

Evaluation of Antidiabetic Activity of *Croton zambesicus* Root Extract: In Vivo Inhibitory Effect on Alpha Amylase and Alpha Glucosidase of Rats

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Abstract

Diabetes mellitus is a global health challenge, necessitating the need for alternative treatments. *Croton zambesicus* Muell Arg. (Euphorbiaceae) a medicinal plant used traditionally in the treatment of some ailments was investigated for its inhibitory potential on alpha-amylase and alpha-glucosidase enzymes in rats. The root extract of *C. zambesicus* (27 - 81 mg/kg) was investigated *in vivo* for the inhibitory effect on alpha amylase and alpha glucosidase enzymes using starch, sucrose, and maltose as substrates. Acarbose was used as a reference drug. Blood glucose levels (BGL) of rats, post administration of the substrate and extract concurrently, were monitored over 3 hours as a parameter to measure the inhibitory potential of the extract. The root extract dose-dependently caused significant ($p < 0.05$) reduction in blood glucose levels of treated rats with the various substrates used. The results suggest that the root extract of *Croton zambesicus* has the potentials to inhibit alpha amylase and alpha glucosidase in rats.

Keywords: Croton zambesicus; Anti-diabetic; Enzyme inhibition; Alpha-amylase; Alpha-glucosidase.

INTRODUCTION

Diabetes mellitus (DM) is one of the major health challenges facing most countries of the world with associated economic and social consequences. The disease has been ranked as the 8th deadly disease causing millions of deaths world over (WHO, 2016). The number of people affected by diabetes is projected to increase to 700 million by 2045 (Saeedi et al., 2019), necessitating urgent steps to remedy this situation. The reliance of the major management approach on the use of conventional medicines has not yielded much positive results due to economic reasons and the associated side effects. Therefore, the search for affordable and safe alternative drugs is inevitable.

Worldwide, herbal preparations have gained significant patronage for DM management. We had previously reported the efficacy of a number of plants extracts indigenous to Nigeria on diabetes such as *Mammea africana* (Okokon et al., 2007), *Hippocratea africana* (Okokon et al., 2010), *Anthocleista djalensis* (Okokon et al., 2012), *Solenostemon monostachyus* (Okokon et al., 2015), cornhusk of *Zea mays* (Okokon & Mandu, 2017), *Setaria megaphylla* (Okokon et al., 2022a) and *Solanum anomalum* (Okokon et al., 2022b). The mechanism of antidiabetic action of only a few of

them concerning their effects on alpha amylase and alpha glucosidase activities has been investigated.

Croton zambesicus Muell Arg. (Euphorbiaceae) (syn *C. amabilis* Muell. Arg. *C. gratissimus* Burch) is a Guineo-Congolese species found in tropical Africa often grown as an ornamental tree in villages and towns in Nigeria. Traditionally, the leaf and root decoctions are used as anti-hypertensive and anti- microbial (urinary infections) (Adjanohoun et al., 1989). The roots are used as antimalarial, febrifuge, laxative and antidiabetic by the Ibibios of Niger Delta region of Nigeria (Okokon & Nwafor, 2009a). Boyom et al. (2002) reported that the essential oils from the root bark of *Croton zambesicus* contain majorly sesquiterpenes. The root and stem bark oils were also found to be rich in oxygen containing compounds, with spathulenol and linalool as major components. Okokon and Nwafor (2009a) reported that the root extract contains alkaloids, saponins, terpenes, tannins, phlobatannins, anthraquinones and cardiac glycosides. The root extract has been reported to possess antimalarial (Okokon & Nwafor, 2009a), anticonvulsant and antiulcer (Okokon & Nwafor, 2009b), anti-inflammatory, analgesic and antipyretic (Okokon & Nwafor, 2010), antidiabetic and hypolipidemic (Okokon et al., 2011a), laxative (Okokon et al., 2020) activities. Moreso, the kidney-protective potential against

gentimicin-induced kidney injury (Okokon et al., 2011b), immunostimulatory, cytotoxicity against HeLa cell line and antileishmanial activities (Okokon et al., 2013) of the root extract of the *Croton zambesicus* has been reported. We report in this study the inhibitory effect of the root extract on the alpha amylase and alpha glucosidase enzymes of rats.

