Alpha Amylase And Alpha Glucosidase Inhibitory Activities Of *Croton zambesicus* Leaf Fractions In Wistar Rats

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Abstract

Croton zambesicus Muell Arg. (Euphorbiaceace) a medicinal plant used traditionally in the treatment of diseases including diabetes was evaluated for effect on alpha amylase and alpha glucosidase enzymes *in vivo*. The leaf fractions (hexane, dichloromethane, ethyl acetate, methanol, 150 mg/kg) of *C. zambesicus* were investigated *in vivo* for inhibitory effect on alpha amylase and alpha glucosidase enzymes using starch, sucrose and maltose as substrates. Acarbose was used as reference drug. The leaf fractions (n-hexane, DCM and ethyl acetate) caused significant ($p<0.05$) reduction in blood glucose levels of treated rats with the various substrates used. DCM fraction exerted the highest inhibitory effect when starch was used as substrate followed by *n*-hexane. *N-hexane* was the most active fraction followed by ethyl acetate when sucrose and maltose were used as substrates. The results suggest that the leaf fractions of *C. zambesicus* have the potentials to inhibit alpha amylase and glucosidase in rats.

***Keywords*:** *Croton zambesicus*; hypoglycaemia, alpha amylase, alpha glucosidase.

Introduction

Diabetes mellitus (DM) is a serious global health problem affecting some 537 million adults worldwide (International Diabetes Foundation, 2021). The WHO reported that people living with diabetes globally increased from 108 million in 1980 to 422 million in 2014, with overweight and obesity being major risk factors (WHO, 2016). Within the same period, diabetes cases in Africa had increased from 4 million to 25 million with worldwide

projected prevalence expected rise to 700 million by 2045 (Saaedi *et al.*, 2019). When functional, the beta pancreatic cells regulate glucose homeostasis. However, when this process is hindered or impaired, glucose-induced insulin production is down-regulated resulting in elevated blood glucose levels. In turn, sustained postprandial hyperglycaemia induces oxidative stress through the generation of reactive oxygen species (ROS) via several molecular pathways including the activation of protein kinase and elevation of cytosolic calcium ions (Yaribeygi *et al.*, 2020). The generated ROS is associated with destruction of microvascular tissues, and other micro-and macro-vascular complications, leading to hypertension, myocardial infarction, diabetes retinopathy, dyslipidaemia, and diabetes nephropathy (Hiyoshi *et al.*, 2019). Prevention of these conditions can be achieved by controlling blood glucose level (Ceriello, 2005), through lowering of carbohydrate absorption by inhibiting alpha amylase and alpha glucosidase, the enzymes that are directly involved in polysaccharides digestion (Proenca *et al.*, 2021). The conventional drugs especially acarbose, used for this purpose are associated with certain adverse effects resulting in gastrointestinal (GI) disturbances (i.e., bloating, abdominal pain, GI cramping, or diarrhea) (Gong *et al.*, 2020; Rosak and Mertes, 2012). Therefore, it is imperative to search for natural products that could inhibit alpha amylase and alpha glucosidase activities without causing unwholesome side effects on the gastrointestinal tract (Gong *et al.*, 2020; Proenca *et al.*, 2021).

