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Antioxidant and Alpha Amylase Inhibitory Potential of Methanol Extract of Unripe *Musa paradisiaca* Fruit with Husk

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ABSTRACT

Diabetes mellitus is a leading cause of death in adults. The study aimed at evaluating the antioxidant and alpha amylase inhibitory potential of methanol extract of unripe *Musa paradisiaca* Linn fruit with husk. Ferric reducing antioxidant power (FRAP) scavenging activity, diphenyl -1- picrlhydrazyl (DPPH) free radical scavenging activity, alpha amylase inhibitory potential and the mode of inhibition of methanol extract of unripe *Musa paradisiaca* Linn fruit with husk were determined using standard methods. The DPPH IC_{50} of the methanol extract of unripe *Musa paradisiaca* Linn fruit with husk was comparable with the standard; ascorbic acid. The percentage inhibition of methanol extract of unripe *Musa paradisiaca* Linn fruit with husk at 2.50 mg/mL and 10.0 mg/mL were greater than 50% (67% and 73%). Acarbose at 1.25 mg/mL, 2.50 mg/mL and 10 mg/mL had a percentage inhibition of 61%, 67% and 70% respectively. The maximum velocity (V_{max}) of the enzyme in the presence of the unripe *Musa paradisiaca* with dried husk extract (inhibitor) was 0.8 mM/min and it was also 0.8 mM/min in the absence of inhibitor (no plant extract). The Michealis-Menten constant (K_m) of the enzyme in the presence of the inhibitor was 1.3 mg/mL, while it was 0.7 mg/mL in the absence of the inhibitor. This study concluded that the methanol extract of unripe *Musa paradisiaca* Linn fruit with husk has high antioxidant capacity. It also inhibits alpha amylase in a competitive way. It can therefore be used to treat diabetes mellitus.

Keywords: Alpha amylase, Diabetes, Antioxidant, Oxidative stress, Unripe *M. paradisiaca*

Introduction

Unpaired electrons are present in molecules known as free radicals.[1] They are highly reactive. Reactive oxygen species (ROS) and reactive nitrogen species are the two main categories of these free radicals (RNS). Normally, biological systems produce them. Increased production of free radicals occurs during long term stress conditions, stressful exercise, use of stimulants, improper diet and excessive exposure to ultraviolet radiation.[2] Free radicals become harmful in biological systems when they are overproduced.[1]

Hyperglycemia, one of diabetes mellitus' main features, produces reactive oxygen species.[3] The development of diabetes is considerably aided by overproduction of reactive oxygen species.[4] In actuality, oxidative stress is one of the main factors that contribute to diabetic complications.[5] Reactive oxygen species (ROS) destroy β-cells because they are low in antioxidant enzymes.[6] The free radical is generated from autoxidation of glucose, polyolyl pathway, mitochondrial respiratory chain and hexosamine pathway.[4] Thus, scavenging for free radicals will prevent the development of complications arising from diabetes. Antioxidants are molecules that can scavenge for free radicals. They are molecules that can inhibit ROS production and upregulate defense from harmful effect of free radicals.[7] Alpha amylase is a catalyst in the reaction which involves the hydrolysis of alpha 1-4 glycosidic linkages of starch and is responsible for starch digestion.[8] It therefore causes blood glucose level to rise.[9]

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Inhibiting alpha amylase can control hyperglycemia and reduce the risk of developing diabetes.[10] Acarbose and miglitol are the two common synthetic alpha amylase inhibitor, however, they can cause bloating, diarrhea, and other unpleasant side effects..[11] Therefore, there is need to search for new alpha amylase inhibitor with little or no side effects. *Musa paradisiaca* Linn fruit commonly called plantain belongs to the family of musaceae. It is grown and eaten mostly in South and North America, Africa and Asia.[12] Unripe *Musa paradisiaca* has antioxidant potential than the ripe one[13]. Unripe *Musa paradisiaca* has been reported to have antidiabetic activity[13] The peel is usually discarded as waste. However, flour made from the peel serves as a good source of fibre and is beneficial in the management and prevention of diseases.[14,15] It is high in polyphenols, carotenoids and other bioactive compounds[16] In order to encourage the bioconsumption of the peels of unripe *Musa paradisiaca*, this study evaluated the antioxidant activity and in vitro alpha amylase inhibitory potential of the methanol extract of the peel with the unripe fruit.

