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Original Article

In vitro α-Glucosidase and α-Amylase Activities of Wild and Cultivated *Amaranthus* spp. and Isolated Compounds

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ABSTRACT

Introduction: Diabetes mellitus is a serious metabolic disorder that affects many South Africans. There is urgent need for alternative and affordable diabetic therapy from edible wild plants which have been used by the indigenous people for many years. This study presents *in vitro* antidiabetic effects of methanol leaf extracts of wild and cultivated *Amaranthus* spp. and isolated compounds. **Methods**: The inhibitory effects against yeast α-glucosidase and porcine α-amylase were determined using a dilution series of ethanolic extracts of wild and cultivated leaf extracts and isolated compounds of *Amaranthus* spp. The aerial parts of the plant material were subjected to silica gel chromatography to yield three compounds. **Results**: Wild *A. hybridus* showed potent α-glucosidase enzyme activity (89.92±0.04%) at the lowest concentration tested (0.125 mg/mL) in comparison to acarbose which exhibited 80.20±0.13% inhibition at the same concentration. Cultivated *A. cruentus* extracts inhibited α-glucosidase enzyme activity (84.95 ± 0.04%) at the lowest concentration compared to cultivated *A. hybridus* which exhibited inhibitory activities of 72.28 ± 0.06%. In addition, three compounds namely, α-spinasterol, palmitic acid and pheophorbide A-methyl ester isolated from wild *A.cruentus* were tested for *in vitro* antidiabetic activity. Palmitic acid demonstrated the highest inhibition against α-glucosidase for all the concentrations tested. Tested separately, the isolated compounds were weaker α-amylase enzyme inhibitors however, when the compounds were combined, inhibition of α-amylase increased to 58.49± 002% at 0.5 mg/mL. **Conclusions**: Extracts and compounds of *Amaranthus* showed strong α-glucosidase activity inhibition and mild α-amylase activity inhibition suggesting that the extracts and compounds slow glucose absorption.

Key words: α-Amylase; α-glucosidase; Blood glucose; Diabetes mellitus; Hyperglycemia; Phytochemicals.

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INTRODUCTION

Amaranthus cruentus L and *Amaranthus hybridus* L which are commonly known as pigweed are widely consumed in South Africa[1] and have, in recent years, garnered interest in the academic community due to their nutritional profile. There is also overwhelming evidence supporting the medicinal claims associated with the use of Amaranth, thus there is a perceived increasing demand for the crop. For an example, the leaves of *Amaranthus hybridus* L are used in folk medicine for the treatment of diabetes in southern India[2]. Furthermore, *A. cruentus* has been reported to treat diabetes in many parts of Zambia[3].

Diabetes mellitus (DM) is a serious, long-term disease that has a significant impact on the lives and well-being of individuals, communities and societies around the world[4]. Pathogenesis of diabetes leads to adverse health problems, such as neuropathy, retinopathy and cardiovascular disorders[5]. Numerous pharmacological agents having a diverse mode of action are being used for the cure and management of DM[6]. While hormonal therapy is effective for the treatment of DM, its use is limited due to its limited actions and adverse side effects including hypoglycemia, weight loss, abdominal pain, liver damage, loss of appetite, lactic acidosis and diarrhea[7,8]. The other diabetes mellitus management strategy is based on the reduction of glucose absorption by interference in the activity of α-glucosidase and α-amylase (carbohydrate-hydrolyzing enzymes)[9]. α-Amylase hydrolyses oligosaccharides to polysaccharides, while α-glucosidase catalyzes the last step of carbohydrate hydrolysis leading to the production of absorbable monosaccharides[10]. To date, only a limited number of α-glucosidase and α-amylase inhibitors are commercially available; perhaps because their synthesis is very complex, and serious gastrointestinal complications have also been reported to be associated with their use[11].

