

ANTIOXIDANT PROPERTIES AND INHIBITORY EFFECT OF ETHANOLIC EXTRACT OF *STRUCHIUM SPARGANOPHORA* (EWURO ODO) LEAF ON α - AMYLASE AND α – GLUCOSIDASE ACTIVITIES

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P.M.B., 704, Akure 340001, Nigeria*E-mail: goboh2001@yahoo.com**Abstract**

Recent studies have shown that vegetables consumption could lower the risk of diabetes mellitus. Therefore, this study sought to investigate the inhibitory effect of *Struchium sparganophora* (Ewuro Odo) 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 this leafy vegetable on these key enzymes. Fresh leaves of *Struchium sparganophora* were blanched in hot water for 10 minutes, and the 70% ethanolic extracts of the fresh and blanched vegetables were prepared and used for subsequent analysis. The antioxidant properties and interaction of the extracts on α - amylase and α - glucosidase activities was determined *in vitro*. The result revealed that *Struchium sparganophora* leaf scavenged DPPH free radical and also inhibited α - amylase and α - glucosidase activities in a dose dependent manner (0.05 -0.2 mg/ml). However, blanching of this leafy vegetables caused a significant ($P<0.05$) increase in the antioxidant properties as typified by the DPPH radical scavenging ability and reducing property but decrease their ability to inhibit α - amylase and α - glucosidase activities. This antioxidant properties and enzyme inhibition could be part of the mechanism by which green leafy vegetables exert their anti-diabetic properties. However, blanching of the vegetable could reduce their ability to inhibit both α - amylase and α - glucosidase activity, but enhance their antioxidant properties *in vitro*.

Keywords: Vegetables; inhibition; blanching; α -amylase; α -glucosidase; type-2 diabetes**Introduction**

Diabetes mellitus is a metabolic disorder in which there is no control of blood sugar (Beverley and Eschwège, 2003). This disease affects approximately 135 million people in the world and is projected to affect about 300 million individuals by the year 2030 (Egede et al., 2011). However, type-2 diabetes (Non-insulin dependent diabetes mellitus, NIDDM) is the most common form of diabetes mellitus, accounting for 90% of all cases, and is mainly caused by insulin resistance, relative insulin deficiency, and an abnormal rise in blood sugar, right after a meal, called postprandial hyperglycemia (Kwon et al., 2007). An effective strategy for NIDDM management has been through the inhibition of α -glucosidase and pancreatic α -amylase (Krentz and Bailey 2005) and many commercially available α -glucosidase inhibitors (acarbose and miglitol) used in the management of the disease has employed this mechanism. Increasing use of natural medicines as a result of consumers seeking complementary and/or alternatives to prescribed drugs has provoked a great interest in research into medicinal plants. Some of these herbal products used to improve overall health, prevent and cure diseases, are likely to act through a stimulation of receptor sites, the immune system, inhibit pathogenesis of disease condition or have a lethal effect on pathogens. The discovery of bioactive compounds from plant origin offers an attractive approach to the control of infectious or non infectious diseases. Hence, natural α -amylase and α -glucosidase inhibitors offer an attractive therapeutic approach to the treatment of postprandial hyperglycemia by ultimately slowing glucose release from starch.

Struchium sparganophora is a culinary herb in most African countries. It is known as “Ewuro Odo” by the Yorubas in Nigeria, belongs to the family of Asteraceae. The plant is found predominantly in Southwestern Nigeria,

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where it is used in the preparation of soup. It occurs more as a cultivated species rather than as a wild plant. It is a shrub that normally grows near the waterside and usually has a tap root; it is greatly branched and fibrous, and grows deep into the soil. It is a green leafy vegetable popularly used in soup preparations in processed and unprocessed forms in Nigeria. It is also widely used medicinally in a number of countries: decoction of the stem and root are employed in the treatment of diabetes mellitus, headaches, gonorrhea (Jakupovic et al., 1987; Akah and Ekekwe, 1995). In the Sao Tome and Principe (STP) island (Gulf of Guinea), it is used for the treatment of malaria (Heywood et al., 1977; Madureira et al., 2002) and in the treatment of measles (Burkill, 1985). The nutritive, antioxidant and antimicrobial and the antimalaria activities of the leaves have been reported by (Obboh, 2006; Obboh et al., 2008; Madureira et al., 2002). Its phytochemical composition and the effect of its aqueous extract on cockroach crude extract- induced airway inflammatory responses in Wistar Rats has been reported by Eko et al. (2008). Sesquiterpine lactone has been isolated from the plant as reported by Jakupovic et al. (1987). Although a lot had been reported on the chemical characterization of phytoconstituents and antioxidant properties of *Struchium sparganophora*, however, limited information is available on the possible mechanism by which *Struchium sparganophora* is being used in traditional medicine in the management/prevention of type-2 diabetes. Hence, this study sought to investigate the inhibitory effect of *Struchium sparganophora* 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) on these key enzymes.

