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Investigating the Antidiabetic Potential of Anthocleista Grandiflora Methanol Extract in Alloxan-Induced Diabetic Albino Rats

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Abstract

Diabetes mellitus is a serious global health problem characterized by hyperglycemia which is caused by absolute or relative deficiency of insulin or by insulin resistance at the cellular level. The aim of this study was to investigate the antidiabetic potential of the methanol stem bark extract of *A. grandiflora* in albino rats. The phytochemical analysis, alpha amylase and alpha glucosidase inhibitory activities as well as glucose concentrations were determined using standard methods. Twenty albino rats were randomly divided into five groups of four rats each in which group 1 was the normal control, group2 was induced with diabetes and not treated, group 3 was induced and treated with glibenclamide, group 4 and 5 were induced and treated with the extract (100 and 200mgk-1 respectively), all for 15 days and blood glucose concentration was checked on three days intervals using a glucometer by cutting the tip of the tail. Result shows presence of phenolic, carbohydrates and tannins in large amounts, flavonoids in moderate amounts while steroids, saponins, terpenes, anthraquinones and cardiac glycosides, were absent. The standard drugs Glibenclamide (98.06%) and Metformin (96.77%) showed higher percentage alpha amylase inhibition activities compared to the *A. grandiflora* extract. The 5.0mg concentration of the sample showed (79.53 %) inhibition. There was significant ($p<0.05$) inhibitory activity of alpha-glucosidase at 30.0mg/ml of the sample (98.70 %) while the standard drugs (Glibenclamide) shown (84.88%) inhibitory activity and Metformin showed (88.22%) inhibitory activity against alpha glucosidase. There was significant ($p<0.05$) reduction of serum glucose across the treated groups while (group 2) showed a sustained diabetic status in all the rats, confirming the antidiabetic properties of the methanol extract.

Keywords: *Anthocleista Grandiflora*; Diabetes Mellitus; Alpha-Amylase; Alpha-Glucosidase; Glucose

Abbreviations: WHO: World Health Organisation; pNPG: P-Nitrophenyl Glucopyranoside; DNS: Dinitro-Salicyclic Acid.

Introduction

Background Information

Hyperglycemia, a severe worldwide health issue associated with diabetes mellitus, is brought on by either a cellular level of insulin resistance or an absolute or relative insulin deficit [1]. According to estimates, this disease affects 25% of the global population [2]. Although oral hypoglycemic treatments have made significant progress in treating diabetes, there is still a need to find new medications due to the drawbacks of the synthetic pharmaceuticals now on the market [3]. For example, the synthetic antidiabetic drugs

that are already on the market cause major adverse effects include hepatorenal abnormalities and hypoglycemia [4]. Because medicinal herbs are relatively safe and inexpensive, they are important in the conventional management of the illness. Research on several of these medicinal plants has revealed that they block the generation of glucose in the liver and the absorption of glucose from the gut, increase insulin secretion, and improve glucose uptake by adipose or muscular tissues [5]. Nevertheless, despite recommendations from the World Health Organisation (WHO), only few of these medicinal plants have been subjected to scientific investigation. We examined A-grandiflora stem bark for its antidiabetic potentials as part of our quest for safer and more effective antidiabetic principles. Plants commonly found in tropical Africa, Cameroon, Sudan, and Sierra Leone are A-grandiflora, members of the Gentianaceae family. In particular, it is found in marshy places close to streams and enclosed woods in Northern, Western, and Eastern Nigeria [6].The stem bark and leaves together are used as an antidiabetic and anti-inflammatory medication, as well as for wound treatment [7]. The hypoglycemic action of the plant's root extract has been shown by Abuh FY, et al. [8] in both hyperglycemic and normal rabbits. The plant's hypoglycemic potential was recently shown by the Anthocleista's amylase inhibitory action in an in vitro model [9]. Plant stem bark has been used to identify steroidal chemicals and derivatives of xanthones [10]. Although the antibacterial potential of these ingredients was assessed, none of them were linked to the plant's hypoglycemic effect. Therefore, the purpose of the current study was to examine the antidiabetic potential of *A. grandiflora's* methanol stem bark in rats in order to give scientific evidence for its application in the treatment and management of diabetes.

Materials

Plant Material

Fresh bark of *A. grandiflora* was gotten from a village in Keana LGA, Nasarawa State, by the help of a local Farmer. It was transported using a poly bag to the laboratory in the department of Biochemistry and Molecular biology, Nasarawa State University, Keffi.

Experimental Animal

The experimental animal used were adult albino rats of both sexes weighing between 100 – 210g They were obtained from the department of Zoology, University of Jos, Plateau State, Nigeria and transported in cages to the animal house in the department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi. They were acclimatized for seven (7) days before commencement of the experiment.