Alternatively, food plants with promising therapeutic potential and limited side effects are now getting a lot of publicity and recognition for DM management. Studies have demonstrated that plant-based foods that naturally contain polyphenolic compounds and flavonoids have been linked to *in vitro* inhibitory activities of intestinal α-glucosidase and pancreatic α-amylase[12-14]. Some of the mostly consumed wild vegetables in South Africa have also played a vital role in the fight against DM. *Cleome gynandra* and *Citrullus lanatus* for an example were found to be potent intestinal α-glucosidase and pancreatic α-amylase inhibitors[15,16] while *Corchorus olitorius* showed higher α-amylase inhibitory ability[17,18]. *Amaranthus* spp. in particular has been subject to investigation and proved effective against DM[19-22].

Efforts should be made to promote the cultivation of Amaranth in order to meet the growing demand, provided that accurate and sufficient information is available on the health and medicinal effects of the plants. Thus, the aim of the current study was to evaluate the *in vitro* inhibitory effects of wild and cultivated *Amaranthus* leaf extracts and isolated constituents on the activities of selected diabetic related carbohydrates metabolizing enzymes, α-amylase and α-glucosidase.

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MATERIALS AND METHODS

Chemicals

α-Glucosidase (*Saccharomyces cerevisiae*), α-amylase (porcine pancreas), 3, 5, di-nitro salicylic acid (DNS), P-nitro-phenyl-α-D-glucopyranoside (p-NPG), sodium carbonate (Na_2CO_3), sodium dihydrogen phosphate, di-sodium hydrogen phosphate, acarbose and potato starch, were purchased from Merck, South Africa.

Collection of plant materials

Collection of wild vegetables from KwaZulu-Natal (KZN) Province, South Africa

Amaranthus hybridus L. leaves were bought from Esikhawini informal market during the month of April of 2018. Esikhawini is a peri-urban settlement situated in the district of Richards bay in KwaZulu-Natal. The area is characterized by 1087 mm of rain per year, with most rainfall occurring mainly during summer. Esikhawini receives the lowest rainfall in June (42 mm) and the highest in March (133 mm) (Esikhawini climate, Map of South Africa, ND)[23]. The leaves of *Amaranthus cruentus* L. were purchased from Stanger which is situated in KwaDukuza. Stanger normally receives about 866 mm of rain per year, most of which occurs during the summer (Stanger climate, Map of South Africa, ND)[24]. Wild Amaranth plants collected from KwaZulu-Natal were confirmed by Prof. Alfred Zobolo, a botanist at the University of Zululand.

Planting of *A. cruentus* and *A. hybridus*

Amaranthus cruentus and *A. hybridus* were grown in November of 2017 at Mothong African Heritage Trust garden in Mamelodi, Pretoria (GPS co-ordinates: 25°41′49.7″S 28°20′17.4″E) a community-based project interested in cultivation of African leafy vegetables (ALVs) and medicinal plants. The seeds were donated by the Agriculture Research Council-Vegetable and Ornamental Plants (ARC-VOP). Prior to planting, the seeds were sown in 98 cavity seedling trays using hygromix as a growth medium and kept under a 40% shade net. Seedlings were transplanted to the field 21 days after emergence. The trial was planted with a spacing of 10cm x 20cm (50 plants m^{-2})[25]. Limestone ammonium nitrate (LAN) (containing 28% nitrogen, calcium and magnesium) fertilizer was applied on the freshly prepared soil on the day of planting the seedlings. Plants were irrigated once a week.

Extraction and purification of chemical constituents

For the analysis of extracts, fifty grams (50 g) of dried plant materials were ground to a fine powder. Each plant sample was soaked overnight at room temperature in 50 mL of 100% methanol (MeOH). The solvent was then removed and replaced with the same solvent volume. This procedure was repeated two times. Extracts were filtered and concentrated to dryness under reduced pressure using a rotary evaporator (BUCHI-Labotech, Switzerland). Dried extracts were stored in the dark at room temperature until analysis.

