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Possible anti-diabetic potentials of *Annona muricata* (soursop): inhibition of α-amylase and α-glucosidase activities

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Abstract

Background: *Annona muricata* has been used in folklore in the management of diabetes. A major strategy in decreasing postprandial hyperglycemia in diabetes involves the inhibition of carbohydrate-hydrolyzing enzymes - α-amylase and α-glucosidase. Thus, this study evaluated the in vivo and in vitro inhibitory potentials of the different parts (fruit-pulp, leaf, stem-bark and root-bark) of *Annona muricata*.

Methods: A total of 120 Wistar rats were treated with methanol extracts for 28 days after which blood and tissue samples were collected for α-amylase assay. In vitro inhibitory properties of methanol, ethyl acetate and dichloromethane extracts of the various parts of the plant on α-amylase and α-glucosidase activities were performed using standard procedures. The mode and mechanism of interactions between the enzymes and extracts (and isolated acetogenin) were determined using various kinetic interpolations and in silico experiments.

Result: The fruit-pulp and root-bark methanolic extracts better -inhibited plasma and tissue amylase in vivo. The in vitro studies revealed that the stem-bark methanolic, fruit-pulp ethyl acetate, and leaf dichloromethane extracts, better inhibited α-amylase activity compared with the standard acarbose. Also, the leaf methanol, fruit-pulp ethyl acetate, and root-bark dichloromethane extract better inhibited α-glucosidase activity. These observations were corroborated with their higher *Bmax* and *Vmax* and lower *Kd* values. All the extracts exhibited an "uncompetitive" type of inhibition pattern. Also, the isolated acetogenin (15-acetyl guanacone) from the fruit-pulp showed a better binding affinity compared to the standard drug, Metformin.

Conclusion: Better natural remedy for diabetics can be obtained from *Annona muricata* with minimal or no adverse side effects.

Keywords: *Annona muricata*, α-Amylase, α-Glucosidase, Acarbose, Metformin

Introduction

Diabetes mellitus is a chronic endocrine disorder of carbohydrate, fat and protein metabolism characterized by an increase in both fasting and postprandial glucose level and it has been reported to be the major cause of mortality worldwide. There is an alarming projection of 471 million people with the disease by the year 2035 [1]. Diabetes is grouped into two forms; type 1, insulin dependent diabetes mellitus and type 2, non- insulin dependent diabetes mellitus. Type 2 is the major form accounting about 90% of cases worldwide [2]. In type 2 diabetes mellitus (DM2), postprandial hyperglycemia is important in the development of the disease; Current treatment for Type 2 diabetes remains inadequate so prevention is preferable [3]. The major strategy for the management of Type 2 diabetes is to decrease postprandial hyperglycemia. The α-glucosidase inhibitors, such as acarbose, have been used in the clinic to control blood glucose increase, especially postprandial, in DM2 [4].

Herbal medicine is predominantly available for the treatment of diabetes and the main advantages of the use of herbal drugs are effectiveness, safety, and

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acceptability [5]. The mechanism of action of these medicinal plant of its products involve retarding the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes, such as pancreatic amylase and α-glucosidase and through the inhibition of these enzymes, medicinal plants can effectively control the postprandial rise in blood glucose. Several medicinal plants have a high potential in inhibiting α-amylase enzyme activity [6].

Annona muricata (*A. muricata*) is a tropical plant species belonging to family Annonaceae and known for its many ethnomedicinal uses. All parts of *Annona* are used in natural medicine in the tropics. It is considered to be a good source of natural antioxidants for various diseases. Traditionally, the leaves are used for headaches, insomnia, cystitis, liver problems, and diabetes, antitumor, anti-inflammatory. The health benefits of this plant have been attributed to their unique phytochemical composition [7–10].

There is, however, a paucity of information that demonstrates the comparative α-amylase and α-glucosidase inhibitory properties of different extracts and fractions, of the various parts of the plant (fruit-pulp, leaf, stem-bark and root-bark) with relation to its anti-diabetic activity. Hence, the present study was carried out to investigate the comparative α-amylase and α-glucosidase inhibitory potentials of methanolic, ethyl acetate and dichloromethane extracts of the fruit-pulp, leaf, stem-bark and root-bark of *Annona muricata* and also to determine the possible modes and mechanisms of inhibition of these enzymes by these extracts. The justification for this research is the severe gastrointestinal side effects such as abdominal pain, flatulence and diarrhea that have been reported in the patients after the use of conventional drugs [11, 12], so that the identification of natural interventions through the use of plants like *Annona muricata*, becomes beneficial due to their very minimal or absence of negative side effects.

Materials and method

Collection of plant materials and preparation of extracts

Fresh parts of the plant which includes the fruit-pulp, leaf, stem-bark, and root-bark were collected from household gardens around the University of Benin, Edo state, Nigeria. The plant was identified by Dr. Bamidele of the Department of Plant Biology and Biotechnology, University of Benin, and authenticated by Professor Mc Idu of the same department. A voucher specimen number, UBHa 0205, was deposited at the Herbarium of Department of Plant Biology and Biotechnology, University of Benin. The properly washed plant samples were pulverized after drying at room temperature (about 25 °C). The pulverized plant parts were extracted by macerating 500 g of each part in methanolic, ethyl acetate and dichloromethane for 72 h after which they were filtered with a muslin cloth and the filtrate was concentrated to dryness using a rotary evaporator. The concentrated extracts were stored in an airtight container (percentage yields of methanolic, 46.30%, ethyl acetate, 31.83% and dichloromethane, 29.44%) and kept in the freezer at 4 °C until use.

In vivo studies using methanolic extracts of the various parts of *Annona muricata*

A total of 120 male albino Wistar rats weighing 190 g–220 g were bought and kept in galvanized cages in the Department of Biochemistry animal house. They were divided into six groups containing 5 rats each. They were allowed access to feed and water ad libitum on a 12 h light / 12 h dark cycle. The animals were acclimatized for 2 weeks before the commencement of the administration of the extract. Approval of the research ethics committee on guidelines and principles for the handling of animals, College of Medical Sciences, University of Benin (CMR/REC/2014/57) was adopted and strictly adhered to. The design for the administration of methanolic extracts of various parts of the plants is shown below:

Doses administered (mg/kg b.w.)	Extract Fruits	administered Leaf	Stem-bark	Root-bark
0(control)	5 rats	5 rats	5 rats	5 rats
100	5rats	5 rats	5 rats	5 rats
200	5 rats	5 rats	5 rats	5 rats
400	5 rats	5 rats	5 rats	5 rats
600	5 rats	5 rats	5 rats	5 rats
800	5 rats	5 rats	5 rats	5 rats

Administration of extracts

The extracts were administered daily with the aid of an orogastric tube. Care was taken not to inflict injuries to the rats.

Biochemical assay

At the end of the 28-day experimental period, the animals fasted overnight and blood samples were collected into plain sample bottles and allowed to clot for 30 min after which it was centrifuged at 3000 rpm for 15 min. The serum was collected separately and used for serum amylase assay. Serum amylase activity was measured using the method of Wallenfels et al. [13]. Pancreatic tissues were also excised and homogenized in ice-cold normal saline (1:4 w/v), centrifuged at 1000 g for 15 min and the supernatant was used for tissue pancreatic amylase assay.

