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In Vitro Comparative Inhibitory Activities of Extracts of *Annona Muricata* on A-amylase and A-glucosidase for Possible Anti-diabetic Remedy

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Abstract

Different parts of the plant Annona muricata has been used in folkloric medicine for the management of diabetes. This study was undertaken to compare the anti-diabetic effects of extracts of different parts of this plant (fruit pulp, leaf, stem bark and root bark). The different parts of the plant were processed into extracts of methanol, water, petroleum ether and hexane prior to enzyme inhibition assay. In vitro inhibitory properties of these extracts on α-amylase and α-glucosidase activities were performed using standard procedures. The mode and mechanism of interactions between the enzymes and extracts were determined using various kinetic interpolations and in silico experiments. The experiments revealed that all the extracts inhibited α-amylase and α- glucosidase dose dependently. The leaf petroleum ether extract gave the highest α-amylase and alpha glucosidase inhibitory effect. Enzyme kinetic studies showed that all extracts of the different part of A. muricata led to a decrease in both Km and Vmax indicating an uncompetitive mode of inhibition of α-amylase and α-glucosidase activities. We therefore concluded that the leaf of A. muricata may serve as a good source of anti-diabetic agents that can reduce post-prandial hyperglycemia.

Key words: Annona muricata, α-amylase, α- glucosidase, mode of inhibition, Diabetes

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Introduction

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia in postprandial and/or fasting state and results in disturbances of carbohydrate, fat, and protein metabolism due to defects in insulin secretion, insulin action, or both (Imam, 2012). The high prevalence of type 2 diabetes mellitus (T2DM) as well as its deleterious effects it poses make type 2 DM a major metabolic disorder receiving a lot of attention (Zimmet, 2011).The early stage of type 2 diabetes is associated with postprandial hyperglycemia due to impaired pancreas after meal. Post prandial hyperglycemia has been shown to induce oxidative stress through extreme production of free radicals that may impair the endogenous antioxidant defense and in the long run leads to various life threatening conditions such as cardiovascular diseases (Johansen *et al.,* 2005). Management of T2DM requires maintaining blood glucose within the normal level through a healthy lifestyle (Midhet *et al.,* 2010). One class of pharmacological intervention for T2DM requires the use of α-glucosidase inhibitors that acts by inhibiting carbohydrate breakdown enzymes such as α-glucosidase and α-amylase, drugs which include, acarbose, miglitol, voglibose, etc. These enzymes inhibit postprandial glucose peaks thereby leading to decreased post-load insulin levels. Due to the disavantages associated with these conventional pharmacological interventions, including deleterious side effects, expensive, etc., there is therefore increased search for alternatives especially from natural sources. Several studies are available on potential α-glucosidase inhibitors from natural sources (Sahere *et al.,* 2017; Anofi *et al.,* 2019; Hind *et al.,* 2017; Sindhu *et al.,* 2013).

Annona muricata commonly called Soursop, graviola or guanabana is an edible tropical fruit tree that belongs to the family of Annonaceae. A number of medicinal uses have been reported from the use of the bark, roots, fruits and leaf and seeds of A. muricata (Bardie and Schauss, 2010). Over 200 bioactive compounds have been isolated from this plant with their structures and biological effects determined. The predominant compound isolated is acetogenins followed by alkaloids and phenols. In our previous study, we established that methanol, dichloromethane, and ethyl acetate

extracts of different parts of A. muricata possess strong inhibitory effects on α- amylase and α-glucosidase. Also, through molecular docking experiment we established that an isolated acetogenin identified as 15-acetyl guanacone (Agu *et al.,* 2017) may have been responsible for the high inhibition of α- amylase and α-glucosidase observed in the fruit pulp of A. muricata (Agu *et al.,* 2019). This study was therefore undertaken to compare the inhibitory effects of the methanol: water, petroleum ether and hexane extracts of different parts of Annona muricata in an attempt to explore on more α-amylase and α-glucosidase inhibitors from plant sources.

Materials and Methods

Plant collection, identification and authentication

Fresh parts of the plant consisting of the fruit-pulp, leaf, stem-bark, and root-bark were collected from Fields around the University of Benin, Edo State, Nigeria. Proper identification and authentication was done at the Department of Plant Biology and Biotechnology, University of Benin by Professor Mc Idu. A voucher specimen (UBHa 0205) was deposited at the Department Herbarium.

Preparation of extract

The carefully separated plant parts were washed and dried at room temperature. The dried plant parts were pulverized. Exactly 500g of the pulverized plant parts were marcerated into 2L of solvent (80% methanol: water, petroleum ether and n-hexane), filtered using Whatman's filter paper and concentrated in vacuo into gel-like extracts using rotary evaporator. The concentrated extracts were then stored in an airtight container and refrigerated at - 4 °C, prior to use.

