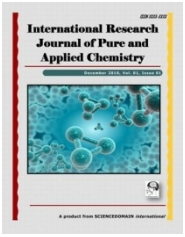

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Assessment of Phytoconstituents and Antidiabetic Activity of the Crude Extract and Partitioned Fractions of *Maytenus senegalensis* (Lam.) Exell (*Celastraceae*) Root Bark

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Authors' contributions

This work was carried out in collaboration between all authors. Author FOR has contributed significantly to acquisition of data, analysis, drafting of the manuscript. Author AM has made substantial contribution to conception and design, interpretation of data, drafting and revising the manuscript for intellectual content. Authors ANS, JY, LAF and EOO have participated in the research design, data analysis and revising the manuscript for intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the antidiabetic activity of the root bark of *Maytenus senegalensis* ethnomedically used in Nupeland for the management of diabetes and isolate phytoconstiteunts responsible for the pharmacological activities.
Study Design: Air-dried root bark *Maytenus senegalensis* ground into a fine powder. The pulverized material was macerated in 70% methanol at room temperature for 72h. The extracts were concentrated *in vacuo* using rotary evaporator at 35ºC. Antidiabetic activity was determined on the crude methanolic extract and partitioned fractions.
Place and Duration of Study: Department of Chemistry and Department of

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Biochemistry, School of Natural and Applied Sciences, Federal University of Technology, Minna, Nigeria between January, 2012 and July, 2013.
Methodology: The present study reports the phytoconstituents evaluation as well as antidiabetic activity determination of the root bark extract of *M. senegalensis* on alloxan-induced diabetic rats. Phytoconstituents analysis and characterization of the root bark extract of *M. senegalensis* were determined using standard phytochemical methods and GC-MS analysis respectively. The crude extract was partitioned using different solvents of varying polarities and the fractions obtained were tested for their phytoconstituents.
Results: The preliminary phytochemical analysis of *M. senegalensis* root bark indicates the presence of alkaloids, flavonoids, saponins, tannins and steroidal compounds. Anthraquinones was completely absent. The root bark of *Maytenus senegalensis* was extracted by 70% methanol which was then partitioned using four solvents of different polarities. The crude extract and solvent soluble fractions were used to determine the antidiabetic activity against alloxan induced (150mg/kg body weight) diabetic rats, after oral administration at a dose of 200mg/kg body weight for two weeks. The result revealed reduction in the elevated blood glucose level by 29.75%. Treatment with the known antidiabetic drug, glibenclamide (5mg/kg body weight) lowered blood glucose level by 27.03% indicating significant improvement in activity. Results obtained indicate that 70% methanolic fraction (aqueous methanol soluble fraction) has highest and most effective activity. This aqueous methanol fraction was then subjected to column and thin layer chromatography as well as GC-MS analysis. The GC-MS spectrum reveals the presence of seven different compounds which may have contributed to the ethnomedicinal properties of this plant.
Conclusion: Scientific research has supported the ethnomedicinal claims that *M. senegalensis* is useful in diabetes management. However, the isolation, purification and characterization as well as mechanism of action of bioactive constituents underlying its curative properties are suggested.

Keywords: Phytoconstituents; Maytenus senegalensis; antidiabetic activity.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder caused by deficiency in production of insulin by the pancreas. Currently there are over 150 million diabetics worldwide and this may double by the year 2030 [1]. Half of people with diabetes are undiagnosed. 4.8 million People died due to diabetes. More than 471 billion USD were spent on healthcare for diabetes. In 2011, 14.7 million adults in the Africa region are estimated to have diabetes, with a regional prevalence of 3.8%. Some of Africa's most populous countries also have the highest number of people with diabetes, with Nigeria having the largest number (3.0 million) in the region. Nigeria with over 250 tribes and different culture and food values show increasing rates of diabetes [2,3]. The progressive increase in the prevalence rates of diabetes is associated with lifestyle changes. Over 30% of our elite population including decision-makers is diabetic [4]. It is a global problem and number of those affected is increasing day by day. According to the report of Oputa and Chinenye [5], diabetes-related deaths in Nigeria in 2011 accounted for 63 340 people, the number of diabetic cases in Nigeria is expected to increase [2]. Currently, diabetes is controlled by diet, exercise, insulin replacement therapy and by the use of herbal hypoglycemic agents [6]. However, available oral hypoglycemic drugs have serious side effects such as nausea and vomiting, jaundice, and hemolytic anemia, generalized hypersensitivity reactions, dermatological reaction and lactic acidosis. Therefore, searching for effective, low cost and less side effected hypoglycemic agents is important. The plants

provide a potential source of hypoglycemic drugs because many plants and plant derived compounds have been used in the treatment of Diabetes mellitus [7-11]. The World Health Organization (WHO) reported in 2001 that the herbal medicine can serve the health need of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries [12]. Many indigenous plants are major ingredients of traditional medicine [13]. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Numerous studies have been conducted on traditional usage of plants which are often substantiated by scientific investigation that resulted in the isolation of bioactive compound for direct use in medicine [14].

