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Full Length Research Article

Activity-Based Investigation of the Possible Anti-Diabetic Potentials of some Nigerian Medicinal Plants

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ABSTRACT
A major therapeutic approach presently used in managing Type 2 Diabetes mellitus is the use of α-glucosidase and α-amylase inhibitors. Hence the growing attention in the quest for medicinal plants of natural sources with inhibitory potentials on these enzymes. This study was done, therefore, to determine the inhibitory potentials of the different parts of three medicinal plants; *Nigella sativum* (seeds), *Spondias mombin* (leaves and stem bark), and *Picralima nitida* (seeds and mesocarp) on α-amylase and α- glucosidase as well as to determine inhibition kinetics. The *in vitro* α-amylase and α-glucosidase inhibitory activities of the plant extract were assessed using 3,5-dinitrosalicylic acid (DNS) and p-nitro-phenyl-a-D glucopyranoside (p-NPG) respectively.The results indicated that all plant extracts assayed exhibited better α- glucosidase inhibitory effects than the reference drug(acarbose), as indicated by the higher IC_{50} (76.10 µg/mL) value of the reference drug, whereas the n-hexane extract of *N. sativum* seeds gave the best α-amylase effect (IC_{50} = 35.83 µg/mL). All the extracts exhibited an "uncompetitive" type of inhibition pattern. Our findings hence support the use of these plants in the management of diabetic conditions.
KEYWORDS: *Nigella sativum*, *Spondias mombin*, *Picralima nitida*, α-amylase, α-glucosidase, Acarbose

INTRODUCTION
Diabetes is a metabolic disease with a growing incidence worldwide. Literature shows that about 1.7 million people in Nigeria are living with diabetes and their ages ranging from 20 and 79 years (Gbolade, 2009). Type 2 Diabetes mellitus is the most prevalent kind of Diabetes and is far more than 80% of the reported cases (Unwin *et al*., 2009). A primary therapeutic target for the management of type 2 diabetes is the use ofα-glucosidase and α-amylase enzyme inhibitors to decrease glucose uptake from the intestine (Sim *et al*., 2010). It is thought that the sudden rise in blood glucose levels after a meal can be efficiently managed by inhibiting these enzymes.

The anti-diabetic potential of many plant species has been reported (Grover *et al.,* 2002). The hypoglycemic properties of some medicinal plants act by slowing down glucose uptake from the intestine by inhibiting enzymes like pancreatic amylase that hydrolyze carbohydrates. Moreover, over two hundred compounds with blood glucose-lowering potentials have been isolated (Marles and Farnsworth, 1994). Therefore, there is the need to screen for α-amylase and α-glucosidase inhibitors from plants. The reliance on plant and plant products for several thousands of years is linked to their acceptability and fewer disadvantages. About eighty percent of the populace is estimated to rely solely on plant-based preparations for their healthcare (Prabhakar *et al*., 2013). Medicinal plants hardly generate any unwanted effects like those observed from conventional drugs. The plant *P. nitida* (Staph) belongs to the Apocynaceae family. The plant has been reported to be beneficial in traditional medicine. Several studies have reported on some of the beneficial effects of the plant (Dzotam and Kuete, 2023; Ubulom *et al*., 2012; Aguwa *et al*., 2001). *N sativa (*Linn). belongs to the Ranunculaceae family and is usually called black cumin or black seeds, and it is a small elegant herb. Aftab *et al*. (2013) have reported on the use of the seeds of this plant as a cure for various disease conditions. Also, it is used to stop vomiting (Sharma *et al*., 2009), and the oils have also been shown to possess radical Scavenging effects (Altan, 2007; Burits and Bucar, 2000). *S. mombin* (Linn) is a fructiferous plant from belonging to Anarcardiaceae family. Several scientific studies exist on the importance of all parts of this plant (Martinez, 2005). Based on the medicinal properties of *P. nitida*, *N. sativa* and *S. mombin*, this study was undertaken to explore their inhibitory effects on α-amylase and α-glucosidase.

