

Full Length Research Paper

Inhibition of α -amylase and α -glucosidase activities by ethanolic extract of *Amaranthus cruentus* leaf as affected by blanching

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This study investigated the inhibitory effect of *Amaranthus cruentus* leaf on key enzyme linked to type-2 diabetes (α -amylase and α -glucosidase) as well as assessing the effect of blanching (a commonly practiced food processing technique) of the vegetable on these key enzymes. Fresh leaves of *A. cruentus* were blanched in hot water for 10 min, and the ethanolic extracts of both the fresh and blanched vegetables were prepared and used for subsequent analysis. The inhibitory effect of the extract on α -amylase and α -glucosidase activities as well as some antioxidant parameter was determined *in vitro*. The result revealed that extract of unprocessed *A. cruentus* leaf reduce Fe^{3+} to Fe^{2+} and also inhibited α -amylase and α -glucosidase activities in a dose dependent manner. However, blanching of the leafy vegetables caused a significant ($P < 0.05$) increase in the antioxidant properties but decreased their ability to inhibit α -amylase and α -glucosidase activities. This antioxidant properties and enzyme inhibition could be part of the mechanism by which they are used in the treatment/prevention of type-2 diabetes. However, the blanched vegetable reduced their ability to inhibit both α -amylase and α -glucosidase activity *in vitro*.

Key words: *Amaranthus cruentus*, blanching, antioxidants, α -amylase, α -glucosidase.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to insulin deficiency and/or insulin resistance resulting in excess blood sugar (Beverly and Eschwège, 2003). Plants and herbal preparations have been used from ancient times for the treatment of diabetes mellitus and are still used in traditional medicine. There are several reports of wide range of plants and plants constituents that are active hypoglycemic agents (Hilary et al., 1998; Jalalpure et al., 2004; Onyeche and Kolawole, 2005). A sudden rise in blood glucose levels, causing hyperglycemia in type 2

diabetes patients happens due to hydrolysis of starch by pancreatic α -amylase and uptake of glucose by intestinal α -glucosidases (Kwon et al., 2007). The inhibition of enzymes involved in the breakdown of starch (α -amylase) and uptake of glucose (α -glucosidase) has been suggested to be a useful approach to the management and prevention of type 2 diabetes and dietary phytochemicals, have promising potential (Kwon et al., 2007). Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starch from being absorbed by the body. Starches are

complex carbohydrates that cannot be absorbed unless they are first broken down by the digestive enzyme amylase and other secondary enzymes (Ranilla et al., 2010; El-kaissi and Sherbeeni, 2011). In recent years, secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as sources of medicinal agents (Asgarpanah and Ramezanloo, 2012; Nasri et al., 2012). Vegetables contain compounds that are valuable antioxidants and protectants; the main protective action of vegetables has been attributed to the presence of antioxidants, especially antioxidant vitamins including ascorbic acid, α -tocopherol, β -carotene and phenolics (Obboh and Rocha, 2007).

However, numerous studies have conclusively shown that the majority of the antioxidant activity may be from compound such as flavonoids, isoflavone, flavones, anthocyanin, catechin and isocatechin, rather than vitamins C, E and β -carotene (Obboh and Rocha, 2007). Several green leafy vegetables with high phenolic contents abound in tropical Africa, they are utilized either as condiments or spices in human diets (Akindahunsi and Obboh, 1999); these vegetables could be harvested at all stages in the process of growth, and could be fed upon in fresh, processed, or semiprocessed forms (Obboh and Akindahunsi, 2004). They are very rich sources of β -carotene, ascorbic acid, minerals and dietary fiber (Makobo et al., 2010). Epidemiological analyses in a large Chinese population have revealed that consumption of vegetables is inversely associated with the risk of type 2 diabetes (Tang et al., 2008).

