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Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
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EVALUATION OF ANTI-HYPERGLYCEMIC EFFECT OF AQUEOUS LEAF EXTRACT OF *ANISOPUS MANNII* (N.E BR) IN ALLOXAN-INDUCED DIABETIC MICE

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ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both.
Aim: The research aimed at evaluating the anti-hyperglycemic effect of aqueous leaf extract of *Anisopus mannii* alone and in combination with metformin in alloxan-induced diabetic mice.
Methodology: Phytochemical screening of the extract was carried out according to the methods of Trease and Evans. The LD_{50} of the extract was determined using OECD Guideline 425. The anti-hyperglycemic effect of the extract was evaluated using the method of Goldener and Gomori. Hyperglycemia was induced by the injection of 150mg/kg of 0.5% alloxan monohydrate in mice. The study was conducted on seven groups of six rats each (n =6). Group I rats were non-diabetic (administered distilled water); while alloxan-induced hyperglycemic rats were assigned accordingly to the remaining six groups. Group II rats served as diabetic control and were administered normal saline (1 mL/kg). Groups III, IV and V were hyperglycemic rats treated with *Anisopus manni* extract at doses of 250, 500 and 1000 mg/kg respectively. Group VI (positive control) were hyperglycemic rats treated with metformin (250 mg/kg). We then added Group VII to observe the anti-hyperglycemic effect of this extract in combination with metformin. The doses were administered orally daily to the various groups for 28 days. Blood glucose levels, food and water consumption were taken for each Group at the end of Day 7, 14, 21 and 28 of the experiment.
Results: The extract contained alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponin, tannins, terpenes and steroids. Anthraquinones were absent. The LD_{50} of the extract was estimated to be greater than 5000 mg/kg. There were significant ($p \leq 0.05$) dose-dependent reductions in blood glucose levels in the extract treated groups when compared to diabetic control group. There were also significant reductions ($p \leq 0.05$) in food and water intake in the treatment groups when compared to the diabetic control group.
Conclusion: Aqueous leaf extract of *Anisopus manni* possess significant anti-hyperglycemic effect in alloxan-induced diabetic mice supporting its traditional use in treatment of diabetes mellitus. Further studies are recommended for isolation and characterization of bioactive compounds responsible for the anti-hyperglycemic activity as well as the possible mechanism(s) of action.
Keywords: Anti-hyperglycemic, *Anisopus mannii*, Alloxan-induced, Diabetic mice

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
APSBJournal

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease that occur either due to decrease in insulin secretion or lack of insulin peripheral activity[1] and causes approximately half of all deaths occurring at the age of 70 years. It has been reported that over 580 million adults aged between 20 and 79 years were diabetic in 2024 with a projected rise to about 850 million by 2050 [2]. The main clinical symptoms related to diabetes include polydypsia (intense thirst), polyuria, polyphagia, weight loss or gain, weakness, dry skin and blurred vision [3]. If left untreated, the disease can lead to various health complications, including disorders of the cardiovascular system, eye, kidney, and nerves, gluconeogenesis and ketogenesis[4,5], increased risk of heart attacks and strokes [6]. There are two main types of Diabetes Mellitus; Type I Diabetes Mellitus (T1DM) and Type II diabetes Mellitus (T2DM). T1DM is also known as Insulin Dependent Diabetes Mellitus (IDDM) or Juvenile onset Diabetes mellitus, which occurs due to pancreatic β-cell destruction, and is mostly characterized by lack of insulin secretion or insufficient production of insulin [7]. It is a long-term autoimmune condition in which the pancreas is unable to produce insulin, a hormone that helps regulate blood glucose levels. The pancreas produces less insulin over time, eventually ceasing to produce any at all. In this type of diabetes the immune system malfunctions, destroying β-cells, which are responsible for producing insulin. This damage to the pancreas can be caused by infection or other external factors. Administration of Insulin is necessary for the survival of individuals with this condition, as high blood glucose levels can cause long-term damage to internal organs [8]. T2DM is characterized by inadequate insulin secretion or insulin resistance whereby pancreatic β-cells do not actively take up glucose leading to hyperglycemia. It is triggered by mutation in the pancreatic β-cell gene or changes in glucokinase and HNF-1 alpha genes [9,10]. T2DM is the most common form of adulthood onset diabetes. Gestational diabetes is another type of diabetes mellitus that occurs in pregnant women [11]. Gestational diabetes mellitus (GDM) is a state of hyperglycemia (fasting plasma glucose ≥ 5.1 mmol/L, 1 h ≥ 10 mmol/L, 2 h ≥ 8.5 mmol/L during a 75 g oral glucose tolerance test according to WHO criteria that is first diagnosed during pregnancy [12]. GDM is one of the most common medical complications of pregnancy, and its inadequate treatment can lead to serious adverse health effects for the mother and child [13]. According to the latest estimates of the International Diabetes Federation (IDF), GDM affects approximately 14.0% of pregnancies worldwide, representing approximately 20 million births annually [14]. Mothers with GDM are at risk of developing gestational hypertension, pre-eclampsia and termination of pregnancy via cesarean section [15]. In addition, GDM increases the risk of complications, including cardiovascular disease, obesity, and impaired carbohydrate metabolism, leading to the development of T2DM in both mother and infant [16]. The increase in the incidence of GDM also leads to a significant economic burden and deserves greater attention and awareness [17]. Despite numerous studies, the pathogenesis of GDM remains unclear, and the results obtained so far indicate a complex mechanism of interaction of many genetic, metabolic and environmental factors [18].