Experimental evidences have confirmed the hypoglycaemic activity of a number of indigenous African medicinal plants. Following the WHO's recommendation for research on the beneficial uses of medicinal plants in the treatment of diabetes mellitus [15] and the increased demand by patients to use the natural product with antidiabetic activities [16], investigations on hypoglycemic agents from medicinal plants have gained momentum. In recent times medicinal plants have been used in the management of diabetes. Many modern drugs are based on molecular prototypes derived from medicinal plants. For instance metformin isolated from *Galega officinalis* is used for lowering glucose-level in humans [17].

Currently, over 400 plants used traditionally to treat diabetes have been reported, even though only a small fraction of these have been scientifically and medically evaluated for their efficacy [17]. Several hypoglycemic effects of some herbal extracts have been confirmed in human and animal studies for type 2 diabetes [17]. The traditional medicinal herbs have been recommended for further investigation by the World Health Organization Expert Committee on diabetes. Presently, the major hindrance in adoption of combination therapy of herbal medicine with modern medical practices is the lack of scientific and clinical data to support their efficacy and safety [17]. There is a need for conducting clinical research in herbal drugs using various animal models for toxicity and safety evaluation.

From the foregoing, it is essential to establish the active component(s) from the efficacious plant extracts. Therefore, search for more effective and safer hypoglycaemic agents has continued to be an important area of active research. Medicinal plants contain a variety of chemical substances and have been reported to possess many useful properties including antidiabetic and antimicrobial activities [11,18-20]. The medicinal value of these plants lies with these chemical substances that produce a definite physiological action on the human body. The most important of these constituents of plants are terpenes, alkaloids, tannins, flavonoids and phenolic compounds [21]. The bioactivity in *Maytenus* species has been attributed to various groups of secondary metabolites such as triterpenes and several types of alkaloids [21]. The genus *Maytenus* is a rich source of quinine methide triterpenes and these compounds seem to have a variety of biological activities such as antimicrobial, cytotoxic, antimalarial and veterinary properties [22-27]. World ethnobotanical information about medicinal plants reports that almost 800 plants could be used to control diabetes mellitus [11]. Several investigations have been conducted and many plants have shown to possess antidiabetic potentials and active principles [20]. Even though active principles have been isolated from some plants [20]; many are yet to be identified. These necessitated the search for new bioactive compounds with proven and effective antidiabetic property from medicinal plants.

Many Nigerian medicinal plants have been found to demonstrate significant antidiabetic activity [20,28]. One of such medicinal plants is *Maytenus senegalensis* which belongs to the *Celastraceae* family and popularly employed in the management of diabetes and other

ailments in Nupeland, North Central Nigeria [29,30]. *M. senegalensis* is a spike-thorny tall shrub or tree with young branches often spiny which grows up to 15m high, unarmed or with spines up to 7cm long (Fig. 1). *M. senegalensis* is commonly called spike thorn. Its synonym is *Gymnosporia senegalensis* [31]. It is found widely distributed in tropical and subtropical regions of both Asia and Africa [32]; and often found in Nigeria; among the Nupe speaking people, it is called “Tsukukporikan”, “Kpakan”, “Ekanshelu” in Nupe [30]; Hausas, called it “Mangaladi”, “Namiji Tsada”, “Bokaroro”, “Kunkushewa”; “Tultulde”, “Yare-lesdi” in Fulani; Yorubas call it “Sepolohun” and “Afor-juru”, “Yabasi” in Igbo [33].

Fig. 1. Showing spike thorny stem, branches with leaves, flowers and fruits of *Maytenus senegalensis* (Photographed by A. Mann)

In Nupeland of North Central Nigeria, the root and stem barks of *Maytenus senegalensis* have been reported as being effective and commonly used for the management of diabetes [30]; but this has not been scientifically investigated for the bioactive single agents. Therefore, the present research work is designed to determine the phytoconstituents and antidiabetic activity of the root bark extract of *M. senegalensis* using 70% methanol in alloxan induced diabetic rats and to isolate active principles single compounds responsible for the antidiabetic activity.

