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Kinetic Studies on the Inhibition Potential of Bitter Leaf (*Vernonia Amygdalin)* in the Management of Diabetes

Uthman H *

Department of Chemical Engineering, Federal University of Technology, P.M.B. 065, Gidan-Kwano Campus, Minna, Nigeria.

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Abstract

The aim of this study was to investigate the inhibition potential of extracts from *Vernonia amygdalina* on α-amylase and α-glucosidase activity, and its useful mechanisms in the treatment of diabetes specifically Type 2. The searchlight is beamed on the potential of the plant extract to inhibit the activity of α-amylase and thereby determining its mechanism or mode on inhibition as well as its IC_{50} value. The inhibition of *alpha-amylase* and *alpha-glucosidase* enzymes is a key strategy in managing diabetes. *Vernonia amygdalina* is a natural plant extract that has been used traditionally to manage diabetes. In this study, the inhibition potential of *Vernonia amygdalina* on *alpha-amylase* and *alpha-glucosidase* was investigated. Kinetic studies were conducted to determine the inhibitory effects of the plant extract on these enzymes. The results showed that *vernonia amygdalina* exhibited significant inhibition of both *alpha-amylase* and *alpha-glucosidase* enzymes. The study also evaluated the kinetic parameters of the inhibition process, including the type of inhibition and the inhibition constant. The findings of this study suggest that *Vernonia amygdalina* has the potential to be used as a natural inhibitor of *alpha-amylase* and *alpha-glucosidase* enzymes, which could lead to the development of new natural remedies for managing diabetes. These results are of interest to researchers studying natural product chemistry and drug development and inhibition of enzyme in bioreactors. *Vernonia amygdalina* inhibits alpha-amylase and alpha glucosidase activities but with greater inhibitory effects experienced in alpha glucosidase shows that plants tend to have higher inhibitory effect on alpha-glucosidase. With the obtain IC_{50} values it helps to provide insight into the mechanism of actions of an inhibitor by helping to determine the nature and strength of the interaction between the inhibitor and the enzyme or the biological process being inhibited. Acarbose which serves as inhibitors of these enzymes with greater effect on alpha-amylase but resulting to gastrointestinal adverse effects due to the accumulation of undigested carbohydrates becoming readily available for bacterial fermentation. With these outcomes the possibility exists that controlling glycemic complication is achievable by combining the inhibitory effect of *Vernonia amygdalina* and acarbose while reducing acarbose dose to alleviate anti-diabetic related adverse effects. Further investigation and studies are warranted in humans to help in glycemic control while in bioreactors this may include the usage of alpha-glucosidase and alpha-amylase as enzyme which similarly employs *Vernonia amygdalina*. It is noteworthy that the activity of these enzymes can be inhibited in bioreactors.

Keywords: Inhibition potential; Kinetic studies; Bitter Leaf (*Vernonia amygdalina); Alpha-amylase* and *alpha-glucosidase* enzymes; Diabetes mellitus

1. Introduction

Diabetes mellitus (DM) commonly and simply called diabetes is a hereditary prevalent disease but it can also result from environment and habits. It is characterized by chronic hyperglycemia which can either be a result of a deficiency in insulin secretion or impaired cellular action of hormones. The deficiency or insufficiency in the secretion of insulin is caused by the total destruction of pancreatic β-cells. It has been of a public and global health concern as it results to numerous complications, poor life, quality and mortality. Although, it is frequently inherited, it can also be brought on

* Corresponding author: Uthman, H

by an individual's surroundings and lifestyle such as genetic susceptibility, sedentary and harmful practices such as alcoholism and smoking. It is a chronic disorder characterized by high blood sugar or glucose levels over an extended period of time because the body is unable to control the amount of glucose in the blood due to either an absence of insulin or its secretion being insufficient, or because the body cells' insulin-sensitive receptors have lost their sensitivity to insulin [1–11].

DM is a chronic heterogeneous metabolic disorder represented by the incidence of hyperglycaemia due to a defect in function or secretion of insulin. Two major types of DM exist namely: type 1 and type 2 diabetes. Type 1 DM is due to an autoimmune disease that leads to the destruction of insulin-producing pancreatic β-cells. This usually results in low or no insulin, causing hyperglycaemia. On the other hand, type 2 DM, the more common type, which is more prevalent, is defined by the inability of the body to use the insulin secreted as a result of insulin resistance [12–13]. DM can also be described as a metabolic disorder characterized by high blood glucose (hyperglycaemia) owing to failure of the pancreatic β cell to secrete insulin and/or inability of the cells to utilize secreted insulin as seen in type 1 diabetes (T1D) and type 2 diabetes (T2D) respectively. T2D accounts for more than 90% of all diabetes types, thus making it a major contributor to diabetic morbidity and mortality [14–15]. Diabetes is one of the major silent killers worldwide is characterized by the body's difficulty in effectively regulating blood sugar and insulin levels. Type 2 diabetes mellitus (T2DM) is brought on by the interaction of a genetic predisposition and a wide range of modifiable and non-modifiable environmental risk factors, including obesity, a poor diet, and inactivity [16–19].