Materials and Method

Materials

Fresh samples of *Struchium sparganophora* (Ewuro Odo) were sourced from the University garden of The Federal University of Technology, Akure. Authentication of the vegetables was carried out in the Department of Biology, Federal University of Technology, Akure, Nigeria. A UV-Visible spectrophotometer (Model 6305; Jenway, Barlo world Scientific, Dunmow, United Kingdom) was used to measured absorbance. All the chemicals used where of analytical grade while the water was glass distilled.

Preparation of 70% Ethanolic 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, chopped into small pieces by table knife. A portion of the chopped vegetables was then blanched for 10 minutes, while the other portion was not blanched. The blanched portion was then drained of water. Both portions were then sun dried and milled to be obtained in a powder form. 10g of each powder was extracted with 70% ethanol then, the extract was filtered with Whatman filter paper and the filtrate was concentrated under reduced pressure to give a solid extract. The concentrated extract was further lyophilized by freeze-drying and the yields were 16% (Unprocessed portion) and 10.6% (Blanched portion). Then, 0.05g of the vegetable extracts were reconstituted in 20ml of 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 dilution (0 - 200 μ l) of the vegetable extracts (stock solution, 2.5 mg/ml) 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 minutes. 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. The α -amylase inhibitory activity was expressed as percentage inhibition.

α -Glucosidase inhibition assay

The α -Glucosidase inhibitory activity was determined according to the method of Apostolidis et al. (2007).

Appropriate dilution (0 - 200 μ l) of the vegetable extracts (stock solution, 2.5 mg/ml) 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. The α -glucosidase inhibitory activity was expressed as percentage inhibition

Determination of Total Phenol Content

The total phenol content was determined according to the method of Singleton et al. (1999). Briefly, appropriate dilutions 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 minutes at 45°C and the absorbance was measured at 765 nm. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of Total Flavonoid Content

The total flavonoid content was determined according to the method of 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 minutes. The absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoid content was subsequently calculated using quercetin as standard.

1,1-diphenyl-2 picrylhydrazyl free radical scavenging ability

The free radical scavenging ability of the vegetable extracts against DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical was evaluated as described by Gyamfi et al. (1999). Briefly, appropriate dilution of the extracts (1 ml) was mixed with 1 ml, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

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 (1986). 2.5ml 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. The ferric reducing antioxidant property was subsequently calculated as ascorbic acid equivalent.

Data Analysis

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

Results and 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).

First, the ability of *Struchium sparganophora* leaf extract to inhibit both α -amylase and α -glucosidase activities *in vitro* was investigated and the result is presented in Figure 1 and 2. The results revealed that *S. sparganophora* leaf extracts inhibited both α -amylase and α -glucosidase in a dose-dependent manner (0–0.2 mg/mL).

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However, as revealed by the EC_{50} (extract concentration causing 50% enzyme inhibition) values (Table 1), unprocessed *S. sparganophora* had a significantly ($P<0.05$) higher α -amylase ($EC_{50} = 0.19$ mg/mL) and α -glucosidase ($EC_{50} = 0.16$ mg/mL) inhibitory

Table 1: α -Amylase and α -Glucosidase inhibitory activities (EC_{50} values) of fresh and processed *Struchium sparganophora* leaf extracts

Samples	α – Amylase (mg/mL)	α – Glucosidase (mg/mL)
Fresh	$0.19^b \pm 0.05$	$0.16^a \pm 0.03$
Blanched	$0.34^c \pm 0.04$	$0.19^b \pm 0.00$

Values represent mean \pm standard deviation of triplicate experiments

Values with the same superscript letter along the same column are not significantly different ($P<0.05$)

Table 2: Total phenol and flavonoid content of fresh and processed *Struchium sparganophora* leaf extracts

Samples	Total phenol (mg/100g)	Total flavonoid (mg/100g)
Fresh	$7.3^b \pm 0.30$	$0.73^a \pm 0.01$
Blanched	$3.1^c \pm 0.32$	$0.70^b \pm 0.00$

