**UNIJERPS**
Unizik Journal of Educational Research and Policy Studies VOL.20 (2);
***July-December, 2025*** https://unijerps.org
***2023 Impact Factor:*** **6.834;** 2022 ***Impact Factor***: 5.156; ***2021 Impact Factor: 5.641;***
***2020 Impact Factor: 5.355.*** *Indexing and Impact Factor URL: http://sjifactor.com/passport.php?id=21363*

# DETERMINATION OF ANTIDIABETIC PROPERTY OF SOME MEDICINAL PLANTS IN STREPTOZOTOCIN (STZ)-INDUCED DIABETIC MALE WISTAR ALBINO RATS

**[1]Onuegbu, P.I., [2]Agbafor, K.N., [1]Igboeli, C.N., [1]Okoye, O.G., [3]Ndife, C.T.**
[1]Department of Biochemistry, Federal Polytechnic Oko.
[2]Department of Biochemistry, Ebonyi State University.
[3]Department of Chemistry, Federal Polytechnic Oko.
**Correspondence Author**: princeifey2010@yahoo.com

## ABSTRACT

*Medicinal plants are effective in the management and treatment of various diseases such as, liver diseases, heart diseases, diabetes. Musa paradisiaca (MP) (plantain) peels, Gongronema latifolium (GLE) (utazi), and Vernonia amygdalina (VA) (bitter leaf) are among the diverse array of medicinal plants traditionally used singly and combined in various cultures for their anti-diabetic properties. Hence, the present study aimed to comparatively assess the antidiabetic property of the leaf extracts of these three plants. Acute toxicity test was done using modified method of Lorke D as there was no mortality death ratio. Twenty-four male wistar albino rats were used for the acute toxicity test while one hundred and five male rats were randomly assigned into 15 groups of 10 rats per group. Diabetes was induced in group 2-15 by intraperitoneal administration of 60mg/kgbw of streptozotocin (STZ) while group 1 was the normal control, group 2 and 3 were treated with normal saline and 500mg/kg of metformin respectively. Groups 4, 5 and 6 were treated with 200, 400 and 600mg/kg of M. paradisiaca peels (MPPE) respectively. Groups 7, 8 and 9 were treated with 200, 400 and 600mg/kg doses of G. latifolium (GLE) extracts. Groups 10, 11 and 12 were also treated with 200, 400 and 600mg/kg of V. amygdalina (VAE) extracts while groups 13, 14 and 15 were treated with combined 200, 400 and 600mg/kg extracts (1:1:1). The treatments lasted for 28 days by oral intubation with different doses of combined extracts of (MPPE, GLE and VAE) respectively. The results showed that blood glucose, amylase activity were significantly higher ($p<0.05$) in group 2 than in group 1. On the other hand, glut1, glut4, AMP kinase, were significantly ($p<0.05$) lower than the group 1. Treatments with the extracts and metformin resulted to a significant reversal in the values of these parameters. The effect of the extracts was found to be significantly dose dependent; the effect produced by the combined extract was significantly ($p<0.05$) higher than the individual extracts. This effect produced by 600mg/kg of combined extract was significantly ($p<0.05$) higher than that of metformin. The findings from this study are indicative that the extracts of MPPE, GAE, VAE and combined form posseses antidiabetic property which may be attributed to their chemical constituents. Furthermore, they also revealed that the antidiabetic property increased when used in combination.*

**Keywords:** *Musa paradisiaca* peels, *Gongronema latifolium*, *Vernionia amygladina*, glut1 and 4, blood glucose, Amp kinase.

## Introduction

Medicinal plants are regarded as rich resources of traditional medicines and from these plants, many of the modern medicines are produced. For thousands of years medicinal plants have been used to treat health disorders, to add flavor and conserve food and to prevent diseases epidemics (Erasto *et al.,* 2000). Medicinal plants play a significant role in the treatment of diabetes mellitus which is a serious metabolic disorder (Ekor, 2014). Traditional plants are reported to have significant anti-diabetic properties with no harmful side effects (Fujimoto *et al.,* 2000). They are rich sources of anti-diabetic compounds such as flavonoids, alkaloids, phenolic, and tannins that improve the efficiency of pancreatic tissues by increasing insulin secretion or decreasing the intestinal absorption of glucose (Eleazu *et al*., 2015).

The ease of access, low side effects, and low cost have made herbal preparations the main player in all available therapies, especially in rural areas (Eleazu *et al*., 2015). Presently, in developing countries where the cost of conventional drugs is a burden on the population, traditional (herbal) medicine is used to treat diabetes (Fadimi *et al.,* 2017). However, there is no biological information about the basic mechanisms of action in the treatment of diabetes even though most plants have been shown to contain substances such as glycosides, alkaloids, terpenoids, flavonoids, and other anti-diabetic compounds (Garlicia *et al.,* 2020). Consistently, many indigenous African medicinal plants are effective in treating diabetes effectively (Schmidt *et al.,* 2008). One of the biggest benefits of medicinal plants is their accessibility and lack of side effects (Fadimi *et al.,* 2017). Existing pieces of the literature suggest that about eight hundred (800) plants that may have antidiabetic potential are recorded in ethnobotanical knowledge but only approximately 410 experimentally proven out of which the complete mechanism has been studied only for 109 plants (Eyo *et al.,* 2010). Importantly, several medicinal plant extracts have been shown to modulate the metabolic pathways such as glycolysis, gluconeogenesis, Krebs cycle, glycogen synthesis and their degradation, synthesis, and release of insulin, cholesterol synthesis, carbohydrate metabolism, and absorption (Eyo *et al.,* 2010).

