

# Antioxidative Properties of *Blighia sapida* K.D. Koenig Stem Bark Extract and Inhibitory Effects on Carbohydrate Hydrolyzing Enzymes Associated with Non-Insulin Dependent Diabetes Mellitus

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## History

- Submission Date: 05-10-2017;
- Review completed: 22-12-2017;
- Accepted Date: 08-01-2018

DOI : 10.5530/pj.2018.2.63

## Article Available online

<http://www.phcogj.com/v10/i2>

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## ABSTRACT

**Background and aim:** In Africa, the fruit, seed, leaf, and stem of *Blighia sapida* K.D. Koenig are used as remedy for a range of diseases. This study characterized the phytochemicals, assessed the antioxidant and inhibitory properties of the stem bark of *Blighia sapida*. **Materials and methods:** Samples were consecutively extracted using ethanol solvent and investigated for phenol content, DPPH radical scavenging abilities, iron chelation, reducing power,  $\alpha$ -amylase and  $\alpha$ -glucosidase activities as markers of *in vitro* anti-diabetic activities. Phytochemicals were analyzed using gas chromatography-mass spectrometry (GC-MS) analysis. **Results:** The extract had radical scavenging capabilities, iron chelation and reducing power. Furthermore, the results reveal the extract had considerably high  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities as the concentration increases with  $IC_{50}$  values of  $4.17 \pm 0.24$  mg/mL and  $5.30 \pm 1.25$  mg/mL, respectively. Enzyme kinetic studies indicated that the extract displayed uncompetitive inhibitor for  $\alpha$ -glucosidase and was a competitive inhibitor of  $\alpha$ -amylase. The ethanolic extract is rich in several aromatic phenolic compounds and long chain aliphatic acids. **Conclusion:** These results reveal the bark possessed antioxidant as well as  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities.

**Key words:** *Blighia sapida* K D Koenig,  $\alpha$ -amylase,  $\alpha$ -glucosidase, Antidiabetic, Antioxidant activity.

## INTRODUCTION

Diabetes mellitus is a metabolic disorder that causes disturbances of carbohydrate, fat and protein metabolism, leading to several complications such as nephropathy, neuropathy, and retinopathy. Current statistics suggest that about 382 million people are living with diabetes worldwide and this number is projected to increase to 552 million by 2035. In Africa, 19.8 million people are diagnosed with the disease while another 12.4 million are undiagnosed.<sup>1</sup> (IDF 2014). The search for the discovery of anti-diabetic drugs from medicinal plants is an important strategy required to combat the widespread nature of diabetes mellitus in the world. This is because present synthetic drugs have many disadvantages ranging from limited efficacy and several side effects such as weight gain and chronic tissue damage.<sup>2</sup> (Kane *et al.* 2005). However, current therapeutic strategy for the control of postprandial hyperglycemia is the inhibition of two key members of exo-acting glycoside hydrolases ( $\alpha$ -glucosidase and  $\alpha$ -amylase), resulting in a protracted delay of carbohydrate digestion to absorbable monosaccharides (Kawamura-Konishi *et al.*

2012). Therefore,  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitors will ultimately reduce the flow of glucose from complex dietary carbohydrates into the bloodstream, diminishing the postprandial hyperglycemia. Therefore, the search for alternative agents with potent antioxidative properties that could also decrease postprandial hyperglycemia, thereby providing a holistic avenue to control hyperglycemia and other diabetic complications resulting from oxidative stress is of utmost importance. One of such plants used in the management of this disease in Africa is *Blighia sapida* K.D. Koenig.

*Blighia sapida* K.D. Koenig, also known as 'Akee apple', belongs to the plant family called *Sapindaceae*. There are different species of this plant, which include *B. sapida*, *B. welwitschii* and *B. unijugata* (Keay 1999). *B. sapida* is a familiar tree often planted to provide shade from hot sun. It is known locally as 'Isin' in Yoruba, 'Gwanja kusa' in Hausa and 'Okpu' in Igbo (Keay 1999). *B. sapida* is about 25m high and 2.5m in girth, with a heavy evergreen crown. *B.*

**Cite this article:** Ojo OA, Ajiboye BO, Imiere OD, Adeyonu O, Olayide I, Fadaka A. Antioxidative Properties of *Blighia sapida* K.D. Koenig Stem Bark Extract and Inhibitory Effects on Carbohydrate Hydrolyzing Enzymes Associated with Non-Insulin Dependent Diabetes Mellitus. Pharmacogn J. 2018;10(2):376-83.

*sapida* is a native of West Africa. It extends from Senegal to Gabon. It is also cultivated in India and tropical America. *B. sapida* is well distributed throughout Nigeria and found in the drier forest of the savannah region (Esuoso and Odetokun 2005). *B. sapida* is a medicinal plant commonly used by traditional healers in Nigeria, and highly valued in Africa for the treatment of various ailments (Owonubi 1996). The fruit has an inhibitory effect against  $\alpha$ -glucosidase and  $\alpha$ -amylase as reported by (Kazeem *et al.* 2014). *B. sapida* root extract has been shown to have hypoglycemic effect in normoglycemic rats (Saidu *et al.* 2012). *B. sapida* bark extract have been shown to ameliorate pancreatic  $\beta$ -cell dysfunction (Ojo *et al.*, 2017). However, an attempt at understanding the pharmacological features of these plants with their different chemical compound that may hitherto poses hypoglycemic potentials necessitated this study. Thus, we conducted a comprehensive and systematic investigation of the *in-vitro* antioxidant as well as  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities of extracts of *B. sapida* K.D. Koenig using complementary *in vitro* models, along with identification of bioactive compounds as well as establishing the mechanisms of  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitions via the enzyme kinetics approach.

