



INHIBITORY EFFECTS OF ORANGE-FLESHED SWEET POTATO EXTRACTS ON CARBOHYDRATE-METABOLIZING ENZYMES IN HYPERGLYCEMIA

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Abstract: The inhibition of carbohydrate metabolizing enzymes is an important strategy in the management of postprandial hyperglycemia. The present study evaluated the anti-hyperglycemic effect of sweet potato by assessing its inhibitory effects on some carbohydrate enzymes linked to type 2 diabetes. The influence of the aqueous extract of orange-fleshed sweet potato tubers (OSPT) and hydromethanolic extract of orange-fleshed sweet potato leaves (OSPL) on the activities of α -glucosidase, α -amylase, sucrase and maltase were assessed using established techniques. The results showed that OSPL had higher polyphenolic content than OSPT. Both extracts showed significantly lesser inhibition and higher IC₅₀ values on the activities of all the enzymes when compared with acarbose. OSPT had the highest IC₅₀ values, 35.03 ± 1.86 , 38.38 ± 2.34 , 51.54 ± 2.76 , and 70.93 ± 1.98 mg/ml, for α -glucosidase, α -amylase, sucrase and maltase, respectively. However, OSPL showed an appreciable inhibition of all the enzymes and a significantly lower IC₅₀, 5.31 ± 0.92 , 5.4 ± 1.82 , 4.14 ± 2.86 , and 5.46 ± 3.01 mg/ml, for α -glucosidase, α -amylase, sucrase and maltase respectively. These findings indicated that the hydromethanolic extract of the leaves of orange-fleshed sweet potato could serve as a potential antidiabetic agent owing to its ability to effectively inhibit carbohydrate metabolizing enzymes but milder than acarbose.

Keywords: Diabetes mellitus, sweet potato, alpha-amylase, alpha-glucosidase, sucrase, maltase and acarbose

INTRODUCTION

Type 2 diabetes is a metabolic disorder characterized by hyperglycemia caused by insulin resistance and/or insulin deficiency (Lebovitz, 1999). One of the strategies that have been employed in the management of type 2 diabetes is the inhibition of carbohydrate metabolizing enzymes that are responsible for the breakdown and digestion of dietary carbohydrates into glucose (Chakrabarti and Rajagopalan, 2002). This therapeutic approach is adopted to control postprandial hyperglycemia, which is a hallmark in type 2 diabetes and its complications. Alpha-glucosidase, alphaamylase, sucrase and maltase are important enzymes involved in the digestion of carbohydrates into glucose (Nair *et al.*,

2013). There are synthetic drugs such as acarbose which are inhibitors of alpha-glucosidase or alpha-amylase, but the use of these synthetic drugs has been associated with gastrointestinal side effects (Kim *et al.*, 2005). There is, therefore, an increasing interest in the search for potent inhibitors of carbohydrate metabolizing enzymes from

plants and its products (Kumar *et al.*, 2011; Mogale *et al.*, 2011; Kazeem *et al.*, 2013; Kazeem *et al.*, 2015; Ramprasad *et al.*, 2016).

Sweet potato is a food crop with different varieties or cultivars being cultivated across the world. Generally, hypoglycemic effects of sweet potato and its extracts have been reported in both *in vivo* and *in vitro* studies (Surayia *et al.*, 2008; Ogunrinola *et al.*, 2015). However, the ability of sweet potato extracts to inhibit carbohydrate digestive enzymes have not been explored to determine if this is one of the mechanisms through which it exerts its hypoglycemic effects. Orange fleshed sweet potato cultivars are particularly rich in carotenoids, polyphenolic compounds and ascorbic acids which are very potent antioxidants (Alam *et al.*, 2016; Rautenbach *et al.*, 2010). Amelioration of oxidative stress by antioxidants is another strategy that is employed in the management of type 2 diabetes and its complications. The combination of antioxidant effects and the ability to inhibit carbohydrate metabolizing enzymes represents desirable attributes in the search for an ideal medicinal plant for managing type 2 diabetes (Mai *et al.*, 2007; Kaskoos, 2013; Dewi and Maryani, 2015). This study investigated the *in vitro* ability of the extracts of orange-fleshed sweet potato cultivar to inhibit alpha-glucosidase, alpha amylase, sucrase and maltase enzymes

MATERIALS AND METHODS

Plant samples: Orange fleshed sweet potato was obtained from the Department of crop science, North-West University, Mafikeng campus, South Africa. The leaves and root tubers were collected fresh from the university farm. The leaves were cut into pieces and tubers into thin chips before being air dried in the laboratory. Air dried samples were ground into fine powder and packed in an airtight container. They were labeled accordingly and stored at 4°C for subsequent use.