Alpha – amylase inhibitory assay:

Serial dilutions of the plant extracts (5.00 mg%, w:v) between 0 to 200 µL were prepared by mixing with 500 µL Sodium phosphate buffer (0.02 M, pH 6.9 with 0.006 M NaCl as the stabilizer), containing pancreatic alpha – amylase (1.0 U/ml) obtained from the mucosal lining of rat proximal small intestine. The mixtures were incubated at 37oC for 5 mins, and then 500 µL of starch solution (1 mg/100mL in 0.02 M

sodium buffer at pH 6.90 with 0.006 M NaCl) was introduced into the reaction mixtures. The reaction mixtures were subsequently, incubated at 37oC for 5 mins in a water bath. The reaction were then stopped using 1.0 ml dinitrosalicylic acid (DNSA) and further incubated in boiling water for 5 mins. The blank sample had no starch solution and enzyme in it, while the control (reference sample) had all the reagents and the enzyme except the starch solution. Acarbose served as the positive control. When the reaction mixtures were cool, absorbance were read at 540nm (Worthington, 1993).

$$\text{Percentage } \alpha\text{-amylase inhibition } (\%) = \frac{Aref - Asample}{Aref} \times 100$$

Serial dilutions of the plant extracts (5.00 mg%, w:v) between 0 to 200 µL were prepared by mixing with 100 µL Sodium phosphate buffer (0.1 M , pH 6.9) containing alpha – glucosidase (1.0 U/mL) and then incubating at 37 °C for 5mins. 0.05 mL of para-nitrophenyl-α-D-glucopyranoside (5.0 mM) solution in Sodium phosphate buffer (0.1 M, pH 6.9) was added to the reaction mixture and incubated at 37 °C for 5 mins. The reaction were then stopped using 1.0 ml dinitrosalicylic acid (DNSA) and further incubated in boiling water for 5mins. The reaction mixtures were allowed to cool and then absorbance read at 405nm (Oboh *et al*., 2009). The blank sample had no starch solution and enzyme in it, while the control (reference sample) had all the reagents and the enzyme except the starch solution. Acarbose served as the positive control.

$$\text{Percentage } \alpha\text{-amylase inhibition } (\%) = \frac{Aref - Asample}{Aref} \times 100$$

Statistical analysis

The data were entered into Microsoft Excel v.13, prior to analyses. The Graph Pad Prism Software, inc., (version 6.01, 2012) was used to analyzed to obtain the means, SEM and IC50, using the data using the One-way analysis of variance and unpaired sample students' T-test. The level of significance was taken as $p \leq 0.05$. The sigmoid (Hill's slope), hyperbola (maximum binding capacity, Bmax, and dissociation constant, Kd), and Michaelis-Menten's (Km and Vmax) were also determined using the Graph Pad Prism Software.

Results

Table 1. Dose-response characteristics of the influence of *Annona muricata* methanol-water (95:5%, v/v) 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}	R^2	*n*	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	Slope
Fruit pulp	0.331	2.142	0.899	4.213	39.80	-0.047	7.045×10^{-13}	41.44	44.56	45.62
Leaf	0.257	1.807	0.992	4.879	47.15	-0.051	1.169×10^{-16}	53.39	53.39	53.20
Stem bark	0.334	2.159	0.988	6.490	47.03	-0.027	1.147×10^{-16}	44.61	44.70	47.38
Root bark	0.336	2.165	0.963	5.359	41.01	-0.035	2.209×10^{-16}	44.39	44.48	46.49
Acarbose	0.236	1.722	1.000	1.481	51.17	-0.079	2.220×10^{-16}	63.07	65.59	41.67
F-value	11.064	18.452	9.445	47.335	55.062	-	-	64.117	39.550	26.470
p-value	0.001	0.013	0.131	0.000	0.000	-	-	0.033	0.039	0.016

Ic50 = fifty percent inhibitory concentration, Bmax = maximum binding (U/L), Kd = concentration (mg/Kg), Km = concentration (mg/Kg) and Vmax = maximum velocity (U/L). Hill's coefficient (KI = $[L_o]$ n, when V_o = ½Vmax; where, KI = sum of individual k-values; $[L_o]$ = initial ligand concentration; V_o = initial velocity; Vmax = final velocity).