## MATERIALS AND METHODS

### Chemicals and reagents

$\alpha$ -Glucosidase from *Saccharomyces cerevisiae*, porcine pancreatic amylase, *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG), *p*-nitrophenol, gallic acid, and potassium ferricyanide were obtained from Sigma-Aldrich, Germany. Starch, dinitrosalicylic acid (DNS), maltose, ethanol, trichloroacetic acid, 1,10-phenanthroline, gallic acid, FeSO<sub>4</sub>, potassium ferricyanide, ferric chloride, dimethylsulfoxide (DMSO), 1,1-diphenyl-2-picrylhydrazil (DPPH), thiobarbituric acid and Folin Ciocalteu reagents were obtained from Merck Chemical Company, Germany.

### Plant material

Fresh stem bark peelings of *Blighia sapida* K.D. Koenig were collected at a local farm Abeokuta, Ogun State. Identification and authentication was previously done by (Ojo *et al.*, 2017).

### Preparation of Extract

Fresh stem bark was air-dried in the laboratory at ambient temperature (30  $\pm$  2°C), pulverized using a laboratory mechanical grinder and the ground powders obtained stored until further use. The powdered sample (100 g) was extracted with a solvent of 70 % ethanol in water for 48 h. The filtrate was concentrated and evaporated to dryness at 60°C, using rotary evaporator. The yield was as shown below and the dry extract was stored at 4 °C until further analysis.

$$\text{Percentage yield} = \frac{\text{Weight of the dry extract}}{\text{Weight of powdered leaves}} \times 100$$

### Determination of total phenol content

The total phenol content of the bark extract was determined (as gallic acid equivalent) as described by McDonald *et al.* (2001) with slight modifications. Briefly, 200  $\mu$  L of the extract dissolved in 10 % DMSO (240  $\mu$  g mL<sup>-1</sup>) was incubated with 1.0 mL of Folin Ciocalteu reagent (diluted 10 times) and 800  $\mu$  L of 0.7 mol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> for 30 min at room temperature. Then, the absorbance was measured at 765 nm on a Shimadzu UV mini 1240 spectrophotometer (Shimadzu, Japan). All measurements were done in triplicates. Results were expressed as mg GAE/ 100 g dry ethanol extracts.

### Determination of Reducing Power

The reducing power of the bark extract was determined by reduction a FeCl<sub>3</sub> solution as described by Pulido *et al.* (2000). A 2.5 mL aliquot of

the extract was mixed with 2.5 mL, 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL, 1% potassium ferricyanide. The solution was incubated at 50 °C for 20 min and then 2.5 mL, 10% TCA was added. The sample was then centrifuged at 650 g for 10 min and 5 mL of the supernatant was mixed with an equal volume of water and 1.0 mL, 0.1% ferric chloride. The same treatments were performed to a standard ascorbic acid solution and the absorbance read at 700 nm. The reducing power was then calculated and expressed as percentage inhibition.

### Determination of Iron Chelation Ability

The Fe<sup>2+</sup> chelating ability of bark extract was determined by employing a modified method of (Minotti and Aust 1987) with a slight modification by Puntel *et al.* (2005). Freshly prepared 500  $\mu$  mol L<sup>-1</sup> FeSO<sub>4</sub> (150  $\mu$  L) was added to a solution containing 168  $\mu$  L of 0.1 mol L<sup>-1</sup> Tris-HCl (pH 7.4), together with 218  $\mu$  L saline and an ethanol extract (1-5 mg/mL). The solution was incubated for 5 min, followed by addition of 13  $\mu$  L of 0.25% 1, 10-phenanthroline (w/v). Absorbance was read at 510 nm. Fe chelating ability was expressed as percentage inhibition.

### DPPH radical scavenging activity

The free radical scavenging activity of the extract was determined using a slightly modified method described by Tuba and Gulcin (2008). A 0.3 mmol L<sup>-1</sup> solution of DPPH was prepared in methanol and 500  $\mu$  L of this solution was added to 1 mL of the extract (dissolved in 10 % DMSO) at different concentrations (1 – 5 mg/mL). These solutions were mixed and incubated in the dark for 30 min at room temperature. Absorbance was read at 517 nm against blank lacking scavenger.

### Determination of $\alpha$ -amylase inhibitory activity

The  $\alpha$ -amylase inhibitory activity was determined as described by Shai *et al.* (2010). A volume of 250  $\mu$  L of ethanol extract at different concentrations (1 – 5 mg mL<sup>-1</sup>) was incubated with 500  $\mu$  L of porcine pancreatic amylase (2 U mL<sup>-1</sup>) in 100 mmol L<sup>-1</sup> phosphate buffer (pH 6.8) at 37 °C for 20 min. 250  $\mu$  L of 1 % starch dissolved in 100 mmol L<sup>-1</sup> phosphate buffer (pH 6.8) was added to the solution and incubated at 37 °C for 1 h. 1.0 mL of DNS color reagent was then added and boiled for 10 min. Absorbance was read at 540 nm and the inhibitory activity was expressed as a percentage of a control sample without inhibitors. All assays were carried out in triplicate.

