FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 13, July, 2026, pp 108 – 113
DOI: https://doi.org/10.33003/fjs-2026-1013-5621

Biochemical Characterization of the Antioxidant and α-Amylase Inhibitory Activities of *Mucuna pruriens* Leaf Extract: Insights from Enzyme Kinetic Analysis

***Chizoba Paul Ugwu, Samuel Chukwuemeka Meludu and Chidiebere Emmanuel Ugwu**

Human Biochemistry Department, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Awka, Nigeria.

*Corresponding authors' email: chizobaugwu7@gmail.com
ORCID iD: https://orcid.org/0000-0001-7018-7219

Received: 24 Jul. 2026
Accepted: 31 Jul. 2026
Published: 6 Aug. 2026

ABSTRACT

The increasing prevalence of type 2 diabetes mellitus has intensified the search for plant-derived therapeutics capable of simultaneously attenuating oxidative stress and regulating carbohydrate metabolism. Although *Mucuna pruriens* is recognized for its medicinal properties, the antioxidant potential and enzyme inhibitory mechanism of its leaf extract remain poorly understood. This study investigated the in vitro antioxidant activity, α-amylase inhibitory potential, and inhibition kinetics of ethanolic leaf extract of *M. pruriens*. Antioxidant capacity was evaluated using hydroxyl radical, lipid peroxidation, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays with butylated hydroxyanisole (BHA) as the reference antioxidant. α-Amylase inhibitory activity was determined across different extract concentrations, while Lineweaver–Burk kinetic analysis was employed to elucidate the mode of enzyme inhibition. The extract exhibited appreciable antioxidant activity in all assay models, demonstrating hydroxyl radical, lipid peroxidation, and DPPH scavenging activities of 55.77 ± 1.66%, 47.00 ± 1.40%, and 45.34 ± 3.72%, respectively, although significantly lower than BHA ($P < 0.05$). The extract inhibited α-amylase in a concentration-dependent manner, increasing from 45.83 ± 1.55% at 1.10 mg/mL to 59.24 ± 1.11% at 2.30 mg/mL, surpassing acarbose (56.24 ± 0.82%) at the highest concentration tested, with an IC_{50} of 1.40 mg/mL. Kinetic analysis revealed a reduction in apparent Vmax (0.27 mM min^{-1}) accompanied by an altered Km (0.39%), indicating a mixed mode of α-amylase inhibition. These findings demonstrate that *M. pruriens* leaf extract possesses dual antioxidant and antihyperglycaemic activities through free radical scavenging and modulation of α-amylase activity, highlighting its potential for future antidiabetic drug development.

Keywords: *Mucuna pruriens,* α-Amylase Inhibition, Antioxidant Activity, Enzyme Kinetics, Mixed Inhibition, Type 2 Diabetes Mellitus

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both (International Diabetes Federation [IDF], 2025). Type 1 DM arises from autoimmune destruction of pancreatic β-cells, whereas Type 2 DM, the most prevalent form, accounting for nearly 90% of cases worldwide, is primarily associated with insulin resistance and progressive β-cell dysfunction. Gestational diabetes mellitus (GDM), on the other hand, manifests as glucose intolerance first recognized during pregnancy, typically in the second or third trimester (IDF, 2021).

The global burden of Type 2 DM has escalated dramatically over the past three decades, affecting populations across all income levels. Recent estimates suggest that 589 million adults aged 20–79 years were living with diabetes in 2024, with projections rising to 853 million by 2050 (IDF, 2021). According to the World Health Organization (WHO), diabetes contributes to 1.5 million deaths annually and is strongly linked to complications such as cardiovascular disease, nephropathy, neuropathy, and retinopathy (WHO, 2024). Oxidative stress, driven by reactive oxygen species (ROS), further exacerbates disease progression by impairing insulin signaling and inducing β-cell dysfunction (Manzor *et al*., 2022).

Conventional management strategies include lifestyle modification, oral hypoglycemic agents, and insulin therapy. However, secondary treatment failure, adverse drug reactions, and high costs remain significant challenges, particularly in resource-limited settings (WHO, 2024; ADA, 2024). In previous study, metformin has been associated with lactic acidosis, while sulfonylureas such as glipizide may cause gastrointestinal disturbances (Dyatlova *et al*., 2023). These limitations have fueled growing interest in plant-derived therapeutics, which are often perceived as safer, more affordable alternatives (Alam *et al*., 2022).