DM is a disease that affects millions of people worldwide. Globally, there has been a continuous rise in its incidence. An unprecedented worldwide health crisis has resulted from chronic hyperglycaemia due to abnormalities in insulin production or activity characterizes diabetes mellitus. It has been reported that 415 million people worldwide were diagnosed with diabetes in 2015, and this figure is expected to rise to 642 million by 2040 [17–27]. DM has been recognized as a global epidemic affecting over 425 million people in 2017, with an estimated 48% increase postulated for 2045. Persistent hyperglycaemia and DM are associated with macrovascular and microvascular complications, resulting in neuropathy, nephropathy, heart disease, stroke and vascular diseases [28–29]. Healthcare systems throughout the world confront enormous problems that need novel and all-encompassing methods of management and prevention due to diabetes's complexity and the severity of its consequences [30, 26].

Sufficient information about the danger of DM is conspicuously absent among the populace. According to the forecast of WHO experts, by 2030 more than 380 million people will suffer from DM in the world. Such a meteoric rise in morbidity is expected mainly due to an increase in the number of patients with type 2 diabetes, developing as a result of growing urbanization and related problems, unhealthy lifestyles, low physical activity, inadequate diet and stress [26].

Plants that are known to possess medicinal properties benefited mankind for ages as they contain essential pharmaceutical ingredients that have aided in the treatment of chronic illnesses. Pharmaceutical corporations produced a large number of synthetic medications for the treatment of various ailments during the previous age, which was thought to be synthetic or artificial [31–36].

The use of these synthetic medications resulted in serious negative effects over time. Aside from the side effects associated with these synthetic drugs, they are known to be very expensive and a large proportion of the population cannot afford to use these medications to their health benefit. As a result of these, in the last decades, there have been global trends with its searchlight beamed on medicinal plants due to its low risk of side effects as well as affordability [37–38]. Similarly, in developed countries, there has been a resurgence of interest in medicinal plants that exhibit hypoglycemic properties [39–41]. One of the major mechanisms involved in the use of medicinal plants in the management of hyperglycemia is the inhibition of α-amylase and α-glucosidase. A wide variety of plants exist that serves useful purposes in the treatment of various ailments that synthetic or orthodox drugs cannot address without causing any side effects [42–53].

Herbal medicine can be of help in this complex and multifaceted work. Insufficient awareness of the population about the causes, prevention, treatment and consequences of diabetes is typical for most countries of the world. The threat of the diabetes epidemic dictates new strategies for the treatment and prevention of the disease. For phyto-dietary purposes, patients with type 1 and type 2 diabetes mellitus may benefit from such wild plants as nettle, dandelion, primrose, plantain, burdock, chicory, St. John's wort, calamus, horsetail, knotweed, cinquefoil, wheatgrass, panicle wormwood, elecampane, oregano and etcetera. The use of Jerusalem artichoke in the dietary recovery of patients with diabetes mellitus which has gathered considerable interest [32].

In modern medicine, medicinal plants not only have not lost their positions, but are attracting more and more attention from scientists and doctors. Of the more than 15 thousand drugs registered in Russia, about 40 % are produced from plant materials. Their number is increasing every year. Currently, the centers for the study of herbal remedies and the development of new dosage forms for the treatment of patients with DM is available in Russia, where Phyto preparations such as novobet, arfazetin, galegamine, topivit, concentrate were created. Jerusalem artichoke, galega-nova, diacor, diabetta and etcetera usage for the treatment of DM was also reported [32].

Many symptoms of the disease can be levelled or weakened if a patient with DM eats rationally, mindful of the diet and resorting to a wide range of herbal remedies. The patient can prepare some therapeutic and dietary products and gets acquainted with certain advice and diet correction from an endocrinologist or phytotherapist [32]. Drugs used to treat type 2 diabetes are currently divided into several classes: insulins, sulphonylurea derivatives, biguanides, alpha-glucosidase inhibitors, PPA RJ agonists (thiazolidinediones), meglitinides (glinides, prandial glycaemic regulators), peptide analogs of glucogone-like 1, gliptins (DPP-IV inhibitors), amylin analogs, combined preparations [32].

