Comparative Study on the $\alpha$-Amylase and $\alpha$-Glucosidase Inhibitory Potential of Different Extracts of \textit{Blighia Sapida} Koenig

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ABSTRACT

The aim of this study was to investigate the inhibitory effect of \textit{B. sapida} leaf extract on the key enzymes linked to diabetes ($\alpha$-amylase and $\alpha$-glucosidase). The inhibitory effect of the leaf extracts on $\alpha$-amylase and $\alpha$-glucosidase activities as well as determination of mode of inhibition was performed. The results revealed that the aqueous extract of \textit{B. sapida} was the most potent inhibitor of $\alpha$-amylase (with IC$_{50}$ 5.80 mg/ml) while ethanolic extract inhibited $\alpha$-glucosidase (with IC$_{50}$ 4.57 mg/ml) most effectively. The curve of Lineweaver-Burke plot revealed that aqueous extract of \textit{B. sapida} exhibited mixed non-competitive inhibition of $\alpha$-amylase while ethanolic extract displayed an uncompetitive inhibition of $\alpha$-glucosidase activities. It can be inferred from this study that the $\alpha$-amylase and $\alpha$-glucosidase inhibitory potential of \textit{B. sapida} may be due to the presence of phytochemicals such as saponins and flavonoids. However, further study is required to isolate the enzyme inhibitory component of this plant.

Keywords: Diabetes, \textit{Blighia sapida}, $\alpha$-amylase, $\alpha$-glucosidase, enzyme inhibition
1. Introduction

Diabetes mellitus is a metabolic disease which is as old as mankind and its incidence is high (4 – 5%) all over the world (Koyuturk et al., 2005). It is also a major cause of disability and hospitalization which results in significant financial burden (Nagappa et al., 2003). The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million people likely to be diabetic by the year 2030 as against 191 million estimated in 2000 (Wild et al., 2004). The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered.

Besides drugs classically used for the treatment of diabetes such as insulin, sulphonylureas, biguanides and thiazolidinediones, several species of plants have been described in the scientific literature as having hypoglycemic activity (Verspohl, 2002; De Sousa et al., 2004; Colca, 2006). Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown (Valiathan, 1998). Some of these plants include Vernonia amygdalina, Zingiber officinale, Ficus exasperata and Blighia sapida (Ogunleye et al., 2003; Ojewole, 2006; Ong et al., 2011)

**Blighia sapida** Koenig is a tree plant which belongs to Sapidaceae family. It is found mainly in some part of West Africa especially in Nigeria, Ghana and Ivory Coast. The tree is identified with different names like Ackee (English), “Ila” (Nupe), “Isin” (Yoruba), “Gwanja kusa” (Hausa) and “Okpu” (Igbo). It is used in traditional medicine for diabetes mellitus management and hypertension. Its fleshy aril fruit is edible and is known to contain saponins, which are haemolytic (Jimoh et al., 2012). The pulp and leaves are used to treat eye conjunctivitis. The seeds are not edible, but the ashes of the dried husks and the seeds are used in
the preparation of soap (Aderinola et al., 2007). It is usually planted as a shade tree and its fruit is relished by humans while the leaves are eaten by ruminants. It is well known for its unripe aril that contains hypoglycin A, a water-soluble phytochemical that inhibits gluconeogenesis thus leading to hypoglycemia (Hassall and Reyle, 1955).

α-amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starch from being absorbed by the body. Thus this could be useful in the treatment of obesity and diabetes mellitus. They exert their blood glucose lowering effect through the inhibition of an enzyme such as salivary and pancreatic amylase (Frantz et al., 2005). α-glucosidase inhibitors also has potential to suppress postprandial hyperglycemia in diabetic patients by completely and reversibly inhibiting α-glucosidase in the intestine. This inhibition reduces glucose absorption through delayed carbohydrate digestion and extended digestion time (Shimabukuro et al., 2006). However, a common side effect of enzyme inhibitory drugs like acarbose is the excessive inhibition of pancreatic α-amylase, which can result in abdominal distention, flatulence and diarrhoea (Lebovitz, 1997). These side effects are caused by abnormal fermentation of unhydrolyzed polysaccharides by gut bacteria (Kwon et al., 2008).

Therefore the aim of this study was to evaluate α-amylase and α-glucosidase inhibitory potential of different extracts of *Blighia sapida* and assesses the mode(s) of inhibition, as a possible source of hypoglycemic agents in the management of diabetes.

2. Materials and Methods

2.1 Plant material

*Blighia sapida* leaves were collected from its tree in Ibarapa, Ibarapa Local Government Area of Oyo State, Nigeria in the month of May 2012. It was identified and authenticated by Dr.
A. B. Kadiri of the Department of Botany, University of Lagos, Nigeria and voucher specimen (LUH 4726) was deposited in the University herbarium.

2.2 **Chemicals and reagents**

α-amylase from *Aspergillus oryzae*, α-glucosidase from *Saccharomyces cerevisiae* and paranitrophenyl-glucopyranoside were products of Sigma-Adrich Co., St Louis, USA while starch soluble (extra pure) was obtained from J. T. Baker Inc., Phillipsburg, USA. Other chemicals and reagents were of analytical grade and water used was glass-distilled.

