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Research Article

Alpha amylase and angiotensin converting enzyme inhibitory potential of aqueous extract of *Azanza garckeana* fruit

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Abstract
Diabetes mellitus and hypertension are common diseases affecting a lot of people. Alpha amylase and angiotensin-converting enzyme (ACE) inhibitors are used to treat type II diabetes and hypertension respectively. This study investigated the alpha amylase and ACE inhibitory potential of *Azanza garckeana* fruits. Phytochemical screening, α-amylase and ACE inhibitory potential of the aqueous extract of *A. garckeana* fruit was determined using standard procedures. The mode of inhibition of α-amylase by *A. garckeana* fruit was determined from the Lineweaver-Burk plot. Alkaloids, flavonoids, anthraquinones, steroids, tannins, phenols and terpenoids were present in the aqueous extract of *A. garkeana* fruit. The percent inhibition of α-amylase was greater than 50%. The IC_{50} values were 2.6 ± 0.02 and 0.04 ± 0.09 for the extract and acarbose (standard drug) respectively. The Lineweaver-Burk plot showed that extract Vmax did not change when compared to the no inhibitor (no extract) but the km increased. The percent inhibition of ACE by *A. garckeana* was also greater than 50%. Its IC_{50} was 0.625 ± 0.03 while that of the standard drug (captopril) was 0.875 ± 0.07. Thus *A. garckeana* inhibited α-amylase and ACE and can be used to treat type II diabetes and hypertension. It is a competitive inhibitor of α-amylase.

Keywords: α-amylase, Angiotensin-converting enzyme, *Azanza garckeana,* Diabetes, Hypertension

INTRODUCTION

Diabetes mellitus is a complex disorder caused by impaired insulin action or no insulin production leading to a rise in glucose in the blood (Agarwal and Gupta, 2016). It is a progressive metabolic disorder of glucose metabolism that causes secondary complications that are difficult to manage (Klein *et al*., 2007). Diabetes usually results in postprandial hyperglycemia (Oboh *et al*., 2012). There are two main types of diabetes mellitus; type 1 and type 2. Type 1 diabetes is characterized by autoimmune destruction of pancreatic beta cells which produce insulin (Atkinsin, 2015). Type 2 diabetes results from ineffective use of insulin leading to high blood glucose (Canivell and Gomis, 2014).

Pancreatic alpha amylase hydrolyses the alpha 1, 4 glycosidic linkages of starch, amylopectin, amylose, glycogen and numerous maltodextrins. It is thus responsible for starch digestion (Agarwal and Gupta, 2016). Inhibition of alpha amylase can therefore delay

the increase in blood glucose levels and keep postprandial hypoglycemia under control (Lebovitz, 1997).

Individuals with type 2 diabetes mellitus often display an array of metabolic derangements termed cardiorenal metabolic syndrome including hypertension (Center for Disease Control Prevention, 2020). A total of 73.6% of people with diabetes aged 18 years and above have hypertension (Naha *et al*., 2021). Hypertension is defined as abnormally high blood pressure. The renin-angiotensin-aldosterone system is important in the regulation of blood pressure (Ruschitzka and Tadder, 2012). The enzyme renin degrades angiotensinogen, a compound released from the liver, to generate inactive angiotensin 1. The C-terminal of angiotensin I is cleaved by angiotensin-converting enzyme (ACE) to angiotensin II (Guang *et al*., 2012). Angiotensin II causes vasoconstriction and increased sodium and water retention, leading to high blood pressure (de Leeuw, 1999). Angiotensin-converting enzyme inhibitors are the cornerstone of blood pressure control (Mancea *et al*., 2007). Synthetic alpha amylase inhibitors such as acarbose have side effects such as gastrointestinal disorders while angiotensin converting enzyme inhibitors have side effects such as hyperkalaemia and dizziness (Van de Kar, 2008; Poovitha and Paran, 2016). Natural herbal drugs have several advantages such as effectiveness with chronic conditions, low risk of side effects, low cost and widespread availability (Subhedar and Goswani, 2011).

