Nigerian Journal of Pharmaceutical and Applied Science Research,
Vol.13 (4): 11-20; December 2024 *ISSN: 2971-737X (Print); ISSN: 2971-7388.*
Available at www.nijophasr.net https://doi.org/10.60787/nijophasr-v13-i4-568

In vivo alpha amylase and alpha glucosidase inhibitory activities of ethanol fruit extract and fractions of *Solanum anomalum* Thonn. ex Schumach. (Solanaceae)

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Sent for review: 2 December 2024 Accepted: 30 December 2024

ABSTRACT

Background: *Solanum anomalum* Thonn. ex Schumach. (family Solanaceae) is an edible shrub whose fruits and leaves are traditionally used to treat various diseases, including diabetes. This study evaluated the effects of its extract and fractions on alpha-amylase and alpha-glucosidase enzymes in vivo.

Methods: The extract (200–600 mg/kg) and fractions (hexane, dichloromethane, ethyl acetate, methanol, 400 mg/kg) of S. anomalum were tested for inhibitory effects on alpha-amylase and alpha-glucosidase enzymes in rats using starch, sucrose, and maltose as substrates. Acarbose was used as the reference drug.

Results: The fruit extract (600 mg/kg) and the ethyl acetate fraction significantly ($p < 0.05$) reduced blood glucose levels in treated rats across all substrate types. The ethyl acetate fraction and the 600 mg/kg extract exhibited the highest inhibitory effects on alpha-amylase and alpha-glucosidase, followed by the dichloromethane fraction.

Conclusion: The findings indicate that the fruit extract and fractions of *S. anomalum* have promising inhibitory effects on alpha-amylase and alpha-glucosidase enzymes, suggesting potential antidiabetic activity *in vivo*.

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

1. INTRODUCTION

Solanum anomalum Thonn. ex Schumach (Solanaceae), is a herbaceous plant whose fruits and leaves are utilised for various purposes, especially in ethnomedicine and nutrition for the preparation of foods and medicine. It is distributed widely in the West and East Africa sub-regions. Different parts of the plant are utilised ethnomedically to treat diabetes, gastrointestinal disorders, malaria, infections, inflammation, and pains [1–4]. Hypoglycemic and antidiabetic activities of the fruits and leaves have been reported [3, 5, 6]. The fruit extract, which has been proven to offer significant protection against lead-induced liver, kidney, and testicular injuries, has an oral LD_{50} confirmed to be above 5,000 mg/kg [7] and an intraperitoneal LD_{50} of 2260 ± 131.78 mg/kg [3]. Antioxidant, genotoxic, cytotoxic [8], and in vivo antiplasmodial activities [9] of the fruit extract and fractions have also been reported. The presence of secondary metabolites such as saponins, cardiac glycosides, anthraquinones, terpenes, flavonoids, tannins, and alkaloids has been reported in the fruit extract [3, 5]. Additionally, compounds such as (E)-9-Octadecenoic acid ethyl ester, tetradecanoic acid, octadecanoic acid, hexadecanoic acid methyl ester, n-

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hexadecanoic acid, myristic acid, palmitoleic acid, (E)-9-octadecenoic acid ethyl ester, 11-octadecenoic acid methyl ester, 9-octadecenoic acid (E)-, octadecanoic acid, heptadecanoic acid, cyclodisilazane-2,2,4,4-tetramine, N,N,N',N'-tetramethyl-1,3-bis[tris(methylamino)silyl]-, 2(3H)-furanone dihydro-5-tetradecyl-, succinic acid 2-(3-nitrophenyl) ethyl nonyl ester, 5-thiazole ethanol 4-methyl-, cyclotrisiloxane hexamethyl, tridecanoic acid 12-methyl- methyl ester, tetradecanoic acid, octadecanoic acid, oleic acid, and methyl stearate have been identified from n-hexane, dichloromethane, and ethyl acetate fractions of the fruits [8, 9]. We report in this study the in vivo alpha-amylase and alpha-glucosidase inhibitory activities of the fruit extract and fractions of *S. anomalum.*

Materials and Methods

2.1 Materials

*2.1.1 Biological Materials:*Albino wistar rats weighing 130-155 g (male and female) were obtained from the University of Uyo's Animal house and used in the study.