Materials and Methods

Chemicals/reagent

Potassium ferric cyanide, trihloroacetic acid (TCA), DPPH, dimethyl sulfoxide, alpha amylase, dinitrosalicyclic acid, maltose and methanol were purchased from Sigma – Aldrich. All reagents were of analytical grade.

Collection and Preparation of the Unripe Musa paradisiaca Linn Fruit and Husk

Fresh pulps of unripe plantain (*M. paradisiaca* Linn) with husk were obtained from Mubi main market, Adamawa State, Nigeria in October, 2021. Authentication of the unripe plantain with dried husk was carried out at the Department of Botany, Adamawa State University Mubi, Adamawa State, Nigeria and was given a voucher number; adsu/001/138. The unripe plantain and the peels were sliced and sun-dried for about two weeks to a constant weight, and grounded into

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flour which was termed 'raw flour'. The flour was passed through a sieve. The plantain flour was stored in an airtight container at room temperature (25℃) for future analysis.

Extraction

The dried plantain flour (500g) was extracted in 2500 mL of absolute methanol for 24 hrs at room temperature, under intermittent shaking. The extraction was repeated three times, and the extract obtained was filtered using Whatman filter paper number 1. The solvent in the filtrate was removed by placing the filtrate in a water bath at 50°C. The extract was stored at 25°C in a covered plastic container for further use.

Antioxidant Activity

Determination of Ferric Reducing Antioxidant Power (FRAP) Assay

The method described by Fejes *et al*.[17] was followed for the assay. Two milliliters of the plant extract at various concentrations were mixed with two milliliters each of 0.2 M phosphate buffer (pH 6.6) and 0.02 mg/mL potassium ferric cyanide. For 20 minutes, the solution was incubated at 50℃. Trichloroacetic acid in an aliquot of 2 mL (0.10 mg/L) was added. After the sample had been centrifuged for 10 minutes at 3000 rpm, the supernatant was collected. It was mixed with 0.4 milliliters of fresh ferric chloride (0.1 percent (w/v)) and two milliliters of distilled water. A blank was prepared. After 10 minutes, the absorbance was measured at 523 nm. Using the following formula, the free radical scavenging activity was estimated;

$$\text{Free radical scavenging activity (\%)} = \frac{1 - A_{sample}}{A_{blank}} \times 100$$

DPPH Free Radical Scavenging Assay

The method described by Katalinic *et al*.[18] was used to determine the DPPH free radical scavenging activity. Six (6) mg of DPPH was dissolved in 50 mL of methanol to produce a DPPH solution. A fraction (2.5 mL) of the extract at 400, 600, 800 and 1000 µg/mL was added to 2.5 mL DPPH solution in a test tube. The test tube was placed in a dark place for 20 mins at room temperature. Absorbance was measured at 517 nm using UV- spetrophotometer. A reference was prepared following the same procedure but replacing the plant extract with ascorbic acid. The percentage inhibition of free radicals by DPPH was calculated using the following formula;

$$\text{\% Inhibition} = \frac{A_{control} - A_{sample}}{A_{Sample}} \times 100$$

A_{sample} is the absorbance of the sample with DPPH solution without extract, and $A_{control}$ is the absorbance of DPPH solution without extract.

