Original Article

**IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS**

Iranian J Pharmacol Ther. 2019 (November);17:1-7.

# Inhibitory effect of aqueous extract of *Ocimum Gratissimum* on digestive enzymes of carbohydrates, α-amylase and α-glucosidase *In-vitro*

Tajudeen A Lawal[1]*, Chimaobi J Ononamadu[1], Tajudeen A Owolarafe[1], Salawu Kailani[1], Abdullahi A Imam[1], Adamu J Alhassan[2]

[1] Department of Biochemistry and Forensic Science, Faculty of Science, Nigeria Police Academy Wudil, Kano State, Nigeria
[2] Biochemistry Department, Faculty of Basic Medical Science, Bayero University Kano, Nigeria

**Please cite this article as:**
Lawal TA, Ononamadu CJ, Owolarafe TA, Kailani S, Imam AA, Alhassan AJ. Inhibitory effect of aqueous extract of *Ocimum Gratissimum* on digestive enzymes of carbohydrates, α-amylase and α-glucosidase *In-vitro*. Iranian J Pharmacol Ther. 2019 (November);17:1-7.

## ABSTRACT

For a long period, ethno medicinal plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of ethno medicinal plant for pharmaceutical purposes has gradually increased in Iran. *Gundelia tournefortii* has been used as an antibacterial, anti-fungal, antipyretic, anti-inflammatory, and antioxidant agent in Iran. In the recent examination, the testicular protective effect of *G. tournefortii* aerial parts aqueous extract on diabetic mice has been evaluated. Seventy mice were used and diabetes was induced by administration of 150 mg/kg of alloxan monohydrate intraperitoneally in 60 mature male mice and they were randomly divided into six groups. The treatment groups received glibenclamide 10 mg/kg and 5, 10, 20 and 40 mg/kg of *G. tournefortii* through gavage for 20 days. Also, one group was considered as the non-diabetic control. At 20$^{th}$ day, the mice were killed, dissected, then blood and testis samples were collected for biochemical and stereological parameters analysis. The data were analyzed by SPSS-21 software. *G. tournefortii* at all doses (especially GT40) and glibenclamide significantly ($p \leq 0.05$) ameliorated the concentrations of fasting blood glucose, testosterone, superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase. Also, multiple doses of *G. tournefortii* (especially GT40) and glibenclamide increased the weight and volume of the testis, the volumes of the tubule and interstitial tissue, the length and diameter of the tubule, the height of the germinal epithelium, and the number of the Leydig cell compared to the diabetic untreated group. According to the obtained results, *G. tournefortii* aerial parts aqueous extract can regulate the concentrations of biochemical parameters and inhibit testicular damages in alloxan monohydrate induced diabetic mice. It seems that *G. tournefortii* can be offered as a testicular protective supplement or drug for prevention, control, and treatment of testicular toxicity in diabetic patients.

Conflicts of Interest: Declared None
Funding: None

**Keywords**
Inhibition,
α-amylase,
α-glucosidase,
Ocimum gratissimum,
Diabetes,
Acarbose

***Corresponding to***:
Tajudeen A Lawal,
Department of Biochemistry and Forensic Science, Faculty of Science, Nigeria Police Academy Wudil, Kano State, Nigeria

Email:
talawal@polac.edu.ng

Received: 20 May 2019
Published:11 Nov 2019

## INTRODUCTION

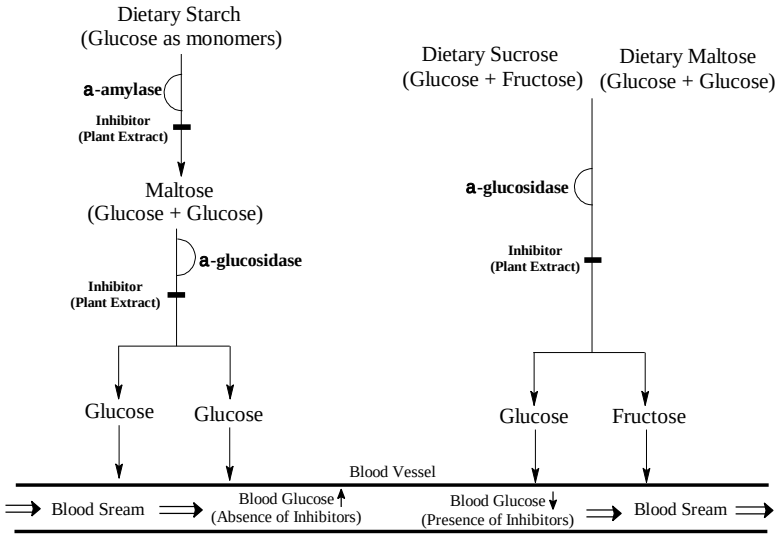
Diabetes mellitus is a lifelong metabolic disease, which is marked by upsurge in blood glucose level that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood sugar) or when the body cannot effectively use the insulin produced [1]. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time may lead to microvascular and macrovascular damage, especially the nerves, kidney, heart,

