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In vitro Hypoglycemic Effect and Antimicrobial Activity of Methanol Extract of Underutilized Leafy Vegetable (*Ipomoea batatas* leaf)

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Abstract

Diabetes mellitus is a chronic condition characterized by major derangements in glucose metabolism and abnormalities in fat and protein metabolism. Diabetes and infection are among the serious health challenges. This study evaluated the in vitro and hypoglycemic effect and antimicrobial properties of methanol extract of *Ipomoea batatas* leaf. The antimicrobial activity was done using agar disc diffusion methods and the *in vitro* hypoglycemic activity was done using Alpha-Glucosidase Inhibitory activities assay, Lipase activity assay, Glucose absorption Capacity assay, Glucose diffusion retardation Index. The result of the percentage alpha glucosidase inhibition ranged from 35.70 to 56.33%. The percentage alpha amylase inhibition ranged from 40.96 to 65.15%. The extract showed significant ($p<0.05$) dose dependent inhibition of alpha glucosidase and alpha amylase. The extract possess inhibition of lipase activity with percentage inhibition ranging from 72.35 to 81.57%. Though there was non-significant ($p<0.05$) difference in the various concentrations of the extract on inhibition of lipase activity. The result of the glucose absorption capacity (0.30 to 2.25) was observed to be dose dependent. The glucose diffusion retardation index was time dependent. The antimicrobial susceptibility screening of extracts showed a zone of inhibition that ranged from 2.70 to 15.73mm. The study showed that *Ipomoea batatas* leaf could be used in the treatment and management of diabetes and infections.

Keywords: Diabetes, antibacterial, hypoglycemic, *Ipomoea batatas*, effect.

INTRODUCTION

The search for novel compounds to alleviate or ameliorate some diseases has been on the increasing sides ever-since health challenge became a global threat to man-kind. Recently and on-going researches has focused on developing new leads from medicinal plants which are major sources of some secondary metabolites that are of health benefits.

Diabetes mellitus is a chronic condition characterized by major derangements in glucose metabolism and abnormalities in fat and protein metabolism. There are several forms of diabetes while spontaneous diabetes is the major form in the West, malnutrition-related diabetes is a major form in Africa and Asia.

Infectious diseases including urinary tract disease (UTIs) and wound infection that are caused by multi-resistant organisms and oxidative stress caused by the presence of free radicals resulting in the imbalance within the body system has been a growing concern to scientists as it affects and has significant impact on the economy (Hadadi et al., 2020).

The abundance of medicinal plants in our environment and the realization that they possess bioactive ingredients with therapeutic values has made the need for their studies imperative. A scientific scrutiny of the ethnobotanical information, chemical constituent and the therapeutic application of plant portend great opportunities for humanity. Plants play role as vessels with chemical constituents that possess pharmacological potentials and their use in management of disease has been a common practice in developing countries for ages. Research into medicinal plants will assist in ascertaining the efficiency of the flora as a remedy, and extend our frontiers of

knowledge, their active principle, dosage and administration (George and Uwakwe, 2014).

Sweet potato, *Ipomoea batatas* (L.) Lam, is a perennial crop which belongs to the morning glory family or Convolvulaceae (Senanayake et al., 2013). Sweet potato Leaf is one of the medicinal plant though underutilized, numerous health-promoting phytochemicals are found in SPL, regular intake of the leaves may provide good health benefits (Islam, 2014).

Ipomoea batatas L. has been indicated to possess a broad folklore relevance in anti-inflammatory, anti-fertility, anti-carcinogenesis, anti-mutagenicity and anti-diabetes properties, including promoting stable blood sugar levels; they are rich in polyphenols such as caffeoylquinic acid derivatives (Konczak-Islam et al., 2003). Considering that the Prevalence of diabetes and infections as the leading cause of death worldwide, it is imperative to evaluate the hypoglycemic effect and antimicrobial properties of methanol extract of underutilized leafy vegetable (*ipomoea batatas* leaf)

MATERIALS AND METHODS

Plant Collection

The leaf of *Ipomoea batatas* was collected from a garden in Owerri, Imo State. The plant was identified by a botanist at the Department of Biology and Environmental Microbiology Federal Polytechnic Nekede, Imo State. The fresh sample of *Ipomoea batatas* leaf was washed with distilled water and then allowed to get dried in a dust-free environment for ten days. The dried sample was blended using an electronic blender.

