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**African Journal of Biochemistry Research**

*Full Length Research Paper*

# Inhibitory potential and antidiabetic activity of leaf extracts of *Vitex doniana*

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**Pancreatic alpha (α)-amylase and intestinal α-glucosidase involved in the enzymatic breakdown of dietary carbohydrates are implicated in postprandial hyperglycemia. The inhibition of these two enzymes has been exploited in the design of antidiabetic drugs. This study was therefore designed to investigate the ability of the leaf extracts of *Vitex doniana* to inhibit α-amylase and α-glucosidase as well as the *in-vivo* antidiabetic potential of the ethanol leaf extract. The inhibitory potentials of the extracts on α-amylase were assayed through the method of starch-iodine colour change while α-glucosidase inhibition was assessed by measuring the release of 4-nitrophenol by p-nitrophenyl-α-D-glucopyranoside (pNPG). Acarbose was used as the standard. The extracts displayed a dose-dependent significant inhibition of the enzymes. The aqueous leaf extract of *V. doniana* was the strongest inhibitor of α-amylase with half-maximal inhibitory concentration ($IC_{50}$) of 3.09 ± 0.22 mg/ml while the ethanol leaf extract was the strongest inhibitor of α-glucosidase with $IC_{50}$ of 17.12 ±1.42 mg/ml. The result also showed that both extracts contain mixed inhibitors of the two enzymes. The result of the *in-vivo* antidiabetic study conducted for 14 days showed a significant (p<0.05) reduction in the fasting blood glucose level of the extract treated groups of animals compared to the untreated diabetic group. These results strongly suggest that *V. doniana* can be exploited in the treatment of diabetes mellitus.**

**Key words:** *Vitex doniana*, antidiabetic, inhibitory potential, Lineweaver-Burk, α-amylase, α-glucosidase.

## INTRODUCTION

Natural products of plant origin are a good source of intermediary metabolites some of which have been found to inhibit some enzymes (Rauf and Jehan, 2017). These plants contain compounds that are rich in polyphenols, which are known to interact with proteins and can inhibit enzyme activity (Oboh et al., 2012). The polyphenols

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**Abbreviations:** VDAQ, Aqueous extract of *Vitex doniana*; VDM, methanol extract of *Vitex doniana*, VDE, ethanol extract of *Vitex doniana*

have been reported to inhibit α-amylase and α-glucosidase associated with lipid peroxidation and type 2 diabetes (Reddy et al., 2010*)*. Natural products from plants have been used for the prevention and management of non-communicable diseases with minimal side effects and toxicity (Bhatia et al., 2019). Sangeetha and Vedasree (2012) reported that some herbal medicines used in treatment of diabetes act on the beta cells to secret insulin which mediates glucose metabolism.

Pancreatic α -amylase hydrolyzes starch to long chain carbohydrate which in turn are degraded by intestinal α-glucosidase to glucose and subsequently into the blood stream (Tundis et al., 2010). The breakdown of starch proceeds rapidly leading to the elevation of level of post-prandial hyperglyceamia (PPHG) (Sudha et al., 2011). These two enzymes have therefore been found to be good targets in drug design for the treatment of diabetes (Subramanian et al., 2008). Some anti-diabetic drugs have been found to competitively inhibit alpha glucosidase by slowing down carbohydrate digestion (Nair et al., 2013).

In type 2 diabetes mellitus there is a dysfunction of endocrine system which is caused by low secretion of insulin by the pancreatic β-cells and insulin resistance or insensitivity of cells to insulin action to regulate blood glucose levels which leads to hyperglycemia (Ardisson Korat et al., 2014). Hyperglycemia is the characteristic feature of type 2 diabetes mellitus, thus, reduction of postprandial hyperglycemia is a good therapeutic approach in treatment of type 2 diabetes (Chipiti et al., 2017).

Diabetic drugs like acarbose, metformin, miglitol and voglibose (Kaissi and Sherbeeni, 2011) though effective for glycemic control can lead to liver disorders, flatulence and abdominal pain (Kwon et al., 2007). Plant products with the potentials of inhibiting α-amylase and α-glucosidase can be used in the treatment of hyperglyceamia with little side effects.