Medicinal plants have long been used in traditional medicine for managing chronic diseases, and numerous studies have demonstrated their potential in diabetes therapy (Ekor, 2024; Modak *et al*., 2007). *Mucuna pruriens* (MP), a leguminous plant widely used in Ayurvedic and folkloric medicine, has demonstrated promising pharmacological properties, including antioxidant, anti-inflammatory, and antidiabetic activities (Yadav *et al*., 2024). Phytochemical analyses reveal that MP contains alkaloids, flavonoids, and phenolic compounds with bioactivity against oxidative stress and carbohydrate-metabolizing enzymes (Bashev *et al*., 2026).

While extracts from MP seeds and leaves have been investigated for their efficacy in managing anemia, liver and kidney disorders, and Type 1 DM, the precise mechanisms underlying its potential role in Type 2 DM remain poorly

understood. Given the central role of oxidative stress and α-amylase activity in diabetes pathogenesis, this study aims to evaluate the antioxidant capacity and α-amylase inhibitory activity of *Mucuna pruriens* leaf extract. Furthermore, enzyme kinetics using Lineweaver–Burk analysis were employed to elucidate the mode of inhibition, thereby providing mechanistic insights into its antidiabetic potential.

MATERIALS AND METHODS

Reagents

The chemicals used in this research work were of analytical grade and were purchased from Sigma-Aldrich Inc., USA. Some of the important chemicals used include α-amylase from human saliva (A1031-1KU), 2, 2-diphenyl-1-picrylhydrazyl, DPPH (D9132-1G), Butylated hydroxyanisole, BHA (B1253-5G) and chicken egg yolk powder (E0625-100G).

Extract Preparation

The fresh leaves of *Mucuna pruriens* (MP) was obtained from Nnewi, Anambra State, Nigeria and was confirmed by Prof. Chinelo A. Ezeabara, in the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The plant leaves specimens was deposited at the Departmental herbarium and a voucher number (257A) was obtained. The leaves were cleaned and air dried for eight days and then pulverized into powder using a grinder. The powder was soaked in ethanol (40% v/v) in an Erlenmeyer conical flask (3000ml) for 48 hours with intermittent shaking, 3-5 times daily. This was filtered using muslin sieve followed by Whatman paper (size no.1). The filtrate was evaporated to dryness using water bath at 55 ºC. The crude extract was obtained in a pasta form, stored at 2 ºC and reconstituted as at when needed for the experiment.

In Vitro Antioxidant Assay

The antioxidant activity of the extract was measured using three different methods: DPPH radical scavenging activity, lipid peroxidation assay and hydroxyl radical scavenging activity. The antioxidant activity of hydrolysates was tested and compared with standard antioxidant, Butylated Hydroxyanisole (BHA).

DPPH Radical Scavenging Activity Assay

The scavenging activity of the samples on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was evaluated using the method originally described by (Gulcin and Alwasel, 2023), with minor modifications. Briefly, 200 µL of sample solution was mixed with 1 mL of 0.1 mM DPPH prepared in ethanol and 450 µL of 50 mM Tris-HCl buffer (pH 7.4). The mixture was incubated at 37 °C for 30 minutes, after which the reduction of DPPH radicals was measured spectrophotometrically at 517 nm. A tube containing DPPH solution without sample served as the control. All assays were performed in triplicate, and the percentage of radical scavenging activity was calculated relative to the control. The percentage of DPPH scavenging activity was calculated according to the following equation:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{\text{Abs of control} - \text{Abs of sample}}{Abs\ of\ control} X\ 100$$

Lipid Peroxidation Scavenging Activity Assay

The lipid peroxidation assay was carried out using the method described previously by (Shahidi and Samarasinghe, 2025). In brief, samples (0.2ml) and distilled water (0.4 ml) was mixed with 0.5ml of egg yolk solution. The solution was vortexed well with 70µl of FeSO4 (10mM) and incubated for 30min at 25oC. After adding 1.5ml of thiobarbituric acid (TBA) solution (0.8% TBA in 1.1% sodium dodecyl sulphate), samples were mixed well and heated for 60min at 37oC. Then, samples were cooled and 5ml of butanol was added. The samples were centrifuged for 10min at 3000rpm. Each Supernatant was transferred into a fresh 5ml sample container and absorbance was measured at 532nm. Ethanol (95%) was used as control. The antioxidant activity was calculated as follows:

$$Lipid\ Peroxidation\ inhibition(\%) = \frac{\text{Abs of control} - \text{Abs of sample}}{Abs\ of\ control} X\ 100$$