MATERIALS AND METHODS

Plants Collection

The plant material *Croton zambesicus* (roots) was collected from compounds in the Uruan area, Akwa Ibom State, Nigeria in August 2024. Dr. Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria, identified and authenticated the plant.

Extraction

The roots were washed clean and shade-dried for two weeks. The dried plants' materials were further chopped into small pieces and reduced to powder. The powdered material was macerated in 50% ethanol for 72 h. The liquid ethanol extract obtained by filtration was evaporated to dryness in a rotary evaporator at 40°C. The extract was stored in a refrigerator at 4°C until used for the experiment reported in this study.

Animals

Albino Wistar rats (125 -142g) of either sex were used for these experiments. The animals were housed in standard cages and maintained on a standard pelleted feed (Guinea feed) and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

In vivo Alpha-amylase and Glucosidase Inhibition Study

Alpha-Amylase Inhibitory Study

Thirty Wistar rats were divided into 6 groups of 5 rats each. The rats in all groups were fasted for 18 hour and fasting blood glucose concentration was first taken at 0 min before administration. Group I, as the normal control, received distilled water (10 mL/kg). Group II rats were orally administered starch at 2 g/kg body weight (orally with distilled water as vehicle) and distilled water (10 mL/kg) simultaneously. Rats in group III were administered starch (2 g/kg) and the standard drug (acarbose) at 100 mg/kg simultaneously. Based on previously determined LD₅₀ and doses (Okokon et al., 2009a), Groups IV, V, and VI were administered with starch (2 g/kg) and *C. zambesicus* root extract at 27, 54, and 81 mg/kg, respectively. All administrations were done orally and blood glucose concentration was monitored at 30, 60, 90, 120 and 180 min (Gidado et al., 2019; Okokon et al., 2023a).

Glucosidase Inhibitory Study

The procedure as described above was used for this study but with sucrose and maltose used as substrates (Okokon et al., 2023a; Okokon et al., 2023b).

Blood Glucose Determination

Drops of blood from the tip of rats' tails were dropped on stripes and glucose concentration was measured using a glucometer according to the manufacturer's specifications (Accu-chek, Indiana). The glucometer works using an electrochemical detection system with the following principle; the biosensor system makes use of disposable dry reagent strip based on glucose oxidase method. Each strip has an electrode impregnated with the enzyme glucose oxidase, which reacts with glucose in the blood sample when dropped on the membrane covering the reagent pad (strip), to produce gluconic acid. During the reaction, in which an electric current is generated, an electrochemical mediator transfers electrons to the electrode surface. This electrode sensor measures the current produced when the enzyme converts glucose to gluconic acid. The magnitude of the generated current is proportional to the amount of glucose present in the drop of blood sample, thus giving an accurate reading of the blood glucose concentration (WHO, 2011).

Statistical Analysis

Data obtained from this work were analysed statistically using one -way ANOVA followed by Tukey-Kramer multiple comparison test using Instat Graphpad software, (San Diego, USA). Differences between means were considered significant at 5% level of significance ie $p \leq 0.05$.

RESULTS AND DISCUSSION

In Vivo Alpha Amylase and Glucosidase Inhibition Assay

Treatment of fasted rats with starch (2 g/kg) elevated blood glucose concentrations of the treated animals after 30 min in various proportions. The proportions were as follows: starch (62.62%), extract-treated groups (ranging from 10.95% to 17.77%), and acarbose-treated groups (17.97%). After 60 minutes, the blood glucose concentrations were lowered with the groups administered with dosages of the extract (27 - 81 mg/kg) having percentage increases ranging from 0.20 to 0.91%. The average blood glucose concentrations of all the groups treated with the extract were lowered to normal levels after 120 minutes except that of the lowest dose (27 mg/kg) with BGL of 0.13%. The BGL of all the extract treated groups was reduced to normal (Table 1).