Bioactive constituents of medicinal plants are used as anti-diabetic, chemotherapeutic, anti-inflammatory, and anti-arthritic agents. Various works has been carried out to determine the potential of some plants to fight against dreadful and deadly diseases including cancer, and diabetes mellitus to mention a few [54–60]. The role of medicinal plants, particularly in developing countries, where many people do not have access to conventional anti-diabetic therapies, is of utmost importance. World Health Organization global report on diabetes (2016) [27, 28] strongly advises the use of medicinal plants for the management of diabetes mellitus as these plants are considered to be less toxic and from side effects. These herbal plants or products from related medicinal plants are quite rich in flavonoids, phenolic compounds, coumarins, terpenoids, and other constituents which help to reduce blood glucose levels [61]. Medicinal plants have now become a major of the world's standard health care which is now founded upon the combination of time-honored traditional usage and ongoing scientific scrutiny of their therapeutic potential and safety [62].

Bitter leaf (*Vernonia amygdalina*) is one important plant commonly grown in the tropical regions of the world with nutritional and health-giving properties. It is also known as ''African bitter leaf'' or 'ndolé' due to the bitter taste of the leaves, it is an African and Asian medicinal plant well known for its use in traditional medicine in various diseases. Furthermore, it is a plant vegetable used for both food and traditional treatment of diseases, such as malaria, wound healing, infertility, diabetes, gastrointestinal problems and sexually transmitted diseases, throughout tropical Africa [63–65, 30]. It possesses anti-diabetic property, anthelmintic activities; antioxidant properties which include: hypolipidemic and anticancer activity. Also, were its cathartic effect; abortifacient; antifertility; antimicrobial; antiplatelet and anticoagulant; antimalarial; hepatoprotective; analgesic activity; anti-inflammatory; anti-pyretic activity; antimutagenicity and effect on $CD4^+$ cell count (HIV/AIDS). Moreover, the safety of bitter leaf had been established through sole administration as well as administration in the presence of toxicants [66]. Studies and analysis of its botanical description, chemical makeup, traditional use and modes of action reveals its possible anti-diabetic properties [30].

In *Vernonia amygdalina*, the distribution of biologically active compounds has not been fully established. But, in general, bitter leaves are rich in nutrients like proteins (up to 20 %), lipids (4.7 %), iodine (35.8 mcg), copper (6–10 mg/100 g), iron (5.14 %), vitamin A and E, thiamine and also a good amount of reducing sugars are present in the leaves. Phytochemicals like saponins, coumarins, flavonoids, lignans, alkaloids, xanthones are also present in the leaf, stem and roots of *Vernonia amygdalina* [67].

The presence of several bioactive compounds in *Vernonia amygdalina*, including flavonoids, saponins, alkaloids, tannins, terpenes, and phenols have been reported [32]. It was reported that aqueous and ethanol extracts of *Vernonia amygdalina's* leaves and roots contain significant amounts of luteolin and vernodalol. The flavonoid luteolin occurs primarily in leaf extracts, while Terpenoid vernodalol is the predominant compound in root extracts [32].

Saponins and alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones and anthraquinone, edotides and sesquiterpenes lasiopus are some of the bioactive compounds present in *Vernonia amygdalina* [68]. Alkaloids and saponins, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, edotides and sesquiterpenes are some of the phytochemicals that have been isolated and extracted from *Vernonia amygdalin*a. These compounds elicit various biological effects including cancer chemoprevention [69].

A number of chemical compounds with potent biological activities have been isolated and characterized by several investigators from the leaves of *Vernonia amygdalina*. Some of the isolated components from *Vernonia amygdalina* include: sesquiterpene lactones, flavonoids like luteolin, luteolin 7-O-glucosides and luteolin 7-O-glucuronide, steroid glycosides and vernonioside A, B, A1, A2, A3, B2, B3 and A4. Recently, a sesquiterpene lactone, epivernodalol, another

elemanolide from the dichloromethane fraction of *Vernonia amygdalina* was isolated and characterized. Epivernodalol compound was isolated from another species of the plant called *Vernonia lasiopus* [67–75]. In this project the aim is to determine the inhibitory potential of *Vernonia amygdalina* on α-amylase for the management of type 2 diabetes disease in human being. This research focuses on the inhibition potential of extracts from *Vernonia amygdalina* on α-amylase and α-glucosidase activity, and its useful mechanisms in the treatment of diabetes specifically Type 2. The searchlight is beamed on the potential of the plant extract to inhibit the activity of α-amylase and thereby determining its mechanism or mode on inhibition as well as its IC_{50} value.

2. Materials and Methods

2.1. Materials

2.1.1. Sample collection and identification

Plant extracts were obtained from Gidan-Kwano Campus of the Federal University of Technology, Minna, Niger State, Nigeria. The plants were identified at the department of Plant biology, Federal University of Technology, Minna, Niger State, Nigeria.