The aerial parts of the plant material (1 kg) were blended and extracted with methanol and water mixture (80:20) (5 L) at room temperature (25 °C) for 24 h. The methanolic water extract was filtered and evaporated to dryness under reduced pressure at 40 °C to yield 63.9 g (6.39%). The total extract (63.9 g) was applied to a silica gel column (10 x 20 cm) and eluted using a gradient of hexane (Hex) and ethyl acetate (EtOAc) in order of increasing polarity: 36 fractions (500 mL each) were collected and combined according to their TLC profiles to yield thirteen fractions. Out of the thirteen combined fractions, fraction one yielded two compounds, palmitic acid (22.2 mg) and α-spinosterol (48.9 mg). In addition, fraction four yielded one compound, pheophorbide A-methyl ester (124 mg), a derivative of chlorophyll which was isolated from *A. cruentus* for the first time.

In vitro antidiabetic assays

α-Glucosidase inhibitory activity

The inhibitory activity of plant extracts against intestinal α-glucosidase was determined following the method proposed by Telagari and Hullatti[5]. The concentration of plant extracts ranged from 0.125 to 2 mg/mL. A negative control was prepared using the same procedure however, the plant extract was replaced with phosphate buffer (100 mM, pH = 6.8). A reaction mixture containing 50 µL phosphate buffer, 10 µL alpha-glucosidase (1 U/mL), and 20 µL of varying extract concentrations was pre-incubated at 37 °C for 15 minutes in a 96-well plate. Acarbose (positive control) and isolated compounds were tested at varying concentrations from 0.062–1 mg/mL. In addition, 20 µL P-NPG (5 mM) was then added as a substrate and further incubated at 37 °C for 20 min. The reaction was stopped with the addition of 50 µL Na_2CO_3 (0.1 M). The assay was performed in triplicate. The absorbance of the released p-nitrophenol was measured at 405 nm with a Thermo Scientific Varioskan Flash Spectrophotometer. The results were expressed as percentage inhibition, which was calculated using the formula,

Inhibitory activity (%) = (1 − As/Ac) ×100

Where,

As is the absorbance in the presence of test substance and Ac is the absorbance of control.

α-Amylase inhibitory activity

The assay was adapted from Telagari and Hullatti[5] wherein the concentration of plant extracts ranged from 0.125 to 2 mg/mL. The concentrations of the positive drug control Acarbose and isolated compounds ranged from 0.062-1 mg/mL. A negative control was prepared using the same procedure however, the plant extract was replaced with phosphate buffer (100 mM, pH = 6.8). The reaction mixture containing 50 µL phosphate buffer, 10 µL α–amylase (2 U/mL), and 20 µL extract was pre-incubated for 20 minutes at 37 °C in a 96-well plate. One percent of a soluble potato starch (100 mM phosphate buffer pH 6.8) was then incorporated as a substrate and further incubated at 37 °C for 30 minutes. A colour reagent, DNS (100 µL) was then added and boiled for 10 minutes. The absorbance of the resulting mixture was measured at 540 nm using a Thermo Scientific Varioskan Flash Spectrophotometer. The results were expressed as percentage inhibition, which was calculated using the formula,

Inhibitory Activity (%) = (1 − As/Ac) ×100

Statistical analysis

All the analyses were carried out in triplicate and the results are expressed in mean ± SD. All of the data analyses were carried out using Microsoft Excel 2010. Moreover, IC_{50} for the extract was calculated by plotting extract concentration against % inhibition. The IC_{50} values were only calculated for only wild *A. cruentus* since all the other samples and a positive control showed activity beyond 50%.

RESULTS

Chemical characterization of the isolated compounds

All the structures of isolated compounds (**1**-**3**) are illustrated in Figure 1. Identification of the isolated compounds was achieved by comparing their spectroscopic data with those reported in literature. All the NMR data of all isolated compounds are illustrated in the Supplementary material.

