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Phytochemical screening, Gas chromatography: Mass spectrometry and antidiabetic properties of aqueous extract of ginger (*Zingiber officinale*) in Alloxan induced diabetic Wistar rats

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Abstract
The study investigates the phytochemistry, Gas-Chromatography–Mass Spectrometry (GC-MS), atomic Absorbtion spectroscopy (AAS) and antidiabetic activities of aqueous ginger (*Zingiber officinale*) extract in diabetic alloxan-induced Wistar rats. Qualitative phytochemical analysis, GC-MS, AAS and antidiabetic properties of the aqueous ginger extract were determined using standard procedures. Phytochemical analysis of the aqueous ginger extract shows that the extract contains tannins, flavonoids, saponins, alkaloids, simple phenolic, glycosides, carbohydrates, reducing sugar and steroids. The GC-MS study of the aqueous *Zingiber officinale* extract revealed the presence of eleven different compounds with 1,3-Cyclohexadiene, 5-(1,5-dimethy 4-hexenyl)-2-methyl-, [S-(R*,S*)]-been the most abundant with peak area of 33.98%. The AAS analysis shows that ginger contain: Ca, Na, Fe, K, P and Zn minerals. Twenty five (25) Wistar rats were grouped into 5 groups and investigated for antidiabetic study for a period of 15 days. Group A and B animals were normal and negative control respectively. Group B rats were induced with alloxan and not treated with drugs or extract. Animals in other groups (C, D and E rats) were diabetic and treated with standard drug (glibenclamide with concentration of 10 mg/kg), 200 and 400 mg/kg body weight of aqueous ginger extract respectively. The group of rats treated with 10, 200 and 400 mg/kg body weight of glibenclamide and aqueous ginger extract showed significant reduction (p<0.0001) in the level of blood sugar level when compared to group B animals. There were significant reduction (p<0.0001) in plasma total cholesterol, triglyceride, low density lipoprotein-cholesterol and an increase in high density lipoprotein-cholesterol and body weight in the treated rats compared to the untreated group B rats.

Keywords: Antidiabetic, GC-MS, ginger (*Zingiber officinale*), phytochemistry, Wistar rats

Introduction
Zingiber officinale (ginger) is a monocotyledonous plant whose root or rhizome contains important secondary metabolites belonging to the Zingiberacea family and is among one of the well-known spices in the world that is cultivated in many countries and has been added to food substances because of its medicinal importance. It is used as fresh paste; slices preserved in syrup, dried powder and candy or crystallized *Zingiber officinale* used for flavouring tea [1]. Ginger grows in most tropical country of the world including Nigeria. *Zingiber officinale* is the botanical name for ginger. The plant is used as a spice to season foods because of its prominent aroma and flavour. The plant has characteristic flavour and odour because it contains a mixture of Shogaols, Gingerols and Zingerone [2]. *Zingiber officinale* is a good flavouring agent that is widely used for food. Ginger medical or health benefits are essential to pharmaceutical and food processing industries for long. The root of the plant is used as a food ingredient, as well as local herbs to treat various diseases such as diarrhea, stomach ache, gastrointestinal, colds and flu, rheumatic disorders and muscular discomfort [3].
Zingiber officinale is used for the treatment of diarrhea, colic, flatulence, spasm, cold and influenza. *Z. officinale* is used as an appetite stimulant, a narcotic antagonist larvicidal activities and an anti-inflammatory agent. Different research works have showed that ginger has anticancer, antioxidant, anti-hyperglycemic, anti-inflammatory, anti-apoptotic, anti-hyperlipidemic and anti-emetic actions [4, 5].
Diabetes mellitus is a chronic metabolic disorder caused by insulin deficiency or insulin resistance, resulting in an abnormal increase in plasma or serum sugar level. Study has shown that increase in plasma or blood sugar concentration could accelerate protein glycation end products (AGEs) formation [6].

The study investigated the phytochemicals, GC-MS, AAS and antidiabetic properties of aqueous ginger extract in alloxan-induced diabetic Wistar rats.

Materials and Methods

Collection and identification of *Zingiber officinale*

The *Zingiber officinale* was bought from a market in Ikorodu, from Lagos State, Nigeria and was authenticated from Department of Biological Sciences, Lagos State University of Science and Technology.

Preparation of aqueous ginger extract

Aqueous ginger extract was prepared according to the method described by Momoh *et al.,* (2016) [7]. The *Zingiber officinale* were cleaned with water to remove soil on their surfaces. 100 g of the ginger was weighted after removing the outer skin surfaces of the plant and later cut into pieces using sterile scalpel. The pieces of the ginger obtained were blended with 200 ml of distilled water using blender for 5 min. The *Zingiber officinale* homogenized was then filtered using white cloth after centrifuging at 2000 × g for 10 min and the clear supernatant was used for the experiment. The filtered aqueous ginger extract was used for the study within 6 hours of preparation.

Qualitative phytochemical analysis of fresh aqueous ginger extract

Phytochemical analyses for the presence of secondary metabolites in the aqueous ginger extract were carried out using standard phytochemical procedures described by Aderele *at al*., 2020 [8] and Momoh *et al*., 2019 [9].