2.1.2. Reagents and Chemicals

All the reagents used were of analytical grade. Reagents used include: Alpha amylase, Alpha-glucosidase, starch soluble, sodium phosphate, dinitrosalicyclic acid (DNSA) and distilled water.

2.1.3. Apparatus and Equipment

The apparatus and equipment used for the analysis include: beaker, test tubes, weighing balance (model GM602), separating funnel (Pyrex), water bath (Griffin 322), hand gloves, sample bottles, EDTA bottles, filter paper (Whatman), pH meter, syringes, pipette and pipette dropper, centrifuge (model LRIO 2.4A) and UV-spectrophotometer (Model 752).

2.2. Methods

2.2.1. Preparation of the Aqueous Extract of Vernonia amygdalina

Aqueous extract of *Vernonia amygdalina* 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. 50 g of the powder was macerated in 500 mL 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^{o}C to yield a dry powdery residue. The extract was reconstituted in buffer solution concentration.

2.2.2. Phytochemical Screening

The phytochemicals compositions of the leaves were determined using various methods. The phytochemical of interest are phenols, flavonoids and tannins in the extract of *Vernonia amygdalina* since it has previously been reported in literatures that they are the bioactive compounds that are responsible for the inhibitory activity of alpha-amylase in medicinal plants [52].

2.2.3. Determination of Total Flavonoids

The method employed in the determination of total flavonoids is the Aluminium chloride calorimetric method, total flavonoids was determined in accordance to the methods and procedures described by [4]. 0.5g of the extract was mixed with 1.5 cm^3 of methanol, 0.1 cm^3 of 10% aluminium chloride, 0.1 cm^3 of 1M sodium acetate and 2.8cm^3 of distilled water. The mixture was incubated at room temperature for 30 minutes before the absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Beckman DU-7) (USA). The calibration curve was plotted by preparing quercetin solution at concentrations 12.5 to 100 mg/cm^3 in methanol

2.2.4. Determination of Total Phenolic Content

Two grams of the extract was heated to boiling in 50 cm^3 of diethyl ether. 5cm^3 of boiled mixture was added to 10cm^3 of distilled water, 2cm^3 of ammonium hydroxide solution and 5cm^3 of concentrated alcohol. The mixture was allowed to stand for 30 minutes for colour development and the absorbance was measured at 505 nm using UV-Vis Spectrophotometer (Beckman DU-7).

2.2.5. Determination of Total Tannins Content

0.2 gram of the extract was measured into a 50 cm^3 beaker, 20cm^3 of 50% methanol was added and the mixture was covered with a para film and then placed in a water bath at 78 °C for 1 hour. It was shaken vigorously to ensure a uniform mixture. The extract was filtered using a double layered Whatman No. 1 filter paper into a 100 cm^3 volumetric flask, 20 cm^3 of water was added, 2.5cm^3 Folin-Denis reagent and 10 cm^3 of 17% Sodium Carbonate were added and mixed properly. The mixture was made up to 100 cm^3 with distilled water mixed well and left undisturbed for 20 minutes for the development of a bluish-green colour. The absorbance for tannic acid standard solution as well as the samples was read after colour development of a UV-Vis (Beckman DU-7) Spectrophotometer model 752, at a wavelength of 760nm.

2.2.6. Invitro Alpha-amylase Inhibition

The inhibition of alpha amylase was performed according to the methods and procedures described by [73]. 0.5cm^3 of 0.2M phosphate buffer (pH 6.8) was pippeted into a beaker and 1 cm^3 of alpha-amylase was added which was immediately followed by the addition of 40μl of the crude extracts of the following concentration 62.5, 125, 250, 500 and 1000 μg/ml. For 10 minutes the mixture was incubated at 37°C before the addition of 580 μl of 1% starch solution. The mixture was further incubated for 15 minutes at 37°C. The reaction was terminated by the addition of 1 cm^3 of DNSA reagent and boiled for 5 minutes. The absorbance was read 540nm using UV Spectrophotometer. Acarbose (50 mg) was used as standard anti-diabetic drug. The alpha amylase inhibitory activity was calculated as percentage inhibition using equation (1):

$$\% \text{ Inhibition} = \frac{\text{Abscontrol} - \text{Absextract}}{\text{Abscontrol}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

IC_{50} value (the concentration of the extract required to inhibit 50% of the amylase activity) of acarbose and the extracts were determined from plots of percentage inhibition versus concentration (μg/ml).