2.1.2 Chemical and Reagents

Ethanol, methanol, n-hexane, dichloromethane, ethyl acetate (Sigma-Aldrich, USA), starch, sucrose and maltose (Sigma-Aldrich,USA)

2.1.3 Equipment and Apparatus

Grinding machine,weighing balance (Ohaus, USA), descicator, measuring cylinder, glucometer and glucometer strips(Accu-chek, Indiana).

2.2 Methods

2.2.1 Plants Collection

Fresh fruits of *Solanum anomalum* were collected in compounds in Afaha Idoro village in the Uyo area, Akwa Ibom State, Nigeria, in August 2023. 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 the Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo (UUH.75a).

2.2.2 Extraction

Fresh fruits of *S. anomalum* were washed and dried under shade for two weeks. The fruits were further pulverized into powder using an electric grinder. The powdered fruit 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). The other part (1.5 kg) was successively and gradiently macerated for 72 hours in each of these solvents (2 × 5 L): 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 at 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.

2.2.3 In vivo Alpha-Amylase and Glucosidase Inhibition Study

2.2.3.1 Alpha-Amylase Inhibitory Study

Forty-five Wistar rats were divided into 9 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 min before administration. Based on a predetermined oral LD50 confirmed to be above 5,000 mg/kg [10] and an intraperitoneal LD_{50} of 2260 ± 131.78 mg/kg [11], the animals were treated as follows:

- **Group I** (normal control): received distilled water (10 mL/kg).
- **Group II**: orally administered starch at 2 g/kg body weight (with distilled water as vehicle) and distilled water (10 mL/kg) simultaneously.
- **Group III**: administered starch (2 g/kg) and the standard drug (acarbose) at 100 mg/kg simultaneously.
- **Groups IV, V, VI**: simultaneously administered starch (2 g/kg) and *S. anomalum* fruit extract at 200, 400, and 600 mg/kg, respectively.
- **Groups VII-IX**: administered starch (2 g/kg) and fractions (n-hexane, dichloromethane, ethyl acetate, and methanol) at 400 mg/kg, respectively.

All administrations were done orally, and blood glucose concentration was monitored at 30, 60, 90, 120, and 180 min [12, 13].

2.2.3.2 Glucosidase Inhibitory Study

The procedure described above was used for this study, but sucrose and maltose were used as substrates [12, 13].

2.2.3.3 Blood Glucose Determination

Drops of blood from the tips of rats' tails were dropped on strips, and glucose concentration was measured using a glucometer according to the 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 color 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 [14].

2.3 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.

3.RESULTS

3.1 *In vivo* alpha-amylase and glucosidase inhibition assay

Administration of starch (2g/kg) caused varying percentages of increase in blood glucose concentrations after 30 mins. The percentages were starch (63.18%), extract/fractions treated groups (7.24 - 26.09%) and acarbose-treated group (17.97%). Prominent reductions in BGL were observed after 90 min with animals treated with the high dose of extract (600 mg/kg) and ethyl acetate fraction having their percentage increases reduced to 12.87 and 13.34 % respectively. These BGL reductions were significant and sustained for 180 min in ethyl acetate fraction-treated group, followed by dichloromethane fraction, n-hexane fraction and extract (600 mg/kg) treated groups with percentage increases in BGL of 0, 0.43, 1.75 and 2.58 % respectively. 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) caused 46.01% increase in blood glucose concentration 30 minutes post-administration of the sucrose in the control group and 31.16- 45.83 % increases in blood glucose concentration of extract/fractions-treated groups. The blood glucose concentrations were significantly reduced in ethyl acetate fraction-treated group (20.21%) and extract high dose (600 mg/kg)-treated group,(29.86) after 60 mins post-administration of sucrose. However,groups treated with the high dose (600 mg/kg) and middle dose (400 mg/kg) of the extract had blood glucose percentage increases of 1.52 and 2.00% respectively, while ethyl acetate fraction, followed by dichloromethane and hexane fractions-treated groups had blood glucose percentages reduced to 4.30, 5.32 and 7.30% respectively after 180 min (Table 2). Maltose administration caused 60.78% increase in blood glucose concentration 30 min after being administered to rats in the control group. However, 38.46 - 58.63% increases were observed in the extract/fractions-treated groups. At 60 and 120 mins, the ethyl acetate fraction-treated group had BGL increment of 19.48 and 4.06% respectively, while the high dose of extract (600 mg/kg) treated group had BGL increment of 26.36 and 6.20% at 60 and 120 min respectively. At 180 min, there was no BGL increment in extract (600 mg/kg) and ethyl acetate fraction treated groups, while BGL percentage increment of methanol fraction and extract (400 mg/kg)-treated groups were reduced to 0.55 and 5.70 % respectively (Table 3).