Sucrose (2 g/kg) administration to fasted rats caused a 41.14% increase in blood glucose level 30 minutes post-administration of the sucrose to the control group. BGL increments of 12.57-31.93 % were also recorded in

groups treated with 27,54 and 81 mg/kg of extract. At 60 min, percentage increases in BGL of groups treated with 27, 54, and 81 mg/kg of extract were 13.85, 0.80, and 0 %, respectively. No increment in BGL was recorded in the extract-treated groups from 120 -180 min (Table 2). There was a 90.94% increase in blood glucose level 30 min following maltose administration in the control group. However, 11.93 - 39.75 % increases in BGL were

observed in the extract-treated groups. At 60 min, groups treated with 27,54 and 81 mg/kg extract had percentage increments of 17.86, 13.23, and 0%, respectively, while percentage increases of 0.41 and 0.18% were recorded for 27 and 54 mg/kg treated groups at 120 min respectively. At 180 min, no increment in BGL was recorded in any of the extract treated groups (Table 3).

Table 1. Effect of ethanol root extract of *Croton zambesicus* on blood glucose level of rat after oral administration of starch load.

Treatment	Dose mg/kg	Blood Glucose Level mg/dL In Min				
		0 min	30 min	60 min	120 min	180 min
Control (normal saline)	-	86.00±11.53	87.66±7.12(1.93)	87.66±7.62(1.93)	91.0±7.50(5.81)	80.00±6.02
Starch		66.0±3.60	107.33±6.36 ^a (62.62)	91.66±2.02(38.87)	77.66±3.71(17.66)	70.66±2.72(6.59)
Acarbose	100	72.33±2.69	85.33±12.97(17.97)	80.33±7.21(11.06)	74.0±1.00(2.30)	72.33±8.68(0)
Extract	27	78.20±2.20	92.10±5.12(17.77)	85.36±5.39 ^a (0.91)	79.23±3.27 ^a (0.13)	76.38±1.24 ^a (0)
	54	76.56±3.56	85.52±3.28(11.70)	82.61±3.55(0.79)	74.10±5.45 ^b (0)	75.32±2.32 ^a (0)
	81	78.60±4.63	87.21±5.84(10.95)	80.20±4.56(0.20)	72.45±5.56 ^a (0)	72.66±3.16(0)

Data are expressed as MEAN ± SEM, Significant at ^ap<0.05, ^bp< 0.01, compared to control (n=6).

Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Table 2. Effect of ethanol root extract of *Croton zambesicus* on blood glucose level of rat after oral administration of sucrose load.

Treatment	Dose mg/kg	Blood Glucose Level mg/dL in Min				
		0 min	30 min	60 min	120 min	180 min
Control (normal saline)		100.00±4.25	88.33±1.85	92.33±4.25	89.0±4.35	87.33±3.84
Sucrose	2000	81.0±4.50	114.33±5.50 ^b (41.14)	112.66±1.45 ^a (39.08)	97.33±1.63(20.16)	94.15±4.81(16.23)
Acarbose	100	90.33±2.48	86.66±2.90	82.0±6.00	71.66±3.75	78.0±3.78
Extract	27	79.66±4.32	105.10±5.29 ^c (31.93)	90.22±4.33(13.25)	75.12±6.88(0)	72.44±4.12
	54	75.34±2.55	91.23±4.18 ^b (21.09)	81.42±4.56(0.80)	74.10±3.48(0)	69.44±4.33(0)
	81	77.20±5.29	86.91±2.34(12.57)	75.46±3.34(0)	69.84±2.26(0)	67.55±4.75(0)

Data are expressed as MEAN ± SEM. Significant at ^ap<0.05, ^bp< 0.01, compared to control (n=6). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Table 3. Effect of ethanol root extract of *Croton zambesicus* on blood glucose level of rat after oral administration of maltose load.

