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# EGGPLANT EXTRACTS ALTER ACTIVITIES of ENZYMES IMPLICATED in TYPE-2 DIABETES and PREVENT $Fe^{2+}$-INDUCED OXIDATIVE STRESS in RAT'S PANCREAS

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## ABSTRACT

Eggplants have been considered a natural therapy for the management of type-2 diabetes, but little is known about biological activities of *Solanum aethiopicum* and *Solanum incanum* in the management of type-2 diabetes. Hence, this study investigated the antioxidant activities and inhibitory actions of *S. aethiopicum* and *S. incanum* on starch-metabolizing enzymes. In addition, the effect of *S. aethiopicum* and *S. incanum* extracts on $Fe^{2+}$-induced oxidative stress in pancreas was examined. Results from this study reveal that extracts of *S. aethiopicum* and *S. incanum* have good antioxidant activities and mild α-amylase and strong α-glucosidase inhibitory activity. Inhibition of these enzymes coupled with antioxidant activities of *S. aethiopicum* and *S. incanum* suggest the possible biochemical basis for their involvement in the management of type-2 diabetes. However, consumption of *S. aethiopicum* and *S. incanum* could be considered a dietary therapy with potential of controlling glucose absorption and hyperglycemia linked to oxidative stress.

Keywords: *S. aethiopicum*; *S. incanum*; oxidative stress; type-2 diabetes.

## INTRODUCTION

Diabetes mellitus is one of major metabolic disorders characterized by inability of pancreas to produce enough insulin (hormone that regulates blood sugar level) or alternatively, due to inability of body to effectively use the insulin produced and associated elevated postprandial hyperglycemia (Gerber and Rutter, 2017). Oxidative stress has been reported to play crucial role in aging and pathogenesis of many disorders including diabetes mellitus particularly type-2 diabetes (Pham-Huy et al., 2008). Oxidative stress set in when there is imbalance between radicals production outweigh their removal by the cellular defense mechanism. The deleterious aspect of oxidative stress is in the reactive oxygen species (ROS) production (Migdal and Serres, 2011). Elevated ROS in diabetes could be as a result of decrease in the endogenous antioxidants defense system. In diabetes, hyperglycemia enhances oxidative stress due to altered glucose metabolism and excessive accumulation of glycosylated compounds and advanced glycation end products (AGEs) (Gerber and Rutter, 2017).

Eggplant, (member of Solanaceae family), is a flowering plant commonly distributed in the tropical and temperate regions (Eun-ju et al., 2011). Depending on the species, eggplants have diverse shapes, size and colour. Various biological activities of eggplant fruits have been reported, these include; antipyretic, anti-inflammatory, antitumor, hepatoprotective and antiallergic (Bhutani et al., 2010). However, these biological activities could be as a result of presence of phytochemicals such as phenolic compounds, flavonoids, anthocyanins and several steroidal alkaloids (Scalzo et al., 2010). Many *Solanum species* have been studied vis-à-vis their biological activities; *S. melongena* and *S. macrocarpon* (Kwon et al., 2008), *S. muricatum* (Hsu et al., 2011), *S. xanthocarpum* (Hussain et al., 2012). Therefore, this study investigated the effect of *S. aethiopicum* and *S. incanum* on activities of enzymes implicated in type-2 diabetes and $Fe^{2+}$-induced oxidative stress in rat's pancreas.

## MATERIALS AND METHODS

### Sample collection and preparation

Two species of Eggplant, *S. aethiopicum* (white with green stripes) and *S. incanum* (green with white stripes) were collected from main market, Akure, South-west Nigeria. The fruits were sliced into small pieces and 30 g of samples were added to 20 ml of distilled water separately and homogenized using laboratory blender. The homogenate was filtered through a Whatman # 2 filter paper. The filtrate was lyophilized and stored in an air-tight container at -4$^{o}$C for subsequent *in vitro* assays.

