

Inhibitory Effect of *Mammea africana* on Alpha-Amylase and Alpha-Glucosidase Enzymes of Rats

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Abstract

Mammea africana Sabine (Guttiferae), 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 stembark extract (30, 60 and 90 mg/kg) of *M. africana* 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 stembark extract caused significant ($p < 0.05$) reduction in blood glucose levels of treated rats with the various substrates used. The results suggest that the stembark extract of *M. africana* have the potentials to inhibit alpha-amylase and alpha-glucosidase in rats.

Keywords: alpha-amylase; alpha-glucosidase; hypoglycaemia; *Mammea africana*.

Abbreviations: Blood Glucose Level (BGL).

INTRODUCTION

Mammea africana Sabine (Guttiferae) (syn. *Ochrocarpus africana* Oliv.) (*M. africana*) is a large forest tree of 50 to 100 feet high with bark often yellow with pale scales and resinous yellow sap (Hutchison and Daziell, 1958). The plant is widely distributed in tropical Africa. Traditionally, the stem bark of the plant is used by the Ibibios, of the Niger Delta region of Nigeria, in the treatment of malaria related fever, diabetes, microbial infections and mental disorders. The stembark is also traditionally used to treat stomach pains, rheumatism pains, scabies, cough and hypertension (Raponda-Walter and Sillans, 1961; Adjanohoun et al. 1996). The stembark extract has been reported to possess cytotoxic activity, *in vitro* (Chapuis et al. 1988; Okokon et al. 2012). Ouahou et al. (2004) reported cytotoxic coumarins with anti-microbial activity against *Staphylococcus aureus* from the plant stembark. The stembark has been reported to have anti-plasmodial (Okokon et al. 2006), cardioprotective (Okokon and Antia, 2007), anti-diabetic, hypolipidaemic (Okokon et al. 2007), vasorelaxant (Dongmo et al. 2007), anti-hypertensive (Nguefack-Mbuyo et al. 2008), anti-inflammatory, analgesic (Okokon et al. 2009), antioxidant (Nguefack-Mbuyo et al. 2010), anti-diarrheal, anti-ulcer (Okokon et al. 2010), immunomodulatory, anti-lesihmanial (Okokon et al.

2012), depressant and anti-convulsant (Okokon and Davis, 2014) as well as nephroprotective (Okokon and Bawo, 2014) and hepatoprotective (Okokon et al. 2016) activities. The stembark has been reported to have 5,7-dihydroxy-8-(12-methyl-butyl)-4-N-pentylcoumarins (Carpenter et al. 1970, 1971; Crichton and Waterman, 1978), 4-phenyl and 4-alkylcoumarins (Games, 1972), mesuxanthone B (Carpenter et al. 1971). Alkaloids have been reported to be absent in the entire plant parts (Gartlands et al. 1980). We report in this study the effect of leaf extract and fractions of the plant on alpha-amylase and alpha-glucosidase of rats.

MATERIALS AND METHODS

Plants Collection

The plant material *Mammea africana* (stembark) were collected in Anwa forest in Uruan area, Akwa Ibom State, Nigeria in January 2022. The plant was identified and authenticated by Dr. Margaret Bassey, at the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria with voucher number FPHUU 381.

Extraction

The stembarks were washed and shade-dried for two weeks. The dried plants' materials were further chopped

into small pieces and reduced to powder using electric grinder. The powdered material (1.5 kg) was macerated for 72 h in 50% ethanol. This was thereafter filtered and the liquid filtrate was concentrated and evaporated to dryness in *vacuo* 40°C using a rotary evaporator (BuchiLab, Switzerland). The extract was 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*.

In Vivo Alpha-amylase and Alpha-glucosidase Inhibition Study

Alpha-amylase Inhibitory Study

Thirty-five Wistar rats were divided into 6 groups of 5 rats each. The rats in all groups were fasted for 18 hours and fasting blood glucose concentration was first taken at 0 minutes 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 and VI were administered simultaneously, starch (2 g/kg) and *Mammea africana* stem bark extract at 30, 60 and 90 mg/kg respectively. All administrations were done orally and blood glucose concentration was monitored at 30, 60, 90, 120 and 180 minutes (Gidado et al. 2019).

Alpha-glucosidase Inhibitory Study

The procedure as described above was used 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 a Light Emitting Diode 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% level of significance i.e. $p \leq 0.05$.

RESULTS AND DISCUSSION

In Vivo Alpha-amylase and Alpha-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 percentages were starch (63.18%), extract-treated groups (6.95-42.73%) and acarbose-treated group (17.97%). These increases were reduced after 60 min to 0%, 15.29% and 20.51% in animal groups treated with 60, 90 and 30 mg/kg of the extract respectively. All the extract-treated groups had their blood glucose level (BGL) reduced to normal without any further increase from 90 to 180 minutes. Also, co-administration of the starch with acarbose prominently inhibited the rise in the blood glucose concentrations (Table 1).

There was 60.78% increase in blood glucose concentration 30 minutes following maltose administration in the control group. However, 17.03-21.05 % increases were observed in the extract-treated groups. At 60 minutes, the BGL of the extract-treated groups were significantly reduced with groups treated with 30, 60, and 90 mg/kg having percentage increments of 6.11, 8.63 and 13.15% in BGL respectively. These reductions were sustained and significant throughout the duration of the study (180 minutes) with no increment in BGL recorded in any group (Table 2).

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 20.95-56.03% increases in blood glucose concentration of extract-treated groups. The blood glucose concentrations were significantly reduced after 60 minutes post-administration of sucrose in all the extract-treated groups and sustained for 180 minutes with the group treated with the lowest dose of the extract (30 mg/kg) having no increment in BGL followed by groups treated with 60 and 90 mg/kg of the extract with 1.16 and 13.35 % increases respectively (Table 3).

