

In-Vivo Alpha-Amylase and Alpha-Glucosidase Inhibitory Activities of *Solanum anomalum* Leaf Extract and Fractions

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Abstract

Solanum anomalum Thonn. ex Schumach. (family Solanaceae), an edible shrub whose fruits and leaves are used medicinally to treat diseases including diabetes was evaluated for effect on alpha amylase and alpha glucosidase enzymes *in vivo*. The leaf extract (70-210 mg/kg) and fractions (hexane, dichloromethane, ethyl acetate, methanol, 140 mg/kg) of *S. anomalum* were evaluated *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 extract especially middle dose (140 mg/kg) and fractions (ethyl acetate and hexane) caused significant ($p < 0.05$) reduction in blood glucose levels of animals treated with the various substrates used. Ethyl acetate fraction exerted the highest inhibitory effect when starch and maltose were used as substrates followed by n-hexane and methanol. n-Hexane was the most active fraction followed by ethyl acetate when sucrose was used as substrate. The results suggest that the leaf extract and fractions of *S. anomalum* have the potentials to inhibit alpha amylase and glucosidase in rats.

Keywords: alpha amylase; alpha glucosidase; hypoglycemia; *Solanum anomalum*.

INTRODUCTION

Solanum anomalum Thonn. ex Schumach, a plant whose fruits and leaves are used medicinally and nutritionally is commonly found growing in West and East Africa sub-regions. Its parts are utilised locally to treat diabetes, gastrointestinal disorders, infections, inflammation and pains (Burkill, 2000; Bukenya and Hall, 1988; Offor and Ubengama, 2015). Hypoglycemic and antidiabetic activities of the fruits and leaves have been reported (Offor and Ubengama, 2015; Okokon et al., 2022). Moreso, *in vivo* and *in vitro* antiplasmodial (Okokon et al., 2016; Okokon et al., 2017a), anti-oedema (Okokon et al., 2017b), antioxidant and antiulcer (Okokon et al., 2019a), anticonvulsant and depressant (Okokon et al., 2019b), analgesic (Okokon et al., 2020) and antidiarrhoeal (Udobang et al., 2022) properties of the leaf extract have also been reported. Phytochemical constituents such as alkaloids, flavonoids, saponins, tanins, diosgenin, a diosgenin glycoside (25(R)-diosgenin-3-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranoside, uracil, 5-methyluracil, 1-octacosanol, and octacosane have been reported on the leaves of the plant (Okokon et al., 2016; Okokon et al., 2022). 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

Fresh leaves of *Solanum anomalum* were collected in Uruan area, Akwa Ibom State, Nigeria in August, 2020. The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo (UUH.75a).

Extraction

Fresh leaves of *S. anomalum* were washed, cut into smaller pieces and dried under shade for two weeks. The leaves were further pulverized to powder using electric grinder. The powdered leaves material was divided into two parts; one part (1.5 kg) was macerated in 50% ethanol (7.5 L) for 72 hours at room temperature (28 ± 2 °C). While the other part, (1.5 kg) was successively and gradually 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. These were thereafter filtered and the liquid filtrates were concentrated and evaporated to dryness *in vacuo* 40°C using a rotary evaporator (BuchiLab, Switzerland). The extract and fractions were stored in a

refrigerator at -4°C , until used for the proposed experiments.

Animals

Wistar rats (138-150 g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets 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*

Fifty Wistar rats were divided into 10 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 minute before administration. Based on predetermined LD_{50} value of 724.56 mg/kg (Okokon et al., 2016), the animals were treated as follows; group I, which served 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 *S. anomalum* leaf extract at 70, 140 and 210 mg/kg respectively while groups VII, VIII, IX and X rats were administered starch (2 g/kg) and fractions (n-hexane, dichloromethane, ethyl acetate and methanol) at 140 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).

▪ *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% and 0.1% level of significance ie $p \leq 0.05$ and 0.001 .

RESULTS AND DISCUSSION

In vivo alpha amylase and glucosidase inhibition assay

Administration of starch (2g/kg) caused varying percentages of increase in blood glucose concentrations after 30 minutes. The percentages were starch (63.18%), extract/fractions treated groups (10.97-32.69%) and acarbose-treated group (17.97%). These increases were reduced after 60 minutes with animals treated with the ethyl acetate fraction (10.53%). These decreases were significant and sustained for 180 minutes in ethyl acetate-treated group, followed by middle dose group, 140 mg/kg, (9.22%) and methanol-treated group (12.15%). However, co-administration of the starch with acarbose prominently inhibited the rise in the blood glucose concentrations (Table 1).