Alpha-amylase inhibitory assay

The assay was done using the procedure described by McCue *et al*.[19] Different concentrations (ranging from 1.25 mg/mL-10 mg/mL) of the extract were prepared using dimethyl sulfoxide (DMSO). Two hundred and fifty (250) µL of each concentration was pipetted into a separate test tube, to which 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing 0.5 mg/mL -amylase solution was then added. At room temperature, the solution was incubated for 10 minutes. The mixture was then mixed with 250 µL of a 0.02 M sodium phosphate buffer solution with a pH of 6.9 and 1 percent starch solution, and the reaction was allowed to stand at room temperature for 10 minutes. To stop the reaction, 500 µL of dinitrosalicylic acid (DNS) reagent were added to the mixture. It was then incubated at 100^0C for 5 minutes. It was allowed to cool to room temperature. Five milliliters of distilled water was added to the mixture. Absorbance was read at 540 nm. The same procedure was followed for the standard drug, acarbose but the plant extract was replaced with acarbose. The control was prepared using the same procedure but methanol extract of *Musa paradisiaca* with husk was not added. The α-amylase inhibitory activity was calculated as

$$\text{\% Inhibition} = \frac{A\,control - A\,sample}{Acontrol} \times 100$$

Mode of α-amylase inhibition

The mode of inhibition of *α*-amylase by the extract was determined according to the modified method of Ali *et al*.[20] described by Ahmed *et al*.[21] An aliquot (250 µL) of the 5 mg/mL methanol extract of *Musa paradisiaca* with husk in dimethyl sulfoxide was preincubated in one set of test tubes with 250 µL of amylase solution for 10 minutes at room temperature. 250 mL of phosphate buffer (pH 6.9) and 250 mL of the -amylase solution were preincubated in a different set of test tubes. An aliquot (250 µL) of a 1 percent starch solution in various concentrations (0.30 - 5.0 mg/mL) were added to each pair of test tubes. Ten minutes at 25°C were spent incubating the mixture. Five hundred (500) microliters of DNS were added, and the reaction was stopped after 5 minutes of boiling. A maltose standard curve was prepared and used to determine the amount of maltose released. This was converted to velocity. Velocity was plotted against substrate concentration to obtain the Michaelis-Menten plot. Additionally, a plot of 1/V against 1/S (where S is the substrate concentration and V is the velocity) was plotted. Parameters obtained from the Lineweaver-Burk plot was used to determine the mode of inhibition of the extract on α-amylase activity.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 24.0 was used to perform the statistical analysis. One-way analysis of variance was used to analyze the data (ANOVA). All findings were presented as the mean ± S.E.M of three determinations. The level of significance was taken at 5 % confidence interval ($p < 0.05$).

Results and Discussion

The ferric reducing power of methanol extract of unripe plantain with dried husk is shown in Table 1. The ferric reducing assay power for ascorbic acid was greater than that of the methanol extract of unripe plantain with dried husk at all concentration assayed for.

The DPPH radical scavenging activity of methanol extract of unripe *M. paradisiaca* Linn fruit with dried husk is shown in Figure 1.

Table 1: Ferric reducing assay power of unripe plantain flour with dried husk

Concentration (µg/mL)	Unripe plantain with husk (% scavenging activity)	Ascorbic acid (% scavenging activity)
400	53 ± 0.03^a	62 ± 0.05^b
600	57 ± 0.12^a	68 ± 0.03^b
800	65 ± 0.22^a	74 ± 0.15^b
1000	77 ± 0.13^a	86 ± 0.07^b

Values are mean ± S.E.M. N = 3.Values with the same superscript along the row are statistically not significant from each other ($p<0.05$).

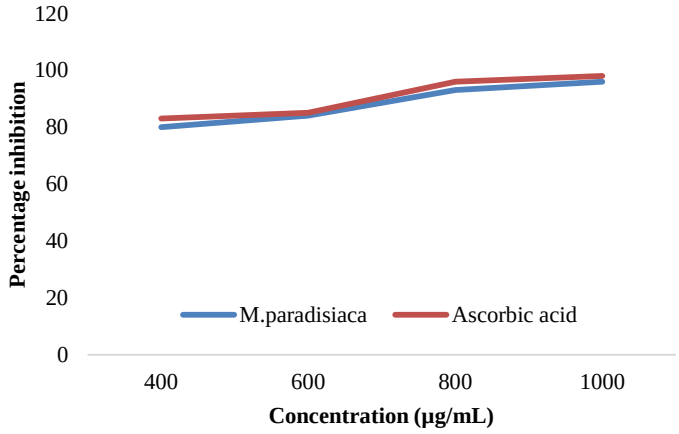

Figure 1: DPPH radical scavenging activity of methanol extract of unripe *M.paradisiaca* with dried husk