*Vitex doniana* of the genus Verbenaceae, also known as black plum, is found in Southern Nigeria (Agbafor and Nwachukwu, 2011). It is the most widespread of the genus commonly found in savannah regions and useful in management of diseases (Dauda et al., 2011). In Nigeria, *V. doniana* is called dinyar in Hausa, galbihi in Fulani, ori nla in Yoruba and uchakoro in Igbo (Adejumo et al., 2013). The young leaves are cooked and prepared as vegetable soup and also added in some sauces. Phytochemical analyses revealed the presence of tannins, anthraquinones, flavonoids, resins, cardiac glycosides, saponins and alkaloids (Ezekwesili et al., 2012). Agbafor and Nwachukwu (2011) reported the antioxidant potential of the leaf extracts. Documented research work shows that *V. doniana* is used in the treatment of diabetes in traditional medicine and the extracts have exhibited anti-diabetic property in animal models (Obasi et al., 2013; Njoku et al., 2019). There is limited information on the *V. doniana* phytoconstituent's α-amylase and α-glucosidase inhibitory activity. This study will help establish the antidiabetic potential of *V. doniana* plant through their ability to inhibit activities of the two enzymes. Patients using α-glucosidase and α-amylase inhibitors such as acarbose suffer from diarrhea, flatulence and meteorism. These side effects may be due to excessive inhibition of α-amylase. Therefore there is a search for a lead substance with a strong inhibitory activity on α-glucosidase, but mild inhibition on α-amylase activity. Therefore, this study was aimed to explore the antidiabetic potential of *V. doniana* sweet through elucidation of its possible α-amylase and α-glucosidase inhibitory activity and mechanism of inhibition. This study will help to validate the traditional uses of the plant in diabetes and also screen its specificity towards the pancreatic digestive enzymes.

## MATERIALS AND METHODS

### Collection of plant material

*V. doniana* leaves were collected from farmlands in Obuoffia Awkunanaw in Nkanu West Local Government Area of Enugu State, Nigeria. It was authenticated in the Department of Botany, Nnamdi Azikiwe University Awka, Nigeria and deposited at the herbarium with a voucher specimen number 2/39.

### Sample preparation

The leaves of *V. doniana* were shade- dried, ground into powder using a manual grinder and stored in air tight containers. The powder was divided into three parts (100 g each) and was extracted in various (500 ml) solvents, viz; methanol, ethanol and aqueous.

### Ethical approval

This study was duly approved and was carried out strictly in compliance with the recommendations in the guide for the Institutional Animal Care and Use Committee of Nnamdi Azikiwe University, Awka, Nigeria in line with the protocols of Animal Care and Use in Research, Education and Testing (ACURET).

### Plant material extraction

#### *Aqueous extract*

One part of the powdered sample was soaked in water. The extract was centrifuged at 10,000 rpm (Centurion centrifuge, Centurion Scientific LTD; West Sussex, United Kingdom, Model K220) for 10 min to get the supernatant from which the aqueous extract of *V. doniana* (VDAQ) was prepared at a concentration of 10 mg/ml.

#### *Methanol extract*

One part of the ground sample was soaked and extracted in methanol by stirring at 150 rpm at room temperature for 72 h. It was filtered using Whatman No.4 filter paper. The filtrate was

evaporated to dryness at 40°C. The extracts were weighed, dissolved in dimethylsulphoxide (DMSO) to obtain methanol extract of *V. doniana* (VDM) at 10 mg/ml concentration, and afterwards kept in the refrigerator until use.

### Ethanol extract

One part of the ground sample was extracted by stirring with ethanol at 150 rpm at room temperature for 72 h. It was filtered using Whatman No.4 filter paper. The filtrate was evaporated to dryness at 40°C. The extracts were weighed, dissolved in dimethylsulphoxide (DMSO) to obtain ethanol extract of *V. doniana* (VDE) at 10 mg/ml concentration, and afterwards kept in the refrigerator until use.

### α-amylase inhibition assay

The α-amylase inhibitory activity was carried out using starch-iodine method as described by Mahomoodally et al. (2012). One hundred microliter (1.25-10 mg/ml) of the plant extracts was pre-incubated for fifteen minutes with 100 µl of the solution of enzyme at room temperature. Three milliliter (3 ml) soluble starch solution (1% starch) and 2 ml phosphate buffer were added to the reaction mixture. The reaction mixture was incubated at room temperature for 1 h. At timed interval, 0.1 ml from the reaction mixture was discharged into 10 ml iodine solution. After mixing, the absorbance of the starch-iodine solution was measured at 565 nm. α-amylase inhibitory activity was calculated as percentage inhibition thus:

% inhibition = $[(A_0 - A_t)/A_0]$ X 100;

$A_0$ and $A_t$ being absorbance of starch-iodine solution at t = 0 min and t = 60 min, respectively.

Controls not containing the plant extracts are said to possess full enzyme activity. Acarbose was used as standard. $IC_{50}$ values (Concentrations of extracts at 50% inhibition) were estimated from the graph and used to compare the plant extracts.

