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Original Research Article

PHYTOCHEMICAL PROFILE AND HYPOGLYCEMIC EFFECTS OF *AZANZA GARCKEANA* (FENZL) F. MUELL, FRUIT PULP: A POTENTIAL NATURAL ALTERNATIVE FOR DIABETES MANAGEMENT

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ABSTRACT

Azanza garckeana is an indigenous medicinal fruit widely used traditionally for the management of diseases, including diabetes mellitus. This study aimed to evaluate the phytochemical constituents, acute toxicity profile, and antidiabetic activity of the aqueous fruit pulp extract of *Azanza garckeana* in experimental rats. Qualitative and quantitative phytochemical analyses were conducted to identify the bioactive constituents in the fruit pulp extract. The acute toxicity of the extract was assessed using experimental Wistar albino rats, while its antidiabetic activity was evaluated by administering graded doses and monitoring blood glucose levels at predetermined time intervals. Phytochemical screening revealed the presence of flavonoids, terpenoids, saponin glycosides, tannins, cardiac glycosides, and free anthraquinones, with flavonoids constituting the predominant bioactive compounds. The aqueous extract produced a dose-dependent reduction in blood glucose levels, with the 800 mg/kg dose producing the greatest hypoglycemic effect after 24 hours ($p< 0.01$). The 400 mg/kg dose significantly reduced blood glucose levels at 12 and 24 hours ($p< 0.01$), while the 200 mg/kg dose showed a significant reduction at 24 hours ($p< 0.05$). Acute toxicity evaluation revealed no mortality even at high doses, suggesting a wide safety margin, although weakness and dizziness were observed at higher doses. The findings demonstrate that *Azanza garckeana* possesses potential phytochemical constituents and exhibits promising antidiabetic activity with a favourable safety profile in experimental rats. These results support its traditional medicinal use and suggest its potential as a natural therapeutic agent for the management of diabetes mellitus.

Keywords: Antidiabetic, *Azanza garckeana* fruit pulp extract, blood glucose, flavonoids, hypoglycaemia effects, pharmacological potential

INTRODUCTION

The plant species *Azanza garckeana* (also called *Thespesia garckeana*), often referred to as Goron Tula in Northern Nigeria, is a member of the *Malvaceae* family. It is a tiny tree indigenous to tropical and Sub-Saharan Africa. The globose, woody fruits of *Azanza garckeana* are usually 2.5–4 cm in diameter and divide into several parts as they ripen. When the fruits reach maturity, their outer woody

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capsule bursts open to reveal edible pulp and seeds, and they have brownish, hairy surfaces.

The fruit's sticky, glutinous feel when chewed is reflected in common colloquial names like tree hibiscus, snot apple, or African chewing gum. It is widely used as a food and nutritional resource in traditional diets throughout its range (Xu *et al*., 2024). Due to its purported therapeutic qualities, it is widely used in traditional medicine in numerous regions of Sub-Saharan Africa. The plant is particularly well-known in West and Central Africa's savannah and semi-arid regions, particularly in Nigeria's Gombe and Adamawa States, where it has long been used medicinally (Mbuya *et al*., 1994; Mulofwa, 1994). The fruit of *Azanza garckeana* is consumed in these areas for its possible health advantages in addition to its sweet, sticky flavour. The fruit, which is frequently chewed like gum, is thought to have aphrodisiac properties and has long been used as a general tonic and to treat a number of illnesses, such as infertility, liver diseases and malaria (Storrs, 1979; Taylor & Kwerepe, 1995). Fruit pulp of *Azanza garckeana's* strong phytochemical composition, which includes flavonoids, saponins, terpenoids, tannins, and other bioactive components, have drawn other interests in addition to its applications in traditional medicine (Nkafamiya *et al*., 2016). These phytoconstituents are believed to play a part in the plant's many medicinal benefits, especially its ability to control blood sugar, lower inflammation, and fight oxidative stress. However, there are few scientific reports on the medicinal benefits despite its extensive use.