Hydroxyl Radical Scavenging Activity Assay

The hydroxyl radical scavenging activity of each hydrolysate was evaluated using the method of (Shahidi and Samarasinghe, 2025), with slight modifications. Briefly, 0.2 mL of sample solution was mixed with 0.45 mL of sodium phosphate buffer (0.2 M, pH 7.0), 0.15 mL of 2-deoxyribose (10 mM), 0.15 mL of EDTA (10 mM), 0.15 mL of $FeSO_4$ (10 mM), 0.15 mL of hydrogen peroxide (10 mM), and 0.525 mL of distilled water. The reaction mixture was incubated at 37 °C for 4 hours. Following incubation, 0.75 mL of 2.8% trichloroacetic acid (TCA) and 0.1% thiobarbituric acid (TBA) were added. The samples were then heated in boiling water for 10 minutes. After cooling, absorbance was measured spectrophotometrically at 520 nm. Absolute ethanol served as the control. The percentage scavenging activity was calculated using the formula:

$$Hydroxyl\ radical\ inhibition(\%) = \frac{\text{Abs of control} - \text{Abs of sample}}{Abs\ of\ control} X\ 100$$

α-Amylase Inhibitory Assay

The α-amylase inhibitory activity of leaf extracts was determined using the method described by Karakaya *et al*. (2018). Briefly, the enzyme substrate was prepared by dissolving potato starch paste using boiled distilled water to achieve 1% (w/v) concentration. The extract was dissolved in 0.02 M sodium phosphate buffer containing 0.006 M NaCl, pH 6. Then, 100µL of the sample (assay concentrations of 1.1–2.3 mg/mL) and 100 µL of α-amylase enzyme solution was added to test tubes and allowed to incubate at 37°C for 10 min. Thereafter, 100 µL of the starch solution was added to test tubes and the reaction mixture incubated at 37°C for 10 min. The reaction was terminated by adding 200 µL of 3, 5-dinitro-salicylic acid (DNS) color reagent followed by incubation in a boiling water bath at 100°C for 5 min. The absorbance was read at 540 nm using spectrophotometer set at 37°C. Standard drug at 1mg/mL was used as the positive control. Inhibition (%) = [Ac − (As − Asb)/Ac] ×100., where Ac = Absorbance of the negative control, As = Absorbance of the sample, and Asb = Absorbance of the sample blank (enzyme omitted). The IC50 value was determined by linear regression analysis of percentage inhibition plotted against the Log10 of sample concentration (mg/mL).

Determination of Mode of Inhibition of α-Amylase

The mode of inhibition of α-amylase by *Mucuna pruriens* leaf extract was determined following the procedure described by Ali *et al*. (2006), with slight modifications. Briefly, 250 µL of the extract (1.40 mg/mL) was pre-incubated with 250 µL of α-amylase solution for 10 minutes at 25 °C in one set of reaction tubes. In parallel, a control set was prepared in which α-amylase was pre-incubated with 250 µL of phosphate buffer (pH 6.9). The reaction was initiated by adding 250 µL of soluble starch solution at varying concentrations (0.2-2.0%) to both sets of mixtures, followed by incubation for 10

minutes at 25 °C. To terminate the reaction, 500 µL of dinitrosalicylic acid (DNS) reagent was added, and the mixtures were boiled for 5 minutes. The amount of reducing sugars released was quantified spectrophotometrically using a maltose standard curve, and the values were converted to reaction velocities. Enzyme kinetics were analyzed by plotting double reciprocal Lineweaver–Burk plots (1/V versus 1/[S]), where V represents reaction velocity and [S] denotes substrate concentration. The mode of inhibition exerted by the crude extract on α-amylase activity was determined based on Michaelis–Menten kinetic parameters (Nelson *et al*., 2021). The kinetic parameters were obtained from the linear equation of the Lineweaver–Burk plot (1/V versus 1/[S]), expressed as $y = mx + c$, where m represents the slope and c represents the y-intercept. The maximum reaction velocity (Vmax) was calculated as the reciprocal of the y-intercept (Vmax = 1/c), while the Michaelis constant (Km) was determined as the product of the slope and Vmax (Km = slope × Vmax). The kinetic parameters obtained for the control and extract-treated reactions were compared to determine the mode of enzyme inhibition.