Gas chromatography–mass spectrometry (GC-MS) Analysis of the aqueous *Zingiber officinale*

Gas chromatography–mass spectrometry **(GC-MS)** analysis of the aqueous ginger was carried out on an Agilent Technology 7890 GC System equipped with a mass spectrometric detector (MSD) using method described by Momoh *et al.,* 2019 [9]. The analysis of the mass spectrum obtained was compared with the database of National Institute Standard and Technique having more than 62,000 patterns. The spectrums of the unidentified compounds were compared with the spectrum of the identified compounds stored in the National Institute Standard and Technique library. The name of the compounds, their molecular weights and structures were identified in the test sample.

Mineral analysis of ginger (*Zingiber officinale*)

2g of ginger was digested with 10 ml of aqua regia (trioxonitrate (v) acid (HNO_3) and hydrochloric acid (HCl) in the ratio 1:3) and the mixture of HNO_3 and HCl was then heated on a crucible until the brown fumes disappeared leaving white fumes. The product obtained was later filtered using filter paper with funnel into beaker. The essential and non-essential elements in the sample were determined using Atomic Absorption Spectrophotometer (Model Perkin Elmer A Analyst 400).

Experimental animals

Twenty five (25) Wistar albino rats with body weight from 175 to 183 g were purchased from the Nigeria Institute of Medical Research (NIMR), situated in Lagos. The rats were acclimatized for two weeks in the animal house at of 23±2 ºC. All the Wistar albino rats were housed in cages in the animal house of Chemical Sciences Department and fed with rodents fed and supply with water *ad-libitum.* The laboratory Wistar rats used in this study were subjected to the rules and regulation of care of laboratory animals (NIH Publication revised, 2011) [10].

Administration of alloxan in the experimental animals

Twenty Wistar albino rats with weight range of 175-183 g were made diabetic by injecting them with alloxan monophosphate (150 mg/kg body weight) intraperitoneally using method described by Momoh *et al.,* 2014 [11]. The developments of diabetes in the experimental animals were confirmed after 72 hours of alloxan administration by using "Accuchek Actie Glucometer" (Roche Diagnostics) and blood sugar test strips. The diabetic rats were later grouped into four groups. Each group contains 5 rats.

Induction of Diabetics

The Wistar rats were fasted overnight and diabetes were induced in the animals by a single intra-peritoneal injection of a freshly prepared solution of alloxan monophosphate (150 mg/kg body weight) in 0.9% sodium chloride solution into 20 of the experimental animals for group B, C, D and E while group A normal control animals were not injected. After seventy two hours, the developments of diabetes were confirmed with animals having high blood sugar level above 500 mg/dl as obtained in the study and they were later taken for ginger treatment.

Grouping of animals

The rats were grouped as follows

Group A animals are normal control rats.

Group B animals are diabetic rats that were not treatment (negative control)

Group C animals are diabetic rats treated with glibenclamide (standard drug) at a dose of 10 mg/kg body weight for 15 days (positive control).

Group D animals are diabetic rats treated with aqueous *Zingiber officinale* extract at a dose of 200 mg/kg body weight for 15 days.

Group E animals are diabetic animals treated with aqueous *Zingiber officinale* extract at a dose of 400 mg/kg body weight for 15 days.

Measurement of body weight

The body weights of the animals were measured daily using weighing balance. The weight values were compared statistically using Graph pad prism 5.01.

Collection of blood samples for plasma preparation

The animals of all the different groups were sacrificed after twenty four hours of fasting. The whole blood cell were collected from the animals in the different groups by ocular puncture into different heparinised tubes and later centrifuge at 3000 rpm for twenty minutes and the plasma stored at -20 °C to estimate lipid profile level.

Determination of plasma lipid profiles

The plasma HDL-Cholesterol (HDL-Chol), Triglyceride (TG) and Total cholesterol (TG) were determined using Randox laboratory diagnostic kit. The low density lipoprotein-Cholesterol. (LDL-C) was calculated using formula from Momoh *et al*. (2021) [12].

LDL-C=TC- HDL-C –TG/5. Momoh *et al*. (2021) [12].

Data Analysis

The data were shown as mean±SD in triplicate. The significant differences between tested groups (A, B, C, D and E) were analyzed using one-way analysis of variance Post Hoc tests for the comparisons between tested groups. A $p< 0.05$ was considered statistically significant.