Diabetes mellitus is a chronic metabolic disorder characterized by persistently high blood glucose levels. Cardiovascular diseases, kidney failure, neuropathy, and retinopathy are amongst the complications of diabetes mellitus (Georg & Ludvik, 2000; Murray *et al*., 2014). Diabetes mellitus is mainly treated with synthetic antidiabetic drugs like insulin and metformin, which are effective but frequently have unfavourable side effects such as hypoglycemia, weight gain, and gastrointestinal problems (Hostalek *et al*., 2015). Medicinal plants are thought to be safer and more readily available, thus; there has been an increase in interest in plant-based therapy. In Africa, where traditional medicine plays a significant role in healthcare, the use of medicinal plants in the treatment of diabetes is not only deeply rooted in cultural practices but also supported by numerous studies highlighting their efficacy in regulating blood glucose levels (Mohammed *et al*., 2014). Plants such as *Moringa oleifera* and *Glycyrrhiza glabra* have been extensively studied for their antidiabetic properties, contributing to the development of herbal medicines for diabetes management (Mbikay, 2012). Given the increasing prevalence of diabetes in Nigeria and other parts of Sub-Saharan Africa, where access to synthetic drugs may be limited or unaffordable, there is need to explore safe and effective; natural alternatives (Akinmoladun *et al*., 2019).

Azanza garckeana, fruit pulp with its documented medicinal properties and traditional use in the management of various ailments, presents a viable option for such an investigation. Preliminary reports on the phytochemical profile of *Azanza garckeana* fruit pulp extract include compounds with the potential to modulate blood glucose levels. Therefore, there is need to scientifically investigate the hypoglycaemic effects of *Azanza garckeana* fruit pulp extract as potential alternative for management of diabetes mellitus.

MATERIALS AND METHODS

PLANT MATERIAL

The *Azanza garckeana* fruits were obtained from Tula in the Kaltungo Local Government Area of Gombe State and were identified and verified by a plant taxonomist at the University of Maiduguri, Nigeria.

PREPARATION OF PLANT EXTRACT

The fleshy portion (pulp) of the *Azanza garckeana* fruit was separated from the seeds and air-dried at room temperature. The dried pulp was then pulverized into a fine powder, yielding 156 g of powdered material. The powdered fruit pulp was soaked in distilled water and stirred thoroughly to obtain a uniform mixture using the decoction method. The mixture was shaken vigorously at 2-hour intervals for 24 hours to facilitate the extraction of the bioactive constituents. Thereafter, the mixture was filtered, and the filtrate was concentrated to dryness using a rotary evaporator. A total of 24.7 g of a brownish, semi-solid crude extract was obtained, corresponding to a percentage yield of 15.8% (w/w). The extract was stored in a refrigerator at 4°C until further use.

QUALITATIVE PHYTOCHEMICAL SCREENING OF THE EXTRACT

The qualitative phytochemical evaluation was done to identify the phytochemical components of the extract using standard test methods. This includes; tannins, saponin glycoside, flavonoids, carbohydrates, cardiac glycosides, cordenolids, terpenoids and free anthraquinone (Brain & Turner, 1975; Trease & Evans, 2002; Yana & Tsani 2021).

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

The phytochemicals were quantified using standard techniques. (Evans, 2009; AOACI, 2019). Alkaloids: Measured using the Harborne Method or colorimetric method by reacting with Dragendorff's reagent and measuring absorbance at a specific wavelength (420 nm). Flavonoids: Quantified using the aluminium chloride method and measuring the absorbance at 415 nm. Cardiac Glycosides: Measured using the Keller-Kiliani test, which involves reaction with a reagent and measurement of absorbance at 520 nm.

Cordenolid: Was quantified using HPLC or UV-Vis spectroscopy depending on the specific method developed

for the compound. Carbohydrates: Measured using the Anthrone method, where absorbance is measured at 620 nm. Terpenoids: Quantified using UV-Vis spectroscopy or Gas Chromatography-Mass Spectrometry (GC-MS).Tannins: Determined using the Folin-Ciocalteu method, where absorbance is measured at 765 nm. Saponin Glycosides: Measured using foam index determination or gravimetric method. Free anthraquinones: Quantified using UV-Vis spectrophotometry at specific wavelengths (480 nm). (Harborne, 1998; Obadoni & Ochuko, 2001; Adams, 2017).