RESULTS AND DISCUSSION

In Fig. 1, the free radical scavenging and antioxidant capacities of the experimental sample were evaluated using three independent *in vitro* assay models: Hydroxyl, Lipid Peroxidation, and DPPH radical assays and directly compared against the commercial antioxidant Butylated Hydroxyanisole (BHA) as a standard reference. The result revealed that the BHA standard consistently exhibited significantly higher radical scavenging percentages across all three assay formats when compared to the experimental sample ($P < 0.05$). In the In Vitro Hydroxyl Radical Assay, the sample demonstrated its highest neutralizing capacity at 55.77 ± 1.66%, which was significantly lower than the 66.44 ± 0.65% inhibition achieved by BHA. A similar trend was observed in the In Vitro Lipid Peroxidation Assay, where the standard reference (64.75 ± 1.25%) statistically outperformed the sample (47.00 ± 1.40%). Lastly, the In Vitro DPPH Radical Assay yielded scavenging rates of 45.34 ± 3.72% for the sample and 59.02 ± 1.42% for BHA, establishing a distinct statistical variation ($P < 0.05$) as confirmed by Duncan's Multiple Range Test. Although the experimental sample showed lower efficacy than the synthetic antioxidant standard, it maintained a notable, moderate-to-high capacity for scavenging diverse free radical species.

The α-amylase inhibitory activity of *the extract* was evaluated across a range of log-transformed concentrations, and the corresponding half-maximal inhibitory concentration (IC_{50}) was calculated. As shown in the linear regression model (Fig. 2), the extract demonstrated a concentration-dependent inhibitory effect against the α-amylase enzyme. Based on the regression equation generated from plotting percentage inhibition against the Log 10 concentration (mg/mL), the IC_{50} value of *the extract* was determined to be approximately 1.40 mg/mL.

The result in Table 1 confirms that the sample's leaf extract exerts a highly robust, concentration-dependent inhibitory effect on α-amylase activity as every incremental increase in extract concentration produced a statistically distinct baseline of enzyme inhibition, progressing sequentially from its lowest efficacy of 45.83 ± 1.55% at 1.10 mg/mL up to 51.48 ± 1.81% at 1.80 mg/mL. Critically, at its peak concentration of 2.30 mg/mL, the crude extract achieved a maximum inhibition of 59.24 ± 1.11%, which is statistically superior to the clinical reference standard, Acarbose, which yielded 56.24 ± 0.82% inhibition at 1.00 mg/mL.

The Lineweaver–Burk plot (Fig. 3) showed that the presence of *the extract* altered both the apparent Vmax and Km of α-amylase compared with the control. The plots generated the linear equations $y = 1.0025x + 1.6114$ for the control and $y = 1.4307x + 3.6581$ for the extract-treated enzyme. The kinetic parameters calculated from these equations showed that the apparent Vmax decreased from 0.62 mM min^{-1} in the control to 0.27 mM min^{-1} in the presence of the extract, while the apparent Km decreased from 0.62% to 0.39%**.** The simultaneous alteration of both kinetic parameters and the intersection of the Lineweaver–Burk plots to the left of the y-axis indicate that the extract inhibits α-amylase through a mixed mode of inhibition with preferential binding to the enzyme–substrate complex**.** This interaction reduces the catalytic efficiency of the enzyme while also affecting substrate binding, thereby slowing starch hydrolysis and supporting the potential antidiabetic activity of the extract.

The present study demonstrated that *Mucuna pruriens* leaf extract possesses significant antioxidant activity, as evidenced by its ability to inhibit hydroxyl radicals, lipid peroxidation, and DPPH radicals. Although the extract consistently exhibited lower scavenging percentages compared to the synthetic antioxidant butylated hydroxyanisole (BHA), its broad-spectrum efficacy across all three assays highlights its potential as a natural source of antioxidant compounds. These findings are consistent with earlier reports showing that plant-derived antioxidants, while generally less potent than synthetic standards, provide substantial free radical scavenging activity due to their diverse phytochemical constituents (Mukherjee *et al*., 2025). The moderate but significant inhibition observed in this study can be attributed to the presence of phenolic compounds, flavonoids, and alkaloids in *Mucuna pruriens*, which are known to donate hydrogen atoms or electrons to neutralize reactive oxygen species (Yadav *et al*., 2024). Similar trends have been reported in other medicinal plants, where natural extracts demonstrated antioxidant activity but did not surpass synthetic antioxidants such as BHA or ascorbic acid (Modak *et al*., 2007; Alam *et al*., 2022). Importantly, the ability of MP leaf extract to inhibit lipid peroxidation is particularly relevant in the context of diabetes, as peroxidized lipids contribute to vascular complications and progression of the disease (Manzor *et al*., 2022). In short, these results reinforce the therapeutic potential of *Mucuna pruriens* leaf extract as a complementary antioxidant agent. While synthetic antioxidants remain more potent, the extract's activity across multiple radical models supports its folkloric use and underscores its relevance in mitigating oxidative stress-related conditions such as Type 2 diabetes. This aligns with previous studies that have highlighted the role of natural antioxidants in reducing oxidative damage and improving metabolic health (Ekor, 2014).

