Research Article

Inhibitory effect of leaf fractions of *Setaria megaphylla* on alpha amylase and alpha glucosidase activities in rats

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Abstract

Objective: *Setaria megaphylla* (Steud) Dur & Schinz (Poaceae), a perennial grass used traditionally in the treatment of various diseases including diabetes was evaluated for effect on alpha amylase and alpha glucosidase enzymes. **Materials and methods:** The leaf fractions (hexane, dichloromethane, ethyl acetate, methanol, 200 mg/kg) of *Setaria megaphylla* 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 referenced drug. **Results:** The leaf fractions caused significant ($p < 0.05$) reduction in blood glucose levels of treated with the various substrates used. *n*-hexane fraction exerted the highest inhibitory effect when starch and sucrose were used as substrates followed by methanol. Methanol was the most active fraction followed by hexane when maltose was used as substrate. The results suggest that the leaf fractions of *S. megaphylla* have the potentials to inhibit alpha amylase and glucosidases in rats.

Keywords: *Setaria megaphylla*; hypoglycemia, alpha amylase, alpha glucosidase

Introduction

Setaria megaphylla (Steud) Dur & Schinz (Poaceae) is a tall, robust, tufted, perennial grass used mainly as pasture grass. It is also called broad leafed brittle grass and occurs in tropical and subtropical areas of Africa, America and India where there is high rainfall (Van Oudtshoorn, 1999). The plant is used traditionally by the Ibibios in Akwa Ibom State, Nigeria in the treatment of various ailments such as malaria, inflammation and diabetes (Okokon et al., 2007). Preliminary report on the leaf extract showed that it possesses antidiabetic and hypoglycaemic activities (Okokon and Antia, 2007). The plant leaves have also been reported to possess antiplasmodial activity *in vitro* (Clarkson et al., 2004; Okokon et al., 2017) and *in vivo* (Okokon et al., 2007), anti-inflammatory and analgesic (Okokon et al., 2006), cytotoxic, immunomodulatory and antileishmanial (Okokon et al., 2013) and antidepressant

(Okokon et al., 2016) activities. The leaf extract as reported by Okokon and Antia, (2007) contains flavonoid, carbohydrate, terpenes, saponins, tannins, anthraquinones and cardiac glycosides with LD₅₀ of 2.4 ± 0.5 g/kg. GCMS analysis of the *n*-hexane fraction of the leaf revealed the presence of 8,11,14-eicosatrienoic acid (Z,Z,Z), phthalic acid, diisooctyl ester, Vitamin E, γ -Elemene, Urs-12-ene, bicyclogermacrene, α -muurolene, germacrene- A, and guaiaol among others (Okokon et al., 2013). We report in this study the effect of solvent fractions of the leaves of *Setaria megaphylla* on alpha amylase and alpha glucosidase activities in rats.

Materials and methods

Plant material

The plant was identified by a taxonomist in the Department of Botany, University of Uyo, Uyo. The leaves were collected from Anwa forest, Uruan in Akwa Ibom State, Nigeria in August, 2021 and were authenticated. A voucher specimen (FPHUU. 221) of the plant was deposited at herbarium of Department of Pharmacognosy and Traditional Medicine, University of Uyo, Uyo.

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Extraction

The leaves were shade dried for 2 weeks. The dried leaves were further chopped into small pieces and reduced to powder using electric grinder. The powder, (1.5 kg) was successively and gradiently macerated for 72 h in each of these solvents, n-hexane, dichloromethane, ethyl-acetate and methanol to give corresponding extract of these solvents. The liquid filtrates obtained were concentrated and evaporated to dryness in vacuo at 40°C using rotary evaporator until they were used for the experiments reported in this study.

Animals

The animals (Swiss albino mice) both male and female that were used for these experiments were obtained from University of Uyo animal house. 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 College of Health Sciences Animal Ethics committee, University of Uyo.

In vivo alpha-amylase and glucosidase inhibition study

Alpha-Amylase inhibitory study

Forty-two Wistar rats were divided into 7 groups of 6 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 solvent leaf fractions (n-hexane, dichloromethane, ethyl acetate and methanol) of *Setaria megaphylla* at 200 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).

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 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 ANOVA (one-way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 5% level of significance ie $p \leq 0.05$.

