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Inhibition of α-amylase and α-glucosidase by *Acanthus montanus* Leaf Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ALO and IAO designed the study and supervised the author OAO in the extraction of the plant sample. Author MIK supervised the author OAO in the anti-diabetic analysis, performed the statistical analysis and wrote the initial draft of the manuscript. Authors AROO and IAO managed the literature searches while author IAO wrote the final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to determine the *in-vitro* anti-diabetic potentials of *Acanthus montanus*. This was done by assessing the inhibitory effect of both methanol and ethylacetate extracts of the plant on the activities of α-amylase and α-glucosidase.
Study Design: The design included extraction of *A. montanus* leaves with methanol and ethanol and subsequent evaluation of the extracts for possible hypoglycemic effect.
Place and Duration of Study: The leaves of *A. montanus* were obtained from Badagry Area of

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Lagos, Nigeria in December 2012. The plant was identified and authenticated by Dr. S. O. Shosanya of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria.
Methodology: The powdered leaves were extracted with ethylacetate and methanol separately for 24 h. The resulting infusions were decanted, filtered and evaporated in a rotary evaporator. Dried extracts were weighed and dissolved in dimethylsulphoxide (DMSO) to yield a stock solution from which lower concentrations were prepared. The inhibitory actions of both extracts against α-amylase and α-glucosidase were determined established procedures.
Results: The results showed that of the two extracts, methanol showed more inhibitory action than ethanol against both α-amylase and α-glucosidase. Lineweaver-Burk plot also depicted that the methanol extract inhibited both α-amylase and α-glucosidase in a non-competitive and competitive manner respectively.
Conclusion: It can be concluded that the hypoglycemic effect of extracts of *A. montanus* may be as a result of the inhibition of these enzymes (α-amylase and α-glucosidase). This observation may be elicited by the presence of some phytochemicals present in the extracts.

Keywords: Acanthus montanus; α-amylase;α-glucosidase; antidiabetic.

1. INTRODUCTION

Diabetes is a metabolic disease which is as old as mankind and its incidence is considered to be high (4–5%) all over the world [1]. It is also a major cause of disability and hospitalization and results in significant financial burden [2]. It is considered a "modern day epidemic" and is rightly recognized as a global public health issue. The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 552 million people likely to be diabetic by the year 2035 as against 382 million estimated in 2014 [3]. There is need for the discovery of anti-diabetic agents from natural sources due to limited efficacy and serious side effects associated with synthetic drugs which include hypoglycaemia, chronic tissue damage and death [4].

Acanthus montanus (Nees) T. Anderson (Acanthaceae) is a small shrub with sparse branches and soft stems. It is commonly known as Mountain Thistle or Bears Breech and is believed to have originated from West Africa [5]. It is used in traditional medicine in the Southern part of Nigeria under the names; 'Mafowokan omomi', 'Agamsoso' and 'Agameru'. It is also used in different parts of Africa in the treatment of various illnesses such as cough, epilepsy, pain, dysmenorrhoea, hypertension, false labour, syphilis, skin infections and diabetes mellitus [6,7]. The pharmacological properties of this plant which include hepatoprotective [8], tocolytic [9], anti-inflammatory, antimicrobial and immunological properties [5] have been reported by several authors. Nana et al. [10] reported the safety of this plant in pregnant rats as well as their offspring while Djami et al. [11] also stated its tolerance in female rats at concentration greater than 1000 mg/kg body weight. Although, there is a study on the hypoglycemic potential of the methanolic extract of this plant [7], there is dirt of information on the possible mechanism by which it elicits its hypoglycemic action.

It is well known that any anti-diabetic agent can act by one or more of the following mechanisms; pancreatic β-cells regeneration, insulin secretion, mimicking the action of insulin, inhibition of carbohydrate metabolizing enzymes as well as slowing down the absorption of sugars from the gut [12]. The aim of this study was to assess the effect of leaf extracts of Nigerian grown *A. montanus* on diabetes-related enzymes (α-amylase and α-glucosidase) as well as its mode of inhibition of these enzymes. In our previously study, the anti-diabetic potentials of some other medicinal plants grown in Nigeria have been reported [13].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Porcine pancreatic α-amylase, rat intestinal α-glucosidase and paranitrophenyl-glucopyranoside were products of Sigma-Adrich Co., St Louis, USA while starch soluble (extra pure) was obtained from J. T. Baker Inc., Phillipsburg, USA. Other chemicals and reagents were of analytical grade and the water used was glass-distilled.

2.2 Plant Sample

The leaves of *Acanthus montanus* were obtained from Badagry Area of Lagos, Nigeria, in December 2012. The plant sample was identified and authenticated by the taxonomist; Dr. S. O.