### Determination of polyphenol content

The total phenol content was determined using Folin-Ciocalteau's method as described by Singleton et al. (1999). The total phenol was subsequently calculated and expressed as milligram gallic acid equivalent per gram of sample (mgGAE/g). Whereas, the total flavonoid content was determined using a slightly modified method reported by Meda *et al.* (2005). The total flavonoid content was subsequently calculated and expressed as milligram quercetin equivalent per gram (mgQUE/g).

### Determination of antioxidant activities

The antioxidant activities of eggplant extracts were assessed through the ability of extracts to scavenge DPPH radical (Gyamfi et al., 1999), ABTS radical (Re et al., 1999), hydroxyl (OH) radical (Halliwell and Gutteridge, 1981) and nitric oxide radical (Marcossi et al., 1994).

### Lipid peroxidation and thiobarbituric Acid Reactive species assay

Pancreas tissue homogenate was prepared according to the method of Belle et al. (2004) and lipid peroxidation and thiobarbituric acid reactive species assays were carried out on pancreas tissue homogenate using the previously described method of Shodehinde et al. (2017).

### Enzyme inhibition assays

Effect of eggplant extracts on α–amylase activity was assayed for using standard method (Worthington, 1993). In addition, inhibitory effect of eggplant extracts on α-glucosidase was determined using method of Apostolidis et al. (2007).

### Statistical analysis

The results of replicate experiments were pooled and expressed as mean±standard deviation (SD). The results were statistically analyzed by ANOVA and Duncan's multiple range tests. Statistical significance was accepted at a level of $p < 0.05$. $IC_{50}$ was determined using linear regression analysis.

## RESULTS AND DISCUSSION

The results of the total phenol and flavonoid distribution in the *S. aethiopicum* and *S. incanum* are presented in Table 1. There was no significance difference between the total phenol content of *S. aethiopicum* (2.53 mgGAE/g) and *S. incanum* (2.99 mgGAE/g), as well as the total flavonoid content of *S. aethiopicum* (1.52 mgQUE/g) and *S. incanum* (1.92 mgQUE/g). Plants are rich source of biologically important chemical compounds, and intake of these plant chemicals has protective potential against degenerative diseases (Pandey and Rizvi, 2009). Polyphenols, a prominent class of phytochemical, are well known for their biological activities (Lawson et al., 2017). Findings in this study revealed that *S. incanum* extract had higher phenol and flavonoid contents than *S. aethiopicum*. However, the values obtained are higher than that of ripe and unripe *Capsicum pubescens* (Oboh and Rocha, 2007) and pepper fruit (Adedayo et al., 2010). The basis for the observed difference in their phenolic contents cannot be categorically stated, however, it may be due to differences in their genetic makeup. Phenolic compounds are capable of protecting biological system against free radicals, whose formation is associated with the normal metabolism of aerobic cells. Phenolic compounds are capable of scavenging free radicals, chelate metal catalysts, activates antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases (Lawson et al., 2017). Their potent antioxidant activity could be due to redox properties of their hydroxyl groups (Eghbaliferiz and Iranshahi, 2016). In addition, flavonoids are well known for their roles in prevention of certain diseases such as cancer, diabetes and cardiovascular complications (Wolfe and Liu, 2008).

**Table 1: Total phenol (mgGAE/g), total flavonoid (mgQUE/g) and ABTS radical scavenging activity (mmolTEAC/g) of *S. aethiopicum* and *S. incanum* extracts**

| | Samples | |
|---|---|---|
| Parameter | *S. aethiopicum* | *S. incanum* |
| Total phenol (mgGAE/g) | $2.53 \pm 0.07^a$ | $2.99 \pm 0.11^b$ |
| Total flavonoid (mgQUE/g) | $1.52 \pm 0.09^a$ | $1.92 \pm 0.15^b$ |
| ABTS* (mmolTEAC/g) | $1.24 \pm 0.07^a$ | $1.50 \pm 0.05^a$ |

Values represent mean±standard deviation of replicate experiments.
Values with the same superscript alphabet along the same row are not significantly different at $p < 0.05$.