MATERIALS AND METHODS
Collection of Plant Materials
Picralima nitida
Mature *P. nitida* fruits were purchased from a local market in Benin City, Nigeria. They were washed thoroughly, after which the seeds were separated from the mesocarp, and both were air-dried separately for 14 days. The dried seeds and mesocarp were pulverized separately using a mechanical blender, and 200 g of the powdered samples wereeach macerated in ethanol (800 mL) to obtain ethanol extracts of the seeds and mesocarp of *P. nitida*, respectively.

Nigella sativum
Seeds of *Nigella sativum* were also bought from a local market in Benin City, Nigeria. They were shade dried and pulverized. The ground seeds (100 g) were macerated in ethanol (400 mL), and another 100 g were macerated in n-hexane (400 mL). The extracts obtained were filtered, and the filtrate was subjected to lyophilization to obtain

powdered extracts, which were used for the following assays.

Spondias mombin

*S. mombin*leaves and stem bark were obtained from gardens around the University of Benin campus, Benin City. They were air-dried, ground and 200 g of the powdered samples were each macerated in ethanol and lyophilized to obtain ethanol extracts of *S. mombin* leaves and stem bark, which were used for the following assays. All plants collected were initially identified and authenticated in the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The voucher specimen was deposited with herbarium numbers UBH_N 506, UBH_P 424, and UBH_S 345for *N. sativum*, P. *nitida* fruit, and *S. mombin*, respectively**.**

Alpha Amylase Inhibitory Assay

The test samples were prepared by adding 200 µL of 0.02 M sodium phosphate buffer, 20 µL of the enzyme, and the plant extracts in the concentration range of 20- 100 mg/mL, after which the test tubes were incubated for 10 min at 25ºC. Exactly 200 µL of starch was then added to all test tubes then 400 µl 3,5-dinitrosalicylic acid (DNS) reagent was added to stop the reaction. The test tubes containing the samples were boiled in a water bath for 5 min. After diluting with 10 mL of distilled water, the mixture was left to cool, and absorbance was read at 540 nm. The control samples were also prepared. Percentage inhibition was determined using the formula:

$$\text{Percentage Inhibition} = \frac{\text{absorbance (control)} - \text{absorbance (extract)}}{\text{absorbance of control}} x\ 100$$

***In vitro* Alpha-Glucosidase Inhibitory Assay**

The alpha-glucosidase enzyme extract was prepared by dissolving in 100 mM phosphate buffer pH 6.8. P-nitrophenyl –α-D-glucopyranoside served as the substrate. Concentrations of 200-1000 µg/mL of the various plant extracts were prepared and added to test tubes containing 320 µL of 100 mM phosphate buffer pH 6.8 at 30ºC for 5 min. NaOH (3 mL, 50 mM) was added to the mixture, and the absorbance was measured at 410 nm. Plant extracts were not added to the control samples. The percentage inhibition was determined as shown below. Acarbose served as a reference drug.

$$\text{Percentage Inhibition} = \frac{\text{absorbance (control)} - \text{absorbance (extract)}}{\text{absorbance of control}} x\ 100$$

Enzyme Kinetics

The mode of Inhibition was determined using sigmoid plot interpolation characteristics (Hill's slope), hyperbola plot interpolation characteristics (viz maximum binding capacity Bmax, and dissociation constant, Kd), as well as Michaelis Mentenkinetics (Km and Vmax). These were used to determine the IC_{50} of the extracts. The IC_{50} shows how potent the extracts are in inhibiting the enzymes. The Bmax and Kd represent the degree of binding and period of Inhibition, which indicates the level of efficacy of the extracts.

Statistical Analysis

The means, SEM, and IC_{50} were determined using Graph Pad Prism Software, inc. (version 6.01, 2012). $P \leq 0.05$ represented a statistically significant difference.