ACUTE TOXICITY TEST

The acute toxicity screening of the *Azanza garckeana* fruit pulp was determined using the up-and-down method (Bruce, 1985), with three (3) rats assigned into three (3) different groups of one (1) rat each. Graded doses of the extract, including 1500 mg/kg, 3200mg/kg and 5000 mg/kg were administered to all groups and monitored for a 24 hours period each for any behavioural changes, toxicity signs and mortality. The geometric mean of the lowest toxic dose and highest tolerated dose was used to determine the oral median lethal dose (LD50). All experiments on animals were carried out according to the biomedical principles involving animals (CIOMS & ICLAS, 2012).

DETERMINATION OF BLOOD GLUCOSE LEVEL

Twenty-five (25) Wistar albino rats of both sexes, weighing between 90 and 280 g, were used for the study. The animals were acclimatized under standard laboratory conditions and were provided Vital® commercial rat feed and clean drinking water ad libitum throughout the experimental period. They were randomly assigned into five groups of five rats each. Group A served as the control and received distilled water together with feed only, while Groups B, C, D, and E were administered the aqueous fruit pulp extract of *Azanza garckeana* orally at doses of 200, 400, 600, and 800 mg/kg body weight, respectively. Blood glucose levels were determined using a OneTouch Basic glucometer (ACCU-CHEK®). A drop of blood was obtained from the tail vein of each rat and applied to the test strip for immediate glucose measurement, with results recorded in mg/dL. Baseline blood glucose concentration was measured before extract administration (0 h), after which subsequent measurements were taken at 1, 6, 12, 18, and 24 hours post-treatment. All readings were recorded and used to assess the effect of the extract on blood glucose levels over a 24-hour period.

STATISTICAL ANALYSIS

All data was subjected to one way ANOVA followed by Duncan's multiple post hoc tests using SPSS version 26. The $p<0.05$ was considered significant. All values are expressed as mean ±Standard deviation (SD).

RESULTS

The dried fruit of *Azanza garckeana* was shown in Figure I. The presence (+) or absence (-) of various bioactive compounds in the methanol extract of *Azanza garckeana* fruit pulp are shown on Table I. Alkaloids was absent, while flavonoids, cardiac glycosides, cordenolid, carbohydrates, terpenoids, tannins, saponin glycosides, and free anthraquinones. Figure II shows the various phytochemicals in varied concentrations. Table II shows the blood glucose levels (mean ± SD) of the different treatment groups at 0, 1, 6, 12, 18, and 24 hours after administration of *Azanza garckeana* fruit pulp extract. Significant reductions in blood glucose levels were observed in all treated groups, with the most pronounced effects in Groups 4 (600 mg/kg) and 5 (800 mg/kg) over the 24-hour period. Table III represents the clinical signs and mortality profile following the administration of varying doses of *Azanza garckeana* aqueous extract (1500 mg/kg, 3200 mg/kg, and 5000 mg/kg) in rats. Clinical signs such as weakness, dizziness, anorexia, droopy eyelids, and whistling movement were observed, but no mortality was recorded in any of the groups (0/1). The LD50 was calculated using the formula $LD50 = \sqrt{a} \times b$, where "a" and "b" represent the dose and the corresponding mortality data, respectively.

QUALITATIVE PHYTOCHEMICAL CONSTITUENTS OF *AZANZA GARCKEANA* FRUIT PULP EXTRACT

Table I shows the qualitative phytochemical constituents of *Azanza garckeana* fruit pulp extract. The analysis revealed the presence of flavonoids, cardiac glycosides, cardenolides, carbohydrates, terpenoids, tannins, and saponin glycosides, whereas alkaloids and free anthraquinones were not detected. The presence of these phytochemicals suggests that the fruit pulp is a rich source of bioactive compounds with potential pharmacological properties. In particular, flavonoids, terpenoids, tannins, and saponin glycosides have been associated with antioxidant and antidiabetic activities through mechanisms involving free radical scavenging, enhancement of insulin sensitivity, and modulation of glucose metabolism. The absence of alkaloids and free anthraquinones indicates that these classes of secondary metabolites are either absent or present below detectable levels in the extract. Overall, the phytochemical profile observed in Table I provides a plausible explanation for the biological activities of *Azanza garckeana* fruit pulp reported in this study.

