effects, cost and inaccessibility of synthetic drugs for some locations in developing countries are limiting factors to the effective management of diabetes. Therefore, the search for new drugs, especially from natural products and manly plants, is of great interest to researchers and pharmacologists in the development of more efficient and better-tolerated drugs. Although *Senna alata* have been reported for several medicinal values, there is a need for scientific authentication of some of these claims. Thus, the current study examined the effectiveness of polar and non-polar solvent extracts of *Senna alata* seed on the STZ-induced diabetic albino rats.

MATERIALS AND METHODS

Study area: This was carried out in the Biochemistry Department of Federal University Wukari, Taraba State, Nigeria between August, 2021 and May, 2022.

Chemicals: Streptozotocin (STZ) and methanol were purchased from Sigma (Sigma-Aldrich, St., Louis, USA). Glibenclamide was purchased from Yola Galbose Pharmacy. Glucose oxidase/peroxidase reactive strips were purchased from Abbott Diabetes Care, Inc., USA. All chemicals and reagents used in this experiment were of analytical grade.

Plant collection and identification: *Senna alata* Seed (SAS) were collected from Michika in Michika Local Government Area of Adamawa State. The leaves were identified and authenticated by the Department of Agriculture Modibbo Adamawa University Yola. The plant was deposited in the herbarium with a voucher number IJU/MAU/ 0413.

Preparation of plant material: *Senna alata* Seed (SAS) was air-dried at room temperature and pulverized into powder for extraction. The powder (1000 g) was macerated in 80% hexane, dichloromethane, ethyl acetate, chloroform and methanol and allowed to stand for 3 days at room temperature. The mixture was filtered with Whatman No. 1 filter paper and the filtrate was concentrated using a rotary evaporator to get a brownish black semi-solid extract. Then the profiling of crude extract from the different fractions of *Senna alata* seed was carried out using the procedures described by Isaac and Fasihuddin[19].

Phytochemical screening: The phytochemical profiling of crude extract of polar and non-polar fractions of *Senna alata* seed was carried out using the procedures described by Umaru *et al*.[20].

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: The GC-MS analysis was carried out in a combined 7890A gas chromatograph system (Agilent 19091-433HP, USA) and mass spectrophotometer, fitted with an HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m×250 µm, film thickness 0.25 µm), interfaced with 5675C Inert MSD with Triple-Axis detector. Helium gas was used as carrier gas and was adjusted to a column velocity flow of 1.0 mL min^{-1}. Other GC-MS conditions are ion-source temperature, 250°C, interface temperature, 300°C, pressure, 16.2 psi, out time, 1.8 mm and 1 µL injector in split mode with split ratio 1:50 with injection temperature of 300°C. The column temperature started at 36°C for 5 min and changed to 150 V at the rate of 4°C min^{-1}. The temperature was raised to 250°C at the rate of 20°C min^{-1} and held for 5 min. The total elution was 47.5 min. The relative percent amount of each component was calculated by comparing its average peak area to total areas. MS solution software provided by the supplier was used to control the system and acquire the data.

Identification of compounds: Identification of components was achieved based on their retention indices and interpretation of the mass spectrum was conducted using the database of the National Institute of Standards and Technology (NSIT). The database consists of more than 62,000 patterns of known compounds. The spectra of the unknown components of the *Senna alata* fraction obtained were compared with the standard mass spectra of known components stored in the NIST Library (NISTII).

DPPH radical-scavenging activity: The scavenging effect for DPPH free radicals was monitored. Briefly, 1.0 mL of 0.16 mM DPPH methanolic solution was added to 1.0 mL of either methanolic solution of extract (sample) or methanol (control). The mixtures were vortexed and then let to stand at room temperature in the dark. After 30 min absorbance was read at 517 nm. Radical-Scavenging Activity (RSA) for DPPH free radical was calculated using the following equation[21]:

$$RSA = \frac{\left(A_{control} - A_{sample}\right)}{A_{control}} \times 100$$

Where:

$A_{control}$ = Absorbance of the methanol control

A_{sample} = Absorbance of the flavonoids

Synthetic antioxidant, BHA, was used as the positive control. The DPPH radical scavenging activity was calculated as the concentration that scavenges 50% of DPPH free radical and thus has RSA = 50% (EC_{50}) $A_{control}$.