Azanza garckeana (F. Hoffm), a member of the Malvaceae family, is known as goron tula in Hausa (Bioltif *et al*., 2020). It is grown in Nigeria in Tula village of Gombe state. It is used in northern Nigeria as an important source of food and medicine (Ahmed *et al*., 2016). It is also used in the treatment of several diseases, such as chest pain, cough, sexually transmitted diseases, infertility, hepatic impairment, liver problems and diabetes (Glew *et al*., 2005; Alfred, 2012; Bioltif *et al*., 2020). Its use in the treatment of diabetes has not been scientifically evaluated. This study evaluated the alpha amylase and angiotensin-converting enzyme inhibitory potential of the fruits of *A. garckeana.*

MATERIALS AND METHODS

Plant collection and identification

The *Azanza garckeana* fruit was obtained from Tula, Kaltungo, Gombe State, Nigeria. The plants were authenticated by a botanist after which the plant was deposited in the herbarium of the Department of Botany, Adamawa State University, Mubi, Nigeria.

Preparation of plant sample extract

The *A. garckeana* fruit was neatly washed and dried at room temperature for two weeks. Mortar and pestle were used to pound it into a fine powder, and then it was sieved and stored in a covered plastic container for further use.

One hundred grams of dried pulverized *Azanza garckeana* was soaked in 500 mL of water for 24 hrs at room temperature, under occasional shaking. The extraction was repeated three times, and the extract obtained was filtered using Whatman filter paper number 1. The solvent in the filtrate was removed by placing the filtrate in a water bath at 50°C. The extract was stored at 25°C in a covered plastic container for further use.

Phytochemical screening

Phytochemical screening of the extract was conducted using standard procedures described by Harbone (1993).

Alpha-amylase inhibitory assay

The alpha amylase inhibitory assay was carried out using the procedure described by McCue *et al*. (2005). Serial dilutions of the extract (ranging from 1.25 mg/mL -10 mg/mL) were prepared using dimethyl sulfur oxide (DMSO). An aliquot amount (250 µL) of the different concentrations was pipetted into separate test tubes, and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing 0.5 mg/mL α-amylase solution was added. The mixture was incubated for 10 minutes at 25°C. Then 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing 1% starch solution was added and incubated at 25°C for 10 minutes. Dinitrosalicylic acid (DNS) reagent (500 µL) was added to terminate the reaction. The mixture was incubated for 5 minutes in boiling water and allowed to cool to room temperature.. Five milliliters of distilled water was added to the reaction mixture and the absorbance was measured at 540 nm. The same procedure was followed for acarbose (the standard drug) but the extract was replaced with acarbose. A control was prepared using the same procedure replacing the extract with DMSO. The α-amylase inhibitory activity was calculated as

Percentage inhibition = (Abs control – Abs sample)/ Abs control × 100 Eq 1

The IC_{50} was determined graphically.

Mode of α-amylase inhibition

The mode of inhibition of *α*-amylase by the extract was performed according to the modified method of Ali *et al.* (2006) described by Ahmed *et al*. (2020). An aliquot amount (250 µL) of α- amylase solution was preincubated with 250 µL of 5 mg/mL extract in DMSO at 25°C for 10 minutes in a set of test tubes. In another set of test tubes, 250 µL of phosphate buffer (pH 6.9) was preincubated with α-amylase solution. To both sets of test tubes, 250 µL of increasing concentration (0.30 – 5.0 mg/mL) of 1% starch solution was added. The mixture was incubated at 25°C for 10 minutes and 500 µL

of DNS was added to terminate the reaction. It was then boiled for 5 minutes**.** A maltose standard curve was prepared and used to determine the amount of maltose released. This was converted to velocity. Velocity was plotted against substrate concentration to obtain the Michaelis-Menten plot. Additionally, a plot of 1/V against 1/S (where S is the substrate concentration and V is the velocity) was plotted. Parameters obtained from the plot (Lineweaver-Burk plot) was used to determine the mode of inhibition of the extract on α-amylase activity.