Table I. Qualitative Phytochemical Constituents of Extract of *Azanza garckeana* Fruit Pulp.

Constituents	Result
Alkaloid	-
Flavonoid	+
Cardiacglycoside	+
Cordenolid	+
Carbohydrate	+
Terpenoid	+
Tannin	+
Saponin glycoside	+
Free anthraquinone	-

Keys: - Negative + Positive

QUANTITATIVE PHYTOCHEMICAL COMPOSITION OF *AZANZA GARCKEANA* FRUIT PULP EXTRACT

Figure II presents the quantitative phytochemical composition of *Azanza garckeana* fruit pulp extract. The results showed that carbohydrates were the most abundant constituent (6.7 mg/g), followed by terpenoids (5.0 mg/g), flavonoids (4.5 mg/g), tannins (3.6 mg/g), saponin glycosides (2.9 mg/g), cardenolides (2.3 mg/g), and cardiac glycosides (1.8 mg/g). Alkaloids and free anthraquinones were not detected in the extract.

Figure I: *Azanza garckeana* fruit

The relatively high concentrations of flavonoids, terpenoids, tannins, and saponin glycosides suggest that these phytochemicals may contribute substantially to the biological activities of the fruit pulp, including its antioxidant and antidiabetic properties. Furthermore, the absence of alkaloids and free anthraquinones may indicate a reduced likelihood of toxicity associated with these classes of secondary metabolites. Overall, the quantitative phytochemical profile supports the potential of *Azanza garckeana* fruit pulp as a valuable source of bioactive compounds with therapeutic relevance.

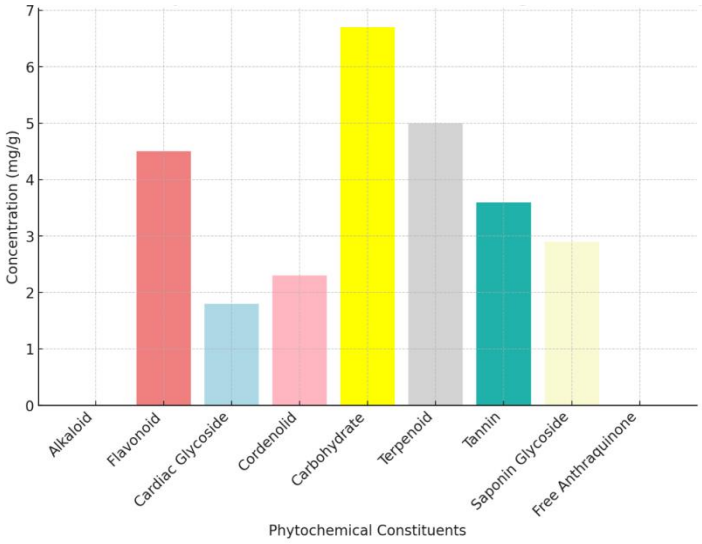

Figure II: Quantitative Phytochemical Constituents of Extract of *Azanza garckeana* Fruit Pulp

THE HYPOGLYCEMIC EFFECTS OF *AZANZA GARCKEANA* EXTRACT AT DIFFERENT DOSES

Table II shows the hypoglycemic effects of *Azanza garckeana* extract at different doses over a 24-hour period. The control group maintained relatively stable blood glucose levels throughout the study, with no significant changes ($p > 0.05$). In contrast, all treatment groups exhibited reductions in blood glucose concentration over time. The 200 mg/kg dose reduced glucose levels from 6.50 ± 0.33 mmol/L at 0 hour to 4.82 ± 0.18 mmol/L at 24 hours. The 400 mg/kg dose produced one of the greatest reductions, decreasing from 5.97 ± 0.80 mmol/L to 4.10 ± 0.82 mmol/L. Similarly, the 600 mg/kg and 800 mg/kg doses lowered blood glucose levels to 4.34 ± 0.98 mmol/L and 4.99 ± 0.74 mmol/L, respectively, at 24 hours. Overall, the extract demonstrated significant hypoglycemic activity, with the most pronounced effects observed at 400 mg/kg and 600 mg/kg doses.

