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***Olax subscorpioidea* Oliv. Leaf Alleviates Postprandial Hyperglycaemia by Inhibition of α -amylase and α -glucosidase**

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ABSTRACT

Diabetes mellitus is a metabolic disease which is characterized by high blood glucose. This study was aimed at evaluating the hypoglycaemic potential of *Olax subscorpioidea* leaf. This was done by assessing the *in vitro* inhibitory effect of different extracts (acetone, ethylacetate and hexane) of the plant on the activities of diabetes-related enzymes (α -amylase and α -glucosidase). Based on this, hexane extract of *O. subscorpioidea* leaf was orally administered to starch-loaded rats and the blood glucose levels of the rats were monitored for 2 h. The results showed that hexane extract of *O. subscorpioidea* leaf displayed the best inhibitory activity against α -amylase (IC_{50} : 0.72 mg mL⁻¹) and α -glucosidase (IC_{50} : 0.10 mg mL⁻¹). Lineweaver-Burk plot of inhibition of α -amylase and α -glucosidase by this extract showed that it is competitive and non-competitive mode, respectively. Administration of hexane extract of *O. subscorpioidea* leaf to starch-loaded rats produced significant reduction ($p < 0.05$) in the blood glucose level of the animals compared to the control. Phytochemical screening also revealed the presence of alkaloids, cardiac glycosides and tannins in the hexane extract. Therefore, it can be concluded that *O. subscorpioidea* leaf possesses hypoglycaemic potential which may be due to the inhibition of pancreatic α -amylase and intestinal α -glucosidase.

Key words: Postprandial hyperglycaemia, *Olax subscorpioidea*, α -amylase, α -glucosidase, uncompetitive inhibition

INTRODUCTION

Diabetes mellitus is a pathological condition characterized by hyperglycaemia due to partial or total loss of insulin secretion (IDF., 2013). It is a metabolic disorder as it causes disturbances of carbohydrate, fat and protein metabolism, leading to several complications such as nephropathy, neuropathy and retinopathy. Current statistics suggests 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 (IDF., 2014). The search for the discovery of antidiabetic 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 hypoglycaemia, weight gain and chronic tissue damage (Kane *et al.*, 2005). One of such plants used in the management of this disease in Africa is *Olax subscorpioidea*.

Olax subscorpioidea is a shrub or tree which belongs to the family of Olacaceae. It is widely distributed in West African countries such as Nigeria, Zaire and Senegal (Ayandele and Adebisi, 2007). Due to the wide usage of the plant, it has many household names in Nigeria. It is referred to as Ifon, Aziza and Gwano kurmi in the Western, Eastern and Northern part of Nigeria, respectively (Ibrahim *et al.*, 2008; Victoria *et al.*, 2010). Ethnobotanical surveys have revealed this plant is used in traditional medicine for the management of asthma (Sonibare and Gbile, 2008), cancer (Soladoye *et al.*, 2012), infectious diseases, mental illnesses (Ibrahim *et al.*, 2008) and diabetes mellitus (Soladoye *et al.*, 2010). Previous studies have shown that the plant displayed antinociceptive (Adeoluwa *et al.*, 2014), anti-ulcer (Victoria *et al.*, 2010), antimicrobial (Ayandele and Adebisi, 2007) and anti-protease (Oyedapo and Famurewa, 1995) activities. Recently, Adebayo *et al.* (2014) also reported the safety of the aqueous extract of the *O. subscorpioidea* leaf in Wistar rats.

Despite the usage of *O. subscorpioidea* leaf in the management of diabetes mellitus, there is no scientific report

on the antidiabetic potential of this plant. Therefore, the present study aimed to assess the hypoglycaemic potential of *O. subscorpioidea* leaf, through the evaluation of inhibitory potential of its extracts on key enzymes linked to diabetes mellitus (α -amylase and α -glucosidase) and on postprandial hyperglycaemia.

MATERIALS AND METHODS

Sample collection: The leaves of *O. subscorpioidea* were obtained from Badagry Area of Lagos in Nigeria in July 2013. It was identified and authenticated by the taxonomist; Dr. A. B. Kadiri of the Department of Botany, University of Lagos, Nigeria and voucher specimen (LUH 5602) was deposited in the University herbarium. The leaves were dried, pulverized and kept in airtight plastic bags.