Results

Table 1: The qualitative phytochemical constituents of aqueous ginger extract

Phytochemical constituent	Test performed	Inference
Tannins	Ferric chloride test	+
Flavonoids	Lead Acetate test	+
Saponins	Froth test	+
Alkaloids	Mayer`s test	+
	Wagner`s test	+
Simple phenolics	Ferric Chloride test	+
Protein and amino acids	Biuret test	-
Glycosides	Liebermann's test	+
Reducing sugar	Fehling`s test	+
Carbohydrate	Molisch`s test	+
Steroids	Salkowski test	+

+ represent presence of constituent, while – represent absence of constituent

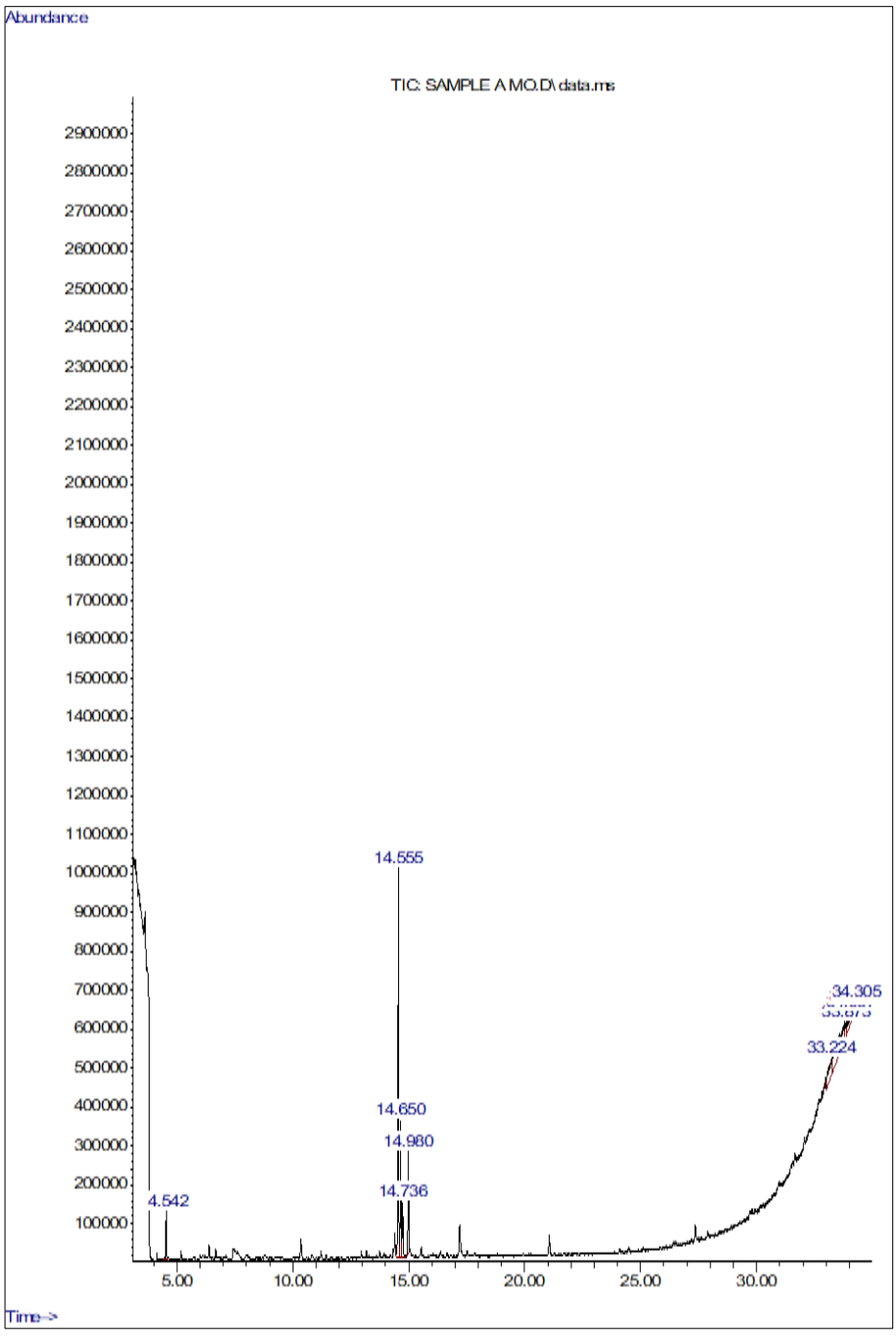

Fig 1: GC-MS Chromatogram of aqueous ginger extract

Table 2: Compound identified in the aqueous ginger extract analysed by GC-MS

SN	Retention Time	Name of the compound	Molecular Formulae	Molecular Weight (g/mol)	Peak Area (%)	Ref#	CAS#	Activity
1	4.540	Propane, 1-chloro-2-nitro-	$C_3H_6ClNO_2$	123.538	3.13	10265	002425-66-3	NF
2	14.554	1,3-Cyclohexadiene, 5-(1,5-dimethy 4-hexenyl)-2-methyl-, [S-(R*,S*)]-	$C_{15}H_{24}$	204.35	33.98	68761	000495-60-3	Responsible for the biological activities of ginger [4, 5].
3	14.651	Cyclopropene, 1-methyl-3-(2-methyl cyclopropyl)-	C_8H_{12}	108.18	11.88	5607	061142-26-5	NF
4	14.737	Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano	$C_{20}H_{27}NO_2$	313.4	6.59	172241	1000294-64-4	NF
5	14.983	(E)-.beta.-Famesene	$C_{15}H_{24}$	204.3511	9.18	68594	018794-84-8	NF
6	33.225	3-Bromo-N-(3,5dichlorophenyl)-benzamide, TMS derivative	C16H16BrCl2NOSi	417.20	8.55	245132	1000331-99-9	NF
7	33.791	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis-[(trimethylsilyl)oxy]-	$C_{12}H_{36}O_4Si_5$	384.8393	18.97	229156	003555-47-3	Antioxidant activity [13].
8	33.871	2,5-Dihydroxybenzoic acid, 3TMS derivative	$C_{16}H_{30}O_4Si_3$	370.6635	2.36	220199	003618-20-0	NF
9	34.060	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	741. 5394	3.09	275509	018772-36-6	NF
10	34.209	3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)-trisiloxane	$C_{12}H_{34}O_4Si_4$	354.74	0.85	208113	072182-11-7	NF
11	34.306	1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethylheptasiloxane	$C_{14}H_{44}O_6Si_7$	505.094	0.42	250650	055319-93-2	NF