2.2.7. Mode of Alpha-Amylase Inhibition

The mode of inhibition of the extract was conducted according to the modified methods and procedures described by [33, 68, 69, 71, 73]. Two different concentrations of the extract were prepared; 100 and 200 μg/ml, and three different concentrations of starch solutions were also prepared; 100, 200 and 500 μg/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 three 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 500 μL 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 540 nm. 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) using equation (2).

$$V = \frac{\text{mg maltose x 100}}{MW_{maltose} \times \text{Incubation Time}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, V is the velocity, '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]) were presented in Figures 2 and 4, 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

2.2.8. Alpha-Glucosidase Inhibitory Assay

The inhibitory effect of *Vernonia amygdalina* on alpha-glucosidase was determined performed according to the methods and procedures described by [45]. Concentrations of the extracts at 10, 30, 50, 70 and 90 μg/ml were prepared in 20mM of sodium phosphate buffer (pH 6.9). An amount of 100 μl of each extract's concentration was pre-incubated with 100 μl of alpha-glucosidase enzyme at 37°C for 10minutes. The mixture was then incubated with 100 μl of 10mM p-nitrophenyl-alpha-glucopyransonide (pNPG) solution at 37°C for another 10 minutes. 2 cm^3 of 0.2M sodium carbonate was added to bring the reacting mixture to a halt. The mixture was diluted with 5 cm^3 of deionised water and the absorbance of the mixture was taken at 405nm. The control sample was without the aqueous extract of *Vernonia amygdalina* was treated in like 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 inhibitory effect was calculated using equation (3):

$$\% \text{ Inhibition} = \frac{\text{Abscontrol} - \text{Absextract}}{\text{Abscontrol}} \times 100 \quad \text{...} (3)$$

2.2.9. Kinetics of Alpha-Glucosidase Inhibition

The kinetic mode of inhibition was determined according to the methods and procedures described by [67]. It was run in triplicates. Two different concentrations of the extract were prepared; 20 and 30 µg/ml, and pNPG in five different concentrations (1.5, 3.0, and 4.5, 6.0 and 7.5 mM) were prepared likewise. 100 µl of each extract concentration was pre-incubated with 100µl of alpha-glucosidase (1.0U) separately for 10 minutes at 37°C in the five pairs of test-tubes. The mixture was then incubated with 100 µl of the different concentration of the substrate (pNPG) at 37°C for reaction to commence for another 10 minutes. The reactions were put to a halt by the addition of 2 cm^3 of 0.2M of sodium carbonate and the absorbance of the mixtures were taken at 405 nm. The control sample was 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) by employing equation (4).

$$V = \frac{\text{mg maltose} \times 1000}{MW_{maltose} \times \text{Incubation Time}} \quad \text{.......................................} (4)$$

3. Results and Discussion

3.1. Phytochemical Analysis

Quantitative phytochemical analysis was carried out on *Vernonia amygdalina* which determine the quantitative presence of Phenols, Flavonoids, and Tannins. The analysis was repeated twice to improve the accuracy of result and increase its precision. The result is shown in Table 1.

Table 1 Phytochemical value of *Vernonia amygdalina*

Phenol	Flavonoids	Tannins
82.965 (mg/g)	3.475(mg/g)	70.965(mg/g)

3.2. Invitro Alpha-amylase Inhibition

3.2.1. Percentage Inhibition of Alpha-amylase by Vernonia amygdalina and acarbose

Table 2 describes the results that were obtained from the inhibition of alpha-amylase by *Vernonia amygdalina* and acarbose standard drug.

Table 2 Inhibition potential of *Vernonia amygdalina* on alpha-amylase

	Extract	Acarbose		
Concentration(µg/ml)	**Absorbance**	**% Inhibition**	**Absorbance**	**% Inhibition**
62.5	**0.704**	12	0.412	48.5
125	0.584	27	0.28992	63.76
250	0.36	55	0.11632	85.46
500	0.184	77	0.07288	90.89
1000	0.16	80	0.02928	96.34

3.3. Kinetics of the mode of inhibition of alpha-amylase by Vernonia amygdalina

Table 3 shows Maltose standard curve concentration parameters, it was observed that the absorbance of the control is 0.8 at all concentrations. Table 4 presents the quantities in milligram of maltose obtained from maltose standard curve. Table 5 shows the reaction velocity of inhibitor concentration of 100µg/m. 200µg/m and control. Figure 1 depicts the percentage Inhibition of potential of *Vernonia amygdalina* on alpha-amylase. Figure 2 portrays the Line-Weaver Burk Plot over the inhibition of Alpha amylase.

3.4. Quantitative Phytochemical Analysis

The presence of phenols, flavonoids and tannin in *Vernonia amygdalina* has been previously establish [52] as such the quantitative amount of these component in the extract used was carried. Which shows a quantitative value of 70.965, 3.475,82.965 respectively.