Experimental animals: Male Wistar rats were obtained from the Animal House of the College of Medicine, University of Lagos, Nigeria. They were housed in plastic cages under a 12 h light/dark cycle at 20-25°C and had free access to standard rat chow and tap water *ad libitum*. The handling of animals conforms to the Guide for the Care and Use of Laboratory Animals.

Chemicals and reagents: Porcine pancreatic α -amylase, rat intestinal α -glucosidase and paranitrophenyl-gluco-pyranoside 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 the water used was glass-distilled.

Preparation of plant extracts: The powdered leaves were divided into three portions of 20 g each and these were extracted with acetone, ethylacetate and hexane. They were all left to steep in covered conical flasks for 24 h, the flasks were shaken and kept still to allow the plant material settle at the bottom of the flask. The resulting infusions were decanted, filtered and evaporated in a rotary evaporator (Cole Parmer SB 1100, Shanghai, China). Dried extracts were weighed and dissolved in dimethylsulphoxide (DMSO) to yield a stock solution from which lower concentrations were prepared.

α -Amylase inhibitory assay: This assay was carried out using a modified procedure of McCue and Shetty (2004). A total of 250 μ L of extract was placed in a test tube and 250 μ L of 0.02 M sodium phosphate buffer (pH 6.9) containing α -amylase (0.5 mg mL⁻¹) solution was added. This solution was pre-incubated at 25°C for 10 min, after which 250 μ L of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then incubated at 25°C for 10 min. The reaction was terminated by adding 500 μ L of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL distilled water and the absorbance was measured at

540 nm using a spectrophotometer (Spectrumlab S23A, Globe Medical England). The control and blank were prepared using the same procedure replacing the extract with DMSO and distilled water respectively. The α -amylase inhibitory activity was calculated as percentage inhibition, thus:

$$\text{Inhibition (\%)} = \frac{\Delta A_{\text{control}} - \Delta A_{\text{extract}}}{\Delta A_{\text{control}}} \times 100$$

Where:

$$\Delta A_{\text{control}} = A_{\text{control}} - A_{\text{blank}}$$

$$\Delta A_{\text{extract}} = A_{\text{extract}} - A_{\text{blank}}$$

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

Mode of α -amylase inhibition: The mode of inhibition of α -amylase by the leaf extract was conducted using the most potent extract according to the modified method described by Ali *et al.* (2006). Briefly, 250 μ L of the (2.5 mg mL⁻¹) extract was pre-incubated with 250 μ L of α -amylase solution for 10 min at 25°C in one set of tubes. In another set of tubes, α -amylase was pre-incubated with 250 μ L of phosphate buffer (pH 6.9). Two hundred and fifty microliter of starch solution at increasing concentrations (0.3-5.0 mg mL⁻¹) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at 25°C and then boiled for 5 min after addition of 500 μ L 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 (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.

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). The substrate solution, p-nitrophenyl gluco-pyranoside (pNPG) was prepared in 20 mM phosphate buffer, pH 6.9. 100 μ L of α -glucosidase (0.5 mg mL⁻¹) was pre-incubated with 50 μ L of the different concentrations of the extracts for 10 min. Then 50 μ L of 3.0 mM pNPG dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 2 mL of 0.1 M Na₂CO₃. The α -glucosidase activity was determined by measuring the yellow coloured para-nitrophenol released from pNPG at 405 nm. The control and blank were prepared using the same procedure by replacing the extract with DMSO and distilled water, respectively. Percentage inhibition was calculated thus:

$$\text{Inhibition (\%)} = \frac{\Delta A_{\text{control}} - \Delta A_{\text{extract}}}{\Delta A_{\text{control}}} \times 100$$

Where:

$$\Delta A_{\text{control}} = A_{\text{control}} - A_{\text{blank}}$$

$$\Delta A_{\text{extract}} = A_{\text{extract}} - A_{\text{blank}}$$

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

Mode of α -glucosidase inhibition: The mode of inhibition of α -glucosidase by the extracts was determined using the extract with the lowest IC_{50} according to the modified method described by Ali *et al.* (2006). Briefly, 50 μL of the (2.5 mg mL^{-1}) extract was pre-incubated with 100 μL of α -glucosidase solution for 10 min 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^{-1}) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at 25°C and 500 μL of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically 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.