Characterization of α-spinasterol (1)

A colorless crystalline solid. ^{1}H-NMR ($CDCl_3$, 600 MHz): 0.53 (3H, s, H-18), 0.78-0.79 (6H, m, H-27, H-29), 0.83 (3H, s, H-19), 0.84 (3H, d, *J*

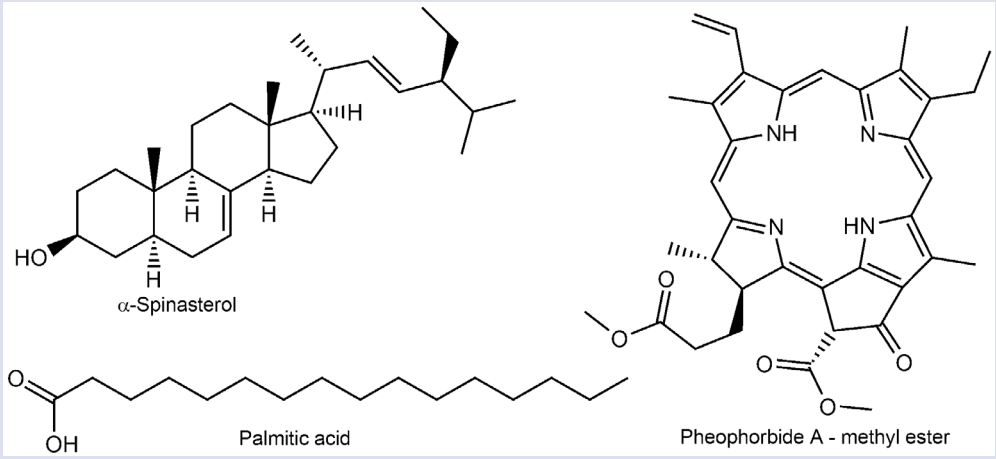

Figure 1: Chemical structures of isolated compounds.

Table 1: Effect of plant extracts and isolated compounds on the inhibition of α-glucosidase enzyme.

α-glucosidase (% inhibition)	IC_{50} mg/mL	0.062 mg/mL	0.125 mg/mL	0.25 mg/mL	0.5 mg/mL	1 mg/mL	2 mg/mL
A.cruentus wild	0.144	N/T	41.85±0.10	55.96±0.13	61.85±0.13	78.12±0.34	87.13±0.18
***A. cruentus*, cultivated**	ND	N/T	84.95±0.09	85.46±0.07	81.63±0.02	77.40±0.11	61.61±0.05
***A. hybridus* wild**	ND	N/T	89.92±0.04	93.98±0.03	90.91±0.24	96.32±0.05	97.10±0.05
***A. hybridus*, cultivated**	ND	N/T	72.28±0.06	76.59±0.23	67.11±0.25	79.19±0.13	63.85±0.16
α-Spinasterol	ND	67.41±0.10	70.82±0.02	72.91±0.04	61.13±0.08	80.06±0.04	N/T
Palmitic acid	ND	91.26±0.01	89.67±0.06	90.51±0.01	85.92±0.02	83.26±0.03	N/T
Pheophorbide A-methyl ester	ND	53.16±0.43	68.08±0.30	70.78±0.11	75.41±0.03	65.22±0.06	N/T
Acarbose	ND	72.72±0.24	80.20±0.13	73.41±0.16	66.31±022	68.18±011	N/T

NT: not tested
ND: not determined

Table 2: Effect of plant extracts and isolated compounds on the inhibition of α-amylase enzyme.

α-amylase (% inhibition)	0.062 mg/mL	0.125 mg/mL	0.25 mg/mL	0.5 mg/mL	1 mg/mL	2 mg/mL
A. cruentus wild	N/T	34.70±0.17	39.63±0.05	34.25±0.05	31.62±0.01	23.47±0.02
***A. cruentus*, cultivated**	N/T	30.46±0.07	25.57±0.14	23.39±0.12	19.74±0.14	24.03±0.12
***A. hybridus*, wild**	N/T	5.67±0.04	20.56±0.06	22.20±0.03	18.87±0.05	27.47±0.13
***A. hybridus*, cultivated**	N/T	33.18±0.14	13.29±0.11	15.24±0.23	24.58±0.46	-7.55±0.29
α-Spinasterol	27.73±0.17	22.86±0.10	28.22±0.12	13.06±0.23	43.37±0.15	N/T
Palmitic acid	18.68±0.07	21.340.08	25.05±0.02	21.99±0.03	21.16±0.07	N/T
Pheophorbide A-methyl ester	49.84±0.04	11.50±0.19	7.23±0.12	14.15±0.15	23.31±0.40	N/T
Combined compounds	57.45±0.04	49.32±0.04	48.69±0.05	58.49±0.02	53.65±0.04	N/T
Acarbose	71.37±0.01	83.41±0.01	86.29±0.02	87.22±0.04	89.00±0.05	N/T