4. DISCUSSION

S. anomalum parts (leaves and fruits) are used in Ibibio traditional medicine for the treatment of diseases such as diabetes, among others. This work focused on the evaluation of S. anomalum fruit extract for in vivo inhibitory effects on alpha-amylase and alpha-glucosidase activities in rats. The extract was found to dose-dependently inhibit increases in blood glucose concentration following starch administration, with the ethyl acetate fraction followed by the dichloromethane 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 [15, 16]. 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 [17–21]. Similarly, the fruit extract and fraction significantly inhibited blood glucose rise when co-administered with maltose and sucrose, with the high dose of the extract (600 mg/kg) and ethyl acetate fraction exerting the highest inhibition. Acarbose, the standard drug used in this study, similarly exerted significant inhibition of blood glucose rise when co-administered with starch, maltose, and sucrose. The results of this study corroborate those reported on the leaves of *S. anomalum* [22] and other species of *Solanum*, such as *S. nigrum, S. melongena depressum, S. gilo, S. melogena, S. melongena L., S. macrocarpo*n, and *S. diphyllum* [23–26], where significant inhibition of alpha-amylase and alpha-glucosidase activities was observed. Okokon et al. [6] had reported the presence of diosgenin in the leaves of this plant. Diosgenin, which has been implicated in the antidiabetic activities of plants [27, 28], exerts its activity through various mechanisms, such as inhibiting alpha-amylase and alpha-glucosidase [29], reducing intestinal glucose absorption, inhibiting the sodium-glucose cotransporter-1 (SGLT-1), and reducing intestinal Na+-K+-ATPase activity [30]. Also, diosgenin glycosides and other steroidal saponins commonly present in Solanum species [32] are reported to exert hypoglycemic activities [31–33]. Some phytochemicals reported to possess antidiabetic potential act by causing α-amylase and glucosidase inhibition [17], including flavonoids, saponins, tannins, and terpenoids [21, 34]. Polyphenolic compounds from plants are known to cause enzyme inhibitions in biological systems [35]. The phenolic compounds, which are biological oxidants, strong metal ion chelators, and protein precipitation agents, form insoluble complexes with proteins [21]. The fruit extract and fractions have been reported to have high total phenolic and flavonoid content, especially in the dichloromethane fraction [36]. Besides, the GC-MS analysis of the fruit extract and fractions revealed that they have high fatty acid content, both saturated and unsaturated [8,37]. Polyunsaturated fatty acids, such as oleic acid, palmitic acid, stearic acid, and linoleic acid, are reported to exert inhibitory effects on alpha-amylase and alpha-glucosidase enzymes in vitro and in silico [18, 20, 38–40]. These compounds, in addition to diosgenin and diosgenin glycosides, may suggest their inhibitory potential on α-amylase and the membrane-bound intestinal α-glucosidase enzymes, which account for the antidiabetic potential of the fruits of *Solanum anomalum*.

5. 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 fruit extract and fractions of *Solanum anomalum* which can be attributed to the activities of its phytochemical constituents.

Acknowledgements

The authors are grateful to Mr Nsikan Malachy of Pharmacology and Toxicology Department for providing technical assistance.

Conflict of interest

The authors have not declared any conflict of interests.

Contribution of the Authors

JEO - Research concept and design; JEO, UUF, CCO Data analysis and interpretation; JEO,UUF, CCO, Writing the article; JEO,CCO and UUF read and approved the final manuscript.