2. MATERIALS AND METHODS

2.1 Plant Material

The root bark of *Maytenus senegalensis* was collected based on ethnobotanical information from Patishin swasun forest, 5 km along Bida-Busu Road, Niger State, Nigeria. The sample was collected in month of January, 2012. Plant was identified and authenticated by Mr Umar

S Gallah, a plant taxonomist at the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria where a voucher specimen with a herbarium number (900199) was deposited.

2.2 Preparation of Samples

The root bark of *M. senegalensis* collected from the forest were thoroughly washed with distilled water, air-dried at room temperature under shade and prepared according to methods described by Mann [34]. It was then pulverized into uniform powder manually. The sample was then sieved (40 mesh sizes), weighed, bottled, sealed, labeled and kept in polyethylene bag for analysis.

2.3 Extract Preparation

The pulverized *M. senegalensis* root bark (249g) was macerated by soaking in 70% methanol (500mL, 72h) at room temperature. This was repeated thrice and the resultant extracts were combined and then concentrated *in vacuo* using rotary evaporator ((NYS RD-205D) at 35ºC to give the crude methanolic extract. The extract was air-dried, weighed, packed in a glass bottle and labeled as the crude methanol extract of *M. senegalensis* and kept under refrigeration away from light by wrapping with aluminum foil prior to further processing and then used to calculate percentage recovering.

2.4 Qualitative Analysis of the Crude Methanolic Extract of *M. senegalensis*

The crude methanolic extract was screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones using standard procedures [35,36].

2.5 Partitioning of the Crude Extract

The crude methanolic extract (69g) was dissolved in 500ml of methanol. The resultant solution was partitioned with 250ml thrice each in order of their increasing polarities (*n*-hexane, chloroform and *n*-butanol) [34]. All the four fractions obtained were filtered one after the other using Whatman No.1 filter paper sheets (Whatman Int. Ltd. Maidstone, England). Each extract was then concentrated over a water bath at 35ºC by the evaporation of various solvents from the extracts after which phytochemical screening was conducted on each fraction and the methanolic extract which was having most of the metabolite was taken for further analysis.

2.6 Experimental Animals

Twenty four young Wisater strain albino rats weighing between 95-130g of both sexes were obtained from Benue State University, Makurdi, Benue State. Before and during the experiment, the rats were allowed free access to standard feed (purchased from Grand Cereal and oil meals Limited, Jos, Plateau State) and water. After randomization of the various groups and before initiation of experiment, the rats were acclimatized to the animal house conditions [37] at the Department of Biochemistry, School of Natural and Applied Science, Federal University of Technology, Minna, Nigeria. Prior to each study, the animals were made to fast for 12-16h [38]. All the animal experiments were conducted at

Biochemistry Laboratory, School of Natural and Applied Sciences, Federal University of Technology, Minna, Nigeria.

2.7 Induction of Experimental Animals

White albino rats were fasted overnight (12-14h) and their weight and fasting blood glucose level were recorded determined using glucometer (Fine test; Infopia Co Ltd, Korea). They were then made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight). Alloxan of 1g was first weighed and then solublized with 10ml distilled water which serves as a stock just prior to injection. Food and water were presented to the animals 30 minutes after administration [37,39]. Two days after alloxan injection, plasma blood glucose level of each animal was determined and animals with a fasting blood glucose range above 190 mg/dl were included in the study. The blood samples were collected from the tail of the rats.

2.8 *In vitro* Antidiabetic Screening of the Crude Extract

During the course of the experiment, the rats were divided into 4 groups for the evaluation of fasting blood glucose level with four animals in each group.

Groups 1 served as negative (took only the vehicle).
Groups 2 served as positive (diabetes) control (diabetic but not treated).
Groups 3 took standard drug (glibenclamide, 5mg/kg per day orally) [37].
Groups 4 were treated with extract of *M. senegalensis* (200mg/kg) in 1ml of DMSO through gavages daily for 2 weeks. Treatment with plant extract started 48h after alloxan injection.

Blood samples were drawn at weekly intervals till the end of the 2 weeks study. Fasting blood glucose levels and body weight measurement of all the rats were recorded on day 1, 7 and 14 during the experimental period [37].

2.9 *In vitro* Antidiabetic Activity Screening of the Partitioned Fractions

The rats were divided into five groups for the evaluation of fasting blood glucose level with two animals in each group as thus;

Group 1 was treated with *n*-hexane fraction.
Group 2 was treated with chloroform fraction.
Group 3 was treated with *n*-butanol fraction.
Group 4 was treated with 70% methanol fraction.
Group 5 served as positive control (diabetic but untreated).

Treatment with the fractions started 48h after induction. Blood samples were drawn at weekly intervals till the end of the study, 2 weeks. Fasting blood glucose levels and body weight measurement of all the rats were recorded on day 1, 7 and 14 during the experimental period [37].