eyes, blood vessels and other cardiovascular diseases [2]. According to WHO 2018 [3], 9% of adults, 18 years and older, had diabetes in 2014. In 2012, diabetes was the direct cause of 1.5 million deaths and more than 80% of diabetes deaths occur in low- and middle-income countries [4]. According to the World Health Statistics (2012), one in ten adults worldwide has diabetes and WHO (2014) projects that diabetes will be the seventh leading cause of death by 2030 [5].

Nigeria has a high population of people suffering from diabetes than those living with HIV/AIDS and about four million Nigerians and 387 million people worldwide have diabetes [6-8]. To date, there is no record of any drug that has potential to cure diabetes permanently and synthetic drugs designed to manage this dreaded disease have some deleterious side effects such as liver disorders, flatulence, abdominal pain, renal tumour, abdominal fullness and diarrhoea [9, 10]. Thus, there is an increasing need for the development of a natural and safe products without or with minimal side effects. Natural products are projected to elicit their anti-hyperglycaemic effects through the ability to restore the function of pancreatic tissues, by causing an increase in insulin production, inducing insulin-receptor interaction, mimic or enhance subcellular effects of insulin or by causing a decrease in the intestinal absorption of glucose through inhibition of digestive enzymes of carbohydrate (α-amylase and α-glucosidase) and may present with no or minimal side effects [10].

Many Nigerian plants including member of the *Ocimum* genus - *Ocimum canum* [11], *Ocimum gratissimum*, *Ocimum basilicum* [12,13], *Ocimum tenuiflorum* [14], *Ocimum sanctum* [13] have been reported to be potentially hypoglycaemic and employed in managing Diabetes mellitus. Extracts of these species was reported to show a satisfactory anti-hyperglycaemia activity due to the possession of bioactive phenolic compoundsl which may be responsible for their glucose reducing effects [15].

*Ocimum gratissimum (Linn.),* a family *Lamiaceae*, is an herbaceous plant generally found in the grassland and tropical rain forest of West Africa [16]. It is also known as *Clove basil* [15] and commonly called 'scent leaf' in Nigeria and it is commonly used as a flavouring agent in cooking [17]. The plant's local names in Nigeria are Efinrin (Yoruba), Daidoyatagida (Hausa), Esewon (Edo-Akoko), Nehonwu, Nchanwu or Ahuji (Igbo), Nton (Ibibio/Efik), Aramogbo (Edo) and in French; Menthesauvage [16-18]. Phytochemical scrutiny of *Ocimum gratissimum* leaf extracts revealed vital components as tannins, alkaloids, saponins, flavonoids, steroids, phlobatannin, terpenoids, cardiac glycosides and phenolic compounds [19, 20]. Wonga *et al.*,[21] (2012) has shown that Ocimum sactum was one of the species of Ocimum family with high total phenolic content. *Ocimum gratissimum* was reported to possess therapeutic properties such as hypoglycaemic, hepatoprotective, anti-tumour, anti-ulcer, anti-helminthic, antifungal, antibacterial, anti-convulsant and analgesic activities [15, 22]. *Ocimum gratissimum* extracts have been reported to be effective against diabetes mellitus [15, 22]. The aqueous extract of the plant according to Okon *et al.*[16] decreased blood sugar level in streptozotocin-induced diabetic rats and ameliorated the associated symptoms namely; polydipsia, polyphagia and weight loss [16]. Mohammed *et al*., [23] (2007) in their review reported that AEOGL decreased blood glucose level by about 81.30% in Streptozocin-induced diabetic rats after 24h of administration. In the same view, aqueous extract of *Ocimum basilicum* was reported to significantly ($P<0.05$) inhibited rat intestinal sucrase, maltase and porcine pancreatic α-amylase [15]. Despite this huge potential in management of Diabetes, there is still dearth of literature on possible hypoglycaemic mode of actions of the extracts of this plant. Thus, this present study investigated the inhibitory activity of aqueous extract of *Ocimum gratissimum* leaves (AEOGL) on the two carbohydrate digestive activity enzymes – α-amylase and α-glucosidase. The hypothesised mechanism of action of the extract is depicted in Diagram 1.