NF stands for not found

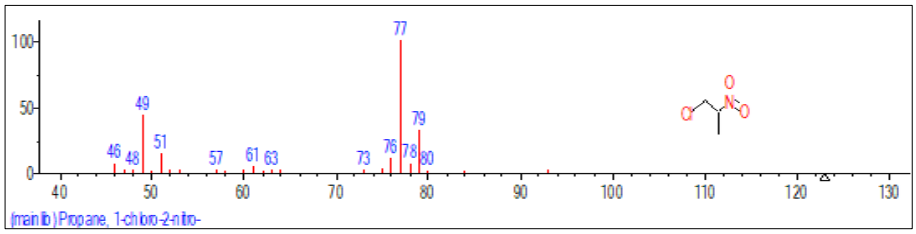

Fig 2a: Mass spectrum of Propane, 1-chloro-2-nitro- (RT 4.540, 3.13%)

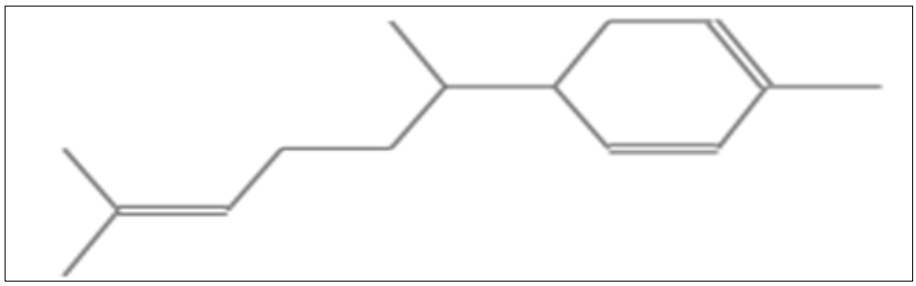

Fig 2b: Structure of 1, 3-Cyclohexadiene, 5-(1, 5-dimethy 4-hexenyl)-2-methyl-, [S-(R*,S*)]-(RT14.554, 33.98%, RT 24.018)

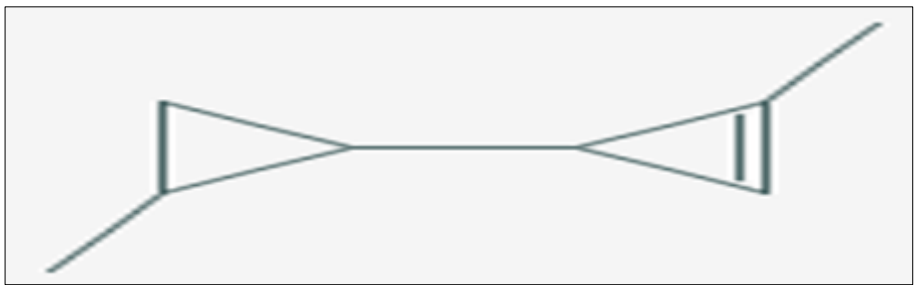

Fig 2c: Structure of Cyclopropene, 1-methyl-3-(2-methyl cyclopropyl)- (RT 14.651, 11.88%)

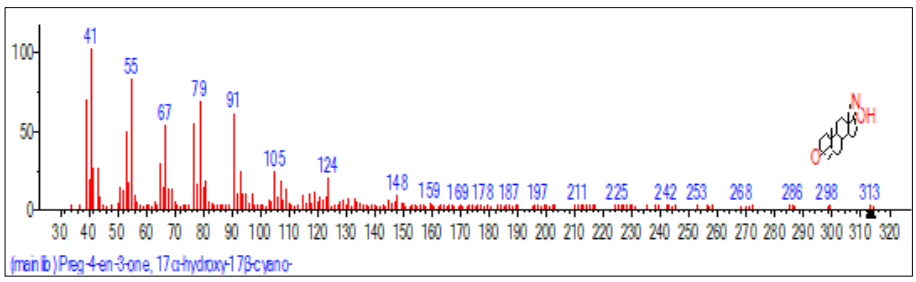

Fig 2d: Mass spectrum of Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano (RT14.737, 6.59%)