Diabetes mellitus (DM), also known simply as diabetes is a complex metabolic disorder characterized by hyperglycemia, a physiologically abnormal condition represented by continued elevated blood glucose levels (Reddy *et al.,* 2003). Hyperglycemia results from anomalies in either insulin secretion or insulin action or both and manifests in a chronic and heterogeneous manner as carbohydrate, fat, and protein metabolic dysfunctions. Diabetes follows a progressive pattern with complex pathogenesis and varied presentation (American Diabetes Association, 2014). Plantain peels, derived from the *Musa paradisiaca* plant, are rich in bioactive compounds such as phenolic acids and flavonoids (Frayling *et al.,* 2007). Traditionally used in various cultures, these peels have garnered attention for their antioxidant and potential therapeutic properties (Fujimoto *et al.,* 2020). Research

suggests that plantain peels may help regulate blood sugar levels, improve insulin sensitivity, and mitigate diabetic complications, making them a promising candidate for diabetes management (Fujimoto *et al.*, 2020).

*Gongronema latifolium,* commonly known as utazi, is a perennial climbing plant native to West Africa. It belongs to the family Apocynaceae and is widely distributed in tropical regions (Eleyinmi *et al.*, 2007). Utazi contains bioactive compounds including flavonoids, alkaloids, and terpenoids (Fujimoto *et al.*, 2020). Widely used in traditional medicine, utazi is known for its antioxidant, anti-inflammatory, antimicrobial, and potentially hypoglycemic effects (Eleyinmi *et al.*, 2007). Incorporating utazi into culinary dishes and herbal preparations may offer various health benefits, including support for diabetes management (Fadimi *et al.*, 2017).

**Background of the Study**

Medicinal plants remain to offer mankind with new medicines. Some of the beneficial properties ascribed to plants have recognised to be flawed and medicinal plant treatment is based on the experimental findings of hundreds to thousands of years (Fadimi *et al.*, 2017). The earliest reports carved on clay tablets in cuneiform date from about 2600 BC are from Mesopotamia; among the materials that were used were oils of Commiphora species (Myrrh), Cedrus species (Cedar), Glycyrrhiza glabra (Licorice), Papaver somniferum (Poppy juice) and Cupressus sempervirens (Cypress) are still used today for the cure of diseases extending from colds and coughs to inflammation and parasitic infections. The traditional medicine practice is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand. About 40% of the total medicinal consumption is attributed to traditional tribal medicines alone by China (Fadimi *et al.*, 2017). In Thailand, herbal medicines make use of legumes encountered in the Caesalpiniaceae, the Fabaceae, and the Mimosaceae. It is estimated that in mid-90s, more than US$2.5 billion have resulted from the sales of herbal medicines (Eleyinmi *et al.*, 2007). The herbal medicinal preparations are more in demand than mainstream pharmaceutical products in Japan. The emphasis on the use of medicinal plants had hitherto been placed on the treatment rather than prevention of diseases (Sung *et al.*, 2006). However, there exists in the literature considerable report in recent times on research work on the use of medicinal plants and their constituents in disease prevention (Fugi *et al*., 2006). A World Health Organisation (WHO) Expert Group defined Traditional Medicine as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental, or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing (Garlicia *et al.*, 2011). For Africa, this may be extended further by including an expression, such as ‘while bearing in mind the original concept of nature which includes the material world, the sociological environment whether living or dead and the metaphysical forces of the universe (Garalicia *et al.*, 2020).