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The percentage inhibition of the methanol extract of unripe *M. paradisiaca* Linn fruit with husk at concentrations assayed for is similar to that of the standard, ascorbic acid. The IC_{50} values are also not significantly different from each other as shown in Table 2. An acceptable method for evaluating the antioxidant activity of plant extracts is DPPH free radical scavenging assay.[22] Antioxidants exert their effect on DPPH by donating hydrogen to free radicals.[23] The methanol extract of unripe *M. paradisiaca* Linn fruit with husk showed good DPPH scavenging activity indicating that it is a good antioxidant. Therefore, the extract exhibited radical scavenging activity by its hydrogen donating ability. Diabetes management has largely focused on control of hyperglycemia but the rising death burden of the disease is mainly due to the vascular complications.[24] Several studies have reported that oxidative stress is essential for the development of diabetes and its related complications, therefore ameliorating oxidative stress with the use of antioxidants might be useful in reducing diabetic complication.[25] Antioxidants are effective in reducing diabetic complications.[24] They are usually recommended alongside anti-diabetic drugs to avoid diabetic complications.[26]

The methanol extract of unripe *M. paradisiaca* Linn fruit with husk is a good antioxidant suggesting that it may be used to reduce diabetic complications and cure diabetes itself. The % inhibition of alpha amylase by methanol extract of unripe plantain flour with husk is shown in Figure 2. The % inhibition of methanol extract of plantain with husk at 2.50 mg/ml and 10.00 mg/ml concentrations were greater than 50% (67% and 73% respectively). Acarbose at 1.25 mg/ml, 2.50 mg/ml and 10.00 mg/ml had a percentage inhibition of 61%, 67% and 70% respectively. The greater than 50% inhibition of alpha amylase by methanol extract of unripe *M. paradisiaca* Linn fruit with husk indicates that the extract is an inhibitor of the enzyme.

Figure 3 shows the Michealis-Menten plot of methanol extract of unripe *M. paradisiaca* with dried husk. The plot shows that the velocity of alpha amylase in the presence of methanol extract of unripe *M. paradisiaca* Linn fruit with husk (inhibitor) was lower than that without inhibitor. The decrease in velocity observed in the Michealis-Menten plot by the methanol extract of unripe *M. paradisiaca* with husk showed that it decreased the activity of alpha amylase, further indicating that the extract is an alpha amylase inhibitor. Inhibition of alpha amylase decreases carbohydrate digestion which in turn significantly reduces the level of blood glucose level. A key strategy in the treatment of diabetes mellitus (a disease that occurs due to high blood glucose level) and its complications is blood glucose level reduction. [27] From this study, unripe *M. paradisiaca* Linn fruit with husk is a good alpha amylase inhibitor. Therefore, diabetes mellitus can be treated with methanol extract of unripe *M. paradisiaca* Linn fruit with husk. Tannins have the potential to inhibit alpha amylase and it also exhibit antioxidant activity.[28] Several studies have reported the presence of tannin in unripe *Musa paradisiaca* and its peels[16, 29-31]. This therefore, suggests that the tannin present in the methanol extract of *Musa paradisiaca* with husk is responsible for the extract's antioxidant activity and alpha amylase inhibitory potential.

By analyzing the Lineweaver-Burk plot, the mode of inhibition of the methanol extract of *Musa paradisiaca* Linn fruit with husk on -amylase activity was identified. In Figure 4, the enzyme's V_{max} is shown to be 0.8 mM/min when the inhibitor (plant extract) is present and the enzyme's V_{max} when the inhibitor is absent (there is no plant extract) is also 0.8 mM/min. The enzyme's Km was 1.3 mg/mL in the presence of the plant extract (inhibitor), while it was 0.7 mg/mL in the absence of the inhibitor (no plant extract). This shows that alpha amylase's V_{max} did not change both in the presence of the inhibitor and in the absence of the inhibitor but the K_m increased. An increase in K_m and the same V_{max} (V_{max} unchanged) are characteristics of competitive inhibition. It therefore follows that unripe *M. paradisiaca* Linn fruit with husk inhibits in a competitive manner. This shows that there is a compound in the extract that competes with the substrate of alpha amylase in binding to the active site of the enzyme. The mode of inhibition of acarbose, the standard drug, is also competitive inhibition.[32] Competitive inhibitors are effective because they are structural analogue of the substrate.[21] They bind to the active site of the enzyme to form an EI (enzyme-inhibitor) complex instead of an ES (enzyme-substrate) complex.[21]