TABLE II: THE HYPOGLYCEMIC EFFECTS OF *AZANZA GARCKEANA*

GROUP	0 HOUR	1 HOUR	6 HOURS	12 HOURS	18 HOURS	24 HOURS
GROUP1 (control)	6.7599±0.6198*	7.3704±0.5472*	6.9375±0.6831*	6.10492±1.007*	6.17156±0.8265*	6.1716±1.194*
GROUP 2 (200mg)	6.5046±0.3269**	6.6822±0.866**	6.5157±2.431**	4.6398±0.4735**	4.4054±0.3601***	4.8174±0.1787**
GROUP 3 (400mg)	5.9718±0.7972**	5.9385±0.8535**	5.4723±0.7432**	4.1736±1.259***	3.996±0.7600**	4.0959±0.8179***
GROUP 4 (600mg)	6.7044±0.5996***	6.5268±0.7125***	6.1716±0.6173***	4.9617±0.9769***	4.7508±0.6550***	4.3401±0.9806***
GROUP 5 (800mg)	7.22±0.3075***	6.7266±0.4499***	6.1826±0.3178***	6.1826±0.3178***	6.6204±0.4010***	4.9858±0.7430***

Key: superscript *(p>0.05) not significant,(p<0.05) very significant, ***(p<0.05) extremely significant**

ACUTE TOXICITY STUDY OF THE AQUEOUS EXTRACT OF *AZANZA GARCKEANA* FRUIT PULP

The acute toxicity study of the aqueous extract of *Azanza garckeana* fruit pulp in table III revealed no mortality in any of the experimental groups throughout the observation period. Animals administered 1500 mg/kg exhibited mild clinical signs such as weakness and dizziness. At 3200 mg/kg, the animals showed weakness, reduced feed intake, dizziness, and droopy eyelids. Similarly, animals treated with 5000 mg/kg displayed weakness, off-feed behaviour, dizziness, droopy eyelids, and whistling movement. The severity of the observed clinical signs increased with increasing dose of the extract, indicating a dose-dependent response. Despite the appearance of these transient toxic signs, no deaths were recorded at any of the administered doses (0/1 mortality in all groups). The absence of mortality at the highest tested dose of 5000 mg/kg body weight suggests that the aqueous extract possesses a wide margin of safety under acute exposure conditions. Consequently, the median lethal dose (LD_{50}) of the extract was considered to be greater than 5000 mg/kg body weight. These findings indicate that the aqueous extract of *Azanza garckeana* fruit pulp is relatively non-toxic when administered orally at the tested doses.

Table III: Clinical signs and mortality profile of aqueous Extract of *Azanza garckeana* fruit pulp

GROUP	DOSE	CLINICAL SIGN	MORTALITY
1	1500	Weakness, dizziness	0/1
2	3200	Weakness, off feed, dizziness, droopy eyelid	0/1
3	5000	Weakness, off feed, dizziness, droopy eyelid, whistling movement	0/1

LD_{50} > 5000 mg/kg body weight

DISCUSSION

Several bioactive compounds which include; flavonoids, terpenoids, saponin glycosides, tannins, cardiac glycosides, and free anthraquinones, were present in varying concentrations in the extract of *Azanza garckeana* fruit pulp which supports its traditional use in treating a variety of ailments. Flavonoids, the most abundant bioactive compounds in the fruit pulp, are recognized for their antioxidant, anti-inflammatory, and antidiabetic effects. (Mbuya *et al.*, 1994). Terpenoids also contribute significantly to the fruit's medicinal properties, demonstrating broad biological activities, including antimicrobial, anti-inflammatory, and anticancer effects (Wang, 2020; Zhang *et al*., 2021). Cardiac glycosides were found to improve heart function by increasing the force of contraction of the heart muscle. (Maroyi, A. 2017; Xu *et al*., 2024). However, their toxicity at high doses requires caution in their therapeutic use (Ohiagu *et al*., 2021). The carbohydrate content of *Azanza garckeana* fruit pulp is notable for its role in energy production and its potential as a precursor for other bioactive compounds (Hayes & Tiwari, 2015; Tiwari & Cummins, 2019). Saponin glycosides demonstrated a significant role in managing metabolic disorders such as diabetes by lowering cholesterol and regulating blood sugar levels. The moderate concentrations of saponins in the fruit pulp suggest its potential as a natural remedy for managing conditions like hyperlipidemia and diabetes (Zhang *et al*., 2024). The hypoglycemic effects of *Azanza garckeana* were evaluated by administering varying doses of the aqueous extract to different groups of rats. The control exhibited relatively stable blood glucose levels throughout the 24-hour period, with only minor fluctuations. This is consistent with previous studies showing minimal changes in glucose levels in untreated control groups (Huang *et al*., 2024).

