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Ethanol Extract of *Aristolochia repens* Mill. Stem Inhibits the Activities of some Enzymes Targeted in the Treatment of Diabetes Mellitus

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ABSTRACT

Herbs are frequently used as alternative medicines in managing diabetes and associated complications in Nigeria with little or no scientific information on the antidiabetic efficacy of some of these plants. *Aristolochia repens* is one of such herbs used by herbalists in South-Western Nigeria in managing diabetes and associated problems. This study investigated the antidiabetic capacity of the stem extracts of *A repens*. A powdered sample of the dried plant was extracted in water as well as ethanol, and the α-amylase, α-glucosidase, and sorbitol dehydrogenase inhibitory activity of the concentrated extracts were determined spectrophotometrically. The ethanol extract of *A. repens* exhibited more effective α-amylase, *α*-glucosidase, and sorbitol dehydrogenase inhibitory potential with IC_{50} values of 33.25, 13.03, and 26.28 mg/mL respectively. Kinetic studies indicated that ethanol extract inhibited *α*-amylase uncompetitively, while α-glucosidase and sorbitol dehydrogenase were inhibited non-competitively. It can be concluded that the use of *A. repens* for managing diabetes mellitus and some of its complications could be due to its ability to inhibit these targeted enzymes usually inhibited in managing diabetes mellitus.

Keywords: Diabetes mellitus, *Aristolochia repens*, Sorbitol dehydrogenase, Enzyme inhibition.

Introduction

A major target in curtailing diabetes mellitus and decreasing the risk of developing complications as a result of diabetes is by regulating glycemic control.[1] Lifestyle modification, change in nutritional habits as well as administration of oral hypoglycemic drugs to regulate glucose uptake are some orthodox methods in managing diabetes.[2] One of the classes of drugs approved for the management of diabetes mellitus are inhibitors of enzymes that take part in the hydrolysis of carbohydrates in the gastrointestinal tract. The accompanying effect of carbohydrate-digestion inhibition leads to reduced glucose absorption in the small intestine resulting in lower postprandial blood glucose levels.[3] Inhibitors of sorbitol dehydrogenase, a major enzyme of the polyol pathway, is also an effective target in the treatment of diabetic complications like eye and kidney damage.[4]

The probe for the discovery of antidiabetic agents from medicinal plants and foods is a veritable strategy that is needed to tackle the widespread nature of diabetes mellitus worldwide.[5] This is because current hypoglycemic drugs are associated with many impediments ranging from limited efficacy and numerous side effects such as hypoglycemia, weight gain, and chronic tissue damage.[6] Consequent upon this, several medicinal plants with folkloric usage as antidiabetics are being verified with the aid of scientific procedures. This is because there is little or no scientific proof on the antidiabetic efficacy of many of these plants.[7] These plants include *Blighia sapida*, *Morus alba*, *Spondias monbin*, *Treculia africana*, and *Aristolochia repens*.

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Aristolochia repens which is known as "Ako-igun" by the "Yoruba" speaking people of Nigeria and "Dumandutsee" in the Northern part of Nigeria, is a climbing bushy plant originally native to tropical America but now cultivated in most countries in West Africa.[8] The plant is used in the management of various ailments in many regions of the world.[9] The root is used in Southwestern Nigeria to treat asthma and diarrhea,[10] while the stem is used for the management of diabetes mellitus and complications associated with it.[11] Phytochemical analysis revealed the presence of different types of aristolochic acid as the main bioactive compound in this plant. These include aristolochic acid I, aristolochic acid II, and aristolochic acid IV.[12]

Despite the various reports on this plant used in treating several diseases, there is a scarcity of information on its hypoglycemic property as well as modulatory potential on relevant enzymes linked to diabetes mellitus. This study evaluated α-amylase, α-glucosidase, and sorbitol dehydrogenase inhibitory activity of *A. repens* stem extracts. The mode of inhibition of the targeted enzymes was also determined, to understand the probable mechanisms of action of the extracts in eliciting their antidiabetic activities.