Table 2: DPPH IC_{50} value of methanol extract of unripe *M.paradisiaca* with dried husk

Unripe *M.paradisiaca*	Ascorbic acid
3.4 ± 0.05^a	3.2 ± 0.02^a

Values are mean ± S.E.M. N = 3. Values with the same superscript along the row are statistically not significant from each other ($p < 0.05$).

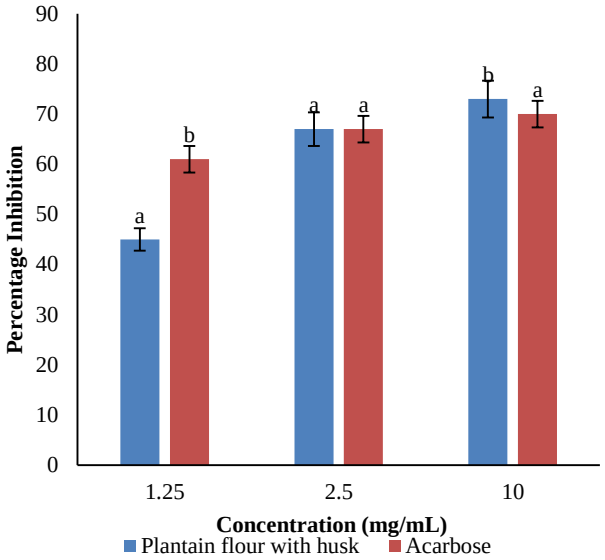

Figure 2: Percentage inhibition of alpha amylase activity by methanol extract of unripe *M. paradisiaca* with dried husk
Values are mean of three replicates ± S.E.M. Bars with different superscript in each category are significantly different ($p < 0.05$).

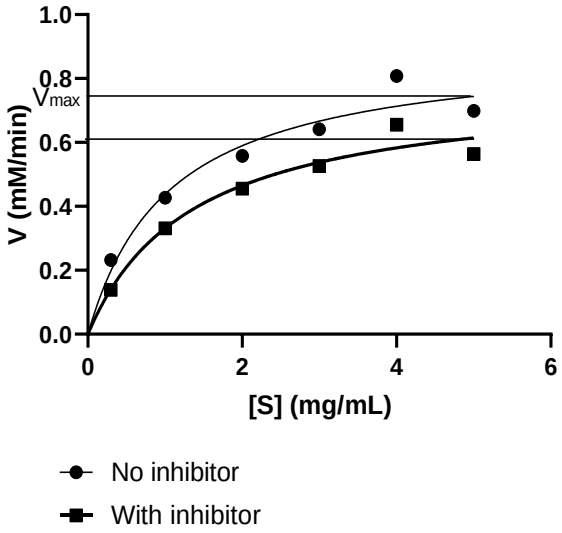

Figure 3: Michealis- Menten plot of methanol extract of unripe *M.paradisiaca* with dried husk

The EI complex prevents the enzyme's substrate to bind to the active site of the enzyme. This therefore infers that methanol extract of unripe *M. paradisiaca* fruit with husk competitively binds to the active site of α – amylase to prevent the binding of its substrate, starch. This will prevent the breaking down of starch to smaller molecules and ultimately prevents the breakdown of starch to glucose which will reduce the post-prandial increase of blood glucose and can thus be a strategy in the management of diabetes mellitus.[33]