Table 1 Dose-response characteristics of the influence of *Annona muricata* methanolic extracts on plasma amylase activity

	Dose-Response characteristics		Sigmoid plot interpolation characteristics		Hyperbola plot interpolation characteristics		Michaelis-Menten's kinetics		Straight line regression interpolation characteristics	
	LogIC50	IC50	R^2	Hill's slope	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	slope
Fruit-pulp	1.606	40.36	0.002	0.064	0.712	− 0.009	2.15×10^{-10}	0.71	0.70	0.005
Leaf	2.409	256.60	0.796	−1.274	0.242	−1.446	1.55×10^{-16}	0.61	1.92	−0.519
Stem-bark	2.478	300.50	0.888	−1.674	0.226	−1.486	1.71×10^{-16}	0.59	2.01	−0.562
Root-bark	2.687	486.20	0.267	0.963	0.847	0.656	0.66	0.85	0.53	0.056

Bmax = maximum binding capacity (U/L), *Kd* = dissociation constant (mg/dL), *Km* = Michaelis-Menten's constant (mg/dL) and *Vmax* = maximum velocity (U/L)

In vitro studies using extracts of the various parts of *Annona muricata*

α – amylase inhibition assay

Serial dilutions of the plant extracts between 0 to 200 μL were prepared by mixing with 500 μL Sodium phosphate buffer (0.02 mol/dm^3, at pH = 6.9 and 0.006 NaCl as the stabilizer), containing pancreatic alpha-amylase (0.50 mg/mL) of Porcine origin (EC 3.2.1.1). The mixtures were incubated at 37 °C for 5 min, and then 500 μL of starch solution (1 mg/100 mL in 0.02 mol/dm^3 sodium buffer at pH of 6.9 with 0.006 NaCl) was introduced into the reaction mixtures. The reaction mixtures were subsequently, incubated at 37oC for 5 min in a water bath. The reaction was then stopped using 1.0 mL dinitrosalicylic acid (DNSA) and further incubated in boiling water for 5 min. 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 positive control. When the reaction mixtures were cool, absorbance was read at 540 nm [14, 15].

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

α – glucosidase inhibition assay

Serial dilutions of the plant extracts between 0 to 200 μL were prepared by mixing with 100 μL Sodium phosphate buffer (0.1 mol/dm^3, at pH = 6.9) containing alpha-glucosidase (EC 3.2.1.2; 1.0 U/mL) and then incubating at 37 °C for 5 min. 0.05 mL of para-nitrophenyl-α-D-glucopyranoside (5.0mmole/dm^3) solution in Sodium phosphate buffer (0.1 mol/dm^3, at pH = 6.9) was added to the reaction mixture and incubated at 37 °C for 5 min. The reaction was then stopped using 1.0 mL dinitrosalicylic acid (DNSA) and further incubated in boiling water for 5 min. The reaction mixtures were allowed to cool and then absorbance read at 405 nm [16]. 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.

$$\text{Percentage α-glucosidase inhibition}\,(\%) = \frac{Aref - Asample}{Aref} \times 100.$$

Investigation of inhibitory concentrations (IC50), modes and mechanisms of inhibition of α-amylase and α-glucosidase activity (enzyme kinetics)

The mode and mechanisms of interactions between the enzymes and extracts (as well as, isolated compound) were studied using the various kinetic interpolations, viz., sigmoid (Hill's slope), a hyperbola (maximum binding capacity, *Bmax*, and dissociation constant, *Kd*), and Michaelis-Menten's (*Km* and *Vmax*). These were used to determine the IC50 of the extracts. The *Bmax* and *Kd* demonstrated the degree of binding and period of inhibition, which gave an idea of the level of efficacy of the extracts. The IC50 gave an idea of the level of potency of the extracts in inhibiting the enzymes.

Table 2 Dose-enzyme response characteristics of the influence of *Annona muricata* methanolic extracts on tissue amylase activity

	Dose-Response characteristics		Sigmoid plot interpolation characteristics		Hyperbola plot interpolation characteristics		Michaelis-Menten's kinetics		Straight line regression interpolation characteristics	
	LogIC50	IC50	R^2	Hill's slope	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	slope
Fruit-pulp	3.726	532.00	0.000	0.029	0.673	−1.11	4.25×10^{-11}	48.00	43.89	1.634
Leaf	2.469	294.60	0.826	−1.467	0.496	− 43.06	2.16×10^{-16}	44.41	232.70	−74.80
Stem-bark	2.462	289.70	0.784	−1.344	0.489	−41.62	1.64×10^{-16}	44.48	221.70	−70.41
Root-bark	2.756	569.80	0.702	−49.47	0.721	10.57	9.77×10^{-19}	2.07×10^{21}	− 117.40	67.06

Bmax = maximum binding capacity (U/L), *Kd* = dissociation constant (mg/dL), *Km* = Michaelis-Menten's constant (mg/dL) and *Vmax* = maximum velocity (U/L)

Table 3 Dose-response characteristics of the influence of *Annona muricata* methanolic extracts on α-amylase activity

	Dose-Response characteristics		Sigmoid plot interpolation characteristics		Hyperbola plot interpolation characteristics		Michaelis-Menten's kinetics		Straight line regression interpolation characteristics	
	LogIC50	IC50	R^2	Hill's slope	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	slope
Fruit-pulp	0.335	2.163	0.927	5.029	40.67	−0.038	1.603×10^{-16}	44.37	34.14	24.40
Leaf	0.266	1.846	0.966	4.832	46.41	−0.049	1.211×10^{-16}	52.17	52.02	27.95
Stem-bark	0.265	1.843	0.983	4.447	45.83	−0.052	1.212×10^{-16}	51.89	52.32	29.08
Root-bark	0.338	2.177	0.947	5.077	40.46	−0.037	1.264×10^{-16}	44.08	37.93	24.22
Acarbose	0.236	1.722	0.996	3.695	51.17	−0.079	2.220×10^{-16}	63.17	65.59	41.67

Bmax = maximum binding capacity (U/L), *Kd* = dissociation constant (mg/dL), *Km* = Michaelis-Menten's constant (mg/dL) and *Vmax* = maximum velocity (U/L)

In silico experiments

The acetogenin, 15-acetyl guanacone, which has previously been isolated from the leaf ethyl acetate extract and characterized by Agu et al. [8], was subjected to molecular docking analyses obtaining their binding affinities (Ba), in an attempt to determine whether the influence of the extracts originated from this compound as the mechanistic molecule. This was compared against a standard anti-diabetic drug, metformin.