RESULTS

α -Amylase Inhibition Assay

The results of α-amylase inhibitory potential of *N. sativum* (seeds), *S. mombin* (leaf and stem bark), and *P. nitida* (seeds and mesocarp) are presented in Table 1, Figures 1 and 2. The result indicated that the n-hexane extract of *Nigella sativum* seed gave the highest α-amylase inhibitory effect (IC_{50} = 35.83 µg/mL); this was followed closely by the ethanol extract of *Nigella sativum* seeds (IC_{50}= 36.13 µg/mL), then by ethanol extract of *P. nitida* seed, *S. mombin* leaves, *S. mombin* stem bark and finally *P. nitida* mesocarp with IC_{50} values of 36.14 µg/mL, 60.35 µg/mL, 64.20 µg/mL and 67.30 µg/mL respectively.

α -Glucosidase Inhibition Assay

The α-glucosidase inhibitory activity results are shown in Table 2, Figures 3 and 4. The n-hexane extracts of *N. sativum* also gave the best α-glucosidase effect (IC_{50} = 44.24 µg/mL) (Table 2). This was closely followed by ethanol extracts of *N. sativum* seeds, *S. mombin* stem bark, *S. mombin* leaf, *P. nitida* mesocarp, and then *P. nitida* seed with IC_{50} values of 44.87 µg/mL, 46.85 µg/mL, 48.99 µg/mL, 49.84 µg/mL, and 64.19 µg/mL respectively.

DISCUSSION

Plants provide valuable substances for managing human diseases, including Diabetes. A likely mechanism for these blood glucose-reducing effects is slowing the absorption of sugars ingested. Several species of plant have been investigated for their hypoglycemic effect, and these plants have varying mechanism of action. The present study clearly shows the anti-diabetic potentials of *N. sativum*, *S. mombin* leaf and stem bark, and *P. nitida* seeds and mesocarp through inhibitory effects on alpha-amylase and alpha-glucosidase enzymes, which are vital targets for a recent therapeutic approach in managing diabetes.

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Table 1: Dose-response characteristics of the influence of extracts on alpha-amylase activity.

	DOSE-RESPONSE CHARACTERISTICS		SIGMOID PLOT INTERPOLATION CHARACTERISTICS		HYPERBOLA PLOT INTERPOLATION CHARACTERISTICS		MICHAELIS-MENTEN'S KINETICS		STRAIGHT-LINE REGRESSION INTERPOLATION CHARACTERISTICS	
	$LogIC_{50}$	IC_{50} (µg/mL)	R^2	Hill's slope	Bmax	Kd	Km	Vmax	Y-intercept	slope
Acarbose	1.703	50.51	0.9749	4.148	-4.14×10^{19}	-1.16×10^{18}	4.25×10^{16}	1.51×10^{18}	-199.80	149.50
***S. mombin* leaf**	1.781	60.35	0.9457	3.737	1.39×10^{23}	4.66×10^{21}	1.37×10^{23}	3.08×10^{24}	-182.00	133.70
***S. mombin* stem**	1.808	64.20	0.9996	9.117	3.08×10^{35}	1.07×10^{34}	1.07×10^{34}	3.08×10^{35}	-217.60	152.90
***Picralima* seed**	1.558	36.14	0.9522	3.901	1.28×10^{18}	3.09×10^{16}	3.09×10^{16}	1.28×10^{18}	-173.50	140.50
***Picralima* mesocarp**	1.827	67.13	0.8956	3.910	1.81×10^{38}	6.79×10^{36}	$>1.07\times10^{34}$	Value too large	-171.40	124.50
***N sativum* (n-hexane)**	1.554	35.83	0.9747	4.377	-1.24×10^{16}	-2.95×10^{14}	5.33×10^{12}	2.28×10^{14}	-175.20	142.10
N. *sativum* seed (ethanol)	1.558	36.13	0.9449	3.766	5.70×10^{14}	1.40×10^{13}	1.40×10^{13}	5.70×10^{14}	-165.10	134.90

Maximum binding capacity, Bmax (µg/mL); Dissociation constant, Kd; Michaelis-Menten's constant, Km (mM) and maximum rate, Vmax (mM/min).