Preparation of Plant extract

One thousand gram (1000g) of powdered leaves was macerated in 2.5L of 95% methanol at room temperature for 72h. It was continuously mixed and then filtered using a filter paper (Whatman size No.1). The filtrate was concentrated using a water bath at 45^0c. The extract of *Ipomoea batatas* leaf was refrigerated in air tight container.

Hypoglycemic Assay

The alpha-glucosidase inhibition assay was determined using a method described by Sancheti et al (2010). The lipase inhibition assay was determined according to the method described by Lewis and Liu (2012). Glucose adsorption capacity and Glucose diffusion retardation index (GDRI) were determined by the method of Ou et al. (2001)

Antimicrobial Assay

Test Bacteria

Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis and Escherichia coli were obtained from Federal medical center, Owerri, Imo State, Nigeria. The organisms were isolated from clinical specimens of the hospitalized patients and maintained on Nutrient Broth for 24 hours.

Standardization of Test Bacteria

The test bacteria was standardized by using a sterile wire loop, to pick 3–5 pure cultures of the test microorganism and emulsified in 3–4 ml of sterile physiological saline. The turbidity reading of the 0.5 McFarland Standard will be recorded as Absorbance in a Spectrophotometer at 540 nm, while the turbidities of the test organisms was adjusted to match the absorbance of the 0.5 McFarland standard at the same wave length, using physiological saline. NB: 0.5 McFarland contains $1.5x10^8$ cfu/ml.

Antimicrobial Susceptibility Test

The antibacterial activities of the extracts against the test bacteria was evaluated by modified disc diffusion methods (Adeyemi et al., 2015). Exactly 25 µl of 0.5 McFarland standardized suspension of test bacteria ($1.5x10^8$ cfu ml^{-1}) were cultured onto the Mueller-Hinton plates by pour plate method. Exactly 50µl of the extracts was used to impregnate the 6mm filter paper discs and placed on two portions of the agar plate. The Inhibition zone diameters of the various plates was measured and recorded in millimeters. All experiments were done in triplicates. Negative controls was set up with sterile physiological saline and positive controls was set up using 50 µg/ml Ciprofloxacin.

Characterization and Identification of bacteria

Identification of the test bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests. The characterization of the bacterial isolates was performed, by employing Gram staining reaction, Catalase test, Citrate test, Sugar fermentation test, Motility test, Oxidase test, Indole test, Methyl Red and lactose fermentation as described by Bergey's Manuel of Determinative Bacteriology, 9th edition.

Statistical analysis

Statistical analysis of the data was carried out with SPSS version 22.0 using One Way Analysis of Variance (ANOVA). The statistically analysed data was reported as Mean+SEM. Significant difference will be accepted at 95% confidence level of probability ($P < 0.05$).

RESULTS AND DISCUSSION

In vitro hypoglycemic potentials of the extract

The results of the alpha glucosidase inhibition, alpha amylase inhibition, lipase activity inhibition, glucose absorption capacity and glucose diffusion retardation index of methanol extract of Ipomoeo batatas leaf are shown in figures 1-5. The result of the percentage alpha glucosidase inhibition ranged from 35.70 to 56.33%. The percentage alpha amylase inhibition ranged from 40.96 to 65.15%. The extract showed significant ($p<0.05$) dose dependent inhibition

of alpha glucosidase and alpha amylase. The extract possess inhibition of lipase activity with percentage inhibition ranging from 72.35 to 81.57%. Though there was non-significant ($p<0.05$) difference in the various concentrations of the extract on inhibition of lipase activity. The result of the glucose absorption capacity (0.30 to 2.25) was observed to be dose dependent. The glucose diffusion retardation index was time dependent.