Over 90% of traditional medicine recipes/remedies contain medicinal plants but this paper will address, specifically, the medicinal plants that have been implicated with preventive measures in disease control strategies (Bhatia *et al.,* 2021). However, it must be noted that only a very thin divide exists between treatment and prevention in some cases. A quick example is the fact that by treating mild elevation of blood pressure renal disease can be prevented (Faselis *et al.,* 2020). In diversified industries the contribution of plants is remarkable such as fine chemicals, cosmetics, pharmaceuticals and drugs and industrial raw materials etc (Faselis *et al.,* 2020). For the development of new drug discovery medicinal plants perform a dynamic part. Medicinal plants have proved their sole role in coping with anumber of deadly diseases including cancer and the diseases associated with viral onslaught viz. Hepatitis, AIDS etc. In the USA drug market approx. 100 plant made new drugs were presented during 1950-1970 which includes vincristine, reseinnamine, vinblastin, deseridine and reserpine which are from different plants. During 1971-1990 fresh medicines i.e., artmisinin, Zguggulsterone, ginkgolides, lectinam, E-guggulsterone, teniposide, ectoposide, plaunotol and nabilone appeared all around the world (Christakov *et al.,* 2018). The 2% medicines which were presented during include irinotecan, toptecan, paclitaxel and gomishin etc. The isolation of serpentine in 1953 from the Indian plant Rauwolfia serpentine root was an innovatory episode in treatment of hypertension and lowering of blood pressure (Christakov *et al.,* 2018). Historically, medicinal plants have been used since ancient times, with many plants being used in traditional medicine for centuries (Aziz *et al.*, 1983). They are a significant part of the global drug market, with many herbal drugs being derived from them. The resurgence in recognition of medicinal plants as a source of drugs is due to their effectiveness, low side effects, and potential as an alternative to high-cost synthetic drugs Fadini *et al.*, (2011). Severally researchers have suggested that medicinal plants owe their therapeutic value to naturally occurring biologically active ingredients, such as primary and secondary metabolites (Anand *et al.,* 2019). These compounds can be found in pure or combined forms and are a pivotal source of drug lead compounds (Emaga *et al.,* 2007). The quality of medicinal plants is influenced by abiotic and biotic factors, and their conservation is crucial to ensure sustainability (Anand *et al.,* 2019). The growing demand for medicinal plants has led to concerns about their availability and potential overharvesting, which can result in biodiversity problems (Calles *et al.,* 2000). *Murraya koenigii* (L.) Spreng, a member of Rutaceae family, commonly known as curry leaf is an important medicinal plant native to India exhibiting diverse biological activities. Leaf decoction mixed with rice flour is used a treatment for indigestion and eye problems by the tribes of the district, Tamil Nadu, India (Faselis *et a*l. 2011). Koenidine, a metabolically stable carbazole alkaloid isolated from the leaves of *M. koenigii* demonstrated a considerable reduction in the postprandial blood glucose level and improved insulin sensitivity in

streptozotocin-induced diabetic rats (Faselis *et a*l. 2011).

Administration of ethanolic leaf extract of *M. koenigii* ameliorated the insulin sensitivity and glucose tolerance in dexamethasone-induced insulin-resistant mice (Chin *et a*l., 2006). The glucose uptake and GLUT-4 translocation was augmented by the stimulatory effect of *M. koenigii* via insulin-mediated phosphorylation of AKT (Balogun *et a*l. 2016). Oral administration of ethanolic extract of the leaves of *M. koenigii* significantly decreased the blood sugar levels in diabetic rats and also rendered protective effects against development of diabetic neuropathy (Balogun *et a*l. 2016). Administration of aqueous extract of the leaves of *M. koenigii* (200 and 400 mg/kg for 30 days) considerably enhanced the antioxidant status and renal function in diabetic rats and also exhibited strong hypolipidemic and hypoglycemic action (Balogun *et a*l. 2016). Aqueous leaf extract of *M. koenigii* demonstrated better anti-hyperglycemic activity over the methanolic extract in treated alloxan-induced diabetes mice. Both the extracts are capable of regulating inflammatory cytokines, oxidative stress and prevention of the pancreatic islet damage in diabetic mice (Eleyinmi *et al.,* 2007).

**Statement of Problem**

The prevalence of diabetes mellitus continues to rise globally posing significant challenges for public health systems and individuals alike (American Diabetes Association, 2013). Despite the availability of pharmacological interventions, many diabetic individuals experience inadequate glycemic control and are at risk of developing complications (Ezuruike *et al.,* 2016). Due to the high cost of drugs used in the management of this disease and the ineffective failure to achieve a desired result using single dose, it gave a threat to the problems for people with the disease. Complementary and alternative approaches, particularly those utilizing natural remedies have gained attention for their potentials to enhance diabetes management.

**Aim/Objectives of study**

The aim is to determination the antidiabetic property of some medicinal plants in streptozotocin (STZ)-induced diabetic male wistar albino rats.

The specific objectives will be:

1 To determine the effect of *Musa paradisiaca* peels, *Gongronema latifolium,* and *Vernonia amygdalina* ethanol extracts on the body weight of the animals.
2 To determine the effect of *Musa paradisiaca* peels, *Gongronema latifolium,* and *Vernonia amygdalina* ethanol extracts on blood glucose levels.
3 To determine the effect of *Musa paradisiaca* peels, *Gongronema latifolium,* and *Vernonia amygdalina* ethanol extracts on alpha amylase activity, in STZ-induced diabetic Wistar albino rats.
4 To determine the effect of *Musa paradisiaca* peels, *Gongronema latifolium,* and *Vernonia amygdalina* ethanol extracts on glucose transporters (1 and 4).

5 To determine the effect of *Musa paradisiaca* peels, *Gongronema latifolium*, and *Vernonia amygdalina* ethanol extracts on AMPK and level in STZ-induced diabetic Wistar albino rats.

## Literature review

### Description of *Musa paradisiaca*

*Musa paradisiaca*, commonly known as the unripe plantain peel, is a large perennial herbaceous plant belonging to the Musaceae family. It is native to Southeast Asia but is now cultivated in tropical and subtropical regions worldwide for its fruit and other uses (Eid *et al.*, 2017). The plantain typically grows from a rhizome, forming a cluster of pseudostems that can reach heights of up to 20 feet or more (Chistakov *et al.*, 2018). These pseudostems are not true stems but rather tightly packed leaf sheaths that enclose the true stem. The leaves are large, elongated, and arranged spirally around the pseudostem, giving the plant its characteristic tropical appearance (Chistakov *et al.*, 2018). *Musa paradisiaca* produces flowers in clusters called inflorescences.