Plants exert their antioxidant activities through preventing free radical generation, scavenging radicals produced and/or chelating metal ions ($Fe^{2+}$ and $Cu^{2+}$) in the system (Lawson et al., 2017; Oboh et al., 2007). Prevention of chain reaction initiated by free radical especially ROS by phytochemicals is one of important antioxidant activities of these plant chemical compounds (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). DPPH is frequently used in the determination of free radical scavenging ability of various food components; however, it has the limitation of colour interference and sample solubility (Dudonné et al., 2009). Therefore, the free radical scavenging ability of the extracts was further elucidated using a moderately stable nitrogen-centered radical species; ABTS radical (Dudonné et al., 2009). However, the antioxidant activities of eggplant extracts used in this study could be a function of their phenolic compounds. The ABTS radical scavenging ability expressed as trolox equivalent antioxidant capacity of the aqueous extracts of *S. aethiopicum* and *S. incanum* was presented in Table 1. The result revealed that *S. incanum* (1.50 mmol TEAC/g) exhibited higher ABTS radical scavenging ability than that of *S. aethiopicum* (1.24 mmolTEAC/g). The DPPH radical scavenging ability of eggplant extracts is presented in Figure 1. The results revealed that both extracts scavenged DPPH radical in a dose-dependent manner (0 – 6.6 mg/ml). However, as revealed by the $IC_{50}$ values (Table 2), *S. incanum* had significantly higher scavenging ability than the *S. aethiopicum* ($p < 0.05$). Furthermore, hydroxyl radical ($OH^{\cdot}$) scavenging ability of *S. aethiopicum* and *S. incanum* extracts is presented in Figure 2. The results revealed that both extracts scavenged hydroxyl radical in a dose-dependent manner (0 –

1.74 mg/ml). However, as revealed by the $IC_{50}$ (Table 2), the $OH^{\cdot}$ scavenging ability of *S. aethiopicum* (3.29 mg/ml) is significantly higher ($p<0.05$) than that of *S. incanum* (8.02 mg/ml) within the range of concentration tested. The nitric oxide (NO) radical scavenging ability of *S. aethiopicum* and *S. incanum* extracts is presented in Figure 3. The results revealed that both extracts scavenged NO radical in a concentration-dependent manner (0 – 4.0 mg/ml). However, as shown in Table 2, *S. incanum* had higher NO radical scavenging ability than *S. aethiopicum.*

