# Inhibition of Alpha-Amylase and Antioxidant Activities of Fractions from The Methanol Extracts of The Stem of *Allanblackia floribunda* Oliv [Guttiferae].

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## ABSTRACT

*Allanblackia floribunda* Oliv. (Guttiferae) is a tree from the tropical rain forest that has been of tremendous benefit in traditional medicine for decades all over Africa. In this study, the antioxidant and hypoglycaemic potential of fractions from the methanol extract of the stem of *Allanblackia floribunda* were investigated. The antioxidant activity was investigated by determining DPPH (1,1-dipenyl-2-picryl hydrazyl) radical scavenging activities and the hypoglycaemic potential was investigated using the α-amylase inhibitory method. All the fractions except the ethyl acetate fraction had potent free radical scavenging activities against DPPH. Chloroform and water fractions were the most active. $IC_{50}$ values of 10.75, 90.15, 91.34 and >100 µg/ml were obtained for Vitamin C, chloroform, water and n-hexane fractions respectively. Alpha amylase inhibition experiment was carried out at concentration of extracts ranging from 50-2000µg/ml. At 2000 µg/ml, Acarbose had 46.04% inhibition of α-amylase while n-hexane, chloroform, ethyl acetate, water fractions had highest inhibitory activities of 45.6, 56.53, 67.00, 72.74% respectively at this concentration. Acarbose had its highest inhibitory activity of 71.4% at 200 µg/ml while all the fractions had highest activity at 2000 µg/ml. The polar fractions were more active compared to the non-polar fractions at inhibiting α-amylase, hence, it is desirable to investigate the bioactive constituents present in the water and ethyl acetate fractions in pursuit of potential anti-diabetic agents.

**Keywords**: *Allanblackia floribunda*, hypoglycaemia, antioxidant, α-amylase

## INTRODUCTION

*Allanblackia floribunda* Oliv. (Guttiferae) is an evergreen dioecious, medium-sized tree that can grow up to 30 m tall. The tree belongs to the Guttiferae family which consists of nine tree species all restricted to the humid forests in West, Central and East Africa. The fruits contain large seeds from which edible high-quality fat can be extracted (Orwa & Munjuga, 2007; Odugbemi, 2008; Orwa et al. 2009;).

*Allanblackia floribunda* (AF) also known as tallow tree and locally called "Orogbo-erin" and "Egba" in the Western and Eastern parts of Nigeria respectively, has tremendous medicinal uses all over Africa. A decoction of the stem is taken for dysentery and mouthwash in Gabon. In Congo, it is taken to relieve stomachache. In Nigeria and Ghana, the pounded bark is used as a pain reliever for toothache and diarrhea. Decoction of the stem or leaves is also taken for cough, asthma, bronchitis and other bronchial infections. Decoctions of the whole fruit are used in Ivory Coast to relieve scrotal elephantiasis (Olowokudejo et al. 2008). Prenylated xanthones have been isolated from the bark and were reported to have moderate in-vitro cytotoxicity (Locksley & Murray, 1971, Nkengfack et al. 2002). We have previously reported, the hypoglycaemic, anti-inflammatory and free radical scavenging activities of extracts from the leaves and fruits of *Allanblackia floribunda* (Ayoola et al. 2008; Ayoola et al. 2009a, b) and we also recently reported that AF is rich in polyphenols (Akpanika et al. 2017). Furthermore, antimicrobial, antitumour and antioxidant potentials of AF have also been demonstrated by Kuete et al. 2011). Free radicals have been implicated in the pathogenesis of diabetes and other diseases and inhibition of pancreatic α-amylase and/or intestinal α-glucosidase is one of the current strategies employed in the treatment of type 2 diabetes to adjust postprandial glucose levels (Lee et al. 2012). Furthermore, polyphenolic fractions from plants have been shown to inhibit α-amylase and α-glucosidase activity and allow for tighter control of blood glucose (McDougall et al. 2005; Yilmazer-Musa et al, 2012). Hence in this present study, we aim to investigate the free radical scavenging activity and hypoglycemic potential of fractions obtained from the methanol extract of AF stem. This will help to reveal fractions that contain bio-active components with the relevant activities.

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## MATERIALS AND METHODS

**Chemicals:** DPPH (1,1-dipenyl-2-picryl hydrazyl), ascorbic acid, acarbose and porcine pancreatic α-amylase were obtained from Sigma Aldrich chemical company, USA. All other reagents and chemicals were of analytical grade and obtained locally from BDH, Fluka and Sigma-Aldrich in Nigeria.

