Antioxidative properties and inhibition of key enzymes linked to type-2 diabetes by snake tomato (*Tricosanthes cucumerina*) and two tomato (*Lycopersicon esculentum*) varieties


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This study sought to compare the antioxidant properties [1,1-diphenyl–2 picrylhydrazyl (DPPH) and hydroxyl (OH) radicals scavenging abilities] and inhibition of Fe$^{2+}$-induced lipid peroxidation and two key enzymes relevant to type-2 diabetes (α-amylase and α-glucosidase) of snake tomato (*Trichosanthes cucumerina*) with two tomato varieties [*Lycopersicon esculentum* Mill. var. *esculentum* (ESC) and *Lycopersicon esculentum* Mill. var. *cerasiforme* (CER)]. Snake tomato (0.84 mg/g) and CER (0.87 mg/g) had significantly (P < 0.05) higher total phenolic content than ESC (0.27 mg/g). However, CER had the highest total flavonoid content of 0.48 mg/g, compared to snake tomato (0.27 mg/g) and ESC (0.15 mg/g). In consonance with the phenolic content, CER and snake tomato had higher DPPH and OH radicals scavenging abilities than ESC. The inhibition of Fe$^{2+}$ induced malondialdehyde (MDA) production in rats pancreas revealed that snake tomato had significantly lower inhibitory effect than CER. Furthermore, snake tomato and CER showed stronger inhibition of α-glucosidase [snake tomato (EC$_{50}$ = 1.65 mg/ml), CER (EC$_{50}$ = 1.32 mg/ml)] than α-amylase [snake tomato (EC$_{50}$ = 2.15 mg/ml), CER (EC$_{50}$ = 2.39 mg/ml)] activity. The antioxidant properties of snake tomato favourably compared with the cultivars of tomatoes, and its stronger inhibition of α-glucosidase activity than α-amylase activities suggests that snake tomato could be an alternative or complement to the use of lycopersicon tomatoes.

**Key words:** Tomato, antioxidant, diabetes, flavonoids, α-amylase, α-glucosidase.

INTRODUCTION

*Tricosanthes cucumerina* (commonly called snake tomato), snake gourd, viper gourd or long tomato is rich in chemical constituents like flavonoids, carotenoids, phenolic acids (Adebooye, 2008; Ojiako and Igwe, 2008) which makes the plant pharmacologically and therapeutically active. Even though some systems of medicine have been exploring some pharmacological potentials of the snake tomato, such as antidiabetic,
hepatoprotective, cytotoxic, anti-inflammatory and larvadic effects, the 'tomato' still remains underutilized as food or as medicinal plant. Snake tomato has been used as a substitute for the common lycopersicon tomatoes in the tropics especially when the prices of the lycoper-sicon tomatoes go up in the off-season. The common tomatoes commonly used as diet almost all over the world, are a major source of antioxidants, and the consumption of fresh lycopersicon tomatoes has been reported to have health benefits such as cancer preven-tion and inhibition of lipid peroxidation (Bub et al., 2000; Ziegler and Vogt, 2002). However, there is dearth of information on some health benefits of snake tomato to justify its use as a substitute to the lycopersicon tomatoes.

The link between free-radicals and development of diabetes has been well established (Ceriello, 2006; Maritim et al., 2003), more so free-radical damage to the pancreas has been implicated in the diabetogenic process (Akbarzadeh et al., 2007). Diabetes is a major health problem worldwide along with its associated complica-tions (Zimmet et al., 1997) and this could be linked to changes in the dietary patterns in both developing and developed countries. The prevalence of type II diabetes is growing at an exponential rate (Zimmet and Lefebvre, 1996) and a lot of attention is been given to natural products for the management of the disease (Covington, 2001).

This study therefore investigated the antioxidant properties and inhibition of key enzymes linked to type-2 diabetes (α-amylase and α-glucosidase) by the under-utilized snake tomato and compare with the common tomatoes so as to find a basis, if any, for the use of snake tomato as a substitute to common tomatoes and also explain the mechanism of action by which the ‘tomatoes’ can be used in the management of type-2 diabetes.

MATERIALS AND METHODS

Sample collection and preparation

Snake tomato (T. cucumerina) and two cultivars of common tomatoes (Lycopersicon esculentum Mill.); Ibadan Local (CER) and Roma VF (ESC) were collected from the main market, identified, washed, weighed and then homogenized by using a blender after distilled water was added (1:3 w/v). The homogenate was centrifuged at 4500 g for 15 min. The supernatant (juice fraction) was recovered and kept in the freezer for subsequent analysis.