Table 1. Effect of ethanol leaf extract and fractions of *Solanum anomalum* 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)
Crude extract	70	70.12±2.00	89.33±1.45(23.11)	100.33±4.05(42.12)	99.66±2.90(42.12)	89.0±2.08(26.92)	80.0±4.16(14.09)
	140	72.33±8.87	89.33±8.68(23.50)	94.66±2.90(30.87)	94.0±0.57(29.95)	84.66±4.41(17.04)	79.0±3.21(9.22)
	210	62.33±1.45	74.33±7.83(19.25)	86.0±10.14(37.97)	96.0±2.64(54.01)	83.0±2.08(33.16)	82.20±2.30(31.87)
n -hexane fraction	140	66.66±1.33	90.33±1.85(35.50)	93.0±3.21(39.52)	108.0±3.60(62.01)	97.00±3.00(45.51)	85.66±4.48(28.50)
Dichloromethane fraction	140	69.33±4.41	92.0±2.51(32.69)	104.3±14.71(34.97)	108.6±12.81(56.64)	107.33±11.78(54.81)	96.66±7.53(39.42)
Ethyl acetate fraction	140	82.00±4.04	91.0±3.21(10.97)	91.66±5.60(10.53)	93.33±1.20(13.81)	91.0±2.30(10.97)	81.66±3.75(0)
Methanol fraction	140	71.33±1.20	90.00±4.35(26.17)	93.33±5.78(30.84)	92.0±5.19(28.97)	85.33±3.18(19.62)	80.0±4.54(12.15)

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.

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 25.95-70.73% increases in blood glucose concentration of extract/fractions-treated groups. The blood glucose concentrations were significantly reduced

in hexane-treated group (13.02%) after 60 minutes post-administration of sucrose. However, n-hexane fraction had the highest effect (0.84%) throughout the duration of the study (180 minutes) followed by middle dose, 140 mg/kg, group (6.47%) (Table 2).

Table 2. Effect of ethanol leaf extract and fractions of *Solanum anomalum* 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	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
Crude extract	70	70.66±5.92	89.0±7.55 ^b (25.95)	92.33±8.83(30.66)	78.33±9.13(10.85)	76.33±2.18(8.02)	77.66±0.66(9.90)
	140	82.33±3.75	117.33±4.80 ^b (42.51)	102.0±2.08(23.89)	88.66±5.45(7.68)	85.66±5.23(4.04)	87.66±1.33(6.47)
	210	72.0±2.64	114.66±6.38(59.25)	113.0±11.24(56.94)	91.33±7.68(26.84)	93.22±9.33(29.47)	83.00±2.08(15.27)
n -hexane fraction	140	79.33±1.45	101.0±3.05(27.31)	89.66±1.45(13.02)	86.0±3.05(8.40)	81.66±1.45(2.93)	80.0±1.00(0.84)
Dichloromethane fraction	140	73.66±7.53	120.33±4.37(55.66)	106.33±7.35(44.35)	113.66±9.38(54.30)	95.00±5.68(28.97)	90.33±2.90(22.63)
Ethyl acetate fraction	140	68.33±3.18	116.66±11.72(70.73)	117.66±6.88(72.19)	110.33±8.53(61.46)	89.66±2.72(31.21)	86.66±1.85(26.82)
Methanol fraction	140	79.33±6.06	106.33±7.86(34.03)	96.66±2.66(21.84)	108.0±2.51(36.14)	91.33±3.33(15.12)	96.66±9.52(21.84)

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.

There was 60.78% increase in blood glucose concentration 30 minutes following maltose administration in the control group. However, 27.08-57.80% increases were observed in the extract/fractions-treated groups. At 60 and 90mins, the ethyl acetate

fraction group had blood glucose level of 18.44 and 0.61% respectively. At 180 minutes, n-hexane fraction and middle dose (140 mg/kg)-treated group had blood glucose level of 2.02% and 2.52% respectively (Table 3).