The IC_{50} value indicates that a concentration of 1.40 mg/mL of the crude leaf extract is required to reduce α-amylase activity by 50% under the assayed conditions. In the context of metabolic health and diabetes management, α-amylase is a key enzyme responsible for breaking down complex carbohydrates into glucose. Inhibiting this enzyme slows down starch digestion, which subsequently reduces postprandial blood glucose spikes.

This study demonstrated that *Mucuna pruriens* leaf extract inhibited α-amylase activity in a concentration-dependent manner, with inhibition increasing significantly from 45.83 ± 1.55% at 1.1 mg/mL to 59.24 ± 1.11% at 2.3 mg/mL (Table 1). This progressive increase suggests that the bioactive constituents of the extract effectively interact with α-amylase, reducing its catalytic activity. Similar concentration-

dependent inhibition of α-amylase has been reported for several medicinal plants rich in phenolic compounds and flavonoids, where increased concentrations enhanced enzyme inhibition through greater occupation of enzyme binding sites (Sales *et al*., 2012). Interestingly, the highest concentration of the extract exhibited significantly higher α-amylase inhibition than the reference drug acarbose (56.24 ± 0.82%). Although acarbose remains the gold standard for delaying carbohydrate digestion, natural inhibitors with comparable activity are increasingly being explored because they may produce fewer gastrointestinal side effects than synthetic drugs (Sales *et al*., 2012). The present findings therefore suggest that leaves extract possess promising antihyperglycemic potential. The observed inhibitory activity may be attributed to the phytochemical constituents present in *Mucuna pruriens* leaves. Our previous GC-MS studies have reported that the leaves contain abundant phenolics and flavonoids. Polyphenols and flavonoids are known to inhibit α-amylase by forming hydrogen bonds and hydrophobic interactions with amino acid residues located within or near the enzyme catalytic site, thereby reducing substrate accessibility and catalytic efficiency.

The findings of this study are consistent with those reported by Bharadwaj *et al.* (2018), in which a novel α-amylase inhibitor was isolated and characterized from *M. pruriens* seeds. Their study confirmed that *M. pruriens* naturally contains bioactive molecules capable of suppressing α-amylase activity. While their investigation focused on purified seed proteins, the present work demonstrates that crude leaf extract also exhibits substantial inhibitory activity, suggesting that different parts of the plant may contribute to its antidiabetic properties.

The Lineweaver–Burk plot revealed that *Mucuna pruriens* leaf extract inhibited α-amylase through a mixed mode of inhibition, as demonstrated by simultaneous changes in both the apparent Michaelis constant (Km) and maximum reaction velocity (Vmax). The reduction in Vmax indicates a decrease in the catalytic efficiency of the enzyme, while the change in Km suggests that the extract also influenced substrate binding. The decrease in both Vmax and Km suggests that the extract binds to both the free enzyme and the enzyme–substrate complex, with a greater affinity for the enzyme–substrate complex. Such behaviour is characteristic of mixed inhibition in which inhibitor binding alters both substrate affinity and catalytic turnover. Previous studies have demonstrated that the antidiabetic activity of *Mucuna pruriens* is largely attributed to its rich phytochemical composition, particularly flavonoids, phenolic compounds, tannins, saponins and alkaloids, which are known inhibitors of carbohydrate-hydrolysing enzymes (Bharadwaj *et al*., 2018). Recent findings by Pangestiningsih *et al*. (2025) further suggested that these phytochemicals exert antihyperglycaemic effects by simultaneously inhibiting α-amylase and α-glucosidase while reducing oxidative stress through their antioxidant properties. Natural products often exhibit multitarget biological activities. In addition to α-amylase inhibition, bioactive compounds have been reported to inhibit components of the renin–angiotensin–aldosterone system, thereby contributing to cardiovascular protection (Mada *et al*., 2020).