$$\alpha - \text{amylase inhibition (\%)} = \frac{A_{540 \text{ control}} - A_{540 \text{ sample}}}{A_{540 \text{ control}}} \times 100$$

### Mode of $\alpha$ -amylase inhibition

The mode of inhibition of  $\alpha$ -amylase by the bark extract was determined by employing a modified method described by Ali *et al.* (2006). Briefly, 250  $\mu$  L of the (2.5 mg mL<sup>-1</sup>) extract was pre-incubated with 250  $\mu$  L of  $\alpha$ -amylase solution for 10 min at 37°C in one set of tubes. In another set of tubes,  $\alpha$ -amylase was pre-incubated with 250  $\mu$  L of phosphate buffer (pH 6.9). Two hundred and fifty  $\mu$  L of the starch solution at increasing concentrations (0.3-5.0 mg mL<sup>-1</sup>) was added to both sets of reaction mixtures to start the reaction. The solution was then incubated for 10 min at 37°C and boiled for 5 min after addition of 500  $\mu$  L of DNS to stop the reaction. The amount of reducing sugars released was determined using a maltose standard curve and converted to reaction velocities. A double reciprocal (Lineweaver-Burk) plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration, was plotted to determine the mode of inhibition.

### Determination of $\alpha$ -glucosidase inhibitory activity

The  $\alpha$ -glucosidase inhibitory activity was determined as described by Ademiluyi and Oboh (2013). Briefly, 250  $\mu$  L of the extract, at different concentrations (1–5 mg mL<sup>-1</sup>), was incubated with 500  $\mu$  L of 1.0 U mL<sup>-1</sup>

$\alpha$ -glucosidase solution in 100 mmol L<sup>-1</sup> phosphate buffer (pH 6.8) at 37 °C for 15 min. Thereafter, 250  $\mu$ L of a pNPG solution (5 mmolL<sup>-1</sup>) in 100 mmol L<sup>-1</sup> phosphate buffer (pH 6.8) was added and the solution was further incubated at 37°C for 20 min more. Absorbance was read at 405 nm and the inhibitory activity was expressed as the percentage of a control sample without inhibitors.

$$\alpha\text{-glucosidase inhibition (\%)} = \frac{A_{405\text{ control}} - A_{405\text{ sample}}}{A_{405\text{ control}}} \times 100$$

#### Mode of $\alpha$ -glucosidase inhibition

The mode of inhibition of  $\alpha$ -glucosidase by the stem bark extract was determined as described by Ali *et al.* (2006). Briefly, 50  $\mu$ L of the (2.5 mg mL<sup>-1</sup>) extract was pre-incubated with 100  $\mu$ L of  $\alpha$ -glucosidase solution for 10 min at 37°C in one set of tubes. In another set of tubes, 100  $\mu$ L  $\alpha$ -glucosidase was pre-incubated with 50  $\mu$ L of phosphate buffer (pH 6.9). 50  $\mu$ L of pNPG at increasing concentrations (0.63-2.0 mg mL<sup>-1</sup>) was added to both sets of reaction mixtures to start the reaction. The solution was incubated for 10 min at 37°C and 500  $\mu$ L of Na<sub>2</sub>CO<sub>3</sub> was added to stop the reaction. The amount of reducing sugars released was determined using a para-nitrophenol standard curve and converted to reaction velocities. A double reciprocal (Lineweaver-Burk) plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted to determine the mode of inhibition.

#### Gas chromatography-mass spectrometric (GC-MS) analysis

The ethanol extract was subjected to GC-MS analysis. The analysis was carried out as described by Ibrahim *et al.* (2014). The carrier gas was ultra-pure helium at a flow rate of 0.7 mL min<sup>-1</sup> and a linear velocity of 37 cm s<sup>-1</sup>. The injector temperature was set at 250°C. The initial oven temperature was 60°C, which was programmed to 280 °C at the rate of 10°C min<sup>-1</sup> with a hold time of 3 min. Injections of 2  $\mu$ L were made in the split less mode with a manual split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230 °C, quadrupole temperature 150 °C, solvent delay 4 min and scan range 50-700 amu. Compounds were identified by direct comparison of the retention times and mass fragmentation pattern with those from the National Institute of Standards and Technology (NIST) library.

#### Data analysis

Results of the three replicates were pooled and expressed as the mean  $\pm$  SD. Differences between groups were assessed by an analysis of variance model and Tukey's test. The level of significance for the analyses was set at  $p < 0.05$ . These analyses were performed by using the free software R version 3.1.1. (R Core Team, 2014).

## RESULTS AND DISCUSSION

#### Total Phenol Content

The ethanol extract of *Blighia sapida* bark had 19.1% as its yield (Table 1). The total phenolic content of ethanol extract of *Blighia sapida* bark increases as the concentration (1-5 mg/ml) of extract increases. At 5 mg/ml, the extract of *Blighia sapida* had high phenol content of 19.92  $\pm$  0.12 mg GAE/100g Table 2. However, ethanolic extract have high yields and contain a high total phenol content as the concentration increases. This is consistent with previous findings that ethanol is the best solvent for the extraction of a maximum yield of polyphenols compared to other solvents (Mohd-Esa *et al.* 2010; Ojo *et al.* 2014). Although no correlation analysis was carried out in this study, previous studies strongly correlate antioxidative effect to total polyphenol contents (Hossain *et al.* 2009).