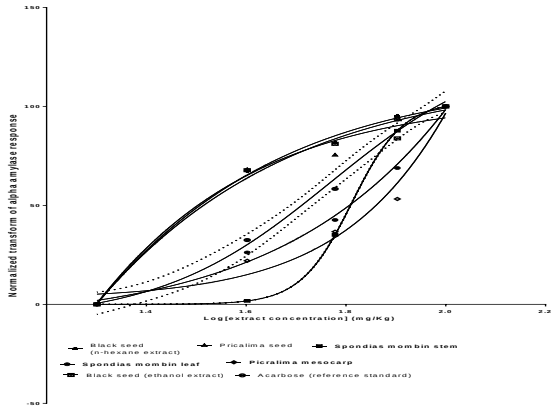

Figure 1: Dose-response curve of alpha-amylase inhibition by the extracts and acarbose (reference standard).

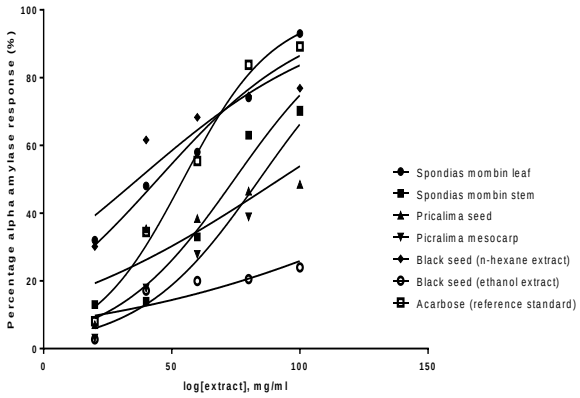

Figure 2: Percentage response curve of alpha-amylase inhibition by the extracts and acarbose (reference standard).

Five concentrations ranging from 20-100 µg/mL of the different plant extracts were tested for their α-amylase and α-glucosidase potentials. For the alpha-amylase inhibition assays, increasing the concentration of the extract led to a dose-dependent Inhibition of alpha-amylase for all extracts of the plants. At the highest concentration (100 µg/mL) of extracts, the n-hexane extract of *P. nitida* seed gave the highest alpha-amylase inhibition of 76.89%. This value was also corroborated by its lower IC_{50} values. All plant extracts did not exhibit a dose-dependent reduction in alpha-glucosidase activity for the alpha-glucosidase assay, as higher inhibition was observed at the lowest concentration (20 µg/mL). Alpha amylase inhibitory potential of the different plant extracts is shown in Table 1. The result indicates that the n-hexane extract of *N. sativum* seed gave the best α-amylase inhibitory effect (IC_{50} = 35.83 µg/mL); this was followed closely by the ethanol extract of Nigella sativum seeds (IC_{50}= 36.13 µg/mL), then by ethanol extract of *P. nitida* seed, *S. mombin* leaves, *S. mombin* stem bark and finally *P. nitida* mesocarp with IC_{50} values of 36.14 µg/mL, 60.35 µg/mL, 64.20 µg/mL and 67.30 µg/mL respectively. We noted that the n-hexane extract of *P. nitida* seeds gave a better α-amylase inhibitory effect than the reference drug. Also, only *P. nitida* seeds, ethanol, and hexane extract of *N. sativum* significantly inhibited alpha-amylase.

