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Alpha amylase and alpha glucosidase inhibitory activities of extracts of *Dialium guineense* stem bark

Abu OD[1*], Onoagbe IO[2], Obahiagbon O[3]
[1, 2] Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria
[3] Physiology Division, Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria

Abstract
Background: *Dialium guineense* belongs to the *Leguminosae* family, and grows in dense forests in Africa along the southern edge of the Sahel. The bark, leaves and fruits of the plant have medicinal properties and are used to treat diseases such as stomatitis, toothache, fever, diarrhoea, palpitations, and microbial infections.
Aim: The present study investigated the α-amylase and α-glucosidase inhibitory activities of extracts of *Dialium guineense* stem bark.
Methods: Aqueous and ethanol extracts of *D. guineense* stem bark were prepared using standard method. Alpha amylase and alpha glucosidase inhibitory activities of the extracts were determined *in vitro*. Acarbose was used as the reference drug.
Results: The results showed that both extracts of *Dialium guineense* possess α-amylase and α-glucosidase inhibitory activities. The aqueous extract (IC_{50} = 76.55 ± 3.01 µg/mL) showed a better α-amylase inhibitory activity when compared with the ethanol extract (IC_{50} = 137.78 ± 5.18 µg/mL) ($p < 0.05$), but there was no significant difference in the corresponding α-glucosidase inhibitory activity between the two extracts ($p > 0.05$). The α-amylase and α-glucosidase inhibitory activities of the extracts were concentration-dependent, and comparable to those of acarbose.
Conclusion: The results of this study indicate that *Dialium guineense* shows great potential in the treatment of Diabetes mellitus.

Keywords: alpha amylase, alpha glucosidase, *Dialium guineense*, acarbose, inhibitory activity

Introduction

Medicinal plants contain wide varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of human diseases (Gowthami *et al.*, 2012a; Gowthami *et al.*, 2012b; Manokaran *et al.*, 2008) [1, 2, 3].

Dialium guineense (Velvet Tamarind), is a tall, tropical, fruit-bearing tree. It belongs to the *Leguminosae* family, and has small, typically grape-sized edible fruits with brown hard inedible shells. In Africa, it grows in dense forests along the southern edge of the Sahel. The bark and leaves have been reported to possess medicinal properties and are used against several diseases. Each fruit typically has one hard, flat, round, brown seed, typically 7 - 8 mm across and 3 mm thick (Dalziel and Hutchison, 1973) [4]. The seed somewhat resembles a watermelon seed *(Citrullus lanatus)*. Some have two seeds. The seeds are shiny, and coated with a thin layer of starch. The pulp is edible and may be eaten raw or soaked in water and consumed as a beverage (Dalziel and Hutchison, 1973) [4]. The bitter leaves are ingredients in a Ghanaian dish called *domoda*. Its wood is hard and heavy, and used for construction. The wood is also used for firewood and charcoal production (Dalziel and Hutchison, 1973) [4].

Prior to the development of oral hypoglycemic agents, the major form of treatment for diabetes mellitus involved dietary manipulation and the use of plant therapies. About 400 plants world-wide have been documented for the treatment of diabetes mellitus and the majority awaits proper scientific and medicinal evaluation. Studies have shown that the antidiabetic mechanisms of traditional herbs include reduced carbohydrate absorption (De Fronzo, 2004) [5], reduced α-glucosidase, α-amylase, and aldose reductase activities (Velho and Froguel, 1997), increased glucose uptake in muscle and adipose tissues (Wang *et al.*, 2013) [7], activation of PPAR γ (Kandra, 2003) [8], increased insulin sensitivity/upregulation of receptor expression (Dewi *et al.*, 2007) [9], exertion of antioxidant effects and decreased β-cell apoptosis (Cheng and Fantus, 2005) [10], stimulation of β-cell insulin secretion (Marles and Farnsworth,1995) [11], inhibition of hepatic gluconeogenesis/glycogenolysis (De Sales, 2012) [12], and prevention of endogenous incretins from degradation/ suppression of glucagon (Wang *et al.*, 2013) [7].

Pancreatic α-amylase, a calcium metalloenzyme that catalyzes the hydrolysis of α-1, 4 glycosidic linkages of amylose, amylopectin, glycogen, and various maltodextrins, is responsible for most of starch digestion in humans. A positive correlation between human pancreatic α-amylase (HPA) activity and increase in postprandial glucose levels has been shown, demonstrating the relevance of suppressing postprandial hyperglycemia (PPHG) in the treatment of type 2 diabetes mellitus (T2DM) (Karthic *et al.*, 2008) [13].