The mixed inhibition observed in the present study is consistent with reports that crude plant extracts commonly exhibit multiple modes of enzyme inhibition because they contain several bioactive compounds with different binding affinities. Studies by Corkovic *et al*. (2022) suggested that dietary polyphenols interact with both catalytic and allosteric sites of α-amylase, resulting in simultaneous alterations in Km and Vmax. Similarly, (Lam *et al*., 2024) showed that naturally occurring flavonoids inhibit α-amylase through competitive, non-competitive and mixed mechanisms depending on their chemical structures and the nature of their interaction with the enzyme. The present findings are also in agreement with the work of Pathania *et al*. (2020), which demonstrated significant α-amylase inhibitory activity of *M. pruriens* extracts and suggested that enzyme inhibition contributes substantially to the plant's antihyperglycaemic effect. Altogether, *M. pruriens* lowers blood glucose through multiple complementary mechanisms, including inhibition of carbohydrate-digesting enzymes, enhancement of antioxidant defence systems and protection against diabetes-induced tissue damage.

Unlike classical competitive inhibitors such as acarbose, where only the apparent Km increases while Vmax remains unchanged, the simultaneous changes in both kinetic parameters observed in the present study indicate that *M. pruriens* leaf extract does not act exclusively by competing with the substrate for the enzyme's active site. Instead, the extract appears to bind at multiple sites on α-amylase, thereby reducing both substrate affinity and catalytic turnover. Such behaviour is expected for crude botanical extracts because they contain numerous phytochemicals acting synergistically (Corkovic *et al*., 2022; Lam *et al*., 2024).

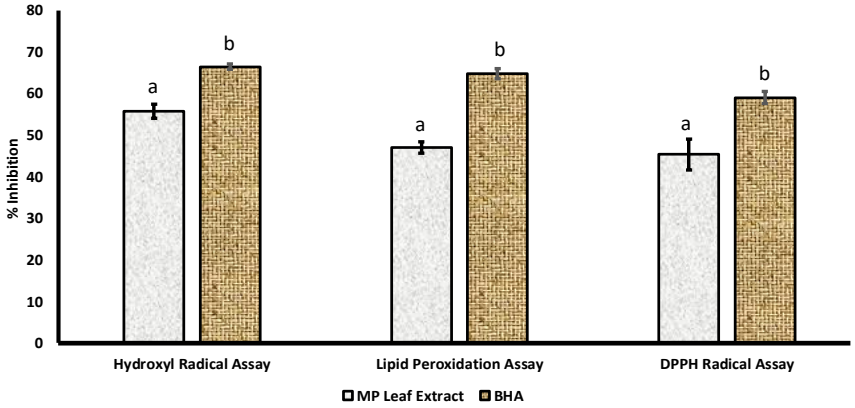

Figure 1: Hydroxyl, Lipid, and DPPH Radical Inhibitory Activities of MP Leaf Extract Using BHA as Standard Antioxidant. Values are Expressed as Mean ± Standard Deviation (n = 3). Means Within the Same Bar Sharing Different Superscript Letters (a, b) are Significantly Different ($P < 0.05$) According to One-Way ANOVA Followed by Duncan's Multiple Range Test

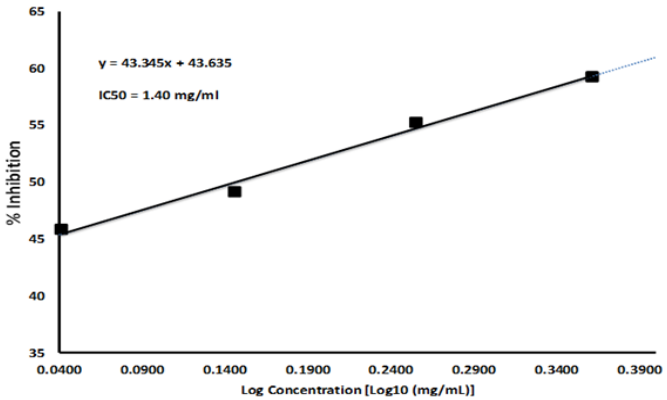

Figure 2: IC50 Determination of α-Amylase Inhibition Assay. IC50 =1.40 mg/mL

Table 1: Inhibitory Effect of Mucuna pruriens Extract on α-Amylase

Treatment Group	Sample Concentration (mg/mL)	α-Amylase Inhibition (%)
Extract	1.1	45.83 ± 1.55^{e}
Extract	1.4	49.13 ± 1.29^{d}
Extract	1.8	51.48 ± 1.81^{c}
Extract	2.3	59.24 ± 1.11^{a}
Acarbose (Standard)	1.0	56.24 ± 0.82^{b}