2.3 **Preparation of plant extracts**

Fresh leaves of *Blighia sapida* were cut and washed with water to remove all contaminants; they were dried under room temperature and grounded to powder. The powdered leaves were divided into three portions and each portion was dissolved in acetone, ethanol or water. They were all left to steep in covered containers for 24 hours; the resulting infusion was decanted, filtered using muslin cloth and evaporated to dryness in a water bath at 40 °C. Dried extracts were weighed and dissolved in 10% dimethylsulphoxide to yield a stock solution from which lower concentrations were prepared.

2.4 **Phytochemical screening**

Phytochemical compositions of the leaves were determined using the methods variously described by Trease and Evans (1996) and Sofowara (2006).

2.5 **α-Amylase inhibitory assay**

This assay was carried out using a modified procedure of McCue and Shetty (2004). A total of 250 µL of extract was placed in a tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution was added. This solution was pre-incubated at 25 °C for 10 mins, after which 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added.
at timed intervals and then further incubated at 25 °C for 10 mins. The reaction was terminated after incubation by adding 500 µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 mins and cooled to room temperature. The reaction mixture was diluted with 5 ml distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition.

\[
\% \text{ Inhibition} = \left[ \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{extracts}})}{\text{Abs}_{\text{control}}} \right] \times 100.
\]

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC\text{50}) were determined graphically.

2.6 Mode of α-amylase inhibition

The mode of inhibition of the leaf extract was conducted using the extract with the lowest IC\text{50} according to the modified method described by Ali \textit{et al.} (2006). Briefly, 250 µL of the (5 mg/mL) extract was pre-incubated with 250 µL of α-amylase solution for 10 minutes at 25 ºC in one set of tubes. In another set of tubes α-amylase was pre-incubated with 250 µL of phosphate buffer (pH 6.9). 250 µL of starch solution at increasing concentrations (0.30 – 5.0 mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 mins at 25 ºC, and then boiled for 5 mins after addition of 500 µL of DNS to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a maltose standard curve and converted to reaction velocities. A double reciprocal plot (1/V versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted. The type (mode) of inhibition of the crude extract on α-amylase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.
2.7 **α-Glucosidase inhibitory assay**

The effect of the plant extracts on α-glucosidase activity was determined according to the method described by Kim *et al.* (2005), using α-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophynyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, pH 6.9. Then 100 µL of α-glucosidase (E.C. 3.2.1.20) was pre-incubated with 50 µL of the different concentrations of the extracts (acetone, ethanol and water) for 10 mins. Then 50 µL of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37 °C for 20 mins and stopped by adding 2 ml of 0.1 M Na₂CO₃. The α-glucosidase activity was determined by measuring the yellow colored para-nitrophenol released from pNPG at 405 nm. The results were expressed as percentage of the blank control.

Percentage inhibition calculated as

\[
\% \text{ Inhibition} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}}\right) \times 100
\]

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC₅₀) were determined graphically.

2.8 **Mode of α-glucosidase inhibition**

The mode of inhibition of the leaf extract was determined using the extract with the lowest IC₅₀ according to the modified method described by Ali *et al.* (2006). Briefly, 50 µL of the (5 mg/mL) extract was pre-incubated with 100 µL of α-glucosidase solution for 10 mins at 25 °C in one set of tubes. In another set of tubes α-glucosidase was pre-incubated with 50 µL of phosphate buffer (pH 6.9). 50 µL of PNPG at increasing concentrations (0.63 – 2.0 mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 mins at 25 °C, and 500 µL of Na₂CO₃ was added to stop the reaction. The amount of reducing sugars released
was determined spectrophotometrically using a paranitrophenol standard curve and converted to reaction velocities. A double reciprocal plot (1/V versus 1/[S]) where V is reaction velocity and [S] is substrate concentration was plotted. The type (mode) of inhibition of the crude extract on α-glucosidase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

3.0 Results

Percentage yield of different extracts of *B. sapida* is shown in table 1. Acetone extract has the highest percentage yield when compared to others. From the analysis carried out (Table 2) on the phytochemical screening of different extracts of the plant, it was observed that all the extracts have flavonoids, reducing sugar and tannins. Also terpenoids were detected from aqueous and ethanolic extract while saponin was present in the aqueous extract only.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Initial weight</th>
<th>Final weight</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>28.31</td>
<td>1.73</td>
<td>6.11</td>
</tr>
<tr>
<td>Ethanol</td>
<td>42.82</td>
<td>1.26</td>
<td>2.94</td>
</tr>
<tr>
<td>Water</td>
<td>41.82</td>
<td>2.1</td>
<td>5.02</td>
</tr>
</tbody>
</table>