Results

In vivo alpha amylase and glucosidase inhibition assay

Administration of starch (2g/kg) caused varying percentages of increase in blood glucose concentrations after 30 min. The percentages were starch (63.18%), fractions-treated groups (32.65-58.20%) and acarbose-treated group (17.97%). Methanol fraction-treated group had the lowest increase in BGL (32.65%) following starch administration. These increases were reduced after 60 min with animals treated with methanol and n-hexane fractions recording 19.28% and 19.67% increases in BGL. These decreases were significant and sustained for 180 min with hexane fraction-treated group having no increase in BGL followed by DCM group with 0.86% and methanol 6.46% 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) produced a 46.01% increase in blood glucose concentration 30 minutes post-administration of the sucrose in the control group and 18.29- 47.82% increases in blood glucose concentration of fractions-treated groups. Ethyl acetate fraction-treated group had the lowest increase with BGL of 18.29%, followed by hexane group with 19.91% increase. The blood glucose concentrations were significantly reduced in hexane and methanol fractions-treated groups after 90 mins post-administration of sucrose. At 90 min there was no increase in BGL of hexane treated group, while at 180 min hexane, dichloromethane and methanol fractions-treated groups had no increase in their BGL (Table 2).

There was 60.78% increase in blood glucose concentration 30 min following maltose administration in the control group. However, 9.20-57.80% increases were observed in the fractions-treated groups with methanol fraction-treated

Table 1. Effect of solvent leaf fractions of *Setaria megaphylla* 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		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)	72.23±8.68(0)
n -hexane fraction	200	81.33±6.93	114.33±7.26 ^a (40.32)	97.33±3.18 ^a (19.67)	89.73±4.80 ^a (10.32)	75.06±7.23	72.66±8.22
Dichloromethane fraction	200	76.0±413.31	108.24±8.62 ^a (42.42)	96.464±7.36(26.92)	88.24±6.24(16.10)	79.66±2.84(0.86)	76.66±2.84(0.86)
Ethyl acetate fraction	200	72.33±6.33	114.43±18.55 ^a (58.20)	104.65±5.60(44.68)	90.0±0.57(24.42)	93.33±3.26(29.03)	80.50±6.24(11.29)
Methanol fraction	200	79.32±1.71	105.22±11.72 ^a (32.65)	94.62±7.29 ^a (19.28)	91.3±7.53(15.10)	88.30±5.84(11.32)	84.45±1.33(6.46)

Data is expressed as MEAN ± SEM, Significant at ^ap<0.05, when compared to control. (n=6). Values in parenthesis are percentage increase in blood glucose concentrations compared to 0 min in the same group. Values with the superscript letter 'a' are significantly different from the zero (0) min value (p < 0.05).

Table 2. Effect of solvent leaf fractions of *Setaria megaphylla* 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
n -hexane fraction	200	87.0±6.11	104.33±1.76 ^a (19.91)	94.62±7.21 ^a (8.75)	81.38±5.89	79.42±9.10	76.34±6.90
Dichloromethane fraction	200	79.37±3.60	117.33±9.93 ^a (47.82)	93.66±6.36 ^a (18.00)	90.66±5.60 ^a (14.22)	83.36±6.22(5.02)	79.32±4.78
Ethyl acetate fraction	200	82.0±1.52	97.0±5.50 ^a (18.29)	110.0±9.71 ^a (34.14)	106.0±3.78(29.26)	100.33±2.90(22.35)	84.33±3.84(2.84)
Methanol fraction	200	81.33±5.29	112.6±7.83 ^a (38.44)	97.0±12.22(19.26)	86.33±2.33(6.14)	84.33±6.00(3.68)	80.71±8.24

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 increase in blood glucose concentrations compared to 0 min in the same group.

group having 9.20% increase in BGL. The various fractions significantly reduced blood glucose level. At 180 min, methanol and hexane fractions-treated groups recorded 4.40 and 7.35% increase in BGL (Table 3).