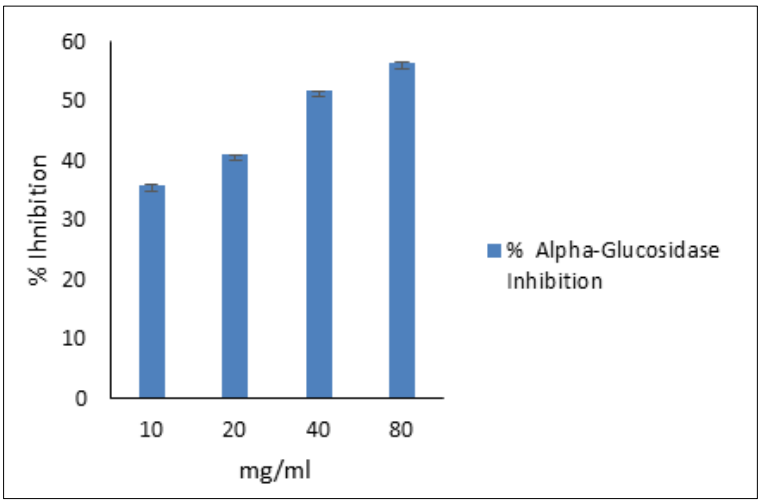

Figure 1: Effect of methanol extract of Ipomoeo batatas leaf on percentage inhibition of alpha amylase
Values are mean±SD of triplicate determination

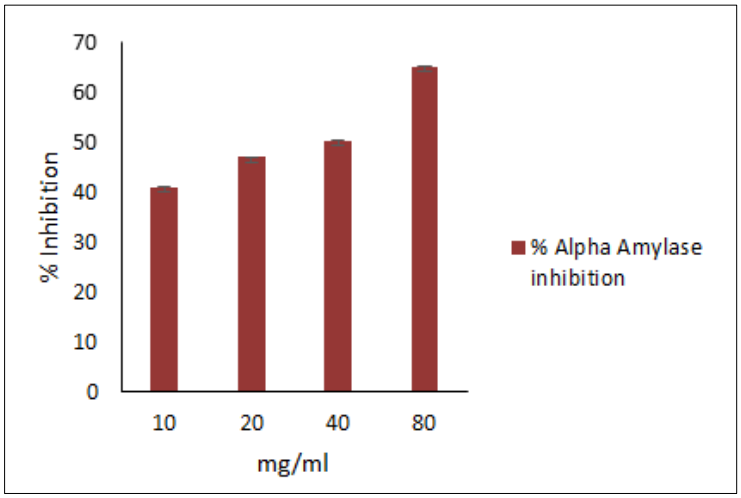

Figure 2: Effect of methanol extract of Ipomoeo batatas leaf on percentage inhibition of alpha glucosidase
Values are mean±SD of triplicate determination

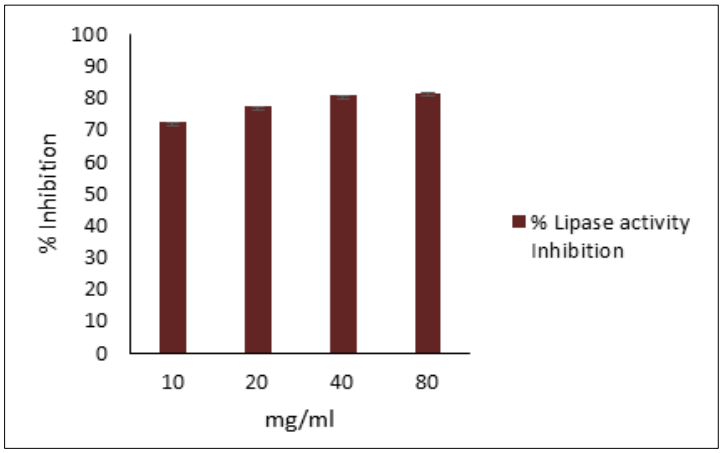

Figure 3: Effect of methanol extract of Ipomoeo batatas leaf on percentage inhibition of lipase activity
Values are mean±SD of triplicate determination