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The highest inhibitory effects observed for the *N. sativum* extract may have been expected as various research has reported the hypoglycemic and anti-diabetic effects of seeds of *N. sativum* seeds *in vivo* (Farah *et al*., 2002; Kanter *et al*., 2008; Matira *et al*., 2008; Najmi *et al*., 2008; Meddah *et al*., 2009; Mohamed *et al*., 2009; Nadia and Taha, 2009). The observed effects may also not be unconnected with the rich active components (Thymoquinone, thymohydroquinone, and other essential compounds) and minerals like Copper, Phosphorus, Zinc, and iron found in the seeds of this plant.

Table 2: Dose-response characteristics of the influence of extracts on alpha-glucosidase activity.

	DOSE-RESPONSE CHARACTERISTICS		SIGMOID PLOT INTERPOLATION CHARACTERISTICS		HYPERBOLA PLOT INTERPOLATION CHARACTERISTICS		MICHAELIS-MENTEN'S KINETICS		STRAIGHT-LINE REGRESSION INTERPOLATION CHARACTERISTICS	
	$LogIC_{50}$	IC_{50} (µg/mL)	R^2	Hill's slope	Bmax	Kd	Km	Vmax	Y-intercept	slope
Acarbose	1.881	76.10	0.9143	6.190	88.49	0.1143	2.395×10^{33}	5.388×10^{34}	-177.20	123.60
***S. mombin* leaf**	1.690	48.99	0.9604	3.545	93.54	0.1977	5.496×10^{25}	1.951×10^{27}	-190.50	144.20
***S. mombin* stem**	1.671	46.85	0.9735	3.258	115.80	0.7469	2.644×10^{26}	9.430×10^{27}	-181.10	139.00
***Picralima* seed**	1.807	64.19	0.9513	4.486	456.20	17.21	1.182×10^{12}	3.375×10^{13}	-185.20	134.20
Picralima mesocarp	1.698	49.84	0.9351	2.863	58.82	0.6444	4.770×10^{25}	1.605×10^{27}	-172.20	131.90
N *sativum* seed (n-hexane)	1.646	44.24	0.9832	3.943	103.70	3.443	2.960×10^{22}	1.135×10^{24}	-193.80	148.90
N *sativum* seed (ethanol)	1.652	44.87	0.9845	3.697	117.50	2.421	1.022×10^{23}	3.790×10^{24}	-185.70	143.10

Maximum binding capacity, Bmax (µg/mL); Dissociation constant, Kd; Michaelis-Menten's constant, Km (mM) and maximum rate, Vmax (mM/min)

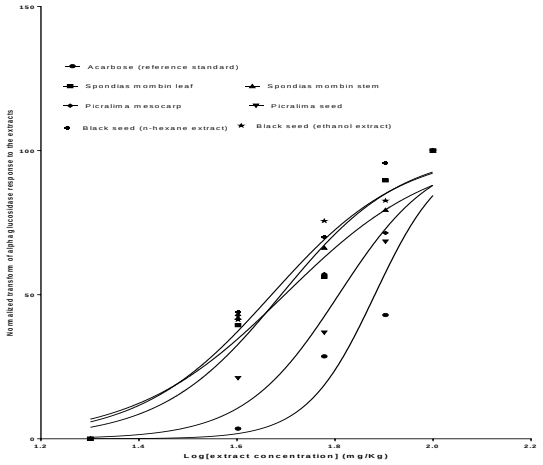

Figure 3: Dose-response curve of alpha-glucosidase Inhibition by the extracts and acarbose (reference standard).

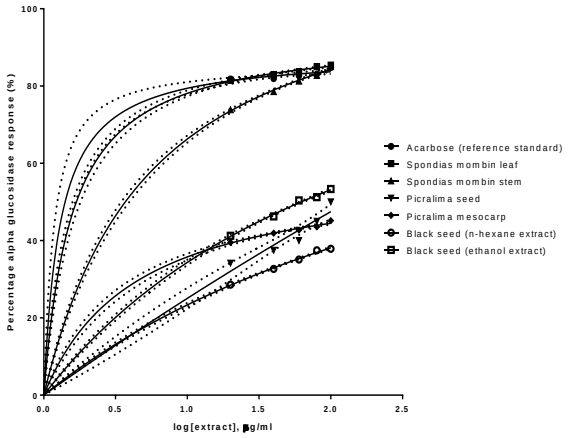

Figure 4: Percentage response curve of alpha-glucosidase Inhibition by the extracts and acarbose (reference standard).