In Nigeria, unlike fruits, green leafy vegetables are not usually consumed in their fresh form; however, they are usually blanched before consumption or in soup preparation (Akindahunsi and Obboh, 1999). Blanching inactivates the enzyme action, sets the colour, and shortens the drying and dehydration time (Obboh and Akindahunsi, 2004). *Amaranthus* spp. leaves are an excellent source of protein (Kadoshnikov et al., 2005), they have also been reported to contain considerable high calcium, iron and phosphorus (Makobo et al., 2010). The vegetable has been reported to have a high concentration of antioxidant components (Hunter and Fletcher, 2002). Losses of antioxidant components from vegetables during cooking have been reported elsewhere (Chu et al., 2000; Yadav and Sehgal, 1995).

Amaranthus extract has been shown to possess antidiabetic activity in both alloxan and streptozotocin diabetic animals (Tang et al., 2008; Nwozo et al., 2004). Although a lot had been reported on the chemical characterization of phytoconstituents and antidiabetic properties of *Amaranthus* spp., limited information is available on the possible mechanism by which they render their antidiabetic properties. Hence, this study sought to investigate the inhibitory effect of *A. cruentus* on key enzyme linked to type-2 diabetes (α -amylase and α -glucosidase) as well as assessing the effect of blanching (a commonly practiced food processing technique) on

these key enzymes.

MATERIALS AND METHODS

Sample collection

Fresh samples of *A. cruentus* were sourced from the University garden of The Federal University of Technology, Akure. Authentication of the vegetables was carried in the Department of Biology, Federal University of Technology, Akure, Nigeria.

Chemicals

Chemicals and reagents used such as Hog pancreatic α -amylase, gallic acid, Folin-Ciocalteu's reagent, dinitrosalicylic acid, α -glucosidase, and *p*-nitrophenyl- α -D-glucopyranoside were procured from Sigma-Aldrich, Inc., (St Louis, MO), trichloroacetic acid (TCA), quercetin, DPPH (1,1-diphenyl-2-picrylhydrazyl) were sourced from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany), sodium carbonate, methanol, $AlCl_3$ (aluminium chloride), potassium acetate, potassium ferricyanide, ferric chloride and starch were of analytical grade while the water was glass distilled.

Preparation of 70% ethanol extract

The inedible parts of the vegetables were removed from the edible parts by hand picking. The edible parts were thoroughly washed in tap water to remove any dirt, and chopped into small pieces by table knife. A portion of the chopped vegetables was then blanched for 10 min at 80°C, while the other portion was not. The blanched portion was then drained of water. Both portions were then sun dried and milled to be obtained in a powder form. The powder was extracted with 70% ethanol then, the extract was filtered with Whatman filter paper and the filtrate was concentrated under reduced pressure using a freeze dryer to give a solid extract. The concentrated extract was further lyophilized. Then, the vegetable extract was reconstituted in distilled water and used for subsequent analysis.

α -Amylase inhibition assay

The α -amylase inhibitory activity was determined according to the method of Bernfield (1951). Appropriate dilutions of the vegetable extracts (500 μ l) and 500 μ l of 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L NaCl) containing Hog pancreatic α -amylase (EC 3.2.1.1) (0.5 mg/ml) were incubated at 25°C for 10 min. Then, 500 μ l of 1% starch solution in 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L NaCl) was added to the reacting mixture. Thereafter, the reaction mixture was incubated at 25°C for 10 min and stopped with 1.0 ml of dinitrosalicylic acid (DNSA). The mixture was then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water, and absorbance measured at 540 nm in the UV-Visible spectrophotometer (Model 6305; Jenway, Barlo world Scientific, Dunmow, United Kingdom). Then, the α -amylase inhibitory activity was calculated as percentage inhibition.

$$\% \text{ Inhibition} = [(Abs_{Ref} - Abs_{Samples}) / Abs_{Ref}] \times 100$$

α -Glucosidase inhibition assay

The α -glucosidase inhibitory activity was determined according to

the method of Apostolidis et al. (2007). Appropriate dilution of the vegetable extracts (50 μ l) and 100 μ l of α -glucosidase solution was incubated at 25°C for 10 min. Thereafter, 50 μ l of 5 mmol/L *p*-nitrophenyl- α -D-glucopyranoside solution in 0.1 mol/l phosphate buffer (pH 6.9) was added. The reacting mixture was then incubated at 25°C for 5 min before reading the absorbance at 405 nm in the UV-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom). Then, the percentage of α -glucosidase inhibitory activity was calculated from inhibition.

$$\% \text{ Inhibition} = [(Abs_{Ref} - Abs_{Samples}) / Abs_{Ref}] \times 100$$

Determination of total phenol content

The total phenol content was determined according to the method of Singleton et al. (1999). Briefly, appropriate dilution of the vegetable extracts were oxidized with 2.5 ml 10% Folin-Ciocalteu's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm in the UV-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom). Gallic acid solution (0.01 to 0.1 mg/ml) was used as standard curve and then, the total phenol content was subsequently calculated as gallic acid equivalent.

Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Meda et al. (2005). Briefly, 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 μ l 10% AlCl₃, 50 μ l 1 M potassium acetate and 1.4 ml water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm in the UV-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom). Quercetin solution (0.01 to 0.1 mg/ml) was used as standard curve and then, the total flavonoid content was subsequently calculated as quercetin equivalent.

Determination of reducing property

The reducing property of the vegetable extracts was determined by assessing the ability of the extract to reduce FeCl₃ solution as described by Oyaizu (1996). 2.5 ml aliquot was mixed with 2.5 ml 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 2.5 ml 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml 0.1% ferric chloride. The absorbance was measured at 700 nm in the UV-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom). Then, the ferric reducing antioxidant property was subsequently calculated as ascorbic acid equivalent.

Statistical analysis

The result of three replicate experiments were pooled and expressed as mean \pm standard deviation. A one-way analysis of variance (ANOVA) and positive analysis was done using Duncan multiple test. Significance was accepted at $P \leq 0.05$ (Zar, 1984).

RESULTS

First, the ability of *A. cruentus* leaf extract to inhibit α -

amylase activity *in vitro* was investigated and the result presented in Figure 1. The results revealed that *A. cruentus* leaf extracts inhibited α -amylase in a dose-dependent manner (0 to 0.2 mg/ml). However, as revealed by the EC₅₀ (extract concentration causing 50% enzyme inhibition) values (Table 1), unprocessed *A. cruentus* (0.32 mg/ml) had a significantly ($P < 0.05$) higher α -amylase inhibitory activity than blanched *A. cruentus* (0.72 mg/ml). Furthermore, the ability of the vegetable extracts to inhibit α -glucosidase activity *in vitro* was also investigated and the result is presented in Figure 2. The results revealed that *A. cruentus* leaf extracts inhibited α -glucosidase in a dose-dependent manner (0 to 0.2 mg/ml). However, as revealed by the EC₅₀ (extract concentration causing 50% enzyme inhibition) values (Table 1), unprocessed *A. cruentus* (0.21 mg/ml) had a significantly ($P < 0.05$) higher α -glucosidase inhibitory activity than blanched *A. cruentus* (0.29 mg/ml).

The result of the total phenol and flavonoid content of *A. cruentus* leaf is presented in Table 2. The result revealed that there was a significant ($P < 0.05$) difference between the total phenol content of unprocessed *A. cruentus* leaf (9.3 mg/100 g) and blanched *A. cruentus* leaf (7.0 mg/100 g). Also, unprocessed *A. cruentus* leaf (3.6 mg/100 g) had a significantly ($P < 0.05$) higher total flavonoid content than blanched *A. cruentus* leaf (1.4 mg/100 g). The reducing power of *A. cruentus* leaf is presented as ascorbic acid equivalent in Figure 3. The result revealed that *A. cruentus* leaf was able to reduce Fe (III) to Fe (II). However, blanched *A. cruentus* (36.2 mg AAE/100 g) had a significantly ($P < 0.05$) higher reducing power than unprocessed *A. cruentus* leaf (29.2 mg AAE/100 g).