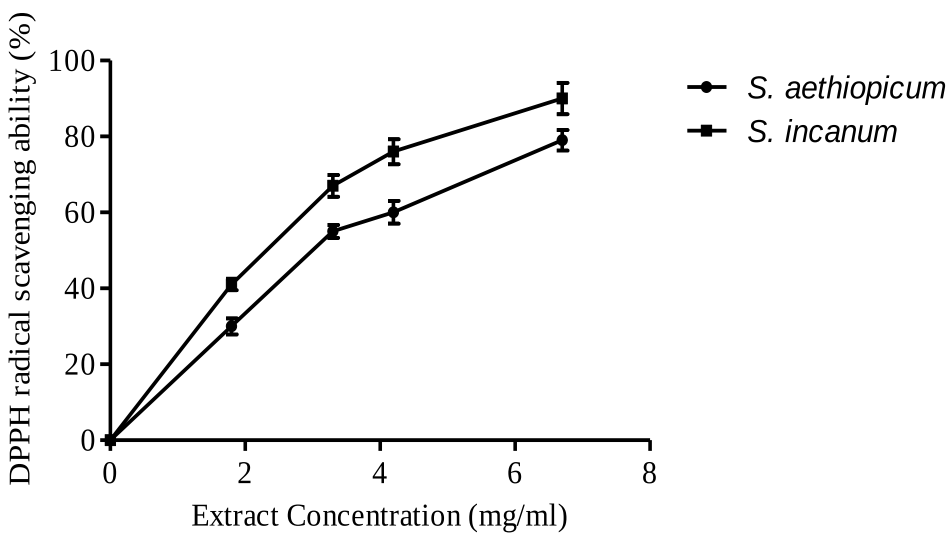


**Figure 1: DPPH radical scavenging activity of *S. aethiopicum* and *S. incanum* extracts**

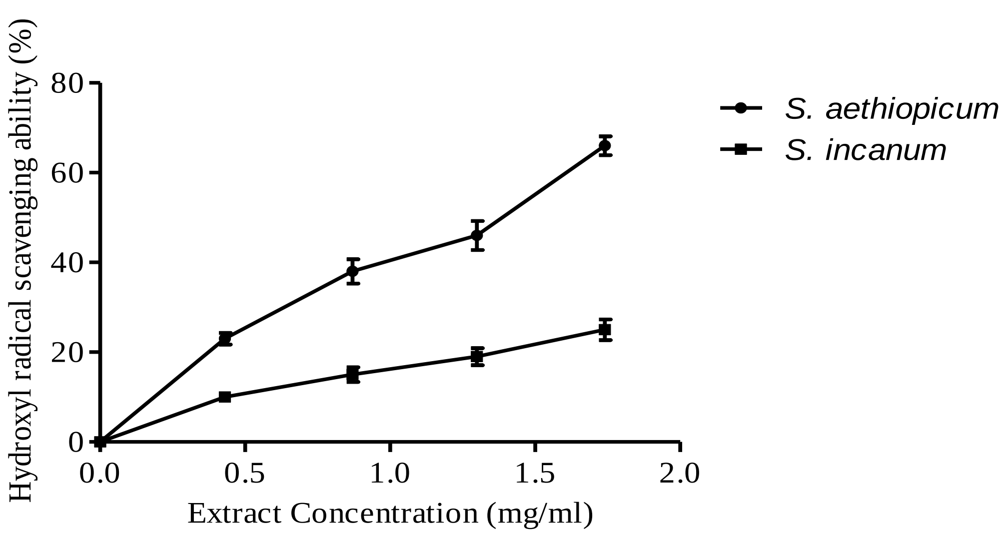


**Figure 2: Hydroxyl (OH) radical scavenging activity of *S. aethiopicum* and *S. incanum* extracts**

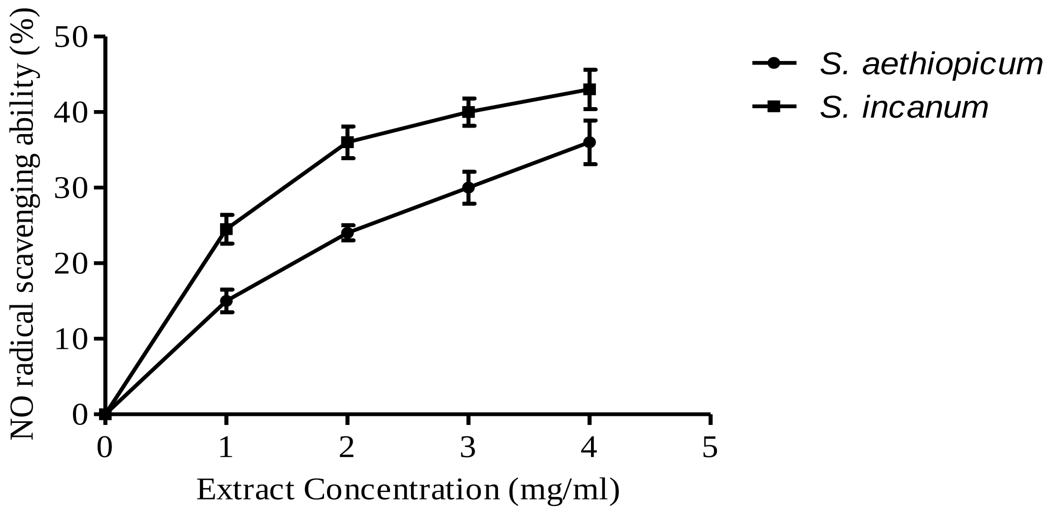


**Figure 3: Nitric oxide (NO) radical scavenging activity of *S. aethiopicum* and *S. incanum* extracts**

**Table 2: $IC_{50}$ values of antioxidant activities (DPPH*, OH*, NO and $Fe^{2+}$-induced lipid peroxidation) and enzyme (α-amylase and α-glucosidase) inhibitory activities of *S. aethiopicum* and *S. incanum* extracts**

| | $IC_{50}$ values | |
|---|---|---|
| Parameter | *S. aethiopicum* | *S. incanum* |
| DPPH* scavenging activity (mg/ml) | $3.64\pm0.7^b$ | $2.84\pm0.31^a$ |
| OH* scavenging activity (mg/ml) | $1.30\pm0.9^a$ | $3.54\pm0.15^b$ |
| NO radical scavenging activity (mg/ml) | $5.33\pm1.8^b$ | $4.10\pm0.05^a$ |
| $Fe^{2+}$-induced lipid peroxidation (mg/ml) | $1.75\pm0.03^a$ | $3.25\pm0.53^b$ |
| α-amylase inhibition (mg/ml) | $2.35\pm0.29^b$ | $1.87\pm0.17^a$ |
| α-glucosidase inhibition (mg/ml) | $1.10\pm0.10^b$ | $0.90\pm0.02^a$ |

Values represent mean±standard deviation of replicate experiment.
Values with the same superscript alphabet along the same row are not significantly different at $p < 0.05$.