2.10 Data Analysis

Data were statistically evaluated by use of one-way ANOVA, followed by post hoc Scheffe's test using version 13 of SPSS software and Microsoft office excel 2003. The values were considered to be significant at $p<0.05$.

2.11 Column Chromatography of the Methanol Fraction

Slurry was prepared by shaking 28.53g of silica gel (particle size 0.040-0.063nm; 70-230 mesh, Merck) with 200ml of hexane and then packed using wet method in a column (3 x 30cm). The silica gel was first activated in the oven for 2h at 105ºC. One gramme (1g) of the extract was re-dissolved in 50ml of acetone and 20g of the activated silica gel was used to absorb the dissolved extract. The dissolved extract was then kept under fan to dry. The dried absorbent (silica gel) with the sample embedded was then introduced to the top of the packed column and some quantities of silica gel were added on top followed by boiling chips. The above prepared column was then eluted using gradient mixtures of *n*-hexane-EtOAc (100:0 →95:5 →20:80 → 0:100) and MeOH. Eluents were collected in 50 ml and were then monitored by TLC behaviour [34].

2.12 Thin Layer Chromatography Analysis of the Eluents

The recovered fractions were dissolved in the appropriate solvent. A line was drawn 2 or 3 cm from the base of each TLC plate (to serve as origin) for spotting. Adopting one dimensional and ascending technique, a fine bore glass capillary tube was used to apply a spot of each of the fraction on the pre-coated TLC plates. Similarly other eluents collected from column chromatography were treated in the same manner. Various solvent systems were tried in order to get good separation or resolution of the constituent spots. Finally the solvent system EtOAc-MeOH, 4:1 that gave the best separation or resolution was adopted and then introduced into the tank and the lid placed until the tank is saturated with the vapour of the solvent system. The plates were then carefully introduced into the tank with the origin about 1.5-2.0cm above the solvent system and allowed to stand for a few minutes. The plates were then removed and allowed to dry under the fan. The developed chromatograms were observed for separation under ultraviolet light (long, 254 nm) and (short, 366nm). Similarly, the plates were also observed under iodine vapour. The Rf values of the spots were then measured [40].

2.13 GC/MS Analysis of the Extract

GC-MS analysis was carried out on a Shimadzu (Kyoto, Japan) GC-MS model QP 2010 at National Research Institute for Chemical Technology, Zaria, according to the EN 14103 standard method [41,42]. The GC column oven temperature (70ºC), injecting temperature (250ºC), flow control mode (linear velocity), total flow (40.8ml/min) column flow (1.80 ml/min), pressure (116.9kpa), linear velocity (49.2cm/sec) and purge flow (3.0ml/min) were employed for this analysis. A sample volume of 8.0µL was injected using split mode (split ratio of 20.0). The peak area, that is, the % amount of every component was calculated by comparing its average peak area to the total areas. Software was used to handle mass spectra and chromatogram.

2.14 Identification of Components from the Extract

Interpretation of mass spectrum GC-MS was conducted by comparing the database peaks of National Institute Standard and technology (NIST) library with those reported in literature, the mass spectra of the peaks with literature data [43]. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Component relative percentages were calculated based on GC peak areas without using

correction factors. The name, molecular weight and structure of the components of the test materials were ascertained.

3. RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Screening

Phytochemical screening was done using colour forming and precipitating chemical reagents on both the crude and fractionated extract to generate preliminary data on the constituents of the plant extracts. The results obtained from the tests were summarized in Tables 1 and 4 for both crude and fractionated extracts. The chemical tests revealed the presence or absence of major secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, saponins, etc in the extracts. The result indicated the presences of flavonoids, alkaloids, saponins and phenolic compounds. Previous study indicated the presence of secondary metabolites like flavonoids, alkaloids, saponins [23]. Plants with hypoglycemic and antihyperglycemic activities may contain one or more chemical constituents. Classes of chemical compounds isolated from plants including alkaloids (vindoline), flavonoids (epicatechin), tannin (cathechin), etc are documented to have the potential to decrease the blood glucose level [38,44,45]. Thus, the significant antidiabetic effect of both the crude and fractionated extract could be due to the possible presence of the aforementioned constituents in the part of the plant used in this particular study, which could act synergistically or independently enhancing the activity.