Treatment	Dose mg/kg	Blood Glucose Level mg/dL in Min				
		0 min	30 min	60 min	120 min	180 min
Normal Control	-	100.00±4.25	88.33±1.85	92.33±4.25(1.80)	89.0±4.35(1.55)	87.33±3.84(3.98)
Maltose	2000	70.00±11.67	133.66±15.44 ^c (90.94)	128.66±8.78 ^a (83.80)	99.36±5.36(41.94)	84.0±7.21(20.0)
Acarbose	100	85.34±1.36	88.22±1.10(3.37)	86.0±2.20(0.77)	84.26±1.14 ^a (0)	82.28±2.26(0)
Extract	27	77.49±6.39	108.30±4.33 ^a (39.75)	91.33±3.68 ^a (17.86)	80.74±4.36 ^a (0.41)	72.58±5.63 ^a (0)
	54	75.46±5.52	94.05±6.64 ^b (24.63)	85.45±2.44 ^a (13.23)	76.85±5.29 ^a (0.18)	72.56±8.34(0)
	81	76.33±3.68	85.44±6.32 ^b (11.93)	75.66±3.56 ^b (0)	73.23±6.18 ^b (0)	70.91±6.36 ^a (0)

Data are expressed as MEAN ± SEM, Significant at ^ap<0.05, ^bp< 0.01, compared to control. (n=6).

Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Discussion

Croton zambesicus parts are used in Ibibio traditional medicine in the treatment of diseases such as diabetes among others. This work investigated the effect of *Croton zambesicus* root extract on alpha amylase and alpha glucosidase activities in rats. The extract was found to inhibit increases in blood glucose concentration following starch administration. α -amylases and α -

glucosidase enzymes act in synergy to digest dietary polysaccharides such as starch. The polysaccharides are reduced to disaccharides by the α -amylase enzyme by breaking the α -bonds of the α -linked polysaccharides. Thereby resulting in disaccharides like maltose, which are also digested by membrane bound α -glucosidase enzymes to monosaccharides (Kalra, 2014; Alongi & Anese, 2018). Inhibitions of these enzymes activities

suppresses the ingested carbohydrates digestion, with associated insignificant elevation in blood glucose concentrations following carbohydrate meals as was observed in this study. As a target for managing Type 2 diabetes mellitus, some medicinal plants have been investigated for α -amylase and α -glucosidase inhibitory potentials. Previously, we had reported on the α -amylase and α -glucosidase inhibitory potentials of *Heinsia crinata*, *Lasianthera africana*, *Setaria megaphylla*, and *Solanum anomalum* (Okokon et al., 2021; Eweh et al., 2022; Etuk et al., 2023) among others.

Similarly, the root extract significantly inhibited blood glucose rise when co-administered with maltose and sucrose. Acarbose, the standard drug used in this study significantly suppresses blood glucose rise when co-administered with starch, maltose and sucrose. The results of this study corroborate the reported activities of the leaf extract and fractions of *C. zambesicus* on alpha amylase and alpha glucosidase activities in rats (Okokon et al., 2022c). Furthermore, the results also corroborate the significant alpha amylase and alpha glucosidase inhibitory activities reported on other species of *Croton* such as *C. bonplandianum* (Qaisar et al., 2014; Karuppiyah et al., 2017), *C. thurifer* (Morocho et al., 2020), and *C. oblongifolius* (Srisongkram et al., 2022) as observed in this study. The inhibitory activities of these species have been linked to their phytochemical constituents especially polyphenols. The root oil from *Croton zambesicus* has been reported to contain majorly sesquiterpenes and is also found to be rich in oxygen containing terpenoid compounds such as spathulenol and linalool. These compounds especially sesquiterpenoids possess alpha glucosidase and alpha amylase inhibitory potentials (Lee et al., 2023; Tan et al., 2023).

The presence of these compounds in the extract could have contributed to the observed activity of this study and therefore explains the antidiabetic mechanism of the roots of *C. zambesicus*.

CONCLUSION

The findings of this research suggest that the root extract of *Croton zambesicus* may exhibit anti-diabetic effects by inhibiting the alpha-amylase and alpha-glucosidase enzymes. This activity may be linked to the presence of phytochemical ingredients in the plant.

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Competing Interests: The authors declare that there are no conflicts of interest concerning this manuscript.

Ethical Approval: Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo. All animal experiments complied with the National Institute of Health Guide for Care and Laboratory Animals (pub. No. 85-23, revised 1985).

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