Table 3. Effect of ethanol leaf extract and fractions of *Solanum anomalum* 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(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 ^a (0.77)	85.33±2.15 ^a (0)	84.26±1.14 ^a (0)	82.28±2.26 ^a (0)
Crude extract	70	83.3±1.20	131.45±3.28(57.80)	125.17±4.39 ^b (50.26)	115.33±1.66 ^a (38.45)	104.35±1.12(25.27)	95.42±2.28(14.54)
	140	81.28±5.28	128.14±1.98(56.05)	105.23±1.52 ^a (29.46)	95.56±3.88(17.56)	90.44±1.86(11.26)	83.33±3.99(2.52)
	210	85.24±7.34	125.19±7.22(46.86)	90.50±4.86 ^a (6.17)	87.25±8.65 ^b (2.35)	81.45±5.60 ^a (0)	77.0±5.21 ^b (0)
n -hexane fraction	140	80.55±4.89	118.24±5.28(46.79)	104.28±2.37 ^a (29.45)	92.22±3.34 ^a (14.48)	87.28±2.28 ^b (8.35)	82.18±3.28(2.02)
Dichloromethane fraction	140	83.82±5.49	122.98±4.29 ^b (46.71)	110.37±3.29(31.67)	102.19±2.19(21.91)	98.28±6.18 ^a (17.25)	91.88±1.83(9.61)
Ethyl acetate fraction	140	82.36±6.54	132.46±7.38(59.61)	97.55±7.44 ^b (18.44)	82.87±9.28 ^b (0.61)	81.65±2.27 ^b	74.33±8.44 ^c
Methanol fraction	140	90.10±10.16	114.50±12.18(27.08)	108.53±5.68 ^a (20.45)	94.66±8.93 ^a (5.06)	82.63±5.68 ^b	73.26±6.79 ^c

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

Various parts of *S. anomalum*, a medicinal plant, is used in Ibibio traditional medicine in the treatment of diseases such as diabetes among others. This work focused on the evaluation of *S. anomalum* leaf extract and fractions for *in vivo* inhibitory effect on alpha amylase and alpha glucosidase activities in rats, as well as isolation and characterization of the active antidiabetic principles from this plant.

The extract was found to non dose-dependently inhibit increases in blood glucose concentration following starch administration with the middle dose, 140 mg/kg, and ethyl acetate fraction exerting the most inhibition. 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 (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 alpha-amylase and alpha-glucosidase inhibitory potential (Esimone et al., 2001; Ibrahim et al., 2014).

Similarly, the leaf extract and 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 that reported on other species of *Solanum*

such as *S. nigrum*, *S. melongena depressum*, *S. gilo*, *S. melogena*, *S. melongena* L., *S. macrocarpon* and *S. diphyllum* (Hossain et al., 2009; Nwanna et al., 2013; Dasgupta et al., 2016; Nwanna et al., 2019), which significant inhibition of alpha –amylase and alpha-glucosidase activities were observed. Diosgenin present in this plant is implicated in antidiabetic activities of plants (Pari et al., 2012; Saravanan et al., 2014). Diosgenin has been reported to exert its activity through various mechanisms such as inhibiting alpha-amylase and alpha-glucosidase (Gosh et al., 2014) to reduce intestinal glucose absorption, inhibiting the sodium-glucose cotransporter-1 (SGLT-1) and reducing intestinal Na⁺-K⁺-ATPase activity (Gan et al., 2020). Also, diosgenin glycosides and other steroidal saponins commonly present in the *Solanum* species (Kaunda and Zhang, 2019) are reported to exert hypoglycaemic activities (Wang et al., 2010; Wang et al., 2012; Elekofehinti, 2015). Similarly, β-sitosterol present in the leaf extract and fraction of this plant has been reported to possess inhibitory potentials on alpha-glucosidase and alpha-amylase enzymes (Kumar et al., 2013). These could have contributed to the observed activity of this study and therefore explains the antidiabetic mechanism of this extract.

Alpha-amylase and alpha-glucosidase inhibitions by plants extracts have been reported severally (Shirwaikar et al., 2005; Ishnava and Metisariya, 2018). Phytochemicals implicated as anti-diabetic agents, do so possibly through alpha-amylase and alpha-glucosidase inhibition. The phytochemicals in question include flavonoids, saponins, tannins and terpenoids (Ortiz-Andrade et al., 2007; Ishnava and Metisariya, 2018). Also, polyphenolic compounds from plants are known to cause enzymes inhibitions on the biological systems (Kaita et al., 2018). The phenolic compounds which are biological oxidants, strong metal ion chelators and

protein precipitation agents form insoluble complexes with proteins (Ishnava and Metisariya, 2018). The presence of the polyphenolic compounds in the leaf extract and fractions in addition to diosgenin, diosgenin glycosides and β -sitosterol may suggest that their inhibitory potential on alpha-amylase and the membrane-bound intestinal alpha-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 extract and fractions of *Solanum anomalum* which can be attributed to the activities of its phytochemical constituents.

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