Croton zambesicus Muell Arg. (Euphorbiaceace) (syn *C. amabilis* Muell. Arg. *C. gratissimus* Burch) is an ornamental tree grown in villages and towns in Nigeria. It is a Guineo–Congolese species widely spread in tropical Africa. Traditionally, the leaf decoction is used as anti-hypertensive, anti- microbial (urinary infections) (Adjanohoun *et al.*, 1989), antimalarial (Okokon *et al.*, 2005a) and antidiabetic (Okokon *et al.*, 2006). The roots are also used as antimalarial, febrifuge and antidiabetic by the Ibibios of Niger Delta region of Nigeria (Okokon and Nwafor, 2009). Boyom *et al.* (2002) reported that the essential oils from the leaves are rich in monoterpenes. Aderogba *et al.* (2011) further isolated quercetin-3-O-*p*-600 (p-coumaroyl) glucopy-ranoside-30-methyl ether, helichrysoside-30-methyl ether, along with kaempferol-3-O-*p*-600 (p-coumaroyl) glucopyranoside, tiliroside and apigenin-6-C-glucoside (isovitexin) as the antioxidant constituents from the leaf of the plant. The ethanol leaf extract has been reported to possess antiplasmodial (Okokon *et al.*, 2005a), anti-inflammatory, analgesic and antipyretic activities (Okokon *et al.*, 2005b). Preliminary report indicated that the leaf extract possesses antidiabetic activity (Okokon *et al.*, 2006). We report in this study the effect of leaf extract and fractions of the plant on alpha amylase and alpha glucosidase of exposed Wistar rats.

Materials and Methods

Plants Collection

The plant material *Croton zambesicus* (leaves) were collected in compounds in Uruan area, Akwa Ibom State, Nigeria in June 2021. The plant was identified and authenticated by Prof. Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Hebarium specimen DPNM.31(c) was deposited at the Department of Pharmacognosy and Natural Medicine Herbarium, Faculty of Pharmacy, University of Uyo, Nigeria.

Extraction

The leaves were washed and shade-dried for two weeks. The dried leaves were further chopped into small pieces and reduced to powder using electric grinder. The powdered leaves material (1.5 kg) was successively and gradiently macerated for 72 h in each of these solvents (2 x 5L), n-hexane, dichloromethane, ethyl-acetate and methanol to give corresponding fractions of these solvents. To ensure exhaustive extraction, the powdered plant material was extracted twice in equal volume of each of the solvents mentioned and rinsed with same solvents to ensure adequate removal of the soluble compounds. Thereafter, the material was air-dried for some time before another solvent was introduced. These solutions were filtered and the liquid filtrates were concentrated and evaporated to dryness in *vacuo* 40°C using a rotary evaporator (BuchiLab, Switzerland). The fractions of which ethyl acetate was more polar were stored in a refrigerator at -4°C, until used for the proposed experiments.

Animals

Albino Wistar rats (120 -135 g) of either sex were used for these experiments. The animals were housed in standard cages and were 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, Nigeria.

In vivo Alpha-Amylase and Glucosidase Inhibition Assays

Alpha-Amylase Inhibitory Study

Thirty-five Wistar rats were divided into 7 groups of 5 rats each. The rats in all groups were fasted for 18 h 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. Groups IV, V, VI and VII were administered simultaneously, starch (2 g/kg) and *C. zambesicus* leaf fractions (n-hexane, dichloromethane, ethyl acetate and methanol) at 150 mg/kg respectively.

All administrations were done orally and blood glucose levels (BGL) of the rats were monitored at 30, 60, 90, 120 and 180 min (Gidado *et al.*, 2019).

Glucosidase Inhibitory Study

Using another set of animals, the procedure described above for α-amylase inhibitory assay was exploited for this study but with sucrose and maltose used as substrates (Gidado *et al.*, 2019).

Blood Glucose Determination

Drops of blood from tip of rats' tails were dropped on stripes and glucose concentration was measured using a glucometer according to manufacturer's specifications (Accu-chek, Indiana). The glucometer works with the following principle; the blood sample is exposed to a membrane covering the reagent pad (strip), which is coated with an enzyme (glucose oxidase, glucose dehydrogenase). The reaction causes a colour change and the intensity of this change is directly proportional to the amount of glucose in the blood sample. Light from an LED strikes the pad surface and is reflected to a photodiode, which measures the light intensity and converts it to electrical signals. An electrode sensor measures the current produced when the enzyme converts glucose to gluconic acid. The resulting current is directly proportional to the amount of glucose in the sample (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% and 0.1% level of significance i.e. $p \leq 0.05$ and 0.001.