Protein preparation and generation of 3-D structure using homology modeling

The starting structure (PDB ID: 4GL7) required for docking was retrieved from the protein data bank repository (HTTP: //www.rcsb.org). Prior to docking, water and ligand coordinates were deleted. α-Amylase and α-glucosidase were downloaded from www.pubmed.org and used to model the starting structure of the elucidated compound used in the current study. Homology modeling was done on Swiss Model Server (http://swiss-model.expasy.org). This requires one sequence of a known 3D structure with significant similarity with the target sequence. The coordinate file of template from protein data bank (PDB ID: 4GL7) was used to model the 3D structure of VEGF2.

Ligand preparation for docking

The 3D structure of the elucidated compound was built using Marvin-sketch and optimized for docking studies. The optimized ligand molecules (the compound) was docked into a refined aromatase model using "Ligand-Fit" in the Auto-Dock 4.2.

Molecular docking calculations

These were carried out through BSP-SLIM and Auto-dock. The modeled structures of VEGF2 and the elucidated compound was loaded on BSP-SLIM server and Auto-dock/Vina and all the water molecules were removed prior to the upload. BSP-SLIM is known as a blind docking method, which primarily uses the structural template match to identify putative ligand binding sites, followed by fine-tuning and ranking of ligand conformations in the binding sites through the SLIM-based shape and chemical feature comparisons [17]. Protein snapshots were taken and analyzed using PYMOL.

Statistical analysis

The data were entered into Microsoft Excel v.13, prior to analysis. The Graph Pad Prism Software, inc., (version 6.01, 2012) was used to analyze to obtain the means, SEM and IC50, using the data using the One-way

Table 4 Dose-response characteristics of the influence of *Annona muricata* ethyl acetate extracts on α-amylase activity

	Dose-Response characteristics		Sigmoid plot interpolation characteristics		Hyperbola plot interpolation characteristics		Michaelis-Menten's kinetics		Straight line regression interpolation characteristics	
	LogIC50	IC50	R^2	Hill's slope	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	slope
Fruit-pulp	0.341	2.196	1.000	4.412	44.75	−0.037	1.341×10^{-16}	48.68	48.77	50.30
Leaf	0.503	3.186	1.000	2.003	46.33	−0.056	1.151×10^{-16}	52.99	53.09	52.56
Stem-bark	0.644	4.409	1.000	1.892	45.13	−0.052	1.704×10^{-16}	51.07	51.17	51.18
Root-bark	0.477	2.997	1.000	3.247	41.72	−0.033	1.275×10^{-16}	45.00	45.08	47.18
Acarbose	0.236	1.722	1.000	1.481	51.17	−0.079	2.220×10^{-16}	63.07	65.59	41.67

Bmax = maximum binding capacity (U/L), *Kd* = dissociation constant (mg/dL), *Km* = Michaelis-Menten's constant (mg/dL) and *Vmax* = maximum velocity (U/L)

Table 5 Dose-response characteristics of the influence of *Annona muricata* dichloromethane extracts on α-amylase activity

	Dose-Response characteristics		Sigmoid plot interpolation characteristics		Hyperbola plot interpolation characteristics		Michaelis-Menten's kinetics		Straight line regression interpolation characteristics	
	LogIC50	IC50	R^2	Hill's slope	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	slope
Fruit-pulp	0.441	2.761	1.000	2.294	46.18	−0.054	1.182×10^{-16}	52.51	52.61	52.32
Leaf	0.328	2.127	1.000	3.863	46.06	−0.044	1.259×10^{-16}	51.07	51.17	51.83
Stem-bark	0.863	7.286	–	1.378	46.86	−0.061	2.098×10^{-16}	54.32	54.42	53.26
Root-bark	0.931	8.528	–	1.418	46.07	−0.059	1.113×10^{-16}	53.22	53.32	52.48
Acarbose	0.236	1.722	1.000	1.481	51.17	−0.079	2.220×10^{-16}	63.07	65.59	41.67

Bmax = maximum binding capacity (U/L), *Kd* = dissociation constant (mg/dL), *Km* = Michaelis-Menten's constant (mg/dL) and *Vmax* = maximum velocity (U/L)

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), a 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

In vivo study (Tables 1 and 2)

In vitro studies (Tables 3, 4, 5, 6, 7 and 8)

Discussion

α -Amylase catalyzes the hydrolysis of α-(1, 4)-D-glycosidic linkages of starch and other glucose polymers. Inhibitors of this enzyme could be of use in the treatment or management of diabetes. In the management of diabetic patients, the inhibition of the enzymes that are involved in the breakdown of carbohydrate e.g., α-amylase and α-glucosidase, leads to inhibition of starch hydrolysis, thus, resulting in a decreased level of glucose available for assimilation into the blood (regulating postprandial glycemic level) . Several In vitro studies have confirmed the inhibitory potential of medicinal plants on α-amylase and α-glucosidase activities and in some cases, the bioactive compounds, which presumably are responsible for this mechanism of action, have been identified. However, studies conducted in animal models are few [18] and even less abundant are the studies performed in human subjects.

In vivo investigations

In this study, methanol extracts of the different part of *Annona muricata* (fruit-pulp, leaf, stem-bark, and root-bark) were investigated (In vivo) for their potential inhibitory effects on plasma and pancreatic tissue amylase activities at varying doses of 0 to 800 mg/kg (Figs. 1 and 2) body weight in male albino Wistar rats. Amongst the parts of the plants tested, the fruit-pulp showed the highest inhibitory effect on plasma and tissue amylase activities. The root-bark showed the second highest inhibitory effect, this was followed by the leaf extract and then the stem-bark extract. As demonstrated in Table 1, the IC50 of the methanol extract of *Annona muricata* fruit-pulp on plasma amylase (40.36 mg/kg) gave a better response compared to the other part extracts. However, on the whole, the fruit-pulp and root-bark methanol extract better inhibited the plasma α-amylase as represented by their *Bmax*, *Kd* and *Vmax* values. Also from Table 2, the methanol extracts of the fruit-pulp and root-bark demonstrated a better *Bmax* compared to leaf and stem-bark extracts that demonstrated better IC50 and *Kd*. Summarily, the IC50, *Kd* and *Vmax* represented how potent the extracts were (i.e., the lower these enzyme kinetic properties, the higher the

Table 6 Dose-response characteristics of the influence of *Annona muricata* methanol extracts on α-glucosidase activity

	Dose-Response characteristics		Sigmoid plot interpolation characteristics		Hyperbola plot interpolation characteristics		Michaelis-Menten's kinetics		Straight line regression interpolation characteristics	
	LogIC50	IC50	R^2	Hill's slope	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	slope
Fruit-pulp	0.828	6.734	0.000	2.726	38.83	−0.030	1.176×10^{-16}	41.59	41.67	44.23
Leaf	0.210	1.623	0.987	5.105	51.49	−0.063	1.825×10^{-16}	60.12	60.23	58.14
Stem-bark	0.318	2.077	1.000	2.116	49.05	−0.067	1.565×10^{-16}	57.93	57.93	55.96
Root-bark	0.812	6.483	0.000	3.002	37.76	− 0.030	1.759×10^{-16}	40.44	40.44	43.14
Acarbose	0.236	1.722	1.000	1.481	51.17	−0.079	2.220×10^{-16}	63.07	63.07	59.18

