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

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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 20th 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.

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Keywords

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 α -amylase,
 α -glucosidase,
Ocimum gratissimum,
Diabetes,
Acarbose

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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 can-

not 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 compounds 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.

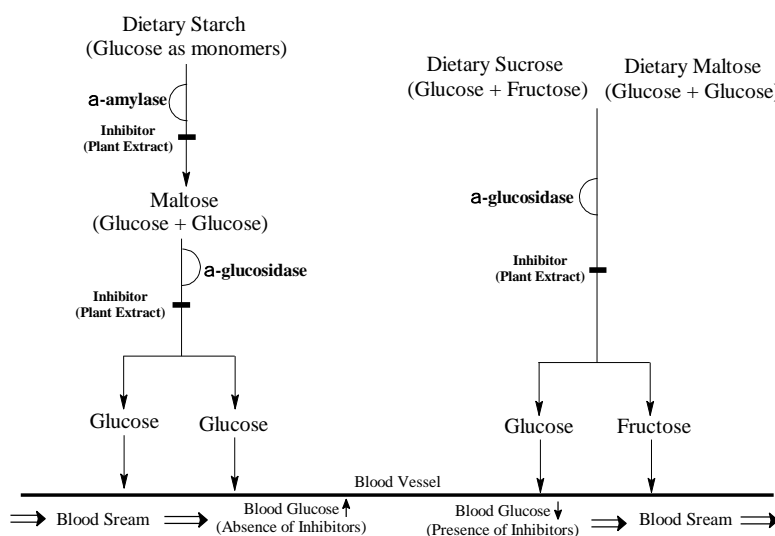


Diagram 1. The hypothesised mechanism of action of the extract

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 μ g/mL were prepared in 20 mM of sodium phosphate buffer (pH 6.9). Aliquot amount (250 μ L) of each extract was pre-incubated with 250 μ L of α -amylase enzyme (0.5mg/mL) at 25°C for 10 minutes. The mixture was then incubated with 250 μ L of 1% w/v starch solution at 25°C for another 10 minutes. The reaction was put to a halt by adding 500 μ L 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;

$$U_a = \frac{\text{mg maltose} \times \text{DF}}{\text{Time} \times \text{mg enzyme}}$$

Where 'U_a' 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:

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

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 (IC₅₀) was determined graphically. IC₅₀ 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 μ g/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 250 μ L of each concentration of the extract was pre-incubated with 250 μ L 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 250 μ L 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 500 μ L 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)

$$v = \frac{\text{mg maltose} \times \text{DF}}{\text{MW}_{\text{maltose}} \times \text{Time} \times \text{Vol. of Enzyme}}$$

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 $\mu\text{g/mL}$ were prepared in 20 mM of sodium phosphate buffer (pH 6.9). Aliquot amount (100 μL) of each extract was pre-incubated with 100 μL 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;

$$U_g = \frac{\text{mg p-nitrophenol} \times \text{DF}}{\text{Time} \times \text{mg enzyme}}$$

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:

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

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 30 $\mu\text{g/mL}$, and five different concentrations (1.5, 3.0, 4.5.0, 6.0 and 7.5mM) of pNPG solutions were prepared too. 100 μL of each extract concentration was pre-incubated with 100 μL 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 100 μL 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).

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

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 305.33 $\mu\text{g/mL}$. 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 ($\mu\text{g/mL}$)	0	80	160	240	320	400	IC_{50} ($\mu\text{g/ml}$)
α -Amylase Activity * (μmol maltose/min/mg enzyme)	45.52 \pm 1.05	37.88 \pm 0.33	31.74 \pm 3.44	27.32 \pm 0.72	22.62 \pm 0.77	17.25 \pm 0.55	305.33 \pm 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 \pm Standard Deviation (of Triplicate)