Table 1. Phytochemical analysis of the crude extract and aqueous methanol fraction of *M. senegalensis*

Phytoconstiteunts	Chemical test	Crude methanol extract	Aqueous methanol fraction
Alkaloids	Dragendorff's reagent	+	+
	Mayer's reagent	+	+
Steroidal	Acetic anhydride and Conc.	+	+
Compounds	Sulphuric acid	+	+
	Chloroform and Conc. Sulphuric acid		
Phenolic Compounds	Ferric chloride and Potassium Ferrocyanide	+	+
Flavonoids	Ammoniacal silver nitrate	+	+
Saponins	Froth	+	+
Tannins	Ferric chloride	-	-
Anthraquinones	Borntrager's	-	-

Key: + → positive, - → negative

3.2 Partitioning of the Crude Extract

The crude extract dissolved in methanol was partitioned using three different solvents (*n*-hexane, chloroform and *n*-butanol) in order of their increasing polarities and the fractions obtained is shown in Table 2 below.

Table 2. Fractions obtained during the partitioning

Fraction	Weight (g)
N-Hexane	2.80
Chloroform	6.90
N-Butanol	12.13
Aqueous Methanol Fraction	16.40

3.3 Column Chromatography Analysis

The eluents obtained from the column using gradient mixtures of n-hexane-ethylacetate and ethylacetate-methanol gave series of fractions which were obtained which were monitored by TLC behavior. Based on the TLC analysis of the fractions, some of the fractions were pooled together as follows: F_{23}, F_{33}, F_{43}, F_{50}, F_{57}, F_{58}, F_{63}, F_{65}, F_{71}, F_{75}, F_{81}, and F_{84}. The Rf values ranges between 0.2-0.8. Most spots are of lower Rf values indicating that most of the component are polar. F_{23} and F_{50} show three spots indicating that there are three likely components in each fraction. The spot(s) of each fraction and their corresponding Rf values are shown in Table 3.

Table 3. Pooled fractions after TLC analysis

Fractions	Spots	Rf Values
F_{1-23}	3	0.3, 0.4, 0.5
F_{24-33}	1	0.3
F_{34-43}	1	0.4
F_{44-50}	3	0.5, 0.6, 0.7
F_{51-57}	2	0.5, 0.6
F_{58-62}	2	0.4, 0.6
F_{63-64}	1	0.5
F_{65-70}	1	0.3
F_{71-74}	1	0.3
F_{75-80}	2	0.6, 0.8
F_{81-83}	1	0.5
F_{84}	2	0.4, 0.6

3.4 Combination of the Fractions from the Column Chromatography

The fractions were combined based on their TLC behaviours of similarities in Rf values of the spots and then were so grouped as follows:

F_{1-23} (F_{23})
F_{34-43} (F_{43})
F_{44-50} (F_{50})
$F_{24-33, 65-70, 71-74}$ (F_{74})
$F_{63-64, 81-83}$ (F_{83})
$F_{58-62, 84}$ (F_{84})

3.5 Phytochemical Analysis of the TLC Fractions

Phytochemical analysis was then carried out on the six fractions and the results were obtained as presented in Table 4.

Table 4. Phytochemical analysis of the TLC combined subfractions

Phytoconstiteunts	Subfractions					
	F_{23}	F_{43}	F_{50}	F_{74}	F_{83}	F_{84}
Alkaloids	-	+	-	-	-	-
Steroids	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Saponins	-	+	+	+	+	-
Phenolics	-	-	-	-	-	-

Key: + →Positive, - → Negative

3.6 Body Weight

The change in the body weight of control and experimental groups of rats treated with *M. senegalensis* is shown in Fig. 2. Alloxan-induced (150 mg/kg body weight) rats showed loss in body weight (from 1.10% to 3.39%), which was reversed by oral administration of crude extract and standard drug. The body weight of the normal control rats (negative control), which took the vehicle only, did not show any significant difference, i.e. 0.14% change on the 14th day. However, the body weight of diabetic untreated rats (positive control) showed a 3.37% decrease after two weeks. Significant body weight improvement was observed starting from day 1 in diabetic rats treated with both the crude extracts and standard drug. The effect was a bit pronounced in case of the crude extract treated rats with 1.95% increment on the 7th day of treatment and on the 14th day, the body weight increased up to 3.05% (from 129.13 to 133.20) during the experimental period .In case of standard drug the body weight increased from114.68 to 115.20 equivalent to 1.45% increment, and on the 14th day the weight improved to 116.21(2.53%). More so, the ANOVA was also conducted and the result which shows that $F(3,44) = 337.230$; $P<0.05$ indicates that there is a high significant difference in change in mean body weights of Normal Control and Alloxan-Induced Diabetic Rats as shown in the Table 4 above.