NT: not tested

= 6.0 Hz, H-26), 1.01 (3H, d, *J* = 6.5 Hz, H-21), 3.58 (1H, m, H-3), 5.01 (1H, dd, *J* = 15.0, 8.5 Hz, H-23), 5.12 (1H, m, H-7), 5.15 (1H, dd, *J* = 15.0, 8.5 Hz, H-22). ^{13}C-NMR ($CDCl_3$, 600 MHz): 12.05 (C-18), 12.22 (C-29), 13.02 (C-19), 19.00 (C-27), 21.06 (C-21), 21.37 (C-26), 21.56 (C-11), 23.02 (C-15), 25.38 (C-28), 28.48 (C-16), 29.69 (C-6), 31.48 (C-2), 31.87 (C-25), 34.23 (C-10), 37.16 (C-1), 38.00 (C-4), 39.48 (C-12), 40.29 (C-5), 40.78 (C-20), 43.30 (C-13), 49.48 (C-9), 51.26 (C-24), 55.13 (C-14), 55.94 (C-17), 71.06 (C-3), 117.45 (C-7), 129.47 (C-23), 138.14 (C-22), 139.57 (C-8).

Characterization of palmitic acid (2)

A slightly yellow coloured oily like semisolid compound. ^{1}H-NMR ($CDCl_3$, 600 MHz): 0.86 (3H, t, *J* = 6.7), 1.26 (28H, overlapping CH_2), 2.33 (2H, t, *J* = 6.7). ^{13}C-NMR ($CDCl_3$, 600 MHz): 14.67, 22.66; 24.68, 29.05, 29.22, 29.33, 29.41, 29.57, 29.57, 29.61, 29.63, 29.64, 29.66, 29.67, 31.90, 34.00, 179.69.

Characterization of pheophorbide A-methyl ester (3)

Pheophorbide A-methyl ester, a dark-green amorphous solid.^{1}H-NMR ($CDCl_3$, 600 MHz): 1.65 (3H, t, *J* = 7.8 Hz, H-31), 1.81(3H, d, *J* = 7.2 Hz, H-25), 2.31 (2H, m, H-2), 2.53 (2H, m, H-1), 3.15 (3H, s, H-29), 3.36 (3H, s, H-26), 3.57 (3H, s, H-36), 3.59 (2H, q, *J* = 7.6 Hz, H-30), 3.65 (3H, s, H-32), 3.87 (3H, s, H-34), 4.20 (1H, m, H-3), 4.45 (1H, dq, *J* = 7.2, 1.8 Hz, H-4), 6.12 (1H, dd, *J* = 11.4, 1.2 Hz, Ha-28), 6.14 (1H, dd, *J* = 17.9, 1.2 Hz, Hb-28), 6.24 (1H, s, H-21), 7.92 (1H, dd, *J* = 17.9, 11.4 Hz, H-27), 8.53 (1H, s, H-6), 9.28 (1H, s, H-11), 9.44 (1H, s, H-16). ^{13}C-NMR ($CDCl_3$, 600 MHz): 11.13 (C-29), 12.02 (C-26), 12.05 (C-32), 17.33 (C-31), 19.38 (C-30), 23.03 (C-25), 29.63 (C-2), 31.06 (C-1), 50.10 (C-4), 51.13 (C-3), 51.61 (C- 36), 52.79 (C-34), 64.70 (C-21), 93.08 (C-6), 97.48 (C-11), 104.36 (C-16), 105.26 (C-22), 122.69 (C- 28), 128.93 (C-19), 129.00 (C-27), 129.02 (C-18), 131.80 (C-8), 136.14 (C-9), 136.19 (C-10), 136.48 (C-13), 137.91 (C-17), 142.03 (C-7), 145.15

(C- 14), 149.64 (C-24), 150.91 (C-15), 155.58 (C-12), 161.18 (C-23), 169.54 (C-33), 172.14 (C-5), 173.30 (C-35), 189.56 (C-20).