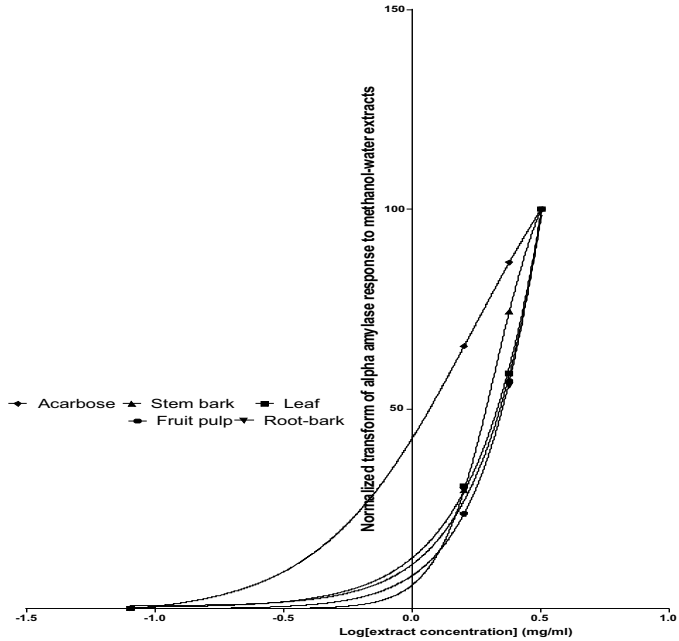

Figure 1. Dose-response curve of alpha amylase inhibition by the *Annona muricata* fruit pulp, leaf and Acarbose (reference standard) methanol-water (95:5%, v/v) extract.

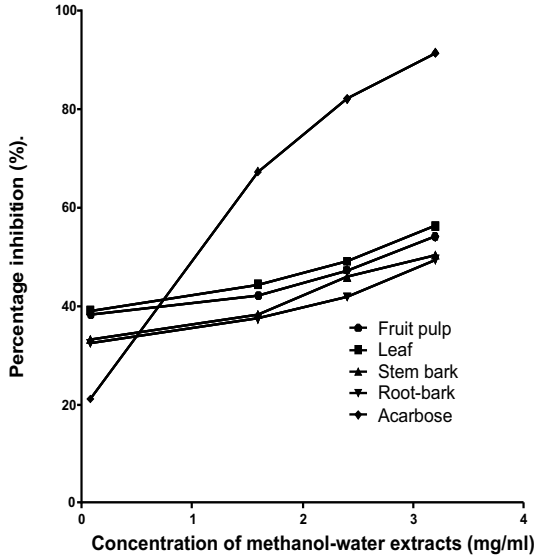

Figure 2. Percentage inhibition of alpha amylase by the *Annona muricata* fruit pulp, leaf and Acarbose (reference standard) methanol-water (95:5%, v/v) extract.

Table 2. Dose-response characteristics of the influence of *Annona muricata* methanol-water (95:5%, v/v) extracts on alpha glucosidaseactivity.

	Dose-Response characteristics		Sigmoid plot interpolation characteristics		Hyperbola plot interpolation characteristics		*Michaelis-Menten*'s kinetics		Straight line regression interpolation characteristics	
	$LogIC_{50}$	IC_{50}	R^2	*n*	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	Slope
Fruit pulp	0.293	1.963	0.735	4.408	43.59	-0.048	1.361×10^{-16}	48.75	48.84	49.45
Leaf	0.231	1.703	0.859	4.285	47.84	-0.060	2.079×10^{-16}	55.50	55.50	54.32
Stem bark	0.265	1.841	0.798	3.475	43.54	-0.060	1.254×10^{-16}	50.48	50.57	49.98
Root bark	0.337	2.173	0.619	5.454	40.94	-0.034	1.538×10^{-16}	44.23	44.32	46.41
Acarbose	0.236	1.722	0.976	1.481	51.17	-0.079	2.220×10^{-16}	63.07	63.07	59.18
F-value	14.660	41.352	47.030	53.039	24.117	-	-	57.394	55.911	56.048
p-value	0.037	0.003	0.000	0.000	0.000	-	-	0.042	0.039	0.018

Ic_{50} = fifty percent inhibitory concentration, *Bmax* = maximum binding capacity (U/L), K_d = dissociation constant (mg/Kg), *Km*= *Michaelis-Menten*'s constant (mg/Kg) and *Vmax* = maximum velocity (U/L). *Hill's coefficient* ($K^I = [L_o]^n$, when $V_o = ½Vmax$; where, K^I = sum of individual k-values; $[L_o]$ = initial ligand concentration; V_o = initial velocity; *Vmax* = final velocity).

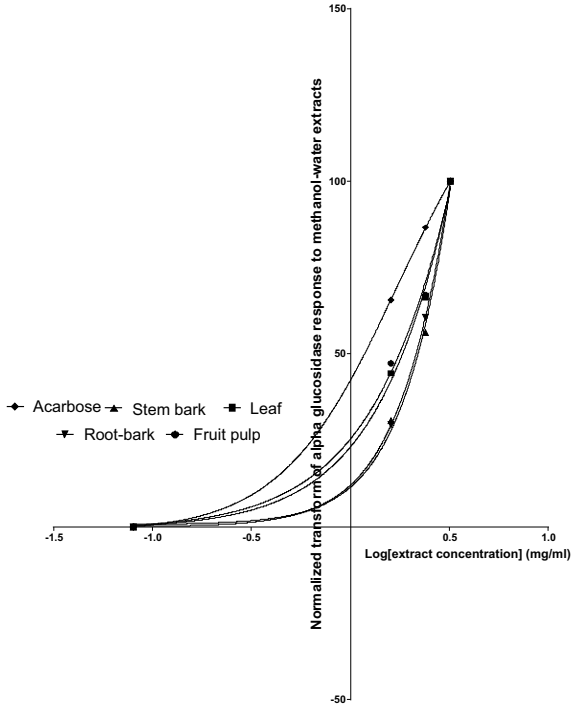

Figure 3. Percentage inhibition of alpha glucosidase by the *Annona muricata* fruit pulp, leaf and Acarbose (reference standard) methanol-water (95:5%, v/v) extract.