The fasting mean blood glucose level values before and after treatment for two weeks in all the four groups are presented in Fig. 3 below. Diabetic control mice were compared with normal control mice and diabetic mice that were treated with the crude extracts were compared with diabetic untreated mice. The fasting mean blood glucose levels did not show a significant difference in normal control (negative control) rats. In the case of diabetic untreated (positive control) rats, the fasting mean blood glucose level showed a statistically significant ($p<0.05$) increase as compared to the normal control rats after two weeks during the experimental period. On treatment with crude extract, the fasting mean blood glucose levels on day 7, i.e. 292.00mg/dl reduced to 251.25mg/dl. This reduction accounts for13.96%%. On 14th day, the fasting BGL reduced to176.50 which is equivalent to 29.75%. The fasting mean blood glucose level of diabetic rats treated with glibenclamide showed a reduction of12.60% after 7 days, i.e. from 304.65 to 266.25mg/dl which further reduced by 27.03% on 14th day (from 266.26 to 194.25mg/dl) as compared with diabetic control (positive control) mice. Conversely, fasting blood glucose of alloxan – induced diabetes rats in standard, diabetic and extract controls are found not to be statistically different from one another other.

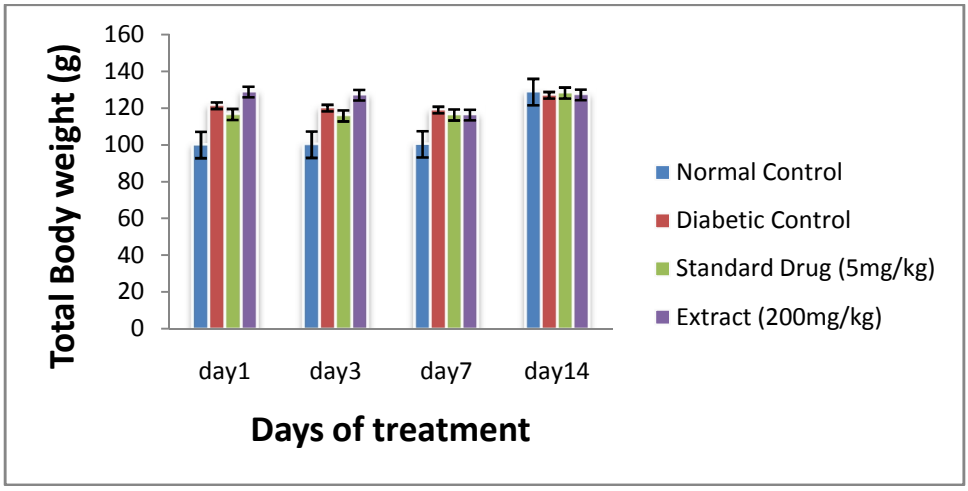

Fig. 2. Effect of crude extract of *M. senegalensis* on body weight in rats

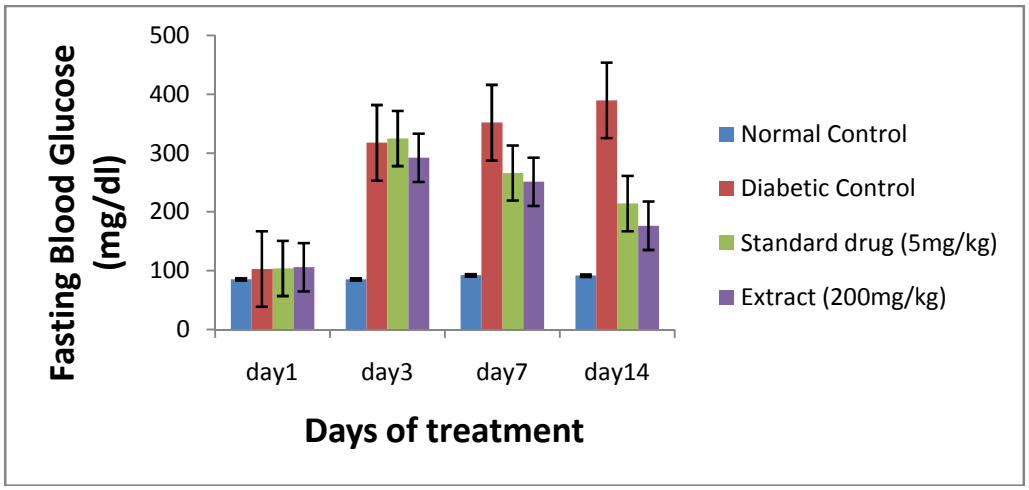

Fig. 3. Effect of Crude extract of *M. senegalensis* on FBS in rats

3.7 Effects of Fractions on Fasting Mean Blood Glucose Level (FBS)