Materials and Methods

Plant material

The stem of *A. repens* was procured from the traditional medicine store in Idumota, Lagos, Nigeria. The sample was confirmed by Dr. A. B. Kadiri, a taxonomist at the University of Lagos, Nigeria, and the voucher sample with reference number LUH 8376 was deposited in the herbarium.

Chemicals and reagents

Experimental grade *A. oryzae* α-amylase, *S. cerevisiae* α-glucosidase, D-sorbitol, NADH, sorbitol dehydrogenase, Tris buffer, Acarbose, maltose, and soluble starch (extra pure) were purchased from Sigma-Aldrich Co, St Louis, USA.

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Preparation of plant extracts

Sample of the stem of *A. repens* was cut, washed, dried, and ground to powder. The powdered sample was shared into two; 5 g of one portion was infused in 100 mL ethanol and the other in 100 mL of distilled water for 48 h. Both extracts were filtered and concentrated appropriately using a rotary evaporator and or freeze dryer. Concentrated samples were dissolved in 1% dimethylsulfoxide (DMSO) to make a stock solution.

α-amylase inhibitory assay

The modified procedure of Mccue and Shetty[13] was used to evaluate the inhibition of α-amylase by graded concentration (125 – 1000 µg/mL) of the extracts. Distilled water substituted the extracts in a control solution following the same procedure.

Mode of α-amylase inhibition

The ethanol extract was selected to assess the kinetics of inhibition of the enzyme using a modified method of Ali *et al*.[14] due to the lowered IC_{50} value.

α-glucosidase inhibitory assay

The method of Kim *et al*.[15] was used to evaluate α-glucosidase inhibition by graded concentration (125 – 1000 µg/mL) of the extracts.

Mode of α-glucosidase inhibition

The ethanol extract was selected to assess the kinetics of inhibition of the enzyme as described by Ali *et al*.[14] due to the lowered IC_{50} value.

Sorbitol dehydrogenase inhibitory assay

Inhibition of sorbitol dehydrogenase activity by different concentrations (125 – 1000 µg/mL) of *A. repens* extracts was evaluated using the modified procedure of Lindstad *et al*.[16]

Mode of sorbitol dehydrogenase inhibition

The mode of inhibition of sorbitol dehydrogenase by the ethanol extract was assessed as described by Gerlach *et al* [17] with slight modification.

Calculation of percentage inhibition of enzymes

The percentages inhibitions of the three enzymes were determined from the equation:

$$\% \text{ Inhibition} = \frac{[(\text{Abscontrol} - \text{Absextract})}{\text{Abscontro}} \text{ x } 100$$

Where: $Abs_{control}$ is the absorbance of control while $Abs_{extract}$ is the absorbance of extracts.

Calculation of IC_{50} values of enzymes' inhibition

The concentration of extract or standard that inhibited 50% of enzyme activities (IC_{50}) was evaluated graphically by plotting percentage inhibition of enzymes against the concentration of extract using Microsoft Excel (2010).

Determination of kinetics of inhibition of the enzymes

The modes of inhibition of the enzymes by the ethanol extract were determined from a double-reciprocal plot using Michaelis Menten kinetics.[18]

Statistical analysis

All results were expressed as mean ± standard error mean (SEM) of triplicate determinations. Data were analyzed using analysis of variance (ANOVA) followed by Bonferroni test, with aid of GraphPad Prism 5.

Results and Discussion

We investigated the *in-vitro* inhibitory properties of *Aristolochia repens* stem on the activities of some enzymes linked to diabetes mellitus. These are α-amylase, α-glucosidase, and sorbitol dehydrogenase. Figures 1 and 2 expresses the level of *α*-amylase and glucosidase inhibition by extracts of *Aristolochia repens* stem at varying concentrations (125-1000 µg/mL). Pancreatic *α*-amylase catalyzes the digestion of starch to disaccharides and oligosaccharides, while α-glucosidase secreted in the intestine is responsible for the hydrolysis of disaccharides to glucose.[19]

Inhibiting these enzymes would obstruct the hydrolysis of carbohydrates in the gastrointestinal tract, thereby ameliorating hyperglycemia. The inhibitory effect of the water extracts on the activity of α-amylase was not significantly different at most concentrations when compared with the ethanol extracts. However, the differences in percentage inhibition of α-glucosidase by both extracts at 125 and 250 µg/mL were significantly different ($p < 0.05$), but at higher concentrations, the percentage inhibitions were similar.