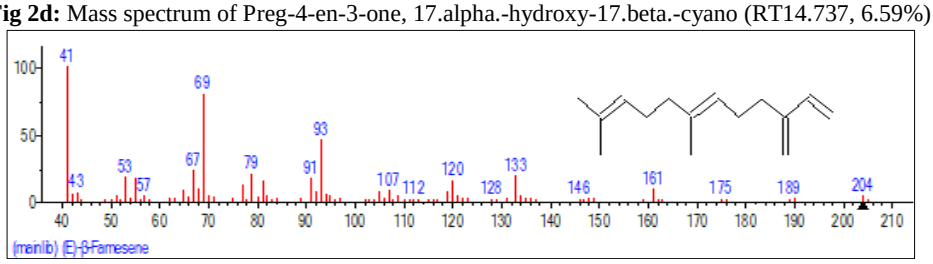

Fig 2e: Mass spectrum of (E)-.beta.-Famesene (RT14.983, 9.18%)

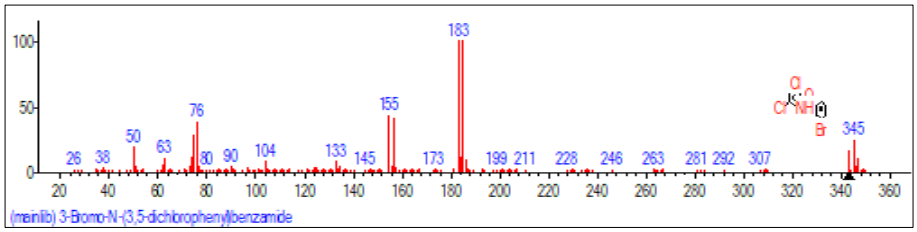

Fig 2f: Mass spectrum of 3-Bromo-N-(3,5dichlorophenyl) –benzamide (RT 33.225, 8.55%

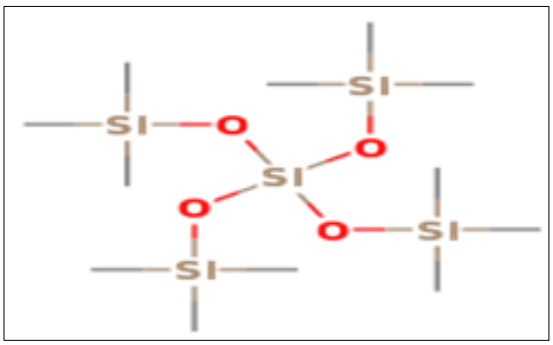

Fig 2g: Structure of Trisiloxane, 1, 1, 1, 5, 5, 5-hexamethyl-3,3-bis-[(trimethylsilyl) oxy]- (RT33.971, 18.97%)

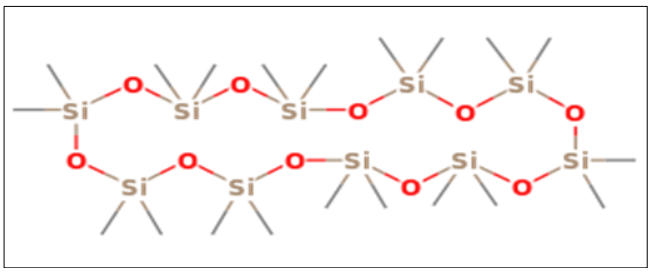

Fig 2i: Structure of Cyclodecasiloxane, eicosamethyl- (RT34.060, 3.09%)

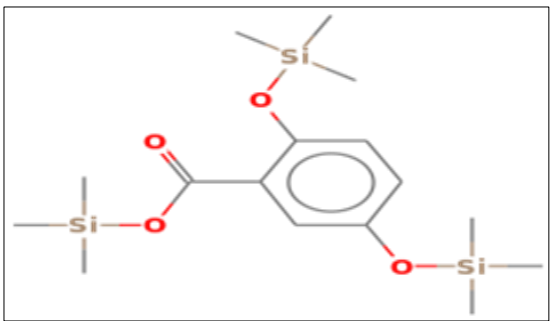

Fig 2h: Structure of 2,5-Dihydroxybenzoic acid, 3TMS derivative (RT33.871, 2.36%)

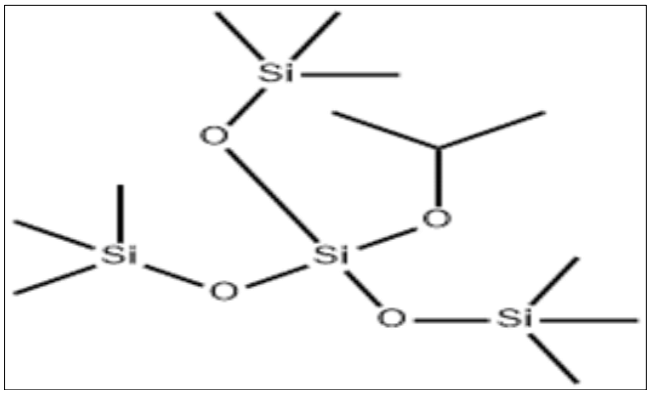

Fig 2j: Structure of 3-Isopropoxy-1, 1, 1, 5, 5, 5-hexamethyl-3-(trimethylsiloxy)-trisiloxane (RT34.209, 0.85%)