Instrument/Equipment

The instrument and equipments used in the study include; Water bath (Dk-420, WOM), Spectrophotometer (752N, China), Rotary Evaporator (RE300, China), AcuCheck Glucometer, Analytical balance (PA214, OHAUS corporation, USA), Weigh balance (G&G Electronic Scale, capacity 300g ×0.01g).

Chemical/Reagents

All the chemicals/reagents used were of analytical grade and products of Sigma Aldrich (USA) and BDH (UK).

Methods

Processing of Plant Material

The Fresh bark of A-grandiflora was dried at room temperature in the Laboratory. After which it was grounded to coarse powder with the aid of mortar and pestle, and also an electronic blender till a fine powdered sample was obtained.

Extraction of the Plant Material

The powdered sample (400g) was macerated in 2 liters of methanol for 72hrs with occasional stirring to facilitate the extraction process. The mixture was then filtered using a muslin cloth followed by what man no. 4 filter paper and, and the filtrate concentrated using a rotary evaporator to get the crude extract at 65°C and stored in containers at about 4°C until use.

Qualitative Phytochemical Analysis

Phytochemicals screening of the methanol bark extract of *A. grandiflora* was carried out to determine the class of secondary metabolites present using standard procedure according to Harbone JB [11]. Active principles tested included tannins, saponins, alkaloids, flavonoids, glycosides, phenols, terpenoids, cardiac glycosides, Anthraquinones, steroids.

- **Test for Tannins:** 0.2mg of sample was added to 2ml of 5% ferric chloride. Formation of dark blue or greenish black indicates the presence of tannins.
- **Test for Saponins:** 0.2mg of sample was added to 2ml of distilled water with continuous shaking in a graduated cylinder for 15mins. The formation of 1cm layer of form indicates the presence of saponins.
- **Test for Alkaloids:** 2ml of concentrated hydrochloric acid was added to 0.2mg of sample. Few drops of Mayer's reagent were added. Presence of green or white color precipitate indicates the presence of alkaloids.

- **Test for Flavonoids:** 1ml of 2N sodium hydroxide was added to 0.2mg of sample. Appearance of yellow color indicates the presence of flavonoids.
- **Test for Glycosides:** 3ml of chloroform and 10% ammonia solution was added to 0.2mg of sample. Formation of pink color indicates presence of glycosides.
- **Test for Phenols:** 2ml of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the sample. Formation of blue or green color indicates the presence of phenols.
- **Test for Terpenoids:** 0.2mg of the sample was treated with 2ml of chloroform and concentrated sulfuric acid. Formation of red brown color at the interface indicates the presence of terpenoids.
- **Test for Cardiac Glycosides:** 2ml of glacial acetic acid and few drops of ferric chloride was added to 0.2ml of the sample. Formation of brown ring at the interface indicates the presence of cardiac glycosides.
- **Test of Anthraquinones:** Few drops of 10% ammonia solution were added to 0.2mg of sample. Appearance of pink color precipitate indicates the presence of Anthraquinones.
- **Test for Steroids:** To 0.2mg of sample, equal volume of chloroform was added and a few drops of concentrated sulfuric acid added appearance of brown ring indicates the presence of steroids and appearance of bluish ring will indicates the presence of steroids.
- **Test for Anthracyanine:** 0.2mg of the sample was added to 1ml 2N sodium hydroxide and heated for 5mins at 100°C. Formation of bluish green color indicates the presence of anthocyanin.

Determination of Alpha Amylase Inhibitory Activity

This assay was carried out using a modified procedure of Mccue P, et al. [12]. A total of 250μL of extract (5.0-90mg/mL) was placed in a tube and 250 μL of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5mg/mL) was added. This solution was pre-incubated at 25°C for 10 min, after which 250 μL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at 250C for 10min. The reaction was terminated by adding 500 μL of Dinitro-salicyclic acid (DNS) reagent. The tubes were then incubated in boiling water for 5min and cooled to room temperature. The reaction mixture was diluted with 5mL distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition:

%Inhibition = [Abs control – Abs extracts / Abs control] × 100

Determination of Alpha Glucosidase Inhibitory Activity

The effect of the plant extracts on α-glucosidase activity was determined according to the method described by Kim YM, et al. [13], using α-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenyl glucopyranoside (pNPG) was prepared in 20mM phosphate buffer, and pH 6.9. 100 μL of α-glucosidase (1.0U/mL) was preincubated with 50 μL of the different concentrations of the extracts (acetone, ethanol, and water) for 10min. Then 50 μL of 3.0mM (pNPG) as a substrate dissolved in 20mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 20min and stopped by adding 2mL of 0.1MNa_2CO_3.Theα-glycosidase activity was determined by measuring the yellow-colored para-nitrophenol released from pNPG at 405 nm. The results were expressed as percentage of the blank control. Percentage inhibition is calculated as