### α-amylase inhibition kinetics

The mechanism of inhibition of α-amylase was carried out with the plant extract with the least $IC_{50}$ (Ali et al., 2006); with some modifications. Thus, 250 µl of the plant extract (2 mg/ml) was pre-incubated for 10 min with 250 µl of α-amylase at room temperature in one group of test tubes. α-amylase was pre-incubated with phosphate buffer (250 µl; pH 6.9) in another group of test tubes. 250 µl of starch solution (0.30 – 5.0 mg/mL) was added to the two groups of mixtures and the mixture was then kept at room temperature for 10 min. 500 µl of DNS was added to the reaction and then boiled for 5 min. The reducing sugars liberated were determined using spectrophotometer and converted to reaction velocities. Linneweaver-Burk plot of 1/V against 1/[S] was plotted:

V = reaction velocity and [S] is the substrate concentration. The mechanism of inhibition on α-amylase activity by the extract was assessed from Lineweaver-Burk plot. Km and Vmax were determined.

### α- glucosidase inhibition assay

The inhibitory activity of the extracts on α-glucosidase was assessed through measurement of the release of 4-nitrophenol by paranitrophenyl α-D glucopyranoside (PNPG) as described by Kim

and Nho (2004). PNPG (5 mM, 0.3 ml) was added to 0.1 M sodium phosphate buffer (1.0 ml, pH: 6.9), 0.5 mg/ml of enzyme solution (0.2 ml) and the extract (0.2ml; 1.25-8 mg/ml), giving a total of 1.7 ml. After 30 minincubation (room temperature), 2.0 ml of 0.2 M sodium carbonate was added to stop the reaction. p-nitrophenol released was determined at 400 nm using spectrophotometer. The rates of inhibition were calculated as:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance Control} - \text{Absorbance Test})}{(\text{Absorbance Control})} \times 100$$

The controls (inhibitor and reagent blank) were also run similarly and then subtracted. Reagent blank contained only substrate, buffer and enzyme and acarbose was used in place of the plant extract in positive controls. $IC_{50}$ values were determined from the graph.

### α-glucosidase inhibition kinetics

The mechanism of inhibition of α-glucosidase by the extracts was assessed by using PNPG in increasing concentration (Ali et al., 2006). The plant extract with the least $IC_{50}$ was used. 50 µl of the extract (8 mg/ml) was added to 100 µl of α-glucosidase solution at 25°C for 10 min in group of test tubes. α-glucosidase was incubated with phosphate buffer (50 µl; pH 6.9) in another group of test tubes. 50 µl of PNPG (0.63 – 2.0 mg/ml) was added to the two groups of the test tubes. The reaction mixture was kept at 25°C for 10 min. 1000 µl of $Na_2CO_3$ was added to terminate the reaction. The liberated reducing sugars was measured spectrophotometrically, the amount determined using a paranitrophenol standard curve and converted to reaction velocities. Lineweaver-Burk plot was plotted. The mechanism of inhibition of the plant extract on α-glucosidase by the extract was determined by analyzing the Lineweaver-Burk plot.

### Animal experiment

Thirty six (36) albino Wistar rats were used for the study. The animals were kept in a well-ventilated part of the animal house of the department of Applied Biochemistry of Enugu State University of Science and Technology under normal laboratory conditions of temperature and humidity in a 12 h light and 12 h dark cycle. They were allowed to acclimatize for a week and were fed with standard feed and distilled water *ad libitum*. At the end of the one week, the animals were weighed and their initial blood glucose were recorded using fine test glucometer.

### Plant material extraction

The ground sample (1 kg) was soaked and extracted in 10 L of 70% ethanol for 48 h. It was first filtered using three layer muslin cloth and then with Whatman No. 4 filter paper with the aid of a vacuum pump. The filtrate was concentrated in a water bath at 60°C, re-dissolved at a concentration of 600 mg/ml and stored in refrigerator (4°C) until further use.