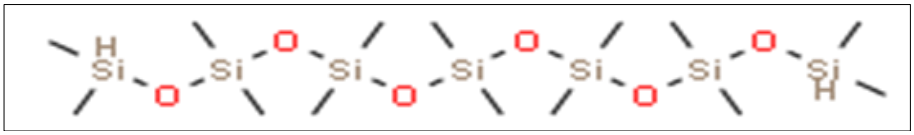

Fig 2k: Structure of 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13-tetradecamethylheptasiloxane (RT34.306, 0.42%)

Fig 2: Mass spectrum and structure of eleven different compounds obtained during GC-MS analysis with their peak area and retention time

Table 3: Mineral composition of ginger (*Zingiber officinale*)

Minerals	Concentration in mg/100g
Calcium	14.89 ± 0.06
Sodium	9.07 ± 0.19
Iron	1.05 ± 0.12
Potassium	15.45 ± 0.76
Phosphorous	19.19 ± 0.34
Zinc	0.23 ± 0.01

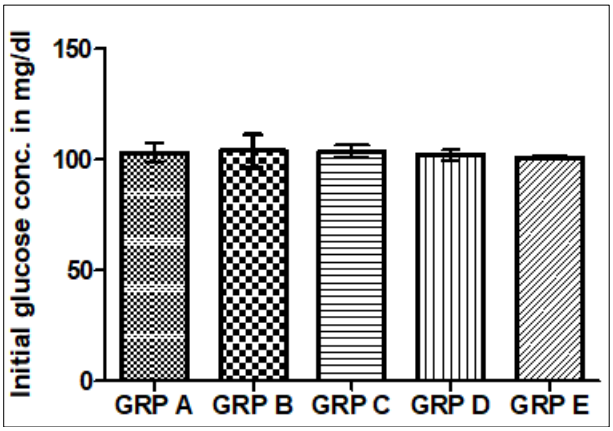

Fig 3: Initial blood glucose concentration values for group A, B and C rats and animals treated with 200 and 400 mglkg body weight of aqueous extract of ginger

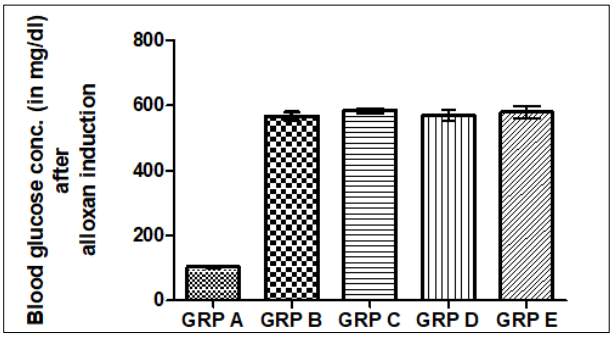

Fig 4: Blood glucose concentration values in mildly after alloxan induction for all the experimental groups. Only group A animals were not induced with alloxan

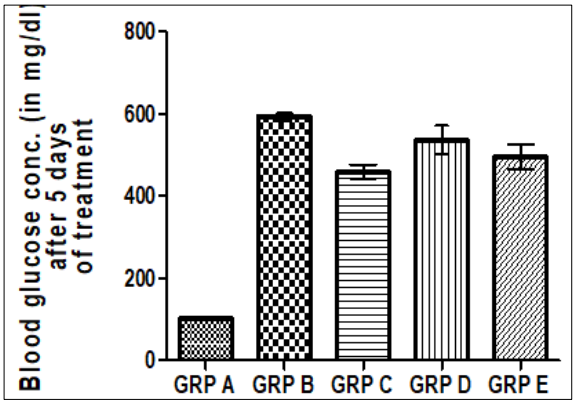

Fig 5: Blood glucose concentration after 5 days of treatment for all the experimental groups

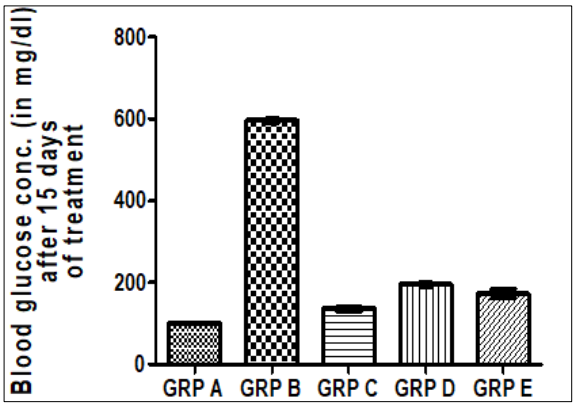

Fig 6: Blood glucose concentration after 15 days of treatment for all the experimental groups

