

Research Paper:

Two Extracts From *Manihot Esculenta* Leaves Efficiently Inhibit α -Glucosidase and α -Amylase: A New Approach for the Management of Diabetes



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ABSTRACT

Background: In Nigeria, the leaves of *Manihot esculenta* (Crantz) are eaten as vegetables. The leaves are rich in phytochemicals, valuable and natural sources of antioxidants, and are highly useful for human health and disease prevention. This study was aimed to evaluate the in vitro inhibitory effects of two extracts derived from the plant leaves on α -glucosidase and α -amylase. The total flavonoid and phenolic contents of the extracts were also assessed.

Methods: The leaves of *M. esculenta* were processed and extracted with ethanol and acetone. The extracts were evaluated for their α -glucosidase and α -amylase inhibitory activities. Also, their total flavonoid and phenolic contents were determined, using standard in vitro assays.

Results: The ethanol extract exhibited a higher α -glucosidase and α -amylase inhibitory activity than the acetone extract, which positively correlated with their total flavonoid and phenolic contents. In addition, the ethanol extract strongly inhibited the α -glucosidase activity (IC₅₀: 0.77±0.03 mg/mL) than Acarbose (IC₅₀: 1.52±0.14 mg/mL). However, both extracts of ethanol (IC₅₀: 1.29±0.02 mg/mL) and acetone (IC₅₀: 1.66±0.05 mg/mL) were less effective in inhibiting α -amylase compared with that of Acarbose (IC₅₀: 0.99±0.02 mg/mL). Comparatively, the percent inhibition of the extracts and Acarbose were in this order: ethanol extract > Acarbose > acetone extract.

Conclusion: The results suggest that the extract of *M. esculenta* leaves have anti-hyperglycemic effects. They are potential alternative antioxidants that could be used to reduce oxidative stress in the body and in the management of diabetes.

Keywords: *Manihot esculenta*; α -amylase; α -glucosidase; Flavonoids; Phytochemicals

Introduction

Diabetes Mellitus (DM) is a heterogeneous group of metabolic disorders, affecting the lipid, carbohydrate and protein metabolism. It is characterized by hyperglycemia and deficiencies in insulin secretion, and/or insulin resistance [1]. Diabetes is believed to be the most prevalent cause of morbidity and mortality

in low to middle-income countries than in rich nations [2]. It is projected that the global incidence of diabetes is escalating yearly, thereby leading to a major burden on the health sector, mainly in developing countries, with the urban population having the highest prevalence [3]. Recent data indicate that about 150 million people worldwide are diabetic, which is expected to double by the year 2025 [4, 5].

The complications arising from diabetes correlate with persistently elevated serum glucose levels, arising from free radicals' production and leading to oxidative stress in tissues [6, 7]. In this context, antioxidants have been effectively employed to lower the severity of DM complications. The effect of diabetes, like most chronic diseases, on both the individuals and society are extensive in terms of personal income, general productivity and burden on the healthcare system [8].

Effective treatment for diabetes is mainly through the use of oral hypoglycemics, such as thiazolidines and sulfonylureas; however, they have side effects, such as hypoglycemia and atherogenesis [9]. That is why efforts are made to search for alternative treatments, such as herbal medicines with low cost and side effects. This has prompted studies, investigating herbal extracts and their efficacies in the management of DM. These efforts have led to the identification of various plant extracts with hypoglycemic effects in animal models and humans [10].

Reducing postprandial blood glucose levels through the inhibition of carbohydrate hydrolyzing enzymes has been one of the therapeutic strategies toward the management of diabetes [11]. Hydrolysis of dietary carbohydrate, or starch, is a major source of blood glucose in humans. This breakdown process occurs through the actions of α -glucosidase and α -amylase in the intestine. Thus, inhibiting these enzymes can drastically lower the postprandial surge of blood glucose, and provide for an effective management of type II diabetes [12].

Manihot esculenta Crantz (*M. esculenta*) known as Cassava in Nigeria, is a tropical perennial plant of the Euphorbiaceae family. Cassava is a major staple in most tropical countries, including Nigeria. The roots of this plant are a major source of carbohydrates, while the leaves are consumed as vegetables. They are rich in vitamins A and C, proteins and minerals, particularly Mg, Zn, Fe and Mn [13, 14]. Also, the leaves are rich in phytochemicals, which are valuable as natural antioxidants, and their consumption has been linked to reducing the risks of cancer, hypertension, diabetes, obesity and heart diseases [14, 15]. The leaves also contain some toxins, such as cyanides; therefore, caution must be exercised in their consumption [16, 17]. The toxicity issue affects the nutritive value and acceptability of the leaves, which could be lowered by drying, boiling and pounding for long periods. It has been stated earlier that when the leaves of cassava are pounded or allowed to stand for a period of 5 hour under shade or when washed thoroughly with water three times, between 72% and 99% of the cyanide content could be reduced [13, 18].