Starch-tolerance test: Ten normoglycaemic rats were divided into two groups of five rats (n = 5) each: Group A was designated as the control rats while Group B is the *O. subscorpioidea* extract treated rats. After an overnight fast (18 h), Groups B rats were orally administered extract of *O. subscorpioidea* leaf (250 mg kg^{-1} body weight) by means of an orogastric tube while Group A received distilled water (vehicle). After 30 min, both groups were orally administered 3 g kg^{-1} body weight of starch solution. Postprandial blood glucose levels were then measured by tail-prick before (0 min) and at 30, 60, 90 and 120 min after oral administration of starch using Glucometer 4 Ames (Bayer Diagnostics, Germany). Postprandial blood glucose curves of experimental rats were plotted and compared with those of control rats (Kazeem *et al.*, 2013).

Phytochemical screening: Phytochemical compositions of the leaf extracts were determined using the methods variously described by Sofowora (2006).

Statistical analysis: Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, USA). All the results were expressed as Mean \pm SEM for triplicate determinations.

RESULTS

Figure 1 showed the result of the α -amylase inhibitory potential of different extracts of *O. subscorpioidea* leaf. At the lowest concentration tested (0.32 mg mL^{-1}), there were significant differences among all the extracts. At higher

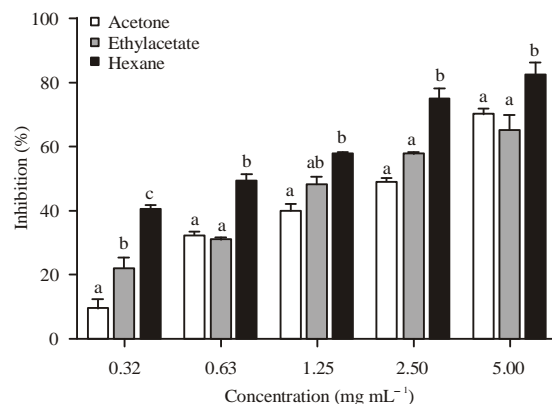


Fig. 1: Percentage inhibition of α -amylase activity by *O. subscorpioidea* leaf extracts. Values are expressed as Means \pm SEM of triplicate determinations. Means not sharing a common letter at the same concentration are significantly different ($p < 0.05$) to each other

Table 1: IC_{50} values for α -amylase and α -glucosidase inhibitory potential of *O. subscorpioidea* leaf extracts

Extracts	IC_{50} (mg mL^{-1})	
	α -amylase	α -glucosidase
Acetone	2.90 \pm 0.03 ^a	3.87 \pm 0.05 ^a
Ethylacetate	2.58 \pm 0.03 ^a	4.92 \pm 0.03 ^a
Hexane	0.72 \pm 0.01 ^b	0.10 \pm 0.00 ^b
Acarbose	2.60 \pm 0.02 ^a	0.63 \pm 0.01 ^c

Values are expressed as Means \pm SEM of triplicate determinations. Means down vertical column not sharing common letter are significantly different ($p < 0.05$) from each other. Acarbose is the standard α -amylase and α -glucosidase inhibitor

concentrations, there were no significant differences between the acetone and ethylacetate extracts. However, the percentage inhibition of the enzyme by hexane extract was significantly higher ($p < 0.05$) compared to other extracts. This is corroborated by the lowest IC_{50} (0.72 mg mL^{-1}) exhibited by the hexane extract compared to other extracts and acarbose (Table 1). The Lineweaver-Burk plot showed that hexane extract of *O. subscorpioidea* leaf displayed competitive inhibition of the enzyme, α -amylase (Fig. 2). Figure 3 showed the inhibitory effect of different extracts of *O. subscorpioidea* leaf on α -glucosidase. Across all the concentrations tested, hexane extract displayed significantly higher ($p < 0.05$) percentage inhibition of the enzyme than acetone and ethylacetate extracts while there was no significant difference between the inhibitions by acetone and ethylacetate except at the lowest concentration.

The higher percentage inhibition exhibited by the hexane extract culminated in its lowest IC_{50} (0.10 mg mL^{-1}). The hexane extract of *O. subscorpioidea* leaf also inhibited α -glucosidase in a mixed non-competitive manner as shown in Fig. 4. The effect of oral administration of hexane extract of *O. subscorpioidea* leaf in starch-loaded Wistar rats is presented in Fig. 5. Throughout the period of investigation (120 min), the extract significantly reduced ($p < 0.05$) blood