DISCUSSION

Management of the blood glucose level is a critical strategy in the control of diabetes complications. Inhibitors of saccharide hydrolysing enzymes (α -amylase and α -glucosidase) have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with type-2 diabetes mellitus. Inhibition of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently reducing the postprandial plasma glucose rise (Kwon et al., 2007). The results as presented in Figure 1 revealed that unprocessed *A. cruentus* had a significantly ($P < 0.05$) higher α -amylase inhibitory activity than blanched *A. cruentus*. This significant ($P < 0.05$) decrease in the inhibition of α -amylase activity as a result of blanching of the vegetable could be attributed to the damage/loss of physiologically active phytochemicals having α -amylase inhibitory activities during the heat processes involved in blanching such as observed in phenol content (Table 2). Nevertheless, the determined α -amylase inhibitory activity of the vegetable agreed with some earlier reports

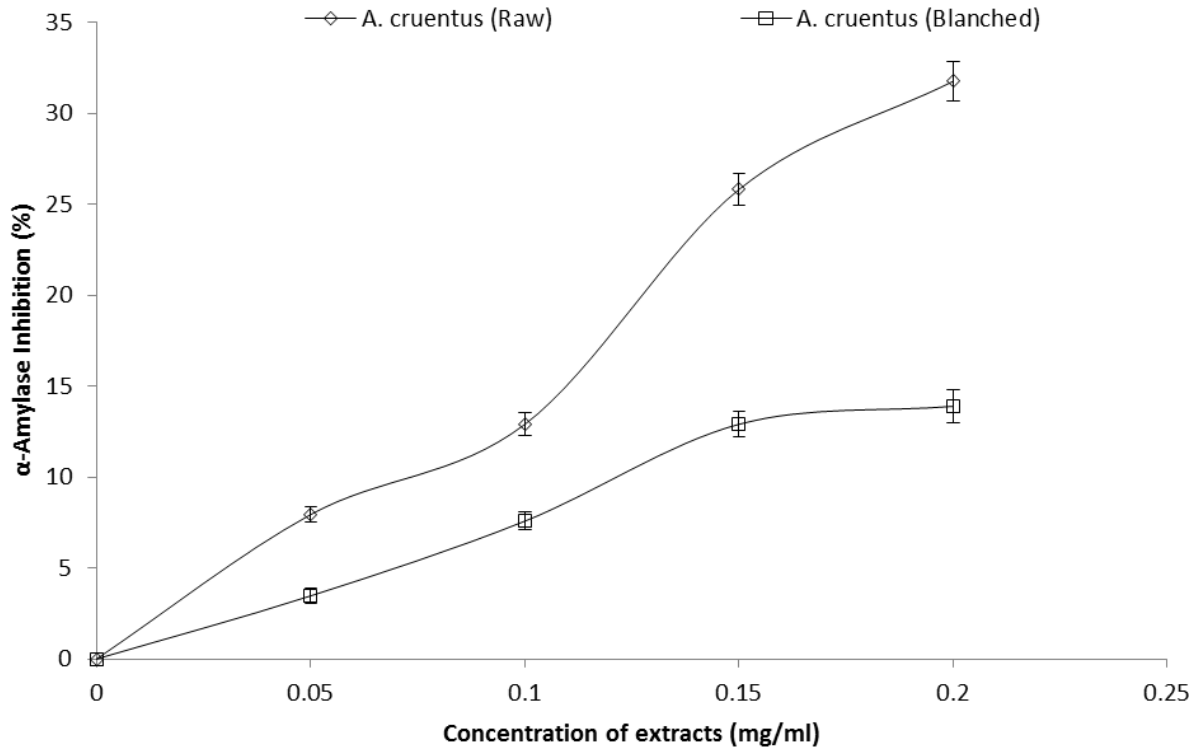


Figure 1. α -Amylase inhibitory activity of *Amaranthus cruentus* leaf extract. Values represent mean \pm standard deviation, n = 3.