### Study design

Six animals were randomly selected and placed separately as the normal control group (A). The remaining 30 animals were administered intraperitoneally with 120 mg/kg body weight of

alloxan dissolved in ice-cold normal saline. Forty-eight (48) hours after alloxan administration, fasting blood glucose levels were measured using Finetest glucometer to confirm diabetes and recorded as post-induction blood glucose level. Rats with a fasting blood glucose concentration greater than 200 mg/dl were considered diabetic and selected for the experiment. The diabetic animals were randomly placed into five groups (B-F) of six (6) animals each:

Group A: The normal control group and was given the standard feed and distilled water only; Group B: This group received 200 mg/kg of *V. doniana* ethanol leaf extract orally, the standard feed and distilled water; Group C: This group received 400 mg/kg of *V. doniana* ethanol leaf extract orally, the standard feed and distilled water; Group D: This group received 600 mg/kg of *V. doniana* leaf extract orally, the standard feed and distilled water; Group E: This group received 5 mg/kg of glibenclamide orally, the standard feed and distilled water; Group F: This group was the positive control, they were diabetic (untreated) and were given standard feed and distilled water.

The treatment was given daily for 14 days and the blood glucose level of the animals in each group was taken daily by drawing blood from the tip of their tail and measured using Finetest glucometer for 14 days. Their weight was also measured on weekly basis using electronic balance.

### Determination of body weight

The weights of the rats were measured before and after induction of diabetes and subsequently during treatment on weekly basis using an electronic weighing balance.

### Statistical analysis

Graphpad prism version 7 was used for the kinetic data analysis while SPSS version 7 was used for other statistical analyses. Values were expressed as mean ± SD. Statistical significance of the results between groups was determined using multiple range test and one way analysis of variance (ANOVA) followed by LSD post hoc test. Differences between means were considered significant at $P < 0.05$.

## RESULTS

### α-Amylase inhibition assay

The effect of the plant extracts of *V. doniana* on the activities of α-amylase are shown in Table 1. All the extracts significantly ($p < 0.05$) inhibited α-amylase. The percentage inhibition increased with increase in concentration. Thus, while the standard acarbose showed an inhibition of 72.45%, the aqueous extract (VDAQ) showed 79.52% inhibition of the enzyme at the highest concentration tested. The inhibition effectiveness of the extracts on α-amylase was compared using $IC_{50}$ values. Aqueous extract of *V. doniana* (VDAQ) with $IC_{50}$ value of 3.09 mg/ml gave the highest inhibition followed by the ethanol extract (VDE), with an $IC_{50}$ value of 3.67 mg/ml while the methanol extract (VDM) was the least

($IC_{50}$ = 4.02 mg/ml). Acarbose inhibited the activity with an $IC_{50}$ value of 4.07 mg/ml (Table 1).

### α-Amylase inhibition kinetic studies

The mechanism of inhibition of α-amylase by the aqueous extract of *V. doniana* was assessed using Lineweaver-Burk plot (double reciprocal). It displayed patterns of mixed inhibition of the enzyme activity as shown in Figure 1. The Km was increased from 0.85 mg/ml without the extract to 1.2 mg/ml with the extract. The value of Vmax was reduced from $4.4 \times 10^{-1}$ to $4.0 \times 10^{-1}$ mg/ml.min$^{-1}$ with and without the extract respectively.

### α-Glucosidase inhibition assay

All the extracts exerted less than 50% inhibition of α-glucosidase activity. The inhibition was found to increase with an increase in the concentration and it reached saturation after 8 mg. $IC_{50}$ values were used to compare inhibition effectiveness of the plant extracts (Table 2). However, at 8 mg/ml, the ethanol extract (VDE) was the most potent and exhibited a maximum of 24.63% inhibition ($IC_{50}$ of 17.12 mg/ml). This was followed by the aqueous extract (VDAQ), with an $IC_{50}$ value of 23.30 mg/ml achieving only 19.18% inhibition. The methanol extract (VDM) displayed an $IC_{50}$ of 23.33 mg/ml and was the least inhibitor (17.96% inhibition). Acarbose inhibited α-glucosidase with an $IC_{50}$ value of 9.0 mg/ml exhibiting 40% inhibition.

### Kinetics of α-glucosidase inhibition

The mechanism of inhibition by the most active plant extract against α-glucosidase (VDE) displayed patterns of mixed inhibition as shown in Figure 2. The Km was increased from $1.9 \times 10^{-2}$ mg/ml in the absence of extract to $6.2 \times 10^{-2}$ mg/ml in the presence of extract while the value of Vmax was reduced from 2.72 to 2.39 mg/ml.min$^{-1}$ with and without the extract respectively.