Determination of angiotensin-converting enzyme inhibitory activity

An angiotensin-converting enzyme inhibitory assay was performed according to a modified spectrophotometric method of Holmquist *et al*. (1979) using FAPGG as substrate. Briefly, 500 µL of 0.5 mM FAPGG (dissolved in 50 mM Tris–HCl buffer containing 0.3 mM NaCl, pH 7.5) was mixed with 20 µL of ACE (0.1 U/mL; final activity of 2 mU) and 100 µL of extract at three concentrations (0.5, 1.0 and 1.5 mg/mL) in 50 mM Tris–HCl buffer. The absorbance at 345 nm was recorded at regular intervals for 5 min at room temperature. A similar procedure was also followed for the standard drug, captopril. Tris–HCl buffer was used for the blank experiment instead of the extract. The percent inhibition was calculated using the equation below:

ACE Inhibition (%) = (Δ Absorbance/min) blank − (Δ Absorbance/min) sample/(Δ Absorbance/min) blank × 100 Eq 2

The concentration of extract that inhibited angiotensin-converting enzyme activity by 50% (IC_{50}) was calculated using a non-linear regression plot of ACE inhibition against sample concentrations.

Statistical analysis

All experiments were performed in triplicate. The results are expressed as the mean ± S.E.M. Data were compared using two-way ANOVA followed by Duncan's test for multiple comparisons. Differences were considered to be statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the phytochemical analysis of the aqueous extract of *A. garckeana* fruits. All the phytochemicals tested were present. Elshiekh and Ali (2020) also reported the presence of alkaloids, flavonoids, tannins, steroids and terpenes in *A.garckeana* fruits.

The percent inhibition of α-amylase by the aqueous extract of *A. garckeana* fruit is shown in Fig. 1. The bar chart shows that the % inhibition of the aqueous extract of *A. garckeana* fruit was 53% and 56% at 0.5 and 5.0 mg/mL while that of the standard drug (Acarbose) was

Table 1. Phytochemical analysis of aqueous *Azanza garckeana* fruit extract

Phytochemical	Inference
Alkaloids	+
Flavonoids	+
Anthraquinone	+
Steroids	+
Phenols	+
Tannin	+
Terpenoids	+

Table 2. IC_{50} values for acarbose and aqueous extract of *Azanza garckeana* fruit

Sample	IC_{50} (mg/mL)
A. garckeana	2.60 ± 0.07
Acarbose	0.64 ± 0.09

61 % and 69% respectively. The % inhibition at this concentration is greater than 50%, indicating that *A. garckeana* fruit is an inhibitor of alpha amylase. Alpha amylase inhibitors are important in the management of type II diabetes mellitus because their activity correlates with an increase in postprandial blood glucose levels (Sudha *et al*., 2011). These inhibitors are effective in lowering postprandial hyperglycemia because they prevent the breakdown of carbohydrates (Wu and Xu, 2014). They impair glucose metabolism without affecting insulin secretion (Vazquez- Armenta *et al*., 2015). The alpha amylase inhibitory potential of *A. garckeana* fruit may be due to the presence of flavonoids. Flavonoids have been reported to inhibit alpha amylase (Li *et al*., 2018).

Table 2 shows the $1C_{50}$ values for the aqueous extract of *A. garckeana* fruit and acarbose. The IC_{50} value for *A. garckeana* fruit is greater than that of the standard drug, acarbose. This indicates that the aqueous extract of *A. garkeana* fruit is not as potent as an inhibitor as acarbose.

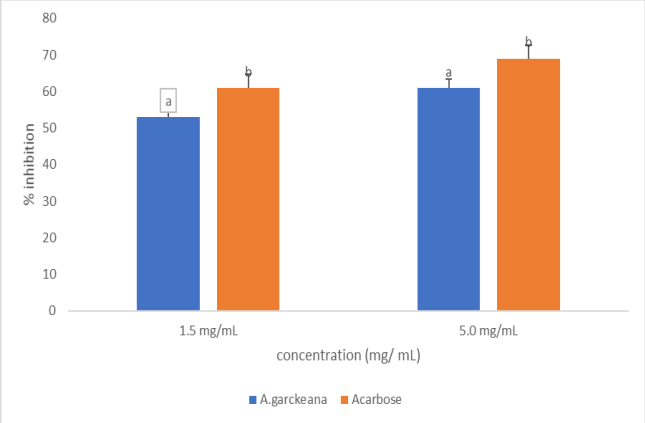

Fig. 1. *Percentage inhibition of alpha amylase by aqueous extract of Azanza garckeana fruit (Bars with different superscripts in each category are significantly different (p < 0.05)*