Sorbitol dehydrogenase is an enzyme in the polyol pathway. The pathway is an alternate route for glucose metabolism in tissues that can take-up glucose without the assistance of insulin.[20] Figure 3 expresses the level of inhibition of the activity of sorbitol dehydrogenase by the extracts of *A. repens*. The pathway is responsible for the conversion of glucose to sorbitol by aldose reductase and then sorbitol dehydrogenase metabolized the sorbitol to fructose.[4] Elevation of fructose through the activity of sorbitol dehydrogenase may promote the production of advanced glycation end-products, which contributes to complications in diabetes. Thus, natural agents that could inhibit the activity of enzymes of the polyol pathway could help mediate diabetic complications.[21]

The inhibitory activity of both aqueous and ethanol extracts was dose-dependent with the aqueous extracts exhibiting higher percentage inhibition compared to the ethanol extracts, although there was no statistical difference ($p > 0.05$) at all concentrations tested. Studies have implicated the polyol pathway in complications of diabetes mellitus.[21]

The major characteristic of diabetes mellitus is an abnormal increase in postprandial blood glucose (hyperglycemia) and some complications including retinopathy, neuropathy, and nephropathy. This is effectively managed by regulating the amount of glucose that gets into the blood by modulating enzymes involved in carbohydrates digestion in the digestive tract.[22] Delaying the digestion of carbohydrates is a convenient clinical treatment for ameliorating postprandial hyperglycemia. This is achieved by inhibiting α-amylase and α-glucosidase activities in the gastrointestinal tract.[23]

This study compared the inhibitory effects of the aqueous and ethanol extracts of *A. repens* on the activities of *α*-amylase, *α*-glucosidase, and sorbitol dehydrogenase. The IC_{50} (concentration of extract responsible for inhibiting 50% enzyme activity) values of the extract that inhibited α-amylase, α-glucosidase, and sorbitol dehydrogenase activity was determined (Table 1). Ethanol extract of the plant had significantly lowered IC_{50} for α-amylase (33.25 mg/mL) and α-glucosidase (13.03 mg/mL), compared to aqueous extract and acarbose. The IC_{50} for the inhibition of sorbitol dehydrogenase is similar for both extracts of the plant. It is evident from this result that the ethanol extract of *A. repens* displayed a better inhibition compared to the aqueous extract and the standard (acarbose). This is because the lower the IC_{50} the better the inhibition of the enzymes.

The IC_{50} value for the inhibition of sorbitol dehydrogenase by ethanol extract is similar to the aqueous extract, it depicts that the ethanol extracts of the plant effectively inhibited the three enzymes. This may indicate that ethanol can extract desirable components of the plants than water. The potent inhibition of α-amylase and α-glucosidase by the ethanol extract of *A. repens* stem suggests that the extract might cause a reduction in the breakdown of carbohydrates to glucose, thereby ameliorating hyperglycemia experienced in diabetes.[24] Similarly, the inhibition of sorbitol dehydrogenase activity by the ethanol extract may mitigate the formation of advanced glycation end-products, thereby contributing to the prevention and or palliation of diabetic complications.[25]

Figures 4, 5, and 6 showed the Double reciprocal plot of the mechanisms by which ethanol extract of *A. repens* inhibits α-amylase,

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α-glucosidase, and sorbitol dehydrogenase. These plots depicted that α-amylase was inhibited in an uncompetitive manner by the ethanol extract, while α-glucosidase and sorbitol dehydrogenase were inhibited non-competitively by the extract.

