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In vitro Assays of Antidiabetic Potential of Ethanolic Leaves Extract of *Bougainvillea spectabilis* Willd.

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ABSTRACT

Diabetes mellitus is a complex disorder characterized by hyperglycemia due to absolute or relative insulin deficiency. A key therapeutic strategy involves reducing postprandial glucose levels by inhibiting digestive enzymes such as α -amylase and α -glucosidase. This study investigated the potential antidiabetic activity of *Bougainvillea spectabilis* Willd. (Paper flower). Ethanol extract of the leaves of *Bougainvillea spectabilis* Willd was subjected to quantitative phytochemical analysis and in vitro assays to assess its inhibitory effects on α -amylase and α -glucosidase, using standard laboratory procedures. The extract demonstrated dose-dependent inhibitory activity against both enzymes. At the highest concentration of 100 $\mu\text{g/ml}$, the plant showed maximum inhibitory effect of α -amylase and α -glucosidase at (77.98 %, 91.66 %) with corresponding IC₅₀ values of $47.50 \pm 0.06 \mu\text{g/ml}$ and $26.23 \pm 0.03 \mu\text{g/ml}$, respectively. These effects were also compared to those of the standard drug voglibose (IC₅₀: Of $45.50 \pm 0.06 \mu\text{g/ml}$ for α -amylase and $33.60 \pm 0.06 \mu\text{g/ml}$ for α -glucosidase). However, it is important to interpret these comparisons with precaution, as pharmacodynamics differences, purity and bioavailability between the crude extract and the synthetic drug were not accounted for. Preliminary screening indicated flavonoids, tannins, and phenolic compounds commonly associated with antidiabetic effects. In conclusion, the ethanol leaves extract of *Bougainvillea spectabilis* Willd demonstrates notable in vitro inhibitory activity against carbohydrate-hydrolyzing enzymes, suggesting its potential as a source of natural antidiabetic agents.

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INTRODUCTION

Diabetes mellitus is a severe metabolic disorder characterized by hyperglycemia and glucose intolerance, either due to a relative deficiency in insulin secretion or impaired effectiveness of insulin action. If left untreated, it can lead to severe complications, which include diabetic neuropathy, renal failure, hyperlipidemia, enzymatic glycation of protein and oxidative stress (Idowu *et al.*, 2018). In recent years, the burden of diabetes mellitus has grown significantly in sub-Saharan Africa. According to the International Diabetes Federation (IDF), approximately 24 million adults aged 20-79 in sub-Saharan Africa were living with diabetes mellitus in 2021, and this number is projected to increase by 129 % by 2045 (IDF, 2021). Nigeria has the highest number of diabetes cases in the region, accounting for over 3 million individuals with many more undiagnosed due to poor access to healthcare and limited public awareness (WHO, 2023). The global increase in diabetes-related morbidity and mortality highlights the urgent need for effective and accessible therapeutic interventions. Given the complexity of the disease and its multifactorial nature, there is a growing interest in developing antidiabetic agents with multiple mechanisms of action (Chimaobi *et al.*, 2019). Medicinal plants have historically played a crucial role in traditional health care systems and continue to serve as valuable drug discovery resources. Their therapeutic intervention depends on their diverse phytochemical constituents such as alkaloids, flavonoids, terpenoids and phenolic compounds known to exert various pharmacological activities (Dias *et al.*, 2012). Recent studies emphasize the importance of scientifically validating these traditional remedies through bioassay-guided isolation and mechanistic studies (Kumara *et al.*, 2020).

Bougainvillea spectabilis Willd, commonly known as Paper flower, is an ornamental plant belonging to the family Nyctaginaceae. Native to South America, this hardy and pest-free plant is widely cultivated for its vibrant, long-lasting blooms. Beyond its decorative use, *Bougainvillea spectabilis* has been reported to possess a wide range of pharmacological properties, including antibacterial, anticancer, antidiabetic, anti-inflammatory, antiulcer,

antioxidant and antifertility effects (Anisa & Wannee, 2017). Preliminary studies suggest its antidiabetic activity may be linked to the inhibition of carbohydrate-digesting enzymes, modulation of glucose uptake and antioxidant mechanisms. However, further studies are needed to substantiate these findings (Ahmed *et al.*, 2020; Rao & Rao, 2019).