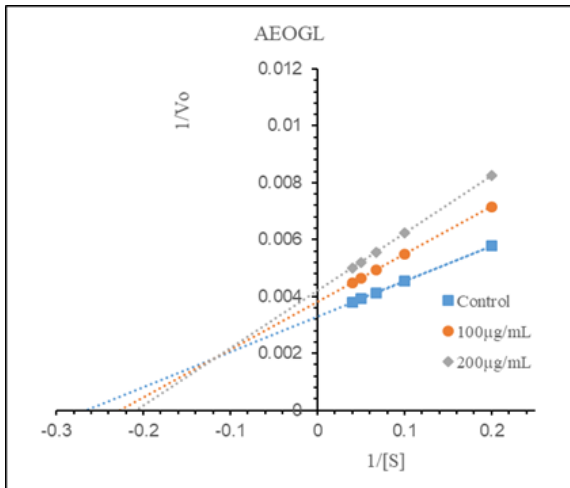


Figure 1. Lineweaver-Burk Plot of α -amylase with and without AEOGL

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 $60.72 \pm 4.00 \mu\text{g/mL}$. 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 $25.42 \pm 2.54 \mu\text{g/mL}$. The

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

Conc. of Acarbose ($\mu\text{g/mL}$)	0	80	160	240	320	400	IC_{50} ($\mu\text{g/mL}$)
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	$60.72 \pm 4.00 \mu\text{g/mL}$
% 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 \pm Standard Deviation (of Triplicate)

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

Conc. ($\mu\text{g/mL}$) of AEOGL	0	10	30	60	80	IC_{50} ($\mu\text{g/mL}$)
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	$25.42 \pm 2.54 \mu\text{g/mL}$
% α -Glucosidase Activity	100	85.98	37.95	15.91	9.92	
% Inhibition	0.00	14.02	62.05	84.09	90.08	

*Mean \pm Standard Deviation (of Triplicate)

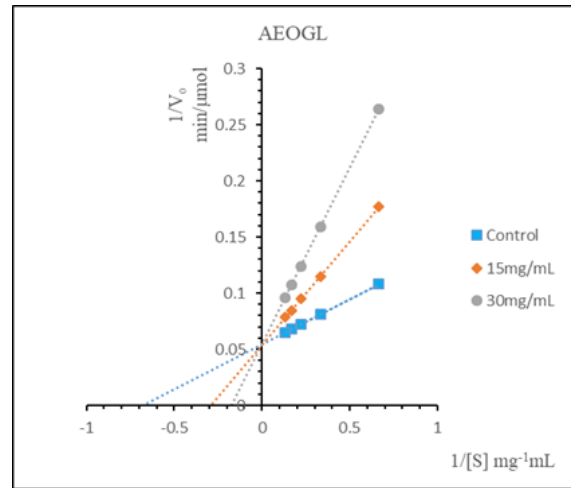


Figure 3. Lineweaver-Burk plot of α -glucosidase with and without AEOGL

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 $(112.23 \pm 7.43 \mu\text{g/mL})$. 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

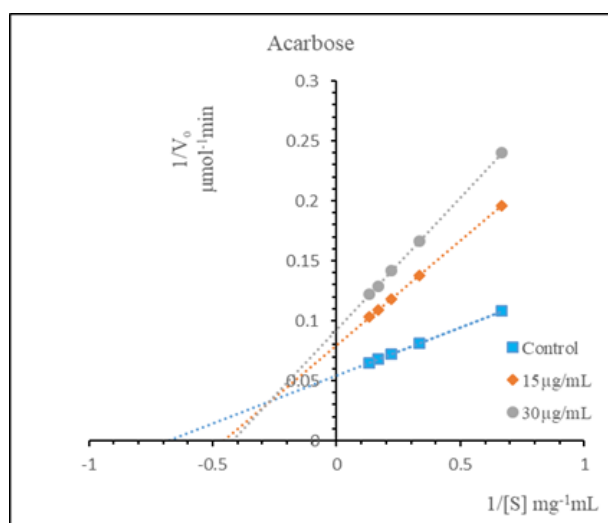


Figure 4. Lineweaver-Burk Plot of α -Glucosidase with or without acarbose

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

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