Results

In vivo Alpha-amylase and Glucosidase Inhibition Assay

Administration of starch (2g/kg) to fasted rats caused varying percentages of increase in blood glucose concentrations of the treated animals after 30 min. The percentage increase of blood glucose levels (BGL) for the control group (administered starch only) was 63.18%, while that of the acarbose-treated group was 17.9%. The fractions (n-hexane, dichloromethane, ethyl acetate and methanol)-treated groups had percentage range of 17.64 - 24.09%. These increases were reduced after 60 min with animals treated with the dichloromethane fraction (10.11%) and ethyl acetate fraction (15.60%) exerting the highest effects. These decreases were significant and sustained for 180 min in dichloromethane fraction-treated group (0%), followed by n-hexane fraction-treated group (5.54%). However, co-administration of the starch with acarbose prominently inhibited the

rise in the blood glucose concentrations (Table 1).

Administration of sucrose (2 g/kg) produced a 46.01% increase in blood glucose concentration 30 minutes post-administration of the sucrose in the control group and 25.05-48.15% increases in blood glucose concentration of fractions-treated groups. The blood glucose concentrations were significantly reduced in ethyl acetate fraction-treated group (20.58%) and hexane fraction-treated group (28.54%) after 60 min post-administration of sucrose. However, n-hexane fraction-treated group had the highest effect (0%) throughout the duration of the study (180 min) followed by ethyl acetate fraction-treated group (Table 2).

There was 60.78% increase in blood glucose concentration 30 min following maltose administration in the control group. However, 70.25-114.75% increases were observed in the fractions-treated groups. At 60 min, the methanol fraction, n-hexane and ethyl acetate fractions-treated groups had percentage increments in BGL of 52.30, 62.93 and 61.02% respectively. At 120 min, n-hexane fraction and ethyl acetate fraction-treated groups had BGL percent increases of 13.35% and 30.74% respectively. The percentage increases at 180 min were 5.17, 22.94 and 28.10% for n-hexane fraction, ethyl acetate-fraction and dichloromethane fraction-treated groups respectively (Table 3).

Table 1: Effect of ethanol leaf extract and fractions of *Croton zambesicus* on blood glucose level of rat after oral administration of starch load

TREATMENT	DOSE	BLOOD GLUCOSE LEVEL mg/dL IN MIN					
	mg/kg	0 min	30 min	60 min	90 min	120 min	180 min
Control normal saline	-	86.00±11.53	87.66±7.12(1.93)	87.66±7.62(1.93)	73.66±6.17	91.0±7.50(5.81)	80.00±6.02
Starch		73.33±8.25	119.66±5.45[a](63.18)	115.66±1.33[a] (57.72)	104.66±2.60[a] (42.72)	95.66±3.75[a] (30.45)	92.0±6.35(25.46)
Acarbose	100	72.33±2.69	85.33±12.97(17.97)	80.33±7.21(11.06)	76.33±3.48(5.53)	74.0±1.00(2.30)	72.33±8.68(0)
n -hexane fraction	150	83.0±1.15	100.6±9.61(21.20)	113.5±2.64(36.74)	105.6±2.18(27.22)	97.6±2.02(17.19)	87.6±3.09(5.54)
Dichloromethane fraction	150	89.0±3.78	109.0±2.33(24.09)	98.0±2.30(10.11)	100.6±2.64(13.03)	99.33±6.00(11.60)	83.0±4.35
Ethyl acetate fraction	150	81.6±4.25	96.0±7.63(17.64)	94.33±6.24(15.60)	92.6±3.75(13.48)	91.0±5.85(11.51)	88.33±4.66(8.24)
Methanol fraction	150	80.6±2.96	96.00±2.08(19.10)	105.0±7.50(30.27)	109.6±2.60(35.98)	112.3±1.76(39.33)	88.3±2.40(9.55)

Data is expressed as MEAN ± SEM, Significant at [a]$p<0.05$, [b]$p< 0.01$, when 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 leaf extract and fractions of *Croton zambesicus* on blood glucose level of rat after oral administration of sucrose load