Table 1. Effect of Ethanol Leaf Extract of *Mammea africana* 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	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	2000	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)
Extract	30	78.0±4.35	111.33±4.97(42.73)	94.0±3.51(20.51)	78.0±1.73(0)	72.33±0.88(0)	66.66±0.88(0)
	60	86.33±3.75	92.33±6.33(6.95)	80.0±2.64(0)	74.00±2.30(0)	70.00±1.15(0)	68.66±2.72(0)
	90	82.10±5.85	100.0±13.15(21.80)	94.66±10.72(15.29)	82.0±4.61(0)	76.66±3.84(0)	71.0±1.73(0)

Data is expressed as MEAN ± SEM, Significant at ^ap<0.05, ^bp< 0.01, when compared to control (n=5). 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 of *Mammea africana* 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	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
Maltose	2000	92.0±4.04	134.33±2.90 ^b (46.01)	128.66±5.45 ^b (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
Extract	30	73.33±1.45	86.0±1.73(17.27)	79.66±0.33(8.63)	74.66±0.33(0)	72.33±1.45	68.33±5.16
	60	76.33±0.66	92.0±3.78(17.03)	81.00±2.51(6.11)	76.0±0.57(0)	70.6±0.33(0)	68.66±0.33(0)
	90	76.0±2.51	92.0±5.03(21.05)	86.0±5.50(13.15)	81.0±5.03(6.57)	74.0±3.05(0)	70.33±2.96(0)

Data is expressed as MEAN ± SEM. Significant at ^ap<0.05, ^bp< 0.01, when compared to control (n=5). 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 of *Mammea africana* 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	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)
Sucrose	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 (0)	84.26±1.14 ^a (0)	82.28±2.26 ^a (0)
Extract	30	84.6±2.60	102.33±8.64(20.95)	94.33±1.20 ^a (11.50)	91.66±2.40 ^b (8.34)	86.66±2.90 ^b (2.43)	81.33±5.69(0)
	60	85.66±5.36	111.6±6.93 ^b (30.28)	98.0±5.29(14.40)	92.0±4.35(7.40)	90.66±4.91 ^a (5.83)	86.66±3.38(1.16)
	90	77.33±1.85	120.66±2.60(56.03)	112.0±3.05 ^b (44.83)	98.0±1.52 ^b (26.72)	93.66±1.85 ^b (21.11)	87.66±1.45 ^c (13.35)

Data is expressed as MEAN ± SEM, Significant at ^ap<0.05, ^bp< 0.01, when compared to control. (n=5). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Discussion

Mammea africana stem bark is used in Ibibio traditional medicine in the treatment of diseases such as diabetes among others. This work investigated the effect of *M. africana* stem bark on alpha-amylase and alpha-glucosidase activities in rats. The extract was found to inhibit increases in blood glucose concentration following starch administration though non-dose-dependently. Complete digestion of dietary polysaccharides like starch is achieved by the combined

action of alpha-amylases and alpha-glucosidase enzymes. The alpha-amylase enzyme digests alpha-bonds of the alpha-linked polysaccharides yielding disaccharides, like maltose, which are further reduced to monosaccharides by membrane bound alpha-glucosidase enzymes (Alongi and Anese, 2018; Kalra, 2014). 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 alpha-amylase and alpha-glucosidase inhibitory potential (Esimone et al. 2001; Ibrahim et al. 2014).

Similarly, the stem bark extract significantly and non dose-dependently inhibited blood glucose rise when co-administered with maltose and sucrose. 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 earlier reported antidiabetic activity of the stem bark extract of *M. africana* in rats (Okokon et al. 2007; Tchamadeu et al. 2010). This further suggest that inhibition of alpha-glucosidase and alpha-amylase activities may be one of the antidiabetic modes of action of the extract. The inhibitory activities of plant extract are linked to their phytochemical constituents especially polyphenols. The stem bark extract has been reported to contain 5,-7-dihydroxy-8(12-methyl-butryl)-4-N-pentylcoumarin (Carpenter et al. ;1970;1971; Crichton and Waterman, 1978), mesuxanthone B (Carpenter et al. 1971), 4-n-propylcoumarins and 4-phenyl coumarins (Ouahouo et al. 2004) have also been isolated from the stem bark. Polyphenolic compounds have been variously reported to inhibit alpha-glucosidase and alpha-amylase activities (Proenca et al. 2017; Su and Tang, 2019; Proenca et al. 2017). Coumarins in particular have been reported to inhibit alpha-glucosidase and alpha-amylase activities (Zhao et al. 2015; Karakaya et al. 2018). Also, xanthenes are reported to possess the potentials to inhibit alpha-amylases and alpha-glucosidase enzymes (Malik et al. 2020).

The presence of these compounds in the stem bark extract could have contributed to the observed activity of this study and therefore explains the antidiabetic mechanism of the stem bark of *M. africana*.

Alpha-amylase and alpha-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 alpha-amylase and alpha-glucosidase inhibition. The phytochemicals implicated include; flavonoids, saponins, tannins and terpenoids (Ishnava and Metisariya, 2018; Ortiz-Andrade et al. 2007; Yoshikawa et al. 1998). Also, polyphenolic compounds from plants are known to cause several effects on the biological systems which include enzymes inhibitions (Funke and Melzig, 2005; Kalita et al. 2018). 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 stem bark extract in addition to the xanthenes may suggests that their inhibitory potential on alpha-amylase and the membrane-bound intestinal alpha-glucosidase enzymes.

CONCLUSIONS

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 stem bark extract of *Mammea africana* which can be attributed to the activities of its phytochemical constituents.

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Competing Interests: The authors declare that there are no competing interests.

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