On treatment of diabetic rats with chloroform fraction, the fasting mean BGL reduced from 218.23 to 207.60 after 7 days which account for about 4.87% and on the 14th day, the fasting BGL showed no significant different. Treatment with hexane fraction brought about 9.15% reduction in fasting BGL i.e. from 215.84 to 195.5 after 7 days and on 14th day the BGL reduced to189.50 which shows reduction of 3.07%. Methanolic fraction gave a reduction of 16.94% in fasting BGL i.e. from 231.17 to 192.00 on 7th day, and on 14th day, the percentage reduction increased to 36.67% i.e. from192.00 to 121.60. Butanol fraction reduced fasting mean BGL from 263.40 to 228.00 (13.44%) on 7th day and on 14th day to 211.00 (7.46%). There was no reduction in fasting mean BGL of the diabetic untreated rats (positive control). From the graph (Fig. 4), it can therefore be inferred that the methanolic fraction shows the

highest activity; hence phytochemical screening was conducted and the result obtained is represented in the Fig. 4 below.

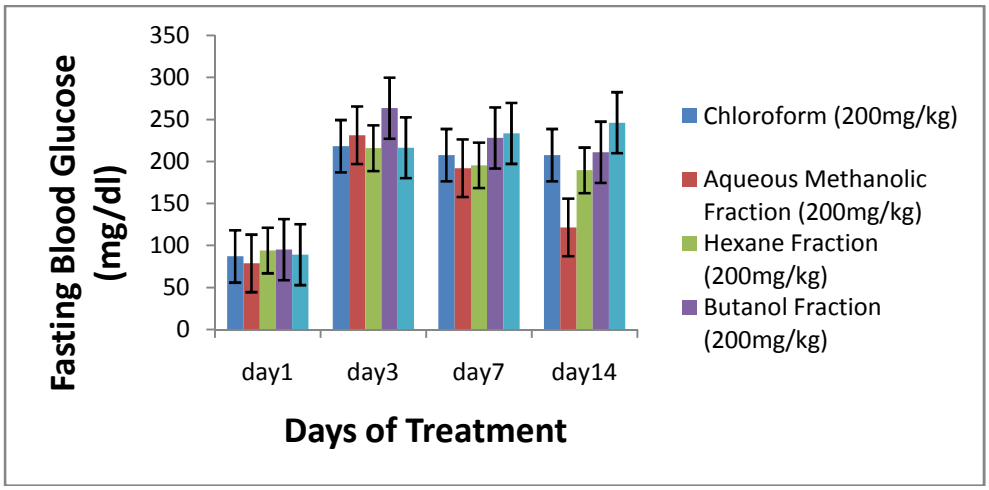

Fig.4. Effects of various solvent soluble fractions of *M. senegalensis* on FBS

3.8 GC-MS for *M. senegalensis* Combined subfraction (F_{43})

The combined subfractions (F_{43}) of *M. senegalensis* on phytochemical screening were observed to have obtained flavonoids, saponins, and trace amount of alkaloids was then taken for GC-MS analysis. The compounds present in the combined subfractions (F_{43}) were identified by GC-MS as shown in Fig. 5. The peaks with their peak area %, molecular formula, molecular mass and individual fragmentation patterns observed for each of the suggested compounds namely: Hexadecanoic acid, 9-octadecenoic acid (Z), 12-hydroxy-9-octadecenoic acid (Z), methyl-1-cyclopentene-1-carbxylate, 12-hydroxy-9-octadecenoic acid, methyl ester (Z); 1, 1-dimethoxyacetone and 13-hexyloxacyclotridec-10-en-2-one are shown in Table 5.

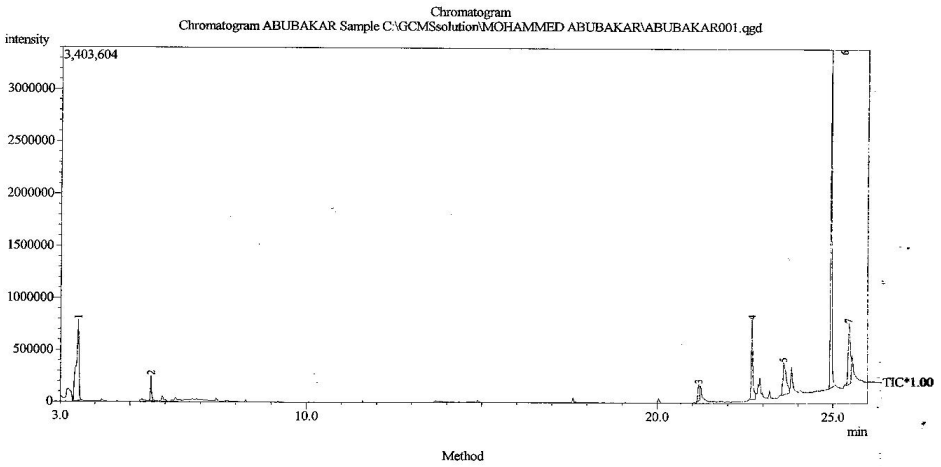

Fig. 5. GC-MS chromatogram for *M. senegalensis* combined subfraction (F_{43})