Alpha glucosidase is an enzyme which is responsible for the conversion of complex carbohydrates into glucose. This enzyme acts by delaying the breakdown of complex carbohydrates into glucose and reduces its absorption from the gut which results in reduction of postprandial blood sugar level (Subramanian *et al.*, 2008) [14]. Although commercially available α-glucosidase inhibitors like voglibose and acarbose are used effectively to control blood glucose levels, they have been associated with serious gastrointestinal side effects. Therefore, there is a need to search for an alternative that can exhibit α-glucosidase inhibitory activity without side effects (Playford *et al.*, 2013) [15]. The

present study investigated the α-amylase and α-glucosidase inhibitory activities of extracts of *Dialium guineense* stem bark.

Materials and Methods

Plant Sample Collection and Preparation

The plant leaves were obtained from Iyekogba area in Benin and identified at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria with voucher number UBH_D330, after which the bark was obtained. Preparation and extraction was carried out using the method of Abu *et al*. (2015) [16]. The aqueous and ethanol extracts were concentrated using rotary evaporator and made into powder by lyophilisation.

Determination of *D. guineense* Alpha-amylase Inhibitory Activity

The assay mixture containing 200 µL of 0.02 M sodium phosphate buffer, 20 µL of enzyme and the plant extracts (20 - 100 µg/mL) were incubated for 10 min at room temperature followed by addition of 200 µL of starch to all test tubes. The reaction was terminated with the addition of 400 µL dinitrosalicylate (DNS) reagent and placed in boiling water bath for 5 min, cooled and diluted with 15 mL of distilled water and absorbance was read at 540 nm. The control samples were prepared without any plant extracts. The % inhibition was calculated according to Equation 1:

$$Inhibition\ (\%) = \frac{A_{control} - A_{extract}}{A_{control}} x\ 100 \ldots\ldots\ldots \quad (1)$$

Where A is absorbance.

The half maximum inhibitory concentration (IC_{50}) values were determined from plots of percentage inhibition versus log of inhibitory concentration and were calculated using nonlinear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha amylase inhibitor. All tests were performed in triplicate (Jung *et al.*, 2006) [17].

Determination of *D. guineense* Alpha-glucosidase Inhibitory Activity

The yeast alpha glucosidase used was dissolved in 100 mM phosphate buffer pH 6.8. P-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Plant extracts (20 - 100 µg/mL) were mixed with 320 µL of 100 mM phosphate buffer pH 6.8 at 30 °C for 5 min. Then, 3 mL of 50 mM NaOH was added to the mixture and the absorbance was read at 410 nm. The control samples were prepared without any plant extracts. The % inhibition was calculated according to Equation 2:

$$Inhibition\ (\%) = \frac{A_{control} - A_{extract}}{A_{control}} x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

The IC_{50} values were determined from plots of percentage inhibition versus log of inhibitory concentration and were calculated using nonlinear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All tests were performed in triplicate (Jung *et al.*, 2006) [17].

Statistical Analysis

Data are presented as mean ± SEM. Statistical analysis was performed using SPSS (21.0). Statistical significance was assumed at $p < 0.05$.

Results

Alpha-amylase and Alpha-glucosidase Inhibitory Activities of Aqueous and Ethanol Extracts of *D. guineense* Stem Bark

The results showed that both extracts of *Dialium guineense* possess α-amylase and α-glucosidase inhibitory activities. The aqueous extract (IC_{50} = 76.55 ± 3.01 µg/mL) showed a better α-amylase inhibitory activity when compared with the ethanol extract (IC_{50} = 137.78 ± 5.18 µg/mL) ($p < 0.05$), but there was no significant difference in the corresponding α-glucosidase inhibitory activity between the two extract groups ($p > 0.05$). The α-amylase and α-glucosidase inhibitory activities of the extracts were concentration-dependent, and comparable to those of acarbose (the standard drug). These results are shown in fig1 and 2).

Concentration of drug or extract (µg/mL)	Percentage inhibition (%) of α-amylase by extracts		
	Aqueous	Ethanol	Acarbose
10	6.00 ± 0.52	4.50 ± 0.11	24.20 ± 1.20
20	9.00 ± 0.51	18.10 ± 1.05	27.20 ± 1.08
30	9.00 ± 0.38	19.60 ± 0.85	30.30 ± 1.50
40	24.20 ± 1.05	21.20 ± 1.25	34.80 ± 1.22
50	39.30 ± 1.05	22.70 ± 1.15	50.00 ± 1.58
60	39.30 ± 0.95	24.20 ± 1.08	54.50 ± 1.55
70	39.30 ± 1.20	28.70 ± 1.80	56.00 ± 1.05
80	54.50 ± 1.19	31.80 ± 1.50	60.60 ± 1.10
IC_{50} (µg/mL)	76.55 ± 3.01	137.78 ± 5.18	58.46 ± 3.08

Data are percentage inhibition of α-amylase by extracts, and are expressed as mean ± SEM (n = 3).