Values represent mean \pm standard deviation of triplicate experiments

Values with the same superscript letter along the same column are not significantly different ($P<0.05$)

activity than blanched *S. sparganophora* [α -amylase ($EC_{50} = 0.34$ mg/mL) and α -glucosidase ($EC_{50} = 0.19$ mg/mL)]. This significant ($P<0.05$) decrease in the inhibition of both α -amylase and α -glucosidase activity as a result of blanching of the vegetable could be attributed to the damage/loss of physiologically active phytochemicals having α -amylase and α -glucosidase 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 where plant phytochemicals from green and black tea inhibited saliva α -amylase activity (Zhang and Kashket, 1998) and inhibitory effects of *Allium* spp. on α -amylase activity (Nickavar and Yousefian, 2009). This also agreed with a recent worked reported by Oboh et al. (2010) where red and white ginger inhibited α -amylase and α -glucosidase activity *in vitro*.

The result of the total phenol and flavonoid content of *S. sparganophora* leaf is presented in Table 2. The result revealed that unprocessed *S. sparganophora* leaf had a significantly ($P<0.05$) higher total phenol (7.3 mg/100g) and flavonoid (0.73 mg/100g) content than blanched *S. sparganophora* leaf [total phenol (3.1 mg/100g) and flavonoid (0.70 mg/100g) content]. The values were lower than what Oboh (2005) reported for some tropical green leafy vegetables (1 - 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 phenolic content due to blanching. The basis of the decrease could be that during blanching some of the phenols 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 - 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 seconds.

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 (Amic et al., 2003). 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 (Marin et al., 2004).

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Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress (Oboh et al., 2007). Polyphenols are considered to be strong antioxidants due to the redox properties of their hydroxyl groups (Materska and Perucka, 2005).

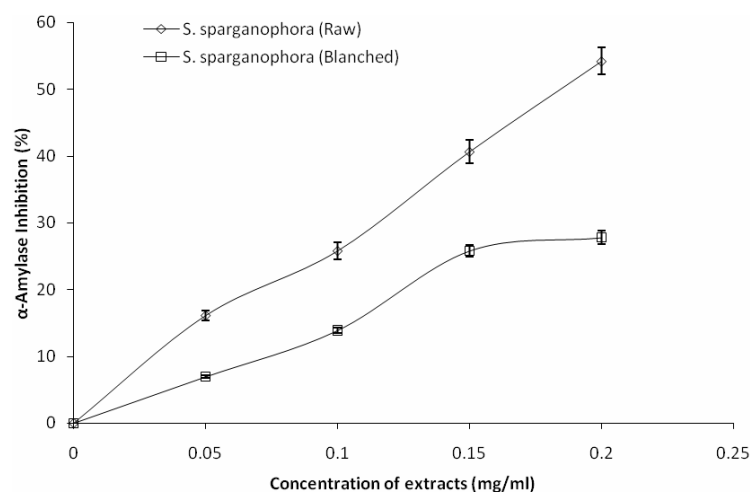


Figure 1: α – Amylase inhibitory activity of *Struchium sparganophora* leaf extracts

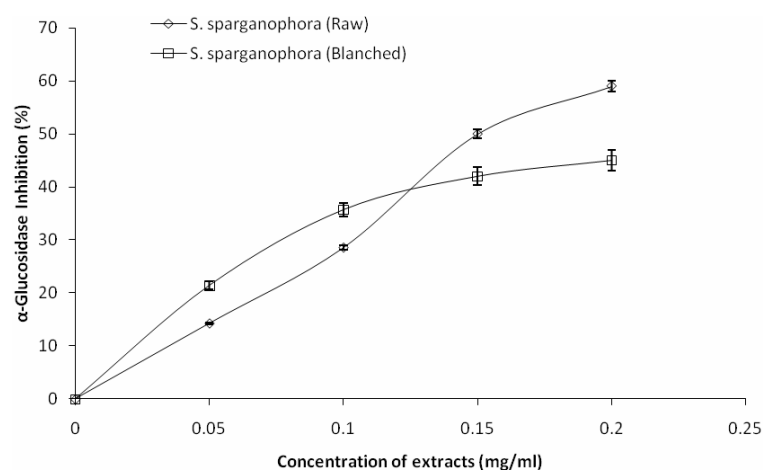


Figure 2: α – Glucosidase inhibitory activity of *Struchium sparganophora* leaf extracts