Medicinally, the leaves extract is used in the management of various disorders, such as rheumatism, fever, diarrhea, headache and loss of appetite. The anti-inflammatory and antimicrobial properties, and the analgesic and anthelmintic effects of Cassava leaves have been reported previously [13, 19-21]. However, there are no reports available on the effects of the Cassava leaves extract on α -glucosidase and α -amylase. The aim of this study was to evaluate the in vitro inhibitory effects of the two extracts derived from Cassava leaves on α -glucosidase and α -amylase, and the total phenolic contents.

Material and Methods

Chemicals & Reagents: Foline Ciocalteu (FC) reagent, Gallic acid, sodium carbonate anhydrous, sodium nitrite, aluminum chloride anhydrous, sodium hydroxide, 3,5 Dinitro Salicylic Acid (DNSA), porcine pancreatic α -amylase and yeast α -glucosidase and catechin were purchased from Sigma-Aldrich (St. Louise, USA). Other chemicals were of analytical grades and obtained from Merck (Darmstadt, Germany).

Plant Collection & Processing: Fresh leaves of *M. esculenta* were collected from site III of the Delta State University and their authenticity were confirmed at the Herbarium of Plant Science Department, University of Benin, Benin City, Nigeria (Voucher # UBHM372). The leaves were thoroughly washed three times with water, air-dried at room temperature for ten days, and were powdered. The leaves (50 g) were extracted in 200 ml of ethanol at room temperature for 24hr. This process was repeated for another 24 h and the extract was concentrated at 40°C under vacuum to dryness and was refrigerated until further use. The extraction with acetone was performed similarly to the procedure used for the ethanol and the material was refrigerated similarly. The dried extracts were dissolved in Dimethyl Sulfoxide (DMSO) to obtain the concentrations required for the subsequent experiments.

α -Glucosidase Inhibition Assay: The α -glucosidase inhibitory effect of *M. esculenta* leaf extracts was achieved following the procedure of Kwon et al. [22] and as described by Okoro et al. [23]. Acarbose was used in this assay as the positive control. The results are stated in percentage as derived from the equation shown below, under α -amylase inhibition assay.

α -Amylase Inhibition Assay: This test was made by the modified method of McCue et al. [24] and as described by Okoro et al. [23]. Acarbose was used in this assay as the positive control. The α -amylase inhibitory

effect was calculated using the following equation: % Inhibition = $[(Ac - Ae) / Ac] \times 100$; where: Ac=absorbance of the control and Ae=sample absorbance.

Total Phenolic Content: The total phenolic content of the *M. esculenta* leaf extract was evaluated spectrophotometrically, using the Folin-Ciocalteu's reagent as described by Singleton et al. [25]. Then, the total phenol content was calculated as the Gallic acid equivalent.

Total Flavonoid Content (TFC) The total flavonoid contents of both extracts were evaluated by the aluminum chloride colorimetric method of Chang, et al. [26]. The TFC was determined based on a calibration curve, in which quercetin was used in the calibration curve and the TFC was expressed as mg of quercetin equivalent per gram *M. esculenta* (mg QCE/g).

Statistical Analysis: The data were statistically analyzed by One-way Analysis of Variance (ANOVA) followed by Sidak's test in Graphpad Prism, version 6.0 (Graph Pad Software, San Diego, CA, USA), expressed as Mean±Standard Deviation (SD) at a confidence limits of 95% ($P \leq 0.05$).

Results

α -Glucosidase Inhibitory Studies: The results of α -glucosidase inhibitory effects are shown in Figure 1. The results revealed a concentration dependent increase as percent inhibition for both acetone and ethanol extracts versus the positive control drug, Acarbose. The α -glucosidase inhibitory activity, expressed as a function of the extract concentration, displayed the inhibition rang-

ing from 13.57 ± 1.00 to 78.83 ± 4.52 percent at 0.25 and 2.00 mg/mL, respectively, for the ethanol extract. Also, the percent inhibition by the acetone extract was from 6.49 ± 1.27 to 70.77 ± 6.87 percent at 0.25 and 2.00 mg/mL, respectively. The reference drug, Acarbose, demonstrated inhibition values of 7.60 ± 0.77 and 64.56 ± 3.63 at 0.25 and 2.00 mg/mL, respectively. Consequently, the highest percent inhibition was noted for the ethanol extract. This was further demonstrated by the lower IC₅₀ value (0.77 ± 0.03 mg/mL), while the acetone extract resulted in a minimal inhibition with the IC₅₀ value of 1.46 ± 0.17 mg/mL (Table 1). The extracts and positive control displayed percent inhibition in the following decreasing order: ethanol extract > Acarbose > acetone extract. Thus, the ethanol extract displayed a significantly higher inhibition percent than the acetone extract ($P < 0.05$).