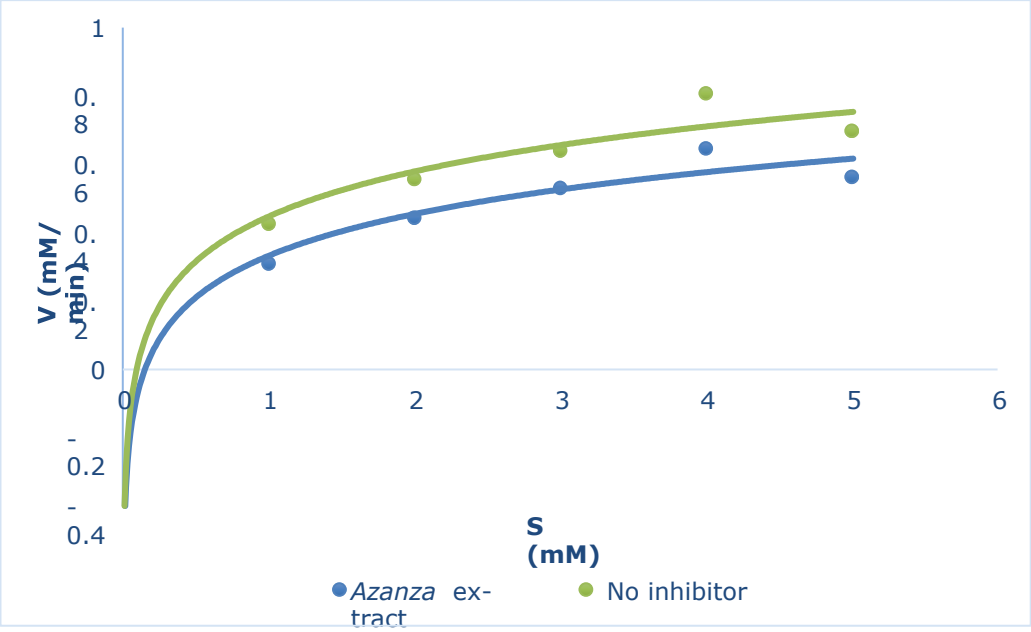

Fig. 2. *Michealis-menten plot of aqueous Azanza garckeana fruit extract*

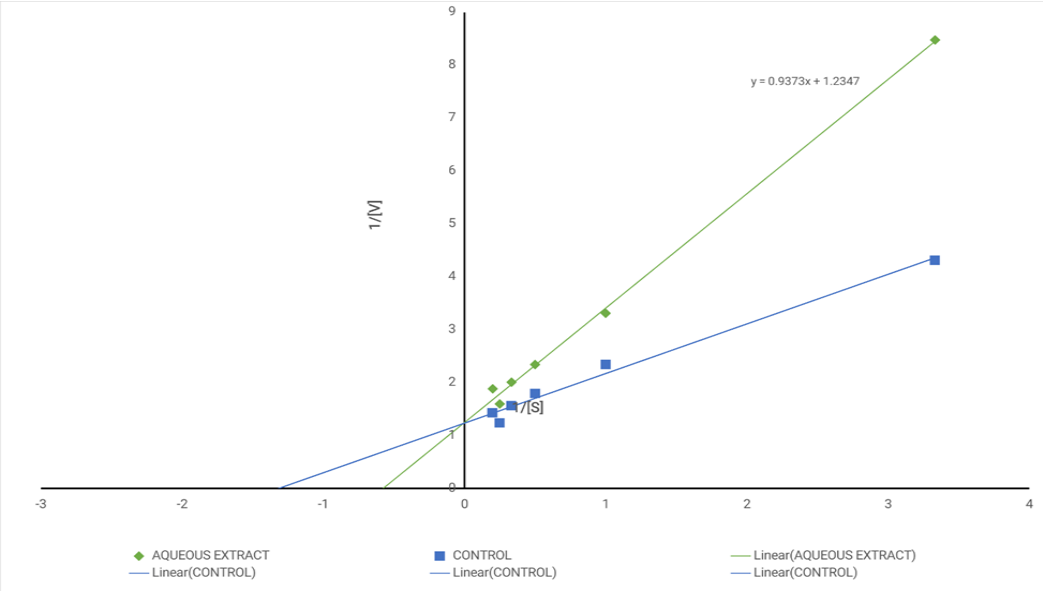

Fig. 3. *Lineweaver-Burk plot of Aqueous extract of A. garckeana fruit*

The Michaelis- Menten plot shows that the aqueous extract of *A. garckeana* fruit decreased velocity at all concentrations assayed when compared to the control (no inhibitor) (Fig. 2). A decrease in velocity indicates decreased activity of the enzyme. This further indicates that *A. garckeana* fruit is an inhibitor of alpha amylase.

The Lineweaver-Burk plot (Fig. 3) shows that the aqueous extract of *A.garkeana* fruit and the no inhibitor (i.e no extract) had the same Vmax and an increase in km. Competitive inhibition is characterized by an increase in km and the same Vmax (Vmax unchanged). It therefore follows that *A. garckeana* fruit inhibits in a competitive manner. Acarbose, the standard drug, also competes in a competitive manner (Rahimzadeh *et al*., 2014). Competitive inhibition is advantageous because when the substrate (the extract) binds to the inhibitor, it forms an enzyme inhibitor (EI) complex instead of an enzyme substrate (ES) complex, which prevents the enzyme from acting on its substrate and thus prevents its breakdown to glucose (Ahmed *et al*., 2020).

Fig. 4 shows the percent inhibition of ACE activity by the aqueous extract of *A. garckeana* fruit. The percent inhibition of aqueous *A. garckeana* fruit was greater than that of the standard drug captopril at all concentrations assayed indicating that the extract is a better inhibitor of ACE than the control. This is also reflected in