Fig 1: Percentage Inhibition of Alpha-amylase by Aqueous and Ethanol Extracts of *Dialium guineense Stem* Bark

Table 2: Percentage Inhibition of Alpha-glucosidase by Aqueous and Ethanol Extracts of *Dialium guineense* Stem Bark

Concentration of drug or extract (µg/ml)	Percentage inhibition (%) of α-glucosidase by extracts		
	Aqueous	Ethanol	Acarbose
10	5.60 ± 0.11	13.00 ± 0.41	12.60 ± 0.64
20	13.70 ± 0.51	16.50 ± 0.45	14.00 ± 0.88
30	15.10 ± 0.22	20.40 ± 1.20	14.90 ± 0.85
40	20.00 ± 1.04	27.10 ± 1.08	24.40 ± 1.41
50	37.70 ± 0.86	35.70 ± 1.33	27.40 ± 1.09
60	42.60 ± 1.64	47.00 ± 1.28	29.40 ± 1.64
70	46.10 ± 1.08	49.80 ± 1.22	37.50 ± 1.85
80	57.70 ± 1.42	52.90 ± 1.08	45.90 ± 1.95
IC_{50} (µg/mL)	72.14 ± 3.86	72.04 ± 3.92	96.28 ± 3.18

Data are percentage inhibition of α-glucosidase by extracts, and are expressed as mean ± SEM (n = 3).

Discussion

Plant food rich in polyphenols have been reported to produce effects similar to insulin in the utilization of glucose, and act as good inhibitors of key enzymes associated with T2DM and lipid peroxidation in tissues (Reddy *et al.*, 2010) [18]. Studies have shown that the bioactivity of polyphenols in plants is linked to their antioxidant properties and many of these plants also possess hypoglycemic effect (Ramkumar *et al.*, 2010) [19]. Higher plants, animals and microorganisms produce a large number of different protein inhibitors of α-amylase and α -glucosidase in order to regulate the activity of these enzymes (Choudhury *et al*., 1996) [20]. Some of these inhibitors act by directly blocking the active site of the enzyme (Kavitha *et al.*, 2012) [21]. In animals, α-amylase inhibitors decrease the high glucose levels that occurs after a meal by slowing the speed with which α-amylase converts starch to simple sugars (Boivin *et al*., 1987) [22]. This is important in diabetic patients where low insulin levels prevent rapid clearance of extracellular glucose from the blood (Mohammed *et al*., 2009) [23]. Hence diabetics tend to have reduced α-amylase

activity in order to keep their glucose levels under control. Plants also use α-amylase inhibitors as defense mechanism against insects. These inhibitors alter the digestive action of α-amylase and proteinases in the gut of insects and inhibit their normal feeding behavior. Therefore, α-amylase inhibitors have potential roles in controlling blood sugar levels and crop protection (Kumanan *et al.*, 2010) [24]. Alpha glucosidase inhibitors are used as oral antidiabetic drugs for treating T2DM. They act by preventing the digestion of carbohydrates such as starch. Alpha glucosidase inhibitors act as competitive inhibitors of α-glucosidase enzyme needed to digest carbohydrates. Intestinal α-glucosidase hydrolyzes complex carbohydrates to glucose and other monosaccharides in the small intestine. Inhibition of these enzyme systems helps to reduce the rate of digestion of carbohydrates (Bhat *et al.*, 2011) [25]. Less amounts of glucose is absorbed because the carbohydrates are not completely broken down into glucose molecules. In diabetics, the short-term effect of these enzyme inhibitor drug therapies is to decrease high blood glucose levels. The presently used synthetic enzyme inhibitors cause gastrointestinal side effects such as diarrhea, flatulence, and abdominal bloating (Bray and Greenway, 1999) [26]. Therefore, natural α-amylase and α-glucosidase inhibitors from dietary plants can be used as an effective therapy for treating post-prandial hyperglycemia with minimal side effects. In this study, aqueous and ethanol extracts of *Dialium guineense* stem bark exhibited good *in vitro* α-amylase and α-glucosidase inhibitory activities which were comparable with those of acarbose the standard drug used.

Conclusion

The results of this study indicate that *Dialium guineense* shows great potential in the treatment of Diabetes mellitus.

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