% Inhibition = [Abs control – Abs extract / Abs control] × 100]

Experimental Design for *In Vivo* Diabetes Study

A total of twenty (20) rats were randomly grouped into 5 groups and treated thus:

- Group 1: Control
- Group 2: induced but not treated.
- Group 3: induced and treated with standard drug (2.5mgkg-1 glibenclamide).
- Group 4: induced and treated with 100mg kg-1 of the chloroform extract.
- Group 5: induced and treated with 200mg kg-1 of the chloroform extract.

Induction of Diabetes with Alloxan

The animals were given a single intraperitoneal injection of 150 mg/kg of alloxan monohydrate in isotonic saline and allowed to stabilize for 3 days before blood glucose levels were measured on three days interval for fifteen (15) days. Blood was drawn from a small cut in the tail and blood glucose level was measured with the aid of a one touch glucometer. Glibenclamide (2.5 mg/kg) was used as reference standard.

Statistical Analysis

The data obtained was analyzed using analysis of variance (ANOVA) in SPSS version 23.0. The result was presented as mean ± standard deviations. The level of significance was

further tested using LSD and Duncan. The acceptable level of significance was set at $p<0.05$.

Results

Qualitative Phytochemical Compositions of *A. grandiflora* Methanol Extract

Table 1 is a presentation of the result of the qualitative phytochemical composition of *A. grandiflora* Methanol extract. Result shows presence of phenolic, carbohydrates and tannins in large amounts, flavonoids in moderate amounts while steroids, saponins, terpenes, anthraquinones and cardiac glycosides, were absent.

Phytochemical	Inference
Phenolics	+++
Steroids	-
Saponins	-
Terpens	-
Anthroquinones	-
Cardiac glycosides	-
Flavanoids	++
Carbohydrates	+++
Tannins	+++

Table 1: Qualitative Phytochemical compositions of *A. grandiflora* Methanol extract.

Key: Presence of phytochemical is denoted by ++ = present in moderate amount +++ = present in high amount, - = absent.

Alpha Amylase Inhibitory Activities of *A. grandiflora* Methanol Extract

As shown in Table 2, below, the standard drugs Glibenclamide (98.06%) and Metformin (96.77%) showed higher percentage alpha amylase inhibition activities compared to the *A. grandiflora* extract. The 5.0mg concentration of the sample showed (79.53 %) inhibition.

Conc.(mg/ml)	% Inhibition of Alpha Analysis
5	79.53 ± 2.51^{C}
25	66.00 ± 14.06^{Bc}
30	64.43 ± 5.73^{Bc}
70	18.03 ± 22.18^{a}
90	38.33 ± 28.63^{ab}
Glibenclamide	98.06 ±15.66
Metformin	96.77 ±9.280

Table 2: Alpha Amylase Inhibitory activities of *A. grandiflora* Methanol Extract.

Result are presented as Mean ± SD (N = 3) Mean value with different letters are considered statistically significant at $P < 0.05$

Alpha Glucosidase Inhibitory Activities of *A. grandiflora* Methanol Extract

Table 3 is a presentation of the results of alpha glucosidase inhibitory activities of *A. grandiflora* methanol extract. The result reveals a significantly ($p<0.05$) higher amount of glucosidase inhibitory activity at 30.0mg/ml of the sample the sample (98.70 %) while the standard drugs (Glibenclamide) shown (84.88%) inhibitory activity and Metformin shown (88.22%) inhibitory activity against alpha glucosidase.

Conc. (mg/ml)	% Inhibition of Alpha Glucosides
5	47.70 ± 14.98^{b}
25	40.27 ± 10.27^{B}
30	98.70 ± 2.06^{C}
70	7.57 ± 6.27^{a}
90	20.73 ± 8.34^{a}
Glibenclamide	84.88± 9.887
Metformin	88.22± 10.631

Table 3: Alpha glycosidase Inhibitory activities of *A. grandiflora* Methanol Extract.
Result are presented as Mean ± SD (N=3) Mean value with different letters are considered statistically significant at $P < 0.05$.

Blood Glucose Concentrations in Alloxan – Induced Diabetes Treated with Methanol Bark Extract of *A. grandiflora*

As shown in Table 4, there was significant ($p<0.05$) reduction of serum glucose across the treated groups while (group 2) showed a sustained diabetic status in all the rats, confirming the antidiabetic properties of the chloroform fraction.

