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Anti-Hyperglycaemic and Antioxidant Properties of *Azanza Garckeana* Aqueous Leaf Extract

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Abstract

Azanza garckeana is an African medicinal plant traditionally used for the management of diabetes among others. The study was conducted to investigate the *in vitro* anti-hyperglycaemic activity using α-amylase and glucosidase inhibition assays and *in vivo* using type II model diabetic rats while DPPH and FRAP were used to evaluate the antioxidant potential of *A. garckeana* aqueous leaf extract (AGALE). Acarbose and vitamin C were used as the standard drugs for the hyperglycaemic assay and antioxidant assay respectively. For the *in vitro* studies, the extract exhibited alpha-amylase inhibitory activity of 2.2±0.02 mg/mL and alpha-glucosidase inhibitory of 2.3±0.03 mg/mL showing a higher IC_{50} value than the control drug acarbose. The DPPH scavenging activity was found to have IC_{50} values of 1.4±0.07 mg/mL and 3.2±0.08 mg/mL for FRAP both higher than the control. For the in vivo studies, twenty rats (20) were grouped into four (4), comprising of five (5) rats each. Diabetes mellitus was induced by intraperitoneal injection of STZ (60 mg/kg). Group A was normal control, Group B Diabetic control, Group C Diabetic rats given 150 mg/kg b.w. and Group D Diabetic rats given 100 mg/kg b.w. AGALE. Blood glucose levels (BGLs) were recorded weekly; it was observed that the oral administration of AGALE extract exhibited a noteworthy decline in blood glucose levels. The dosage of 150 mg/kg resulted in a more considerable reduction ($P<0.05$) in blood glucose levels starting from the second week. When compared to the untreated diabetic rats that had a continous increase in the BGL, the treated groups showed a significant decrease ($P<0.05$) in BGLs. The present study demonstrated that AGALE exerted potent anti-hyperglycaemic and antioxidant activity and exhibited appreciable inhibition against alpha amylase and glucosidase hence its use in the management of diabetes mellitus may be justified.

Introduction

Hyperglycaemia is defined as an increase in blood glucose levels above the physiologic range (Fasting 70-99mg/dL), if left untreated hyperglycemia can lead to many serious life-threatening complications that include damage to the eye, kidneys, nerves, heart, and peripheral vascular system (Mouri & Badireddy, 2023). Factors contributing to

hyperglycaemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. Glucose homeostasis is a balance between hepatic glucose production and peripheral glucose uptake and utilization. Insulin is the most important regulator of glucose homeostasis (Yari et al., 2020). Diabetes is the most common and primary cause of hyperglycaemia. As of 2019, 8.2 million Nigerians were estimated to have impaired glucose tolerance, with the number projected to increase to 11.5 million by 2030 (Adeleye, 2021).

An antioxidant is a substance that significantly delays or prevents the oxidation of a substrate. This protection can occur through various mechanisms, including inhibition of generation and scavenging capacity against reactive oxygen or nitrogen species (ROS or RNS); reducing capacity; metal chelation; singlet oxygen quenching activity; and hydrogen peroxide decomposition. Antioxidants serve as a defensive factor against the harmful effects of free radicals in the body (Gulcin, 2025). Currently, a variety of synthetic antioxidants are widely used. However, the use of these compounds has been restricted by regulations due to concerns about their toxicity and carcinogenic effects (Gulcin, 2025). Plants are potential sources of natural antioxidants, such as vitamin C, α-tocopherol, carotenoids, flavonoids, and phenolic acids, which help prevent free radical damage. They provide a phenolic hydroxyl group that reacts with free radicals, thereby inhibiting oxidative mechanisms that contribute to degenerative diseases (Altay et al., 2019).

Azanza garckeana is known as goron tula in Nigeria, it is a valuable edible indigenous fruit widely distributed in Tula, Kaltungo Local Government Area (LGA) of Gombe State and in Michika in Adamawa State (Ibrahim et al., 2023b). It generally grows naturally in all types of woodlands from sea level to about 1700m above sea level. It also grows in semi-arid regions, receiving lowest annual rain fall of 250mm and highest rain fall of 1270mm (Maroyi, 2017). It is a shrub or small tree, growing up to 10 m tall, with stem diameter at breast height of upto 25 cm. The leaves are alternate, simple, round, 3-5 lobe upto 20×20 cm in size, subcircular in outline and palmately

with 3-5 lobes (Maroyi, 2017). Pharmacological studies indicate that the species have a diverse range of pharmacological activities which include but not limited to anti-hyperglycaemic, antimalarial, antioxidant, antibacterial, antifungal, and iron absorption (Maroyi 2017).