Experimental animals: Adult albinos Wistar rats (160-200 g) of both sexes were used for this study. All animals were obtained from an animal farm, Natural Product Research Laboratory (NPRL) Bajubure No. 14 Sanitation Rd Federal Housing Estate. Adamawa State, Nigeria and housed in cages under controlled conditions of 12 hrs light/and 12 hrs without light and 25°C. They received pellets of food enriched with 20% protein and water ad libitum. They were deprived of food for 15 hrs (but with access to drinking water) and weighed before the experiments. Each experiment on the animals were performed according to the protocols already approved by the Department of Biosafety, Quality Control Federal University Wukari, Nigeria and met the international standards for animal study (Internationally Established Principles of the US Guidelines: NIH publication #85-23, revised in 1985).

Induction of diabetes in experimental animals: Induction of diabetes in experimental animals was done after 8-10 hrs of fasting by intraperitoneal injection of STZ dissolved in 0.1 M cold citrate buffer, pH 4.5, at a single dose of 60 mg kg^{-1} [22]. After STZ injection, the animals were allowed to drink a 10.0% glucose solution overnight to overcome the initial drug-induced hypoglycaemia. After 7 days, rats with glycaemia $\geq$250 mg dL^{-1} were selected for further experimentation.

Experimental grouping and treatments: All animals received a standard laboratory diet and drinking water ad libitum during the experimental period. With the common routine of diabetic studies all the rats' body weights and fasting blood glucose levels were measured on weekly basis and monitored for any behavioural changes during the study period. Table 1 shows the two categories of the experimental grouping and treatments. Hyperglycaemic non-diabetic rats consist of rats that were fed with high glycaemic index feed while the hyperglycaemic diabetic rats were the rats induced with STZ. Treatment with extract and the standard drug was carried out for 28 days.

Table 1: Animal grouping

Groups	Hyperglycaemic non-diabetic rats	Hyperglycaemic diabetic rats
1	Normal control	Normal control
2	Glibenclamide control (10 mg kg^{-1})	Diabetic control
3	100 mg kg^{-1} extract	Glibenclamide control (10 mg kg^{-1})
4	200 mg kg^{-1} extract	100 mg kg^{-1} extract
5	400 mg kg^{-1} extract	200 mg kg^{-1} extract
6	800 mg kg^{-1} extract	400 mg kg^{-1} extract
7	1000 mg kg^{-1} extract	800 mg kg^{-1} extract
8	Nil	1000 mg kg^{-1} extract

Evaluation of anti-hyperglycaemic effect of extract in rats: An Oral Glucose Tolerance Test (OGTT) was performed to investigate the effects of the *Senna alata* extract on hyperglycaemia in rats. The blood glucose levels were measured in overnight-fasted rats at a time point designated as 0 min. The animals were then orally dosed with glucose solution (2 g kg^{-1}) using a gavage needle and their glucose tolerance was measured hourly for 6 hrs. Blood glucose levels were estimated by glucose oxidase/peroxidase reactive strips.

Statistical analysis: All results were represented as the Mean±Standard Deviation (SD) and SPSS (Statistical Package for the Social Sciences, Version 16.0, IBM Corporation, New York, USA). All the data from the treatment groups were compared with the results from the diabetic control group using a One-way ANOVA. A $p<0.05$ was considered statistically significant.

RESULTS

Phytochemicals: The phytochemical study of extracts from *Senna alata* seed revealed a broad variety of phytochemicals. The key phytochemical components, such as flavonoids, anthocyanins, tannins, carbohydrates, alkaloids, cardiac glycosides, steroids, phenols, anthraquinones, leucoanthocyanidin, diterpenes and saponins, were qualitatively present in the extract in Table 2.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: The GC-MS chromatogram of methanol seed extracts of *Senna alata* in Fig. 1 recorded a total of 30 peaks corresponding to the bioactive compounds that were recognized by relating their peak retention time, peak area (%), height (%) and mass

Table 2: Phytoconstituents of *Senna alata* seed methanolic extract

Phytochemical	Level
Flavonoids	+++
Anthocyanins	+
Tannins	+
Alkaloids	++
Cardiac glycosides	+
Steroids	+
Anthraquinones	+
Leucoanthocyanidin	+
Diterpenes	++
Saponins	++