Preparation of extracts: Preparation of crude aqueous and aqueous-methanol (1:1) extracts of the tubers and leaves' extracts respectively was done by soaking 5g of the powder in 100ml of the respective solvents for 24 hours at room temperature with constant shaking. Extracts were recovered through lyophilization and evaporation using a freeze dryer (Alpha 1-4 LSC Plus) and a rotary evaporator (RE-52A) respectively. Dried extracts of the tubers (OSPT) and the leaves (OSPL) were stored in the dark at -20 °C for subsequent experiments.

Determination of flavonol content: The flavonol content was determined according to the method of Mazza *et al.* (1999) with slight modifications. A sample volume (12.5µl) of each extract was added to 12.5µl of 0.1% HCl in 95% ethanol and 225µl of 2% HCl in designated wells of a 96 well plate. The absorbances of the resulting solutions were read at 360nm with Multiskan™ spectrophotometer (Thermo scientific) after incubation for 30 minutes at room temperature. Quantification was based on standard curve generated from 0, 5, 10, 20, 40, 80 mg/L solutions of quercetin in 95% ethanol. Total flavonol content was calculated and expressed as mg of quercetin equivalent /g of extract.

Determination of total flavanol content: A modified method of Treutter (1989) was adopted to estimate the total flavanol content of the samples using 4-(Dimethylamino)cinnamaldehyde (DMACA) reagent. DMACA solution (0.05%) was prepared in HCl-MeOH mixture (1:3). A sample volume of 50 µl of each extract was added to 250µl of DMACA solution in the wells. Next, the plate was incubated for 30 minutes at room temperature and absorbances were read at 640nm using Multiskan™ spectrophotometer (Thermo scientific). A standard curve was prepared using 0, 1.36, 2.72, 6.8, 13.6, 27.2mg/L solutions of catechin in methanol. Total flavanol contents of the samples were extrapolated from the standard curve and expressed as mg catechin equivalent /g of extract.

Determination of total flavonoid content: The total flavonoid content was estimated spectrophotometrically (Taie *et al.* 2010) by adding 500µl of 2% aluminum chloride (AlCl₃) in ethanol to 500µl of the samples. The mixtures were incubated for 60 min at room temperature and the absorbances were measured at 420nm. Quantification of the total flavonoid contents of the samples was based on standard curve generated from 0, 10,

20, 40, 80 and 160 mg/L solutions of quercetin in 95% ethanol. The results were expressed as mg of quercetin equivalent /g of extract.

Determination of total polyphenol content: The total polyphenol contents of the cultivars were determined spectrophotometrically using Folin-Ciocalteu method with slight modification (Swain and Hillis, 1959). A sample volume of 25 µl of the reconstituted extracts of the leaves and the tubers were added to 125 µl of 10 % folin reagent in a 96well plate. After 5 min, 100 µl of 7.5 % Na₂CO₃ was added to the wells and incubated for 2 h at room temperature. The absorbances of the resulting solution were read at 765 nm. Standard curve was generated using 0, 20, 50, 100, 250, and 500 mg/l of gallic acid in 10 % ethanol. The total polyphenol content was expressed as mg of gallic acid equivalent (GAE) /g of extract.

Alpha-glucosidase, sucrase and maltase inhibition assays: The effect of the extracts on α-glucosidase activity was determined by using α-glucosidase enzyme from rat intestinal acetone powder (Adisakwattana and Chanathong, 2011). The substrates; 5mM p-nitrophenyl glucopyranoside (pNPG), 25 mM maltose and 50mM sucrose were prepared in 0.1M sodium phosphate buffer. Rat intestinal acetone powder was homogenized in 0.9% NaCl (0.3 mg/ml). The mixture was centrifuged at 12,000g for 30 min and 100µl of the crude enzymes was pre-incubated with different concentration of the leave (1, 2, 3, 4 and 5) mg/ml and tuber (10, 20, 30, 40 and 50) mg/ml extracts for 10 min. Fifty microliters (50µl) of the substrates were added to initiate the reaction while the mixture was incubated at 37°C for 30 min. The reaction was terminated by adding 2 ml of 0.1 M Na₂CO₃ and the enzymes activities were determined by measuring the absorbance of the resulting products at 405 nm (α-glucosidase) and 540 nm (maltase and sucrose). The results were expressed as percentage of the blank control. Percentage inhibition was calculated as $\% inhibition = [(A_0 - A_s)/A_0] \times 100$. A₀ and A_s are the absorbances of the control and extracts respectively. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC₅₀) were determined graphically.