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in the study are of analytical grade and were purchased from BDH Chemicals Ltd., Poole, England.

Plant Collection and Identification

B. Willd (also known as Paper flower) fresh leaves were obtained from the natural surroundings of the Faculty of Science, University of Maiduguri, located in Borno State, Nigeria, in September 2021. A botanist at the Department of Biological Sciences, University of Maiduguri, Nigeria, identified and authenticated the sample.

Preparation of Plant Material

The fresh leaves of *Bougainvillea spectabilis* Willd were washed to remove any unwanted adhering materials and shade-dried at room temperature. It was pulverized using a pestle and mortar into fine powder. The fine powder was sieved with a 0.01mm sieve size and stored in a container for further analysis.

Plant Extraction

Five hundred grams (500 g) of powder of *Bougainvillea spectabilis* Willd leaves was extracted with 70 % ethanol using the cold maceration method. The product was evaporated to dryness and then stored in a refrigerator until used.

Quantitative Phytochemical Analysis of Ethanolic Leaves Extract of *Bougainvillea spectabilis* Willd (Paper flower)

Determination of Total Saponin Contents

To estimate the saponin levels, 10 grams of the plant sample were mixed with 100 ml of 20% ethanol solution. This mixture was placed on a water bath shaker at 55°C for four hours to facilitate extraction. The residue was then filtered and re-extracted using the same procedure to ensure maximum recovery. Both extracts were combined and concentrated to about 40 ml using a water bath at 90°C. The concentrate was transferred into a separating

funnel, and 10 ml of diethyl ether was added. After shaking thoroughly, the aqueous layer was collected and the ether layer discarded. This step was repeated once more. Next, n-butanol was added to the aqueous layer and the resulting solution was washed twice using 10 ml of 5%

sodium chloride solution. The upper layer was collected and evaporated using a water bath, then oven-dried to a constant weight. The percentage of saponins was calculated based on the dry weight obtained (Rasool *et al.*, 2010).

$$\% \text{ Saponins} = \frac{\text{Weight of saponins}}{\text{Weight of sample}} \times 100$$

Determination of Total Tannin Contents

Quantity of tannin was determined using the spectrophotometer method where 0.5 g of sample was weighed into a 50 ml plastic bottle and 50 ml of distilled water was added and stirred for 1hr. The sample was filtered into a 50 ml volumetric flask and 50ml of the filtered sample was then pipetted out into test tube and mixed with 2 ml of 0.1M FeCl₃ in 0.1M HCl and 0.008M K₄Fe(CN)₆·3H₂O. The absorbance was measured with a spectrophotometer at 395nm

wavelength within 10min (Krishnaiah *et al.*, 2009). To quantify the tannins, a calibration curve was prepared using tannic acid standards at concentrations of 0, 20, 40, 60, 80 and 100 mg/L. Each standard was treated identically to the samples and absorbance readings were plotted against their respective concentrations. The sample tannin content was then extrapolated from the curve and expressed as tannic acid equivalents per 100 grams of the plant sample.

The following formula was used to calculate the total tannin contents:

$$\text{Tannic acid (mg/100g)} = \frac{C \times \text{Extract volume} \times 100}{\text{Aliquot volume} \times \text{weight of sample}}$$

Where C is concentration of tannic acid read off the graph.