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 pastes were oxidized with 2.5 ml 10% Folin-Ciocalteau’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 spectrophotometer. 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 appropri-ately diluted sample was mixed with 0.5 ml methanol, 50 μl 10% AlCl₃, 50 μl 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; the total flavonoid content was subsequently calculated. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

1,1-diphenyl-2-picyrlyldrazyl radical scavenging ability

The free radical scavenging ability of the pastes against 1,1-diphenyl-2-picyrlyldrazyl (DPPH) 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.

Hydroxyl radical scavenging ability

The method of Halliwell and Gutteridge (1981) was used to determine the ability of the pastes to prevent Fe²⁺/H₂O₂ induced decomposition of deoxyribose. The extract 0 to 100 μl was added to a reaction mixture containing 120 μl of 20 mM deoxyribose, 400 μl of 0.1 M phosphate buffer, 40 μl of 500 μM of FeSO₄, and the volume were made up to 800 μl with distilled water. The reaction mixture was incubated at 37°C for 30 min and the reaction was then stopped by the addition of 0.5 ml of 28% trichloroacetic acid. This was followed by addition of 0.4 ml of 0.6% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in a spectro-photometer.

Lipid peroxidation assay

Preparation of tissue homogenates

Adult male rats weighing 220 to 240 g (10 to 12 weeks old) were decapitated under mild diethyl ether anaesthesia and the pancreas was rapidly isolated and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1/10 w/v) with about 10-up-and down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenate was centrifuged for 10 min at 3000 x g to yield a pellet that was discarded, and a low-speed supernatant (S1) was kept for lipid peroxidation assay (Belle et al., 2004).

Lipid peroxidation and thiobarbituric acid reactions

The lipid peroxidation assay was carried out using the modified method of Ohkawa et al. (1979), briefly 100 μl S1 fraction was mixed with a reaction mixture containing 30 μl of 0.1 M pH 7.4 Tris-HCl buffer, extract (0 to 100 μl) and 30 μl of 250 μM freshly prepared FeSO₄. The volume was made up to 300 μl by water before incubation at 37°C for 1 h. The colour reaction was developed by adding 300 μl 8.1% Sodium dodecyl sulphate (SDS)
to the reaction mixture containing S1, this was subsequently followed by the addition of 600 μl of acetic acid/HCl (pH 3.4) mixture and 600 μl 0.8% Thiobarbituric acid (TBA). This mixture was incubated at 100°C for 1 h. Thiobarbituric acid reactive species (TBARS) produced were measured at 532 nm and the absorbance was compared with that of standard curve using malondialdehyde (MDA).

α-Amylase inhibition assay

This was measured using the dinitroalicyclic acid method adapted from Bernfeld (1955). Appropriate dilution of the pastes (500 μl) and 500 μl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M 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 μl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube. The reaction mixtures were incubated at 25°C for 10 min and stopped with 1.0 ml of dinitroalicylic acid colour reagent. Thereafter, the mixture was 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 EC50 (the extract concentration inhibiting 50% of the α-amylase activity) of the pastes was calculated.

α-Glucosidase inhibition assay

Appropriate dilution of the pastes (50 μl) and 100 μl of α-glucosidase solution (1.0 U/ml) in 0.1 M phosphate buffer (pH 6.9) was incubated at 25°C for 10 min. Then, 50 μl of 5 mM p-nitrophenylα-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25°C for 5 min before reading the absorbance at 405 nm in the spectrophotometer. The α-glucosidase inhibitory activity was expressed as percentage inhibition. The EC50 of the pastes was calculated (Apostolidis et al., 2007).

Data analysis

The results of three replicates were pooled and expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and least significance difference (LSD) were carried out (Zar, 1984). Significance was accepted at p ≤ 0.05.

RESULTS

The results of the total phenolic and flavonoid contents are presented in Table 1. ESC had significantly lower total phenolic content than snake tomato and CER, while CER had significantly higher total flavonoid content than snake tomato and ESC. Figures 1, 2 and Table 2 reveal that CER and snake tomato had higher radicals scavenging abilities than ESC. The inhibition of Fe2+ induced MDA production in rats pancreas is presented in Figure 3 and Table 2. Snake tomato had significantly lower inhibitory effect than CER, but higher inhibitory effect than ESC. Furthermore, as presented in Figures 4, 5 and Table 2, snake tomato and CER showed stronger inhibition of α-glucosidase than α-amylase activity.