The inhibition of $Fe^{2+}$ induced lipid peroxidation in rat's pancreas by aqueous extracts of *S. aethiopicum* and *S. incanum* is presented in Figure 4. Incubation of rat's pancreas tissue homogenate in the presence of $Fe^{2+}$ caused a significant increase in the pancreas malondialdehyde (MDA) level (103.48%). However, both extracts reduced MDA level in pancreas tissue homogenate in a dose-dependent manner (0.31 – 1.25 mg/ml). Nevertheless, at 1.25 mg/ml, *S. aethiopicum* showed greater inhibition of lipid oxidation by lowering MDA level in rat's pancreas to 65.22% than *S. incanum* which reduce MDA level to 78.26%. Polyphenols chelate and deactivate transition metals, thereby preventing such metals from initiating lipid peroxidation and oxidative stress through metal catalyzed reaction (Oboh and Rocha, 2007). $Fe^{2+}$ catalyzes one-electron transfer reactions that generate reactive oxygen species such as $OH^*$, which is formed from $H_2O_2$ through the Fenton's reaction (Oboh et al 2007). Overproduction of ROS can directly attack polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation (Uttara et al., 2009). Findings revealed that eggplant species used in this study chelated $Fe^{2+}$ and scavenged radicals that capable of inducing oxidative stress in biological system. Results also revealed that *S. aethiopicum* had higher radicals scavenging ability than *S. incanum*. It is important to note that both eggplant species had higher radicals scavenging ability than the various varieties of pepper (Oboh et al., 2007; Oboh and Rocha, 2007) and pepper fruit (Adedayo et al., 2010*)* earlier reported.