Bmax = maximum binding capacity (U/L), *Kd* = dissociation constant (mg/dL), *Km* = Michaelis-Menten's constant (mg/dL) and *Vmax* = maximum velocity (U/L)

Table 7 Dose-response characteristics of the influence of *Annona muricata* ethyl acetate extracts on α-glucosidase activity

	Dose-Response characteristics		Sigmoid plot interpolation characteristics		Hyperbola plot interpolation characteristics		Michaelis-Menten's kinetics		Straight line regression interpolation characteristics	
	LogIC50	IC50	R^2	Hill's slope	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	slope
Fruit-pulp	0.235	1.717	0.999	6.582	38.83	−0.030	1.176×10^{-16}	41.59	41.67	44.23
Leaf	0.283	1.919	0.999	7.390	51.49	−0.063	1.825×10^{-16}	60.12	60.23	58.14
Stem-bark	0.286	1.930	0.993	5.534	49.05	−0.067	1.565×10^{-16}	57.93	57.93	55.96
Root-bark	0.336	2.168	0.951	5.089	37.76	−0.030	1.759×10^{-16}	40.44	40.44	43.14
Acarbose	0.236	1.722	1.000	1.481	51.17	−0.079	2.220×10^{-16}	63.07	63.07	59.18

Bmax = maximum binding capacity (U/L), *Kd* = dissociation constant (mg/dL), *Km* = Michaelis-Menten's constant (mg/dL) and *Vmax* = maximum velocity (U/L)

potencies and abilities to delay the speed of the reactions catalyzed by the enzymes), while the *Bmax* described the possible efficacies of the extracts (i.e., the higher this property, the higher the efficacies and the abilities of the moieties involved the active sites of the enzymes to bind actively, firmly and efficiently to inhibit the speed of catalysis and hydrolysis of the carbohydrate substrate).

However, since these plant extracts (especially, the fruit-pulp extract) were observed to be effective in inhibiting α-amylase and α-glucosidase activities, which is one of the mechanisms involved in controlling postprandial glycemia, these extracts may be helpful in the management of obesity and diabetes.

In vitro investigations

One of the major strategies used in the treatment and management of diabetes which has been proven to be most effective is to decrease post-prandial hyperglycemia, at the intestinal-blood interphase, by targeting the point of carbohydrate hydrolysis and mobilization into the blood. The biochemical argument is that, if this point can be effectively checked, then the major underlying cause of diabetes ab initio (hyperglycemia) can be prevented. This can be achieved by using inhibitors such as acarbose, miglitol, and voglibose [4].

However, severe gastrointestinal side effects such as abdominal pain, flatulence, and diarrhea seen in the patients [11, 12] have been linked to the use of these drugs. These side effects can be explained due to the fermentation of undigested carbohydrates by resident bacteria in the colon which they are able to reach as a result of the complete inhibition of α-amylase [4]. In addition, it is thought that some of these drugs may increase the incidence of renal tumors, hepatic injuries, acute hepatitis and pancreatitis [19].

Therefore, there is a need to identify and explore the amylase inhibitors from natural sources having fewer side effects.

Several studies have revealed that α-amylase and α-glucosidase activity have a great influence on blood glucose level and their inhibition could significantly decrease the postprandial rise in blood glucose [20]. Several inhibitors of *α*-amylase and *α*-glucosidase have been isolated from medicinal plants to serve as an alternative drug with increased potency and lesser adverse effects than existing synthetic drugs [21, 22]. α-amylase inhibitory activity has been demonstrated in a number of plant extracts including *Hibiscus sabdariffa* L. (Malvaceae) [23], *Artocarpus heterophyllus* Lam. (Moraceae) [24], *Amaranthus hypochondriacus* L. (Amaranthaceae) [25], *Punica granatum* L. (Punicaceae), *Mangifera indica* L. (Anacardiaceae) [6], *Arecae* seeds (Palmaceae) and *Corni* fruits (Cornaceae) [26].

The obtained results of the In vitro α-amylase inhibitory potential of methanolic, ethyl acetate and dichloromethane

Table 8 Dose-response characteristics of the influence of *Annona muricata* dichloromethane extracts on α-glucosidase activity

	Dose-Response characteristics		Sigmoid plot interpolation characteristics		Hyperbola plot interpolation characteristics		Michaelis-Menten's kinetics		Straight line regression interpolation characteristics	
	LogIC50	IC50	R^2	Hill's slope	*Bmax*	*Kd*	*Km*	*Vmax*	Y-intercept	slope
Fruit-pulp	1.445	27.86	–	1.068	46.18	−0.068	1.486×10^{-16}	54.79	54.89	53.00
Leaf	0.857	7.188	–	1.325	47.43	−0.061	2.000×10^{-16}	55.05	55.15	53.74
Stem-bark	1.253	17.91	–	1.144	46.69	−0.065	1.878×10^{-16}	54.79	54.88	53.25
Root-bark	0.629	4.254	1.000	1.375	48.50	−0.065	1.642×10^{-16}	57.02	57.12	55.15
Acarbose	0.236	1.722	1.000	1.481	51.17	−0.079	2.220×10^{-16}	63.07	63.07	59.18

Bmax = maximum binding capacity (U/L), *Kd* = dissociation constant (mg/dL), *Km* = Michaelis-Menten's constant (mg/dL) and *Vmax* = maximum velocity (U/L)

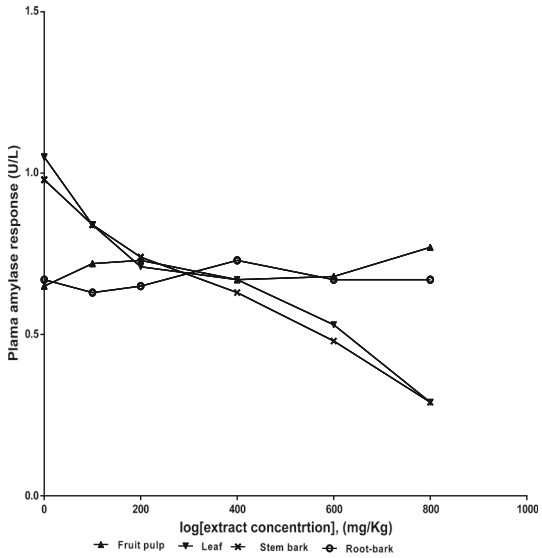

Fig. 1 Dose-response line plot of the influence of *Annona muricata* methanolic extract on plasma amylase activity

