

INHIBITORY POTENTIAL OF AN AFRICAN VERNONIA AMYGDALINA DEL. (ASTERACEAE) LEAVES ON A GLUCOSIDASE ENZYME

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ABSTRACT. Extracts of medicinal plants origin have been reported in several studies to regulate postprandial hyperglycemia by impeding the rate of carbohydrate digestion in the small intestine thereby hampering the diet associated acute glucose excursion. This study describes the inhibitory activity of an African *Vernonia amygdalina* leaves on a glucosidase enzyme. Powdered sample (100g) was extracted by cool maceration with acetone. Acetone crude extract was fractionated by column chromatography. *In vitro* α -glucosidase activity was evaluated at varying concentrations using standard method. One way analysis of variance was used to compare the data. Qualitative phytochemical analysis was carried out to identify bioactive constituents present in the fractions. Column chromatographic separation resulted in eight fractions that were pooled together to give three fractions (PF₁ - PF₃). *In vitro* α -glucosidase inhibitory studies showed potent inhibitory activity with PF₃ exhibiting a strong inhibitory activity having an IC₅₀ = 70 ± 0.03 μ g/ml. PF₁ had the highest percentage inhibition (75.95%) at 20 μ g/ml. The least inhibitory activity (6.33%) at 120 μ g/ml was observed in PF₃. The fractions showed a concentration dependent inhibitory activity except for the CE. Inhibitory activity varied significantly ($p < 0.05$) between the crude extract and fractions tested. Qualitative phytochemical analysis of the fractions showed the presence of triterpenes, phenolics, sugars and sugar phenylhydrazones in varying quantities. The results of this study showed that the leaf of *Vernonia amygdalina* possessed potent inhibitory activity on α -glucosidase and can serve as a source of lead compounds for isolation and optimization as an antidiabetic agent.

Keywords: *Vernonia amygdalina*, Column chromatography, α -glucosidase, Inhibitory activity

INTRODUCTION

Diabetes mellitus is a disease of metabolic disorder, reported to have multiple etiology that is characterized by hyperglycemia. It has affected a number of population worldwide and these number is on the increase. Zimmet et al [1] reports that worldwide, 371 million people are affected and by 2035 the number is predicted to go up to almost 600 million. In Africa, the World Health Organization Diabetes program on Country and Regional Data, 2017, estimated that there will be 18,234,000 diabetes patients by 2030 out of which around 4,835,000 will be coming from Nigeria alone. This makes Nigeria the most

vulnerable for diabetes in African region [2]. Available treatments including drug therapies have not been that successful in stemming the tide probably due to adverse side effects, low cost effectiveness, decreased efficacy over time and limited mode of action.

To prevent the progression of diabetes, especially Type 2 Diabetes Mellitus and for the treatment of prediabetes conditions, one of the most effective therapeutic approaches is the reduction of post-prandial hyperglycemia by inhibiting the carbohydrate hydrolyzing enzymes, α -glucosidase and α -amylase. Inhibition of these enzymes reduces the rate of digestion of carbohydrates, resulting in less absorption of glucose [3], consequently reducing the risk of hyperglycemia. Varieties of therapeutic drugs, all synthetic and of microbial origin [4] are currently used in the management of postprandial hyperglycemia. While these drugs are effective, they are largely associated with adverse side effects which have made them less attractive as therapeutic agents [5]. Hence, the need for an alternative source of therapeutic agents having similar mode of action and devoid of these side effects.

Vernonia amygdalina belongs to the family asteraceae. This plant has been traditionally regarded as an indispensable medicinal plant for ages, particularly amidst sub-Saharan Africans to treat various diseases including malaria, stomach disorder, hiccups, diabetes, inflammation, fertility problem and bacteria infections [6]. Several studies [2, 6, 8], demonstrated the antidiabetic potential of the African *Vernonia amygdalina* leaves. However, there is dearth of information on the inhibitory activity of the African *Vernonia amygdalina* leaves on glucosidase enzymes that may suggest one of the possible pathways by which the plant exhibits its antidiabetic activity. Therefore, this study was designed to evaluate the inhibitory activity of crude extract and fractions from *Vernonia amygdalina* leaves on a glucosidase enzyme.

This research is limited to evaluating the inhibitory potential of the crude extract and column fractions from the African *Vernonia amygdalina* leaves. It does not involve complete purification and isolation of specific compounds with inhibitory potential on the glucosidase enzyme. The enzyme used in the study was a crude enzyme that did not under go any form of purification.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Fresh leaves of *Vernonia amygdalina* were collected from a household garden at Hadejia town, Jigawa state, Nigeria in April, 2019. The plant was authenticated at the Herbarium unit, Department of Plant science, Bayero University Kano, Nigeria with voucher number, BUKHAN 143. The plant material was then shade dried for 14 days and pulverized into powder using mechanical grinder.

Preparation of Extract from Vernonia amygdalina Leaves

Powdered sample (100 g) was extracted by cool maceration in 500 ml of acetone for 72 hours with occasional shaking. The extract obtained was concentrated and dried to a constant weight, then stored in an air-tight container [9].