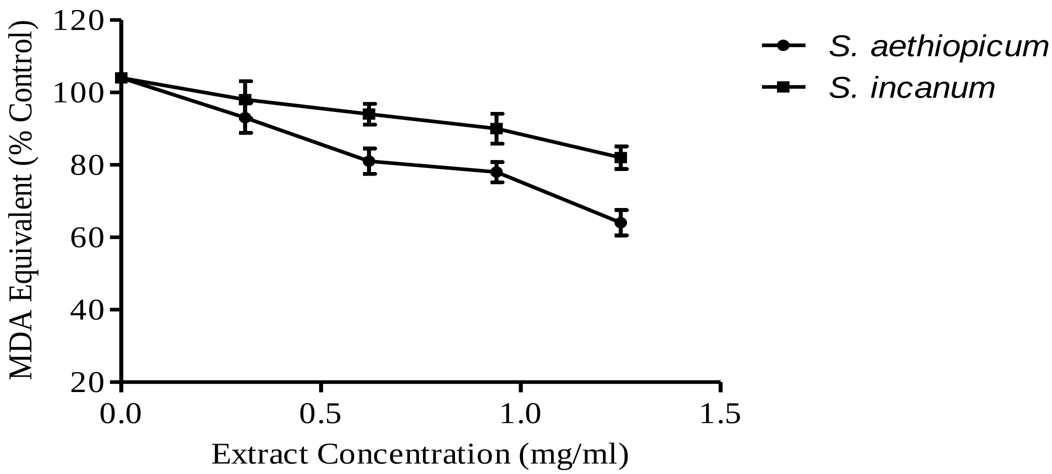


**Figure 4: Effect of *S. aethiopicum* and *S. incanum* extracts on $Fe^{2+}$-induced lipid peroxidation in rat's pancreas**

The control of postprandial blood glucose levels is critical in the early treatment of diabetes mellitus and also in preventing other chronic vascular complications (Ortiz-Andrade et al., 2007). Inhibition of enzymes involved in the hydrolysis of carbohydrates such as α-amylase and α-glucosidase is one of the therapeutic approaches for reducing postprandial hyperglycemia (Lee et al., 2010). In this study, eggplant extracts inhibited α-amylase in a concentration-dependent manner. However, *S. incanum* had higher inhibitory effect on α-amylase than *S. aethiopicum* extract. Inhibition of α-amylase by eggplant extracts observed in this study agreed with earlier report where soybean phenolic-rich extract inhibited α-amylase activity *in vitro* (Ademiluyi and Oboh, 2013). Inhibition of α-amylase reduces the hydrolysis of dietary starch to disaccharides and trisaccharides which could be converted by other enzymes to glucose (Perry et al., 2007). The α-amylase inhibitory effect of *S. aethiopicum* and *S. incanum* is presented in Figure 5. Both extracts inhibited α-amylase activities in a concentration-dependent manner. However, the α-amylase inhibitory effect was higher in *S. aethiopicum* than *S. incanum*, as revealed by Table 2. Similarly, findings in this study revealed that both extracts inhibited α-glucosidase activities in a dose-dependent manner (Figure 6). However, there was no significance difference in their α-glucosidase inhibitory properties (Table 2).