There is growing interest in natural and safer anti-hyperglycaemic and antioxidant compounds. This research work investigated the *in vitro* and *in vivo* anti-hyperglycaemic and antioxidant activities of *Azanza garckeana* aqueous leave extract (AGALE).

Materials and Methods

Collection and identification of plant material

The leaves of *Azanza garckeana* (AG) was collected from Tula village, Kaltungo L.G.A, Gombe state of Nigeria (9.846° N, 11.474° E) and identified at the Herbarium Unit Biological Science Department, B. U. K. Kano with accession number BUKHAN 650.

Extraction of the plant material

Azanza garckeana leaves procured were dried under shade for 5 days. They were pulverized into powder form using an electric blender.

The extraction of *Azanza garckeana* leaves was conducted using distilled water through maceration. About 1.2 kg of the powdered sample was packed into glass bottles and soaked in distilled water for 24 hours and then filtered. The obtained filtrate was then evaporated using a water bath and dried in a desiccator.

In vitro anti-hyperglycaemic assays

Determination of alpha amylase inhibitory activity of aqueous extracts of *Azanza garckeana (AG)* leaf.

The alpha-amylase inhibitory activity of the extract was carried out according to the method used by Etsassala et al., (2019). In a 96-well plate, the reaction mixture containing 50µL of phosphate buffer (100 mM, pH = 6.8), 10µL alpha amylase (2 U/mL), and 20µL of varying concentrations of the extracts were pre-incubated at 37°C for 20 min. Next, 20µL of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and incubated further at 37°C for 30 min. A volume

of 100µL of the colour reagent (DNS) was added and then boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using a Multiplate Reader (Multiskan thermo scientific, version 1.00.40). Acarbose at various concentrations was used as a standard. Each experiment was performed in triplicates. The results were expressed as the percentage inhibition, which was calculated using the formula:

$$\text{Inhibitory activity (\%)} = (1 - A/B) \times 100$$

where A is the absorbance in the presence of the test substance and B is the absorbance of the control.

Determination of α- Glucosidase inhibitory activity of aqueous extracts of *Azanza garckeana (AG)* leaf.

Alpha-glucosidase inhibitory activity of the extract was carried out according to the method used by Etsassala et al. (2019). In a 96-well plate, the reaction mixture containing 50µL of phosphate buffer (100 mM, pH = 6.8), 10µL alpha-glucosidase (1 U/mL), and 20µL of varying concentrations of the extracts was pre-incubated at 37°C for 15 min. Next, 20µL of p-NPG (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 50µL of sodium carbonate Na_2CO_3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using a Multiplate Reader (Multiskan thermo scientific, version 1.00.40, Vantaa, Finland). Acarbose at various concentrations was included as a standard. Each experiment was performed in triplicates. The results were expressed as a percentage inhibition, which was calculated using Formula;

$$\text{Inhibitory activity (\%)} = (1 - A/B) \times 100$$

where A is the absorbance in the presence of the test substance and B is the absorbance of the control.

***In vitro* antioxidant assay**

1,1-Diphenyl-2-picrylhydrazyl (DPPH)Assay

The assay was performed as described previously (Alam et al., 2013). DPPH reagent was (8 mg) dissolved in MeOH (100 mL) for a solution concentration of 80 μL/mL. To determine the scavenging activity, 100 μL DPPH reagent was mixed with 100 μL of sample in a 96-well microplate and was incubated at room temperature for 30 min. After incubation, the absorbance was measured 514 nm using a microplate reader, and 100% methanol was used as a control. The DPPH scavenging effect was measured using the following formula:

$$\%\ \text{DPPH scavenging} = (A0 - A10)/A0) * 100$$

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP solution was prepared as described previously by Benzie & Szeto (1999). The FRAP working solution was prepared by mixing 10-volumes of acetate buffer (300 mM, pH 3.6) with 1-volume of TPTZ (40mM dissolved with 40mM HCl) and 1-volume of ferric chloride (20 mM in water). Microplate FRAP assay was performed according to previous reports (Jimenez-Alvarez et al., 2008; Firuzi et al., 2005; Tsao et al., 2003). Sample solutions (20 µl) were added directly to the 96-well microplate followed by 280 µl of working FRAP solution. The mixtures were shaken, incubated at 37°C in the dark for 30 minutes and then A593 readings were recorded using a microplate reader.