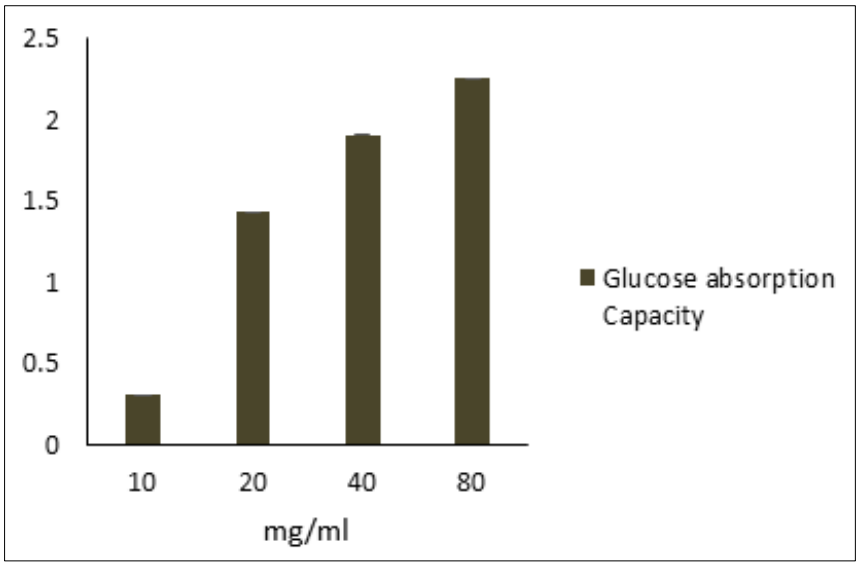

Figure 4: Effect of methanol extract of Ipomoeo batatas leaf on glucose absorption capacity
Values are mean±SD of triplicate determination

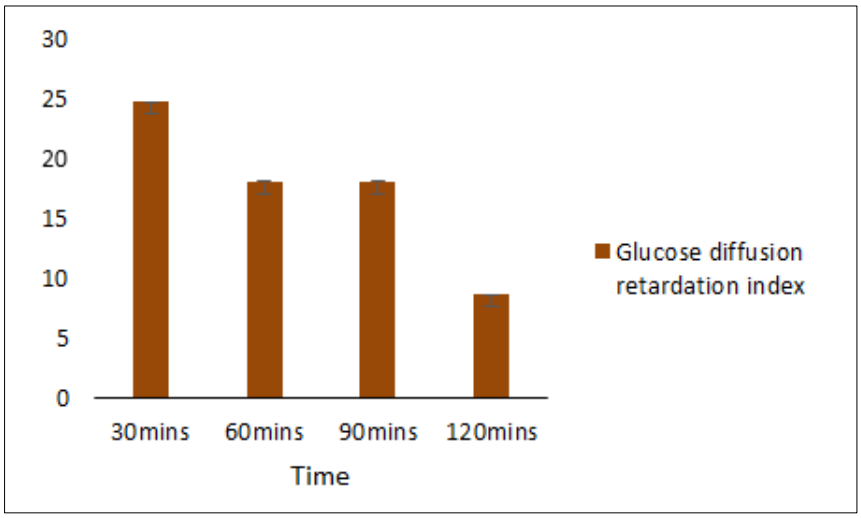

Figure 5: Effect of methanol extract of Ipomoeo batatas leaf on glucose diffusion retardation index
Values are mean±SD of triplicate determination

Antibacterial activities of the extract

The result of the morphological and biochemical identifications of the various isolates of the test bacteria was shown in Table 1. The antimicrobial susceptibility screening of extracts was shown in Table 2. The zone of inhibition ranged from 2.70 to 15.73mm.

Table 1: Morphological and Biochemical Identifications of the Various Isolates of the Test Bacteria.

Isolate	Form	Surface	Colour	Gram	Cat	Mot	Ind	MR	Cit	Lac	Glu	Identity
PA	Circular	Smooth	Whitish	- Rod	+	+	-	-	+	AG	A	*Pseudomonas aeruginosa*
ST	Circular	Smooth	Greyish/ white	-Rod	+	+	-	+	-	-	+	*Salmonela typhimurium*
SA	Circular	Smooth	Yellowish	+ cocci	+	+	-	+	-	AG	AG	*Staphylococcus aureus*
EC	Circular	Smooth	Whitish	-Rod	+	+	+	+	-	+	+	*Escherichia coli*
EF	Circular	Smooth	Cream	+coccus	-	-	-	-	-	+	+	*Enterococcus faecalis*

Gram: Gram reaction, Cat: Catalase test, Mot: Motility test, Ind: Indole test, MR: Methyl-red test, Cit: Citrate Utilization test, Lac: Lactose Fermentation, Glu: Glucose Fermentation