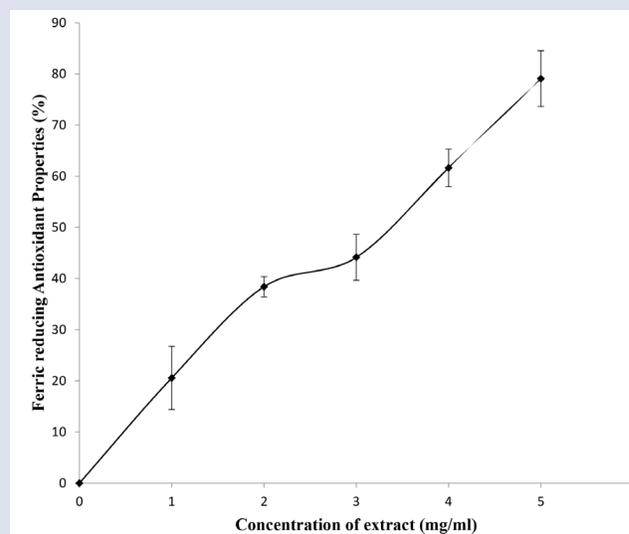
**Table 1: Percentage yield of Ethanol extract of *Blighia sapida* bark.**

Extract	Initial weight (g)	Final weight (g)	% Yield
Ethanol	100	19.1	19.1

**Table 2: Total Phenol contents of the Ethanol Extract of *Blighia sapida* Stem bark.**

Concentration (mg/ml)	(mg GAE/100g)
1	7.45 $\pm$ 0.09
2	9.80 $\pm$ 0.13
3	12.75 $\pm$ 0.11
4	14.85 $\pm$ 0.01
5	19.92 $\pm$ 0.12

\* Results are expressed as mean of three determinations  $\pm$  standard deviation.



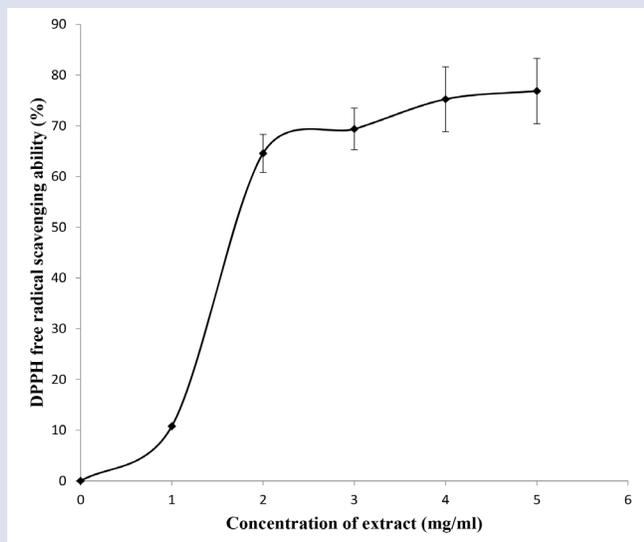
**Figure 1:** Ferric reducing antioxidant properties of ethanol extracts of *Blighia sapida* stem bark.

#### Reducing Power

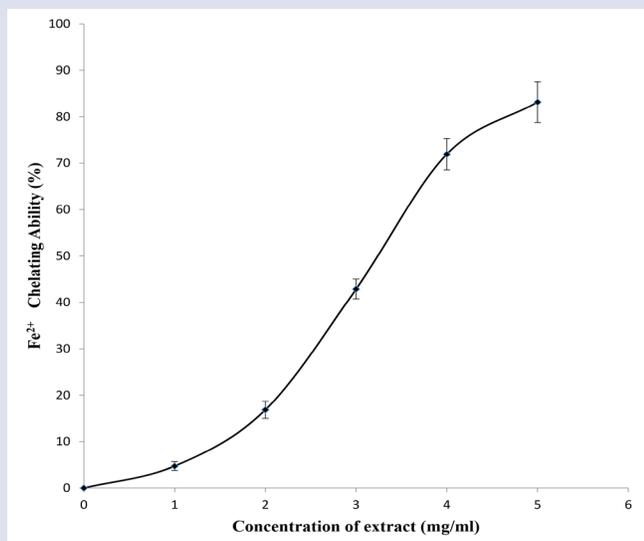
Ethanol extracts of *Blighia sapida* bark showed significantly ( $P < 0.05$ ) higher reducing power Figure 1. The trend of the reducing capacity of the extracts was concentration dependent (1-5 mg/ml). Reducing power is an antioxidant defense system and the two mechanisms known to affect this property are electron transfer and hydrogen atom transfer (Dastmalchi *et al.* 2007; Ojo *et al.* 2014). The reducing capacity of the extracts could also indicate potential antioxidant activities (Obboh and Rocha 2007).

#### DPPH radical scavenging ability

The scavenging effect of extracts in the range 1 – 5 mg mL<sup>-1</sup> on the DPPH radical increased with an increasing concentration of *B. sapida* extracts Figure 2. IC<sub>50</sub> value for DPPH scavenging by the ethanol extracts of *B. sapida* was 2.53 mg mL<sup>-1</sup>. In the present investigation, *B. sapida* at different concentration demonstrated significant DPPH scavenging activity indicating its ability to act as radical scavengers. This study revealed that ethanol extracts of *B. sapida* stem bark scavenged DPPH free radicals. Thus, the observed DPPH radical scavenging ability might



**Figure 2:** DPPH free radical scavenging ability of ethanol extracts of *Blighia sapida* stem bark.



**Figure 3:** Iron chelating ability of ethanol extracts of *Blighia sapida* stem bark.

be attributed to the abundant phenols in the extracts. This is consistent with previous studies (Gyamfi and Yonamine 1999; Shim *et al.* 2003).