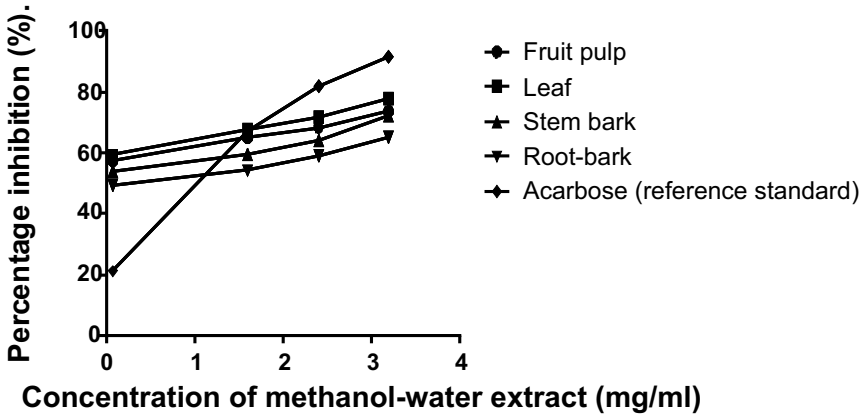

Figure 4. Percentage inhibition of alpha glucosidase by the *Annona muricata* fruit pulp, leaf and Acarbose (reference standard) methanol-water (95:5%, v/v) extract

Table 3. Dose-response characteristics of the influence of *Annona muricata* petroleum ether 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}	R^2	*n*	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	Slope
Fruit pulp	0.046	1.113	0.899	4.213	39.80	-0.047	7.045×10^{-13}	41.44	44.56	45.62
Leaf	0.035	1.084	0.992	4.879	47.15	-0.051	1.169×10^{-16}	53.39	53.39	53.20
Stem bark	0.319	2.086	0.988	6.490	47.03	-0.027	1.147×10^{-16}	44.61	44.70	47.38
Root bark	0.328	2.127	0.963	5.359	41.01	-0.035	2.209×10^{-16}	44.39	44.48	46.49
Acarbose	0.236	1.722	1.000	1.481	51.17	-0.079	2.220×10^{-16}	63.07	65.59	41.67
F-value	83.705	24.088	6.185	37.511	44.009	-	-	38.082	38.152	44.106
p-value	0.001	0.007	0.094	0.000	0.000	-	-	0.035	0.029	0.017

Ic_{50} = fifty percent inhibitory concentration, *Bmax* = maximum binding (U/L), *Kd* = concentration (mg/Kg), *Km* = concentration (mg/Kg) and *Vmax* = maximum velocity (U/L). *Hill's coefficient* ($K^l = [L_o]^n$, when $V_o = ½Vmax$; where, K^l = sum of individual k-values; $[L_o]$ = initial ligand concentration; V_o = initial velocity; *Vmax* = final velocity).

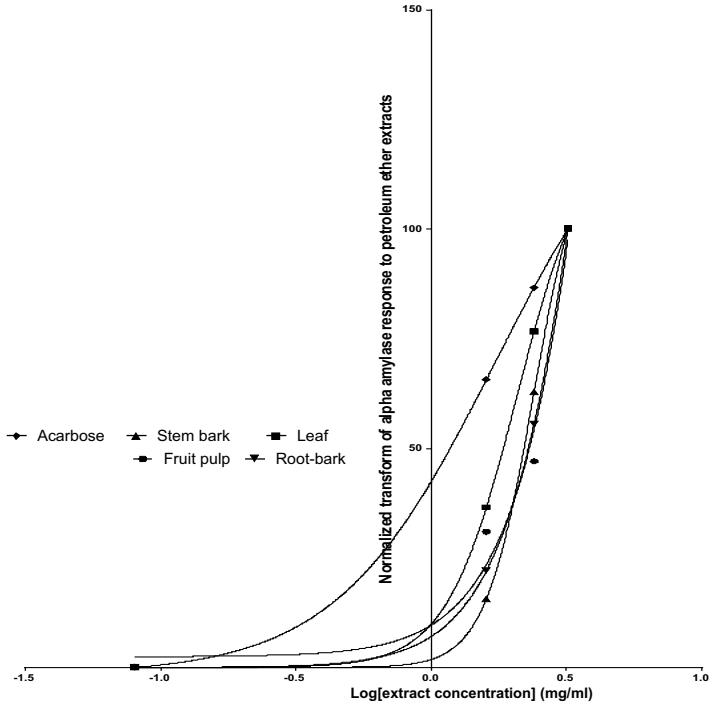

Figure 5. Dose-response curve of alpha amylase inhibition by the *Annona muricata* fruit pulp, leaf and Acarbose (reference standard) petroleum ether extract.