α -Amylase Inhibitory Studies: The results of α -amylase inhibitory effects are shown in Figure 2. The percent α -amylase inhibition results, expressed as a function of extract concentration, shows the inhibition trend of both extracts of Cassava leaves and Acarbose (standard). The percent inhibition ranged from 10.80 ± 0.74 to 65.81 ± 2.53 at 0.25 and 2.00 mg/mL for the ethanol extract, respectively. The percent inhibition for the acetone extract ranged from 6.42 ± 1.11 to 55.93 ± 1.85 at 0.25 and 2.00 mg/mL, respectively. Similarly, the standard drug displayed inhibition values of 9.70 ± 1.31 and 86.07 ± 2.14 at 0.25 and 2.00 mg/mL, respectively. Thus, the highest percent inhibition was shown by the ethanol extract (IC₅₀ = 1.29 ± 0.02 mg/mL) and the least inhibition was recorded for the acetone extract (IC₅₀ = 1.66 ± 0.05 mg/mL), as represented by the data in Table 1. Although, the positive control drug displayed the highest inhi-

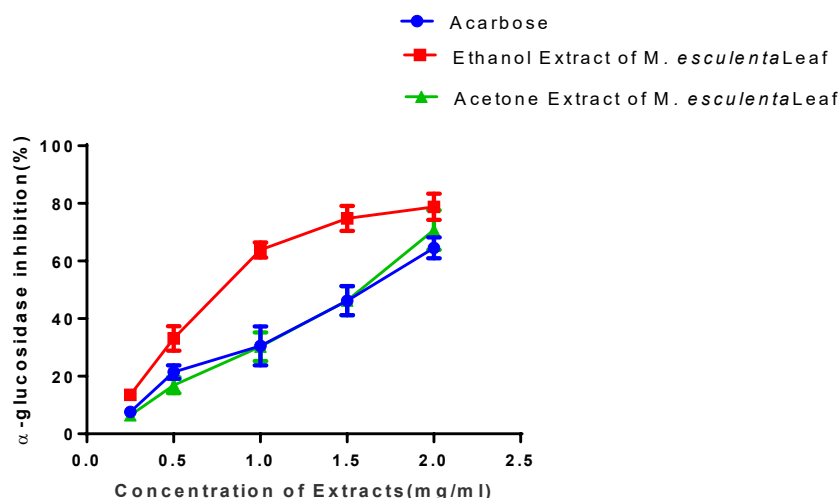


Figure 1. α -Glucosidase inhibitory activity of Acarbose and two extracts of *M. esculenta* leaves

* Values represent Mean±Standard Deviation; N=3

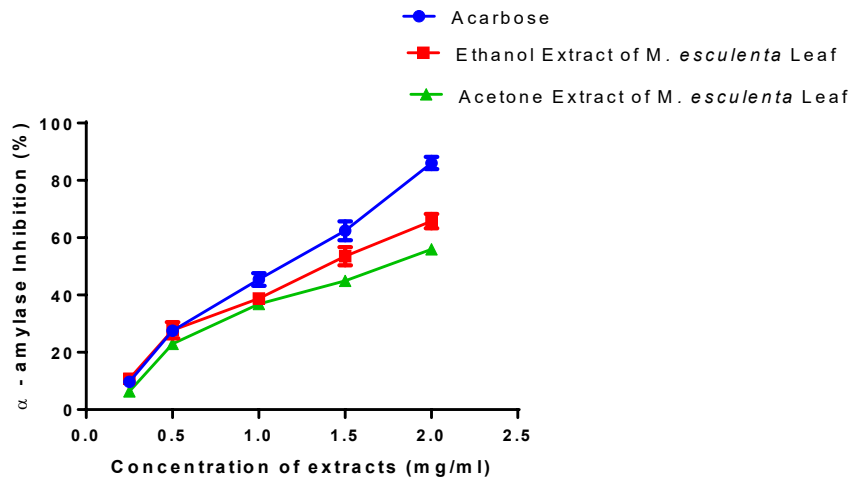


Figure 2. α -Amylase inhibitory activity of Acarbose and both extracts of *M. esculenta* leaves

* Values represent Means \pm Standard Deviations; N=3.

tion percent overall, the ethanol extract was more effective than the other extract, as it displayed a significantly higher percent inhibition of α -amylase than the acetone extract did ($P<0.05$).