α-Amylase inhibition assay: The effect of the extracts on the activity of α-amylase was determined according to the modified method of McCue and Shetty (2004). Alphaamylase solution (0.5mg/ml) was prepared in 0.02 M sodium phosphate buffer (pH 6.9). Three hundred microliters (300 µl) of the leaves (1, 2, 3, 4, and 5) mg/ml and tuber (10, 20, 30, 40, and 50) mg/ml extracts were added to 300 µl of α-amylase solution in Eppendorf tubes. The reaction mixture was incubated at 25 °C for 10 min, after which 300 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added. The solution was then incubated again at 25 °C for 10 min. The reaction was stopped by the addition of 600 µl of dinitrosalicylic acid (DNS) reagent. The tubes were incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted with 5 ml distilled water and absorbance was measured at 540 nm using a spectrophotometer. Distilled water and acarbose were used as control and standard, respectively. The α-amylase inhibitory activity was calculated as follows:

$$\% inhibition = [(A_0 - A_s)/A_0] \times 100.$$

A₀ and A_s are the absorbances of the control and extracts respectively. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC₅₀) were determined graphically.

Statistical analysis: Statistical analysis was done using GraphPad Prism 5 statistical package (GraphPad Software, USA). The data were analysed by one-way analysis of variance (ANOVA) followed by turkey's test. All the results data were expressed as mean ± standard deviation.

RESULTS

The total flavonol, total flavanol, total flavonoid, and total polyphenol contents of the extracts are shown in Table 1. The total flavonol content in OSPT was 1.01 ± 0.24 mgQE/g extract, while it was 52.53 ± 3.12 mgQE/ g extract in OSPL. The total flavanol content in OSPT was 0.82 mg CE/g extract, while in OSPL, it was 0.28 mg CE/g

extract. Furthermore, the total flavonoid contents in OSPT and OSPL were 3.31 ± 0.13 mg QE/g extract and 85.28 ± 3.27 mg QE/g extract, respectively, while in OSPL, the total polyphenol contents for OSPT and OSPL were 10.96 ± 0.66 mg AE/g extract and 152.20 ± 3.52 mg AE/g extract, respectively.

In this study, acarbose was used as the standard antidiabetic drug. Figures 1, 2, 3 and 4 show the percentage inhibition of α -glucosidase, sucrase, maltase, and α -amylase in OSPT, OSPL, and acarbose. Generally, OSPL showed a considerable inhibition, which was significantly stronger than that of OSPT ($P < 0.05$). However, both extracts showed significantly weaker inhibition of all the enzymes, compared with the standard acarbose ($P < 0.05$). The IC₅₀ values of OSPT, OSPL, and acarbose for all the enzymes are shown in Table 2. The IC₅₀ values for α -glucosidase, sucrase, and maltase in OSPT were 35.03 mg/ml, 51.54 mg/ml, and 70.93 mg/ml, while that of OSPL were 5.31 mg/ml, 4.14 mg/ml, and 5.46 mg/ml, respectively. The IC₅₀ values of α -glucosidase, sucrase and maltase activities were significantly lower in OSPL than in OSPT ($P < 0.05$).

Acarbose showed significantly lower IC₅₀ values of 0.194 mg/ml, 0.11 mg/ml, and 0.04 mg/ml for α -glucosidase, sucrase and maltase activities, respectively, compared with both extracts. Similarly, OSPL showed a more potent inhibition of the α -amylase than OSPT. The IC₅₀ values were 0.47 mg/ml, 38.38 mg/ml, and 5.40 mg/ml for acarbose, OSPT and OSPL respectively.