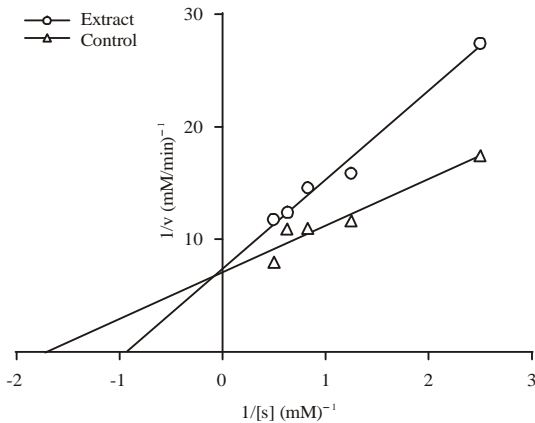


Fig. 2: Mode of inhibition of α -amylase by hexane leaf extract of *Olax subscorpioidea*

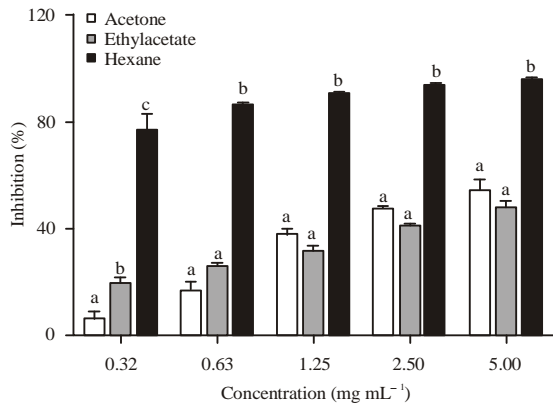


Fig. 3: Percentage inhibition of α -glucosidase activity by *Olax subscorpioidea* leaf extracts. Values are expressed as Means \pm SEM of triplicate determinations. Means not sharing a common letter at the same concentration are significantly different ($p < 0.05$) from each other

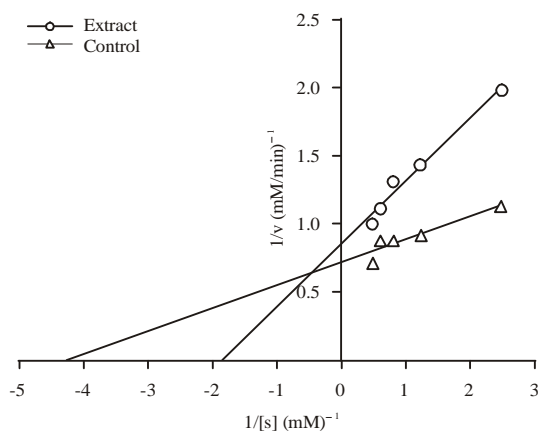


Fig. 4: Mode of inhibition of α -glucosidase by hexane leaf extract of *Olax subscorpioidea*

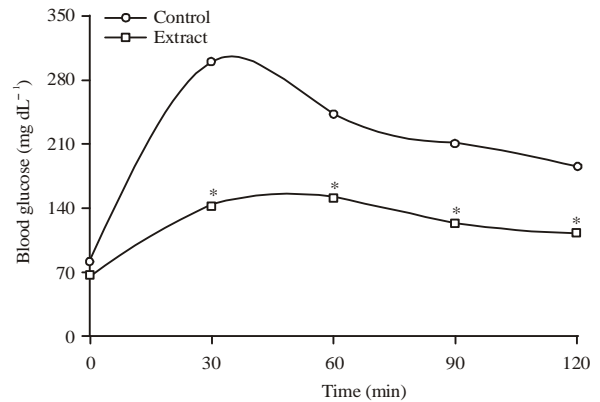


Fig. 5: Effect of administration of hexane extract of *Olax subscorpioidea* leaf on blood glucose level of starch-loaded rats. *Values are significantly different from the control ($p < 0.05$)

Table 2: Pharmacokinetic parameters of control and *Olax subscorpioidea* treated rats after starch ingestion

PK parameters	Groups	
	Control	Extract
C_{max} (mg mL ⁻¹)	300.70 \pm 7.300	152.30 \pm 3.400*
T_{max} (min)	30.00 \pm 2.500	60.00 \pm 4.100*
AUC (mg dL ⁻¹ min)	26690.20 \pm 25.70	15285.50 \pm 12.30*

Values are expressed as Means \pm SEM of triplicate determinations. *Mean values are significantly different from the control. C_{max} : Maximum blood glucose level of the rats, T_{max} : Time at which the maximum blood glucose level was attained, AUC: Area under the curve