Discussion

The leaf of *Setaria megaphylla* is use in ethnomedicine for the treatment of various ailments such as malaria, inflammation and diabetes (Okokon et al., 2007). Preliminary report on the leaf extract showed that it possesses antidiabetic and hypoglycaemic activities (Okokon and Antia, 2007). This work was designed to explore the effect of leaf fractions of *Setaria megaphylla* 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 hexane and ethyl acetate fractions 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

Table 3. Effect of solvent leaf fractions of *Setaria megaphylla* 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 ^b (46.60)	115.0±2.46(39.73)	106.22±4.24(29.06)
Acarbose	100	85.34±1.36	88.22±1.10 ^c (3.37)	86.0±2.20 ^c (0.77)	85.33±2.15 ^c	84.26±1.14 ^a	82.28±2.26 ^b
n -hexane fraction	200	81.04±3.21	127.3±6.38(57.08)	121.3±2.40 ^b (49.67)	98.33±3.66 ^a (21.33)	93.02±7.34(14.78)	87.0±4.00(7.35)
Dichloromethane fraction	200	79.35±6.33	122.6±1.45(54.50)	111.6±6.54 ^b (40.64)	96.31±6.36 ^a (21.37)	105.60±3.71 ^a (33.08)	90.10±4.18(13.54)
Ethyl acetate fraction	200	89.0±1.73	121.33±4.48(36.32)	107.6±8.14(20.89)	100.66±7.86(13.10)	106.3±6.26(19.43)	98.65±5.23(10.50)
Methanol fraction	200	83.33±3.38	91.0±5.77 ^b (9.20)	104.0±8.66 ^b (24.80)	93.33±5.50(12.00)	85.33±10.71(2.40)	87.0±6.08(4.40)

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.

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 fractions significantly inhibited blood glucose rise when co-administered with maltose and sucrose with hexane and methanol 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.

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 in this activity include flavonoids, saponins, tannins and terpenoids (Ortiz-Andrade et al., 2007; Ishnava and Metisariya, 2018; Yoshikawa et al., 1998). Phytochemical screening of the leaf extract revealed the presence of saponins, tannins, flavonoids, alkaloids and terpenes (Okokon et al., 2007). Also, GCMS analysis of the n-hexane fraction of the leaf revealed the presence of 8,11,14-eicosatrienoic acid (Z,Z,Z), phthalic acid, diisooctyl ester, Vitamin E, γ -Elemene, Urs-12-ene, bicyclogermacrene, α -muurolene, germacrene-A, and guaiol among others (Okokon et al., 2013), while GCMS analysis of ethyl acetate fraction revealed the presence of (E)- β -ocimene, P-metha-1(7),8-diene,D:A-friedooleanan-3-ol,(3a)-,Stigmastone-3,6-dione,(5a), Bicyclo [2.2.1] heptan-2-ol, 4,7,7-trimethyl, P-cymene (Okokon et al., 2017). Polyphenolic compounds from plants are known to cause several effects on the biological systems which include enzymes inhibitions (Kaita 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 and terpenes in the leaf

fractions may suggests that their inhibitory potential on α -amylase and the membrane-bound intestinal α -glucosidase enzymes may be similar to the mechanism proposed generally for the polyphenolic compounds.

The inhibitory potentials of the leaf fractions on α -amylase and α -glucosidase activities as was observed in this study in addition to enhanced the secretion of insulin in response to glucose load and increase peripheral utilization of glucose, which are the major proposed mechanisms of antidiabetic action (Kar et al., 1999; Andrikopoulos et al., 2008), maybe responsible for the reported antidiabetic activity of *S. megaphylla* leaves.

Conclusion

The results of this study suggest that the leaf fractions of *Setaria megaphylla* have inhibitory effect on alpha amylase and alpha glucosidase activities which can be attributed to the activities of their phytochemical constituents.

Conflict of interest.

We declare that we have no conflict of interest regarding the publication of this paper.

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References

- Alongi M, Anese M. 2018. Effect of Coffee Roasting on *in vitro* α -Glucosidase activity: inhibition and Mechanism of Action. Food Research International, 111:480-487.
- Andrikopoulos S, Blair AR, Deluca N, Fam BC, Proietto J. 2008. Evaluating the glucose tolerance test in mice. American Journal of Physiology-Endocrinology and Metabolism, 295:E1323-E1332.
- Clarkson C, Maharay VJ, Crouch NR, Grace OM, Pillay P,