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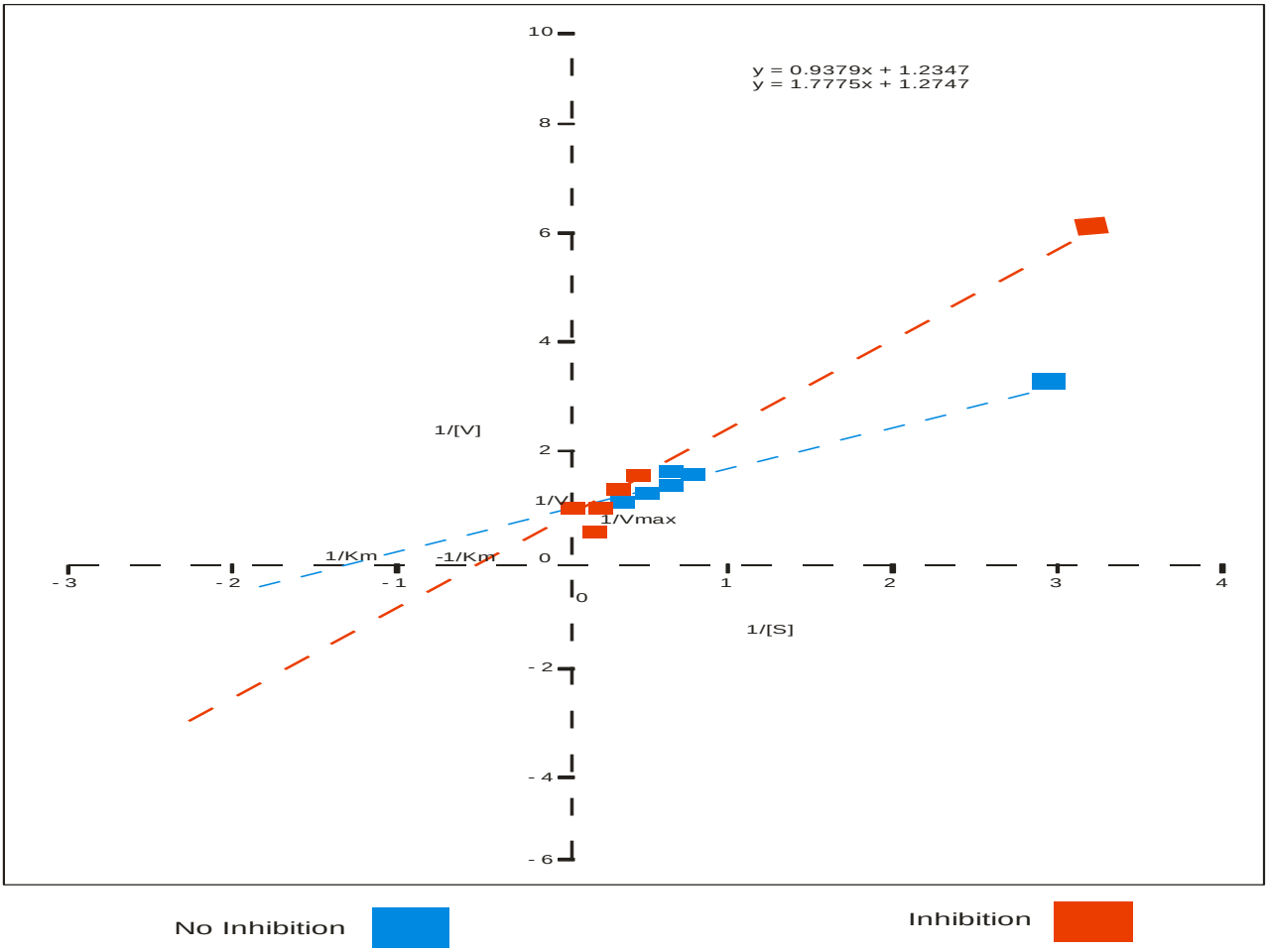

Figure 4: Lineweaver-Burk plot of methanol extract of unripe *M. paradisiaca* with dried husk

Conclusion

Unripe M. paradisiaca Linn fruit with husk is a good antioxidant and it can serve as a competitive inhibitor of alpha amylase. It can therefore be used in the treatment of diabetes and can be used to manage complications arising from diabetes mellitus.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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