Conventional drugs are effective in the management of diabetes to some extent, but are far from being satisfactory because of

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
APSBJournal

their high costs, undesirable side effects and poor disease prognosis [19,20]. Thus, searching alternative anti-diabetic natural products from plants has received great attention.

Traditional medicinal plants have been used from time immemorial for treating diabetes in many countries like China, India, Southeast Asia, and African countries [21,22]. Plant-based medicine acts not only as a pharmacological approach but also considered a plant-based diet as veganism to treat many human ailments [21-23]. More than 800 plants have been reported to possess hypoglycemic effects and many of the plants are highly significant with antidiabetic properties which make them scrutinized for antidiabetic molecule isolation [24].

Anisopus manni (*A. manni*) is a perennial herbaceous shrub and belongs to the family Apocynaceae. The species is native to Africa, especially prominent in the central and western tropical regions. It spans the modern-day countries of Cameroon, Central African Republic, Democratic Republic of the Congo, Equatorial Guinea, Gabon, Ghana, Ivory Coast, Liberia, Nigeria, and Senegal. Species in the genus Anisopus are generally found in closed rain forest environments [25]. *Anisopus mannii* has been used in treatment of diabetes mellitus in humans as herbal medicine in different localities [26] but scientific evidence to validate this claim is scanty. A recent study shows its efficacy compared to glibenclamide [27]. In northern Nigeria, *A. mannii* is commonly used to treat elevated blood sugar levels. The plant is known as "kashe zaki" in Hausa, which means "sweet-killer" or sometimes Sakayau [28]. The aim of this study was to evaluate the anti-hyperyglycemic effect of the aqueous leaf extract of *A. mannii* in alloxan-induced diabetic mice.

MATERIALS AND METHOD

Experimental Animals

Albino mice (19-25g) of both sexes were acquired from the Animal House Facility of the Department of Pharmacology and Toxicology, Kaduna State University, Kaduna. Nigeria. The animals were kept in clean, dry cages and maintained under ambient conditions of temperature and humidity and 12h/12h light/darkness cycle in the Animal House. Standard feed (Vital Feed, Jos) and water *ad libitum* were provided for the animals, except when fasting was necessary in the course of the study.

The mice were acclimatized for five days in the laboratory prior to commencement of the experiment. The animals were handled in accordance with the National Institute of Health (NIH) Guidelines for the care and use of laboratory animals (Production no. 85-23, revised 1996). All efforts were made to minimize the number of animals used for the study and their suffering. Only the minimum numbers of animals necessary to produce reliable scientific data were used.

Drugs, Chemicals and Equipment

Alloxan monohydrate (Sigma-Aldrich, Germany), Metformin hydrochloride (Emzor Pharmaceuticals Ltd, Nigeria.), 0.9% Normal saline (Dana Drugs limited, Nigeria), D glucose, Dragendoff's reagent (BDH, Poole Ltd, U.K), Concentrated Sulphuric acid, Concentrated Hydrochloric acid, Lead sub-acetate solution, Chloroform, 10% Ammonium solution, Glacial Acetic Acid containing traces of ferric chloride, 10% Sodium hydroxide, Ferric chloride solution, Acetic anhydride, 1% aqueous hydrochloric acid, Molisch's reagent (BDH, Poole Ltd, U.K), Wagner's reagent (BDH, Poole Ltd, U.K), Meyer's reagent (BDH, Poole Ltd, U.K), Sterile Syringes and Needles, Mixer,

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
APSBJournal

Whatman Filter Paper No 1, Stopwatch, Digital Weighing Balance, Animal Cages, Markers, Rotary Evaporator (Searchtech Searchtech Instruments, England. RE 52-3), Water Bath (Model DK-420, No L-606382), Glucometer (Accu-Chek).