3.5. Alpha-amylase Inhibition Assay

Data from these studies shows the variable inhibitory effect of tested plants extracts on alpha-amylase activity invitro. IC_{50} values of the extract is found to be at 245µg/ml while that of acarbose is 60.0µg/ml. From Table 1 and Figure1, the extract inhibitory potential was found to be lower compared to standard acarbose.

3.6. Alpha-amylase Kinetic Studies

Since inhibition was noted in the extracts, kinetic studies were further carried out to determine the mode of inhibition. From the Line-Weaver-Burk plot showed that the extract of this plant inhibits alpha-amylase competitively. This points to the fact that the active components in *Vernonia amygdalina* compete with the substrate for binding to the active site of the enzyme thereby preventing the breakdown of oligosaccharides to disaccharides [73].

3.7. Alpha-glucosidase Assay

In the inhibition of alpha-glucosidase the extracts exhibit stronger inhibition compared to alpha-amylase. Kwon *et al*. *(*2015) [21] reported that plant alpha-glucosidase inhibitors show higher inhibition and therefore can be used as effective therapy for hyperglycemia with minimal side effects.

Table 3 Maltose standard curve concentration parameters

Conc(µg/ml):	**100**	**200**	**300**	**400**	**500**	**600**	**700**	**800**	**900**	**1000**
Absorbance:	0.096	0.192	0.288	0.384	0.48	0.48	0.672	0.768	0.864	0.96

Table 4 Quantity in milligram of maltose obtained from maltose standard curve

		100µg/ml		**200µg/ml**		**Control**
Substrate Conc (µg/ml)	Absorbance	mg of maltose	absorbance	mg of maltose	absorbance	mg of maltose
100	0.05699	63.33	0.0353	39.3105	0.0138	14.2496
200	0.1055	117.3311	0.04364	55.1611	0.0446	49.565
500	0.02139	244.2858	0.1053	110.3227	79.515	0.0716

Table 5 Reaction velocity of inhibitor concentration of 100µg/m. 200µg/m and control

Substrate			**100µg/ml**		**200µg/ml**		**Control**
Concn. (µg/ml)	**1/S Velocity,V**		**1/V Velocity,V**		**1/V Velocity,V**		**1/V**
500	0.002	71.4286	0.014	35.7143	0.028	4.16656	0.024
200	0.005	34.4828	0.029	17.5439	0.057	23.2500	0.043
100	0.01	23.8095	0.042	11.9048	0.084	14.4927	0.069

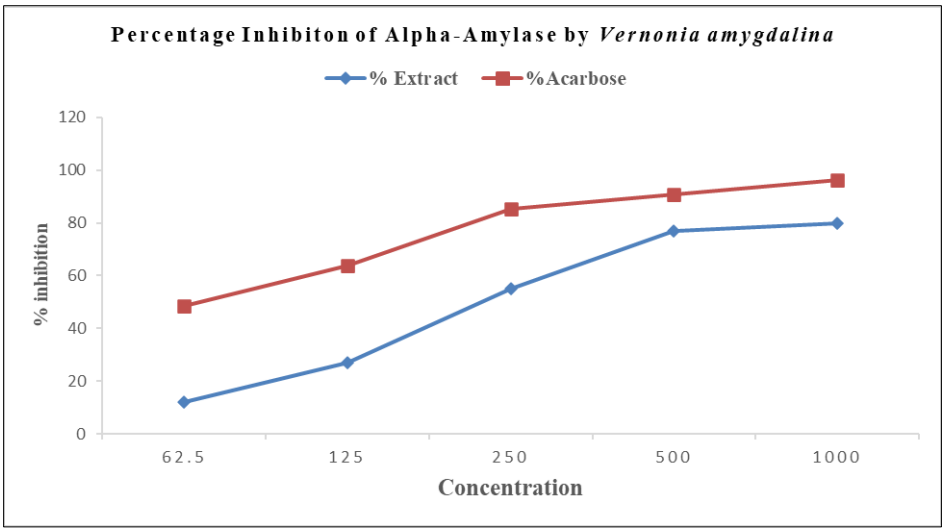

Figure 1 Percentage Inhibition of potential of *Vernonia amygdalina* on alpha-amylase