### Phytochemical constituents of unripe plantain peels

Unripe plantain peels contain various polyphenols, including flavonoids and phenolic acids, which possess antioxidant and anti-inflammatory properties (Chin *et al.*, 2006). These compounds have been studied for their potential to manage diabetes by improving insulin sensitivity, reducing oxidative stress, and lowering blood sugar levels (Anand *et al.*, 2019). Dopamine, a neurotransmitter found in plantain peels, has been shown to have insulin-like effects, promoting glucose uptake in cells (Emaga *et al.*, 2007). Studies have suggested that dopamine may help regulate blood glucose levels and improve insulin sensitivity, making it a potential therapeutic agent for diabetes management (Erasto *et al.*, 2006).

### Description of *Gongronema latifolium*

*Gongronema latifolium*, commonly known as "Utazi" or "Arokeke" in Nigeria, is a perennial climbing plant belonging to the Apocynaceae family (Eleyinmi, 2007). Native to the tropical regions of West Africa, particularly Nigeria and Ghana, it is widely cultivated for its culinary and medicinal uses (Eid *et al.*, 2017). The plant features dark green, glossy leaves with a broad elliptic to ovate shape and a slightly serrated margin. Its stems are slender and twining, allowing it to climb and spread easily (Eleyinmi, 2007).

### Description of *Vernonia amygdalina*

*V. amygdalina*, commonly known as bitter leaf, is a perennial shrub native to tropical Africa (Calles *et al.*, 2000). It belongs to the Asteraceae family and is widely distributed across regions with tropical and subtropical climates. Bitter leaf is characterized by its dark green, serrated leaves that are highly bitter, hence its name (Calles *et al.*, 2000).

### AMPK

Skeletal muscle predominately expresses α2β2 complexes with low levels of α1 and β1 (Grame *et al.*, 2011). AMPK γ1 and γ2 are expressed in multiple tissues whereas γ3 appears to be restricted to skeletal muscle, where it's association with α and β isoforms differ depending on the fiber composition of

the muscle (Bahtia *et al.*, 2016).

**Glut 1**

Glut1 expression occurs in almost all tissues, with the degree of expression typically correlating with the rate of cellular glucose metabolism. In the adult it is expressed at highest levels in erythrocytes and also in the endothelial cells of barrier tissues such as the blood–brain barrier (Aziz *et al.*, 2021).

**Glut4**

Glucose transporter type 4 (GLUT4), also known as solute carrier family 2, facilitated glucose transporter member 4, is a protein encoded, in humans, by the *SLC2A4*gene. GLUT4 is the insulin-regulated glucose transporter found primarily in adipose tissues and striated muscle (skeletal and cardiac) (Anal *et al*.,2014). The first evidence for this distinct glucose transport protein was provided by David James in 1988. The gene that encodes GLUT4 was cloned and mapped in 1989 (Anal *et al*., 2014).

**Materials and Methods**

Materials:

Sreptozotocin [2deoxy 2 (3methyl 3nitrosoureido) Dglycopyranoside] as a diabetogenic agent, were purchased from Sigma Aldrich Chemical Co., St Louis, MO, USA. All other chemicals were of analytical grade and were obtained from standard commercial supplies. Glucose and lipid profile kits were purchased from Spinreact (Spain). The insulin Sandwich ELISA kit were purchased from Linco Research (USA). Teflon homogenizer (GlasCol, Terre Haute, USA) were used for the homogenization of the pancreas. UV spectrophotometer (ERBA Chem 7; ERBA Diagnostics Mannheim GmbH, Mallaustrasse 69–73 68,219 Mannheim, Germany) were used for the detection of biochemical parameters and oxidative stress parameters. Cooling centrifuge (Centurion Scientific K3 Series Centrifuges; Core Life Sciences, Irvine, 92,623 California, USA) were used for centrifugation of blood and pancreas homogenate. ELISA STAT FAX2100 washer, shaker, and microplate reader were used for the determination of insulin level.

**Chemical analysis**

The proximate, mineral, vitamin, and phytochemical contents were determined using the method of A.O.A.C. (1997).

**Acute Toxicity Test**

The acute toxicity ($LD_{50}$) of MPP, GL and VA leaves in male wistar albino rats were determined by a modified method of Lorke (1983). Twenty-four (24) rats were randomized into six (6) groups of four rats each, Group A- Control, Group B- 1000mg/kg, Group C- 2000mg/kg, Group D- 3000mg/kg, Group E- 4000mg/kg and Group F- 5000mg/kg.

**Induction of Diabetes**

Experimental DM were induced in overnight fasted rats using a single intraperitoneal (i.p.) injection of STZ (60 mg/kg b.w.) which was dissolved in 0.09 M citrate buffer (pH 4.5), Fifty male Wistar rats were injected with NA and STZ. Seven days after, the STZ was injected and the rats were screened for a hyperglycemic state. Rats that have 2-hr serum glucose ranging from level 200 to 300 mg/dl were used as diabetic rats in the experiment.