Shosanya of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria and voucher specimen (FHI 109720) was deposited in the Institute's herbarium. The leaves were air-dried, pulverized and kept in airtight plastic bags.

2.2.1 Preparation of extracts

The powdered leaves were divided into two portions of 10 g each and these were extracted with ethylacetate and methanol respectively. The mixtures were left to steep in covered conical flasks for 24 h, the flasks were shaken at interval and kept still to allow the plant material to settled at the bottom of the flask. The resulting infusions were decanted, filtered and evaporated in a rotary evaporator (Cole Parmer SB 1100, Shangai, China). Dried extracts were weighed and dissolved in dimethylsulphoxide (DMSO) to yield a stock solution from which lower concentrations were prepared. All extracts were stored at 4ºC prior to analysis.

2.3 α-Amylase Inhibitory Assay

This assay was carried out using a modified procedure of McCue and Shetty [14]. A total of 250 µL of extract was placed in a test tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution was added. This solution was pre-incubated at 25ºC for 10 min, after which 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then incubated at 25ºC for 10 min. The reaction was terminated by adding 500 µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL of distilled water and the absorbance was measured at 540 nm using a spectrophotometer (Spectrumlab S23A, Globe Medical England). The control and blank solutions were prepared using the same procedure by replacing the extract with DMSO and distilled water respectively. The α-amylase inhibitory activity was calculated as percentage inhibition as follows;

% Inhibition = $[(\Delta A_{control}-\Delta A_{extract})/A\Delta_{control}] \times 100$

where $\Delta A_{control} = A_{control} - A_{blank}$ and $\Delta A_{extract} = A_{extract} - A_{blank}$

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

2.3.1 Mode of α-amylase inhibition

The mode of inhibition of α-amylase by the extract was conducted using the most potent extract according to the modified method described by Ali et al. [15]. Briefly, 250 µL of the (5 mg/mL) extract was pre-incubated with 250 µL of α-amylase solution for 10 min at 25ºC in one set of tubes. In another set of tubes α-amylase was pre-incubated with 250 µL of phosphate buffer (pH 6.9). Then, 250 µL of starch solution at increasing concentrations (0.3–5.0 mg/mL) was added to both sets of reaction mixtures to enable the reaction to commenced. The mixture was then incubated for 10 min at 25ºC, and then boiled for 5 min after addition of 500 µL of DNS to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a maltose standard curve and converted to reaction velocities. A double reciprocal (Lineweaver-Burk) plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted to determine the mode of inhibition.

2.4 α-Glucosidase Inhibitory Assay

The effect of the plant extracts on the activity of α-glucosidase was determined according to the method described previously by Kim et al. [16]. The substrate solution, p-nitropheynyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer (pH 6.9). Also, 100 µL of α-glucosidase (E.C. 3.2.1.20) was pre-incubated with 50 µL of the different concentrations of the extracts for 10 min. Then, 50 µL of 3.0 mM pNPG dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37ºC for 20 min and stopped by adding 2 mL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow coloured para-nitrophenol released from pNPG at 405 nm. The control and blank were prepared using the same procedure by replacing the extract with DMSO and distilled water respectively. Percentage inhibition was calculated thus;

% Inhibition = $[(\Delta A_{control}-\Delta A_{extract})/A\Delta_{control}] \times 100$

where $\Delta A_{control} = A_{control} - A_{blank}$ and $\Delta A_{extract} = A_{extract} - A_{blank}$

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

2.4.1 Mode of α-glucosidase inhibition

The mode of inhibition of α-glucosidase by the extracts was determined using the extract with the lowest IC_{50} according to the modified method described by Ali et al. [15]. Briefly, 50 µL of the (5 mg/mL) extract was pre-incubated with 100 µL of α-glucosidase solution for 10 min at 25ºC in one set of tubes. In another set of tubes, α-glucosidase was pre-incubated with 50 µL of phosphate buffer (pH 6.9). Thereafter 50 µL of pNPG at increasing concentrations (0.63 - 2.0 mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at 25ºC after which 500 µL of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a para-nitrophenol standard curve and converted to reaction velocities. A double reciprocal (Lineweaver-Burk) plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted to determine the mode of inhibition of the enzyme.

2.5 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, USA). The data were analysed by one way analysis of variance (ANOVA) followed by Bonferroni test. All the results were expressed as mean ± SEM for triplicate determinations.

3. RESULTS AND DISCUSSION

Fig. 1 showed the percentage inhibition of α-amylase by methanol and ethylacetate extracts of *A. montanus*. There were no significant differences between the extracts at low concentrations (0.32 - 0.63 mg/mL). However at higher concentrations, the ethylacetate extract exhibited significantly higher percentage inhibition of the enzyme. The higher percentage inhibition of the enzyme displayed by the ethylacetate extract was corroborated by its lower IC_{50} value compared to that of methanol extract (Table 1).