Table 1:

Phenolic profile of the extracts of orange-fleshed sweet potato

Extra Flavonols Flavanols Flavonoids Polyphenols

CTS (mgQE/g (mgCE/g (mgQE/g (mg AE/g extract) extract) extract) extract)

OSPT	1.01 ± 0.24	0.82 ± 0.01	3.31 ± 0.13	10.96 ± 0.66
OSPL	52.53 ± 3.12	0.28 ± 0.01	85.28 ± 3.27	152.20 ± 3.52

Values are expressed as mean $\pm$ SD ($n = 3$).

OSPT: Aqueous extract of orange fleshed sweet potato tubers OSPL: Hydromethanolic extract of orange fleshed sweet potato leaves.

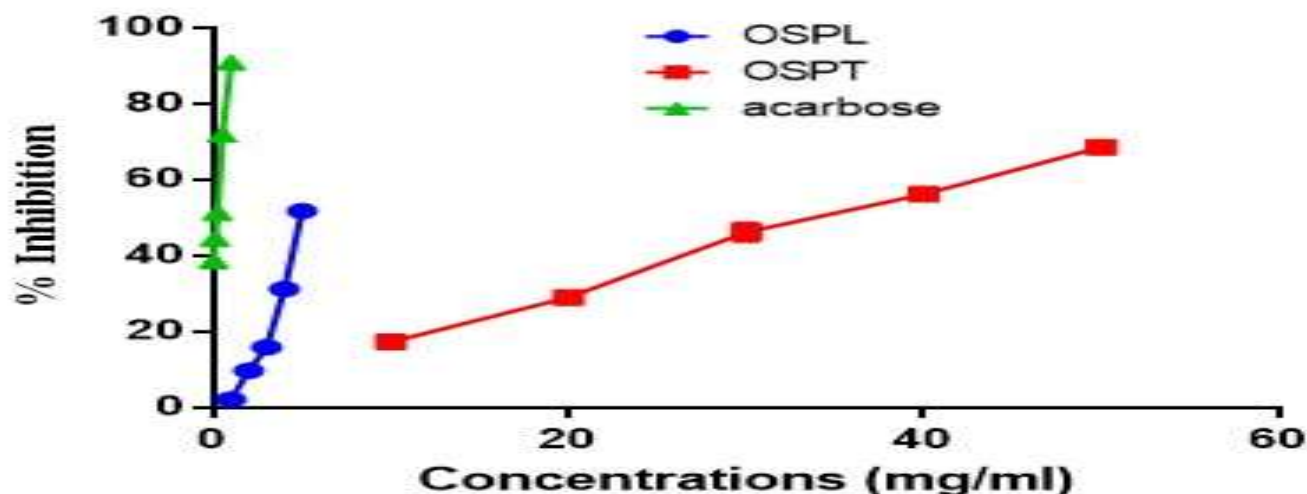


Figure 1:

Percentage inhibition of orange fleshed sweet potato extracts and acarbose on α -glucosidase.