TREATMENT	DOSE	BLOOD GLUCOSE LEVEL mg/dL IN MIN					
	mg/kg	0 min	30 min	60 min	90 min	120 min	180 min
Control normal saline	-	100.00±4.25	88.33±1.85	92.33±4.25	90.33±2.33	89.0±4.35	87.33±3.84
Sucrose	2000	92.0±4.04	134.33±2.90[b](46.01)	128.66±5.45[a] (39.84)	117.33±4.66[a](27.53)	97.66±0.66(6.15)	104.16±2.48(13.21)
Acarbose	100	90.33±2.48	86.66±2.90	82.0±6.00	79.33±2.96	71.66±3.75	78.0±3.78
n -hexane fraction	150	94.6±8.17	118.3±5.20(25.05)	121.6±5.36(28.54)	106.0±2.51(12.05)	89.0±7.02()	79.0±3.78()
Dichloromethane fraction	150	95.0±5.50	138.6±13.11(45.89)	127.0±6.65(33.68)	117.6±7.63(23.78)	100.0±7.00(5.26)	85.3±2.33()
Ethyl acetate fraction	150	94.6±2.00	126.3±5.89(33.50)	114.0±2.88(20.50)	107.3±3.38(13.42)	97.0±2.15(2.53)	79.6±3.38()
Methanol fraction	150	89.3±4.97	132.3±7.31(48.15)	113.8±4.66(27.43)	104.6±2.40(17.13)	95.3±1.45(6.71)	86.6±1.76()

Data is expressed as MEAN ± SEM. Significant at [a]$p<0.05$, [b]$p< 0.01$, when 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 leaf extract and fractions of *Croton zambesicus* on blood glucose level of rat after oral administration of maltose load

TREATMENT	DOSE	BLOOD GLUCOSE LEVEL mg/dL IN MIN					
	mg/kg	0 min	30 min	60 min	90 min	120 min	180 min
Control normal saline	-	100.00±4.25	88.33±1.85	92.33±4.25(1.80)	90.33±2.33(3.62)	89.0±4.35(1.55)	87.33±3.84(3.98)
Maltose	2000	82.30±2.14	132.33±1.90[b](60.78)	130.22±2.45(58.22)	120.66±3.22[a](46.60)	115.0±2.46(39.73)	106.22±4.24(29.06)
Acarbose	100	85.34±1.36	88.22±1.10(3.37)	86.0±2.20[c](0.77)	85.33±2.15[c]()	84.26±1.14[a]()	82.28±2.26[a]()
n -hexane fraction	150	77.33±6.36	131.66±3.93(70.25)	126.33±1.85[a](62.93)	108.0±7.21[a](39.66)	87.66±1.20[b] (13.35)	81.33±2.90(5.17)
Dichloromethane fraction	150	72.33±3.75	155.33±10.91[b](114.75)	127.66±7.21(76.49)	100.00±3.60(38.25)	98.33±2.90[a](35.94)	92.66±6.36(28.10)
Ethyl acetate fraction	150	72.66±1.20	142.66±6.17(96.33)	117.0±2.00 [b](61.02)	105.33±1.20[b] (44.96)	95.0±6.08 [b](30.74)	89.33±0.83[c](22.94)
Methanol fraction	150	72.0±3.51	132.0±9.07(83.33)	109.66±1.45[a](52.30)	105.0±6.02[a](45.83)	102.66±3.00 [b](42.58)	101.66±5.36[c](41.19)

Data is expressed as MEAN ± SEM, Significant at [a]$p<0.05$, [b]$p< 0.01$, when compared to control. (n=6). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Discussion