Ethical Approval

The experiments were carried out in accordance with the National Institute of Health (NIH) Guidelines for the care and use of laboratory animals (Production no. 85-23, revised 1996).

Collection and Identification of Plant Material

Anisopus manni leaves were collected in May, 2024 at Buruku farmland in the Chikun Local Government Area of Kaduna State, Nigeria. The plant was identified and authenticated by Mallam Umar S. Gallah, a taxonomist in the Department of Biological Sciences, Kaduna State University, Kaduna, and it was assigned a voucher specimen number of KASU/BSH/1102.

Preparation of *Anisopus mannii* aqueous leaf extract

Anisopus mannii leaves were air-dried under shade in the laboratory until a constant weight was achieved. The dried leaves were size reduced using a wooden pestle and mortar, and then ground into fine powder using an electrical mixer (Binatone). Then, 120g of *A. mannii* powder was weighed and macerated with 500 mL of water for 48 hours, with periodic shaking. The mixture was filtered using a Muslin cloth followed by Whatman filter paper No 1 and then dried on a water bath at a temperature of 60-70^{o}C. The dried extract was transferred into an air-tight container, labeled as *A. mannii* aqueous leaf extract and stored until required.

Percentage yield of the extract was calculated as follows;

$$\% \text{ Yield} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of powdered plant material (g)}} \times 100$$

Acute toxicity study (LD_{50} Determination) of *Anisopus mannii* aqueous leaf extract

The acute toxicity study of the aqueous leaf extract of *A. mannii* was carried out using OECD Procedure [29]. Female nulliparous and non-pregnant mice were used and their weights fell within the interval of ± 20% of the mean weight of the sample population obtained. The mice were housed individually in plastic cages in the laboratory at ambient temperature and humidity and 12 hours light and 12 hours darkness. The mice were fed with standard feed (Vital Feeds, Jos) and water *ad libitum*. The mice were kept in their cages for at least 5 days prior to dosing to allow for acclimatization to laboratory conditions.

The mice were deprived of food for 3-4 hours prior to dosing, but they were given water *ad libitum*. The mice were then weighed and the extract was administered orally in a single dose according to the body weight obtained after fasting. After the extract was administered, food (and not water) was withheld for another 1-2 hours. Limit test dose of 5000 mg/kg body weight was used in the experiment.

One mouse was administered a single dose 5000 mg/kg of the extract orally using an oral feeding cannula. After administration of the extract, food (and not water) was withheld for 1-2 hours. The mouse was observed for any signs of toxicity such as sedation, vomiting, inactivity/hyperactivity, diarrhoea, piloerection, or mortality in the first 4 hours and over a period of 24 hours. There was no

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
APSBJournal

mortality recorded after 24 hours, and two additional mice were dosed with the extract at 5000 mg/kg body weight orally. The two mice were observed for any signs of toxicity or mortality for the first 4 hours and over the period of 24 hours. Absence of mortality led to the termination of the Limit Test and all the three treated mice were further observed for signs and symptoms of toxicity or mortality for 14 days without further administration of the extract.

Qualitative Phytochemical Screening of *Anisopus mannii* aqueous leaf extract

Qualitative phytochemical screening of the aqueous leaf extract of *A. mannii* was carried out in the Department of Pharmacognosy and Drug Development, Kaduna State University, Kaduna. Nigeria. The standard methods of Trease and Evans [30] were used to screen for the presence or absence of alkaloids, flavonoids, saponins, tannins, glycosides, carbohydrates, steroids/triterpenes and anthraquinones.