### Animal experiment

#### *Weights of the animals*

The weights of the animals recorded before the treatment, 48 h post-induction of diabetes and on weekly basis following treatment are presented in Table 3. Alloxan caused a reduction in the weight of the diabetic animals as seen 48 h post-induction. There was a significant improvement ($p < 0.05$) in the weights of the animals following treatment with the ethanol leaf extract
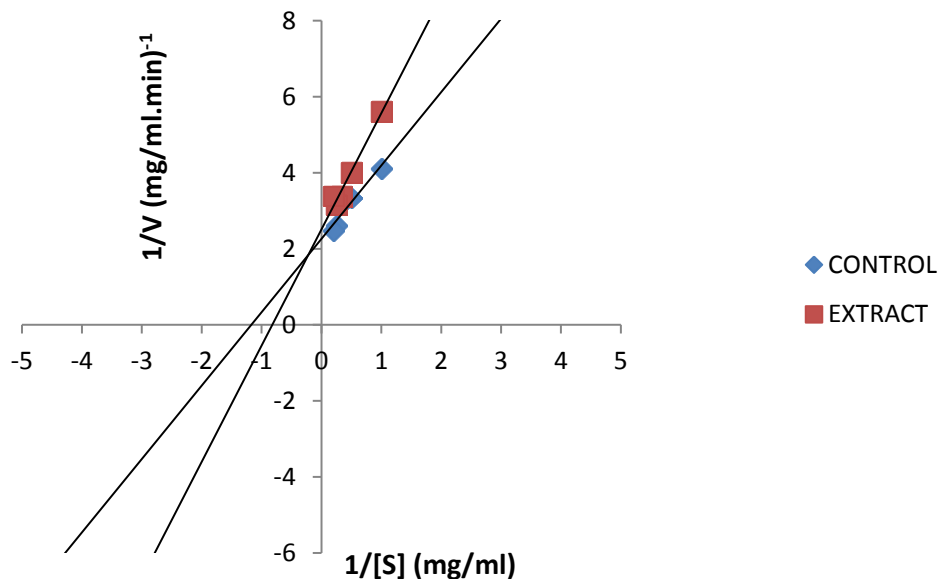
**Figure 1.** Mixed inhibition of α- amylase by the aqueous extract of *V. doniana.*

**Table 1.** Percentage inhibition of α- amylase by different leaf extracts of *V. doniana* and the standard; acarbose at varying concentrations.

| Concentration (mg/ml) | % Inhibition Acarbose | % Inhibition Methanol | % Inhibition ethanol | % Inhibition Aqueous |
|---|---|---|---|---|
| 2 | 59.51±10.1[a] | 60.38±6.27[a] | 57.71±1.91[a] | 67.37±4.27[bc] |
| 4 | 62.89±9.01[a] | 63.35±6.04[a] | 68.06±9.43[b] | 71.02±1.09[bc] |
| 6 | 66.75±3.11[a] | 66.89±6.59[a] | 72.34±4.60[b] | 74.84±1.59[bc] |
| 8 | 69.00±3.31[a] | 70.72±5.46[a] | 75.37±4.52[b] | 78.19±2.94[bc] |
| 10 | 72.45±3.26[a] | 72.23±0.83[a] | 76.91±0.26[b] | 79.52±3.04[bc] |
| $IC_{50}$ (mg/ml) | 4.07±0.83 | 4.02±0.71 | 3.67±0.36 | 3.09±0.22 |

Values are presented as means ± SD of the tests in triplicate. [a] Significant reduction in comparison with other groups. [b] Significant increase in comparison with Acarbose; [c] Significicnat increase in comparison with all extract groups.

of *V. doniana* and maximum improvement was observed in the second week of the experiment compared to the weight in the first week and at the commencement of the experiment. The weight of the untreated diabetic group reduced when compared with the extract treated groups and their initial weight.

**Antidiabetic activity**

The *in-vivo* antidiabetic study was done using the ethanol leaf extract of *V. doniana.* The effects of treatment with all ethanol extract doses and glibenclamide on the blood glucose level of the diabetic rats in comparison with the normal control and diabetic control are presented in Table 4. The blood glucose levels were reduced considerably within 14 days of daily treatment. There was

dose-dependent reduction in the blood glucose level of the treated groups. The diabetic groups treated with ethanol leaf extract of *V. doniana* showed significant (p<0.05) reduction in blood glucose levels when compared with the untreated group at all the doses tested. There was significant difference between the groups treated with the ethanol leaf extract of *V. doniana* and that treated with glibenclamide, a standard anti-diabetic drug. The standard drug showed a better glucose lowering ability compared to the extract.