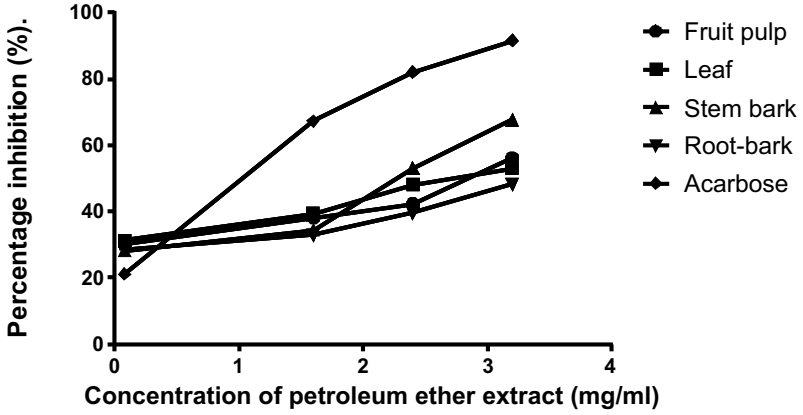

Figure 6. Percentage inhibition of alpha amylase by the *Annona muricata* fruit pulp, leaf and Acarbose (reference standard) petroleum ether extract

Table 4. Dose-response characteristics of the influence of *Annona muricata* petroleum ether 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}	R^2	*n*	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	Slope
Fruit pulp	0.148	1.405	0.735	4.408	43.59	-0.048	1.361×10^{-16}	48.75	48.84	49.45
Leaf	0.239	1.733	0.859	4.285	47.84	-0.060	2.079×10^{-16}	55.50	55.50	54.32
Stem bark	0.249	1.774	0.798	3.475	43.54	-0.060	1.254×10^{-16}	50.48	50.57	49.98
Root bark	0.352	2.250	0.619	5.454	40.94	-0.034	1.538×10^{-16}	44.23	44.32	46.41
Acarbose	0.236	1.722	0.976	1.481	51.17	-0.079	2.220×10^{-16}	63.07	63.07	59.18
F-value	18.533	22.094	15.388	42.710	30.522	-	-	45.037	42.110	33.076
p-value	0.041	0.035	0.049	0.000	0.005	-	-	0.000	0.003	0.001

Ic_{50} = fifty percent inhibitory concentration, *Bmax*= maximum binding capacity (U/L), K_d= dissociation constant (mg/Kg), *Km*= *Michaelis-Menten*'s constant (mg/Kg) and *Vmax*=maximum velocity (U/L). *Hill's coefficient* ($K^I = [L_o]^n$, when $V_o = ½Vmax$; where, K^I = sum of individual k-values; $[L_o]$ = initial ligand concentration; V_o = initial velocity; *Vmax* = final velocity).

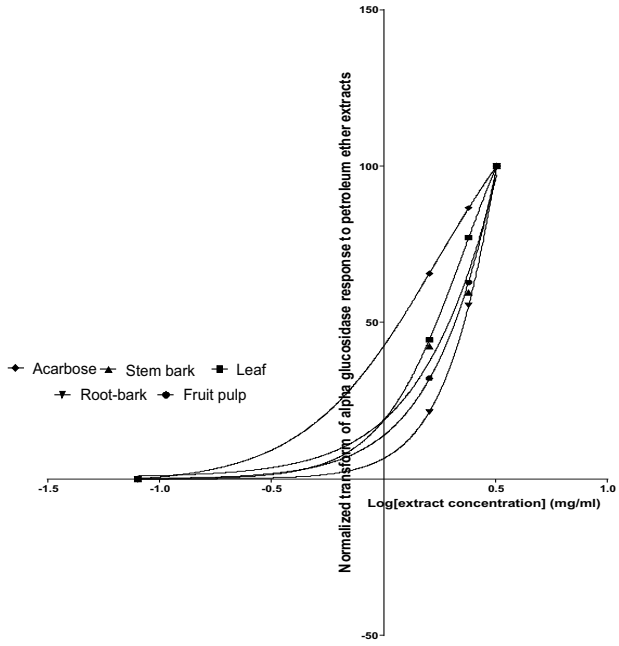

Figure 7. Percentage inhibition of alpha glucosidase by the *Annona muricata* fruit pulp, leaf and Acarbose (reference standard) petroleum ether extract.