Anti-hyperglycemic effect of *Anisopus mannii* aqueous leaf extract in alloxan-induced diabetic mice

Induction of hyperglycemia

Hyperglycemia was induced by adopting the method described by Goldener and Gomori[31]. Sixty (60) mice of both sexes (weight 19-25 g) were injected intraperitoneally with 150 mg/kg of 0.5% alloxan monohydrate dissolved in 10 mM sodium citrate (pH 4.5) [32]. One hour after administration of alloxan, the mice were treated with 5% glucose for 24 hours to prevent fatal hypoglycemia that may occur due to massive pancreatic release of insulin. The mice were then given access to food and water and observed for 72 hours for signs of hyperglycemia. Thereafter, they were examined for hyperglycemia using one-touch glucometer (Accu-Chek® Active, Mannheim, Germany) with compatible strips, which adhere to the glucose oxidase principle [33]. This was achieved by pricking the tail tip of each mouse and squeezing its tail to obtain a drop of blood. Mice with fasting blood glucose >200 mg/dL (11.1 mmol/L) were selected and used for the study.

Experimental Design

The study was conducted on seven groups (19-25 g) of six mice each ($n=6$). Group I mice were normal mice/Non-diabetic control (administered distilled water, 1 mL/kg); while alloxan-induced hyperglycemic mice were assigned accordingly to the remaining six groups. Group II mice served as hyperglycemic control/diabetic control and were administered normal saline (1 mL/kg). Groups III, IV and V were hyperglycemic mice treated with *A. mannii* leaf extract (AMLE) at doses of 250, 500 and 1000 mg/kg respectively. Group VI (positive control) were hyperglycemic mice treated with metformin (250 mg/kg) and Group VII were hyperglycemic mice treated with a combination of AMLE (250 mg/kg) and metformin (250 mg/kg). All treatments were given orally for 28 days. The blood glucose levels were taken at Day 7, 14, 21 and 28[34].

Statistical Analysis

Data were expressed as Means ± Standard Error of Mean (Mean ± SEM). Differences between Means were analyzed using One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. SPSS Version 20.0 (2011) was used as the statistical software package. Differences in Means with values of $p \leq 0.05$ were considered statistically

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
APSBJournal

significant. The results were presented as Tables.

RESULTS

Percentage Yield of Extract

Percentage yield of the extract was calculated to be 26.5% as shown below:

Weight of dried extract (g)/Weight of Powdered plant material (g) X 100

= 26.5/100 X 100 = 26.5%

Qualitative phytochemical screening of *Anisopus mannii* aqueous leaf extract

Qualitative phytochemical screening of the aqueous leaf extract of *A. mannii* indicated the presence of carbohydrates, flavonoids, saponins, tannins, steroids/terpenes, alkaloids and cardiac glycosides. Anthraquinones were absent (Table 1).

Table 1: Phytochemical Constituents of the Aqueous Leaf Extract of *Anisopus mannii*

Constituents	Test	Observation	Inference
Carbohydrates	(Molisch's test)	Reddish coloured ring at the interphase	+
Flavonoids	(Ferric chloride test)	Greenish precipitation	+
Saponins	(Frothing test)	Occurrence of a honeycomb froth	+
Alkaloids	(Mayer's test)	Formation of cream coloured precipitate	+
Terpenes/steroids	(Libermann-Burchard's test)	Reddish-pink or brown ring at the interphase and a bluish-green or violet upper layer	+
Tannins	Lead sub acetate test	Cream coloured precipitate	+
Cardiac glycosides	Keller killian's test	Brown ring at the interphase and a pale green upper layer	+
Anthraquinones	(Bontrager's test)	No formation of bright pink color at the upper layer	-

Key: + = Present - = Absent

Acute toxicity study (LD_{50} determination) of *Anisopus mannii* aqueous leaf extract

The extract was well tolerated by the mice at an oral dose of 5000 mg/kg in the first and second phases of the test as there were no signs and symptoms of toxicity and no deaths were recorded. The LD_{50} was estimated to be greater than 5000 mg/kg.

Anti-hyperglycemic Effect of *Anisopus mannii* aqueous leaf extract in alloxan-induced diabetic mice

The anti-hyperglycemic effect of AMLE in alloxan-induced diabetic mice revealed a progressive decrease in blood glucose levels over 28 days, with the most significant reduction observed in the group treated with AMLE 1000 mg/kg when compared with the diabetic control group (Alloxan +Normal saline). The reduction in blood glucose level

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
APSBJournal

in the group of mice that received AMLE 1000 mg/kg was also significantly greater than that observed in the group of mice that received metformin 250 mg/kg and AMLE 250 mg/kg combination ($P \leq 0.05$) (Table 2).