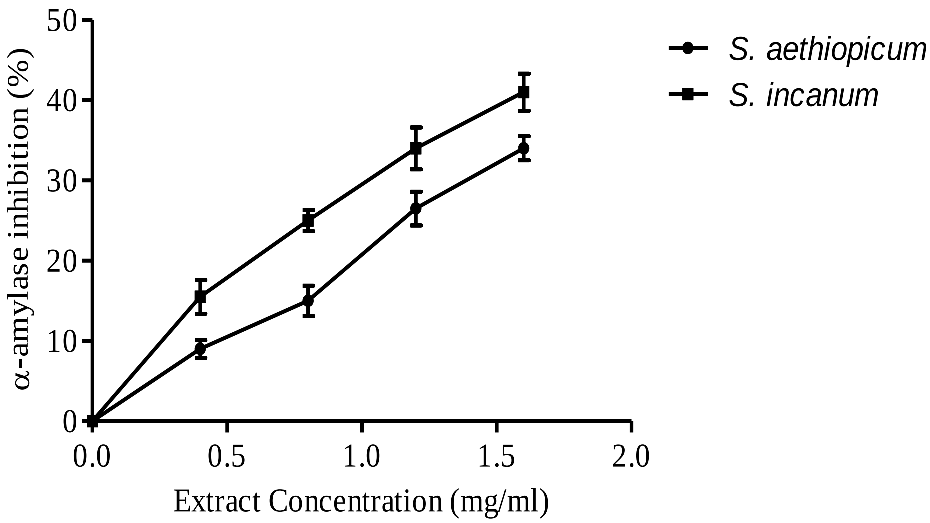


**Figure 5: Effect of *S. aethiopicum* and *S. incanum* extracts on α-amylase activity *in vitro***

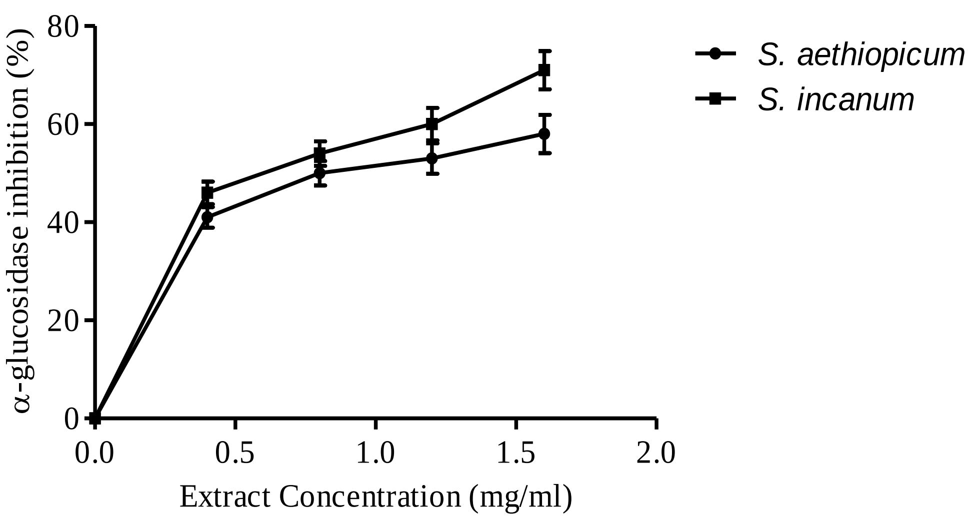


**Figure 6: Effect of *S. aethiopicum* and *S. incanum* extracts on α-glucosidase activity *in vitro***

Attenuation of α-glucosidase activity slows down the breakdown of disaccharide to simple sugar which in turn reduces postprandial glucose level and this has been considered an important therapeutic approach in diabetic subjects (Kwon et al., 2007). The inhibition of α-amylase and α-glucosidase activities by eggplant extracts could be attributed to their inherent phytochemicals which have shown to possess α-amylase and α-glucosidase inhibitory properties (Bhandari et al., 2008). In diabetes management, equal inhibition of α-amylase and α-glucosidase activity or a stronger inhibition of α-amylase than α-glucosidase activities by drugs that are currently in use for diabetes management, results in side effects such as abdominal dissention, bloating, flatulence and diarrhoea (Kwon et al., 2007). The excessive inhibition of the pancreatic α-amylase leads to bacterial fermentation of undigested carbohydrate in the colon (Dong et al., 2012). A mild inhibition of α-amylase coupled with a stronger inhibition of α-glucosidase prevents the occurrence of side effects. Interestingly, *S. aethiopicum* and *S. incanum* showed mild α-amylase inhibition and strong α-glucosidase inhibition, the property that could be advantageous over most conventional drugs use in the management of type-2 diabetes.

## CONCLUSION

The results from this present study indicate the positive effect of two eggplant species, *S. aethiopicum* and *S. incanum*, on enzymes relevant to hyperglycemia. It was revealed that *S. aethiopicum* and *S. incanum* have strong antioxidant activities and good inhibitory action on the activities of enzymes relevant to carbohydrate metabolism (α-amylase and α-glucosidase). Hence, findings in this study provide a strong biochemical basis for the use of eggplant especially *S. aethiopicum* and *S. incanum* as one of natural remedies for people suffering from type-2 diabetes.

### Conflict of interest

Authors declare no conflict of interest.

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