Experimental animals

The experimental animals used in this study were twenty (20) Wistar rats weighing approximately 120-150g, procured from Ahmadu Bello University (ABU) Zaria. These animals were housed under standardized conditions and given 2 weeks to acclimate to their new environment. They had unrestricted access to water and food, in compliance with internationally recognized guidelines for the utilization and care of laboratory animals, as outlined in the US guidelines (NIH, 1992) and as approved by the Federal University Dutsin-Ma Ethical Committee on the use of experimental animals and registered as FUDMA/CEA/2023/078.

Induction of Type 2 Diabetes

The induction procedure for Type 2 diabetes as carried out as previously described by Okoduwa et al., 2017. In brief, commercially available Normal Diet Feed (NDF) was fortified with margarine in a ratio of 10 g NDF per gram of margarine. This was administered, along with 20% fructose solution as drinking water, to the rats ad libitum for six weeks after which they were fasted overnight and injected

(ip) with STZ (dissolved in a citrate buffer pH 4.5) at a single low dose of 55 mg/kg b.w. In the first 24 h after STZ induction, the rats were provided with 5% glucose solution as drinking water.

Confirmation of Diabetes

Pre-confirmation of diabetes was done three days after STZ induction, with Accu-Chek glucometer using blood samples obtained via tail puncture of the rats. On day 10 following the pre-confirmation, animals with fasting blood glucose (FBG) ≥ 300 mg/dL were confirmed diabetic and incorporated in the study as diabetic animals.

Anti-hyperglycaemic study

Twenty rats (20) were grouped into four (4), each group comprising of five (5) rats each.

Group A – Normal control

Group B – Diabetic control

Group C – Diabetic rats were given 150 mg/kg b.w. *Azanza garckeana* aqueous leaves extract

Group D – Diabetic rats were given 100 mg/kg b.w. *Azanza garckeana* aqueous leaves extract

Extract administration to the animals was done orally at 24 hours interval for 28 days. Blood glucose level was recorded weekly.

Statistical Analysis

The results were presented as mean ± standard error of mean. SPSS (version 20; Chicago, Illinois, USA) was used to conduct an ANOVA. Significance was attributed to data with a P-value of less than 0.05 when compared to the control group.

Results

Determination of alpha amylase and alpha glucosidase inhibitory activity of aqueous extract of *Azanza garckeana* (AG) leaves.

In this study, the inhibitory activity of aqueous extract of *Azanza garckeana* leaves was investigated against α amylase and α glucosidase. The results showed that aqueous extract demonstrated appreciable inhibitory activity but had a higher IC_{50} value than the control drug acarbose as shown in Table 1.

Table 1: Inhibitory activity of aqueous extract of *Azanza garckeana* (AG) leaves against alpha amylase and alpha glucosidase.

Samples	IC_{50} (mg/ml)	
	α amylase inhibition	α glucosidase inhibition
Acarbose	1.3 ± 0.08^a	2.0 ± 0.03^a
Aqueous	2.2 ± 0.02^b	2.3 ± 0.03^b

Data are presented as mean ± SD values of triplicate determinations. Different superscripts within a column are significantly different from each other.

Determination of *in vitro* antioxidant assay of aqueous extract of *Azanza garckeana* (AG) leaf.

The *in vitro* antioxidant activities of the aqueous extract of *A. garckeana* were investigated by evaluating the Ferric reducing antioxidant potential (FRAP) and 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) antioxidant capacity. Table 2 shows the DPPH radical scavenging activity and FRAP of aqueous extract against vitamin C as standard.

Table 2: *In vitro* antioxidant activity of aqueous extract of *Azanza garckeana* leaves.

Samples	IC_{50} (mg/ml)	
	DPPH scavenging activity	FRAP
Vitamin C	0.4 ± 0.18^a	1.7 ± 0.07^a
Aqueous	1.4 ± 0.07^b	3.2 ± 0.08^b

Data are presented as mean ± SD values of triplicate determinations. Different superscripts within a column are significantly different from each other ($p < 0.05$).

Anti-hyperglycaemic activity

It was observed that the diabetic control group experienced a steady elevation in blood glucose levels. Conversely, the oral administration of AGALE extract exhibited a significant ($P<0.05$) decline in blood glucose levels. Our research outcomes suggest that the dosage of 150 mg/kg resulted in a considerable reduction in blood glucose levels starting from the second week of the experiment (Figure 1).