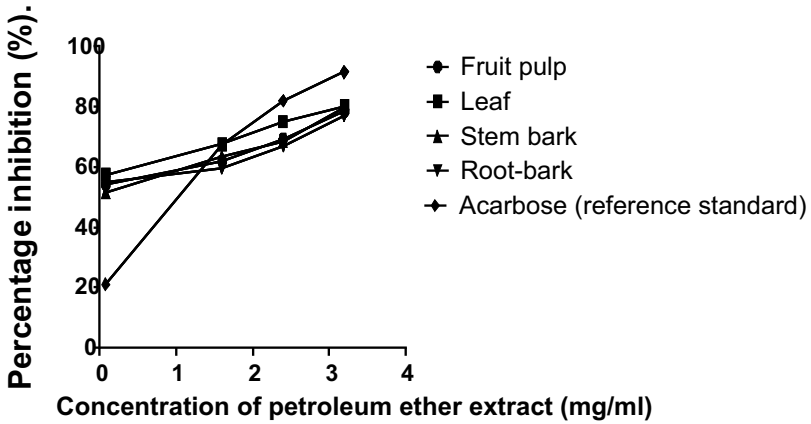

Figure 8. Percentage inhibition of alpha glucosidase by the *Annona muricata* fruit pulp, leaf and Acarbose (reference standard) petroleum ether extract

Table 5. Dose-response characteristics of the influence of *Annona muricata* hexane 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}	R^2	*n*	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	Slope
Fruit pulp	0.273	3.072	0.850	4.021	23.16	-0.041	4.007×10^{-13}	36.18	42.37	43.58
Leaf	0.101	1.186	0.947	4.439	27.59	-0.047	4.001×10^{-16}	35.09	43.08	44.36
Stem bark	0.209	2.113	0.991	5.853	22.43	-0.044	4.035×10^{-16}	29.14	42.11	43.60
Root bark	0.331	5.220	0.970	5.826	27.08	-0.047	4.062×10^{-16}	29.20	43.90	44.71
Acarbose	0.236	1.722	1.000	1.481	51.17	-0.079	2.220×10^{-16}	63.07	65.59	41.67
F-value	27.106	33.155	17.007	40.822	41.063	-	-	55.311	31.007	7.353
p-value	0.000	0.000	0.064	0.000	0.000	-	-	0.000	0.000	0.085

Ic_{50} = fifty percent inhibitory concentration, *Bmax* = maximum binding (U/L), Kd = concentration (mg/Kg), Km = concentration (mg/Kg) and *Vmax* = maximum velocity (U/L). *Hill's coefficient* ($K^I = [L_o]^n$, when $V_o = ½Vmax$; where, K^I = sum of individual k-values; $[L_o]$ = initial ligand concentration; V_o = initial velocity; *Vmax* = final velocity).

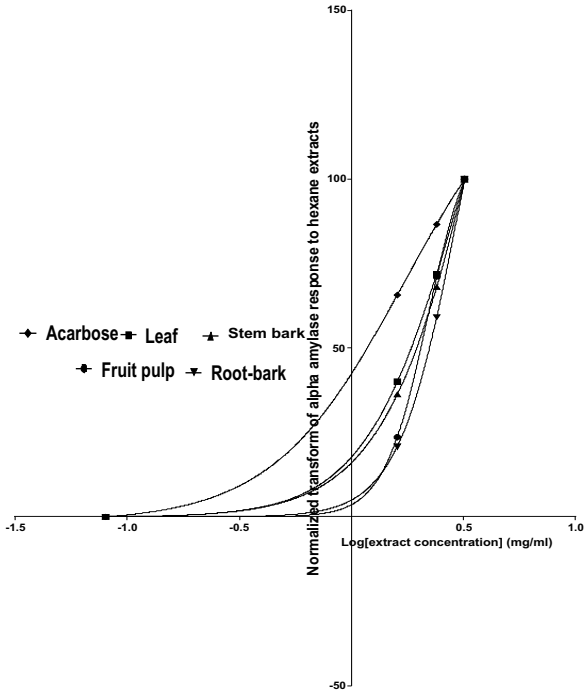

Figure 9. Dose-response curve of alpha amylase inhibition by the *Annona muricata* fruit pulp, leaf and Acarbose (reference standard) hexane extract.

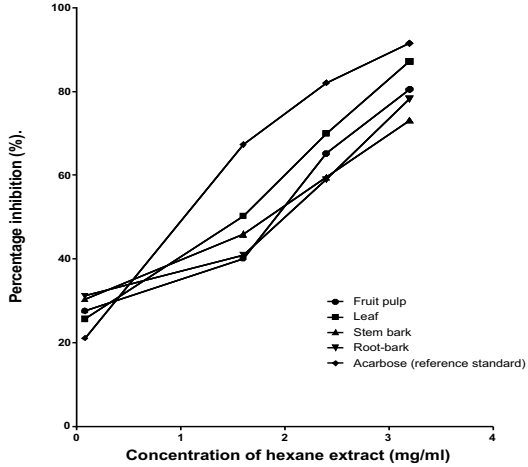

Figure 10. Percentage inhibition of alpha amylase by the *Annona muricata* fruit pulp, leaf and Acarbose (reference standard) hexane extract.