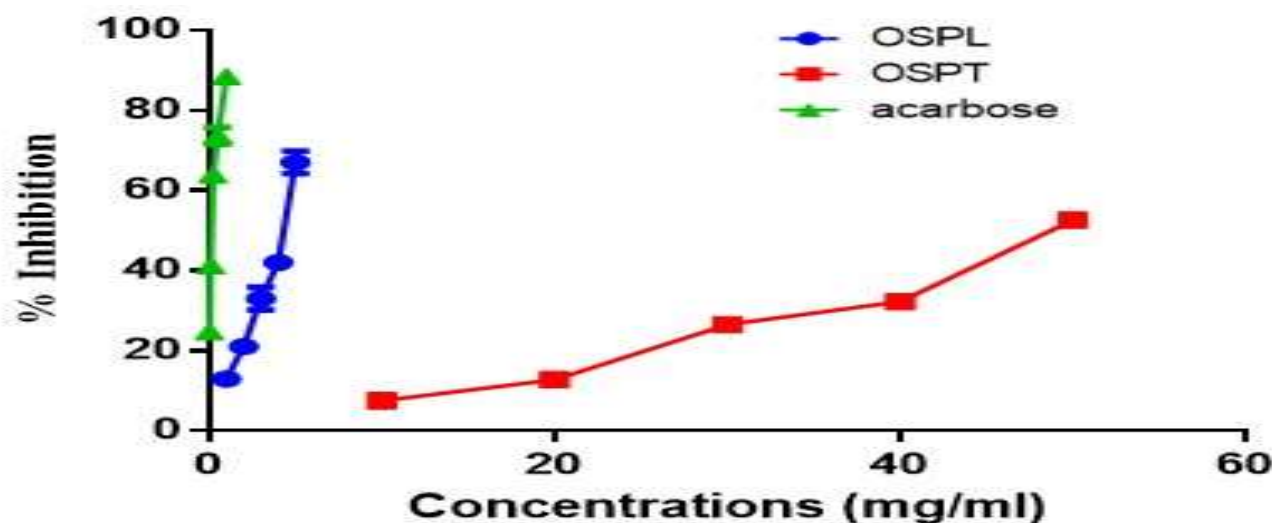


Figure 2

Percentage inhibition of orange fleshed sweet potato extracts and acarbose on sucrose.

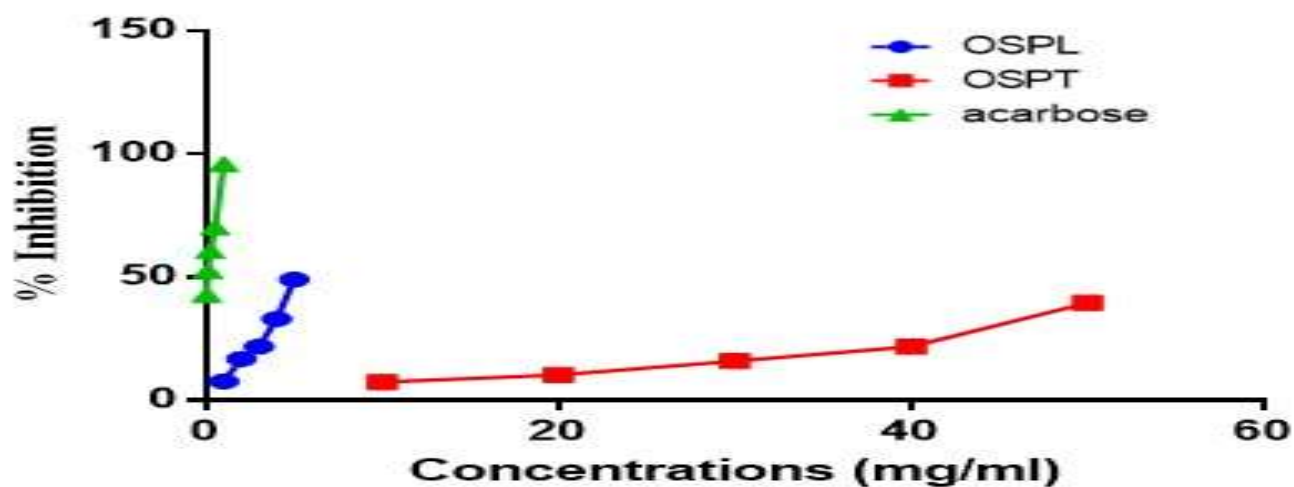


Figure 3:

Percentage inhibition of orange fleshed sweet potato extracts and acarbose on Maltase.