#### Iron chelating ability

*Blighia sapida* ethanol bark extract had a high chelating effect. IC<sub>50</sub> values for the chelating effect of ethanol extracts of *B. sapida* bark was 3.29 mg/mL. The trends of the chelating activity increased with increasing extract concentration of *B. sapida* bark (Figure 3). Furthermore, chelating iron could help prevent the generation of hydroxyl radicals. Iron serves as a metal catalyst in producing hydroxyl radicals from hydrogen peroxide (Obboh *et al.* 2008; Ojo *et al.* 2013a). The bark extract significantly chelates Fe<sup>2+</sup> in a concentration dependent manner (1–5 mg/mL). By chelating

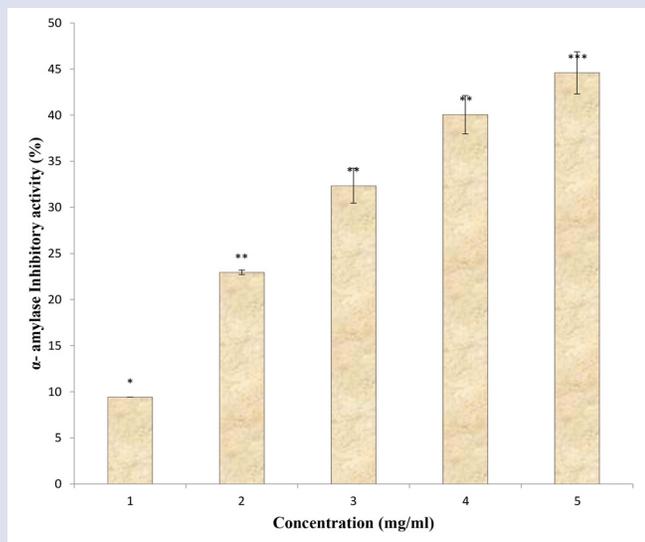
Fe<sup>2+</sup>, the generation of hydroxyl radicals in the Fenton reaction can be attenuated and thus prevent possible damage of hydroxyl radicals to biomolecules. Accumulation of iron has been reported to lead to an increase in free radicals and development of oxidative stress (Shim *et al.* 2003).

#### $\alpha$ -amylase inhibitory activity

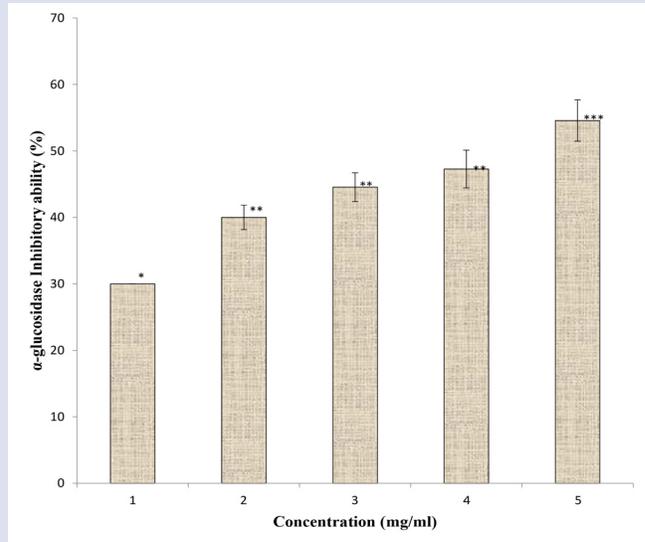
Figure 4 shows the percentage inhibition of  $\alpha$ -amylase ethanol extract of *B. sapida* bark. The fraction had appreciable *in vitro* inhibitory activity against  $\alpha$ -amylase. The IC<sub>50</sub> value for the ethanol extract against  $\alpha$ -amylase activity was determined from a dose-response curve to be  $0.053 \pm 0.25$  mg/mL (Table 3). The Lineweaver-Burke plot was generated to determine the mode of inhibition of the enzyme and the result showed that ethanol extract of *B. sapida* inhibited  $\alpha$ -amylase in a competitive manner (Figure 6). It is an established fact that  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors from natural sources play a significant role in diabetic management. This is achieved via a decrease in postprandial hyperglycemia through inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase actions (Adefegha and Obboh 2012). Furthermore, for effective control of postprandial hyperglycemia, moderate  $\alpha$ -amylase inhibition and potent  $\alpha$ -glucosidase inhibition provide better options for controlling the availability of dietary glucose for absorption in the intestinal tract (Kajaria *et al.* 2013). This is due to adverse effects associated with strong  $\alpha$ -amylase inhibition such as abdominal distension, flatulence, and diarrhea (Al-Zuhair *et al.* 2010). In this study, *B. sapida* ethanol extract demonstrated  $\alpha$ -amylase inhibition and  $\alpha$ -glucosidase inhibition, indicating a potential role as an anti-diabetic agent. The competitive inhibition of  $\alpha$ -amylase by the extract of *B. Sapida* bark suggests that the active inhibitory component(s) of the extract are structurally similar to the normal substrate of the enzyme. Therefore, it binds reversibly to the active site of the enzyme and occupies it in a mutually exclusive manner with the substrate (Shai *et al.* 2010). These high  $\alpha$ -amylase inhibitory activity, might suggest the presence of more active compounds that could inhibit the breakdown of complex carbohydrates to oligosaccharides, thereby diminishing the effect of carbohydrate consumption on postprandial hyperglycemia. This is because some of the side effects associated with the currently available drugs for the management of T2D are linked with excessive inhibition of  $\alpha$ -amylase activity (Ademiluyi and Obboh 2013).