Values are Expressed as Mean ± Standard Deviation (n = 3). Means Within the Same Column Sharing Different Superscript Letters (a, b, c, d, e) are Significantly Different ($P < 0.05$) According to One-Way ANOVA Followed by Duncan's Multiple Range Test

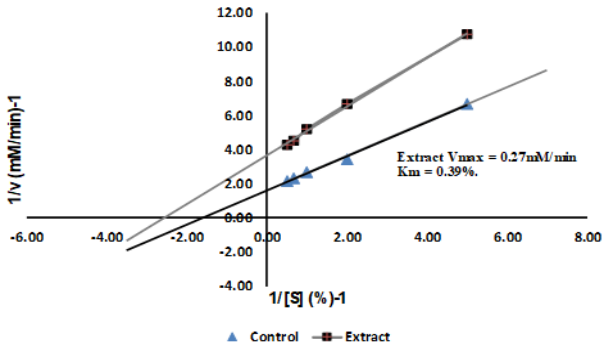

Figure 3: Line-Weaver Burk Plot of Mucuna Pruriens Extract

CONCLUSION

The present study demonstrates that *Mucuna pruriens* leaf extract possesses appreciable in vitro antioxidant and α-amylase inhibitory activities, highlighting its potential as a natural therapeutic agent for the management of type 2 diabetes mellitus. The extract effectively scavenged hydroxyl, DPPH, and lipid peroxidation-derived free radicals while exhibiting concentration-dependent inhibition of α-amylase, with inhibitory activity comparable to and, at the highest concentration, exceeding that of acarbose. Furthermore, Lineweaver–Burk kinetic analysis revealed a mixed mode of α-amylase inhibition, indicating that the extract modulates both substrate binding and enzyme catalytic activity. Collectively, these findings suggest that *M. pruriens* leaf extract may help mitigate oxidative stress and reduce postprandial hyperglycaemia, supporting its potential as a promising source of plant-derived antidiabetic agents. However, further in vivo studies are warranted to validate these biological activities, elucidate the underlying mechanisms of action, and establish the efficacy and safety of the extract under physiological conditions before its therapeutic application can be considered.

ACKNOWLEDGEMENTS

The authors would like to gratefully acknowledge the department of Human Biochemistry, Nnamdi Azikiwe University Awka for making their laboratory facilities available for some of this work.

REFERENCES

Alam, S., Sarker, M. M. R., Sultana, T. N., Chowdhury, M. N. R., Rashid, M. A., Chaity, N. I., Zhao, C., Xiao, J., Hafez, E. E., Khan, S. A., & Mohamed, I. N. (2022). Antidiabetic phytochemicals from medicinal plants: Prospective candidates for new drug discovery and development. *Frontiers in Endocrinology, 13*, 800714. https://doi.org/10.3389/fendo.2022.800714

Ali, H., Houghton, P.J., & Soumyanath, A. (2006). *α*-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *Journal of Ethnopharmacology*, 107 (3): 449–455. https://doi.org/10.1016/j.jep.2006.04.004

American Diabetes Association. (2024). Standards of medical care in diabetes. *Diabetes Care.47 (Suppl. 1)*, S1–S154. https://doi.org/10.2337/dc24-S001

Bashev, Z., Karcheva-Bahchevanska, D., Ardasheva, R., & Ivanova, S. (2026). A comprehensive review of the therapeutic potential of *Mucuna pruriens*. *Molecules, 31*(5), 868. https://doi.org/10.3390/molecules31050868

Bharadwaj, R. P., Raju, N. G., & Chandrashekharaiah, K. S. (2018). Purification and characterization of α-amylase inhibitor from the seeds of underutilized legume, *Mucuna pruriens*. *Journal of Food Biochemistry, 42*(7), e12686. https://doi.org/10.1111/jfbc.12686

Ćorković, I., Gašo-Sokač, D., Pichler, A., Šimunović, J., & Kopjar, M. (2022). Dietary Polyphenols as Natural Inhibitors of α-Amylase and α-Glucosidase. *Life*, *12*(11), 1692. https://doi.org/10.3390/life12111692

Dyatlova, N., Tobarran, N. V., Kannan, L., North, R., & Wills, B. K. (2023). *Metformin-associated lactic acidosis (MALA). In StatPearls. StatPearls Publishing*. https://www.ncbi.nlm.nih.gov/books/NBK580485/