- Matsabisa MG, Bhagwandin N, Smith PJ, Folb PI. 2004. In vitro antiplasmodial activity of Medicinal Plants native to or naturalized in South Africa. *Journal of Ethnopharmacology*, 92:177-191.
- Esimone CO, Okonta JM, Ezugwu CO. 2001. Blood sugar lowering effect of *Anacardium occidentale* leaf extract in experimental rabbit model. *Journal of Natural Remedy*, 1:60-63.
- Funke I, Melzig MF. 2005. Effect of different phenolic compounds on α -amylase activity: screening by microplate-reader based kinetic assay. *Pharmazie*, 60(10):796-797.
- Gidado A, Watafua M, Sa'ad RS, Tagi HS, Abdullahi S. 2019. Alpha-amylase, maltase and sucrase inhibitions by aqueous leaf extracts of *Anacardium occidentale* (Anacardiaceae) and *Piliostigma reticulatum* (Caesalpiniaceae) in rats. *Tropical Journal of Natural Product Research*, 3(6):210-215
- Ibrahim MA, Koorbanally NA, Islam S. 2014. Antioxidative activity and Inhibition of Key Enzymes linked to Type 2 diabetes (α -glucosidase and α -amylase) by *Khaya senegalensis*. *Acta Pharm*, 64:311-324.
- Ishnava KB, Metisariya DM. 2018. In vitro study on α -amylase Inhibitory activity of selected ethnobotanical plant extra its and its herbal formulations. *International Journal of Pharmacognosy and Chinese Medicine*, 2(3):000136.
- Kalita D, Holm DG, LaBarbera DV, Petrash JM, Jayanty SS. 2018. Inhibition of α glucosidase, α -amylase, and aldose reductase by potato polyphenolic compounds. *PLoS ONE*, 13(1):e0191025.
- Kalra S. 2014. Alpha Glucosidase Inhibitors. *The Journal of Pakistan Medical Association*, 64(4):474-476.
- Kar A, Choudhary BK, Bandyopadhyay NG. 1999. Preliminary studies on the inorganic constituents of some indigenous hypoglycemic herbs on oral glucose tolerance test. *Journal of Ethnopharmacology*, 64(2):179-184.
- Okokon JE, Antia BS, Ita BN. 2006. Antiinflammatory and antinociceptive effects of ethanolic extract of *Setaria megaphylla* leaves in rodents. *African Journal of Biomedical Research*, 9:229 - 233.
- Okokon JE, Antia BS. 2007. Hypoglycaemic and Antidiabetic effect of *Setaria megaphylla* on normal and alloxan induced diabetic Rats. *Journal of Natural Remedy*, 1:134-138.
- Okokon JE, Dar A, Choudhary MI. 2013. Immunomodulatory, cytotoxic and antileishmanial activities of *Setaria megaphylla*. *International Journal of Phytomedicine*, 4:155-160
- Okokon JE, Okokon PJ, Sahal D. 2017. In vitro antiplasmodial activity of some medicinal plants from Nigeria. *International Journal of Herbal Medicine*, 5(5):102-109.
- Okokon JE, Otuikor JE, Davies K. 2016. Psychopharmacological study on ethanol leaf extract of *Setaria megaphylla*. *African Journal of Pharmacology and Therapeutics*, 6(4):166-172.
- Okokon JE, Ubulom PM, Udokpoh AE. 2007. Antiplasmodial activity of *Setaria megaphylla*. *Phytotherapy Research*, 21:366-368.
- Ortiz-Andrade RR, García-Jiménez S, Castillo-España P, Ramírez-Aviila G, Villalobos-Molina R, Estrada-Soto S. 2007. Alpha-Glucosidase Inhibitory Activity of the Methanolic Extract from *Tournefortia hartwegiana*: An Anti-hyperglycemic Agent. *Journal of Ethnopharmacology*, 109:48-53.
- Shirwaikar A, Rajendran K, Punitha ISR. 2005. Antidiabetic Activity of Alcoholic Stem Extract of *Coscinium fenestratum* in Streptozotocin Nicotinamide Induced Type 2 Diabetic Rats. *Journal of Ethnopharmacology*, 93:369-374.
- Van Oudtshoorn FP. 1999. Guide to Grasses of South Africa. Briza Publications, Cape Town.
- World Health Organization. Glucose analyser. Core medical equipment information. 2011. www.who.int/medical_devices/en/index.html. Assessed on 3rd June, 2019.
- Yoshikawa M, Murakami T, Yashiro K, Matsuda H. 1998. Kotalanol, a potent alpha-glucosidase inhibitor with thiosugar sulfonium sulfate structure from antidiabetic agurvedic medicine *Salacia reticulata*. *Chem Pharm Bull (Tokyo)*, 46:1339-1340.