#### $\alpha$ -glucosidase inhibitory activity

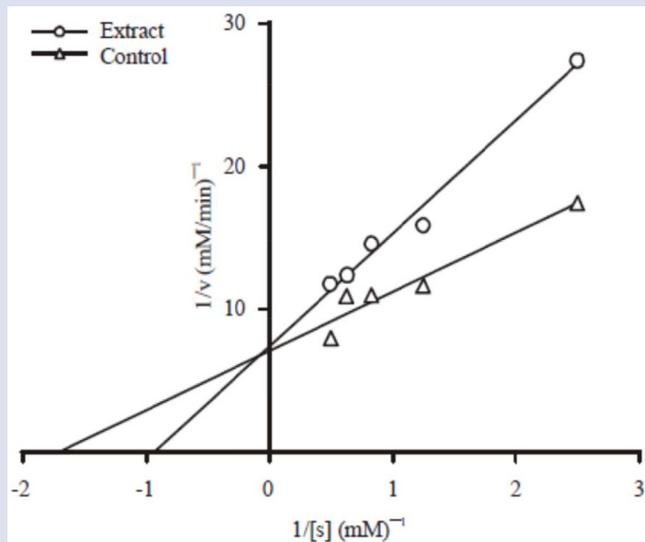
Figure 5 shows the percentage inhibition of  $\alpha$ -glucosidase by ethanol extract of *B. sapida* bark. The fraction inhibited  $\alpha$ -glucosidase activities *in vitro* in a dose dependent manner. The concentration of the extract required to cause 50% inhibition (IC<sub>50</sub>) was  $0.0417 \pm 0.24$  mg/ml (Table 3). Figure 5 depicts that the mode of inhibition of  $\alpha$ -glucosidase by the ethanol extract of *B. sapida* is an un-competitive type of inhibition (Figure 7). For  $\alpha$ -glucosidase inhibition, the strong inhibition of the enzyme displayed by the bark extract as shown by the IC<sub>50</sub> values suggests that this extract is a potent  $\alpha$ -glucosidase inhibitor. Further study to ascertain the mode of inhibition  $\alpha$ -glucosidase by the extract of *B. sapida* bark showed the extracts inhibited the enzyme competitively. This suggests that the inhibitor binds exclusively to the enzyme substrate complex yielding an inactive enzyme-substrate-inhibitor complex (Bachhawat *et al.* 2011; Kazeem *et al.* 2013). Thus, the strong inhibition of  $\alpha$ -glucosidase by the extract would slow down the breakdown of disaccharides to liberate glucose, thereby reducing glucose absorption from the small intestine (Kwon *et al.* 2007). Furthermore, in spite of abundant reports on the  $\alpha$ -glucosidase inhibitory activities of extracts and pure compounds from plants from stem bark, only a few authors (Kang *et al.* 2012) to the best of our knowledge reported such a low IC<sub>50</sub> value as found with the ethanol extract in our study. This observation might be connected to the phenol content in this extract, because polyphenolic fractions from plants have



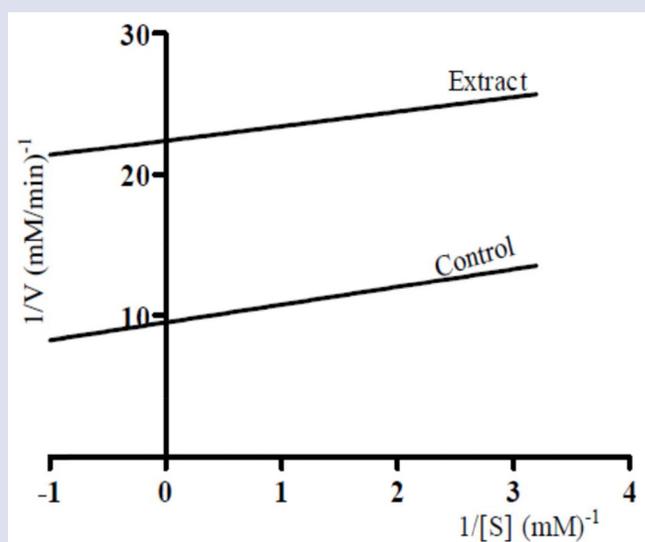
**Figure 4:** α-amylase inhibitory activities of ethanol extract of *Blighia sapida* bark. Data are presented as mean ± SD of triplicate determinations. (\*-\*\*\*). Different symbol over the bars for a given concentration for each extract indicate a significant difference from each other (Duncan multiple range post hoc test,  $p < 0.05$ ).



**Figure 6:** α-glucosidase inhibitory activities of ethanol extract of *Blighia sapida* bark. Data are presented as mean ± SD of triplicate determinations. (\*-\*\*\*). Different symbol over the bars for a given concentration for each extract indicate a significant difference from each other (Duncan multiple range post hoc test,  $p < 0.05$ ).