Digram 1. The hypothesised mechanism of action of the extract

## MATERIALS AND METHODS

### *Materials*

*Equipment:* Equipment used for this study includes, centrifuge, spectrophotometer, water bath, weighing machine, heater, oven, micropipettes, test-tubes, test-tube rack

*Medicinal Plant Used: Ocimum gratissimum* leaves were obtained locally from Ilorin, Kwara state, Nigeria. The leaf was identified in herbarium unit of Biological department of Bayero University Kano, Nigeria and was assigned herbarium number BUKHAN00306. A sample of the leaves was deposited in the herbarium for record purpose.

*Reagents:* Porcine pancreatic α-amylase, saccharomyces cerevisiae α-glucosidase, p-nitrophenyl glucopyranoside (pNPGP) and Dinitrosalicylic acid (DNSA) were purchased from Sigma-Aldrich USA, Soluble starch, Maltose monohydrate, D-glucose, p-Nitrophenol, sodium dihydrogen phosphate dihydrate and disodium hydrogen phosphate dodecahydrate were purchased from Hopkin & Williams Chadwell Health Essex England. Sodium hydroxide and sodium carbonate solution were purchased from BDH Chemicals Ltd Poole England, while potassium sodium tartrate was from Fisons Plc Loughborough LE II ORG, England. Acarbose (Glucobay 100mg/tablet by Bayer -antidiabetic) was purchased from Bayer AG Kaiser-Wilhelm-Allee 51368 Leverkusen Germany.

### *Methodology*

*Preparation of the Aqueous Extract of Ocimum gratissimum Leaves (AEOGL):* Aqueous Extract of *Ocimum gratissimum* Leaves (AEOGL) was prepared by washing the leaves and thereafter air-dried in the shade for seven days and then ground to powder using mortar and pestle. 50g of the powder was macerated in 500mL of deionised water. The mixture stood for 48 hours with intermittent shaking and thereafter filtered with mesh cloth. The liquid mixture was then centrifuged at 8000 rpm for 10 minutes. The crude extract was then oven-dried at a temperature of 45°C to yield a dry powdery residue. The extract was reconstituted in buffer solution in appropriate concentration before administration.

*Preparation of Acarbose Solution:* Weight of 5 tablets of acarbose (Glucobay 100mg/tablet) was taken and the average weight determined. Equivalent weight of 50 mg as acarbose was weighed and dissolved in 50mL of sodium phosphate buffer, pH 6.9, agitated for 30 minutes, filtered and the filtrate was refrigerated and used within 2 weeks.

*α-Amylase Inhibitory Assay:* This assay was carried out using a modified method of Apostolidis and Lee [24]. Different concentrations of extracts; 80, 160, 240, 320 and 400 µg/mL were prepared in 20 mM of sodium phosphate buffer (pH 6.9). Aliquot amount (250µL) of each extract was pre-incubated with 250µL of α-amylase enzyme (0.5mg/mL) at 25°C for 10 minutes. The mixture was then incubated with 250 µL of 1% $^{w}/_{v}$ starch solution at 25°C for another 10 minutes. The reaction was put to a halt by adding 500µL of DNSA reagent to the reacting mixture, boiled for 5 minutes in water bath and allowed to cool to room temperature. The cooled mixture was diluted with 10mL of deionised water and the absorbance of the mixture was taken at 540nm. The control sample without AEOGL (negative control) was treated in the same manner but buffer was substituted for the extract. Acarbose was also used in place of extract as positive control and treated in the same way.

The experiments were run in triplicates and the absorbance was converted to the amount of maltose released using a maltose standard curve and the enzyme activities were determined by using the formula;

$$Ua = \frac{\text{mg maltose x DF}}{\text{Time x mg enzyme}}$$

Where 'Ua' is the unit of α-amylase activity
'DF' is dilution factor
'Time' is the time of incubation
'mg enzyme' is the amount of enzyme in the reaction mixture

Percentage of enzyme activities was determined from the formula:

$$\% \text{ Enzyme Activity} = \frac{A_{\text{with extract}}}{A_{\text{without extract}}} \text{ x } 100$$

Where 'A' is the activity of enzyme

A plot of percentage enzyme activity was made against the concentrations of the extracts and the concentration of the extract that corresponds to 50% enzyme activities ($IC_{50}$) was determined graphically. $IC_{50}$ is the inhibitory concentration of 50% enzyme activities.

*Mode of α-Amylase Inhibition:* The mode of inhibition of the extract was conducted according to the modified method of Ali *et al* [25]. Two different concentrations of the extract were prepared; 100 and 200 µg/mL, and five different concentrations of starch solutions were also prepared; 5.0, 10.0, 15.0, 20.0 and 25.0 mg/mL. A volume of 250µL of each concentration of the extract was pre-incubated with 250µL of α-amylase (0.5mg/mL) separately for 10 minutes at 25°C in five pairs of test-tubes. The mixtures were then incubated with 250µL of the different concentration of substrate (starch) at 25°C for catalysis to commence for another 10 minutes. The reactions were put to halt by the addition of 500µL of DNSA and boiled for 5 minutes in a water bath. The reaction mixtures were allowed to cool to room temperature and the absorbance of the mixtures were taken at 540nm. The control sample was also prepared and treated likewise, but the extract was replaced by buffer solution.