**Plant material collection and extraction**

*Allanblackia floribunda* stems were collected fresh from forest sources in Oke-igbo, Ondo state, South-west Nigeria in February 2016. The stems were identified at the Forestry Research institute of Nigeria (FRIN), and given a voucher number (FHI107929). The whole stems were cut into pieces, air dried and milled. The powdered *Allanblackia floribunda* (500 g) was then subjected to cold maceration in a total of 4,500 ml methanol (Analar grade) over 72 h. The crude extract was filtered first through cotton wool, then through Whatman's filter paper No. 42 (125 mm). The filtrate was then concentrated using rotary evaporator at 40ºC and further dried in the oven at 40°C

The dry extract was weighed and stored in a sample bottle.

**FRACTIONATION OF THE METHANOL EXTRACT OF THE STEM OF ALLANBLACKIA FLORIBUNDA**

10 g of the methanol extract was dissolved in 250 ml of distilled water and placed in a separating funnel to be fractionated with 3 × 250 ml of each solvent in order of polarity, starting with the least polar, n-Hexane, followed by chloroform, ethyl acetate and the water. The fractions were dried in the oven at 40°C. The dried fractions were weighed and stored in a glass sample bottle.

**Phytochemical screening**

The methanol extract of *Allanblackia floribunda* was screened for the presence of alkaloids, tannins, saponins, flavonoids, anthraquinones, cardiac glycosides, steroids and reducing sugars using standard procedures (Trease and Evans, 1983, Sofowora, 1993).

**Antioxidant screening**

*DPPH Rapid Screening method*

The DPPH rapid screening was carried out to determine which of the fractions had the ability to inhibit DPPH free radical scavenging activity. Each of the fractions were spotted onto the chromatographic plate using a capillary tube. A solution of DPPH in methanol solution was prepared and poured into a spray gun. The DPPH solution was sprayed onto the chromatographic plate and the spots were observed for change in colouration. The fractions that changed the colour of DPPH from purple to yellow were further investigated for DPPH radical scavenging activity.

*DPPH Radical Scavenging Activity*

The radical scavenging activity of the fractions against DPPH was determined by a previously reported method (Brand-Williams et al, 1995). Graded concentrations of each fraction (20-1000 µg/ml) were prepared. Ascorbic acid and Vitamin E (20-100 µg/ml) used as standards. All the solutions were prepared in methanol. Two mL of each prepared concentrations were placed into test tubes and 0.5 mL of 1mM DPPH solution in methanol was added. The test tubes were incubated for 15 min at room temperature, and the absorbance was read at 517 nm. A blank solution was prepared and measured containing the same amount of methanol and DPPH. The experiments were carried out in triplicates. The lower the absorbance of the reaction mixture the higher the free radical scavenging activity of the sample. Radical scavenging activity was calculated using the following formula:

DPPH scavenging effect (%) = $\frac{AB-AA}{AB}$ x 100

Where AB is the absorption of the blank sample and AA is the absorption of the extract solution.

**Alpha-amylase inhibitory activity**

The, α-amylase inhibition assay was carried out as reported by Adesegun et al. 2013. The dried fractions were dissolved in methanol to make different concentrations (50-2000 µg/ml). A 500 µl aliquot of each fraction or acarbose at concentrations between (50-2000 µg/mL were added to 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing porcine pancreatic α-amylase (0.5 mg/mL) and mixed at 32°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 at timed intervals. The reaction mixtures were incubated at 32°C for 10 min. 1 mL of Dinitrosalicylic acid colour reagent was added and the test tubes were incubated in a boiling water bath for 5 min and then cooled to room temperature. The reaction mixture was diluted with 10 mL distilled water and absorbance was measured at 540 nm. Acarbose was used as a positive control. The inhibition of α-amylase was calculated as follows:

% inhibition= $[(Abs_{control} - Abs_{samples}) / Abs_{control}] \times 100$

**DETERMINATION OF $IC_{50}$ VALUES**

$IC_{50}$ values (Concentrations of the inhibitor needed to give 50% inhibition) were determined using Microsoft Excel from plots of percent inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values.

All the experiments were carried out in the Laboratory of Phytomedicines, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos.

**RESULTS**

**Extraction and Phytochemical Composition**

The crude stem extract was obtained with a percentage yield of 4.37% while solvent fractions were obtained with the following yield: n-hexane (9.2%), Chloroform (3.4%), ethyl acetate (2.2%), water (1.8%).

Phytochemical screening of the methanol extract of AF indicated the presence of saponins, alkaloids, cardiac glycosides, reducing sugars and steroids. Tannins anthraquinones and flavonoids were absent.