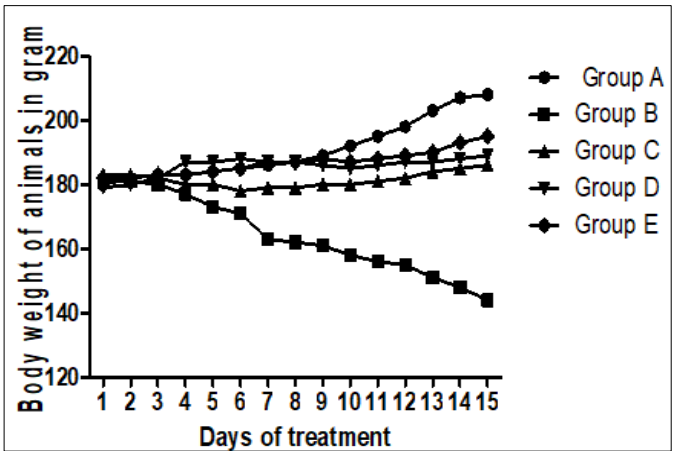

Fig 7: Body weight of animals after treatment with glibenclamide, 200 and 400 mg/kg B.W of aqueous ginger extract

Table 4: The effect of *Zingiber officinale* (ginger) extract on lipid profiles in alloxan–induced diabetic rats

Parameters	Group A	Group B	Group C	Group D	Group E
Total Cholesterol (mg/dl)	97.36 ±4.28^{c}	158.09±7.77^{a}	144.31±6.18^{b}	145.02 ±6.17^{b}	147.32±5.06^{ab}
Triglyceride (mg/dl)	78.09±3.47^{b}	103.17±6.22^{a}	83.63±5.39^{b}	82.67 ±3.28^{b}	81.43 ±4.78^{b}
High-density lipoprotein (mg/dl)	48.53 ±2.34^{b}	20.88 ±2.28^{d}	41.84 ±2.74^{c}	49.95±4.37^{b}	61.43 ±4.53^{a}
Low-density lipoprotein (mg/dl)	33.21±2.73^{d}	116.58±5.53^{a}	85.74±3.18^{b}	78.54±3.94^{b}	69.60±3.82^{c}

Discussion

The phytochemical analysis of aqueous *Zingiber officinale* (ginger) extract showed that the plant contains phenolic compounds, glycosides, alkaloids, flavonoids, steroids, tannin, saponins, reducing sugar, carbohydrates, while amino acids and proteins were absence in the extract. Different studies have shown the occurrence of some of these phytochemicals in the extract of ginger [14, 15]. These phytochemicals possess numerous medicinal functions: Saponin has hypoglycemic activity [16]. Study has shown that secondary metabolite like saponins that are produced by plants are natural antibiotics that act as a deference

mechanism to stop the attacks of foreign pathogens [17]. Saponins have cholesterol lowering properties and can kill or inhibit cancer cells [17, 18-20]. The presence of saponin in the ginger maybe responsible for the reduction of cholesterol in the experimental animals studied. Favonoids demonstrate significant anti-inflammatory [21], hypoglycemic and anti-diabetic properties [22], antioxidant and free radical-scavenging activities [23, 24]. Study has shown that tannins may accounted for the sharp taste of both pepper and ginger and will hasten the healing of inflamed mucous membranes and wounds [17].

From Gas chromatography–mass spectrometry analyses of aqueous extract ginger, it was observed that the extract of ginger has 11 compounds. The chromatogram of aqueous extract of ginger shows 2 significant peaks (Figure 1). 1, 3-Cyclohexadiene, 5-(1,5-dimethy l-4-hexenyl)-2-methyl-, [S-(R*,S*)]- have retention time (RT) of 14.554 with the highest peak area of 33.98% among all the compounds detected in the aqueous extract of ginger. The relative molecular weight and chemical structure of the most abundant compound of the aqueous extract of ginger has been shown in Figure 2 above. 1,3-Cyclohexadiene, 5-(1,5-dimethy 4-hexenyl)-2-methyl-, [S-(R*,S*)]- is also called (-)-Zingiberene and is found in ginger. *Zingiber officinale* contains essential oil fragrant of about 3% whose main components are sesquiterpenoids compounds, with (-)-zingiberene compound been the main constituents. It contains little amounts of other sesquiterpenoids compounds (β-sesquiphellandrene, bisabolene and farnesene) and a small fraction of monoterpenoid compounds (β-phelladrene, cineol, and citral) have been identified in the plant [4]. Some of the properties of ginger have been confirmed in literature, these include essential oil, zingiberene, zingiberone, zingiberol and pungent compounds like: [6]-shogaol and [6]-gingerol [5]. Phytochemical analysis reports have shown that the main components of ginger are zingerone, paradol, shogaols and gingerols [5]. The pungent non-volatile ingredients from ginger include gingerol. shogaol and zingerone. These components are known to have the ability to suppress inflammation, has high rate of proliferative, and transformation processes of carcinogenesis [5]. The second major components of the extract with peak area of 18.97% and retention time of 33.791 was 1,1,1,5,5,5-hexamethyl-3,3 bis[trimethylsilyl)oxy] trisiloxane compound which is an oxygenated diterpenes and possess antioxidant properties [13].