Table 6. Dose-response characteristics of the influence of *Annona muricata* hexane 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}	R^2	*n*	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	Slope
Fruit pulp	0.417	2.611	0.261	3.118	11.28	-0.017	1.507×10^{-16}	24.05	38.05	26.33
Leaf	0.352	2.250	0.250	3.075	10.65	-0.013	1.443×10^{-16}	24.11	41.83	20.81
Stem bark	0.416	2.609	0.233	3.106	11.03	-0.017	1.486×10^{-16}	23.75	40.22	25.70
Root bark	0.329	2.133	0.227	3.015	10.39	-0.015	1.442×10^{-16}	23.59	39.51	27.55
Acarbose	0.236	1.722	0.976	1.481	51.17	-0.079	2.220×10^{-16}	63.07	63.07	59.18
F-value	41.255	26.701	17.350	23.044	35.116	-	-	37.220	21.514	26.077
p-value	0.003	0.001	0.015	0.096	0.040	-	-	0.000	0.000	0.000

Ic_{50} = fifty percent inhibitory concentration, *Bmax*= maximum binding capacity (U/L), K_d= dissociation constant (mg/Kg), Km= *Michaelis-Menten*'s constant (mg/Kg) and *Vmax*=maximum velocity (U/L). *Hill's coefficient* ($K^I = [L_o]^n$, when $V_o = ½Vmax$; where, K^I = sum of individual k-values; $[L_o]$ = initial ligand concentration; V_o = initial velocity; *Vmax* = final velocity).

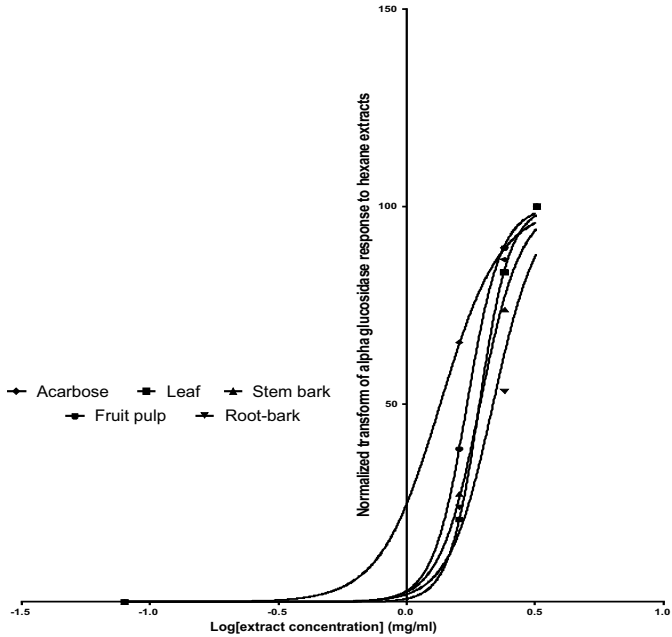

Figure 11. Dose-response curve of alpha glucosidase inhibition by the *Annonamuricata* fruit pulp, leaf and Acarbose (reference standard) hexane extract.

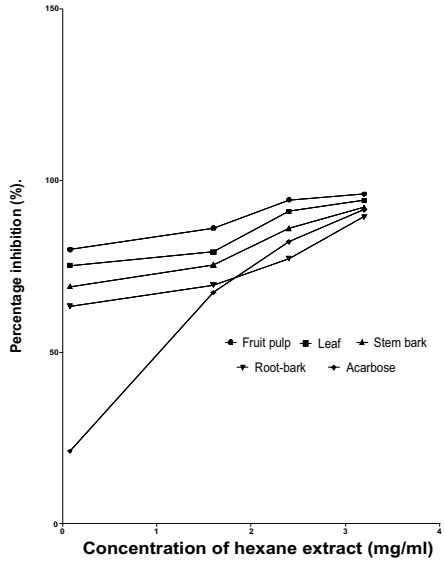

Figure 12. Percentage inhibition of alpha glucosidase by the *Annona muricata* fruit pulp, leaf and Acarbose (reference standard) hexane extract.

Discussion

In recent times there has been growing interest in the use of medicinal plant for the treatment of various disease conditions due to their availability, affordability, and little or no side effects. Although the antidiabetic effects of several species of Annona such as Annona squamosa, Annona muricata, Annona glabra, and Annona cherimola have been reported (Shirwaikar *et al.,* 2004; Andrade-Cetto and Heinrich, 2005; Adeyemi *et al.,* 2009), no detailed comparative studies exist between the different parts of the plant. This present study investigated the inhibitory effects of methanol: water, petroleum ether and hexane extracts of different parts of Annona muricata (fruit pulp, leaf, stem bark and root bark) on α-amylase and α-glucosidase. To determine the potency and effectiveness of the various extracts, kinetic properties such as IC50, Kd and Vmax (which helps determines the potency of the extracts) and Bmax which describes the possible efficacies of the extracts (i.e., the higher these kinetic parameters, the higher the efficacies of the ligand, molecule or extract) were determined.