Determination of Total Cyanogenic Glycoside Contents

Cyanogenic glycosides were analyzed following the method described by Amadi *et al.* (2004) and later cited by Ejikeme *et al.* (2014). One gram of the sample was placed into a 250 ml round-bottom flask and combined with 200 ml of distilled water. The setup was left to stand for two hours to allow enzymatic hydrolysis

(autolysis). The mixture was then distilled into a conical flask containing 20 ml of 2.5% sodium hydroxide solution. To prevent foaming during distillation, tannic acid was added. The distillate was treated with 8 ml of 6 M ammonium hydroxide and 2 ml of 5% potassium iodide. The resulting solution was titrated with 0.02 M silver nitrate using a micro burette. The endpoint was determined by the formation of a steady turbidity, indicating the presence of cyanide ions.

$$\text{Cyanogenic Glycosides (mg/100g)} = \frac{\text{Titre Value (Cm3)} \times 1.08 \times \text{exact volume}}{\text{Aliquot volume (Cm3)} \times \text{sample weight (g)} \times 100}$$

Preparation of Crude Enzyme Solution

Crude α -amylase and α -glucosidase enzyme solutions were prepared from the small intestinal brush border membranes of male Wistar rats. The use of crude intestinal enzymes was intentionally chosen to replicate the physiological enzymatic environment more closely than commercial or purified enzymes, which may not fully reflect *in vivo* activity

dynamics (Kessler *et al.*, 1978). To account for biological variability and ensure experimental reproducibility, six (6) healthy adult male Wistar rats were used. All animal handling procedures complied with internationally recognized ethical standards for laboratory animal use and care, as outlined in the NIH Guidelines (NIH, 1992). Ethical approval for this study was granted by the Animal Use and Ethics Committee (AUEC)

of the Faculty of Veterinary Medicine, University of Maiduguri (Approval No.: REP/FP/092021/PGVP/10).

Following humane euthanasia, the small intestines were carefully excised and rinsed with ice-cold phosphate buffer (pH 6.9). The mucosal layer was gently scraped off using a sterile glass slide and homogenized in 10 ml of phosphate buffer (pH 6.8). The homogenate was centrifuged at 5000 rpm for 20 minutes at 4°C and the resulting supernatant containing the crude enzyme extract was collected. To allow for standardization and accurate enzyme activity comparison, the protein concentration of the crude extract was determined using the Bradford assay, following standard protocols. The enzyme extracts were aliquoted and stored appropriately until use to preserve enzymatic integrity.

***In vitro* Antidiabetic Studies of Ethanolic Leaves Extract of *Bougainvillea spectabilis* Willd (Paper flower)**

***In vitro* α -amylase Inhibition Activity Assay**

The α -amylase inhibitory activity of the ethanol leaf extract was evaluated using a modified version of the method described in the Worthington Enzyme Manual (Worthington, 1993) and by Kwon *et al.* (2006), with necessary adjustments to ensure reproducibility and reliability of results. To assess enzyme inhibition, varying concentrations (20, 40, 60, 80 and 100 μ g/mL) of the plant extract were prepared. As positive controls, both voglibose and acarbose clinically established α -amylase

inhibitors were included at the same concentrations to allow comparative benchmarking. Prior to the assay, substrate linearity and enzyme kinetics were validated. A standard curve using different starch concentrations was plotted to confirm that the reaction conditions operated within the linear range of Michaelis-Menten kinetics, ensuring accurate quantification of enzyme activity.

For the assay, 500 μ L of phosphate buffer (pH 6.9) containing crude α -amylase enzyme (prepared from rat small intestinal brush border membranes) was incubated with 500 μ L of each extract or control drug solution at room temperature for 10 minutes. Following this, 500 μ L of 1% soluble starch solution (also in phosphate buffer, pH 6.9) was added to initiate the enzymatic reaction. The mixture was further incubated for 10 minutes at room temperature. The reaction was terminated by adding 1 mL of 3,5-dinitrosalicylic acid (DNS) reagent, followed by heating the tubes in a boiling water bath for 5 minutes to allow color development. After cooling to room temperature, each sample was diluted with 10 mL of distilled water and the absorbance was measured at 540 nm using a spectrophotometer. A blank containing phosphate buffer instead of starch served as the reagent background control. A negative control (containing enzyme but without extract or drug) represented 100% enzymatic activity. The percentage inhibition of α -amylase activity was calculated using the following formula:

$$\text{Inhibition of } \alpha\text{-amylase activity (\%)} = ((\text{Abs C} - \text{Abs S}) / \text{Abs C}) \times 100$$

Where Abs C is the absorbance of the control (100 % enzyme activity) and Abs S is the absorbance of the tested sample (plant extract).