Column Chromatography of Crude Extract

The adsorbent, silica gel (15 g, 60 - 120 μ m) was carefully packed in a column using wet slurry method. The crude extract, 0.4 g was loaded on to the packed adsorbent and eluted with 100 % hexane. Polarity of the elution solvent was adjusted with chloroform,

acetone and ethanol in different ratios and collected at a volume of 20 ml per fraction. Fractions collected were monitored on TLC plates, similar fractions were pooled together and coded [10, 11].

Experimental Animals

Adult Wister rats of mixed sexes were obtained from the animal house, Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. Adequate environmental conditions were provided. The rats were starved for 12 hours before being sacrificed. The National Institutes of Health Principles of Laboratory Animal Care were observed and the protocol of the experiment was duly approved by Ahmadu Bello University animal ethics committee.

Extraction of Intestinal α -Glucosidase

Small intestines from adult Wister rats were removed and washed with 30 ml of 0.9 % sodium chloride solution. It was then minced with a surgical knife and homogenized (1:4 w/v) in a tissue grinder with 0.1 M potassium phosphate buffer, pH 6.8. After 30 min, the homogenate was centrifuged for 30 min at 10,000 rpm at 4°C. The Supernatant was designated as crude α -glucosidase source [12].

α -Glucosidase Assay

α -Glucosidase Inhibition assay was carried out according to the method adopted from [13] with modification. Briefly, 20 μ L of crude enzyme (α -glucosidase) was pre-incubated with 20 μ L of the crude extract and fractions (20 – 120 μ g/ml) at 37°C. Then 70 μ L of 2 % maltose (w/v) was added to start the reaction. The mixture was incubated at 37°C for 30 mins and stopped by incubating in boiling water for 10 mins. The concentration of glucose released from the reaction mixture was determined using glucose oxidase kit (Agappe glucose kit).

The reaction sample (10 μ l) was added to 1000 μ l of reagent, mixed well and then incubated for 10 minutes at 37°C and absorbance measured at 550 nm. The inhibitory activity of the crude extract and fractions from *Vernonia amygdalina* leaves was calculated as:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Qualitative Analysis

The fraction with the best inhibitory activity was subjected to qualitative analysis using TLC plates developed in Ethyl Acetate: Ethanol (H:E:E) (8:1.9:0.1) and sprayed with specific spray reagents.

Data Analysis

Results were expressed as mean \pm standard errors of the mean for all values. Statistical package for social sciences (SPSS) was used for the Analysis of Variance (ANOVA). Duncan Multiple Range Test was used to separate the means.

RESULTS AND DISCUSSION

Extraction Yield

Extraction of the powdered plant material with acetone after 72 hours resulted in a dark green coloured crude extract having a percentage yield of 11.68%. The crude extract obtained was coded CE.

Column Chromatography of Crude Extract

Column chromatographic separation of the crude extract resulted in eight (8) fractions. These fractions were monitored on TLC plates using p-Anisaldehyde as a detecting reagent. Fractions collected from column with similar TLC profiles were pooled together to give three combined fractions and coded PF₁, PF₂, and PF₃.

α -Glucosidase Inhibition

The management strategies of some syndromes are central topics in scientific and medicinal areas. Recently, the theory stating that the inhibition of key enzymes [14, 15] could be a valuable strategy for disease treatment, particularly with the use of natural derived products in order to avoid the side effects of synthetic inhibitors, was accepted [16]. This study reports the inhibitory activity of *Vernonia amygdalina* leaves crude extract and fractions on α -glucosidase. At 60 $\mu\text{g/ml}$, CE was observed to have the highest inhibitory activity while the least inhibitory activity (55.72 %) was recorded at 120 $\mu\text{g/ml}$. The inhibitory activity of CE was rather biphasic in nature (Figure 1). This could be as a result of the interaction between CE and the complex macromolecules present along with the crude enzymes or that the different bioactive molecules present in CE could be responsible for such activity since crude extracts are known to be composed of many bioactive molecules interacting to bring about an activity. This is similar to the findings of Zakariya et al [17] and co-workers. An IC₅₀ value of 569.28 $\mu\text{g/ml}$, that was censored to the right was calculated for CE, hence, considered a weak inhibitor of α -glucosidase (Table 1).