α-Glucosidase inhibitory activity

Wild *A. hybridus* and cultivated *A. cruentus* showed strong inhibitory activities when compared to the positive control Acarbose (Table 1). The inhibition percentage of extracts against α-glucosidase enzyme ranged from 61.61±-97.10±% to 97.10±0.05% at the highest concentration tested (2 mg/mL). The extracts of wild grown *A. cruentus* showed weakest inhibition (41.85±0.10%) against α-glucosidase at the lowest concentration tested (0.125 mg/mL) whereas wild grown *A. hybridus* showed potent inhibition (89.92±0.04%) at the same concentration. Acarbose, which was used as a standard reference drug exhibited 80.20±0.13% and 72.72±0.24% inhibition against α-glucosidase at 0.125 mg/mL and 0.062 mg/mL respectively (Table 1). All isolated compounds inhibited α-glucosidase enzyme beyond 50%. Palmitic acid showed the highest inhibitory activity towards α-glucosidase at 83.26±0.03% and 91.26±0.01% at both high and low concentrations respectively.

α-Amylase Inhibitory Activity

In vitro α-amylase inhibitory results demonstrated that none of the crude *Amaranthus* spp. leaf extracts screened inhibited pancreatic α-amylase beyond 50% (Table 2). The percent inhibition of all tested extracts ranged from -7.55±0.29 to 34.70±0.17% at 2 mg/mL and 0.125 mg/mL respectively. Amongst the cultivated species, although not significant, *A. cruentus* had the highest α-amylase inhibition of 23.47±0.02% at 2 mg/mL. Wild grown *A. cruentus* had the highest α-amylase inhibition of 34.70±0.17% at 0.125 mg/mL compared to wild *A. hybridus* which only inhibited α-amylase enzyme by 5.67±0.04%. Acarbose, a known antidiabetic drug, had an enzyme inhibition ranging from 71.37±0.01 (0.062 mg/mL) to 89.00±0.05% (1 mg/mL) (Table 2). α-Spinosterol, palmitic acid and pheophorbide A-methyl ester were tested as individual compounds and none of these isolated compounds inhibited α-amylase enzyme beyond 50%. On the contrary, when the compounds were combined and tested against the activity of α-amylase, the compounds showed α-amylase inhibition (58.49±002%) at 0.5 mg/mL. At the same concentration (0.5 mg/mL), Acarbose, a positive antidiabetic drug, exerted the high inhibitory activity of 87.22±004% (Table 2).

DISCUSSION

Amaranth is widely used for its nutritional as well as medicinal properties, including its antidiabetic activity. The effect of genetic makeup and geographical distribution, however have not received attention, which is crucial for promoting cultivation of Amaranth species to improve nutritional status amongst communities.

This study is the first report on *A. hybridus*'s high α-glucosidase inhibitory activity. The highest α-glucosidase enzyme activity observed in *A. cruentus* in this study is in tandem with the work reported by Oboh[26] where A. *cruentus* inhibited α-glucosidase activity *in vitro*. Other species of *Amaranthus* have also been evaluated for α-glucosidase inhibitory activity. For example; the methanol extract of *A. spinosus* was a strong α-glucosidase inhibitor at 8.49 μM/mL[27]. This study also compared the behavior of wild and cultivated *Amaranthus* spp. on α-glucosidase enzyme inhibition. Wild *A. cruentus* was less active at lowest concentration compared to its cultivated counterpart with inhibitory activities of 41.85±0.10% and 84.95±0.09 at 0.125 mg/mL respectively. The effective dose required for wild *A. cruentus* was concentration dependent which meant that the plant's effectiveness increased with an increase in concentration. As a result, it was possible to calculate the plant's IC_{50} = 0.144 mg/mL. This was not the case with cultivated *A. cruentus*. There was no relationship between the concentration level and inhibition which means, as the concentrations of cultivated *A. cruentus* increased, the enzyme inhibition presented no increase. Similar findings were presented in another study which examined α-glucosidase inhibitory effects of different fractions of three species of Labiatae extracts. The study reported no relationship between the concentration level (in some fractions) and enzyme inhibition[28]. *Amaranthus hybridus* showed opposite results whereby wild samples showed potent α-glucosidase inhibitory activity at all concentrations tested, with 89.92±0.04% inhibition recorded at the lowest concentration (0.125 mg/mL). In contrast, the best activity for the cultivated *A. hybridus* (79.19±0.13%) was obtained at a high extract concentration of 1 mg/mL. The results for *A. hybridus* correlate with findings from Chauhan and colleagues[29] which also showed that the wild variety of the plant shows more inhibition as compared to the cultivated *Rauwolfia serpentine*.