***In vitro* of α -glucosidase Inhibition Activity Assay**

Inhibitory activity of α -glucosidase was determined by incubating 1ml of 2 % starch solution in phosphate buffer (pH 6.8) and various concentration of ethanol leaves extract and standard drug voglibose (20, 40, 60, 80 and 100 μ g/ml) for 5 min at room temperature. The reaction was initiated by adding 1ml of alpha-glucosidase enzyme to it followed by incubation for 40 min at room temperature. Then the

reaction was terminated by adding 2 ml of 6N HCl. After terminating the reaction 2 ml of glucose reagent was added to each test tubes and further incubated for 10 min at room temperature. The absorbance was measured using spectrophotometer at wavelength of 540 nm. As for the blank, buffer solution (phosphate buffer) was used instead of substrate. The tube with enzyme solution but without plant extracts or standard drug served as the control with total enzyme activity (Sundar and Vijey, 2017). The enzyme inhibition rate was expressed as percentage of inhibition, was calculated using the following formula:

Inhibition of α -glucosidase activity (%) = ((Abs C - Abs S)/Abs C) \times 100

Where Abs C is the absorbance of the control (100 % enzyme activity) and Abs S is the absorbance of the tested sample (plant extract).

Data Analysis

The results obtained from the data generated are presented as mean \pm standard error of mean

(SEM). Differences between the means was determined by student t-test using SPSS software version 21.0 where $P < 0.05$ was considered statistically significant. The IC_{50} values of the sample and that of standard was calculated using MS Excel (2013 version).

RESULTS

Table 1: Quantitative Phytochemical constituents of the ethanolic leaves extract of *Bougainvillea spectabilis* Willd (Paper flower)

Phytochemicals	Constituents
Saponins (%)	5.20 \pm 0.01
Tannins (mg/100g)	0.138 \pm 0.02
Cyanogenic glycosides (mg/100g)	19.5 \pm 0.86

Values are presented as mean \pm SEM of three determinations.

Table 2: IC_{50} (μ g/ml) Values for Ethanol Leaf Extract of *Bougainvillea spectabilis* Willd and Standard (Voglibose)

Sample	IC_{50} (α -glucosidase)	IC_{50} (α -amylase)
<i>Bougainvillea spectabilis</i> (μ g/ml)	26.23 \pm 0.03*	47.50 \pm 0.06
Voglibose (μ g/ml)	33.60 \pm 0.06	45.50 \pm 0.06

Values are presented as mean \pm SEM, of three determinations. Values with asterisk (*) along the column are significantly different ($P < 0.05$). The statistical evaluation using an unpaired Student's t-test revealed that the observed differences were not statistically significant ($P < 0.05$).