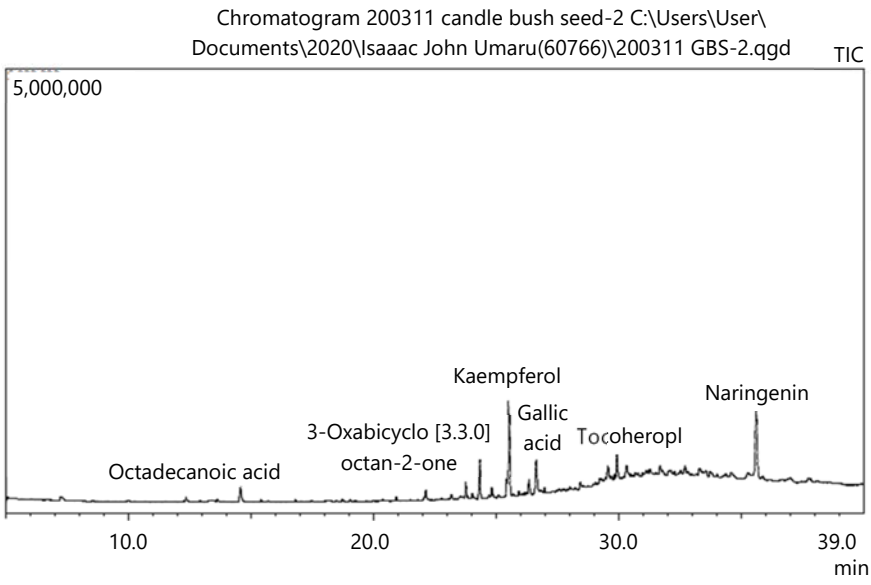

Fig. 1: GC-MS Chromatogram of phytoconstituents of *Senna alata* seed methanolic extract

Table 3: Chemical composition of methanol seed extract of *Senna alata*

Peak#	R-Time	Area	Area (%)	Height	Height (%)	Name
Peak Report TIC						
1	14.568	801987	3.74	167649	3.25	Quercetin
2	22.134	274082	1.28	87262	1.69	1-Decanol, 2-hexyl-
3	23.764	442471	2.06	170742	3.31	3,5-Dimethoxy-4,6-dimethyl-(6S)-((2R)-2-met h
4	24.038	175817	0.82	54645	1.06	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
5	24.328	1512271	7.05	442866	8.59	3-Oxabicyclo[3.3.0]octan-2-one, 7-isopropylid e
6	24.828	394121	1.84	108057	2.10	Cyclo(L-prolyl-L-valine)
7	25.433	660519	3.08	209805	4.07	Tocopherol
8	25.518	4093395	19.09	1085156	21.04	Kaempferol
9	26.348	659762	3.08	166687	3.23	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-
10	26.636	1779083	8.30	369141	7.16	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-
11	26.976	154789	0.72	61427	1.19	Heneicosane
12	28.442	243995	1.14	73506	1.43	Eicosane
13	29.230	331453	1.55	64105	1.24	Isoquinoline
14	29.445	307191	1.43	52365	1.02	Hexadecane, 2-methyl-
15	29.563	979212	4.57	180252	3.50	Octadecanoic acid
16	29.705	209733	0.98	59118	1.15	Luteolin
17	29.839	192169	0.90	65854	1.28	Heneicosane
18	29.932	1263314	5.89	311110	6.03	Gallic acid
19	30.321	736026	3.43	134030	2.60	Nonadecane, 9-methyl-
20	30.712	196220	0.91	26390	0.51	Limonene
21	30.970	226775	1.06	35986	0.70	cis-1-Chloro-9-octadecene
22	31.120	150466	0.70	52436	1.02	n-Heptadecylcyclohexane
23	31.190	159356	0.74	52452	1.02	Tetracontane
24	31.288	186953	0.87	46986	0.91	Tetrapentacontane
25	31.687	431294	2.01	95845	1.86	Pentatriacontane
26	31.830	147018	0.69	35269	0.68	Octatriacontane, 3,5-dimethyl-
27	32.520	152928	0.71	34891	0.68	Nonyl tetracosyl ether
28	32.714	368758	1.72	86446	1.68	Astragalin
29	33.298	262503	1.22	57568	1.12	Anthrone
30	35.615	3952119	18.43	769098	14.91	Naringenin
		21445780	100.00	5157144	100.00	

spectral fragmentation patterns to that of the known compounds described by the National Institute of Standards and Technology (NIST) library. Results in Table 3 revealed major compounds as 21.04 Kaempferol, 14.91 Naringenin, 8.59 3-Oxabicyclo[3.3.0]octan-2-one, 7-isopropylid, 7.16 Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-, 6.03 Gallic acid, 4.07 Tocopherol, 3.25 Quercetin and 3.50 Octadecanoic acid.