The amount of maltose released was determined spectrophotometrically using a maltose standard curve and converted to reaction velocities (v)

$$v = \frac{\text{mg maltose x DF}}{MW_{\text{maltose}} \text{ x Time x Vol. of Enzyme}}$$

Where v is the velocity, DF is dilution factor, 'Time' is

the time of incubation and $MW_{maltose}$ is molecular weight of maltose.

A double reciprocal or Lineweaver-Burk plot (1/v versus 1/[S]), where v is reaction velocity and [S] is substrate concentration, was plotted and the mode of inhibition determined from the nature of the graph obtained.

*α-Glucosidase Inhibitory Assay:* The effect of each plant extract on α-glucosidase activity was determined according to the modified method of Kang *et al*.,[26]. Various concentrations of extracts; 10, 30, 60, 80 and 100 µg/mL were prepared in 20 mM of sodium phosphate buffer (pH 6.9). Aliquot amount (100µL) of each extract was pre-incubated with 100µL of α-glucosidase enzyme (1.0U) at 37°C for 10 minutes. The mixture was then incubated with 100 µL of 10 mM p-nitrophenyl-α-glucopyranoside (pNPG) solution at 37°C for another 10 minutes. The reaction was put to halt by adding 2 mL of 0.2M $Na_2CO_3$ solution to the reacting mixture. The mixture was diluted with 5mL of deionised water and the absorbance of the mixture was taken at 405 nm. The control sample without AEOGL (negative control) was treated in the same manner but buffer was substituted for the extract. Acarbose was also used in place of extract as positive control and treated in the same way.

The experiments were run in triplicates and the absorbance was converted to the amount of p-nitrophenol released using p-nitrophenol standard curve and the enzyme activities were determined by using the formula;

$$Ug = \frac{\text{mg p-nitrpphenol x DF}}{\text{Time x mg enzyme}}$$

Where 'Ug' is the unit of α-glucosidase activity

'DF' is dilution factor

'Time' is the time of incubation

'mg enzyme' is the amount of enzyme in the reaction mixture

Percentage of enzyme activities was determined from the formula:

$$\% \text{ Enzyme Activity} = \frac{A_{\text{with extract}}}{A_{\text{without extract}}} \text{ x } 100$$

Where 'A' is the activity of enzyme

A plot of percentage enzyme activity was made against the concentrations of the extracts and the concentration of the extract that inhibits 50% enzyme activities ($IC_{50}$) was determined graphically.

*Mode of α-Glucosidase Inhibition:* The mode of inhibition of the extract was determined using the modified method of Ali *et al* [25]. It was run in triplicate. Two different concentrations of the extract were prepared; 15 and 30µg/mL, and five different concentrations (1.5, 3.0, 4.5.0, 6.0 and 7.5mM) of pNPG solutions were prepared too. 100µL of each extract concentration was pre-incubated with 100µL of α-glucosidase (1.0U) separately for 10 minutes at 37°C in the five pairs of test-tubes. The mixtures were then incubated with 100µL of the different concentration of substrate (pNPG) at 37°C for catalysis to commence for another 10 minutes. The reactions were put to halt by the addition of 2mL of 0.2M $Na_2CO_3$ and the absorbance of the mixtures were taken at 405 nm. The control sample was also prepared and treated likewise, but the extract was replaced by buffer solution.

The amount of p-nitrophenol released was determined spectrophotometrically using a p-nitrophenol standard curve and converted to reaction velocities (v).

$$v = \frac{\text{mg p-nitrophenol x DF}}{MW_{\text{p-nitrophenol}} \text{ x Time x Vol. of Enzyme}}$$

Where v is the velocity, DF is dilution factor, 'Time' is the time of incubation and $MW_{\text{p-nitrophenol}}$ is molecular weight of p-nitrophenol.

A Lineweaver-Burk (double reciprocal) plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted. The type (mode) of inhibition of the crude extract on α-glucosidase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

## RESULTS

The result of inhibitory activity of AEOGL on α-amylase is presented in Table 1. The result reveals that the activity of α-amylase reduces significantly ($P < 0.05$) as the concentration of the extract increases when compare to their control group (i.e. extract concentration of zero). The extract concentration that inhibit 50% α-amylase enzyme activities ($IC_{50}$) was determined to be 305.33µg/mL. The result also showed that as the concentration of the extract increases, percentage α-amylase activity reduces, and percentage inhibition increases.