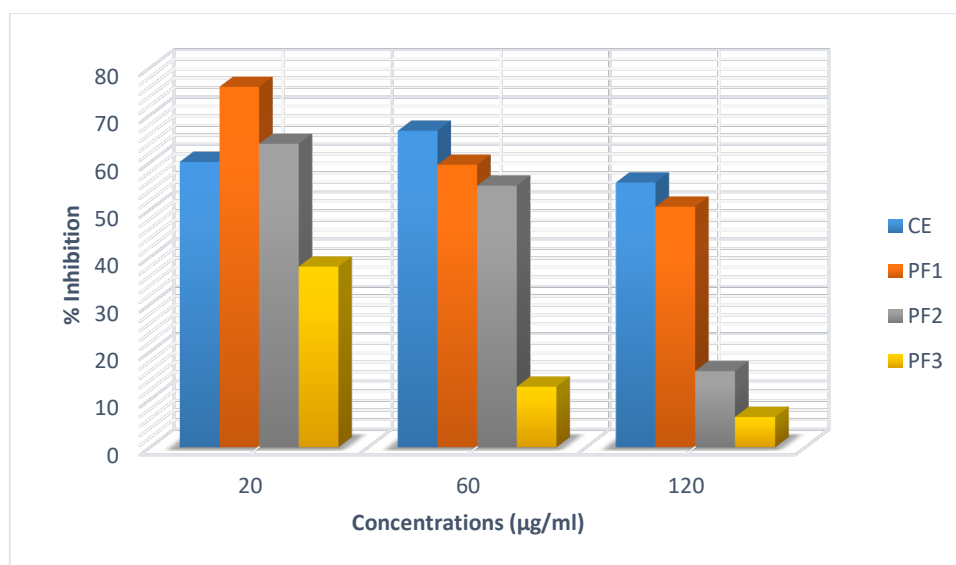


Fig. 1. α -Glucosidase inhibitory activity of *Vernonia amygdalina* leaves. Values are expressed as % means of triplicate tests. CE= Crude Extract, PF₁= Pooled Fraction one, PF₂= Pooled Fraction two, PF₃= Pooled Fraction three.

The highest inhibitory activity (75.95 %) and (63.92 %) was observed in PF₁ and PF₂ at 20 µg/ml respectively. A low inhibitory activity (15.92 %) was recorded at 120 µg/ml for PF₂ compared to PF₁ at same concentration (Fig. 1). The IC₅₀ value calculated for PF₁ was censored to the right while a point value was determined for PF₂ (Table 1). Generally, PF₃ had a low inhibitory activity across the concentrations compared to those of PF₁ and PF₂ (Figure 1). At 120 µg/ml, the least inhibitory activity (6.33 %) was recorded for PF₃. A point value of 70 µg/ml was determined as the IC₅₀ value of PF₃ and was considered a strong inhibitor of α-glucosidase (Table 1). Statistically, PF₃ varied significantly ($p < 0.05$) from PF₁ and CE but did not vary significantly ($p > 0.05$) from PF₂. Furthermore, PF₁ and CE did not vary significantly ($p > 0.05$) from PF₂. There was no significant difference ($p > 0.05$) among the concentrations used in this study.

Table 1. IC₅₀ values for α-glucosidase treated with crude extract and fractions of *Vernonia amygdalina* leaves

S/N	Extract and Fractions*	IC ₅₀ ± SEM** (µg/ml)
1	CE	569.28 ± 0.04
2	PF ₁	160 ± 0.01
3	PF ₂	90 ± 0.04
4	PF ₃	70 ± 0.03

*Crude Extract (CE); Partial Fraction one (PF₁); Partial Fraction two (PF₂); Partial Fraction three (PF₃).

**SEM=Standard Error of Mean

Several studies have reported the inhibitory potential of crude extracts, fractions or pure compounds of medicinal plant origin on glucosidase enzymes. However, none have reported the inhibitory potential of fractions from *V. amygdalina* on α-glucosidase. α-Glucosidase inhibitors have been grouped to be the third category of hypoglycemic agents [18]. Enzymes remain prime targets for drug design because altering enzyme activity has immediate and defined effects. Even with the increase in the use of drugs for receptors to modulate signals from outside the cell, 47 % of all current drugs inhibit enzyme targets [19]. This study, demonstrated a potent inhibitory activity of an African *Vernonia amygdalina* leaves on α-glucosidase, and these findings corroborates other reports [6, 16, 20], on the use of medicinal plants as inhibitors of α-glucosidase. This is geared towards finding new therapeutic agents devoid of side effects for the management of diabetes mellitus.

Qualitative Analysis

Qualitative phytochemical analysis of the fractions on TLC plates visualized with specific detecting reagents (Lieberman-Buchard, Ferric chloride and p-Anisaldehyde) showed the presence of triterpenes, phenolics, sugars and sugar phenylhydrazones in varying quantities based on the intensity of the spots (results not shown). The presence of these bioactive constituents acting singly or in synergy is responsible for the reported activity.

CONCLUSION

This study demonstrated a potent inhibitory activity by an African *Vernonia amygdalina* leaves on α -glucosidase. This may suggest that one of the possible pathways by which *Vernonia amygdalina* leaves exhibits its antidiabetic activity is through the inhibition of α -glucosidase in the digestive tract. Bioactive constituents present in the leaves of the plant is clearly responsible for this activity, hence, can serve as a source of lead compounds for isolation and optimization as an antidiabetic agent.

Therefore, further purification, isolation and characterization of pure compound(s) from the African *Vernonia amygdalina* leaves having potent antidiabetic activity by inhibiting α -glucosidase enzyme in the digestive tract holds a lot of prospect.

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