**Figure 5:** Mode of inhibition of α-amylase by ethanol extract of bark of *Blighia sapida*.



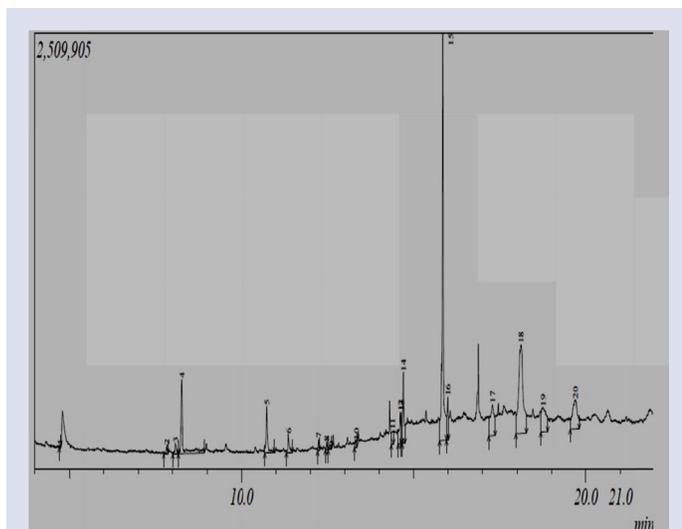
**Figure 7:** Mode of inhibition of α-glucosidase by ethanol extract of bark of *Blighia sapida*.

**Table 3:** IC<sub>50</sub> values for the inhibition of α-glucosidase and α-amylase by ethanol extract of *Blighia sapida* bark.

Fraction	IC <sub>50</sub> (mg/ml)	
	α-glucosidase	α-amylase
Ethanol	0.0417 ± 0.24 <sup>a</sup>	0.0530 ± 0.25 <sup>b</sup>

Data are presented as mean ± SD of triplicate determinations. a-b Different letters over the bars for a given concentration for each extract indicate a significant difference from each other (Duncan multiple range post hoc test,  $p < 0.05$ ).

been shown to inhibit α-glucosidase activity, allowing for tighter control of blood glucose (Kamiyama *et al.* 2010). The inhibitory effects of the extract of the *B. sapida* stem bark on the α-amylase and α-glucosidase activities may be attributed to the presence of phytochemicals such as polyphenols, tannins and saponins. However, 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 contributes to the plant hypoglycemic properties (Hassall and



**Figure 8:** GC-MS Chromatogram of the ethanol extract of *Blighia sapida* bark.

Name: 2-Methoxy-4-vinylphenol

Name: 2,4,6-Trimethylanisole

Name: 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl) Name: 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)

Name: 1,2-Benzenedicarboxylic acid

Reyle 1955). This could also give the reason behind the inhibitory activity

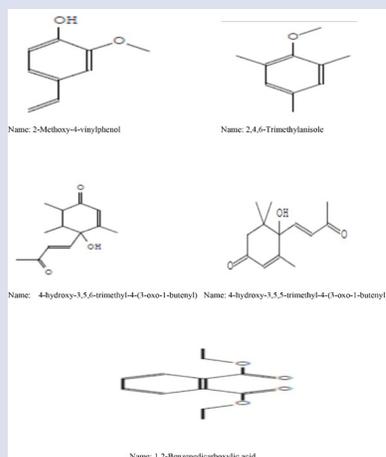
shown by the ethanol extract of *B. sapida* bark towards these enzymes.

#### GC-MS analysis

The GC-MS chromatogram of the fraction is presented in Figure 8. Twenty peaks were visible in the chromatogram and the various chemical constituents at those peaks were identified from the NIST library Table 4 and the compounds detected correspond to aromatic phenols, aromatics containing ether, ester and acid moieties and long chain aliphatic acids. The retention time and molecular mass of the detected compounds are provided in Table 4. 2-Methoxy-4-vinyl phenol, 1-eicosanol, (-)-mellein, diethyl phthalate, 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol, phenol, 3-isopropoxy-5-methyl-, phthalic acid, heptyl 4-nitrophenyl ester, 9,9-dimethoxy bicyclo [3.3.1] nona-2,4-dione, di-dodecyl phthalate, 9,9-di-methoxybicyclo [3.3.1]nona-2,4-dione, phytol, 9,12-cctadecadienoic acid (Z,Z)-, 1,2-9,9-dimethoxybicyclo[3.3.1]nona-2,4-dione, 9-octadecenamamide, (Z)-, 9-octadecenamamide (Z)-, citronellol epoxide (R or S), 9,19-cyclolanostan-3-ol, acetate, (3.beta.)-, cholest-4-en-3-one, 9,9-dimethoxybicyclo [3.3.1]nona-2,4-dione, cis-1,2-cyclododecanediol, were present in the ethanol extracts of *B. sapida*. The most abundant phytochemicals (> 50%) in the extract as identified by the library were phenolic (2-methoxy-4-vinyl phenol) and phthalic acid (1, 2-benzenedicarboxylic acid) (Figure 9). The possible bioactive compounds in the ethanol extract of *B. sapida* bark were detected using GC-MS analysis. Although, identification of pure compounds has not been recorded. Hence, the anti-diabetic action of the ethanol extract of *B. Sapida* bark in our study could possibly be attributed to the presence of these compounds as well, while not discounting the possible contributions of the other detected phytochemicals. Phytochemical analysis of the bark resulted in the identification of compounds with potential medicinal usage (Singh *et al.* 2012). For example, 2-Methoxy-4-vinylphenol, has already been implicated in a wide array of therapeutic applications such as antioxidant, anti-microbial and anti-inflammatory. Phytols are said to be cancer preventive. Other antioxidants present were phenol, 3-isopropoxy-5-methyl-, 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol. Hypo-cholesterolemic com-