However, the Lineweaver-Burk plot of the mode of inhibition of α-amylase by the methanol extract of this plant showed that it is a non-competitive inhibitor of the enzyme (Fig. 2).

The percentage of inhibition of α-glucosidase by the extracts of *A. montanus* is shown in Fig 3. At all concentrations tested, methanol extract exhibited significantly higher (P=.05) percentage inhibition of this enzyme compared to ethylacetate extract. However, the inhibition of the enzyme by both extract was dose-dependent. This is supported by the lower IC_{50} value for ethanol extract compared to methanol extract. Kinetic analysis of the mode of inhibition of the enzyme with the aid of Lineweaver-Burk plot showed that the ethanol extract inhibited the enzyme in a competitive manner (Fig. 4).

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

Extracts	IC_{50} (mg/mL)	
	α-Amylase	α-Glucosidase
Methanol	2.87±0.02[a]	1.65±0.02[a]
Ethylacetate	2.39±0.04[b]	7.10±0.15[b]
Acarbose	2.60±0.01[a]	0.63±0.00[c]

The management of hyperglycemia is the hallmark of treatment in diabetes. A convenient therapeutic approach for decreasing postprandial hyperglycemia is to retard the digestion and absorption of carbohydrates. This is done through the inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract [17]. Though, synthetic α-glucosidase inhibitors such as acarbose and voglibose are presently in use but are bedeviled by undesirable side effects such as nausea, hypoglycaemia, diarrhoea and liver failure [14], which necessitated this study.

The present study showed that the ethylacetate extract of *A. montanus* produced stronger inhibition of α-amylase than methanol extract. However, methanol extract will be more suitable to be used as anti-diabetic agent because of its mild inhibition of the enzymes, possessing higher IC_{50} than ethylacetate extract and acarbose. Previous studies have shown that any prospective anti-diabetic agent should be a mild inhibitor of α-amylase so as to prevent the drawback of synthetic drugs (like acarbose), which occur due to the excessive inhibition of the enzyme resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon [18,19]. Therefore, the Lineweaver-Burk plot of the inhibition depicted that methanol extract of *A. motanus* inhibited the enzyme in a non-competitive manner. This implies that the active components in the extract binds to a site other than the active site of the enzyme and combines with either free enzyme or the enzyme-substrate complex, possibly interfering with the action of both [20].

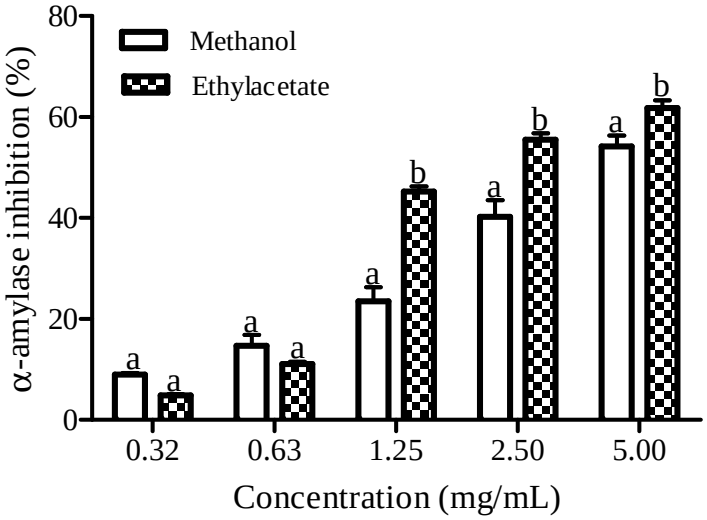

Fig. 1. Inhibitory potency of *A. montanus* leaf extracts against α-amylase activity. The values are expressed as means ± SEM of triplicate determinations. Means not sharing a common letter at the same concentration are significantly different (*P* = .05)