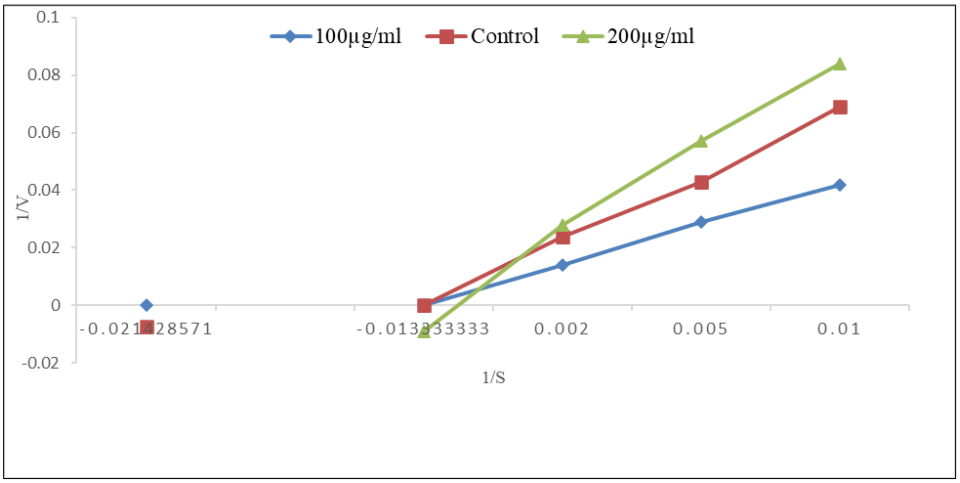

Figure 2 Line-Weaver Burk Plot over the inhibition of Alpha amylase

3.8. Invitro Alpha-Glucosidase Inhibition

The percentage inhibition of alpha-glucosidase is presented in Table 6. Table 7 shows the p-nitrophenol absorbance value. Table 8 depicts the quantities in milligram of maltose formed at 20, 30µg/ml and acarbose (50 mg). Table 9 portrays the reaction velocity at different concentration of substrate. Figure 3 shows the percentage inhibition of alpha-glucosidase by *Vernonia amygdalina* and Figure 4 presents the Lineweaver-Burk Plot.

Table 6 Percentage inhibition of Alpha-Glucosidase

Inhibition of alpha-glucosidase (absorbance of control = 0.8)				
	Absorbance		**% Inhibition**	
Concentration	Extract	Acarbose	Extract	Acarbose
10	0.679	0.751	15.125	6.125
30	0.32	0.537	60	32.875
50	0.13	0.401	83.75	49.875
70	0.209	0.216	97.3875	73
90	0.0078	0.11	99.025	86.25

Table 7 p-nitrophenol absorbance value

Conc (mM):	0.01	0.02	0.03	0.04	0.05	0.06
Absorbance	0.015	0.0304	0.0455	0.384	0.759	0.09108

Table 8 Quantity in milligram of maltose formed at 20, 30µg/ml and acarbose (50mg)

Concentration	**Absor-bance**	**20 mg Maltose**	**Absor-bance**	**30 mg Maltose**	**Absor-bance**	**Acarbose mg maltose**
100	0.1103	72.5658	0.0866	56.9737	0.1566	103.0263
80	0.0543	35.7237	0.04851	31.9145	0.0777	51.1184
60	0.037	24.3421	0.0313	20.5921	0.07147	47.0197

Table 9 Reaction velocity at different concentration of substance

Extract	**20**			**30**			Control
Substrate, S	**1/S**	**Velocity**	**1/V**	**Velocity**	**1/V**	**Velocity**	**1/V**
100	0.0100	21.3400	0.0469	16.7830	0.0595	31.8980	0.0313
80	0.0125	10.4370	0.0958	9.3510	0.1069	14.9432	0.0669
60	0.0167	7.2982	0.1370	6.1320	0.1630	10.8839	0.0919