With regard to α-amylase enzyme inhibition, all the extracts (wild and cultivated) of *A. hybridus* and *A. cruentus* were weak inhibitors. This is in line with earlier reports which showed that plant phytochemicals of *A. cruentus* and *Zea mays* were mild α-amylase inhibitors [26,30]. Contrary to this study, although the extracts were tested at higher concentrations (3 mg/mL), Odhav and colleagues[31] reported that *A. hybridus* inhibited α-amylase enzyme by 52.76%[31]. Moreover, in another study, *A. cruentus* exhibited the highest inhibitory value of 55.40% at plant extract concentration of 100 mg/Ml[32]. In a separate study, although the authors reported on different species than the ones utilized in this study, Conforti and colleagues[33] demonstrated that methanol, ethyl acetate and hexane extracts from two varieties of *A. caudatus* seeds showed α-amylase inhibitory activity (above 80% inhibition rate) at 0.25-1 mg/mL[33].

The combined ethyl acetate extract of the leaves of *A. cruentus* yielded α-spinasterol[34], palmitic acid[35], and pheophorbide A-methyl ester[36]. Pheophorbide A-methyl ester has been isolated from *A. cruentus* for the first time. α-Spinasterol inhibited α-glucosidase (80.06±0.04%) at highest concentration tested (1 mg/mL). Several studies have shown promising results for α-spinasterol in various antidiabetic assays. In a study by Chukwujekwu and co-workers[37], α-spinasterol isolated from the leaves of *Buddleja saligna* showed α-glucosidase enzyme inhibition with IC_{50} value of 10.7 μg/mL. In another study, α-spinasterol was reported as a moderate inhibitor of α-glucosidase (IC_{50} = 200 μM)[38]. In a different antidiabetic assay model, α-spinasterol was found to ameliorate the development and progression of diabetic nephropathy in streptozotocin-induced diabetic mice.

There are no previous reports, about the inhibitory activity of pheophorbide A-methyl ester on α-glucosidase and α-amylase *in vitro*. The best activity (75.41±0.03%) was obtained at 0.5 mg/mL against α-glucosidase. This is in support of results reported by Kim et al. (2019) that pheophorbide A, an unesterified analog isolated from *Gelidium amansii* caused significant decrease in the activity of α-glucosidase and α-amylase when compared to Acarbose[40]. Palmitic acid showed the best α-glucosidase activity in all the concentrations tested ranging from 324.69-355.89 μM. These results are comparable with findings reported by Cherigo and Martínez-Luis (2018) in which palmitic acid showed α-glucosidase inhibitory activity of 237.5 μM[41]. The authors reported that palmitic acid inhibited the activity of α-glucosidase enzyme and its activity was similar to acarbose (241.6 μM). In the same study, the authors established that palmitic acid acted as a competitive inhibitor and that the compound binds to the same site as acarbose does in the human intestines[41]. In another study, it was found that palmitic acid showed a moderate inhibitory effect on both α-amylase and α-glucosidase activities at concentrations of 3000 μM and 750 μM respectively[42].