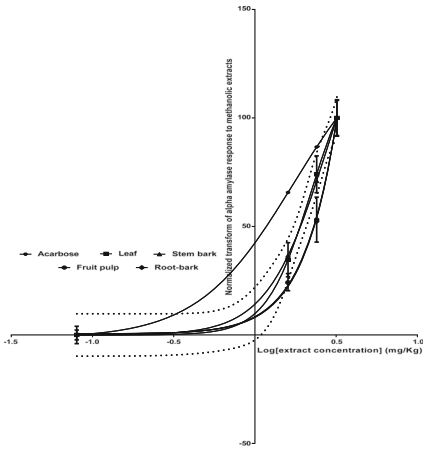

Fig. 3 Dose-response curve of α-amylase inhibition by methanolic extract of *Annona muricata* fruit-pulp, leaf, stem-bark and root-bark and acarbose (reference standard)

extracts of the fruit-pulp, leaf, stem-bark, and root-bark of *A. muricata* is shown in Figs. 3, 4, 5, 6, 7 and 8. For methanolic extract, the stem-bark (IC50 = 1.843 mg/dL) showed the highest inhibitory effect followed by leaf extract, then fruit-pulp and root-bark with IC50 of 1.846 mg/dL, 2.163 mg/dL and 2.177 mg/dL, respectively, compared to Acarbose (1.722 mg/dL). At the highest concentration of 3.20 mg/dL, the methanol extract of fruit-pulp, leaf, stem-bark and root-bark showed a 63.46% and 77.49%, 79.22% and 67.33% inhibitory effect respectively on α- amylase activity as against the 91.51% shown by Acarbose. The high total phenol content of the methanolic leaf and fruit-pulp extracts of *A. muricata* [7] may be responsible for this high inhibitory effect. This is consistent with earlier studies where α-amylase and α-glucosidase inhibitory effects of plant foods were attributed to their phenolic constituents [27–29]. The observed effects may also be due to the presence of more chemical constituents such as acetogenins, lignans (phyllanthin and hypophyllanthin), terpenes, flavonoids (quercetin, quercetin, rutin), and alkaloids in the methanolic extracts of the fruit-pulp and leaves. This observed higher inhibitory potencies of the leaf and stem-bark was corroborated by their higher *Bmax* and *Vmax* values (Table 3).

The result of the α-amylase inhibitory activities of the ethyl acetate extracts of the fruit-pulp, leaf, stem-bark and root-bark showed that the fruit-pulp and root-bark extracts gave the higher inhibitory effect with IC50 of 2.196 mg/dL and 2.997 mg/dL, respectively, compared with the standard Acarbose (IC50 = 1.722 mg/dL), and also the higher *Kd*

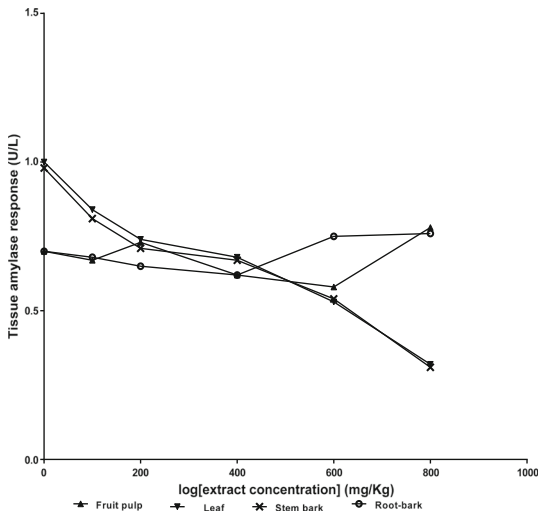

Fig. 2 Dose-response line plot of the influence of *Annona muricata* methanolic extract on tissue amylase activity

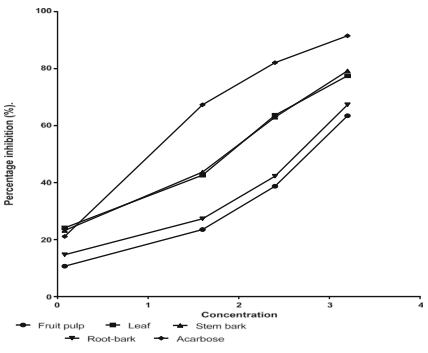

Fig. 4 Percentage inhibition of α-amylase by methanolic extract of *Annona muricata* fruit-pulp, leaf, stem-bark, root-bark and acarbose (reference standard)

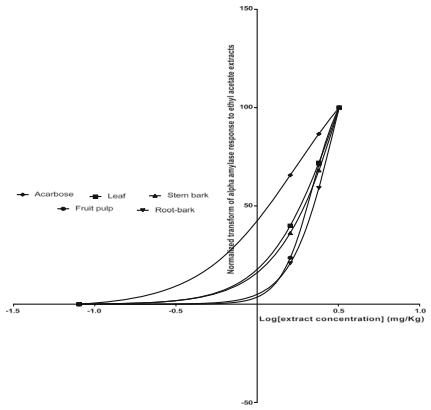

Fig. 5 Dose-response curve of α-amylase inhibition by ethyl acetate extract of *Annona muricata* fruit-pulp, leaf, stem-bark, root-bark and acarbose (reference standard)

value compared with the leaf and stem-bark extracts with the higher *Bmax* and *Vmax* (Table 4). For the dichloromethane extracts (Table 5), the leaf and fruit-pulp extracts had the highest inhibitory effects (IC50 of 2.127 mg/dL and 2.761 mg/dL, respectively; *Kd* of – 0.044 and – 0.054 mg/dL, respectively) as against that of Acarbose (IC50 of 1.722 mg/dL; *Kd* of – 0.079).

The result of the α-glucosidase inhibitory activity is demonstrated by Figs. 9, 10, 11, 12, 13 and 14. For the methanolic extracts, the leaf extract (IC50 of 1.623 mg/dL) showed the highest α-glucosidase inhibitory effect; this was closely followed by the stem-bark, root-bark and lastly fruit-pulp (IC50 of 2.077 mg/dL, 6.483 mg/dL and 6.734 mg/dL, respectively); this observed higher potency of the methanolic leaf extract against α-glucosidase is strongly corroborated by the higher *Bmax* (51.49 U/L), lower *Kd* (– 0.030 mg/dL) and higher *Vmax* (60.12 U/L) compared to the other methanolic extracts and acarbose (Table 6). For the ethyl acetate extract, the fruit-pulp gave the highest inhibitory potency against α-glucosidase activity compared to the leaf extract, then the stem-bark and root-bark; fruit-pulp had an IC50 of

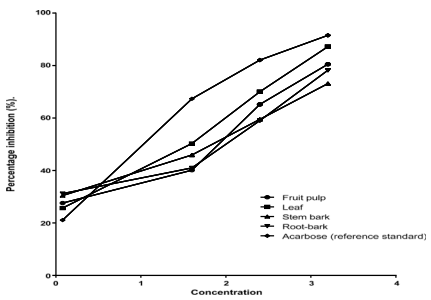

Fig. 6 Percentage inhibition of α-amylase by ethyl acetate extract of *Annona muricata* fruit-pulp, leaf, stem-bark, root-bark and acarbose (reference standard)