Table 2: Anti-hyperglycemic effect of *Anisopus mannii* N.E Br. aqueous leaf extract in alloxan-induced diabetic mice

Treatment (mg/kg)	Mean Blood Glucose Levels (mmol/L)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Non-diabetic control	4.64±1.2	5.21±1.9	4.79±2.1	5.51±3.1	5.68±2.6
Diabetic control	16.43±2.91^{a}	19.15±7.24^{a}	21.61±2.88^{a}	24.56±3.87^{c}	23.01±4.09^{c}
AMLE(250)	17.91±3.25^{a}	18.06±5.00^{a}	16.91±4.25^{a}	16.63±4.23^{a}	14.85±4.22^{b}
AMLE(500)	20.36±8.51^{a}	22.35±8.57^{a}	20.85±7.87^{a}	17.46±7.90^{b}	15.80±7.37^{b}
AMLE(1000)	23.03±7.43^{a}	21.93±7.47^{a}	18.80±6.07^{a}	16.20±4.65^{a}	13.11±4.37^{a}
Metformin(250)	19.66±8.22^{a}	18.68±8.20^{a}	19.33±9.41^{a}	17.50±7.37^{b}	16.33±7.81^{b}
AMLE+ Metformin(250/250)	19.93±4.51^{a}	19.15±4.00^{a}	16.60±2.77^{a}	16.66±2.41^{a}	14.86±2.86^{b}

Values are mean±SEM. One way Analysis of Variance (ANOVA) followed by Tukey post hoc test. Mean with the same superscript in the column are not significantly different ($P>0.05$), n=6,AMLE = *Anisopus mannii* leaf extract

Effect of *Anisopus mannii* aqueous leaf extract on food intake in alloxan-induced diabetic mice

The food intake of alloxan-induced diabetic mice varied significantly across the different treatment groups over four weeks (Table 3). Normal saline treated mice consistently had the highest food intake, while metformin (250 mg/kg) and AMLE at various doses significantly reduced food intake. The reductions in food intake in the extract treated groups, metformin alone and metformin combined with AMLE 250 mg/kg were significant ($p \leq 0.05$) when compared to the food intake in diabetic control group (alloxan + normal saline). Notably, AMLE 500 mg/kg had the most pronounced effect in reducing food intake, comparable to or even greater than metformin in some cases. The combination of AMLE 250 mg/kg with metformin 250 mg/kg showed a moderate reduction in food intake, suggesting a possible interaction between the two treatments.

Table 3: Effect of *Anisopus mannii* aqueous leaf extract on food intake in alloxan-induced diabetic mice

Treatment (mg/kg)	Weight of food intake (g)			
	Day 7	**Day 14**	**Day 21**	**Day 28**
Non-diabetic control	20.4±0.01	19.6±0.03	21.7±0.05	18.7±0.02
Diabetic control	31.13±0.18^{e}	35.63±0.08^{f}	33.99±0.01^{f}	36.42±0.00^{f}
AMLE(250)	20.56±0.00^{b}	16.86±0.01^{a}	19.71±0.00^{a}	22.42±0.01^{d}
AMLE(500)	20.56±0.01^{b}	22.85±0.01^{d}	23.64±0.09^{d}	23.78±0.10^{e}

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
APSBJournal

AMLE(1000)	19.70±0.01[a]	22.14±0.00[c]	21.13±0.01[b]	18.00±0.00[a]
Metformin(250)	22.27±0.01[c]	20.43±0.01[b]	22.13±0.01[c]	20.71±0.00[c]
AMLE+ Metformin(250/250)	23.85±0.00[d]	25.15±2.01[e]	24.42±0.00[e]	20.13±0.01[b]

Values are means ±SEM. One Way ANOVA followed by Tukey post hoc test. Means with the same superscript in the column are not significantly different (P>0.05). AMLE = *Anisopus mannii* leaf extract, n=6

Effect of *Anisopus manii* aqueous Leaf Extract on water intake in Alloxan-induced Diabetic Mice

The results of the effect of AMLE on water intake in alloxan-induced diabetic mice as presented in Table 4 indicate significant differences across the treatment groups over 28 days.

Overall, the results suggest that AMLE, either alone or in combination with metformin, significantly ($p \leq 0.05$) reduced water intake in alloxan-induced diabetic mice. The effect appeared to be dose-dependent, with AMLE 500 mg/kg and AMLE 1000 mg/kg showing the most pronounced reductions. The combination of AMLE 250 mg/kg and metformin 250 mg/kg also demonstrated a significant ($p \leq 0.05$) reduction in water intake, suggesting possible additive effect.