Total Phenolic and Flavonoid Contents: Figure 3 presents the Total Phenolic Content (TPC) of the two extracts of *M. esculenta* leaves, expressed as mg Gallic acid equivalents per grams of the extract. The crude ethanol extract of *M. esculenta* leaves contained 22.81 ± 0.82 mg Gallic acid equivalent per gram of the extract. The acetone extract produced 15.13 ± 0.58 mg Gallic acid equivalent per gram of extract. Thus, both extracts displayed varying contents of total phenol with the ethanol extract producing a significantly higher content than did the acetone extract ($P<0.05$). Similarly, the influence of

the solvents on the Total Flavonoid Content (TFC) was comparable to that of the TPC. The maximum TFC of 10.36 ± 0.64 expressed as the quercetin equivalent (mg QCE/g) was found for the ethanol extract, while lower (7.71 ± 0.45) content was observed for the acetone extract. The ethanol extract exhibited the highest contents of both phenols and flavonoids between the two extracts.

Discussions

Diabetes mellitus is a metabolic disease characterized by hyperglycemia. A carbohydrate-rich diet usually leads to a sharp rise in blood glucose level, because the complex carbohydrates from food are rapidly absorbed, aided by α -amylase and α -glucosidase enzymes in the intestine. These enzymes break down carbohydrates

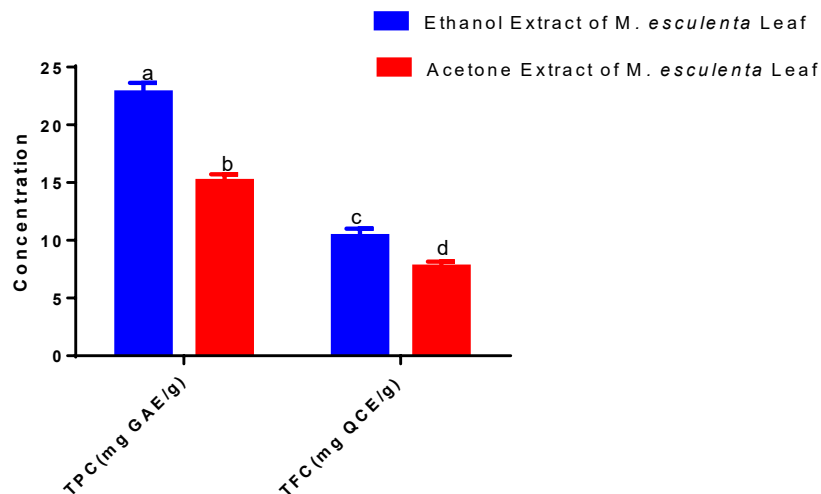


Figure 3. Total phenolic and Flavonoids contents of two extracts of *M. esculenta* leaves. * Values represent Means \pm Standard Deviations; N=3; * Bars with letters differ significantly ($P<0.05$)

Table 1. The IC50 values for the inhibitory activity of the extracts against yeast α -glucosidase and porcine α -amylase

Standard & Extract	IC50 (mg/ml)	
	α -Glucosidase	α -Amylase
Acarbose	0.99±0.02	1.52±0.14
Ethanol	1.29±0.02	0.77±0.03
Acetone	1.66±0.05	1.46±0.17

into simple, absorbable monosaccharides. By inhibiting these enzymes, the absorption of glucose is delayed, and the surge in blood glucose is precluded, thus making this a different therapeutic approach in the management of diabetic patients [22].

The regularly used synthetic drugs, such as Miglitol and Acarbose have been broadly used in the management of DM as standard, clinical drugs. They act by inhibiting α -amylase and

α -glucosidase, thus lowering the blood glucose level. However, they have adverse effects, such as liver toxicity, flatulence and diarrhea [11]. Hence, using plant-based inhibitors of α -glucosidase may be a promising therapeutic approach for the treatment of patients with type II diabetes [27]. This study was undertaken to evaluate the in vitro α -glucosidase and α -amylase inhibitory effects, plus their total phenolic and flavonoid contents.