**Table 4: Identified compounds of the ethanol extract of *Blighia sapida* bark by GC-MS.**

Peak No	Compound	Retention time	Molecular mass
1	2-Methoxy-4-vinylphenol	4.73	150
2	1-Eicosanol	7.84	298
3	(-)-Mellein	8.08	178
4	Diethyl Phthalate	8.26	222
5	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	10.73	180
6	Phenol, 3-isopropoxy-5-methyl-	11.36	222
7	Phthalic acid, heptyl 4-nitrophenyl ester	12.23	385
8	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	12.48	212
9	Didodecyl phthalate	12.61	502
10	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	13.33	212
11	Phytol	14.37	296
12	9,12-Octadecadienoic acid (Z,Z)-	14.61	280
13	1,2-9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	14.65	212
14	9-Octadecenamamide, (Z)-	14.71	281
15	9-Octadecenamamide, (Z)-	15.84	281
16	Citronellol epoxide (R or S)	15.98	172
17	9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-	17.29	470
18	Cholest-4-en-3-one	18.11	384
19	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	18.75	212
20	cis-1,2-Cyclododecanediol	19.69	200



**Figure 9:** Structures of components identified by GC-MS.

pounds such as 9, 12-octadecadienoic acid (Z, Z) - were also present. The presence of various bioactive compounds in the *B. Sapida* justifies the use of the plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to the biological activity assays will definitely give useful results.

Furthermore, the phenolic hydroxyl group present in compounds 2-methoxy-4-vinylphenol and 4-hydroxy-3, 5, 6-trimethyl-4-(3-oxo-1-butenyl) could directly or indirectly be the key feature that contributed to the higher antioxidative and antidiabetic effects depicted by the stem extracts. The low reduction potentials of phenolics, hydroxyls and other related compounds inactivate and terminate the initiation and propagation of chain reactions associated with oxidative damage (Nagababu *et al.* 2010). In a similar way, phenolics and hydroxyls were reported to interfere with some surface amino acid side chains in both  $\alpha$ -amylase and  $\alpha$ -glucosidase structures (Ludwig 2002). This causes some conformational changes in the enzyme structure, thereby decreasing their actions and causing a reduction in blood glucose levels and subsequently reduced postprandial hyperglycemia.

## CONCLUSION

Based on the findings of this study, it can be concluded that ethanol stem bark extract of *Blighia sapida* K.D. Koenig possesses antioxidative as well as effective  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory effects. Hence, it is recommended that bioassay-guided fractionation of the ethanol extracts could be done in order to fully investigate the *in vivo* antidiabetic and antioxidative effects of this extract, isolating the active principle and subjecting it to *in vivo* antidiabetic evaluation.

## ACKNOWLEDGMENT

The Authors wish to acknowledge the Department of Biochemistry, Afe Babalola University for Providing the necessary facilities to carry out this study.

## COMPETING INTERESTS

The authors declare no conflict of interest.

## ABBREVIATIONS USED

***Blighia sapida*:** *B. sapida* g; gram; **GAE:** Gallic acid equivalents; **GC-MS:** gas chromatography-mass spectrometry; **DMSO:** dimethylsulfoxide

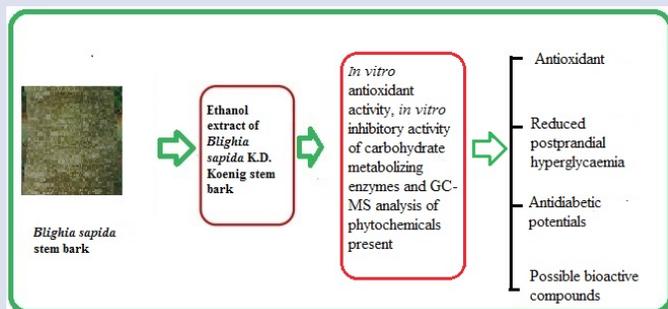
(DMSO), **DNS:** Dinitrosalicylic acid; **DPPH:** 1,1-diphenyl-2-picrylhydrazil; **pNPG:** p-nitrophenyl- $\alpha$ -D-glucopyranoside; **NIST:** National Institute of Standards and Technology.

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## GRAPHICAL ABSTRACT



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**Cite this article:** Ojo OA, Ajiboye BO, Imiere OD, Adeyonu O, Olayide I, Fadaka A. Antioxidative Properties of *Blighia sapida* K.D. Koenig Stem Bark Extract and Inhibitory Effects on Carbohydrate Hydrolyzing Enzymes Associated with Non-Insulin Dependent Diabetes Mellitus. *Pharmacog J*. 2018;10(2):376-83.

## SUMMARY

- *Blighia sapida* stem extract possess strong anti-diabetic activity via
- Inhibition of carbohydrate hydrolyzing enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase activity.
- Possess good antioxidant activity which supports its uses in the management of hyperglycaemia and its associated complications in type-2 diabetes mellitus
- This inhibitory activity may be attributed to the action of their inherent polyphenols.
- GC-MS analysis reveals bioactive compounds such as phenolics