Table 4: Effect of *Anisopus mannii* aqueous leaf extract on water intake in alloxan-induced diabetic mice

Treatment (mg/kg)	Volume of water intake (ml)			
	Day 7	Day 14	Day 21	Day 28
Non-diabetic control	22.81±0.02	25.11±0.01	21.45±0.04	22.13±0.01
Diabetic control	57.13±0.01[e]	45.99±0.01[f]	47.99±0.01[d]	63.13±1.02[c]
AMLE(250)	33.42±0.01[b]	24.99±0.01[c]	20.70±0.09[a]	20.71±0.00[ab]
AMLE(500)	33.70±0.01[c]	27.42±0.01[e]	23.56±0.01[c]	19.27±0.01[a]
AMLE(1000)	36.13±0.01[d]	22.42±0.01[b]	23.27±0.01[c]	22.85±0.01[b]
Metformin(250)	30.77±0.08[a]	23.13±0.01[a]	23.42±0.00[c]	21.28±0.00[ab]
AMLE+ Metformin(250/250)	33.85±0.01[c]	25.85±0.01[d]	21.85±0.01[b]	19.92±1.72[ab]

Values are means ±SEM. One Way ANOVA followed by Tukey post hoc test was used to analyze data. Means with two of the same superscripts in the column are not significantly different (P>0.05). AMLE = *Anisopus mannii* leaf extract, n=6.

DISCUSSION

The phytochemical screening of *A. mannii* aqueous leaf extract (AMLE) revealed the presence of several bioactive compounds, including carbohydrates, flavonoids, saponins, alkaloids, terpenes/steroids, tannins, and cardiac glycosides, while anthraquinones were absent. Many of these phytochemicals have been reported to possess hypoglycemic properties [35]. The

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
APSBJournal

presence of carbohydrates suggests the availability of glycosidic compounds, which may contribute to the plant's biological activity, particularly its role in energy metabolism. Carbohydrate-containing plant extracts play a significant role in glucose homeostasis [36]. Flavonoids are well documented for their antioxidant and anti-hyperglycemic activities. Studies indicate that flavonoids enhance insulin secretion and glucose uptake while reducing oxidative stress in diabetic conditions [37]. The presence of flavonoids in AMLE supports its potential role in managing diabetes because flavonoids have been shown to inhibit α-amylase and α-glucosidase, thus reducing postprandial hyperglycemia. Furthermore, saponins are known for their anti-diabetic and cholesterol-lowering properties. They enhance glucose transport by increasing the expression of GLUT4 in peripheral tissues, thereby improving insulin sensitivity [38]. The presence of alkaloids in AMLE may also have contributed to its anti-hyperglycemic effect as berberine, an alkaloid has been reported to enhance glucose uptake [39]. A study by Adewale *et. al.*, [40] found that terpenoid-rich extracts enhance insulin secretion and protect pancreatic β-cells from oxidative stress. The presence of these compounds supports its potential as an antidiabetic agent. Cardiac glycosides are known for their primary role in heart function. In addition, recent studies have suggested their potential in glucose homeostasis as they enhance insulin release and glucose uptake [40] and their presence in this extract may have contributed to the anti-hyperglycemic effects observed in this study.

The result of the acute oral toxicity test indicated that the LD_{50} was estimated to be greater than 5000 mg/kg body weight of the extract as there were no reported cases of signs and symptoms of toxicity and there were no deaths at the tested limit dose. According to the OECD [29], this result shows that the extract is non-toxic and is safe for use. The findings of this study align with previous research on the safety profile of medicinal plants used in diabetes management. A similar high safety margin has been reported for *Vernonia amygdalina*, with no observable toxic effects up to 5000 mg/kg [37]. Likewise, no acute toxicity was found with *Moringa oleifera* at doses exceeding 4000 mg/kg [42], supporting the use of these plants in traditional medicine.

The anti-hyperglycemic effect of AMLE in alloxan-induced diabetic mice revealed a progressive decrease in blood glucose levels over 28 days, with the most significant reduction observed in the group treated with AMLE 1000 mg/kg when compared with the diabetic control group (Alloxan +Normal saline). Blood glucose levels of mice given a combination of AMLE and metformin (14.86±2.86 mmol/L) compared to metformin 250 mg/kg alone (16.33±7.81 mmol/L), suggests possible additive effect between metformin and the extract.