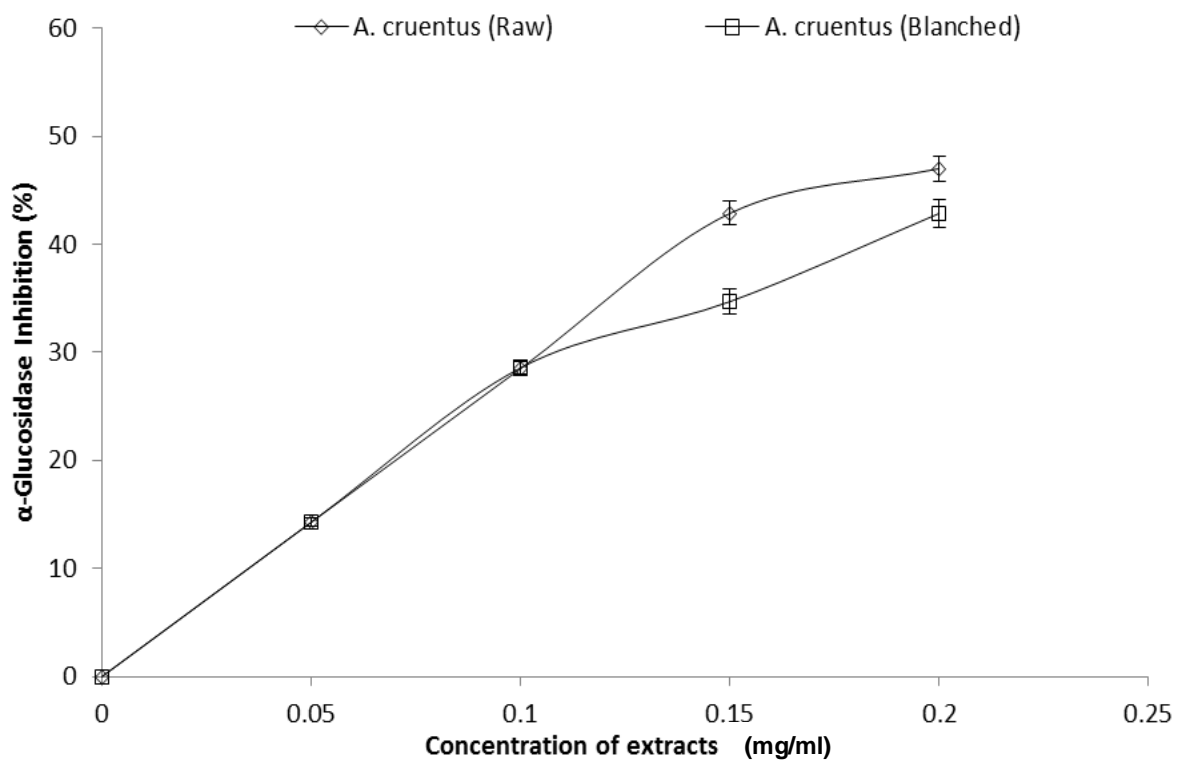


Figure 2. α -Glucosidase inhibitory activity of *Amaranthus cruentus* leaf extract. Values represent mean \pm standard deviation, n = 3.