Radical scavenging activity: In the present study, different concentrations of *Senna alata* seed extracts were subjected to 2, 2-Diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging method and the absorbance and the percentage inhibition obtained at different concentrations are presented in Table 4 and 5, respectively.

At concentrations of 5, 10, 20, 30 and 40 μg mL^{-1}, ascorbic acid displayed absorbance of 0.2380, 0.1719, 0.0469, 0.0415 and 0.0410 respectively. At concentrations of 5, 10, 20, 30 and 40 μg mL^{-1}, hexane extract displayed absorbance of 0.2521, 0.2528, 0.2480, 0.2434 and 0.2317 respectively. At concentrations of 5, 10, 20, 30 and 40 μg mL^{-1}, methanol extract displayed absorbance of 0.2460, 0.2430, 0.2170, 0.1629 and 0.1430, respectively.

At concentrations of 5, 10, 20, 30 and 40 μg mL^{-1}, ascorbic acid showed percentage inhibitions of 2.59, 29.56, 82.11, 85.04 and 86.23%, respectively. At concentrations of 5, 10, 20, 30 and 40 μg mL^{-1}, hexane showed percentage inhibitions of 0.89, 0.72, 0.19, 2.68 and 9.23%, respectively. At concentrations of 5, 10, 20, 30 and 40 μg mL^{-1}, methanol showed percentage inhibitions of 0.19, 0.87, 12.83, 35.65 and 43.349%, respectively.

Table 4: Absorbance of different extracts of *Senna alata* seed and standard ascorbic acid

Concentration (µg mL^{-1})	Ascorbic acid (Abs)	Hexane (Abs)	Methanol (Abs)
5	0.2380	0.2521	0.2460
10	0.1719	0.2528	0.2430
20	0.0469	0.2480	0.2170
30	0.0415	0.2434	0.1629
40	0.0410	0.2317	0.1430

Table 5: Percentage inhibition of different extracts of *Senna alata* seed and ascorbic acid

Concentration (µg mL^{-1})	Ascorbic acid (%)	Hexane (%)	Methanol (%)
5	2.59	0.89	0.19
10	29.56	0.72	0.87
20	82.11	0.19	12.83
30	85.04	2.68	35.65
40	86.23	9.23	43.349

Table 6: Effect of *Senna alata* seed extract on glucose-induced hyperglycaemia in rats

Groups	0 hr (mg dL^{-1})	1 hr (mg dL^{-1})	2 hrs (mg dL^{-1})	3 hrs (mg dL^{-1})	4 hrs (mg dL^{-1})	5 hrs (mg dL^{-1})	6 hrs (mg dL^{-1})
Control	106.11±14.56	105.03±12.34	108.22±12.11	125.10±3.45	113±12.56	110.13±7.89	104.54±11.32
G.amide 10 mg kg^{-1}	106.11±3.78	61.23±3.56*	74.34±44.12*	76.47±3.67*	64.67±6.22*	56.76±3.12*	49.77±31.17*
Ext. 100 mg kg^{-1}	124.32±4.58	118.78±5.48	120.63±4.84	133.12±4.24	116.31±7.09	114.26±23.42	107.48±15.67
Ext. 200 mg kg^{-1}	103.76±1.77	98.12±6.87*	102.68±5.46	114.25±23.34	112.23±11.42	105.12±33.42	102.37±12.73
Ext. 400 mg kg^{-1}	101.12±11.24	98.62±10.34*	99.23±34.21*	100.13±34.22*	95.46±33.24*	92.45±41.23*	89.23±22.09*
Ext. 800 mg kg^{-1}	102.56±32.12	84.74±6.53*	98.67±44.56*	110.12±23.32	112.16±33.24	99.76±12.46*	98.56±34.48*
Ext. 1000 mg kg^{-1}	102.36±11.38	80.45±14.23*	100.24±23.07	105.12±9.34*	99.98±12.23*	96.34±11.24*	86.54±25.36*

Data are represented as the mean±SD (n = 8), *denotes a significant difference ($p<0.05$) between the treatment groups and the normal control group and the comparisons were made by one-way ANOVA

Table 7: Effect of *Senna alata* seed extracts on STZ-induced hyperglycaemia in rats