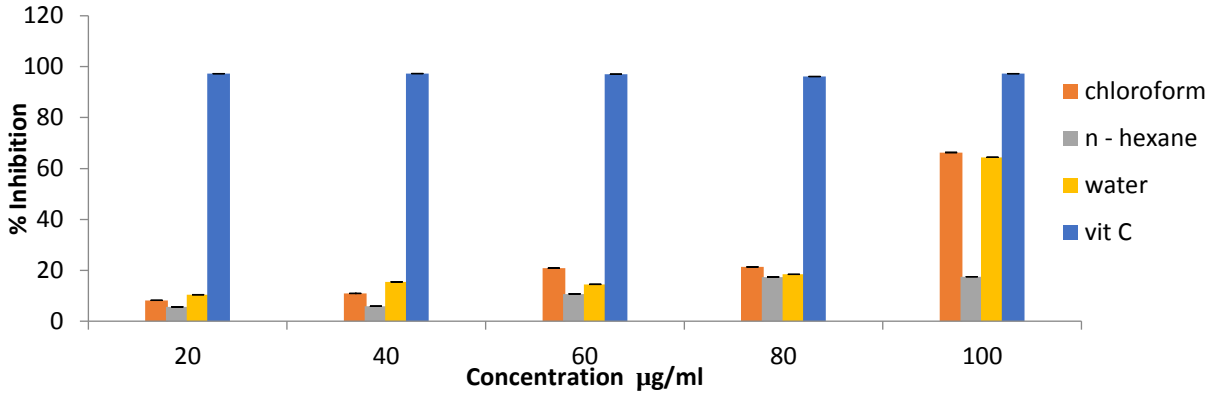


Figure 1: Percentage inhibition of DPPH for ascorbic acid and fractions from the methanol extract of AF stem

Table 1: Concentration of acarbose and solvent fractions versus percentage inhibition of alpha amylase for different solvent fractions from the methanol extract of the stem of *Allanblackia floribunda.*

| Concentration (µg/ml) | Acarbose %Inhibition | Hexane fraction %Inhibition | Chloroform fraction %Inhibition | Ethyl acetate fraction %Inhibition | Water Fraction %Inhibition |
|---|---|---|---|---|---|
| 50 | 54.6±0.001 | 6.67±0.003 | 20.38±0.001 | 8.78±0.001 | 13.74±0.001 |
| 100 | 64.13±0.001 | 16.44±0.00 | 29.3±0.001 | 42.23±0.001 | 44.59±0.001 |
| 200 | 71.41±0.01 | 21.51±0.036 | 36.82±0.001 | 48.76±0001 | 54.84±0.001 |
| 500 | 64.78±0.001 | 28.33±0.001 | 50.68±0.001 | 56.31±0.001 | 55.63±0.001 |
| 1000 | 53.31±0.001 | 38.40±0.001 | 54.05±0.001 | 62.50±0.001 | 67.45±0.001 |
| 2000 | 46.04±0.001 | 45.61±0.001 | 56.53±0.001 | 67.00±0.001 | 72.74±0.001 |

Table 2: $IC_{50}$ values for alpha-amylase inhibiton by acarbose and solvent fractions from *Allanblackia floribunda* stem.

| Sample | $IC_{50}$ for α-amylase inhibition (µg/ml) |
|---|---|
| Acarbose | 45.5 |
| Hexane fraction | >200 |
| Chloroform fraction | 500 |
| Ethyl acetate fraction | 209.1 |
| Water fraction | 126.1 |

**DPPH Free Radical Scavenging Activity**

Rapid TLC screening for antioxidant activity was positive for the crude extract, n-hexane chloroform and water fractions only as the colour of the DPPH spray changed from purple to yellowish spots. The ethyl acetate fraction of the stem of AF was not active as a free radical scavenger as it did not change the colour of DPPH. $IC_{50}$ values of 10.75, 90.15, 91.34 and >100 µg/ml were obtained for Vitamin C, Chloroform, water and n-hexane fractions respectively. The highest percentage DPPH inhibition recorded were 97.3% for Vitamin C at 20 µg/ml, 66.3, 64.4 and 17.5% for chloroform, water fraction and n-hexane fractions respectively at 100 µg/ml (Figure 1).