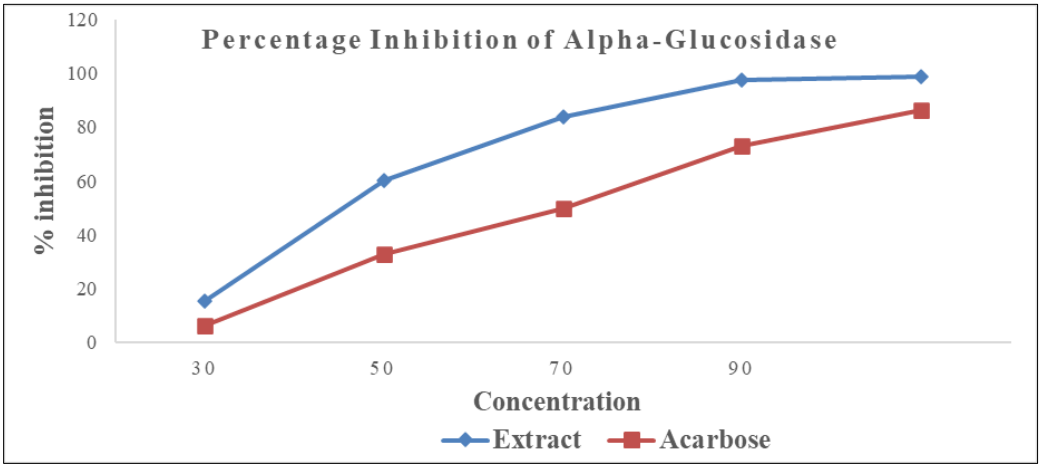

Figure 3 Percentage Inhibition of alpha-glucosidase by *Vernonia amygdalina*

3.9. Alpha-Glucosidase Kinetics Studies

From Figure 4, it was observed that the value of Km equivalent to 10.44828 remains constant at concentration of inhibitor which from the double reciprocal plots, it shows a noncompetitive mode of inhibition leading to the fact that the active components in the extracts do not compete with substrate for binding to the active site rather the inhibitors bind to a separate site on the enzyme retarding the conversion of disaccharides to monosaccharides. This result is in close agreement with those reported by [46].

The present study shows the inhibitory potential of *Vernonia amygdalina* against alpha-amylase and alpha glucosidase consequently determining their mechanism of inhibition. The knowledge of this mechanism is vital for clinical

implication for individuals with impaired glycemic control by altering the rate of digestion of carbohydrates. This result agrees very well with similar result reported by [70, 74]. According to Satosh *et al*. (2015) [72], plants generally have shown to be better inhibitors of alpha-glucosidase than alpha amylase. *Vernonia amygdalina* exhibited considerable inhibition potential against both alpha-amylase (IC_{50} of 63µg/m) and alpha-glucosidase (IC_{50} of 45µg/m) with a stronger inhibition on alpha-glucosidase. This shows that it is *Vernonia amygdalin* is more potent against alpha-glucosidase than against alpha-amylase.

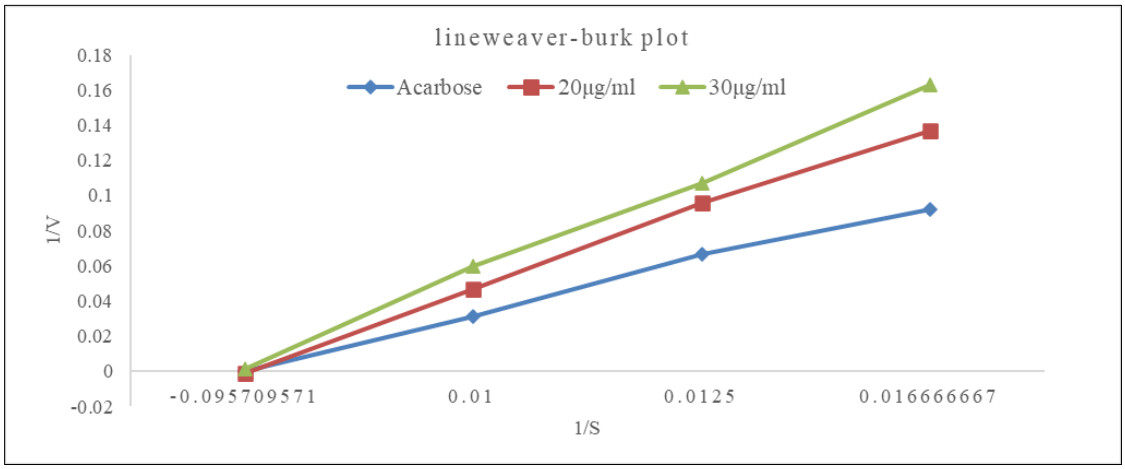

Figure 4 Lineweaver-Burk Plot

4. Conclusion

Vernonia amygdalina inhibits alpha-amylase and alpha glucosidase activities but with greater inhibitory effects experienced in alpha glucosidase shows that plants tend to have higher inhibitory effect on alpha-glucosidase. With the obtain IC_{50} values it helps to provide insight into the mechanism of actions of an inhibitor by helping to determine the nature and strength of the interaction between the inhibitor and the enzyme or the biological process being inhibited. Acarbose which serves as inhibitors of these enzymes with greater effect on alpha-amylase but resulting to gastrointestinal adverse effects due to the accumulation of undigested carbohydrates becoming readily available for bacterial fermentation. With these outcomes the possibility exists that controlling glycemic complication is achievable by combining the inhibitory effect of *Vernonia amygdalina* and acarbose whilst reducing acarbose dose to alleviate anti-diabetic related adverse effects. In humans on further studies, it can help in glycemic control while in bioreactors that might include the usage of alpha-glucosidase and alpha-amylase as enzyme which similarly employs *Vernonia amygdalina*, it is noteworthy that the activity of these enzymes can be inhibited in bioreactors.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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