Groups	0 hr (mg dL^{-1})	1 hr (mg dL^{-1})	2 hrs (mg dL^{-1})	3 hrs (mg dL^{-1})	4 hrs (mg dL^{-1})	5 hrs (mg dL^{-1})	6 hrs (mg dL^{-1})
Normal control	110.41 ±4.66*	114.31 ±3.47*	116.31 ±12.54*	115.31 ±23.12*	114.31 ±23.67*	111.21±34.23*	110.31 ±11.26*
Diabetic control	424.34±4.38	458.48±6.23	454.63±14.34	433.14±4.26	416.31±7.34	414.25±25.62	407.45±13.64
Diabetic+G.amide 10 mg kg^{-1}	336.11±3.74*	361.23±3.56	354.34±44.16*	356.47±3.67*	354.67±6.22*	346.76±3.15*	329.77±32.16*
Diabetic+Ext. 100 mg/kg/b.wt.	445.46±12.34	438.12±5.77	432.68±5.23	424.23±23.36	412.24±11.47	405.13±32.12	402.37±12.77
Diabetic+Ext. 200 mg/kg/b.wt.	347.12±15.27*	367.62±10.37*	363.23±35.26*	358.17±36.21*	345.46±13.44*	332.45±41.24*	329.27±32.12*
Diabetic+Ext 400 mg/kg/b.wt.	332.56±42.15*	354.74±4.66*	348.67±44.52*	345.12±25.35*	333.16±32.66*	326.76±11.67*	318.56±34.28*
Diabetic+Ext 800 mg/kg/b.wt.	322.36±11.34*	344.45±14.44*	336.24±23.13*	329.12±9.36*	319.98±11.18*	306.34±11.19*	286.54±26.19*
Diabetic+Ext. 1000 mg/kg/b.wt.	316.11±3.72*	331.22±4.43*	328.34±36.16*	326.47±13.23*	319.67±6.33*	216.76±3.15*	259.75±22.34*

Data are represented as the mean±SD (n = 6), *denotes a significant difference ($p<0.05$) between the treatment groups and the diabetic control group and the comparisons were made by one-way ANOVA

The methanol seed extract revealed maximum DPPH activity of 43.349%, while Hexane which is the non-polar recorded about 9.23%, respectively (Table 4). The methanol (polar) seed extract exhibited the highest DPPH radical scavenging activity.

Effect of *Senna alata* seed methanolic extract on hyperglycaemia: The effects of *Senna alata* seed extracts and glibenclamide on glucose-induced hyperglycaemia in rats and the effect of *Senna alata* seed extracts and Glibenclamide on STZ-induced hyperglycaemia in rats are represented in Table 6 and 7. Administration of the extract at various doses significantly ($p<0.05$) lowered the blood glucose level of the treated hyperglycaemic rats when compared with the untreated hyperglycaemic rats. In Table 6, the animals treated with the standard drug, glibenclamide after being fed with a high glycaemic-index diet

howed significant ($p<0.0$) reduction in blood glucose levels, 106.11±3.78, 61.23±3.56, 74.34±44.12, 76.47±3.67, 64.67±6.22, 56.76±3.12 and 49.77±31.17 mg dL^{-1} at as the time increases from 0-6 hrs respectively. This reduction was also noticed in the groups treated administered with 100, 200, 400, 800 and 1000 mg kg^{-1} of the extract when compared with the standard drug. A similar experience was seen in Table 7 where glibenclamide significantly ($p<0.0$) reduced the blood glucose levels across times 0, 1, 2, 3, 4, 5 and 6 hrs. Equally, several of the extract-treated rats also displayed such signification ($p<0.05$) decrease in the blood sugar level compared to the standard drug and the normal control. However, there was a significant ($p<0.05$) increase (424.34±4.38, 458.48±6.23, 454.63±14.34, 433.14±4.26, 416.31±7.34, 414.25±25.62 and 407.45±13.64 mg dL^{-1} at time 0, 1, 2, 3, 4, 5 and 6 hrs respectively) in the STZ-induced but not treated rats when compared with the extract and standard drug-treated rats.

DISCUSSION

The result of phytochemical screening of the seed of candle bush (*Senna alata*) extract revealed the presence of flavonoids, triterpenoids, alkaloids, tannins, saponins and phenolic compounds. The presence of some of these phytochemicals in this plant was previously revealed by Lahare *et al.*[23], who demonstrated that its methanol and aqueous-based leaf, stem and root extracts showed good antioxidant activity due to the presence of flavonoids and phenolic compounds. Also, *Senna alata* was found to contain saponins, alkaloids, tannins, phlobatannins, anthraquinones, cardenolides, steroidal rings and flavonoids[24]. The present finding collaborates the antioxidant potentials of this plant since flavonoids and phenolic compounds were visible in the extract.