**Experimental design**

A total of 150 male rats were assigned into 15 groups of 10 rats per group. Group 1 received feed and water only. Diabetes was induced in Groups 2-15 by administration of 60mg/kg of STZ. Then, Group 2 (negative control) was not treated whereas other Groups were treated. Group 3 (positive control) diabetic rats were treated with Metformin (standard drug). Group 4 diabetic rats were treated with 200mg of *Musa paradisiaca* peel ethanol extracts (MPPE). Group 5 diabetic rats were treated with 400mg of MPPE. Group 6 diabetic rats were treated with 600mg of MPPE. Group 7 diabetic rats were treated with 200mg of *Gongronema latifolium* ethanol extracts (GLE). Group 8 diabetic rats were treated with 400mg of GLE. Group 9 diabetic rats were treated with 600mg of GLE. Group 10 diabetic rats were treated with 200mg of extracts *Vernonia amygdalina* ethanol extracts (VAE). Group 11 diabetic rats were treated with 400mg of VAE. Group 12 diabetic rats were treated with 600mg of VAE. Group 13 diabetic rats were treated with 200mg of Comb 1 (200mg MPPE+200mgGLE+200mgVAE). Group 14 diabetic rats were treated with 400mg of Comb 2 (200mg MPPE+200mgGLE+200mgVAE). Group 15 diabetic rats were treated with 600mg of Comb 3 (600mg MPPE+600mgGLE+600mgVAE).

**Sample collection**

At the end of the induction, rats were deprived of food for 10–12 hr overnight fast and were anesthetized with ethyl ether solution through inhalation. Blood samples were collected from the jugular vein and kept at room temperature to coagulate and then centrifuged at 3,000 rpm for 15 min. The clear sera were stored at −20°C for biochemical parameters (Babayemi *et al.*, 2010).

**Tissue preparation**

After decapitation and dissection, the pancreas of each rat were quickly excised. The pancreas were divided into two portions. One portion was homogenized at 4°C in isotonic sterile saline (0.9% NaCl) at a concentration of 10% (1 g/10 ml). The pancreas homogenate was centrifuged at 3,000 r.p.m. for five minutes and cellular debris removed. The supernatant of pancreas homogenate of each rat were separated and stored at −20°C for oxidative stress biomarkers while others were used to assay glucose transporters and AMPK.

**Determination of blood glucose level**

Blood glucose level were determined using an Accu-Chek glucometer as described by Aziz and Hsiang, (1983).

**Principle**

The principle behind the Accu-Chek glucometer and similar devices is based on the enzymatic reaction involving glucose oxidase. When a blood sample is applied to the test strip, glucose oxidase catalyzes the oxidation of glucose to gluconic acid, producing hydrogen peroxide as a byproduct. The hydrogen peroxide then reacts with a chromogen in the strip to produce a color change. The intensity of the color change is proportional to the concentration of glucose in the blood, and this is measured by the

glucometer to provide a numerical glucose reading.

**Procedure**
The Accu-Chek glucometer were cleaned and inserted with a test strip. The rat's tail were cut to obtain a small drop of blood. The edge of the test strip were touched to the blood drop allowing it to be drawn in by capillary action. The glucometer automatically measures it and displayed blood glucose levels.

**Determination of α-amylase activity**
The activity of α-amylase were assayed by the method of Emaga *et al.*,. (2012) following the protocol outlined in the kit used.

**Principle**
The determination of α-amylase activity were based on its ability to hydrolyze starch into simpler sugars such as maltose and glucose. This enzymatic activity can be measured by monitoring the decrease in starch concentration or the increase in reducing sugars over time. One common method involves using a chromogenic substrate, which releases a colored product upon hydrolysis by α-amylase.

**Procedure**
Two ml of the glucose solution were added to a test tube or microplate well. A specific 1 ml of the enzyme sample were added to the same tube or well. It was mixed thoroughly and incubated at 37°C for 10 minutes. After the incubation period the enzymatic reaction was by addition of a denaturation agent. Measured the absorbance of the reaction mixture at the appropriate wavelength using a spectrophotometer. Compare the absorbance readings of the samples to a standard curve prepared using known concentrations of glucose. Calculate the α-amylase activity in the sample. That is convert the absorbance to enzyme units based on the standard curve.

**Determination of the Glucose transporters (1 and 4) levels**
The expression levels of glucose transporters 1 and 4 were assayed by the western blotting method adopted by Edward *et al.* (2019) following the assay Kit manufacturer's protocols.

**Principle**
The principle involves separating proteins by size using SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), transferring them onto a polyvinylidene difluoride (PVDF) membrane, and probing with specific antibodies to visualize target proteins. Initially, cultured cells are lysed in a buffer containing a Mammalian Protein Extraction Reagent and a protease inhibitor cocktail to prevent protein degradation. The lysate is then loaded onto a precast polyacrylamide gel, where proteins are separated based on molecular weight. After electrophoresis, the proteins are transferred onto a PVDF membrane. Non-specific binding sites on the membrane are blocked using a solution of 5% skim milk powder in Tris-buffered saline. The membrane is then incubated with primary antibodies specific to the proteins of interest (GLUT1, GLUT4, and AMPK). Following this, the membrane is incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies that

bind to the primary antibodies. The immune complexes formed are detected using enhanced chemiluminescence (ECL) reagents, which produce a luminescent signal upon reaction with the HRP. This signal is captured and visualized using an imaging system such as the Fusion Solo, allowing for the identification and quantification of the target proteins.