Table 1. Concentration Effect of AEOGL on α-Amylase Activity

| Conc. of AEOGL (µg/mL) | 0 | 80 | 160 | 240 | 320 | 400 | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|---|---|
| α-Amylase Activity * (µmol maltose/min/mg enzyme) | 45.52±1.05 | 37.88±0.33 | 31.74±3.44 | 27.32±0.72 | 22.62±0.77 | 17.25±0.55 | 305.33±10.22 |
| % α-Amylase Activity | 100 | 83.23 | 69.74 | 60.02 | 49.70 | 37.91 | |
| % Inhibition | 0.00 | 16.77 | 30.26 | 39.98 | 50.30 | 62.09 | |

*Mean ± Standard Deviation (of Triplicate)

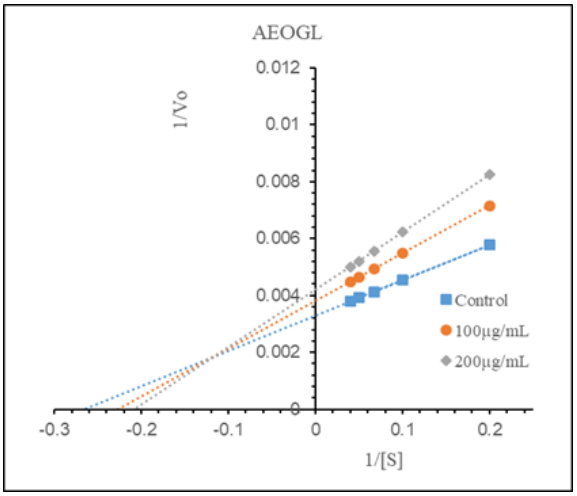



Figure 1. Lineweaver-Burk Plot of α-amylase with and without AEOGL

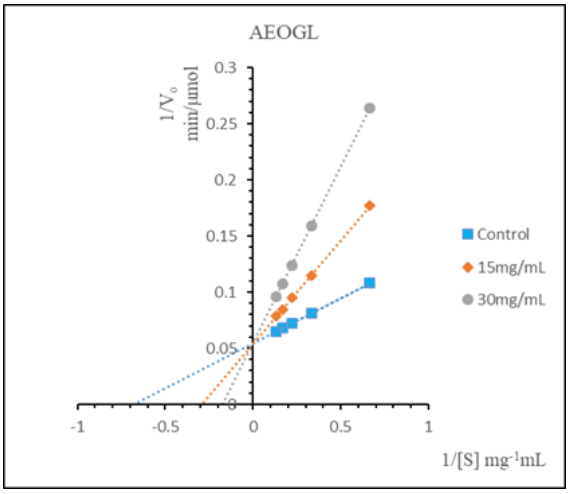



Figure 3. Lineweaver-Burk plot of α-glucosidase with and without AEOGL

Figure 1 presents the Lineweaver-Burk plot of α-amylase with and without AEOGL. The mode of inhibition was determined from the nature of the graph to be mixed competitive inhibition.

The inhibitory activity of acarbose on α-amylase catalysis shows that activity of α-amylase enzyme reduces significantly ($P < 0.05$) as the concentration of the drug increases (Table 2). The result shows that as the concentration of AEOGL increases, percentage α-amylase activity reduces, and percentage inhibition increases. The $IC_{50}$ was determined to be 60.72±4.00µg/mL. This was significantly ($P < 0.05$) higher than that of AEOGL.

Table 3 shows the effect of AEOGL on activity of α-glucosidase. AEOGL reduces the activity of the enzyme significantly ($P < 0.05$) as the concentration of the extract increases. Percentage α-glucosidase activity reduces, and percentage Inhibition increases as the concentration of AEOGL increases (Table 3). The concentration of AEOGL that reduces the activity of the enzyme by 50% ($IC_{50}$) was determined from the graph to be 25.42±2.54µg/mL. The mode of inhibition of AEOGL against α-glucosidase was determined from Lineweaver-Burk plot was determined to be competitive (Fig. 3).

Table 4 shows the concentration effect of acarbose on activity of α-glucosidase. Acarbose showed a significantly poor inhibition of the activity of α-glucosidase as the concentration of acarbose increases (Table 4). The concentration of acarbose that reduced the activity of the enzyme by 50% ($IC_{50}$) was determined to be (112.23±7.43µg/mL). The mode of inhibition of acarbose was determined from the nature of the Lineweaver Burk graph to be mixed competitive inhibition (Fig. 4).