Table 3. IC_{-50} of *A. garckeana* fruit and captopril

Sample	Concentration (mg/ml)
A.garckeana	0.625 ± 0.03
Captopril	0.875 ± 0.07

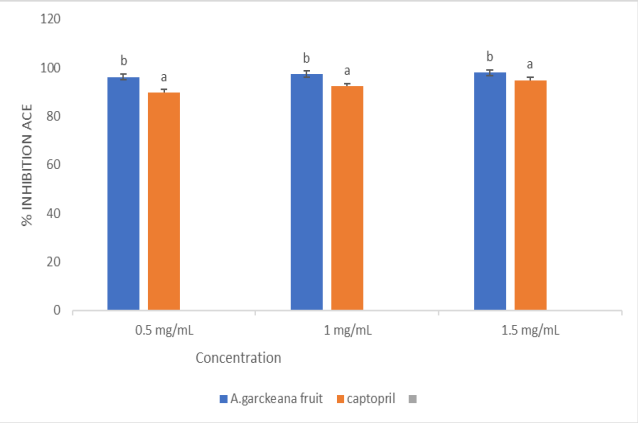

Fig. 4. *Percent inhibition of ACE activity by A. garckeana fruit*

the IC_{50} (Table 3) where the IC_{50} of *A. garckeana* fruit (0.625 ± 0.03) is lower than the IC_{50} of the standard drug (captopril) (0.875 ± 0.07). Angiotensin-converting enzyme inhibitors prevent the formation of angiotensin II by ACE and thereby reduce peripheral vascular resistance and blood pressure (Kouchmeshky *et al*., 2012). Therefore, *A. garckeana* can be used in the treatment of hypertension.

Conclusion

The results from this study show that *Azanza garckeana* fruit is an inhibitor of both alpha amylase and angiotensin-converting enzyme. *A. garckeana* is not as potent as acarbose (standard antidiabetic drug). It is a competitive inhibitor of alpha amylase. The inhibitory potential of *A. garckeana* fruits may be due to the presence of flavonoids. It can therefore be used in the treatment of type II diabetes and hypertension. This study justifies the folklore use of *A. garckeana* in the treatment of diabetes mellitus.

Conflict of interest

The authors declare that they have no conflict of interest.

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