The 200 mg/kg, showed a marked reduction in blood glucose level starting at 6 hours, with a significant decrease observed at 24 hours post treatment ($p < 0.05$), and 400 mg/kg demonstrated even more pronounced hypoglycemic effects, with significant reductions in blood glucose observed at 6 hours post treatment ($p < 0.05$) and the most significant decreases at 12 and 24 hours post treatment ($p < 0.01$). Studies have shown that higher concentrations of bioactive compounds such as flavonoids, saponins, and terpenoids, which are present in *Azanza garckeana*, have a stronger impact on blood glucose regulation and insulin sensitivity (Dawud *et al*., 2023). Also, the 600 mg/kg dosage showed a consistent decrease in blood glucose at all-time points, with the most significant reduction at 12 and 24 hours post treatment ($p < 0.01$ and $p < 0.001$). The dose-dependent nature of the extract's effect supports previous findings on the role of plant-based extracts in regulating glucose metabolism (Saadeldeen *et al.,* 2020). At the dosage of 800 mg/kg, blood glucose levels were significantly reduced at 1 hour post treatment ($p < 0.05$), with a consistent reduction seen up to 24 hours post treatment ($p < 0.01$). The rats in group 5 (800 mg/kg) showed the most significant reduction in blood glucose at 24 hours post treatment. This suggests a plateau effect, implying that higher doses of *Azanza garckeana* may offer the most substantial benefit, but further optimization of the dose may be needed to achieve a maximum, sustained effect. This aligns with studies indicating that while higher doses show the strongest effects, careful dose optimization is essential for long-term efficacy (Saadeldeen *et al.,* 2020; Faloye *et al*., 2024). The reduction in blood glucose across all treatment groups can be attributed to the presence of several bioactive compounds, particularly flavonoids, saponin glycosides, and terpenoids, which have been shown to improve insulin sensitivity and inhibit glucose absorption (Singh *et al*., 2022). These compounds may act synergistically to enhance glucose metabolism and reduce oxidative stress, which plays a crucial role in the pathophysiology of diabetes (Singh *et al*., 2022). The LD_{50} of *Azanza garckeana* aqueous extract was calculated based on clinical observations following the administration of different doses (1500 mg/kg, 3200 mg/kg, and 5000 mg/kg) in rats. The rats in these groups exhibited clinical signs of weakness, dizziness, and off-feed behaviour but showed no mortality (0/1 mortality rate). These signs, though indicative of some level of toxicity, were not lethal within the observed time frame. The absence of fatalities at these doses suggests that the extract is relatively safe, even at higher doses, and may be suitable for therapeutic use. These findings are consistent with other plant-based extracts, where the determination of LD_{50} serves as an important step in assessing the safety and therapeutic potential of natural products (Xu *et al*., 2024). The lack of mortality at the tested doses suggests that *Azanza garckeana* has a favourable safety profile. However, clinical application should be approached with caution, as the observed signs of toxicity, such as weakness and dizziness, point to the need for careful dosage and monitoring, particularly for long-term use (Dawud *et al*., 2023).

CONCLUSION

In conclusion, the observed hypoglycaemic effect of *Azanza garckeana* fruit pulp extract indicates a promising natural alternative for the management of diabetes mellitus.

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CONFLICT OF INTEREST

The authors declare no conflict of interest associated with the study.

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