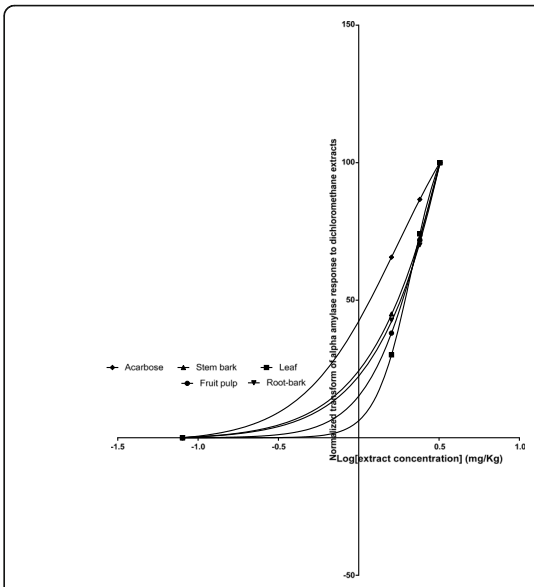

Fig. 7 Dose-response curve of α-amylase inhibition by dichloromethane extract of *Annona muricata* fruit-pulp, leaf, stem-bark, root-bark and acarbose (reference standard)

1.717 mg/dL (*Kd* of – 0.030 mg/dL) better than acarbose with an IC50 of 1.722 mg/dL (*Kd* of – 0.079 mg/dL). However, the ethyl acetate leaf extract demonstrated a better efficacy with *Bmax* of 51.49 U/L and *Vmax* of 60.12 U/L, compared to acarbose with *Bmax* of 51.17 U/L and *Vmax* of 63.07 U/L (Table 7). For the dichloromethane extract, the root-bark gave the highest α-glucosidase inhibitory effect (IC50 0f 4.254 mg/dL), closely followed by leaf extract, stem-bark and fruit-pulp with IC50 of 7.188 mg/dL, 17.91 mg/dL, and 27.86 mg/dL, respectively (Table 8). The root-bark dichloromethane extract demonstrated the highest *Bmax* (48.50 U/L) and *Vmax* (57.02 U/L), compared to the other dichloromethane extracts. These results obtained for the extracts of the different parts of *Annona muricata*

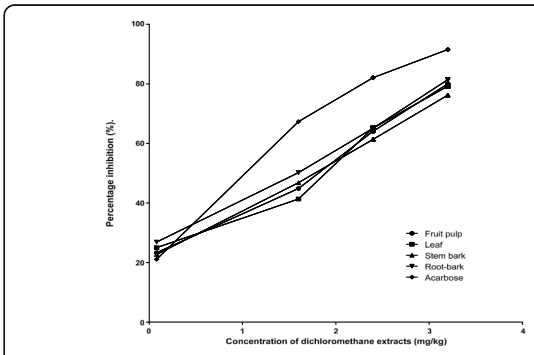

Fig. 8 Percentage inhibition of α-amylase by dichloromethane extract of *Annona muricata* fruit-pulp, leaf, stem-bark, root-bark and acarbose (reference standard)

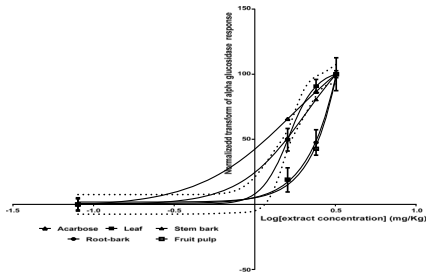

Fig. 9 Dose-response curve of α-glucosidase inhibition by methanolic extract of *Annona muricata* fruit-pulp, leaf, stem-bark, root-bark and acarbose (reference standard)

clearly shows that leaf and fruit-pulp exhibited a better inhibitory effect than the standard drug acarbose and therefore may be used as natural sources of management of post-prandial hyperglycemia. Also observed in this study was that the extracts exhibited a better α-glucosidase inhibitory activity (potency and efficacy) compared to the α-amylase activity. Earlier reports by Stephen et al. [30] on the distribution of phenolic contents, anti-diabetic potentials, anti-hypertensive properties, and anti-oxidative effects of Soursop (*Annona muricata* L.) fruit parts in vitro showed that Soursop extracts significantly inhibited α-glucosidase more than α-amylase. Kwon et al. [31] had earlier suggested that natural *α*-glucosidase inhibitors from plants had been shown to have strong inhibitory activity against *α*-glucosidase and therefore can be potentially used as an effective therapy for the management of postprandial hyperglycemia with minimal side effects.

To suggest or predict the nature of inhibition (competitive, non-competitive, uncompetitive, or mixed exhibited by the extracts) data are often analyzed by a set of techniques that linearize inherently non-linear relationships such as the Lineweaver-Burke's plot. We tried to understand the inhibition mechanism utilized against α-amylase and α-glucosidase by extracts of different parts of *the Annona muricata*. When compared

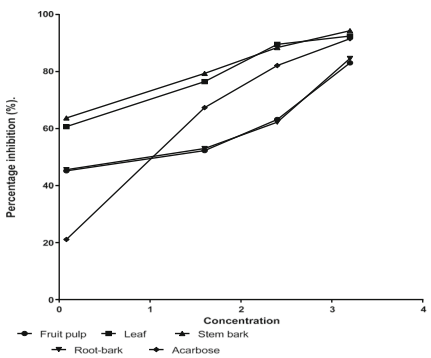

Fig. 10 Percentage inhibition of α-glucosidase by methanolic extract of *Annona muricata* fruit-pulp, leaf, stem-bark, root-bark and acarbose (reference standard)

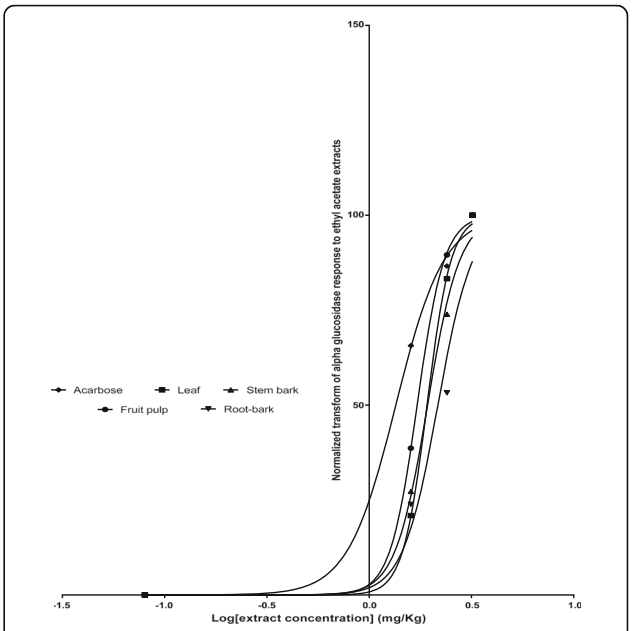

Fig. 11 Dose-response curve of α-glucosidase inhibition by ethyl acetate extract of *Annona muricata* fruit-pulp, leaf, stem-bark, root-bark and acarbose (reference standard)

with the standard drug (acarbose), all the extracts showed decreases in *Km* and *Vmax*, thus, suggesting that all extracts may have exhibited an uncompetitive inhibition pattern. This means that the active compound(s) in the extracts bind only to the enzyme-substrate complex (the inhibitor binding is only assessable when the carbohydrate binds to α-amylase and α-glucosidase) and that, the inhibition cannot be reversed by increase in the substrate concentration, i.e., intestinal carbohydrate; these confer a high level of benefits for