C. zambesicus parts are used in Ibibio traditional medicine in the treatment of diseases such as diabetes among others. This work investigated the effect of *C. zambesicus* leaf fractions on alpha amylase and alpha glucosidase activities in rats. The fractions were found to inhibit increases in blood glucose concentration following starch administration with the dichloromethane fraction followed by n-hexane fraction, exerting the most inhibition. Complete digestion of dietary polysaccharides like starch is achieved by the combined action of α-amylases and α-glucosidase enzymes. The α-amylase enzyme digests α-bonds of the α-linked polysaccharides yielding disaccharides, like maltose, which are further reduced to monosaccharides by membrane bound α-glucosidase enzymes (Kalra, 2014; Alongi and Anese, 2018). Inhibitions of these enzymes delay the digestion of ingested carbohydrates thereby resulting in a small rise in blood glucose concentrations following carbohydrate meals as was observed in this study. As a target for managing Type 2 diabetes mellitus, many medicinal plants have been reported to possess α-amylase and α-glucosidase inhibitory potential (Ibrahim *et al.*, 2014; Esimone *et al.*, 2001).

Similarly, the leaf fraction significantly inhibited blood glucose rise when co-administered with maltose and sucrose with n-hexane and ethyl acetate fractions exerting the highest inhibition. Acarbose, the standard drug used in this study significantly inhibited blood glucose rise when co-administered with starch, maltose and sucrose. The results of this study corroborate the activities reported on other species of *Croton* such as *C. bonplandianum* (Qaisar *et al.*, 2014; Karuppiah *et al.*, 2017), *C. thurifer* (Morocho *et al.*, 2020), and *C. oblongifolius* (Srisongkram *et al.*, 2022) which significant inhibition of alpha – amylase and alpha-glucosidase activities were observed. The inhibitory activities of these species were linked to their phytochemical constituents especially polyphenols. The leaf of *C. zambesicus* have been reported to be rich in flavonoids and other phenolic compounds such as quercetin-3-O-*p*-600 (p-coumaroyl) glucopy-ranoside- 30-methyl ether, helichrysoside-30-methyl ether, along with kaempferol-3-O-*p*-600 (p-coumaroyl) glucopyranoside, tiliroside, apigenin-6-C-glucoside and isovitexin (Boyom *et al.*, 2002; Aderogba *et al.*, 2011). These compounds have been variously reported to inhibit alpha glucosidase and alpha amylase activities (Proenca *et al.*, 2017; Su and Tang, 2019) More so, monoterpenes which are richly found in the leaves of this plant similarly have been reported to inhibit alpha amylase and alpha glucosidase (Oboh *et al.*, 2017). The presence of these compounds in the fractions could have contributed to the observed activity of this study and

therefore explains the antidiabetic mechanism of the leaves of *C. zambesicus*.

Alpha-amylase and α-glucosidase inhibitions by plants extracts have been reported severally (Ishnava and Metisariya, 2018; Shirwaikar *et al.,* 2005). Phytochemicals implicated as anti-diabetic agents, do so possibly through α-amylase and glucosidase inhibition. The phytochemicals implicated include; flavonoids, saponins, tannins and terpenoids (Ortiz-Andrade *et al.*, 2007; Ishnava and Metisariya, 2018; Yoshikawa *et al.*, 1998). Also, polyphenolic compounds from plants are known to cause several effects on the biological systems which include enzymes inhibitions (Kalita *et al.*, 2018; Funke and Melzig, 2005). The phenolic compounds are known to be strong metal ion chelators and protein precipitation agents forming insoluble complexes with proteins as well as acting as biological oxidants (Ishnava and Metisariya, 2018). The presence of the polyphenolic compounds in the leaf extract and fractions in addition to the monoterpenes may suggests that their inhibitory potential on α-amylase and the membrane-bound intestinal α-glucosidase enzymes.

Conclusion

The results of this study suggest that inhibition of alpha amylase and alpha glucosidase enzymes maybe one of the modes of antidiabetic activity of the leaf fractions of *C. zambesicus* which can be attributed to the activities of its phytochemical constituents.

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Conflict of Interest

Authors have declared that no competing interests exist

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