The uncompetitive inhibition of α-amylase by *A. repens* is an indication that the active compound from the plant binds only the enzyme-substrate (ES) complex to interfere with the enzyme function.[26] Conversely, the non-competitive inhibition of α-glucosidase and sorbitol dehydrogenase suggest that the bioactive compounds from the extract do not contend with the substrate in binding to the active site of the enzyme, but interact with the enzyme at a separate site to inhibit the conversion of disaccharides to monosaccharides.[27,28] The benefits of the non-competitive and uncompetitive type of inhibitors as drugs are that they decrease the turnover rate of the enzyme rather than disturbing the extent of substrate binding to the enzyme. Instead of the reaction of the enzyme being stopped, it is reduced and the reaction cannot be reversed by increasing the concentration of the substrates.[29] This is additional proof that ethanol extract of *A. repens* could be a veritable source of hypoglycemic agents.

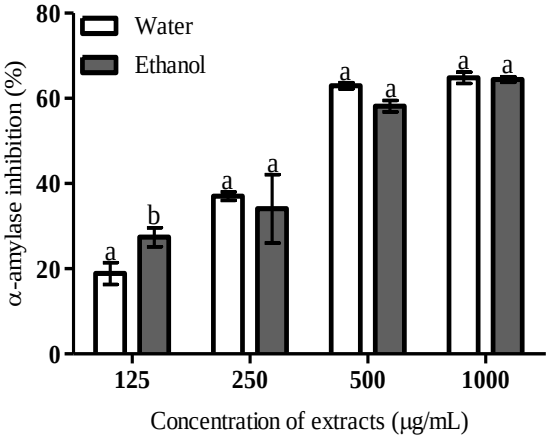

Figure 1: Inhibitory potential of aqueous and ethanol extracts of *Aristolochia repens* stem on α-amylase activity.
Values presented are mean ± SEM of experiments carried out in triplicates. Values not sharing the same alphabet at the same concentration are significantly different ($p < 0.05$).

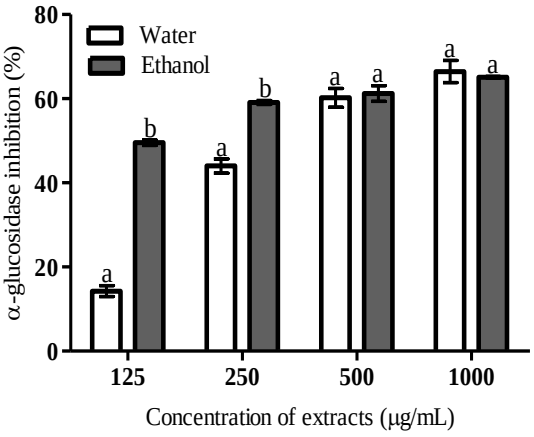

Figure 2: Inhibitory potential of aqueous and ethanol extracts of *Aristolochia repens* stem on α-glucosidase activity.
Values presented are mean ± SEM of experiments carried out in triplicate. Values not sharing the same alphabet at the same concentration are significantly different ($p < 0.05$).

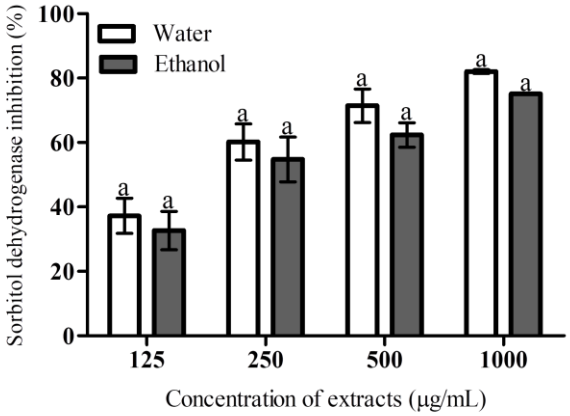

Figure 3: Inhibitory potential of aqueous and ethanol extracts of *Aristolochia repens* stem on sorbitol dehydrogenase activity.
Values presented are mean ± SEM of experiments carried out in triplicate. Values not sharing the same alphabet at the same concentration are significantly different ($p < 0.05$).