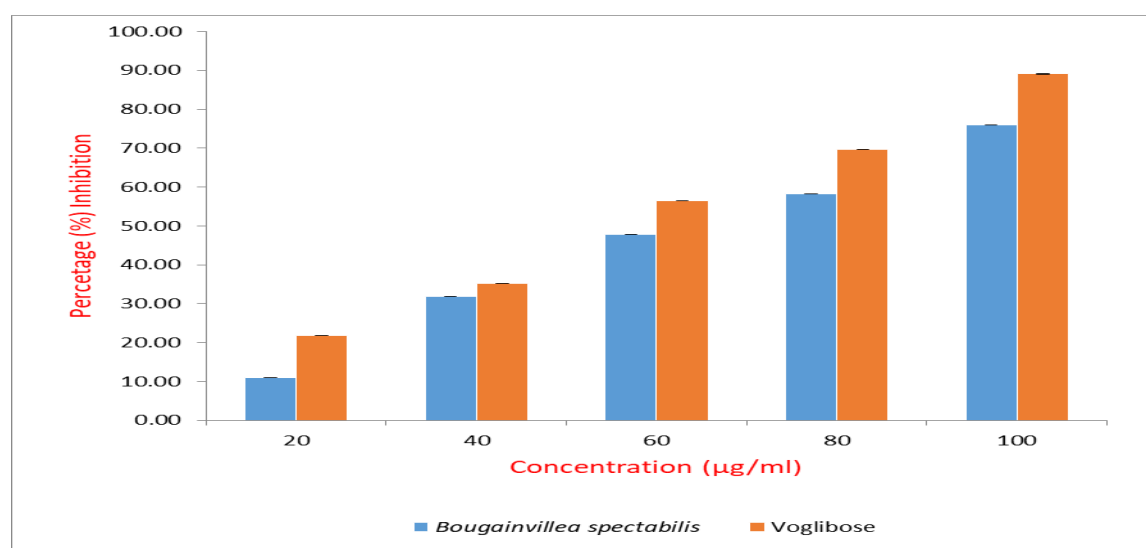


Figure 1: Percentage Inhibition of α -Amylase Activity by Ethanolic Leaf Extract of *Bougainvillea spectabilis* Willd and Standard (Voglibose)

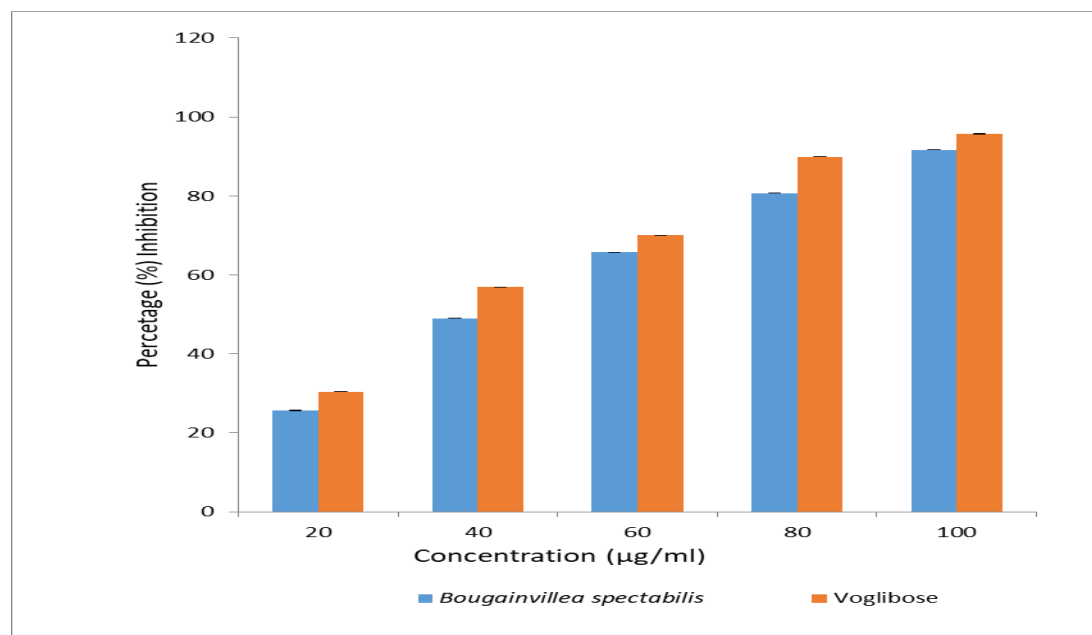


Figure 2: Percentage Inhibition of α -Glucosidase Activity by Ethanol Leaf Extract of *Bougainvillea spectabilis* Willd and Standard (Voglibose)

DISCUSSION

Diabetes mellitus remains a pressing global health concern, with prevalence steadily rising due to carbohydrate-rich diets forming a substantial part of daily intake. Despite significant research efforts, a definitive cure has yet to be established for this metabolic disorder (Luka *et al.*, 2013; International Diabetes Federation, 2023). Among the therapeutic strategies employed in the management of early-stage type 2 diabetes is the inhibition of carbohydrate-hydrolyzing enzymes, specifically α -amylase and α -glucosidase to slow glucose absorption and attenuate postprandial hyperglycemia (Augustine *et al.*, 2019; Kumar *et al.*, 2022).