**Procedure**

Briefly, cultured cells were homogenized in lysis buffer containing Mammalian Protein extraction and were loaded on precast polyacrylamide gel (Bio-Rad, Laboratories, Inc). GLUT1, GLUT4, and AMPK were then transferred to Immuno-Blot polyvinylidene difluoride membrane (Bio-Rad). Non-specific binding sites were blocked using 5% skim milk powder in Tris-buffered saline for 1 h at room temperature. Blots were then incubated overnight at 4 °C with rat polyclonal GLUT1 antibody (1:1000; Abcam), rat polyclonal GLUT4 antibody (1:400; Abcam) or rat monoclonal AMPK antibody (1:5000; Sigma-Aldrich) as primary antibody; and later with anti-rat immunoglobulin (Ig) G peroxidase-label secondary antibody (1:10000; GE Healthcare Life Science) or anti-rat IgG peroxidase-label secondary antibody (1:10000; Invitrogen), respectively. Immune complexes were visualized by enhanced chemiluminescence plus western blotting detection reagents (GE Healthcare Life Science) and visualized using FUSION Solo S (VilberLourmat).

**Statistical Analysis**

Data was reported as mean ± standard deviation, where appropriate. One–way analysis of variance (ANOVA) and correlation analysis was used to analyze the data results using GraphPad Prism. The group Mean obtained after each treatment was compared with controls and the difference is considered significant when the results are $p<0.05$.

**Results**

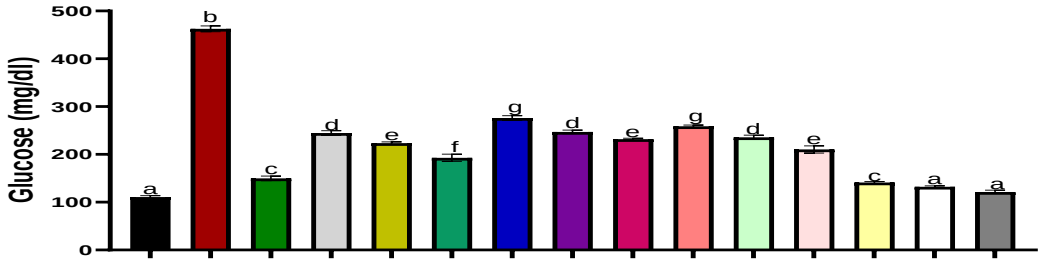



**Figure 1:** Serum Glucose level in streptozocin (STZ)-induced diabetic rats. Data are shown as mean ± S.D (n=4). Mean values with the different letters are significantly different at $P<0.05$. **Comb 1**= [GLE (200mg/kg) + VAE (200mg) + MPPE (200mg/kg)]; **Comb 2** = [GLE (400mg) + VAE (400mg) + MPPE (400mg/kg)]; **Comb 3** = [GLE (600mg) + VAE (600mg) + MPPE (600mg)].

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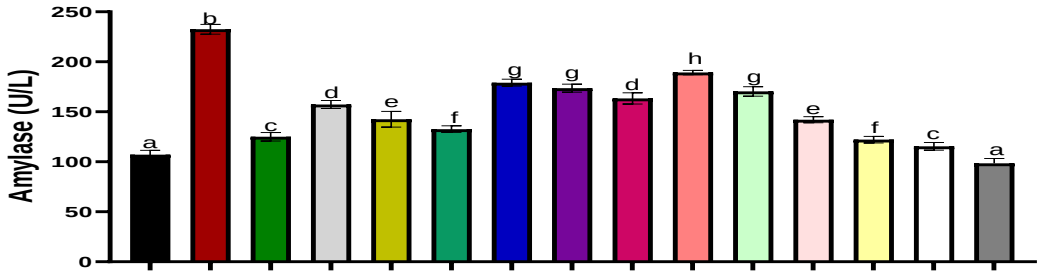



**Figure 2:** Serum Amylase level in streptozocin (STZ)-induced diabetic rats. Data are shown as mean ± S.D (n=4). Mean values with the different letters are significantly different at P<0.05. **Comb 1**= [GLE (200mg) + VAE (200mg) + MPPE (200mg)]; **Comb 2** = [GLE (400mg) + VAE (400mg) + MPPE (400mg)]; **Comb 3** = [GLE (600mg) + VAE (600mg) + MPPE (600mg)].