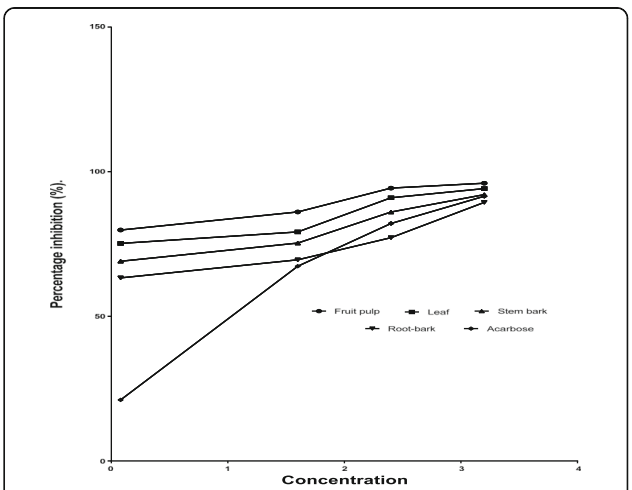

Fig. 12 Percentage inhibition of α-glucosidase by ethyl acetate extract of *Annona muricata* fruit-pulp, leaf, stem-bark, root-bark and acarbose (reference standard)

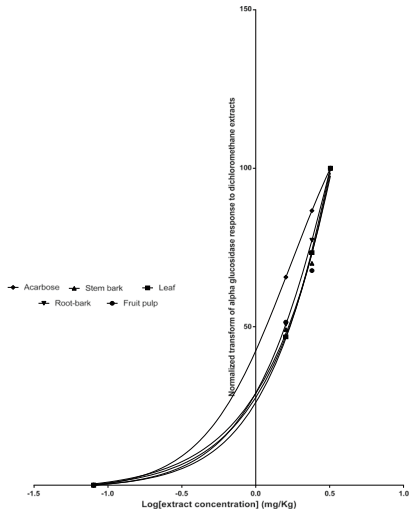

Fig. 13 Percentage inhibition of α-glucosidase by dichloromethane extracts of *Annona muricata* fruit-pulp, leaf, stem-bark, root-bark and acarbose (reference standard)

diabetics indicating that no matter the consumed concentration of carbohydrate, α-amylase, and α-glucosidase will be inhibited preventing or reducing glucose mobilization into the blood postprandial. Thus, to increase α-amylase and α-glucosidase affinity for carbohydrate, the enzyme-substrate complex must be decreased, but with the presence of the inhibitors present in *Annona muricata*, this becomes unachievable especially during the fed state of the diabetic; uncompetitive inhibition works best when the substrate concentration is high ("a light at the end of the tunnel for diabetics").

In silico investigations

In an attempt to narrow this observed potency and efficacy of the leaf and fruit-pulp extracts in uncompetitive inhibition of α-amylase and α-glucosidase, an isolated acetogenin [8] identified as 15-acetyl guanacone was subjected to molecular docking experiments to ascertain its affinity levels of these enzymes compared to a standard, metformin (Figs. 15, 16, 17, 18 and 19).

The strong α-amylase and α-glucosidase inhibitory potentials observed in the ethyl acetate extracts of fruit (and leaf) may be linked to the presence of 15-acetyl-guanacone (a compound isolated from the ethyl acetate fraction of fruit-pulp of *Annona muricata*) [8].

The molecular docking tool has been used to study the inter-relationship between a small molecule and a receptor at the atomic level, which may give the insight to characterize the behavior of small molecules in the binding site of target proteins, as well as to elucidate biochemical processes (amino acids bonded to an active site) [32]. The knowledge of the interaction between compounds and digestive enzymes may be an initial stage toward the synthesis of drug, nutraceuticals or functional foods [32].

It was observed that the binding of metformin (a standard drug) to the active site of α-amylase and α-glucosidase gave binding energies of − 4.90 kcal/mole (α-amylase) and − 5.40 kcal/mole (α-glucosidase), while the binding of 15-acetyl guanacone to the active site of α-amylase and α-glucosidase gave binding energies of − 6.80 kcal/mole and − 7.00 kcal/mole, respectively. Thus the isolated compound demonstrated a better ability to bind to the enzymes than the standard drug, metformin. These observations corroborate the observed significantly higher *Bmax* and *Kd* values observed for the fruit-pulp and leaf extracts.

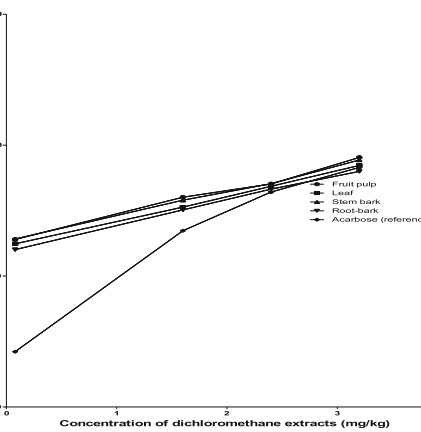

Fig. 14 Percentage inhibition of α-glucosidase by dichloromethane extract of *Annona muricata* fruit-pulp, leaf, stem-bark, root-bark and acarbose (reference standard)

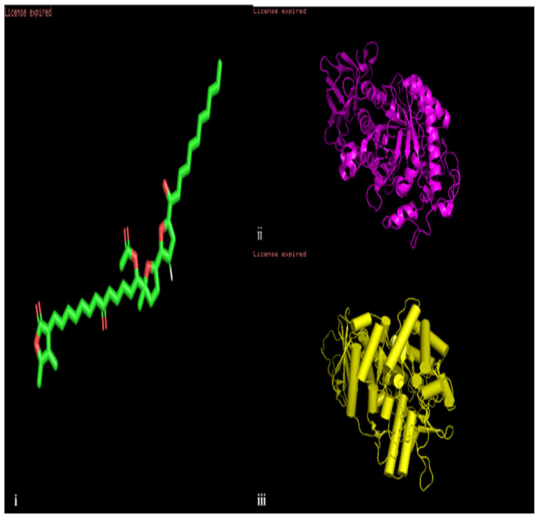

Fig. 15 2D structure of 15-acetyl guanacone (i), the 3D structure of α-amylase (ii), and 3D structure of α-glucosidase (iii)