## DISCUSSION

The result of the study showed that the aqueous extract of *Ocimum gratissimum* leaves (AEOGL) have a significant potential to competitively inhibit α-glucosidase better than α-amylase. This is an indication that the AEOGL may contain phytochemicals that are structurally analogues to the natural substrates of the two enzymes which consequently competes

Table 2. Concentration Effect of Acarbose on α-Amylase Activity

| Conc. of Acarbose (µg/mL) | 0 | 80 | 160 | 240 | 320 | 400 | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|---|
| α-Amylase Activity * (µmol maltose/min/mg enzyme) | 39.98±0.50 | 16.76±0.50 | 9.84±0.55 | 6.08±0.55 | 3.76±0.44 | 3.10±0.06 | 60.72±4.00µg/mL |
| % α-Amylase Activity | 100 | 41.91 | 24.62 | 15.21 | 9.41 | 7.75 | |
| % Inhibition | 0.00 | 58.09 | 75.38 | 84.79 | 90.59 | 92.25 | |

*Mean ± Standard Deviation (of Triplicate)

Table 3. Concentration Effect of AEOGL on α-Glucosidase Activity

| Conc. (µg/mL) of AEOGL | 0 | 10 | 30 | 60 | 80 | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|---|
| α-Glucosidase Activities* (µmol p-nitrophenol/min/mg enzyme) | 19.48±0.31 | 16.75±0.49 | 7.39±1.75 | 3.10±0.95 | 1.93±0.74 | 25.42±2.54µg/mL |
| % α-Glucosidase Activity | 100 | 85.98 | 37.95 | 15.91 | 9.92 | |
| % Inhibition | 0.00 | 14.02 | 62.05 | 84.09 | 90.08 | |

*Mean ± Standard Deviation (of Triplicate)

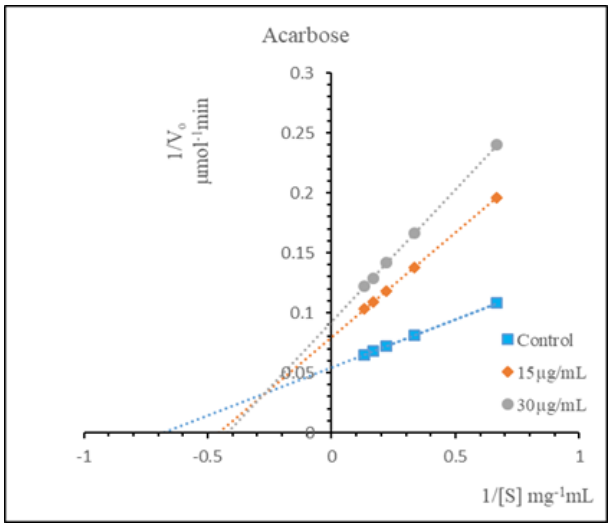



Figure 4. Lineweaver-Burk Plot of α-Glucosidase with or without acarbose

for the binding site of the enzymes. The study furthermore revealed that the extract is a more potent on α-glucosidase than α-amylase when compared to the standard drug, acarbose. This is of huge therapeutic significance, because this pattern of inhibition -relative lower inhibition of amylase and higher glucosidase inhibition of the extract is an indication that the extract may not present the gastrointestinal side effects associated with the use of the standard drug, acarbose. These results are in line with the reports of previous studies. Alhassan *et al*., [10, 27] (2017a&b) and` Lawal *et al.*, [28](2017) showed that aqueous extracts of some medicinal plants are better inhibitors of α-amylase and α-glucosidase. Ononamadu *et al.* [29] in similar study reported that aqueous-methanol solvent fraction of crude extract of *Dacryodes edulis* leaves significantly reduces fasting blood glucose in diabetic Wistar rat and significantly inhibited α-glucosidase *in vitro* relative to amylase. Research conducted by Egesie *et al*., [30] (2006) and Mohammed *et al*., [23] (2007) revealed that AEOGL given orally to streptozotocin-induced diabetic rats decreased plasma glucose level significantly ($P < 0.05$). Oguanobi *et al.*, [31](2102) showed that AEOGL significantly ($p<0.05$) reduce postprandial hyperglycaemia in type-2 diabetic model rats. Methanolic extract of *Ocimum gratissimum* was also reported to significantly ($p<0.05$) reduced plasma glucose levels in normal and alloxan-induced diabetic rats by 56 and 58% respectively [32]. Synergetic effect of decoction of *Ocimum gratissimum* with *Vernonia amygdalina* and *Gonronema latifolium* was reported to be more pronounced in lowering blood glucose level than their individual activity or combination of two [33]. Akpan and Effiom [34] (2015) reported that AEOGL significantly ($P < 0.05$) lower intestinal glucose uptake in Streptozotocin-induced diabetic rats and this might be due to the hypoglycaemic effect of this leaves. Lawal *et al.*,[35] (2019) reported that AEOGL significantly ($P < 0.05$) lowered glycaemic index of starch meal from 82.11% to 19.50% and this might be the manifestation of the extract's ability to inhibit the digestive enzymes of carbohydrates, α-glucosidase and α-amylase. The findings of all the previous studies on this plant demonstrated that the plants possesses a significant potential to ameliorate hyperglycaemia in diabetic models. However, detailed mechanism of how this extract may reduce postprandial glucose has not got much of the research attention. This present study was able demonstrate in vivo the inhibition of carbolytic enzyme as well as the kinetics. This further corroborates and elaborates the results of the previous studies.