DISCUSSION

The total phenol content of snake tomato was not significantly (P > 0.05) different from that of CER, but higher than that of ESC. However, the total flavonoid content of snake tomato was significantly (P < 0.05) lower than that of CER, but higher than that of ESC. The antioxidant capacities of the ‘tomato’ samples were assessed not only for comparison between the species but also because free radicals are involved in the development and complications of diabetes in a number of ways: the white blood cell production of reactive oxygen species mediates the autoimmune destruction of the beta cells in the islets of langerhans in the pancreas (Yoon and Jun, 2005). Abnormalities in transition metal metabolism are postulated to result in the establishment of diabetes (Parameshwar et al., 2012). Diabetes can be induced in animals by the drugs alloxan and streptozotocin; the mechanism of action of these two drugs is different, but both result in the production of reactive oxygen species and scavengers of oxygen radicals have been found to be effective in preventing diabetes in these animal models (Moussa, 2008).

The DPPH* scavenging ability of snake tomato was not significantly (P > 0.05) different from ESC, but lower than that of CER. However, the total flavonoid content of snake tomato was significantly (P < 0.05) lower than that of CER, but higher than that of ESC. The antioxidant capacities of the ‘tomato’ samples were assessed not only for comparison between the species but also because free radicals are involved in the development and complications of diabetes in a number of ways: the white blood cell production of reactive oxygen species mediates the autoimmune destruction of the beta cells in the islets of langerhans in the pancreas (Yoon and Jun, 2005). Abnormalities in transition metal metabolism are postulated to result in the establishment of diabetes (Parameshwar et al., 2012). Diabetes can be induced in animals by the drugs alloxan and streptozotocin; the mechanism of action of these two drugs is different, but both result in the production of reactive oxygen species and scavengers of oxygen radicals have been found to be effective in preventing diabetes in these animal models (Moussa, 2008).

The DPPH* scavenging ability of snake tomato was not significantly (P > 0.05) different from ESC, but lower than that of CER. This trend in the results agrees with the total phenolic and antioxidant capacity of snake tomato having the highest scavenging ability.

The antioxidant properties of the samples could be

### Table 1. Total phenol and total flavonoid content of snake tomato and two tomato varieties (ESC and CER) (mg/g).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenol</th>
<th>Total flavonoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snake tomato</td>
<td>0.84±0.11</td>
<td>0.27±0.06</td>
</tr>
<tr>
<td>ESC</td>
<td>0.27±0.06</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>CER</td>
<td>0.87±0.08</td>
<td>0.48±0.04</td>
</tr>
</tbody>
</table>

Values represent means of triplicate (n=3). Values with the same alphabet (a,b,c) along the same column are not significantly different (P>0.05). ESC - Lycopersicon esculentum Mill. var. esculentum. CER - Lycopersicon esculentum Mill. var. cerasiforme.
attributed to a combination of carotenoids and other phenolic compounds. Lycopene, which is the major carotenoid in tomatoes has been shown by cellular and molecular studies to exhibit potent antioxidative properties (Khachik et al., 2002; Sahasrabuddhe, 2011), and correlations have been established between the phenolic content and antioxidant properties of many samples (Jayaprakasha et al., 2008; Kedage et al., 2007).

MDA is increased when Fe^{2+} induces lipid peroxidation by catalyzing the decomposition of hydrogen peroxide to generate hydroxyl radical via the fenton reaction (Bayir et
Table 2. EC\textsubscript{50} of DPPH\textsuperscript{*} and OH\textsuperscript{*} scavenging abilities, inhibition of Fe\textsuperscript{2+} induced MDA Production, \(\alpha\)-amylase and \(\alpha\)-glucosidase activities of snake tomato and two tomato varieties (ESC and CER).

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH\textsuperscript{*} (mg/ml)</th>
<th>OH\textsuperscript{*} (mg/ml)</th>
<th>MDA   (mg/ml)</th>
<th>(\alpha)-amylase (mg/ml)</th>
<th>(\alpha)-glucosidase (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snake tomato</td>
<td>0.35±0.09\textsuperscript{a}</td>
<td>0.52±0.07\textsuperscript{a}</td>
<td>5.51±0.09\textsuperscript{a}</td>
<td>2.15±0.09\textsuperscript{a}</td>
<td>1.65±0.14\textsuperscript{a}</td>
</tr>
<tr>
<td>ESC</td>
<td>0.36±0.08\textsuperscript{a}</td>
<td>0.84±0.08\textsuperscript{b}</td>
<td>6.71±0.06\textsuperscript{b}</td>
<td>1.81±0.06\textsuperscript{b}</td>
<td>1.93±0.11\textsuperscript{b}</td>
</tr>
<tr>
<td>CER</td>
<td>0.30±0.06\textsuperscript{b}</td>
<td>0.46±0.05\textsuperscript{a}</td>
<td>4.31±0.07\textsuperscript{c}</td>
<td>2.39±0.07\textsuperscript{c}</td>
<td>1.32±0.10\textsuperscript{c}</td>
</tr>
</tbody>
</table>

Values represent means of triplicate (n=3). Values with the same alphabet (a,b,c) along the same column are not significantly different (P>0.05). OH\textsuperscript{*} - hydroxyl radical, MDA – malondialdehyde, DPPH\textsuperscript{*} - 1,1-diphenyl-2-picrylhydrazyl radical, ESC - Lycopersicon esculentum Mill. var. esculentum, CER - Lycopersicon esculentum Mill. var. cerasiforme.