Pheophorbide A-methyl ester; palmitic acid and α-spinasterol were weak inhibitors of the α-amylase enzyme. However, when the

compounds were tested as a mixture, their activity was enhanced. Thus, the study proposes that the synergistic effects as well as individual activity of phytochemicals in vegetables are responsible for their potent antidiabetic activities, and that the benefit of a diet rich in vegetables is attributed to the complex mixture of phytochemicals present in whole foods. Although the mechanism of action for enzyme inhibition was not determined in this study, it is possible that compounds isolated from *A. cruentus* can either bind to the enzyme at a catalytic site[43] or can act as a non-competitive inhibitor[44,45]. In other cases, compounds can act as competitive inhibitors[46].

CONCLUSION

In line with an old adage by Hippocrates, "Let food be thy medicine, and medicine thy food", an inclusion of *Amaranthus* crop in people's diets, not only provides minerals and micronutrients, but according to the findings of this study, the crop has medicinal properties. Strong α-glucosidase activity inhibition and mild α-amylase activity inhibition of *Amaranthus* extracts and compounds could address the major disadvantages of currently used α-glucosidase and α-amylase inhibitors which are associated with side effects such as abdominal discomfort, flatulence, and possibly diarrhea. The high α-glucosidase and low α-amylase enzyme inhibition observed in this study is beneficial since excessive inhibition of α-amylase enzyme might result in abdominal discomfort due to undigested starch linked to high α-amylase inhibitory activity. The findings of this study showed that effective, cost-saving therapy using traditional vegetables could be a means of reducing untreated diabetes problems. This study is the first to report the isolation of pheophorbide A-methyl ester from *Amaranthus* spp. as well as its α-glucosidase inhibiting activity.

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APPENDIX

Supporting information consisting the NMR spectra of the isolated compounds are included in the appendix file.

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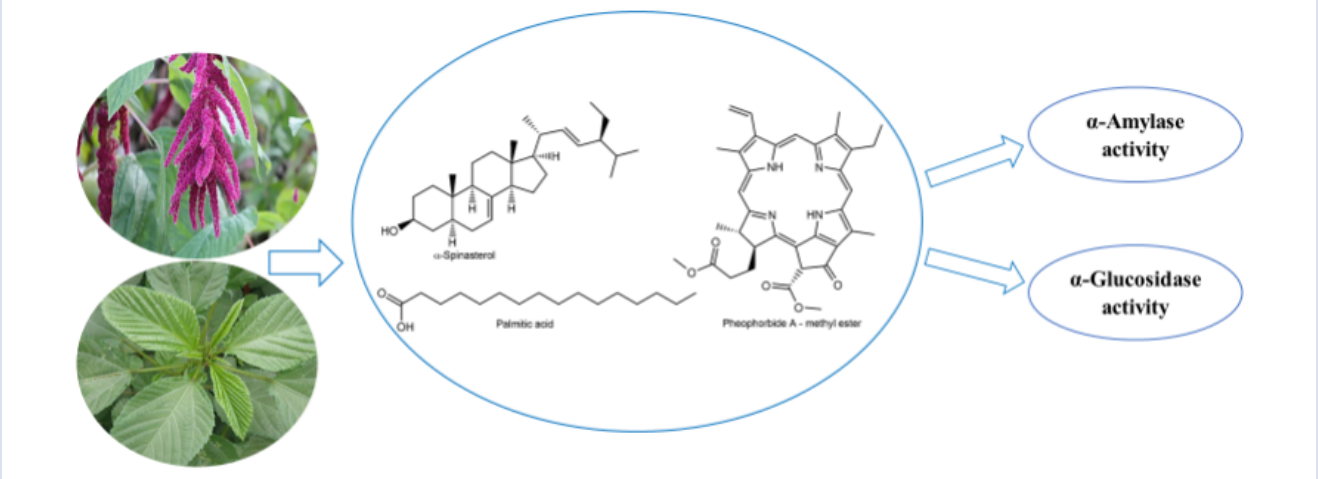
GRAPHICAL ABSTRACT



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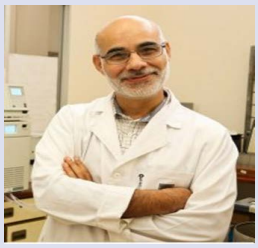

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