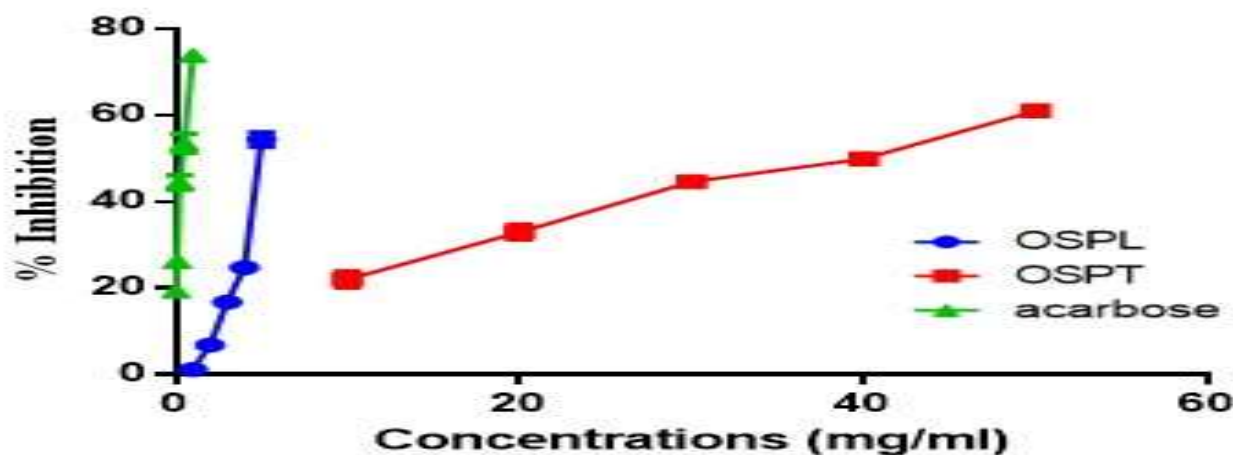


Figure 4:

Percentage inhibition of orange fleshed sweet potato extracts and acarbose on α -amylase.

Table 2:

Inhibitory potential of the extracts of orange-fleshed sweet potato and acarbose on α -glucosidase, sucrase, maltase and α -amylase.

Extracts	α -glucosidase	Sucrase	Maltase	α -amylase
OSPT	35.03 $\pm 1.86^a$	51.54 $\pm 2.76^a$	70.93 $\pm 1.98^a$	38.38 $\pm 2.34^a$
OSPL	5.31 $\pm 0.92^b$	4.14 $\pm 2.86^b$	5.46 $\pm 3.01^b$	5.4 $\pm 1.82^b$
Acarbose	0.194 $\pm 0.45^c$	0.11 $\pm 0.87^c$	0.04 $\pm 1.56^c$	0.47 $\pm 1.34^c$

The values are expressed as means $\pm$ SD of triplicate tests. Means down vertical column with different letters are significantly different ($P < 0.05$). OSPT: Aqueous extract of orange fleshed sweet potato tubers

OSPL: Hydromethanolic extract of orange fleshed sweet potato leaves

DISCUSSION

The study showed that the leaves of orange-fleshed sweet potato contained a higher level of phenolic contents than the tubers. This was consistent with previous reports that sweet potato leaves had higher phenolic content than any other part of the plant (Islam et al., 2002; Truong et al., 2007). The inhibition of carbohydrates digestive enzymes is an important strategy in the management of type 2 diabetes. The concentrations of sample to inhibit 50 % of enzyme activity were calculated as IC₅₀. IC₅₀ is a standard measure of the efficacy and performance of a therapeutic agent or candidate. It is the concentration of a drug that results into 50 % inhibition of a particular biological process (Sebaugh, 2011). The extracts of orange-fleshed sweet potato showed a considerable inhibition of the carbohydrate metabolizing enzymes, with the leaf extract demonstrating superior inhibitory activity, compared with the tuber extract. The inhibition of alpha-glucosidase, alpha amylase, sucrase, and maltase by plants have been linked to the presence of phenolic compounds (Mai et al., 2007; Miao et al., 2016). Guava (Deguchi et al., 1998; Mai et al., 2007) and green teas (Hara and Honda, 1992; Mai et al., 2007) are some of the edible plants that have shown inhibitory activities against digestive enzymes. The rate of inhibition of the enzymes is also directly correlated with the concentration of phenolic compounds present in these plants (Mai et al., 2007).

The stronger inhibition of the activities of enzymes in the leave extract could be attributed to the presence of higher concentration of phenolic compounds in the leaves than in the tubers. Orange- fleshed sweet potato cultivars are rich in flavonoids and phenolic acids that have been linked to the inhibitory actions of medicinal plants on carbohydrate metabolizing enzymes (Tadera et al., 2006; Kwon et al., 2008). Generally, the results of this study indicate that the extract of orange-fleshed sweet potato is a milder inhibitor of the carbohydrate metabolizing enzymes than acarbose. This outcome could be a desirable, as it may lead to fewer side effects typically associated with synthetic drugs, such as acarbose.

In conclusion, this study showed that the leaf extract of orange-fleshed sweet potato effectively inhibited the activities of carbohydrate metabolizing enzymes associated with diabetes mellitus. This activity can be attributed to the high content of polyphenolic compounds present in the leaves, and hence, isolating and characterizing the specific compounds responsible for this inhibition would be an important next step in exploring the antidiabetic potential of sweet potato.

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