Fred-Jaiyesimi *et al*. (2009) have established the *in vitro* hypoglycemic effects of the leaf of *S. mombin*. For the α-glucosidase inhibitory study, the n-hexane extracts of black seed also gave the best α-glucosidase result (IC_{50} =44.24 µg/mL) (Table 2). This was closely followed by ethanol extracts of *N. sativum* seeds, *S. mombin* stem bark, *S. mombin* leaf, *P. nitida* mesocarp, and then *P. nitida* seed with IC_{50} values of 44.87 µg/mL, 46.85 µg/mL, 48.99 µg/mL, 49.84 µg/mL, and 64.19 µg/mL respectively. All plant extracts had better alpha-glucosidase inhibitory effects than the standard acarbose. The IC_{50}, Kd, and Vmax give us an idea of the potency of a plant (meaning that the higher these kinetics characteristics, the lower the capacity to delay the rate of the enzyme-catalyzed reactions). In contrast, the Bmax gives information about the effectiveness of the extracts (i.e., the lower this value, the lower the efficacy of the extracts). Although we did not record the highest Bmax value for the n-hexane extract of *N. sativum* seeds when compared with other plant extracts, the observed Bmax for α-amylase and α-glucosidase study respectively was nonetheless higher than that of the standard acarbose (Tables 1, 2).

The enzyme kinetics were determined to obtain further information regarding the type and mode of inhibition of the different plant extracts on alpha-amylase and alpha-glucosidase. For the kinetic model of the plant extracts on alpha-amylase, all plant extracts except the ethanol extract of *P. nitida* seed showed a mixed non-competitive mode of inhibition as evidenced by their different Km (the affinity of the enzymes for the substrate) and Vmax (the velocity of reaction). The ethanol extract of *P. nitida* seeds resulted in a decrease in both Km and Vmax. Therefore, depicts an uncompetitive manner of inhibition. Uncompetitive inhibitors bind to the ES complex by forming an ES-inhibitor complex (Bisswanger, 2008; Cornish-Bowden, 2013). This complex decreases the affinity of the substrate to attach to the active site of the enzyme, thus reducing the reaction rate (Cornish-Bowden, 1974; Bachhawat *et al*., 2011).

We also noted that the ethanol extract of *P. nitida* seeds, *N. sativum* seeds, and the n-hexane extract of *Nigella sativum* seeds inhibited alpha-glucosidase uncompetitively. In contrast, ethanol extract of *P. nitida* mesocarp, *S. mombin* leaves, and stem bark exhibited a mixed type of inhibition on alpha-glucosidase. This type of inhibitor binds to the enzyme in its free and bound state, thus hindering the substrate from binding (Bisswanger, 2008; Cornish-Bowden, 2013) or enhancing substrate binding affinity and reducing reaction rate (Cornish-Bowden, 1974). Enzyme inhibition and kinetic studies are vital tools that help differentiate the inhibitory mechanism types. Several studies have reported that polyphenolic compounds from plants showed competitive, non-competitive, and mixed inhibition patterns on α-amylase α-glucosidase enzymes (Williamson *et al*., 1992; Oates, 2008; Yao *et al*., 2010; Ghosh *et al*., 2014).

CONCLUSION

This research has revealed the ability of three anti-diabetic medicinal plants to inhibit alpha-amylase and alpha-glucosidase and also gave insight into their mode and type of inhibition. Among the three plants, *N. sativum* showed the highest α-amylase and α-glucosidase inhibitory effects, suggesting and supporting this plant's use as a new anti-diabetic agent of natural source.

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