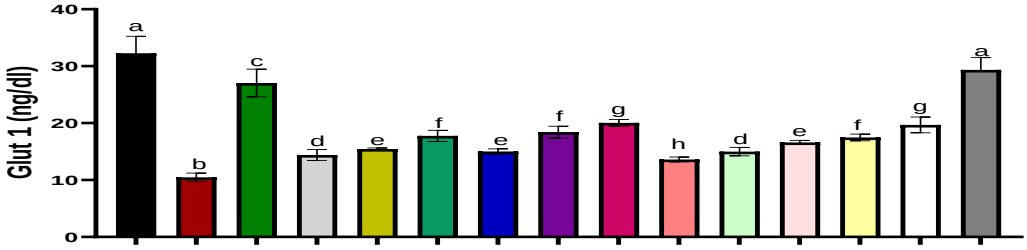



**Figure 3:** Glucose Transporter 4 (Glut 4) level in streptozocin (STZ)-induced diabetic rats. Data are shown as mean ± S.D (n=4). Mean values with the different letters are significantly different at P<0.05. **Comb 1**= [GLE (200mg) + VAE (200mg) + MPPE (200mg)]; **Comb 2** = [GLE (400mg) + VAE (400mg) + MPPE (400mg)]; **Comb 3** = [GLE (600mg) + VAE (600mg) + MPPE (600mg)].

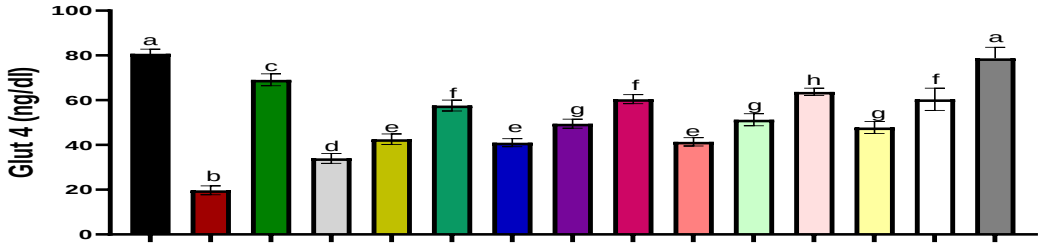



**Figure 4:** Glucose Transporter 4 (Glut 4) level in streptozocin (STZ)-induced diabetic rats. Data are shown as mean ± S.D (n=4). Mean values with the different letters are significantly different at P<0.05. **Comb 1**= [GLE (200mg) + VAE (200mg) + MPPE (200mg)]; **Comb 2** = [GLE (400mg) + VAE (400mg) + MPPE (400mg)]; **Comb 3** = [GLE (600mg) + VAE (600mg) + MPPE (600mg)].

**Discussion**

Serum glucose parameters have been established as significant biomarkers to decipher disease conditions including

manifestations of diabetes. In this study, the antidiabetic effects of extracts of *Musa paradisiaca* peel (MPPE), *Gongronema latifolium* (GLE), and *Vernonia amygdalina* (VAE) were investigated. Results on serum glucose biomarkers showed that administration of STZ in rats resulted in an increase in blood glucose levels and amylase activity. This model aligns with the pathophysiology of diabetes in humans, where insulin deficiency or resistance leads to hyperglycemia and altered enzymatic activity associated with glucose metabolism (Eleyinmi, 2007; Wang*et al.*, 2022). However, treatment with the plant extracts (MPPE, GLE, and VAE) at various doses (200mg/kgBW, 400mg/kgBW, and 600mg/kgBW) revealed a significant reduction in serum glucose levels and amylase activity. These results suggest that MPPE, GLE, and VAE have potent antihyperglycemic properties. For instance, *Musa paradisiaca* has been documented for its antihyperglycemic and insulin-enhancing effects (Eleazuand Okafor, 2015 Abdel Aziz*et al.*, 2020), likely due to the presence of phytochemicals such as flavonoids and alkaloids, which are known to modulate glucose metabolism and improve insulin sensitivity. *Gongronema latifolium* has also been reported to possess antidiabetic and antioxidant properties (Ojo *et al.*, 2020). Its bioactive components, including saponins and polyphenols, may enhance insulin secretion or action, protect pancreatic β-cells from oxidative damage, and inhibit glucose absorption in the intestine, contributing to lower blood glucose levels (Schneider*et al.*, 2003).

Similarly, *Vernonia amygdalina* has been reported to have hypoglycemic effects and ability to improve insulin sensitivity (Nkono *et al.*, 2022) which aligns with the current findings. As observed in this study, the similar efficacy observed among different doses of these extracts implies that the active compounds within these plants may have reached a threshold of effectiveness, beyond which no additional benefits are observed. The biochemical significance of these findings can be attributed to the modulation of key enzymes and hormones involved in glucose homeostasis. For example, the reduction in amylase activity observed with the extract treatments suggests an inhibition of carbohydrate digestion, leading to decreased glucose availability and absorption (Gong *et al.*, 2020). This mechanism helps mitigate postprandial hyperglycemia, a common challenge in diabetes management (Abdel Aziz *et al.*, 2020). Additionally, the increase in serum insulin levels indicates that these extracts might stimulate the pancreas to release more insulin or protect pancreatic β-cells from STZ-induced cytotoxicity.