Our result showed that for the methanol: water extract, the leaf extract gave the significantly highest inhibitory effect on alpha amylase and alpha glucosidase as evidenced by its significant lower IC50 values of 1.807 mg/dL ($p=0.01$) and 1.703 mg/dL respectively ($p=0.003$) (Table 1, 2). This observed effect was also corroborated with a significantly high Bmax and Kd value of 47.15 U/L and -0.051 respectively ($p=0.0001$). Although this value was not higher than the reference standard acarbose ($p<0.05$). From our previous study (Agu et al., 2019) we reported the highest inhibitory effect from the methanol extract of stem-bark (IC50, 1.843 mg/dL). For a plant to be regarded as a good anti diabetic agent, it should be able to exhibit a mild α-amylase inhibitory (lowest IC50 value) and strong α-glucosidase inhibitory (lowest IC50 value) activities (Kazeem et al., 2016). This was clearly and significantly recorded by the leaf methanol: water extract of the Annona muricata.

For the petroleum ether extracts of the different parts of Annona muricata, we observed that the extract of the leaf gave the significantly highest inhibitory effect - IC50 - of 1.084 mg/dL, even better than the reference standard acarbose, 1.722 mg/dL ($p=0.007$).

This was followed by the fruit pulp, stem bark and root bark having the least inhibitory effect. The observed effects was also corroborated with their higher Bmax and Kd values ($p<0.000$). On the other hand, for the alpha glucosidase inhibitory effect, the fruit pulp had the best inhibitory effect on alpha glucosidase. Followed by the leaf, stem bark and root bark (IC50; 1.405 mg/dL, 1.733 mg/dL, 1.744 mg/dL, 2.250 mg/dL, respectively) (table 3, 4 and figures 5, 6, 7, 8).

For the hexane extract, only the leaf extract, gave a significant alpha amylase inhibitory effect when compared with the standard acarbose ($p<0.05$). Whereas we recorded that the root bark better inhibited alpha glucosidase, IC50 of 2.133. This was followed by the leaf, stem bark and fruit pulp IC50 of 2.250 mg/dL, 2.609 mg/dL, and 2.611 mg/dL, respectively ($p<0.001$). Also, we observed a higher Bmax value for the fruit pulp (11.28 U/L) as compared to the 10.39 U/L value recorded for the root bark. This could imply that the active moieties in the fruit pulp extract binds firmly to the active site of the enzyme thereby inhibiting the speed of catalysis.

In an attempt to understand the inhibition mechanism utilized against α-amylase and α-glucosidase, we determined the type of inhibition exhibited by the different extract of the various parts of Annona muricata using the dose – respose relationships. All extracts of the different parts of A. muricata led to a decrease in both Km and Vmax suggesting an uncompetitive mode of inhibition of α-amylase and α-glucosidase activities. Uncompetitive inhibitors can only bind to the enzyme – substrate (E-S) complex to form enzyme – substrate – ligand (E-S-L) complex. Therefore, these inhibitors decrease Km because of increased binding efficiency and decrease Vmax because they interfere with substrate binding and hamper catalysis in the E-S complex. The uncompetitive inhibition of both α-amylase and α-glucosidase by the different extract of the various parts of Annona muricata suggest the binding of the active chemical entities to the enzyme-substrate complex which adversely lowers the substrate affinity for the active site, thus ultimately hinders the continuous hydrolysis of oligosaccharides to monosaccharides (Bachhawat, 2011). These plant extracts are not affected by higher concentrations of the substrate as does the acarbose (competitive inhibitor)

indicating that at a high carbohydrate intake, a high concentration of the extract would necessarily not be needed to present the same effect. Earlier reports by various researchers have shown that poly phenolic compounds from plants showed competitive, noncompetitive and mixed inhibitors tested for the inhibition of α-amylase α-glucosidase (Ghosh et al., 2014; Yao *et al.,* 2010; Wang *et al.,* 2010; Williamson et al., 1992; Oates, 2008). All the extracts displayed positive cooperativity, i.e., the Hill's coefficient (n), with the stem bark and root bark demonstrating greater magnitudes in this respect (Agu *et al.,* 2017; Adefegha and Oboh, 2012; Shobana *et al.,* 2009).

In conclusion, the observed effect may be linked to the presence of phenolics and other important compounds (annonaceous acetogenins) found to be present in rich amount in different parts of Annona muricata. Earlier reports of research by, have established the association between phenolic compounds and inhibition of carbohydrate hydrolyzing enzymes.

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