The anti-hyperglycemic effect of AMLE can be attributed to the presence of carbohydrates, flavonoids, alkaloids, tannins, saponins and cardiac glycosides which have been reported to play various roles as anti-diabetic agents [42]. These compounds may lower blood glucose by stimulating insulin secretion, enhancing glucose uptake, or inhibiting carbohydrate digestion and absorption in the intestine [43]. The result of this study is similar to that reported for *Vernonia amygdalina* which significantly reduced blood glucose levels in alloxan-induced diabetic rats, with effects comparable to metformin [44] and that of Bello *et al.* (2021) [42] who reported that

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
APSBJournal

Moringa oleifera leaf extract exhibited a strong anti-hyperglycemic effect in diabetic animal models by enhancing insulin sensitivity and glucose uptake in peripheral tissues.

The reductions in food intake for the extract treated groups (Table 3) showed a familiar pattern to that demonstrated by *Garcinia kola* extract which reduced excessive food intake in diabetic animals by modulating leptin and ghrelin levels, the hormones responsible for appetite regulation [45]. The decrease in food intake observed in the AMLE-treated groups in this study may also be attributed to similar mechanisms, where bioactive compounds such as flavonoids and alkaloids interact with metabolic pathways to suppress excessive hunger. The reduction in food intake observed in AMLE-treated mice suggests that *A. mannii* may help control hyperphagia in diabetes. This effect is beneficial as excessive food intake exacerbates hyperglycemia and metabolic complications in diabetic individuals.

Polydipsia (excessive water intake) is one of the common symptoms of diabetes mellitus. In this study, there was a consistent increase in water intake in the diabetic control mice throughout the 28-day period of study (Table 4). The reduction in water intake observed with AMLE is similar to the findings of other studies on medicinal plants with anti-hyperglycemic properties. For instance, a study by Oloyede *et al*. [46] reported that *Azadirachta indica* (neem) leaf extract significantly reduced water intake in diabetic rats by modulating blood glucose levels. Similarly, a study by Yusuf *et al.* [47] showed that *Cymbopogon citratus* (lemongrass) extract reduced polydipsia in diabetic rats, which was linked to its hypoglycemic and anti-inflammatory effects. These studies, like the current one, suggest that plant extracts with hypoglycemic activity can alleviate the symptoms of diabetes, such as excessive thirst. Additionally, Akinmoladun *et al.*, [48] observed a reduction in water intake in diabetic rats treated with *Morinda lucida* leaf extract, attributing the effect to its ability to regulate blood glucose and improve kidney function. The results of this study align with these findings, indicating that *A. mannii* may also exert similar effects on the kidneys and fluid balance, reducing the need for excessive water intake. The significant reduction in water intake in the AMLE-treated groups suggests that *A. mannii* has a beneficial effect on diabetes-related symptoms such as polydipsia. This finding is particularly relevant because excessive water consumption and urination are distressing symptoms in diabetes, often leading to dehydration and electrolyte imbalances. By reducing water intake, *A. mannii* may help restore fluid balance and alleviate one of the major complications associated with uncontrolled diabetes.

CONCLUSION AND RECOMMENDATION

The result of this study has shown that the aqueous leaf extract of *A. mannii* possess significant anti-hyperglycemic effect in alloxan-induced diabetic mice. Thus, supporting the traditional use of this plant in the management of Diabetes mellitus. Further studies are recommended for the isolation and characterization of bioactive compounds responsible for this anti-hyperglycemic effect as well as the possible mechanism(s) of action.

CONFLICT OF INTEREST

The authors declare that there was no conflict of interest in this research work, writing and publication of the manuscript. The whole project was self-financed.

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
APSBJournal

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AUTHORS CONTRIBUTION
Jimoh, A A and Is'haq, H designed the study and performed the experiments. Bashir, A I-J did the literature search, Jimoh, AA wrote the initial draft of the manuscript. Yakubu, M I performed statistical analysis of the data. Oloyede, R B did the phytochemical analysis of the plant extract. Mallam, D, Timothy, M and Sambo, SS did the acute toxicity test. All the authors read and approved the final manuscript.

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Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
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Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
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Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 38 - 52 Submitted 9/10/2025 Accepted 21/11/2025
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