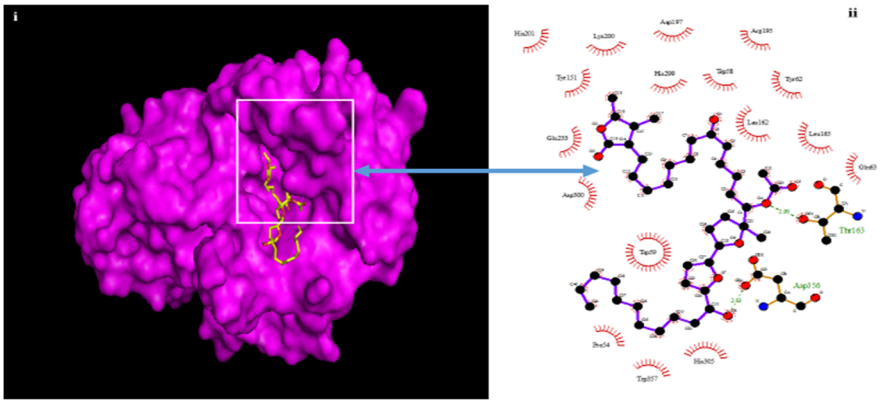

Fig. 16 The Binding pose of 15-acetyl guanacone at the active site of α-amylase with a binding energy of − 6.80 kcal/mole (i), and molecular interaction of 15-acetyl guanacone with amino acid residues within the active site of α-amylase (ii)

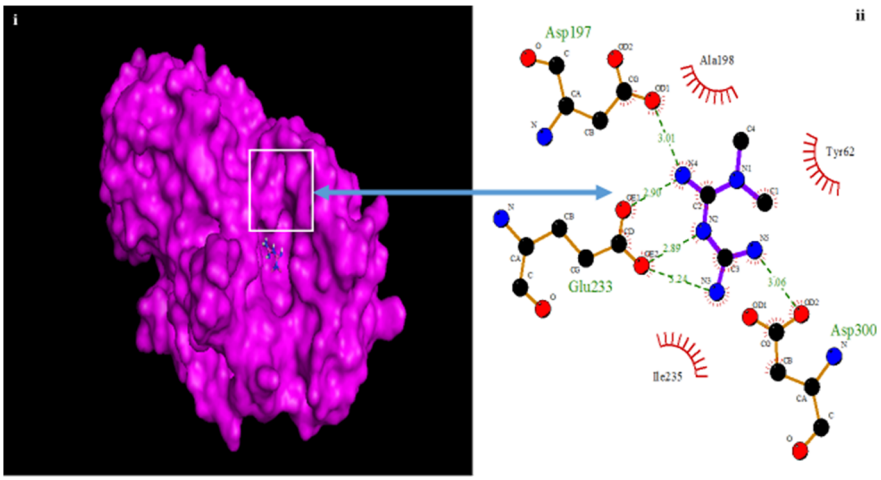

Fig. 17 The Binding pose of metformin at the active site of α-amylase with a binding energy of − 4.90 kcal/mole (i), and molecular interaction of metformin with amino acid residues within the active site of α-amylase (ii)

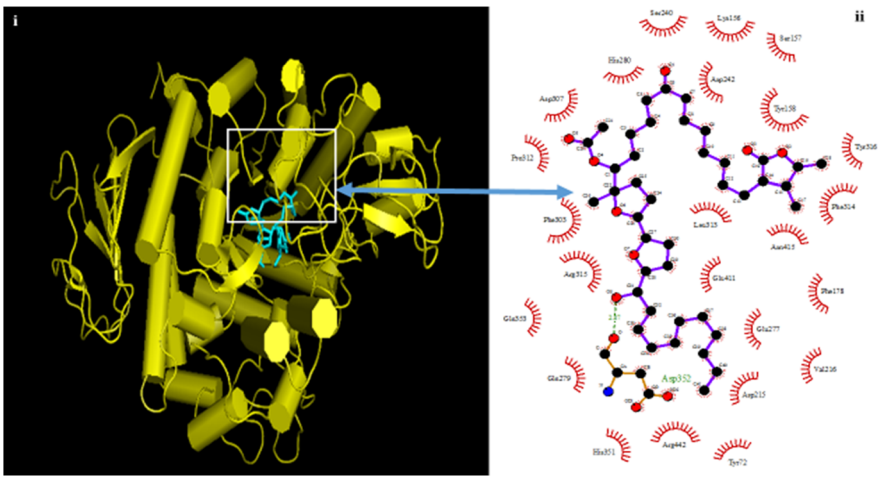

Fig. 18 The Binding pose of 15-acetyl guanacone at the active site of α-glucosidase with a binding energy of − 7.00 kcal/mole (i), and molecular interaction of 15-acetyl guanacone with amino acid residues within the active site of α-glucosidase (ii)

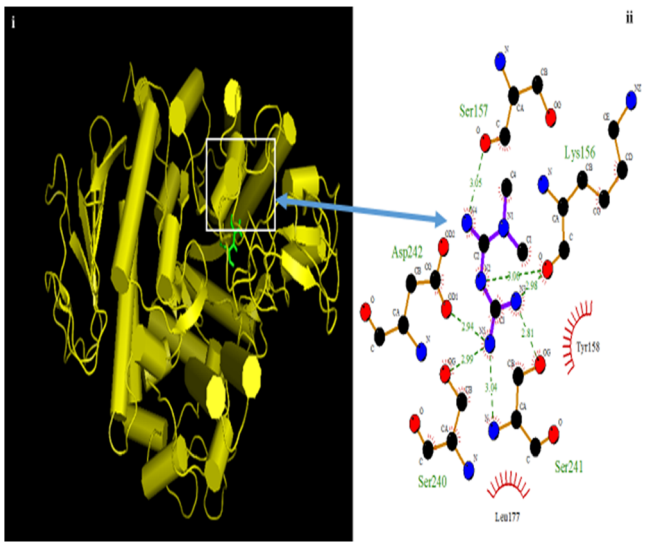

Fig. 19 The Binding pose of metformin at the active site of α-glucosidase with a binding energy of − 5.40 kcal/mole (i), and molecular interaction of metformin with amino acid residues within the active site of α-glucosidase (ii)

Conclusion

The fruit-pulp and leaf of *Annona muricata* (Soursop) demonstrated significantly high abilities to inhibit α-amylase and α-glucosidase and minimize the rate of glucose assimilation into the blood after feeding. This is evident in the obtained IC50, *Bmax*, *Kd* and *Vmax* values which further suggested that the mechanism of inhibition is that of uncompetitive inhibition, thus conferring an appreciable potency and efficacy for the plant compared to standard drugs. The research also suggested that these acetogenins may be responsible for these remarkable observations as was demonstrated by in silico studies using 15-acetyl guanacone. Thus, *Annona muricata* can be very beneficial in the treatment and management of hyperglycemia, diabetes, overweight, and obesity, etc. This suggests that better natural remedies for diabetics can be obtained from *Annona muricata* with minimal or no adverse side effects.

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Authors' contributions

KCA provided the funding and designed the research protocol, KCA and NE wrote the manuscript, ROO and DA assisted during the in vivo studies, GI, MOO and POO assisted during the in vitro studies, while OOE assisted during the in silico studies. All authors read and approved the final manuscript.

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Availability of data and materials

All the data for the research have been provided within the research paper.

Ethics approval and consent to participate

Written approval of the research ethics committee guideline principles on the handling of animals of the College of Medicine, University of Benin (CMR/REC/2014/57) was adopted and strictly adhered to.

Consent for publication

All the authors participated in developing the manuscript and grant their consent for onward publication.

Competing interests

All the authors declare no competing interests.

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