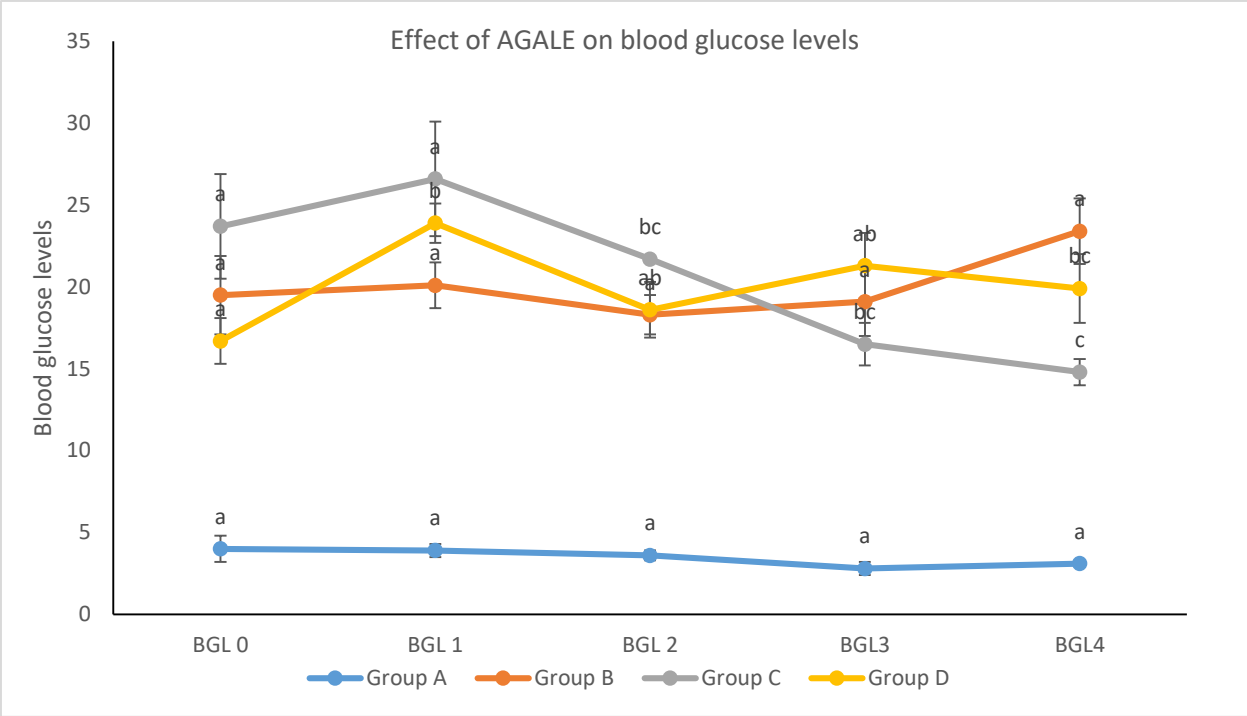

Figure 1: Blood glucose levels of rats administered *Azanza garckeana* aqueous leaves extract for four weeks

Values are expressed as the mean ± SD (n=4) values with different letters on the same line are significantly ($p<0.05$) different from each other. Group A: Negative control, Group B: Positive control Group C: 150mg/kg *Azanza garckeana* aqueous leaves extract, Group D: 100mg/kg *Azanza garckeana* aqueous leaves extract.

Discussion

The increasing prevalence of Type II Diabetes (T2D) threatens the quality of human life and hence qualitative and extensive research that will lead to the development of efficient anti-diabetic agents free of adverse effects is imperative. Medicinal plants are continually studied in animal models of disease with the aim of developing new and relatively safer plant-based antidiabetic therapies (Okoduwa et al., 2017).

The inhibition of carbohydrate-metabolizing enzymes and regulation of blood glucose levels are very critical to the management of diabetes mellitus (DM) (Owoade et al., 2018).Various studies have suggested the role of plant extracts as alpha-amylase and glucosidase inhibitors clearly indicating the potential of these extracts to manage hyperglycaemia. The activity of these plant extracts can be attributed to phyto-constituents present in them, such as flavonoids, alkaloids, terpenoids, anthocyanins, glycosides, phenolic compounds (Ibrahim et al., 2023b; Bhatia et al., 2019). In the present study, the extract showed appreciable inhibition potential against the alpha-amylase and glucosidase enzymes (Table 1). Phytochemical analysis of aqueous extract of *Azanza garckeana* leaves has shown the presence of terpenes, alkaloids and flavonoids which could be responsible for the inhibitory activity of the plant against both alpha amylase and alpha glucosidase (Ibrahim et al., 2023b).