**DISCUSSION**

High blood sugar is the main characteristic feature of type 2 diabetes mellitus, thus, reduction of postprandial hyperglycemia is a good therapeutic approach in
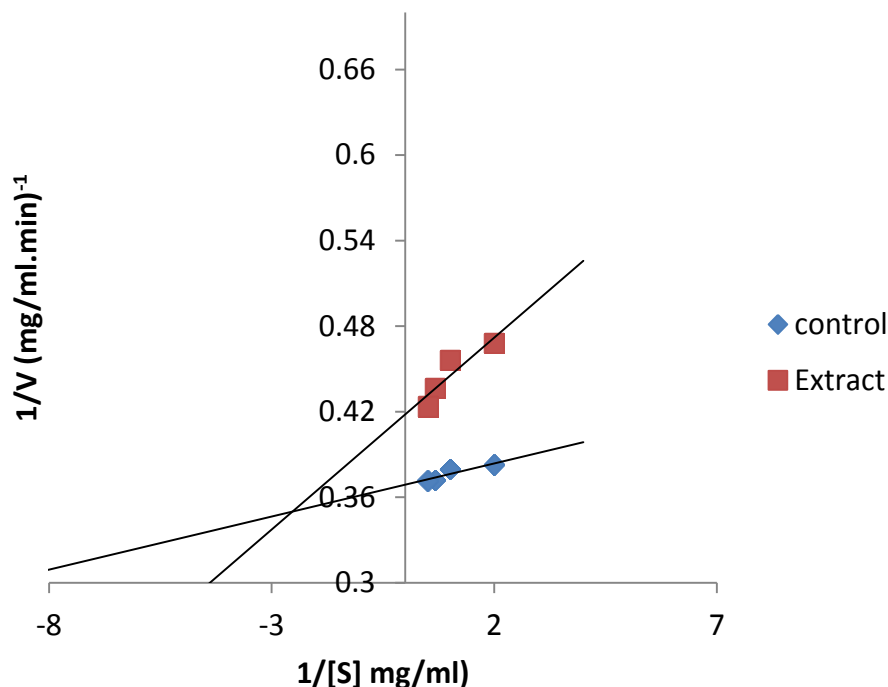
**Figure 2.** Mixed inhibition of α-glucosidase by ethanol extract of *V. doniana.*

**Table 2.** Percentage inhibition of α-glucosidase by different plant extracts of *V. doniana* at varying concentrations with their $IC_{50}$.

| Concentration (mg/ml) | % Inhibition Acarbose | % Inhibition Methanol | % Inhibition ethanol | % Inhibition Aqueous |
|---|---|---|---|---|
| 2 | 5.95±0.14 | 7.83±0.46 | 9.20±0.35[c] | 5.56±0.41[b] |
| 4 | 32.23±0.05[a] | 12.62±2.4[b] | 12.44±0.82[b] | 7.25±0.33[b] |
| 6 | 34.66±0.09[a] | 13.87±2.34[b] | 17.17±0.81[c] | 10.24±0.35[b] |
| 8 | 40.35±0.13[a] | 17.96±0.84[b] | 24.63±2.09[c] | 19.18±0.18[c] |
| IC50 (mg/ml) | 9.0±0.03 | 23.33±1.67 | 17.12±1.4 | 23.30±0.41 |

Values are presented as means ± SD of the tests in triplicate. [a]Significant increase in comparison with the extract groups; [b] Significant decrease in comparison with other groups; [c]Significant increase in comparison with other extract groups.

treatment of type 2 diabetes (Chipiti et al., 2017). The leaf extracts of *V. doniana* showed potent inhibition of α-amylase activity with a significant (p<0.05) increase in inhibition activity as the concentration increases. The aqueous extract was the most potent inhibitor of α-amylase. The inhibitory activity increased significantly when compared with the standard. This might be one of the possible reasons for the reported traditional use of the plant in diabetes and their hypoglycemic activity. It might also be as a result of several phytochemicals such as flavonoids, tannins and saponins as reported (Sama et al., 2012; McEwan et al., 2010). Furthermore, the mechanism of inhibition of α-amylase by the aqueous

extract of *V. doniana* was determined using Lineweaver-Burk plot. A pattern of mixed inhibition of the enzyme activity was displayed as shown in Figure 1. The Km was increased from 0.85 mg/ml with the extract to 1.2 mg/ml without the extract while the value of Vmax was reduced from 4.4 x 10^{-1} to 4.0 x 10^{-1} mg/ml.min^{-1} with and without the extract respectively. A mixed inhibitor prevents binding of the enzyme to its substrate thereby reducing efficiency. It is so because binding of the inhibitor at sites other than on the active site interferes with binding of the enzymes and subsequently, the rate at which products are formed. Mixed inhibition can lead to either a reduction in the affinity of the enzyme for the substrate (Km value

**Table 3.** Effect of ethanol leaf extract of *V. doniana* on body weight of diabetic rats in comparison with the normal control group.