The general composition of the chemical constituents were found to be 30 compounds which includes 3.25 Quercetin, 1.69 1-Decanol, 2-hexyl-, 3.31 3,5-Dimethoxy-4,6-dimethyl-(6S)-((2R)-2-met h, 1.06 Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-, 8.59 3-Oxabicyclo[3.3.0]octan-2-one, 7-isopropylide, 2.10 Cyclo(L-prolyl-L-valine), 4.07 Tocopherol, 21.04 Kaempferol, 3.23 Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-, 7.16 Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3 -, 1.19 Heneicosane, 1.43 Eicosane, 1.24 Isoquinoline, 1.02 Hexadecane, 2-methyl-, 3.50 Octadecanoic acid, 1.15 Luteolin, 1.28 Heneicosane, 6.03 Gallic acid, 2.60 Nonadecane, 9-methyl-, 0.51 Limonene, 0.70 cis-1-Chloro-9-octadecene, 1.02 n-Heptadecylcyclohexane,1.02 Tetracontane, 0.91 Tetrapentacontane, 1.86 Pentatriacontane, 0.68 Octatriacontane, 3,5-dimethyl-, 0.68 Nonyl tetracosyl ether, 1.68 Astragalin, 1.12 Anthrone, 14.91 Naringenin as shown in Table 2. The result is concomitant with Kolawole *et al.*[24] GC-MS analysis of an ethanolic extract of *Senna alata* that led to the identification of different compounds in the classes of xylene, alcohol, aldehydes, alkanes, alkenes, fatty alcohol, acetic acid, ketones and ester. Similarly, Victor, and David[25] revealed the presence of 6-Octadecenoic acid 2, 3-Dihydroxypropyl-9-octadecenoate and Octadecanoic acid (18.08%) in the chloroform-methanol extract.

Seed of candle bush (*Senna alata*) methanol extract was evaluated for scavenging activities and it was observed that at a lower concentration of 20 µg mL^{-1}, hexane and methanol extracts revealed scavenging potential of 0.19 and 12.83% respectively. However, the scavenging activities were increased at 40 µg mL^{-1} for both hexane and methanol extracts, which is 9.23 and 43.35%, respectively. Previously, the *in vitro* DPPH assay of ethyl acetate-DCM, methanol-DCM and oil fractions of *Senna alata* leaves significantly inhibited the free radicals at 500 g mL^{-1} due to polyphenol and flavonoid presence[26,27], whereas, the same inhibition was experienced at concentrations of between 5-40 µg mL^{-1} in the present study. Also, the scavenging activity revealed here is in agreement with Chatatikun and Chiabchalard[28] work where *Senna alata* was shown to have high antioxidant content and activity and significantly inhibited both tyrosinase and collagenase.

The high glycaemic index feed and STZ caused a significant increase in the blood glucose level of the rats, meanwhile, the normal group of animals showed normal glucose levels. However, the blood glucose level of both animals that were on feed with high glycaemic index and the ones that were induced with STZ progressively decrease upon the administration of the extract. This decrease was dose-dependent and was

comparable with the effect of the standard drug (glibenclamide). The result indicates the effectiveness of *Senna alata* seed extracts on hyperglycaemia and may be used as a remedy for diabetes. The present finding has been validated by the identification and characterization of ant-diabetic principles in *Senna alata* by Uwazie *et al*.[29]. Also, upon the administration of *Senna alata*, the hyperglycaemia induced by a high-fat diet was significantly reversed by restoring the insulin sensitivity[30].

CONCLUSION

Senna alata seed extracts exhibited an anti-hyperglycemic action against hyperglycaemia induced by a high glycaemic index diet as well as STZ-induced diabetics. This action may be related to the antioxidative and phytochemical constituents of the extract. This reduction could be associated with the improvement of functions of insulin and enzymes in the liver and kidneys. These properties justify the empirical use of *Senna alata* seed extracts in the treatment of hyperglycaemia. However, more studies need to be undertaken to identify, isolate and purify specific compounds responsible for the anti-hyperglycaemic effect of this extract.

SIGNIFICANCE STATEMENT

This study discovered the anti-hyperglycaemic effect of *Senna alata* which can be beneficial for diabetic patients. This study will help the researchers to uncover the critical areas of the action mechanism of this plant that many researchers were not able to explore. Thus a new theory on diabetic management may be arrived at.

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