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology, 4*(177). https://doi.org/10.3389/fphar.2013.00177

International Diabetes Federation. (2025). *IDF Diabetes Atlas* (11th ed.). Brussels: IDF. Retrieved from: https://idf.org/about-diabetes/what-is-diabetes/

Karakaya, S., Gözcü, S., Güvenalp, Z., Özbek, H., Yuca, H., Dursunoğlu, B., Kazaz, C., & Kılıç, C. S. (2018). The α-amylase and α-glucosidase inhibitory activities of the dichloromethane extracts and constituents of *Ferulago bracteata* roots. *Pharmaceutical Biology, 56*(1), 18–24. https://doi.org/10.1080/13880209.2017.1414857

Lam, T. P., Tran, N. V., Pham, L. H. D., et al. (2024). Flavonoids as dual-target inhibitors against α-glucosidase and α-amylase: A systematic review of in vitro studies. *Natural Products and Bioprospecting, 14*, 4. https://doi.org/10.1007/s13659-023-00424-w

Manzoor, M.F., Arif, Z., Kabir, A., Mehmood, I., Munir, D., Razzaq, A., Ali, A., Goksen, G., Coşier, V., Ahmad, N., Ali, M & Rusu, A. (2022). Oxidative stress and metabolic diseases: Relevance and therapeutic strategies. *Frontiers in Nutrition*. 9:994309. https://doi.org/10.3389/fnut.2022.994309

Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., & Devasagayam, T. P. A. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of Clinical Biochemistry and Nutrition, 40*(3), 163–173. https://doi.org/10.3164/jcbn.40.163

Mukherjee, B., Hoque, A. A., Hota, S. H., Gope, S., Ray, M., Barman, M., Bhattacharya, S., Chakraborty, S., & Das, L. (2025). Antioxidants and their physiological role in free radical scavenging. In *Dietary Supplements and Nutraceuticals* (pp. 397–440). Springer. https://doi.org/10.1007/978-981-96-2375-6_14

Nelson, D. L., Cox, M. M., & Hoskins, A. A. (2021). *Lehninger principles of biochemistry* (8th ed.). W. H. Freeman.

Pangestiningsih, T.W., Nuraini, D.M., Andityas, M., & Ariana, A. (2025). Histological response and blood glucose level in a diabetic animal model after the oral administration of *Mucuna pruriens*: A systematic review and meta-analysis. Vet World. 2025 May; 18(5):1377-1388. https://doi.org/10.14202/vetworld.2025.1377-1388

Pathania, R., Chawla, P., Khan, H., Kaushik, R., & Khan, M.A. (2020). An assessment of potential nutritive and medicinal properties of *Mucuna pruriens*: a natural food legume. *3 Biotech* 10, 261 (2020). https://doi.org/10.1007/s13205-020-02253-x

Sales, P. M., Souza, P. M., Simeoni, L. A., & Silveira, D. (2012). α-Amylase inhibitors: A review of raw material and isolated compounds from plant source. *Journal of Pharmacy & Pharmaceutical Sciences, 15*(1), 141–183. https://doi.org/10.18433/J35S3K

World Health Organization (2024). Health topics: Diabetes. https://www.who.int/health-topics/diabetes#tab=tab_1 . Accessed June 3, 2026.

Yadav, J. P., Pathak, P., Yadav, S., Singh, A., Palei, N. N., & Verma, A. (2024). In-vitro evaluation of antidiabetic, antioxidant, and anti-inflammatory activities in *Mucuna pruriens* seed extract. *Clinical Phytoscience,* 10, 21. https://doi.org/10.1186/s40816-024-00381-y

Gulcin, İ., & Alwasel, S. H. (2023). DPPH Radical Scavenging Assay. *Processes*, *11*(8), 2248. https://doi.org/10.3390/pr11082248

Shahidi, F., & Samarasinghe, A. (2025). How to assess antioxidant activity? Advances, limitations, and applications of in vitro, in vivo, and ex vivo approaches. *Food Prod Process and Nutr* **7**, 50. https://doi.org/10.1186/s43014-025-00326-z

Bello Mada, S., Paul Ugwu, C., Musa Abarshi, M. ., & Saliu, M. A. (2020). Renin-Inhibitory Bioactive Peptides With Antihypertensive Property: A Review . *Fudma Journal OF Sciences*, *4*(2), 478-489. https://doi.org/10.33003/fjs-2020-0402-140