| Sample | Weight variation in gram | | | | | |
|---|---|---|---|---|---|---|
| | Normal | 200 mg/kg | 400 mg/kg | 600 mg/kg | Glibenclamide | Untreated |
| Initial | 165.81±3.24 | 195.25±5.50 | 241.50±3.20 | 191.00±3.98 | 175.00±5.01 | 189.50±1.28 |
| Post-Induction | 171.75±2.16 | 183.16±3.21[b] | 221.50±2.10[b] | 183.50±4.01[b] | 172.00±2.43 | 187.00±0.12[b] |
| 1st week | 170.00±4.13 | 198.50±3.00[ac] | 244.00±3.50[ac] | 174.50±5.31[b] | 165.00±1.19 | 182.50±3.56[b] |
| 2nd week | 186.00±4.87 | 222.00±1.15[ac] | 250.00±2.10[ac] | 194.50±3.43[a] | 176.00±3.89 | 178.50±0.00[b] |

Values are presented as means ± SD of the tests in triplicate. [a]Significant increase in comparison with the extract groups; [b] Significant decrease in comparison with other groups; [c]Significant increase in comparison with other extract groups.

**Table 4.** Fasting blood glucose levels of the treated rats, untreated rats and the control groups measured daily for 14 days.

| Days | Normal Group A | 200 mg Group B | 400 mg Group C | 600 mg Group D | Glibenclamide Group E | Untreated Group F |
|---|---|---|---|---|---|---|
| Initial | 86.00±3.01 | 112.00±4.38 | 82.25±3.18 | 86.25±2.11 | 77.00±2.32 | 82.00±1.14 |
| Post Induction | 124.00±2.10 | 303.67±13.43 | 330.00±6.55 | 600.00±12.56 | 600.00±14.12 | 600.00±13.15 |
| Day 1 | 107.00±1.00 | 334.33±15.51[ab] | 376.50±3.17[ab] | 522.50±8.71[ab] | 600.00±12.23[b] | 523.00±7.81[bc] |
| Day2 | 89.00±2.00 | 303.00±6.89[ab] | 355.00±5.19[ab] | 377.50±11.21[ab] | 600.00±8.67[b] | 523.00±9.13[bc] |
| Day3 | 93.00±3.10 | 283.67±3.99[ab] | 301.00±5.43[ab] | 329.00±9.22[ab] | 523.00±11.1[ab] | 489.00±12.31[bc] |
| Day4 | 101.00±2.21 | 268.67±3.54[ab] | 297.50±4.20[ab] | 360.50±2.65[ab] | 368.00±4.31[ab] | 490.00±6.12[bc] |
| Day5 | 89.00±2.20 | 255.00±6.15[ab] | 292.50±7.17[ab] | 366.50±4.36[ab] | 364.00±3.12[ab] | 490.00±4.10[bc] |
| Day6 | 76.00±0.89 | 247.00±2.13[ab] | 286.50±3.10[ab] | 359.00±1.87[ab] | 361.00±3.10[ab] | 471.00±3.11[bc] |
| Day 7 | 81.00±2.10 | 242.00±0.00[ab] | 280.00±5.34[ab] | 350.50±2.12[ab] | 282.00±5.19[ab] | 461.00±5.02[bc] |
| Day 8 | 87.00±4.21 | 242.67±2.27[ab] | 272.50±2.21[ab] | 334.50±3.46[ab] | 276.00±4.21[ab] | 410.00±3.15[bc] |
| Day 9 | 98.00±2.20 | 230.67±4.17[ab] | 262.50±6.17[ab] | 308.00±1.09[ab] | 160.00±2.20[abd] | 400.00±1.09[bc] |
| Day 10 | 100.00±3.12 | 213.33±2.22[ab] | 247.50±2.18[ab] | 286.00±5.96[ab] | 151.00±3.70[abd] | 398.00±3.98[bc] |
| Day 11 | 90.00±3.30 | 203.00±4.12[ab] | 230.00±8.12[ab] | 260.00±2.12[ab] | 111.00±3.11[ad] | 390.00±2.20[bc] |
| Day 12 | 85.00±2.10 | 193.00±0.19[ab] | 216.50±0.00[ab] | 240.50±5.23[ab] | 90.00±3.21[ad] | 390.00±0.00[bc] |
| Day 13 | 89.00±2.20 | 183.67±2.22[ab] | 186.00±0.00[ab] | 220.50±4.51[ab] | 101.00±1.13[ad] | 388.00±0.00[bc] |
| Day 14 | 90.00±1.90 | 167.33±7.16[ab] | 170.00±2.12[ab] | 197.00±0.00[ab] | 90.00±1.10[ad] | 282.00±0.00[bc] |