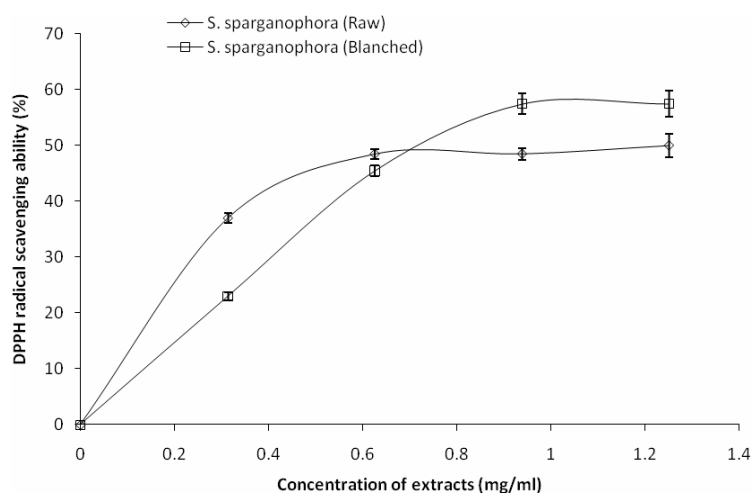


Figure 3: DPPH free radical scavenging ability of *Struchium sparganophora* leaf extracts

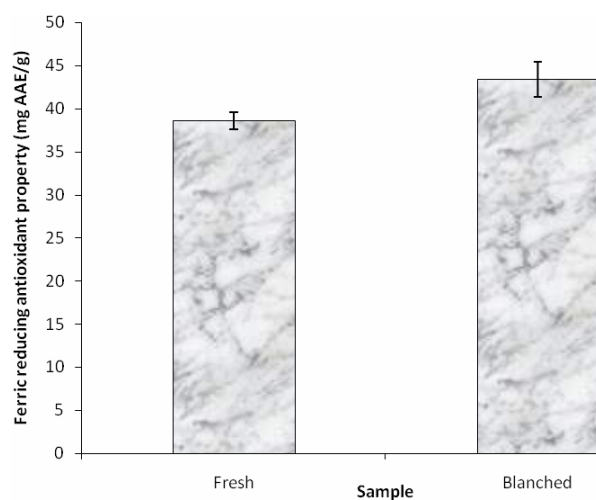


Figure 4: Ferric Reducing Antioxidant Properties (FRAP) of *Struchium sparganophora* leaf extracts

The prevention of the chain initiation step by scavenging various reactive species such as free radicals is considered to be an important antioxidant mode of action (Dastmalchi et al., 2007). DPPH is a free radical donor that accepts an electron or hydrogen to become a stable diamagnetic molecule (Je et al., 2009). The tendencies of electron or hydrogen donation are critical factors in characterizing antioxidant activity that involves free radical scavenging (Hu et al., 2000). The result of the DPPH free radical scavenging ability of *S. sparganophora* leaf is presented in Figure 3. The results revealed that *S. sparganophora* leaf extract scavenged DPPH radicals in a dose-dependent pattern (0 – 1.25 mg/ml), however, the blanched vegetable cause a significant increase ($P < 0.05$) in the DPPH free radical scavenging ability. The basis for the significant increase in the DPPH free radical scavenging ability could be reasoned out that the temperature at which blanching is carried out would have enhance the activity of the phenolic compound or other DPPH free radical-scavenger components in the blanched vegetable to the extent that the high phenol content observed in the unprocessed vegetable could not shield their effect.

Reducing power is a novel antioxidation defence mechanism; the two 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 (Halvorsen et al., 2002). The reducing power of *S. sparganophora* leaf is presented as ascorbic acid equivalent in Figure 4. The result revealed that *S. sparganophora* leaf was able to reduce $\text{Fe}(\text{III})$ to $\text{Fe}(\text{II})$. However, blanched *S. sparganophora* (43.4 mg AAE/100 g) had a significantly ($P < 0.05$) higher reducing power than unprocessed *S. sparganophora* leaf (38.6 mg AAE/100 g). This result agrees with DPPH free radical scavenging ability where blanching cause an increase in the scavenging ability.

Conclusion

In conclusion, *S. sparganophora* 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 mechanism by which green leafy vegetables exert their anti-diabetic properties. However, unprocessed *S. sparganophora* leaf extract had higher inhibitory on both α - amylase and α - glucosidase activity, but blanched *S. sparganophora* leaf extract had higher antioxidant properties *in vitro*.

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