As reported by other researchers, the phytochemicals in these plants could enhance insulin gene expression, improve β-cell function, or act as insulin mimetics, thereby lowering blood glucose levels (Truscheit *et al.*, 2010; Alam *et al.*, 2022). Furthermore, the combination treatments of these extracts demonstrated noteworthy results. In this study, when administered in equal ratios, the combined doses showed no significant difference compared to the standard drug metformin regarding glucose levels at a dose of 200mg/kgBW. Also, for amylase,

combination treatment at 400mg/kgBW was comparable to metformin, which shows the potential of these plant extracts as a natural alternative or adjunct to conventional diabetes treatments. This synergistic effect suggests that the combined actions of the different phytochemicals present in the extracts offer a multifaceted approach to diabetes management. As formally opined for single doses of the extract, the complementary mechanisms might include enhanced inhibition of carbohydrate digestion, more robust protection and stimulation of pancreatic β-cells, and improved insulin action, thereby providing a comprehensive strategy for managing hyperglycemia (Alam *et al.*, 2022). Therefore, this investigation provides compelling evidence that MPPE, GLE, and VAE can effectively manage hyperglycemia and improve insulin levels in STZ-induced diabetic rats. Effectively, the extracts demonstrate significant antihyperglycemic and insulin-enhancing properties, which are comparable to those of metformin, a widely used antidiabetic drug. We propose that the combination treatments were evident in the potential for synergistic effects, making these plant extracts promising candidates for natural diabetes therapies. Results showed that administration of STZ significantly reduced the levels of glucose transporters (Glut 1 and Glut 4) and AMPK in the rats' pancreatic homogenates.

The observed reduction following the STZ administration is consistent with the impaired glucose transport and metabolic dysregulation observed in diabetes, where insulin resistance and deficient insulin signaling hamper the activity and expression of glucose transporters and metabolic regulators. Previous studies have reported that reduced AMPK activity further exacerbates metabolic disturbances by diminishing the cell's ability to respond to low energy states, thereby impairing glucose uptake and utilization (Lyons *et al.*, 2018; Szewczuk *et al.*, 2020). However, treatment with MPPE, GLE, and VAE at doses of 200mg/kgBW, 400mg/kgBW, and 600mg/kgBW respectively, significantly elevated the levels of Glut 1, Glut 4, and AMPK in the rats' pancreatic homogenates. This elevation suggests that these extracts can restore the expression and function of key proteins involved in glucose transport and metabolism. These extracts' ability to enhance Glut 1 and Glut 4 levels in the current research indicates an improvement in glucose uptake efficiency, which is crucial for lowering blood glucose levels.

Moreover, the increase in AMPK levels signifies a restoration of energy balance and metabolic regulation, potentially facilitating better glucose homeostasis and insulin sensitivity (Stein *et al.*, 2000; Hardie, 2004). Generally, Glut 1 is responsible for basal glucose uptake in most tissues (Klip*et al.*, 2019), while Glut 4 is the primary glucose transporter in muscle and adipose tissue, regulated by insulin (La Flamme *et al.*, 2005; Wang *et al.*, 2020). This investigation upholds that enhancing the levels of these transporters implies that the extracts may improve insulin sensitivity and glucose utilization in peripheral tissues (Hardie, 2004). Additionally, the increase in AMPK levels suggests that these extracts activate

AMPK pathways, promoting glucose uptake and fatty acid oxidation while inhibiting lipid synthesis, thus improving overall metabolic health. Furthermore, the present study compared the efficacy of single extract treatments with combined doses. The combined doses of MPPE, GLE, and VAE in equal ratios also caused a significant elevation of Glut 1, Glut 4, and AMPK levels, comparable to the effects of individual extracts. This finding indicates a potential synergistic or additive effect, where the combined action of the bioactive compounds from different extracts provides a more holistic approach to restoring metabolic balance. Interestingly, the similar trends observed among single and combined extract treatments across the test groups suggest that the active components in these extracts might share common metabolic pathways or mechanisms of action in enhancing glucose transporter expression and AMPK activity. This consistency underscores the effectiveness of the therapeutic effects of these plant extracts. Therefore, our finding in this study demonstrates that MPPE, GLE, and VAE extracts effectively counteract the adverse effects of STZ-induced diabetes by elevating the levels of Glut 1, Glut 4, and AMPK in pancreatic homogenates. We theorized that this improvement in glucose transport and metabolic regulation points to the potential of these extracts as powerful natural interventions for diabetes management. In furtherance, the results suggest that these extracts, whether used individually or in combination, can restore critical aspects of glucose homeostasis, likely through mechanisms involving the upregulation of glucose transporters and activation of AMPK pathways.

## Conclusion

This study has demonstrated the potential antidiabetic properties of *Musa paradisiaca* (plantain) peels, *Gongronema latifolium* (utazi), and *Vernonia amygdalina* (bitter leaf) ethanol leaf extracts in streptozotocin-induced diabetic male Wistar albino rats. Findings have shown that administration of these MPPE, GLE, and GAE significantly improved glucose homeostasis, amylase activity and glut1 and 4. Furthermore, combined doses of the extracts exhibited comparable efficacy to standard drug treatment (metformin).

## Recommendations

Based on the findings from this study, we recommend to:

1. Conduct further studies to elucidate the molecular mechanisms underlying the antidiabetic effects of the plant extracts, including their interactions with glucose transporters and signalling pathways.
2. Investigate the long-term effects of the extracts on diabetic complications and their safety profile through comprehensive toxicological studies.
3. Explore the potential synergistic interactions among the compounds present in the combined doses of the extracts to optimize their therapeutic efficacy.

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