The basic functions of minerals elements are: they help to maintain acid-base balance and in the maintenance of body fluids, in the movement of gases between tissues in the body and also in muscle contractions.

The result of this experiment shows that phosphorous is the most abundant element followed by potassium and calcium (Table 3). Magnesium, phosphorus and calcium are used for the formation of strong bone and teeth [18]. Magnesium is a risk factor for ischemic heart disease, hypertension, atherogenesis, cardiac arrhythmias and sudden cardiac death [25]. Calcium ions help in converting prothrombin to thrombin during blood coagulation and are also involve in milk clotting. Calcium helps in enzyme activation and activates numerous enzymes activities. Examples of such enzymes are: succinic dehydrogenase, lipase**,** adenosine triphosphatase. Phosphorus helps in the following functions: It functions as a constituent of teeth, bones, phosphorylated metabolic intermediates, nucleic acids and adenosine triphosphate (ATP). Phosphorus plays buffering roles. High dietary intake of sodium is responsible for rising blood pressure, cardiovascular and renal disorders in human. Hence, high dietary sodium intake should be prevented in human diets and in patients who suffer from hypertension. Chromium and Zinc elements are used as cofactors for insulin productions [26].

This research work studies the effect of aqueous *Zingiber officinale* on blood sugar level, body weight and lipid profile in alloxan-induced diabetic rats. Figure 3 shows the initial blood sugar concentration of all animals before induction and this shows that all the rats used for the experiment are healthy animals ($p>0.05$). Figure 4 shows the level of blood sugar concentration of all the experimental animals after induction. Group A rats were not induced with alloxan and they are all healthy animals. The result obtained showed that all the rats from group B–E were diabetic after inducing them with 150 mg/dl alloxan. Momoh *et al*., (2014) [11] study shows that alloxan compound induce diabetes in experimental animal by damaging the insulin secreting cells of the pancreas leading to high blood sugar. Figure 5 and 6 show that the standard drug and aqueous extract of *Z. officinale* has hypoglycemic properties because there were significant ($p<0.0001$) reduction of blood sugar concentration of animals in group C to E compared to group B animals after periods of five (5) and fifteen (15) days of treatment. The study has shown that ginger reduces blood glucose level in diabetic animals and could protect against diabetes mellitus and its complications. Sampath *et al*., 2017 study elucidate the antidiabetic properties of ginger and its constituents [27]. The consumption of aqueous *Z. officinale* reduces the concentration of blood glucose level, glycated hemoglobin A, triglyceride and total cholesterol in diabetic mellitus type 2 patients [28]. The hypoglycemic properties of aqueous *Z. officinale* maybe due to their potential to improve the function of pancreatic cell damage by helping in the production of more insulin. Hence, the use of the plant in the treatment of diabetic may have a positive effect on protecting beta-cells and reducing high blood glucose concentration.

As shown in figure 7, the induction of diabetes in animals, prevented increase in the body weight of the diabetic animals compared to the weight gain found in group A rats ($p<0.05$). The loss in body weight of the diabetic rats have been shown to be caused by increased in the blood sugar that resulted in gradual lack of sugar concentration in the cells; forcing, the cells in the body to use fatty acids and amino acids as an alternate source of energy for the body which eventually leads to the reduction of body fats and proteins which causes weight loss [29]. The study shows that the extract causes increase ($p<0.05$) in the body weight of the Wistar rats after treatment with the aqueous ginger extract when compared to group B animals.

There were significant ($p<0.0001$) increase in TG, LDL-C, TC and a decrease in HDL-C levels for group B rats compared to other rats in other groups. This an indication that treatment with ginger reduces TC, LDL-C, TG concentration and increases HDL-C level in diabetic animals and may be used for the reduction of dyslipidemia in diabetic patients. Arablou *et al.*, 2014 [30] study shows that ginger reduces fasting plasma glucose, total cholesterol, triglyceride, CRP and prostaglandin-E_2 (PGE_2) significantly in patients with type 2 diabetes mellitus when compared with placebo group ($p<0.05$). They also showed that *Z. officinale* is effective in the prevention of diabetes complications. Another study shows that low concentration of *Z. officinale* at 50 mg/kg have no effect on the reduction of serum cholesterol levels and thromboxane-B_2 levels, however oral administration leads to significant changes in the serum PGE_2. High intake of *Z.*

officinale with concentration of 500 mg/kg body weight lowers serum cholesterol and PGE_2 level [31]. The results of this work suggest that *Zingiber officinale* may be a good source of cholesterol-lowering agent. The diabetic experimental Wistar rats treated with two hundred (200) and four hundred (400) mg/kg body weight of ginger aqueous extract had significant ($p<0.05$) increase in high density lipoprotein-Cholesterol level, decrease in low density lipoprotein-Cholesterol level (group E only) and no difference ($p>0.05$) were observed for TC and TG levels when compared with rats treated with glibenclamide.

Conclusion

This study shows that aqueous ginger (*Zingiber officinale*) extract possess important secondary metabolites, minerals, hypoglycemic and antihyperlipidemic effects in alloxan-induce diabetic Wistar rats.

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