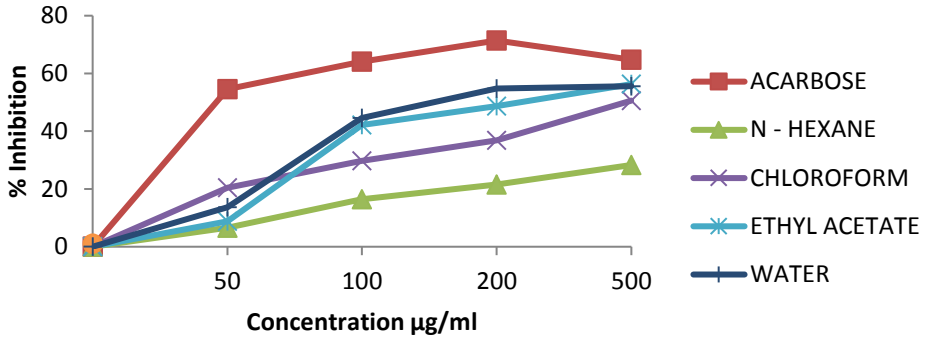


Figure 2: Graph showing percentage inhibition of α-amylase for acarbose and fractions from the methanol extract of AF stem

**Alpha-amylase inhibitory activity**

Solvent fractions from the methanol extract of *Allanblackia floribunda* stem inhibited the α-amylase enzyme with $IC_{50}$ values of 126.14, 209.1, 500 and >2000 µg/ml for water, ethyl acetate, chloroform and n-hexane fractions respectively. $IC_{50}$ value of 45.45 µg/ml was obtained for Acarbose. (Table 2). Acarbose had the highest activity of 71.4% at 200 µg/ml. Lower activities were recorded for Acarbose concentrations above 200 µg/ml. At 2000 µg/ml Acarbose had 46.04% inhibition of α-amylase while n-hexane, chloroform, ethyl acetate, water fractions had highest activities of 45.6, 56.53%, 67.00%, 72.74% respectively at the same concentration. Also at 1000 µg/ml, all fractions except n-hexane fraction showed higher α-amylase enzyme inhibition than the standard drug, Acarbose (Table 1 and Figure 2)

**DISCUSSION**

The phytochemical screening of the methanol extract showed that the stem contained alkaloids, steroidal nucleus, saponin, cardiac glycosides and reducing sugars. These compounds are known to have medicinal properties (Sofowora, 1993). Interestingly, tannins, flavonoids and anthraquinones were absent in the extract. In another report, aqueous and ethanol extracts of the stem bark of AF were found to contain flavonoids and anthraquinones while only the aqueous extract contained saponins. The difference can be due to environmental factors and soil composition.

The presence of steroidal nucleus, saponin and reducing sugar (polysaccharide) in the plant stem is likely to be responsible for the antidiabetic property. In a previous study, saponins have been found to be a probable α-amylase inhibitor (Dou *et al*., 2013; Elekofehinti, 2015)

The DPPH test showed the ability of the test compound to act as a free radical scavenger. Half-maximal inhibitory concentration ($IC_{50}$) of 10.75, 90.15, 91.34 and >100 µg/ml were obtained for Vitamin C, Chloroform, water and n-hexane fractions respectively. Hence chloroform and hexane fractions were at least 8 times less potent while the n-hexane fraction is more than ten times less active as a free radical scavenger compared to Vitamin C.

The reaction between the extract, α-amylase and starch solution results in the generation of maltose which is quantified by the reduction of the colorimetric reagent 3, 5- Dinitrosalicyclic acid (DNS).

*Allanblackia floribunda* stem extract is shown to inhibit α-amylase enzyme. At a concentration of 2000 µg/ml, Acarbose had 46.04% inhibition of α-amylase while n-hexane, chloroform, ethyl acetate and water had inhibitory activities of 45.6, 56.53, 67.0 and

72.7% respectively. At concentrations above 1000µg/ml, all plant extract fractions except n-hexane fraction were more active compared to acarbose as α-amylase inhibitors. Acarbose was more active at concentrations of 200µg/ml and below. It exhibited highest inhibitory activity of 71.4% at 200 µg/ml. Half-maximal inhibitory concentration ($IC_{50}$) showed that the water fraction was the most potent fractions and it is about three times less potent compared to acarbose (Table 2). Interestingly, though the ethyl acetate fraction of the stem extract did not show DPPH radical scavenging activity but was active at inhibiting the alpha amylase enzyme and the chloroform fraction that showed potent DPPH scavenging activity was not as potent as an α-amylase inhibitor. Isolation and characterization of the constituents in the polar fractions (water and the ethyl acetate fractions) is desirable in obtaining potent hypoglycaemic compounds.

**CONCLUSION**

Most of the fractions obtained from of methanol extract of the stem of *Allanblackia floribunda* showed both hypoglycaemic and antioxidant properties. All the fractions except ethyl acetate fractions exhibited potent free radical scavenging activities against DPPH, while solvent fractions except n-hexane exhibited potent α- amylase inhibitory properties. Isolation and characterization of active fractions is desirable in order to obtain novel compounds with potent antidiabetic activity.

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