In this study, the ethanolic leaf extract of *Bougainvillea spectabilis* Willd demonstrated significant *in vitro* inhibitory activity against both α -amylase and α -glucosidase enzymes. The IC_{50} value (Table 2) for α -glucosidase inhibition by the extract was 26.23 ± 0.03 μ g/mL, which was lower than that of the standard drug voglibose (33.60 ± 0.06 μ g/mL), indicating a potentially stronger inhibitory effect.

Conversely, the IC_{50} value (Table 2) for α -amylase was 47.50 ± 0.06 μ g/mL, slightly higher than voglibose (45.50 ± 0.06 μ g/mL), suggesting a comparatively weaker inhibition. While these findings indicate a concentration-dependent inhibition, such trends, although expected, should not be over-interpreted in the absence of kinetic modeling to confirm the type and specificity of enzyme interaction.

The enzyme inhibition results align with earlier studies where plant extracts demonstrated similar enzyme inhibition trends. For instance, Sundar and Vijey (2017) reported dose-dependent inhibition of both enzymes using *Amaranthus tristis*, although without kinetic validation. However, it is important to consider non-specific mechanisms such as tannin-induced protein precipitation or pH alteration that may artificially enhance or distort enzyme inhibition *in vitro* (Yin *et al.*, 2014; Borrelli & Izzo, 2000). Phytochemical screening of the extract revealed the presence of secondary metabolites known for their biological activities (Table 1). Quantitatively, the extract contained $5.20 \pm 0.01\%$ saponins, 0.138 ± 0.02 mg/100g tannins

and 19.5 ± 0.86 mg/100g cyanogenic glycosides. These compounds are known to affect carbohydrate metabolism. For instance, flavonoids (likely present, although not quantified here) are known to enhance insulin sensitivity and modulate glucose uptake (Peterson & Dwyer, 2022). Tannins, while inhibitory to digestive enzymes, may act non-specifically due to their ability to bind and precipitate proteins (Scalbert, 1991) and should therefore be interpreted with caution when included in bioactivity claims. The relatively high level of cyanogenic glycosides (19.5 ± 0.86 mg/100g) also warrants attention. These compounds can release hydrogen cyanide upon hydrolysis and are potentially toxic if consumed in significant quantities (EFSA, 2019). Their presence highlights the need for detailed toxicity assessments before considering the extract for therapeutic applications.

Furthermore, the inhibitory strength of the extract was visualized through enzyme activity plots (Figures 1 and 2), which showed a clear concentration-dependent inhibition of both α -amylase and α -glucosidase. However, without kinetic characterization (e.g., Lineweaver-Burk analysis), the exact mechanism of inhibition (competitive, noncompetitive, or uncompetitive) remains speculative. This limitation constrains the ability to predict *in vivo* efficacy or pharmacodynamic behavior. While the ethanolic extract of *Bougainvillea spectabilis* exhibited promising *in vitro* inhibitory effects on α -amylase and α -glucosidase enzymes supported by IC_{50} values and phytochemical content, the findings must be interpreted within the context of their limitations. The potential influence of non-specific inhibitory effects, lack of kinetic modeling and presence of potentially toxic compounds such as cyanogenic glycosides underscore the need for further *in vivo* validation, mechanistic enzyme kinetics and comprehensive toxicological assessments to confirm its utility as a safe and effective antidiabetic agent.

CONCLUSION

The findings of this study demonstrate that the ethanolic leaf extract of *Bougainvillea spectabilis* Willd possesses notable *in vitro* inhibitory activity against α -amylase and α -glucosidase, key enzymes involved in

postprandial glucose metabolism. This enzymatic inhibition suggests a potential role for the plant extract in managing early-stage hyperglycemia associated with type 2 diabetes mellitus. However, while the results are promising, they are limited to *in vitro* assays and do not confirm clinical efficacy or safety. Future research should include detailed enzyme kinetics, *in vivo* efficacy trials and comprehensive safety profiling to establish the therapeutic relevance, dosage limits and potential side effects of *Bougainvillea spectabilis* extracts. These steps are essential to move from preliminary laboratory data to safe and effective clinical applications.

CONFLICT OF INTEREST:

The authors do hereby declare that there are no conflicts of interest on this work.

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