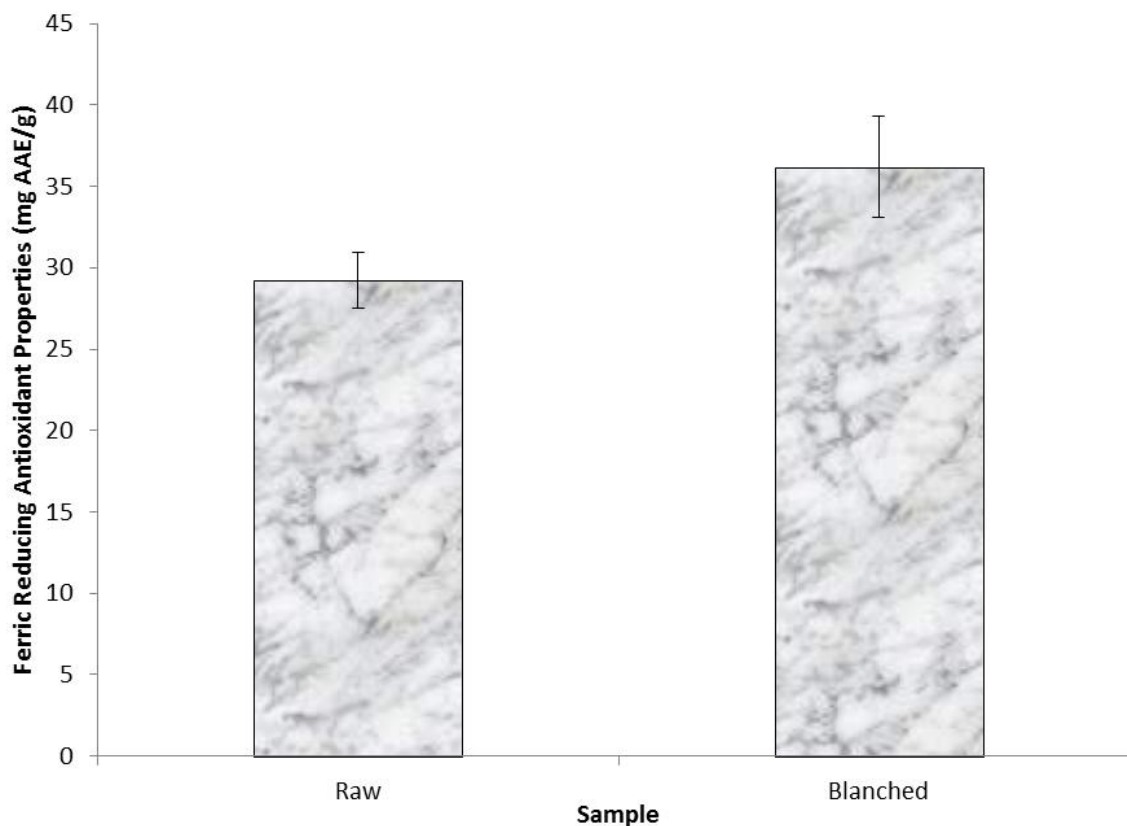


Figure 3. Ferric reducing antioxidant properties (FRAP) of *Amaranthus cruentus* leaf. Values represent mean \pm standard deviation, $n = 3$.

where plant phytochemicals from pepper inhibited saliva α -amylase activity (Kwon et al., 2007) and inhibitory effects of *Allium* spp. on α -amylase activity (Nickavar and Yousefian, 2009). This also agreed with a recent worked where with bitter leaf inhibited α -amylase activity *in vitro* (Saliu et al., 2012).

Furthermore, the vegetable extracts inhibited α -glucosidase activity *in vitro* as presented in Figure 2. The results revealed that unprocessed *A. cruentus* had a significantly ($P < 0.05$) higher α -glucosidase inhibitory activity than blanched *A. cruentus*. This significant ($P < 0.05$) decrease in the inhibition of α -glucosidase activity as a result of blanching of the vegetable could not be categorically stated, however, it could be attributed to the excessive loss of physiologically active phytochemicals as a result of blanching such as observed in Table 2. The determined α -glucosidase inhibitory activity follows the same pattern as observed in Figure 1. This result is in agreement with a recent work reported by Saliu et al. (2012) where bitter leaf inhibited α -glucosidase activity *in vitro*.

The results of the enzyme (α -amylase and α -glucosidase) inhibitory assays showed that ethanolic extract of the *A. cruentus* leaves were strong inhibitors of α -glucosidase but mild inhibitors of α -amylase as shown

in Figures 1 and 2. This however, is in agreement with earlier reports that showed that plant phytochemicals are mild inhibitors of α -amylase and strong inhibitors of α -glucosidase activity (Kwon et al., 2007), a property that confers advantage over synthetic drugs such as acarbose; used by diabetics in the management of postprandial blood glucose, which strongly inhibit α -amylase. Stronger inhibition of α -glucosidase activity and mild inhibition of α -amylase activity of the ginger extracts could address the major drawback of currently used α -glucosidase and α -amylase inhibitor drugs with side effects such as abdominal distention, flatulence, meteorism and possibly diarrhea (Pinto et al., 2009).

It has been suggested that such adverse effects might be caused by the excessive pancreatic α -amylase inhibition resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Kwon et al., 2007). Therefore, this study buttress the claim that natural inhibitors from dietary plants have mild inhibitory effect on α -amylase activity but strong α -glucosidase inhibitory activity, and could be used as effective therapy for the management of postprandial hyperglycemia with minimal side effects (Kwon et al., 2007). This agrees with the finding on eggplant phenolics, which have been recommended as a choice diet for the management of

Table 1. EC₅₀ values (mg/ml) of α -amylase inhibitory and α -glucosidase activity of *Amaranthus cruentus* leaf as affected by blanching.