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TABLE 1: Effect of ethanol fruit extract and fractions of *Solanum anomalum* 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	2000	73.33±8.25	119.66±5.45a(63.18)	115.66±1.33a (57.72)	104.66±2.60a (42.72)	95.66±3.75a (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	200	71.52±4.26	90.18±3.20(26.09)	99.55±3.24(39.19)	95.42±4.34(33.41)	88.24±2.45(23.37)	80.13±5.28(12.03)
	400	72.33±8.87	85.24±4.23(17.84)	90.39±3.24(24.96)	88.24±1.65(21.99)	80.22±2.41(10.90)	76.25±3.29(5.41)
	600	70.38±2.66	75.48±3.78(7.24)	81.66±5.16(16.02)	79.44±4.86(12.87)	76.10±2.38(8.12)	72.20±3.25(2.58)
n -hexane fraction	400	74.54±3.56	89.44±5.22(19.98)	95.19±1.32(27.70)	85.29±1.63(14.42)	78.33±1.56(5.08)	75.85±3.23(1.75)
Dichloromethane fraction	400	76.23±1.46	91.54±2.51(20.08)	94.19±4.71(23.56)	88.24±4.16(15.75)	81.65±5.29(7.11)	76.56±3.45(0.43)
Ethyl acetate fraction	400	73.42±3.28	85.68±5.35(16.69)	90.25±5.60(22.92)	83.22±2.86(13.34)	79.26±0.39(7.95)	71.28±1.45()
Methanol fraction	400	71.47±2.20	89.46±5.38(25.17)	98.39±4.29(37.66)	90.33±9.37(26.38)	88.22±6.82(23.43)	80.23±2.18(12.25)

Data is expressed as MEAN ± SEM, Significant at ap<0.05, bp< 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 fruit extract and fractions of *Solanum anomalum* 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±2.90b(46.01)	128.66±5.45a (39.84)	117.33±4.66a(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	200	73.25±3.56	106.13±6.75b(44.88)	101.33±6.48(38.33)	92.43±8.26(26.18)	86.43±4.38(17.99)	78.66±2.28(7.38)
	400	76.33±7.16	108.30±6.37b(41.88)	103.4±2.67(35.64)	88.48±8.34(15.91)	80.56±6.54(5.54)	77.86±3.10(2.00)
	600	72.0±2.64	94.65±9.36(31.45)	93.50±4.19(29.86)	83.20±5.78(15.55)	80.36±6.19(11.61)	73.10±3.29(1.52)
n -hexane fraction	400	75.66±4.51	110.34±5.20(45.83)	99.20±5.14(23.54)	91.29±3.05(20.65)	88.66±2.66(17.18)	81.19±1.45(7.30)
Dichloromethane fraction	400	74.68±4.37	108.29±2.18(45.00)	100.20±4.11(34.17)	90.02±7.36(20.54)	85.38±6.28(14.32)	78.66±8.24(5.32)
Ethyl acetate fraction	400	73.38±3.34	96.25±2.71(31.16)	88.29±4.35(20.31)	85.33±6.64(16.28)	80.66±6.74(9.92)	76.54±4.03(4.30)
Methanol fraction	400	72.35±4.38	105.42±6.38(45.70)	100.39±3.34(38.75)	95.39±2.65(31.84)	90.20±3.55(24.67)	85.55±5.36(18.24)

Data is expressed as MEAN ± SEM, Significant at ap<0.05, bp< 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 fruit extract and fractions of *Solanum anomalum* 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±1.90b(60.78)	130.22±2.45(58.22)	120.66±3.22a(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±2.20c(0.77)	85.33±2.15c()	84.26±1.14a()	82.28±2.26a()
Crude extract	200	73.33±2.36	116.33±6.39(58.63)	110.21±9.28b(50.29)	101.57±4.12a(38.51)	93.19±5.18(27.08)	87.53±5.32(19.36)
	400	71.28±3.45	108.30±3.22(51.93)	102.29±2.44a(43.50)	91.55±8.28(28.43)	80.54±4.13(12.99)	75.35±4.44(5.70)
	600	75.60±7.34	100.34±3.65(32.72)	95.53±3.82a(26.36)	88.71±3.43b(17.34)	80.29±4.57a(6.20)	72.39±4.46 b ()
n -hexane fraction	400	70.35±2.35	111.55±2.58(58.56)	101.23±4.37a(43.89)	95.39±2.54 a(35.59)	85.43±1.14 b(21.43)	75.24±3.56(6.95)
Dichloromethane fraction	400	73.55±6.26	112.24±3.26b(52.60)	100.34±4.18(36.42)	95.25±7.84(29.50)	90.34±6.38a(22.82)	81.35±8.24(10.60)
Ethyl acetate fraction	400	72.38±5.64	100.22±3.24(38.46)	86.48±5.38 b(19.48)	80.23±5.82b (10.84)	75.32±4.28 b(4.06)	72.33±5.48c
Methanol fraction	400	75.03±6.91	109.30±4.86(45.67)	105.11±2.24a(40.09)	96.47±6.28a(28.57)	85.45±6.34 b(13.88)	75.45±7.36c(0.55)

Data is expressed as MEAN ± SEM, Significant at ap<0.05, bp< 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.