Table 3: Phytochemical composition of different leaf extracts of *Olax subscorpioidea*

Phytochemicals	Inference		
	Acetone	Ethylacetate	Hexane
Alkaloids	-	+	+
Cardiac glycosides	+	+	+
Flavonoids	+	+	-
Phenolics	+	-	-
Phlobatannins	+	-	-
Reducing sugar	-	-	-
Saponins	-	-	-
Steroids	-	+	-
Tannins	-	+	+
Terpenoids	+	-	-

+: Detected, -: Not detected

glucose level of the animals compared to the control. This resulted in the reduction in the Area Under Curve (AUC) and other pharmacokinetic parameters (Table 2). Table 3 showed the presence of cardiac glycosides in all the extracts while alkaloids and tannins were detected in the ethylacetate and hexane extracts only. Phenolics, phlobatannins and terpenoids were only detected in the acetone extract while steroids were present in the ethylacetate extract only.

DISCUSSION

It was found that all the extracts of *O. subscorpioidea* leaf inhibited both α -amylase and α -glucosidase. The highest

percentage inhibition of the α -amylase by the hexane extract culminated in its low IC_{50} value which implies that it is the most potent inhibitor of the enzyme out of the three extracts and acarbose (Kazeem *et al.*, 2014). However, it is undesirable of an antidiabetic agent to be a strong inhibitor of α -amylase so as to prevent some of the drawbacks of synthetic drugs which might be due to excessive inhibition of the enzyme (Kwon *et al.*, 2008). The hexane extract also displayed the most potent inhibition of α -glucosidase with an IC_{50} which is about six-folds lesser than the standard drug acarbose. This is in conformity with previous reports that antidiabetic agents from plants are strong inhibitors of α -glucosidase (Kwon *et al.*, 2008). This implies that hexane extract of *O. subscorpioidea* leaf offer better pharmacological effect than the common synthetic drugs.

The competitive inhibition of α -amylase by the hexane extract of *O. subscorpioidea* leaf 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). Conversely, the mixed non-competitive inhibition of α -glucosidase by the extract implies the α -amylase inhibitory components of the extract do not compete with the substrate for binding to the active site of the enzyme but bind to another site (allosteric site) of the enzyme or the enzyme-substrate complex (Mogale *et al.*, 2013). The binding of these inhibitors to the allosteric site changes the conformation of the enzyme so that the affinity of the substrate for the active site is reduced, leading to a change in the kinetic properties of the enzyme (Dixon and Webb, 1999). The inhibition of the activities of both enzymes ultimately leads to reduction in the digestion of carbohydrates which prevents hyperglycaemia.

Since there are no previous scientific reports on the hypoglycaemic potential of *O. subscorpioidea* leaf, the effect of oral administration of hexane extract of *O. subscorpioidea* leaf in starch-loaded rats was evaluated. The significant reduction in the blood glucose level of the extract loaded rats throughout the duration of the experiment is a pointer to the fact that the extract possesses antihyperglycaemic potential. Therefore, It was inferred that the antihyperglycaemic effect of the extract may be due to the inhibition of the pancreatic α -amylase and intestinal α -glucosidase in the rats, thereby lowering their blood glucose levels (Akkarachiyasit *et al.*, 2011).

It is a known fact that pharmacological activities of medicinal plants are as a result of their chemical components. This is what informed the phytochemical screening of the three extracts of *O. subscorpioidea* leaf used in this study. The presence of only alkaloids, cardiac glycosides and tannins was detected in the hexane extract. Alkaloids have been shown to display wide spectrum antidiabetic potentials-by inhibiting diabetes-related enzymes like α -glucosidase and preventing protein glycation (Choudhary *et al.*, 2011). Tannins on the other hand, are exhibits insulin-mimetic effect by improving the sensitivity of insulin receptors (Huang *et al.*, 2005) while cardiac glycosides prevent diabetes-related complications by

inhibiting Na^+/K^+ pump, thereby improving cardiac output and preventing cardiovascular diseases (Krishnaiah *et al.*, 2009).

CONCLUSION

This study showed that *O. subscorpioidea* leaf possesses antidiabetic potential in Wistar rats and the possible mechanism of action is the inhibition of pancreatic α -amylase and intestinal α -glucosidase, thereby slowing down the absorption of carbohydrates and preventing hyperglycaemia. The hypoglycaemic activity of this plant may also be due to the presence of alkaloids, tannins and cardiac glycosides.

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