Values are presented as mean ± SD. [a]Significant reduction in comparison with untreated diabetic group; [b]Significant increase in comparison with the normal control group; [c]significant increase in comparison with the treated groups; [d]Significant reduction in comparison with the extract treated groups.

increases); that is where the free enzyme binds favorably to the inhibitor or to an increase in the affinity of the enzyme for the substrate (Km value decreases) if the inhibitor binds favorably to the enzyme-substrate complex. In any case, the apparent maximum rate of the enzyme reaction is reduced by the inhibition (Storey, 2004). In this study, the Vmax decreased with an increase in Km. This result shows that the inhibitory component of the plant extract do not bind on the active site of the enzyme competitively with the substrate rather the inhibitors bind to other site on the enzyme delaying the breakdown of carbohydrate (Mogale et al., 2001). A good strategy used in the treatment of type 2 diabetes is delaying glucose production and uptake (Picot et al., 2014).

Alpha-glucosidase inhibitors slow down hydrolysis of carbohydrate and reduce the postprandial glucose uptake in the diabetic (Kwon et al., 2007). With the low α-glucosidase inhibition by *V. doniana,* it suggests that the plant is a mild α-glucosidase inhibitor but can still be used in treatment of postprandial hyperglycemia albeit not effectively. The ethanol extract of *V. doniana* was the strongest inhibitor of α-glucosidase. The inhibitory activity decreased significantly ($p < 0.05$) in comparison with the standard (Acarbose). Acarbose, inhibited α-glucosidase with an $IC_{50}$ of 9.0 mg/ml and exhibited 40% inhibition. This finding is in line with other reports where acarbose exerted little inhibition on α-glucosidase (Shai et al., 2010). Furthermore, to ascertain the mechanism of inhibition of *V.* doniana on α-glucosidase, the ethanol leaf

extract displayed mixed competitive inhibition. The Km was increased from $1.9 \times 10^{-2}$ mg/ml in the absence of extract to $6.2 \times 10^{-2}$ mg/ml in the presence of extract while the value of Vmax was reduced from 2.72 $mg/ml.min^{-1}$ to 2.39 $mg/ml.min^{-1}$ with and without the extract respectively. This also suggests that the inhibitor do not competitively bind on the active site of the enzyme with the substrate but binds to other site on the enzyme delaying turnover.

From the animal experiment, induction of alloxan produced loss of body weight in all the diabetic groups as seen 48 h post induction when compared to the normal control in Table 3. Following treatment with the ethanol leaf extract of *V. doniana,* there was significant improvement in body weight of the animals compared to the untreated diabetic control.

The result of the antidiabetic study showed that there was a significant ($p < 0.05$) increase in the blood glucose levels of all the animals following administration of alloxan compared to the normal control as seen in Table 4. However, following treatment with ethanol leaf extract of *V. doniana,* there was significant reduction ($p < 0.05$) in the fasting blood glucose level of the diabetic animals (groups B-D) compared to the untreated diabetic group (F). The blood glucose level was reduced significantly within 14 days of daily extract administration. The potent anti-diabetic effect of the ethanol leaf extract of *V. doniana* suggests that the plant extract contains potent antidiabetic active components, which produced anti-hyperglycemic effect in the diabetic rats. The reduction in the blood glucose level also led to increase in the body weight of the animals.

Therefore, leaf extracts of *V. doniana* have antidiabetic activity and the mechanism through which it exerts its antidiabetic effect is through the inhibition of the two carbohydrate metabolizing enzymes; α-amylase and α-glucosidase.

## Conclusion

Enzyme inhibitors disrupt biochemical processes and activities of enzymes and are thus produced as drugs. Poisons are natural inhibitors that protect plants and animals from predators. *V. doniana* leaves extracts have displayed effective inhibition of α-amylase and a moderate inhibition of α-glucosidase. From the *in-vivo* study, the ethanol leaf extract has also confirmed that the plant has antidiabetic ability. This study has proven that one of the mechanisms by which *V. doniana* plant exerts its antidiabetic activity is through the inhibition of these two carbohydrate metabolizing enzymes. Diabetes is known as one of the world's greatest health problems and has become a serious medical concern worldwide. This prompts efforts in exploring for new antidiabetic agents. In adopting an alternative treatment approach to curb the

problems of drug resistance and its associated side effects, the leaf of *V. doniana* may be exploited as a good treatment option.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENT

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