Table 1: Percentage yield of different extracts of *B. sapida*
Table 2: Phytochemical composition of different extract of *Blighia Sapida*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Extracts inference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetone</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 1 shows the percentage inhibition of α-amylase by various extracts of *B. sapida*. At low concentration (1.25 – 10 mg/mL) there were no significant differences among all the extracts tested. However, at 10 mg/mL acetone extract significantly reduced (p < 0.05) when compared to others. From the IC₅₀ extrapolated graphically, it shows that aqueous extract of *B. sapida* has lowest IC₅₀ value which gives more inhibitory potential compared to ethanol and acetone extracts (table 3). The Lineweaver-Burke plot was generated to determine the mode of inhibition of the enzyme and the result showed that aqueous extract of *B. sapida* inhibited α-amylase in a mixed non-competitive manner (Figure 2).
Fig. 1 Percentage inhibition of α-amylase by different extracts of *Blighia Sapida*

Fig. 2. Mode of inhibition of α-amylase by aqueous extract of *Blighia sapida*
Table 3: IC₅₀ value of various extracts of *Blighia sapida* against alpha Amylase

<table>
<thead>
<tr>
<th>Extracts</th>
<th>IC₅₀ (mg/ml)</th>
<th>α-amylase</th>
<th>α-glucosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>13.25 ± 2.10</td>
<td>5.15 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>8.50 ± 1.31</td>
<td>4.57 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>5.80 ± 0.70</td>
<td>5.20 ± 0.41</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3 shows the percentage inhibition of α-glucosidase by different extracts of *B. sapida*. The values from all the extracts were not significantly different (p > 0.05) from one another at the same concentration. However, the IC₅₀ values obtained from the dose-response curves of the extracts showed that ethanolic extract has the lowest value (Table 3). Figure 4 depicts that the mode of inhibition of α-glucosidase by the ethanolic extract of *B. sapida* is an uncompetitive type.

![Graph showing percentage inhibition](image)

**Fig. 3** Percentage inhibition of α-glucosidase by different extracts of *Blighia Sapida*
4.0 Discussion

Management of blood glucose level is a critical strategy in the control of diabetes and its complications (Dicarli et al., 2003). α-amylase and α-glucosidase inhibitors 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 carbohydrates digestion time causing a reduction in the rate of glucose absorption and consequently reducing postprandial plasma glucose rise (Kimura et al., 2001).

The low percentage inhibition of α-amylase by the extracts of B. sapida is a pointer to the fact that the plant is a mild inhibitor of the enzyme, which is desirable in order to prevent some of the side effects produced by synthetic drugs. This is also in agreement with the report of Pinto et al. (2009) that dietary management of hyperglycemia linked to diabetes can be targeted through foods or botanical supplements that have moderate α-amylase inhibition. The characteristic mixed non-competitive inhibition by the aqueous extract of B. sapida suggest that...
the active components in the extract do not compete with the substrate for the active site of the enzyme, rather the inhibitors bind to a separate site on the enzyme to retard the conversion of substrate to product (Mayur et al., 2010).

As for $\alpha$-glucosidase inhibition, the strong inhibition of the enzyme displayed by all the extracts especially ethanolic extract as shown by the lowest IC$_{50}$ (4.57) suggests that this extract contains potent $\alpha$-glucosidase inhibitor. This is because any plant or drug which is a strong inhibitor of $\alpha$-glucosidase could serve as effective therapy for postprandial hyperglycemia with minimal side effects (Kwon et al., 2006). Further study to ascertain the mode of inhibition of $\alpha$-glucosidase by the ethanolic extract of this plant showed the extracts inhibited the enzyme uncompetitively. This suggests that the inhibitor binds exclusively to the enzyme-substrate complex yielding an inactive enzyme-substrate-inhibitor complex (Bacchawat et al., 2011).

The inhibitory effects of aqueous and ethanolic extract of $B$. sapida on the $\alpha$-amylase and $\alpha$-glucosidase activities respectively may be attributed to the presence of phytochemicals such flavonoids, tannins and saponins. Previous studies attributed the medicinal property of the aqueous extract of this plant to the presence of saponins (Abolaji et al., 2007; Aderinola et al., 2007). Moreover, a water-soluble pure compound named hypoglycin was isolated from this plant which also suggests that the plant possesses hypoglycemic properties (Hassall and Reyle, 1955). This also explains the reason behind the effective inhibitory activity displayed by the aqueous extract towards these enzymes when compared to that exhibited by acetone and ethanolic extract. The $\alpha$-amylase and $\alpha$-glucosidase potential of this plant may also be attributed to the presence of flavonoids which have been reported to preserve $\beta$-cell integrity and function by mopping up free radicals in the system and therefore protect against the progression of insulin resistance of type 2 diabetics (Sama et al., 2012).
It can be concluded that the aqueous extract of *B. sapida* effectively inhibited the activity of α-amylase in a non-competitive manner while ethanolic extract inhibited α-glucosidase in an uncompetitive manner respectively. This inhibitory property of the extract maybe attributed to the presence of phytochemicals such as saponins and flavonoids. However, further study is required to isolate the active enzyme inhibitory component from this plant.

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