Table 2: Preliminary Antimicrobial Susceptibility Screening of Extracts

Test Bacteria	Zone of inhibition diameter (mm)
Gram positive bacteria	
Staphylococcus aureus	2.70±0.00[a]
Enterococcus faecalis	11.17±0.76[b]
Gram negative bacteria	
Salmonella typhi	11.00±1.00[a]
Escherichia coli	15.73±1.74[b]
Pseudomonas aeruginosa	14.63±1.76[c]

Values are mean±SD of triplicate determination. Different alphabets in each column differs significantly at p< 0.05

DISCUSSION

The methanol extract of *Ipomoea batatas* leaf (MEIBL) was evaluated for its *in vitro* hypoglycemic activities using Alpha-glucosidase inhibition activity, Alpha amylase inhibition activity, Lipase inhibition activity, glucose absorption capacity, and glucose diffusion retardation index. The antimicrobial efficacy of the extract was also evaluated against selected clinical isolates. Diabetes mellitus was characterized by hyperglycemia and glucose intolerance, either due to the relative deficiency in insulin secretion or impaired effectiveness of insulin's action to enhance glucose uptake (Sudan and Karthikeyan, 2022). The methanol extract of *Ipomoea batatas* leaf inhibited the activity of α-amylase, α-glucosidase, and lipase. Reducing post-prandial hyperglycemia is one treatment strategy for the management of diabetes. α-Amylase is one of the main enzymes in the human body that is responsible for the breakdown of starch into more simple sugars. α-Amylases hydrolyze complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolyzed by α-glycosidase to monosaccharides which are absorbed through the small intestines into the hepatic portal vein and increase postprandial glucose levels" (Uddin et al., 2014). The digestive tracts amylase and glucosidase, which break down carbohydrates, are inhibited in order to delay the absorption of glucose. Inhibitors of these enzymes cause slower carbohydrate digestion and longer total carbohydrate digestion times, which slows down the rate of glucose absorption and dampens the post-prandial rise in plasma glucose (Awad et al., 2012; Kifle et al., 2020). The MEIBL produced a significant inhibition of α-amylase, α-glucosidase and lipase enzymes.

A helpful in vitro metric for predicting the impact of extracts on the delay in glucose absorption in the gastrointestinal system is the glucose diffusion retardation index (GDRI) (Maktoof et al., 2019). The glucose diffusion retardation index was used to ascertain the inhibitory impact of the MEIBL on the transport of glucose across a membrane. In this study, the GDRI appears to have saturated after 120 minutes of incubation which was in line with the report of Cura et al. (2021). The findings showed that the extracts could bind to glucose efficiently even at low glucose concentrations, hence lowering the quantity of available glucose in the small intestine. Based on the aforementioned findings, MEIBL have a high retardation index and may contribute to the hypoglycemic impact (Maktoof et al., 2019). Glucose absorption capacity of MEIBL was proportional to the concentration of glucose. As the concentration increased, glucose adsorption capacity of MEIBL increased significantly. Dietary fibers can bind glucose (Harish et al., 2014), and the glucose absorption capacity of the extract could be as a result of it dietary fibers.

Antimicrobial activity of MEIBL was observed against gram negative and gram-positive bacteria. The result shows that the MEIBL had some significant inhibitory potential on both gram negative and gram-positive organisms. The findings of this study is in agreement with the report of Islam (2008) where the lyophilized leaf powder of sweet potato strongly suppressed the growth of Gram positive and Gram negative bacteria. Ayeleso et al. (2016) reported that acetone and ethanol extracts of sweet potato leaves showed antimicrobial activity against *Salmonella typhimurium* and *Pseudomonas aeruginosa* respectively. Hence, the outcome of this study suggests that *Ipomoea batatas* could be a potential source for antimicrobial agent.

CONCLUSION

Findings from the present study highlighted the hypoglycemic and antibacterial activities of *Ipomoea batatas* leaf as assessed by various in-vitro methods. The study provided insight by which the extract of *Ipomoea batatas* leaf may contribute in lowering the postprandial glucose levels. The hypoglycemic effect exhibited by the extracts was mediated by inhibiting alpha glucosidase, alpha amylase, lipase activity, as well as increasing glucose adsorption and decreasing glucose diffusion rate. The extract of *Ipomoea batatas* leaf also revealed appreciable zone of inhibition against the test bacteria isolates.

Conflict of interest

The authors declare that no conflict of interest exists with respect to this work.

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