Figure 3. Inhibition of Fe\textsuperscript{2+} induced MDA production by snake tomato and two tomato varieties (ESC and CER). ESC - Lycopersicon esculentum Mill. var. esculentum, CER - Lycopersicon esculentum Mill. var. cerasiforme.

Incubation of rat’s pancreas in the presence of 250 \(\mu\)M Fe\textsuperscript{2+} caused a significant increase in the malondialdehyde (MDA) content of the pancreas. Free-radicals induced pancreatic damage has been linked to the development of diabetes (Akbarzadeh et al., 2007). Nevertheless, the ‘tomato’ samples significantly (P<0.05) inhibited MDA production in the pancreas in a dose-dependent manner as shown in Table 2. However, CER and snake tomato had significantly (P < 0.05) stronger inhibitory effect on MDA production in the pancreas (\textit{in vitro}) than ESC. The reason for the higher inhibitory ability of CER and snake tomato cannot be categorically stated, but it could be due to other antioxidant mechanisms, since CER and snake tomato had higher phenolic contents, DPPH\textsuperscript{*} and OH\textsuperscript{*} scavenging abilities.

The MDA inhibitory ability of snake tomato and the lycopersicon tomatoes cannot be ascribed to the lycopene content alone as a combination of purified lycopene with \(\alpha\)-tocopherol was reported to result in a significant greater inhibition of \textit{in vitro} low density lipoprotein (LDL) oxidation, than the expected additive individual inhibitions. Purified lycopene was also shown to act synergistically with other natural antioxidants like the flavonoid glabridin, the phenolics rosmarinic acid and carnosic acid, and garlic in inhibiting LDL oxidation \textit{in vitro} (Furhman et al., 2000). Thus, the combination of lycopene and other natural antioxidants present in the samples may be responsible for the potent inhibition of lipid peroxidation.

\(\alpha\)-Amylase hydrolyzes starch to maltose, while \(\alpha\)-glucosidase enzymes are responsible for the breakdown of oligo- and/or disaccharides to monosaccharides, and an inhibition of these enzymes therefore leads to a decrease in blood glucose level, and this is one of the therapeutic approaches for reducing postprandial blood glucose levels.
glucose values in a bid to prevent/manage diabetes (Shim et al., 2003). The samples inhibited α-amylase activity in a dose-dependent manner, however the EC50 (Table 2) revealed that the trend of the result was different from the earlier results as ESC had the highest inhibitory effect. It is noteworthy however that snake tomato had higher inhibitory effect on α-amylase activity than CER. Furthermore, the α-glucosidase inhibitory activity showed that the samples inhibited the enzyme activity in a dose-dependent manner.

A comparison of the α-amylase and α-glucosidase inhibition by the samples revealed a stronger inhibition of...
α-glucosidase activity, but milder inhibition of α-amylase activities by snake tomato and CER. A stronger inhibition of α-glucosidase activity, but milder inhibition of α-amylase activities is desirable as this could address the main drawback of currently used α-glucosidase and α-amylase inhibitors drugs which is caused by the excessive inhibition of pancreatic α-amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Rao and Jamil, 2011). These results confirms the claim that natural inhibitors from dietary plants could show lower inhibitory effect against α-amylase activity and a stronger inhibitory activity against α-glucosidase and can be used as effective therapy for postprandial hyperglycemia with minimal side effects (Kwon et al., 2006, 2007). Furthermore, Kar et al. (2003) showed that crude ethanolic extract of snake tomato showed significant blood glucose lowering activity in alloxan induced diabetic albino rats and Arawawala et al. (2009) using hot water extract of aerial parts of snake tomato also noted the improved glucose tolerance and tissue glycogen in non insulin dependent diabetes mellitus induced rats.

Conclusion

The inhibition of key enzymes linked with type-2 diabetes (α-amylase and α-glucosidase) and antioxidative properties of the "tomatoes" used in this study could make them good dietary means for the management and/or prevention of type-2 diabetes. The antioxidiant properties of snake tomato which favourably compares with the other lyopersicon tomatoes (except CER), combined with its stronger inhibition of α-glucosidase activity, but milder inhibition of α-amylase activities suggests that snake tomato could be an alternative or complement to the use of lyopersicon tomatoes.

REFERENCES