## CONCLUSION

In conclusion, the results of this study showed that AEOGL possess potent inhibitor(s) against α-glucosidase and α-amylase and the effect was more pronounced against α-glucosidase. When compare to the standard drug acarbose, AEOGL is a better candidate against α-glucosidase. Thus, this extract provides a potential source for candidate glucosidase inhibitors for control of postprandial sugars. However, further studies are required to characterise the extract for active principles.

## CONFLICTS OF INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article

## REFERENCES

1. WHO (1999). World Health Organization: Definition Diagnosis and Classification of diabetes Mellitus and its Complications. Part 1: Diagnosis and Classification of Diabetes Mellitus. 1999 Geneva, WHO/NCD/99.2
2. Parra-Naranjo A, Delgado-Montemayor C, Fraga-López A, Castañeda-Corral G, Salazar-Aranda R, Acevedo-Fernández JJ, et al. Acute hypoglycemic and antidiabetic effect of Teuhetenone A isolated from Turnera diffusa. Molecules 2017;22(4):599.
3. WHO (2018). Diabetes. https://www.who.int/news-room/fact-sheets/detail/diabetes.
4. World Health Statistics (2012). World Health Organization. ISBN: 9789241564441.
5. WHO (2014). World Health Organization; Global Health Estimates: Death by Cause, Age, Sex and Country, Geneva 2002 – 2012.
6. NACA (2017). National Strategic Framework on HIV and AIDS. 2017 – 2021.
7. Fasanmade OA, Dagogo-Jack S. Diabetes Care in Nigeria. Ann Global Health 2015;81(6):821-829
8. Bukola A. More Nigerians die of diabetes than HIV/AIDS: Punch Newspaper, November, 27th 2013.
9. Adefegha SA, Oboh G. In vitro inhibition activity of polyphenol-rich extracts from Syzygium aromaticum (L.) Merr & Perry (Clove) buds against carbohydrate hydrolysing enzymes linked to type-2 diabetes and $Fe^{2+}$-induced lipid peroxidation in rat pancreas. Asian Pac J Trop Biomed 2012;2(10):774-781.
10. Alhassan AJ, Lawal TA, Dangambo MA. Antidiabetic Properties of thirteen local medicinal plants in Nigeria -A review. J Pharma Res 2017a;6(8):2170-2189.
11. Ononamadu CJ, Alhassan AJ, Aminu A, Imam AA, Ihegboro GO, Owolarafe TA, et al. In vitro and in vivo evaluation of the antidiabetic and antioxidant activity of methanol extract/ solvent fractions of Oci-