Groups	Day1	Day3	Day6	Day9	Day12	Day15
Group 1	173.00±0.00^{a}	168.00±0.00^{b}	148.00±0.00^{b}	101.00±0.00^{ab}	145.00±0.00^{ab}	110.00±0.00a
Group 2	437.00±78.51d	470.50±42.14^{d}	339.00±106.80^{d}	366.0±51.96^{d}	384.00±55.42^{d}	313.0±66.97^{d}
Group 3	348.00±27.10^{c}	157.00±19.18^{b}	135.00±30.75^{b}	131.25±27.95^{b}	155.75±42.35^{b}	140.5±13.30^{a}
Group 4	325.00 ± 0.00^{b}	89.00 ± 0.00^{a}	199.00 ± 0.00^{a}	135.00 ± 0.00^{a}	172.00 ± 0.00^{a}	164.00 ± 0.00^{b}
Group 5	465.00 ± 0.00^{C}	105.00 ± 0.00^{a}	52.77 ± 0.00^{C}	361.00 ± 0.00^{b}	141.00 ± 0.00^{a}	151.00 ± 0.00^{b}

Table 4: Blood glucose concentration in alloxan – induced diabetes treated with methanol bark extract of *A. grandiflora*.

Result are presented as Mean ± SD (N=4) mean values with different letters as superscripts are considered to be statistically significant at $P < 0.05$. Group 1 Normal control, Group 2 Negative control, Group 3 Induced treated with standard drugs, Group 4 Induced treated with plant extract 100mg/kg^{-1}, Group 5, Induced treated with plant extract 200mg/kg^{-1}.

Discussion

The development of diabetes mellitus and its consequences, including micro and macrovascular illnesses, have been linked to postprandial hyperglycemia, which has been identified as an independent risk factor [14,15]. Thus, it is hypothesised that managing postprandial hyperglycemia is critical to managing diabetes and averting cardiovascular problems [16]. Therefore, compounds that inhibit amylase and glycosidase have been studied, and some of them have been developed as drugs to treat diabetes mellitus. Inhibiting glucose uptake in the intestines may help diabetic patients to control the blood glucose level in the postprandial state [17]. Reductions in postprandial glycemic levels and the entire spectrum of postprandial glucose levels are the primary advantages linked to aglucosidase inhibitors [18]. However, the negative side effects of synthetic aglucosidase inhibitors, including diarrhoea, cramping in the abdomen, and flatulence, are well-documented. Furthermore, some of them may raise the risk of acute hepatitis, major hepatic injury, and kidney tumours [19-21]. As a result, natural sources of aglucosidase inhibitors may become necessary. The precise mode of action underlying Anthocleista djalonensis's hypoglycemic activity has not been determined. However, the extracts' capacity to suppress alpha amylase activity may have contributed to the rats' reported anti-diabetic effect.

In this study, the phytochemical composition of *A. grandiflora* methanol extract was determined, the result shown that the extract is rich in phytochemical compound. Such as the presence of tannins, flavonoid, phenolics, carbohydrate fehling's in large amounts. While protein, starch, gardiac glycoside, anthroquinone, steroid, saponins terpanoid, where absent. These compounds have been reported to have health promoting effects. Flavanoid and fehling's shows positive, which is an indication to possess a number of beneficial medication including anti-cancer, antioxidant, anti-inflammatory and antiviral properties.

Also, in the determination of the inhibitory activity of *A. grandiflora* methanol bark fraction on the activity of α-amylase, the methanol fraction showed potent inhibition of α-amylase of 79.53% inhibitory activity, compared to both glibenclamide and metformin (standard drugs) that shows similar inhibitory percentage of 98.06% and 96.77% respectively. This result is in agreement to Kazeem MI, et al. [22], which indicated that the inhibition of α-amylase could be due to abnormal fermentation by bacteria of the carbon source. As for the α-glucosidase, the methanol bark fraction the result revealed a significant difference of ($p<0.05$) high amount of glucosidase activity at 30.0 mg/ml of the sample (98.70%) while the standards howed 84.88 % inhibitory activity and metformin showed about 88.22% inhibitory activity against -glucosidase.

Conclusion

The study demonstrated the hypoglycemic properties of *A. grandiflora* methanol bark extract in alloxan-induced diabetic rats. The significant reduction in blood glucose levels indicates its potential as an anti-diabetic agent. The findings align with the traditional use of *A. grandiflora* in folk medicine for treating diabetes. This study provides scientific validation for its effectiveness in managing hyperglycemia as indicated by the high percentage alpha amylase and alpha glucosidase inhibitory activities coupled with reduced glucose concentration in blood after fifteen days of treatment.

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