Sample	α -Amylase (mg/ml)	α -Glucosidase (mg/ml)
Fresh	0.32 ^b ±0.03	0.19 ^a ±0.01
Blanched	0.72 ^c ±0.05	0.29 ^b ±0.01

Values represent mean \pm standard deviation of triplicate experiments. Values with the same superscript letter along the same column are not significantly different ($P < 0.05$).

Table 2. Total phenol and flavonoid content of *Amaranthus cruentus* leaf (mg/100g) as affected by blanching.

Sample	Total phenol (mg/100 g)	Total flavonoid (mg/100 g)
Fresh	9.3 ^a ±0.05	3.6 ^a ±0.30
Blanched	7.0 ^b ±0.30	1.4 ^b ±0.00

Values represent mean \pm standard deviation of triplicate experiments. Values with the same superscript letter along the same column are not significantly different ($P < 0.05$).

type 2 diabetes (Pinto et al., 2009). Also, this agrees with Saliu et al. (2012) for bitter leaf extract.

The result of the total phenol and flavonoid content of *A. cruentus* leaf revealed that there was a significant ($P < 0.05$) difference between the total phenol and flavonoid contents of unprocessed *A. cruentus* leaf and blanched *A. cruentus* leaf. The values obtained were lower than what Oboh and Akindahunsi (2004) reported for some tropical green leafy vegetables (1 to 3 mg/g). The difference in phenolic value is as a result of the extraction medium used in the study. However, there was a decrease in the flavonoid content due to blanching. The basis of the decrease could not be categorically stated, however, it could be that during blanching, some of the flavonoids would have been leached into the water. However, the result was in agreement with Chen and Lin (2007) that phenolics content in cooked yams prepared at different temperatures (50 to 100°C) was lower compared to the raw ones. Also, this result was in line with Chung et al. (2008) that more than 40% of phenolic content in yam peels were lost after blanching at 85°C for 30 s.

Phenolic compounds can protect the human body from free radicals whose formation is associated with the normal metabolism of aerobic cells. They are strong antioxidants capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α -tocopherol radicals and inhibit oxidases (Oboh and Rocha, 2007). The presence of derivatives of flavonoids has been found in many fruits and vegetables; moreover, numerous studies have conclusively shown that the majority of the antioxidant activity maybe from compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechin and isocatechin rather than from vitamins C, E and β -carotene (Rong, 2010). Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress (Rong, 2010).

Polyphenols are considered to be strong antioxidants due to the redox properties of their hydroxyl groups (Rong, 2010). Reducing power is a novel antioxidation defence mechanism; the mechanisms available to affect this property are by electron transfer and hydrogen atom transfer (Dastmalchi et al., 2007). This is because the ferric-to-ferrous ion reduction occurs rapidly with all reductants, with half reaction reduction potentials above that of $\text{Fe}^{3+}/\text{Fe}^{2+}$, the values in the ferric reducing antioxidant property (FRAP) assay will express the corresponding concentration of electron-donating antioxidants (Dastmalchi et al., 2007). The reducing power of *A. cruentus* leaf revealed that blanched *A. cruentus* had a significantly ($P < 0.05$) higher reducing power than unprocessed *A. cruentus* leaf. The basis for the significant increase in the reducing power could not be categorically stated, however, it could be reasoned out that the temperature at which blanching is carried out would have enhance the activity of the phenolic compound or other Fe^{3+} reducing agents in the blanched vegetable to the extent that the high phenol content observed in the unprocessed vegetable could not shield their effect.

Conclusion

A. cruentus leaf exhibited antioxidant properties and inhibited α -amylase and α -glucosidase (key enzyme linked to type-2 diabetes) activities. This antioxidant properties and enzyme inhibition could be part of the possible mechanism by which *A. cruentus* leaf is used in the management/prevention of type-2 diabetes. However, blanching of the vegetable could reduce their ability to inhibit both α -amylase and α -glucosidase activity, but could enhance their antioxidant properties *in vitro*.

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