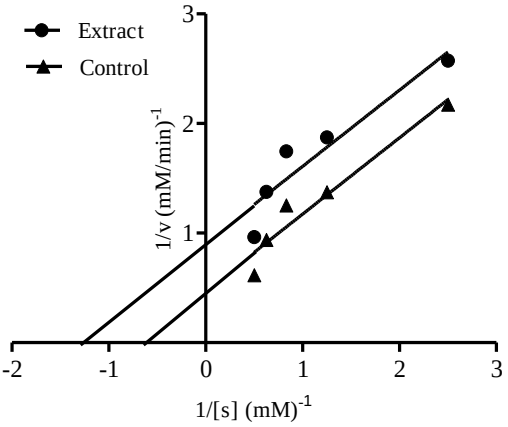

Figure 4: Double reciprocal plot of the inhibition of α-amylase activity by ethanol extract of *A. repens* stem

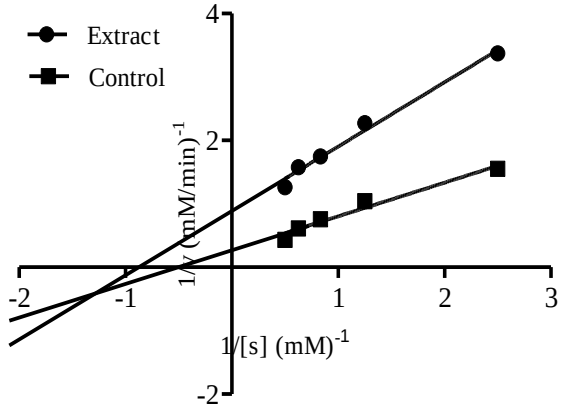

Figure 5: Double reciprocal plot of the inhibition of α-glucosidase activity by ethanol extract of *A. repens* stem

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Table 1: IC_{50} values for the inhibition of α-amylase, α-glucosidase, and sorbitol dehydrogenase by aqueous and ethanol extracts of *Aristolochia repens* stem.

Extract	IC_{50} (mg/mL)		
	α-amylase	α-glucosidase	Sorbitol dehydrogenase
Water extract	46.78 ± 0.78[a]	40.06 ± 1.23[a]	25.87 ± 1.12[a]
Ethanol extract	33.25 ± 1.05[b]	13.03 ± 0.36[b]	26.28 ± 0.50[a]
Acarbose	67.5 ± 2.56[c]	129.0 ± 4.05[c]	Not determined

Values presented are mean ± SEM of experiments carried out in triplicate. Columns with different alphabetical superscripts are significantly ($p < 0.05$) different.

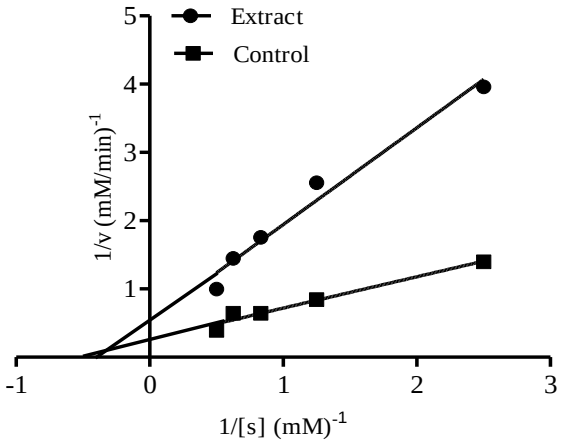

Figure 6: Double reciprocal plot of the inhibition of sorbitol dehydrogenase activity by ethanol extract of *A. repens* stem

Conclusion

This study demonstrated that *α*-amylase, α-glucosidase, and sorbitol dehydrogenase activities could be inhibited by aqueous and ethanol extracts of *Aristolochia repens*. However, ethanol extract displayed better inhibitory activities of all the three enzymes tested. The ethanol extract of *A. repens* inhibited the activity of α-amylase in an uncompetitive manner while it displayed non-competitive inhibition towards both α-glucosidase and sorbitol dehydrogenase. Based on this study, it can be concluded that *Aristolochia repens* could be a source of a drug candidate for the treatment of diabetes and its associated complications. The inhibitory property of the ethanol extract of *A. repens* on the activities of diabetes-related enzymes may be due to the presence of bioactive compounds such as aristolochic acid I, II, and IV in the plants.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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