Table 5. Parameters deduced from GC-MS spectrum of *M. senegalensis* combined subfraction (F_{43})

Line No	IUPAC Name	Molecular formula	Molar mass	R.T(S)	% Area	Fragmentation peaks
1	1,1-Dimethoxy acetone	$C_5H_{10}O_3$	118	3.51	20.75	55, 57, (75), 87, 118
2	Methyl-1-cyclopentene-1-carboxylate	$C_7H_{10}O_2$	126	5.60	2.73	51, (67), 95, 111, 126
3	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	21.18	2.85	55, (73), 85, 98, 115, 129, 149, 213, 256
4	13-Hexyloxa cyclo tridec-10-en-2-one	$C_{18}H_{32}O_2$	280	22.69	11.81	55, 67, 81, 96, (98), 123, 137, 166, 280
5	9-Octadecenoic acid (Z)	$C_{18}H_{34}O_2$	282	23.60	10.50	(55), 69, 83, 97, 112, 264
6	12-Hydroxy-9-octadecenoic acid, methyl ester (Z)	$C_{19}H_{36}O_3$	312	24.94	38.05	(55), 69, 74, 96,124, 137, 148, 166, 195, 263, 294
7	12-Hydroxy-9-octadecenoic acid (Z)	$C_{18}H_{34}O_3$	298	25.46	13.31	(55), 69, 83, 97, 124, 137, 148, 166

3.9 Phytoconstituent Identification of *M. senegalensis* Combined Subfraction (F_{43}) by GC-MS

Based on the results of antidiabetic activity, the methanolic fraction of *M. senegalensis* was fractionated using column chromatography to give six combined subfractions namely: (F_{23}), (F_{43}), (F_{50}), (F_{74}) (F_{83}) and (F_{84}). Similarly, based on the results of the phytochemical analysis of the six combined subfractions of *M. senegalensis* above, only combined subfractions (F_{43}) was analysed using GC-MS spectrometer [104]. In present study, seven different compounds were identified with the help of standard library search software and the constituents identified belong to the class of saturated and unsaturated fatty acids, fatty acid esters and ketones family (Table 5). The GC-MS spectrum of the combined fractions (F_{43}) of *M. senegalensis* after fractionation using column chromatography in conformity with TLC analysis revealed the presence of many components namely: Hexadecanoic acid, 9-octadecenoic acid (Z), 12-hydroxy-9-octadecenoic acid (Z), methyl-1-cyclopentene-1-carbxylate, 12-hydroxy-9-octadecenoic acid, methyl ester (Z); 1, 1-dimethoxyacetone and 13-hexyloxacyclotridec-10-en-2-one as shown in Table 5. The major peaks are fatty acids such as hexadecanoic acid, 9-octadecenoic acid (Z) and 12-hydroxy-9-octadecenoic acid (Z) as well as fatty acid derivatives such as methyl-1-cyclopentene-1-carbxylate and 12-hydroxy-9-octadecenoic acid, methyl ester (Z). The fraction also contained 1, 1-dimethoxyacetone and 13-hexyloxacyclotridec-10-en-2-one. The presence of these fatty acids in a considerable amount might serve to recognize the potential pharmacological importance of this plant.

4. CONCLUSION

Phytochemical investigation of *Maytenus senegalensis* revealed the presence of flavonoids, saponins and alkaloids, while the GC-MS analysis identified compounds such as 1, 1-dimethoxyacetone, 13-hexyloxacyclo-tridec-10-ene and 12-hydroxy-9-octadecenoic acid, methyl ester (Z) as the major constituents present. These metabolites are chemical substances known to possess potentials in the management of diabetes. Compounds belonging to these chemical groups are known to be bioactive for the management of diabetes. A significant antidiabetic activity was observed with the root bark methanolic

extract of *M. senegalensis* having the highest activity of 36.67% reduction of blood glucose level in rats. Their classes of compounds were earlier associated with antidiabetic, antimalarial and analgesic activities. This is therefore in support of the traditional use of this plant in Nupe ethnomedicine for management of diabetes in humans. Further studies are required to identify, isolate and spectrally elucidate the active components present in the root bark extract of *M. senegalensis* using NMR analysis.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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