mum canum leaves. Caspian J Intern Med 2019;10:162-175.
12. El-Beshbishy HA, Bahashwan SA. Hypoglycemic effect of basil (Ocimum basilicum) aqueous extract is mediated through inhibition of a-glucosidase and a-amylase activities: An in vitro study. Toxicol Indust Health 2012;28(1):42-50.
13. Malapermal V, Botha I, Krishna SBN, Mbatha JN. Enhancing antidiabetic and antimicrobial performance of Ocimum basilicum and Ocimum sanctum (L) using silver nanoparticles. Saudi J Biol Sci 2015;24(6):1294-1305.
14. Antora RA, Rabeta MS, Norazatul-Hanim MR. Evaluation of in vitro sucrase Inhibitory and non-enzymatic glycation properties of Ocimum tenuiflorum (L). leaves. Food Res 2012;2:1-10.
15. Antora RA, Salleh RM. Antihyperglycemic effect of Ocimum plants: A short review. Asian Pac J Trop Biomed 2017;8(7):755-759.
16. Okon UA, Owo DU, Udokang NE, Udobang JA, Ekpenyong CE. Oral administration of aqueous leaf extract of Ocimum gratissimum ameliorates polyphagia, polydipsia and weight loss in streptozotocin induced diabetic rats. Am J Med Med Sci 2012;2(3):45-49.
17. Ugonna EV. Effect of Ocimum Gratissimum and Gongronema Latifolium on Fertility Parameters: A case for Bi-Herbal Formulation. Standard Res J Med Plants 2013;1(1):1-5.
18. Nwinyi OC, Chinedu NS, Ajani OO, Ikpo CO, Ogunniran KO. Antibacterial Effects of Extracts of Ocimum gratissimum and Piper guineense on Escherichia coli and Staphylococcus aureus. Afr J Food Sci 2009;3(1):22-25.
19. Afolabi C, Akinmoladun EO, Ibukun I, Emmanuel A, Obuotor EM, Farombi EO. Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. Sci Res Essay 2007;2(5):163-166.
20. Abdullah M. Phytochemical constituents and antimicrobial and grain protectant activities of Clove Basil (Ocimum gratissimum L) Grown in Nigeria. Int J Plant Res 2012;2(1):51-58.
21. Wongsa P, Chaiwarit J, Zamaludien A. In vitro screening of phenolic compounds, potential inhibition against a-amylase and a-glucosidase of culinary herbs in Thailand. Food Chem 2012;131(3):964-971.
22. Orafidiya OO, Elujoba AA, Iwalewa FO, Okeke IN. Evaluation of antidiarrhoeal properties of Ocimum gratissimum volatile oil and its activity against entero-aggregative Escherichia coli. Pharmacol Lett 2000;10:9-12.
23 Mohammed A, Tanko Y, Okasha MA, Magaji RA, Yaro AH. Effects of aqueous leaves extract of Ocimum gratissimum on blood glucose levels of streptozocin-induced diabetic Wistar rats. Afr J Biotechnol 2007;6(18):2087-2090.
24. Apostolidis E, Lee CM. In vitro potential of Ascophyllum nodosan phenolic antioxidant-mediated alpha-glucosidase and alpha-amylase inhibition. J Food Sci 2010;75:H97-102.
25. Ali H, Houghton PJ, Soumyanath A. Alpha-amylase inhibitory activity of some Malaysian Plants used to treat diabetes; with particular reference to phyllanthus amarus. J Ethnopharmacol 2006;107:449-455.
26. Kang WY, Song YL, Zhang LC. Glucosidase inhibitory antioxidant properties and antidiabetic activity of Hypericum ascyron L. Med Chem Res 2011;20:809-816.
27. Alhassan AJ, Sule MS, Et-ta'alu AB, Lawal AT. In vitro inhibitory activities of Persea americana seed extracts on α-amylase and α-glucosidase. Bayero J Pure Appl Science 2017b;10(1):546-552.
28. Lawal AT, Alhassan AJ, El-ta'alu AB, Dangambo MA. Effect of Persea americana seed aqueous extract on glycaemic index of a standard food. Yusuf Maitama Sule University, Kano, Faculty of Science, 3rd YUMSCIC Nov. 2017.
29. Ononamadu CJ, Alhassan AJ, Ibrahim A, Imam AA, Ihegboro GO, Owolarafe TA, et al. Methanol-Extract/Fractions of Dacryodes edulis Leaves Ameliorate Hyperglycemia and Associated Oxidative Stress in Streptozotocin-Induced Diabetic Wistar Rats. J Evid -Bas Integrat Med 2019;24:1-12.
30. Egesie UG, Adelaiye AB, Ibu JO, Egesie OJ. Safety and hypoglycaemic properties of aqueous leaf extract of Ocimum gratissimum in streptozotocin induced diabetic rats. Niger J Physiol Sci 2006; 21(1-2):31-35.
31. Oguanobi NI, Chijioke CP, Ghasi S. Antidiabetic effect of crude leaf extracts of Ocimum gratissimum in neonatal streptozotocin-induced type-2 model diabetic rats. Int J Pharma Sci 2012;4(5):77-83.
32 Aguiyi JC, Obi CI, Gang SS, Igweh AC. Hypoglycaemic activity of Ocimum gratissimumin rats. Fitoterapia 2000;71(4):444-446.
33. Ejike CECC, Awazie SO, Nwangozi PA, Godwin CD. Synergistic postprandial blood glucose modulatory properties of Vernonia amygdalina (Del.), Gongronema latifolium (Benth.) and Occimum gratissimum (Linn.) aqueous decoctions. J Ethnopharmacol 2013;149(1):111-116.
34. Akpan OU, Effiom OE. Ocimum gratissimum impairs gut glucose absorption but enhances water absorption in Streptozotocin-induced diabetic rats. Gaziantep Med J 2015;21(1):43-50.
35. Lawal TA, Salawu K, Imam AA, Wudil AM, Alhassan AJ. Effect of Aqueous Extract of Ocimum Gratissimum on Glycaemic Index of Starch Meal. Glob J Med Plants Res 2019;5(3):009-015.