The inhibitory effect of α -amylase of the two extracts is likely to be due to the flavonoid and phenolic contents. The polyphenols have been reported to reduce oxidative stress and inhibit carbohydrate hydrolyzing enzymes by binding to proteins [28]. Flavonoids are polyphenolic compounds that have been described to have both α -glucosidase and α -amylase inhibitory activity [29, 30]. The inhibitory effects of flavonoids have been linked to interactions that obstruct the reaction between starch and α -amylase, thus inhibiting the digestion of starch [31]. The results of this study are in agreement with earlier reports that suggested a positive association between the flavonoid content, total polyphenols, and the ability to inhibit α -amylase and α -glucosidase [29, 32].

The findings of this study confirmed the inhibitory effects of the extracts on α -glucosidase. This study also demonstrated a trend similar to that of α -amylase where the ethanol extract gave the lowest IC50 value, suggesting a higher inhibitory activity of the extract against the enzymes compared to those for the acetone extract. Overall, both extracts demonstrated strong inhibitory activities similar to Acarbose. Thus, it may be inferred

that the extracts, especially the ethanolic one, would be a promising plant source of hypoglycemic agent with lower side effects than Acarbose for the management of diabetic patients.

A review of literature revealed that the leaves of *M. esculenta* plant are rich in terpenes, flavonoids, tannins, alkaloids, saponins and phenols, all of which are believed to be effective inhibitors of α -glucosidase [33]. This study found a weak α -amylase and a considerable α -glucosidase inhibitory activity by both extracts tested. An obvious association between polyphenols and the anti-diabetic effects of herbal extracts has previously been reported [34]. Similarly, studies have revealed that the antioxidant properties of plants are directly proportional to their phenolic contents. The α -glucosidase and α -amylase inhibitory effects of flavonoids and tannins have also been demonstrated [35]. In the current study, both extracts of *M. esculenta* leaves displayed varying contents of total phenolic agents with the ethanol extract, having a significantly higher phenolic content than the acetone extract. This finding is not consistent with an earlier report by Suresh, et al. [21], where a higher total phenolic content was found in the acetone extract of *M. esculenta* leaves than in the ethanolic extract, but was consistent with those reported by Khan, et al. [36].

Phenolic compounds are known secondary metabolites which act as antioxidants as a result of their ability to release hydrogen, quench singlet oxygen, and act as chelators of metal ions [37, 38]. Oxidative stress plays a major role in the development of diabetic complications, and free radicals are known to be generated in diabetes due to glucose oxidation and the successive oxidative breakdown of glycosylated proteins [39].

Flavonoids are the secondary metabolites with antioxidant activity, and their potency depends upon the number and position of the free OH groups [40, 41]. It is suggested that genetic diversity, environmental, biological, and seasonal differences largely affect the flavonoid contents in plants [42]. Flavonoids are a group of poly-

phenolic compounds with a benzo- γ -pyrone structure, and are beneficial in various ways to the plants [43]. The usefulness of flavonoids as antimicrobial, anti-angiogenic, antiulcer, anti-arthritic and anticancer agents have been reported [44]. The preventive roles of beverages and phenolic-rich foods against diseases, like cancer, inflammation, heart disease, neurodegenerative diseases, arthritis, immune related diseases, and diabetes have also been confirmed [45]. In this study, the presence of flavonoid and phenolic compounds in the extracts of *M. esculenta* leaves correlated with the health values of the plant, particularly their anti-hyperglycemic effects.

Limitations of the Study: The isolation of active components responsible for the observed inhibitory activities of the extracts against α -glucosidase and α -amylase, and its structural elucidation that might be useful to developing new anti-diabetic agents were not carried out. This was because the study was aimed at an in-vitro assessment of α -glucosidase and α -amylase inhibitory activity of the ethanol and acetone extracts of *M. esculenta*.

Recommendations for Future Research: Based on our results, we recommend that further research on the extracts be undertaken to elucidate the molecular components responsible for the observed inhibition of the tested enzymes.

Conclusion

The in vitro α -glucosidase and α -amylase inhibitory effects of both extracts of *M. esculenta* leaves, as investigated in this study, indicated that they possessed significant inhibitory activities against both enzymes dose dependently, with the ethanol extract demonstrating the most effective inhibition of α -glucosidase and α -amylase. The inhibitory effects of the extracts were also positively related with their total flavonoid and phenolic contents. Thus, the extracts have anti-hyperglycemic properties and are potential candidates to be used in the management of diabetes and the resultant oxidative stress.

Ethical Considerations

Compliance with ethical guidelines

The technical support provided by Mrs. Edith Omozefe Okoro of Emmanuel Medical Laboratory in Abraka, Nigeria, is greatly appreciated. This study did not use animals or human subjects; however, the Ethical principles in the use of chemicals and plants were followed as set by Delta State University, Abraka, Nigeria.

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