Several previous studies have reported that various terpenes, including ursolic, corosolic, and oleanolic acids, exhibit α-amylase inhibitory activity (Alqahtani et al., 2019). Zhang et al. (2017) reported that pentacyclic triterpenes with an ursane skeleton significantly inhibited α-amylase activity. Additionally, numerous terpenoids—including betulinic, ursolic, corosolic, and oleanolic acids, as well as betulin—have been identified as α-glucosidase inhibitors. The inhibition of α-amylase by diethyl ether extract (Alozieuwa et al., 2022) and

methanolic extract (Lawal et al., 2022) of *T. garckeana* pulp has been reported and is believed to be one of the anti-hyperglycemic mechanisms of the plant.

Oxidative stress caused by free radicals is considered a major contributing factor in the development of various disorders, including diabetes mellitus, arthritis, cancer, cardiovascular diseases, and aging. Reports on T2DM indicated an increase in the concentration of free radicals (Alqahtani et al., 2019). The DPPH free radical assay and FRAP have been used extensively as a model scheme to evaluate the scavenging activity of antioxidants *in vitro* (Oyaizu, 1986). From Table 2 it was noted that the extracts had appreciable activity although lower than that of vitamin C. This could be due to the presence of various phytochemical found in the plant such as phenolic compounds. In addition, the hydrogen donating properties of the extract as a means of radical scavenging has been demonstrated by the DPPH and FRAP assay. Consequently, the high flavonoid and phenolic contents of *A. garckeana* as reported by Ibrahim et al., (2023a) may be responsible for the antioxidant activity of the extracts.

Phenolic compounds have been reported to be responsible for the antioxidant activity of numerous plant species by stabilization of radicals by donating electrons or metal ion complexation, among other mechanisms (Etsassala et al., 2019). Diterpenes exhibit potent antioxidant activity, primarily due to the presence of ortho-dihydroxyl or vicinal hydroxyl groups on the aromatic ring, which act as hydrogen or electron donors to reactive species, resulting in the formation of stable quinone derivatives. In general, phenolic compounds are expected to transfer electrons or donate protons to the reactive radicals because of the resonance stability of the phenoxy radical (Brewer, 2011). Studies have shown that *A. garckeana* fruit extract have exhibited appreciable DPPH scavenging activities which increases with increase in concentration (Salih et al., 2022; Maroyi, 2017). *In vitro* studies have also shown significant and dose-related antioxidant activities of the diethyl ether extract of *T. garckeana* pulp in four different *in vitro* models of antioxidant assays; DPPH, FRAP, LPO, and ABTS (Alozieuwa et al., 2022).

In this study, the anti-hyperglycaemic potential of aqueous extracts of *A. garckeana* in a model of T2D was carried out. *A. garckeana* is used for the traditional remedy of diabetes mellitus in Nigeria and other African countries (Ibrahim et al., 2023a), the anti-hyperglycaemic potential of the leaf extract in T2D model is either not available or scarce in the published literature.

The anti-hyperglycaemic activity of aqueous extracts of *A. garckeana* in a model of T2D showed significant ($P<0.05$) blood glucose reduction from the 2nd week of administration. However, the 150 mg/kg group had a more significant ($P<0.05$) effect of lowering the blood glucose level (BGL) proving the ameliorative effects of the extract on experimentally induced diabetes. The reduction in fasting blood glucose observed in this study may be attributed to the presence of some bioactive compounds in the plant (Osibemhe et al., 2019). Phytochemicals such as alkaloids, phenols, polysaccharides, steroids and flavonoids, have been reported to have glucose lowering capacity through various mechanisms such as inhibition of alpha glucosidase, decrease glucose transport, stimulation of insulin secretion and sensitivity (Ibrahim et al., 2023b, Ezeonu & Ejikeme, 2016; Gaikwad et al., 2014). Another possible mechanism by which the plant might exert its anti-hyperglycaemic effect may be by aiding the body's antioxidant system. Phytochemicals such as flavonoids and tannins are well known to scavenge free radicals (Berenguer et al., 2006) and also build up immunity, thus taking care of the stimulation of free radicals and oxidative stress as a result of the high glucose levels. Alozieuwa et al. (2022) reported that treatment of the STZ-induced diabetic rats with diethyl ether extract of *T. garckeana* pulp caused significant and progressive decreases in the fasting blood sugar (FBS) levels in a dose-related manner.

Conclusion

The antihyperglycaemic and antioxidant activities of the aqueous extract of *A. garckeana* leaves in T2D animal model was confirmed after daily

administration of the extract for four weeks (reduction in blood glucose levels) which could be attributed to the phytochemicals presence in the plant extract.

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Author Contributions

Ibrahim, M. drafted the research, analysed and interpreted the data, Idoko, A. S. and Ganiyu, A. I. revised the work. All authors reviewed the findings and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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