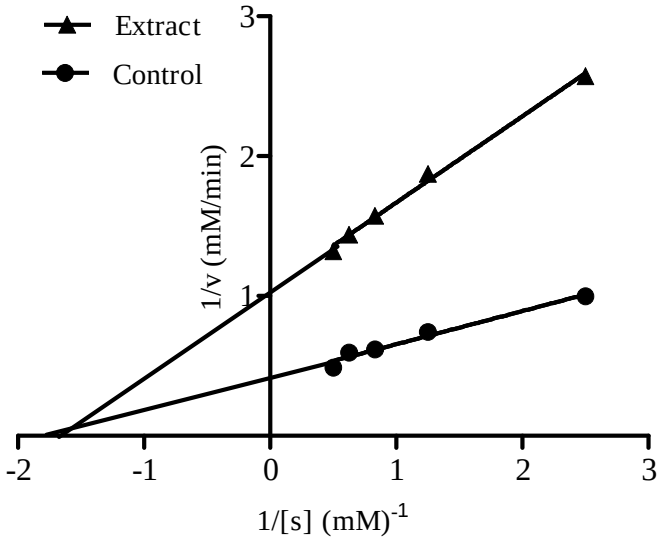

Fig. 2. Mode of inhibition of α-amylase by methanol extract of *A. montanus*

The stronger inhibition of α-glucosidase by the methanol extract of *A. montanus* at all concentrations tested compared to ethylacetate extract, culminated into having low IC_{50} which is also desirable of a good antidiabetic drug. The competitive inhibition of the enzyme by methanol extract of *A. montanus* suggest that the inhibitory component(s) in the plant binds reversibly to the active site of the enzyme and occupies it in a mutually exclusive manner with the substrate [21,22]. This may due to structural similarity between the inhibitor and the normal substrate (disaccharides), thereby slowing down the production of glucose and reducing hyperglycemia.

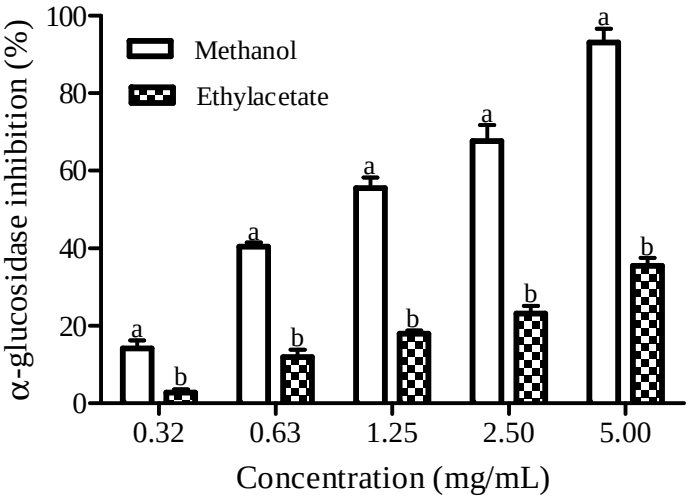

Fig. 3. Inhibitory potency of *A. montanus* leaf extracts against α-glucosidase activity. The values are expressed as means ± SEM of triplicate determinations. Means not sharing a common letter at the same concentrations are significantly different (*P* = .05)

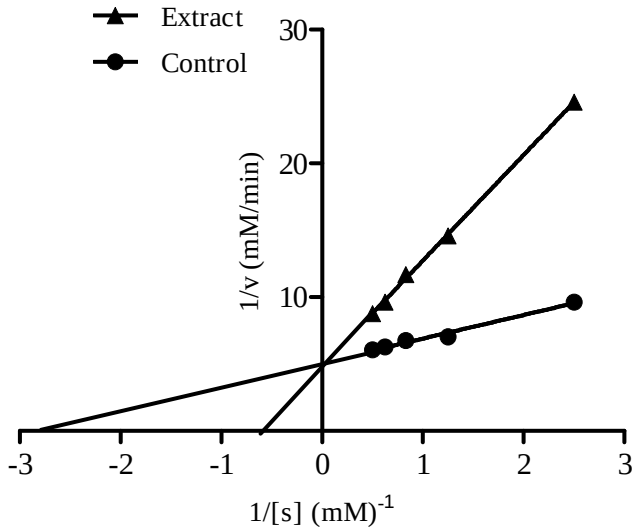

Fig. 4. Mode of inhibition of α-glucosidase by ethylacetate extract of *A. montanus*

Tannins are phenolic compounds which have been found to induce phosphorylation of insulin receptors and translocation of glucose transporter, thereby helping in the reduction of blood glucose level [23]. Studies have also shown the antioxidant and antidiabetic properties of saponins from different medicinal plants [24,25]. Therefore, it is probable that the inhibitory effect of *A. montanus* extracts on the activities of α-amylase and α-glucosidase may be due to the presence of these kinds of phytochemicals present in the extracts.

4. CONCLUSION

This study showed that methanol extract of *A. montanus* is a more potent inhibitor of α-amylase and α-glucosidase than ethylacetate extract. However, this methanol extract proved to be a non-competitive and competitive inhibitor of both

α-amylase and α-glucosidase respectively. It can therefore be concluded that the hypoglycemic action of this plant may be due to the inhibition these diabetes-related enzymes studied.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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