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Antihyperglycemic Activity of Anthocleista Vogelli And Anthocleista Djalonensis (Gentianaceae) Leaves Methanol Extracts and Fractions in Alloxan-induced Diabetic Rats

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Abstract

The present study was undertaken in other to scientifically appraise the ethnomedical use of Anthocleista vogelli Planch and Anthocleista djalonensis A. Chev (Gentianaceae) leaves in the management of diabetes. The antidiabetic property of A. vogelli and A. djalonensis leaves methanol extracts and fractions wereexaminedin alloxan-induced diabetic rats using acute and chronic studies. In the acute study, hypoglycaemia activity was determined every 30 minutes for 3 hours after oral administration of methanol extracts of A. vogelli and A. djalonensisleaves separately at a dose of 1g/Kg body suspended in 1.5 ml distilled water. Whereas, in the case of chronic study hypoglycaemia activity was estimated daily for seven days after oral administration of leaves methanol extract of A. vogelli and its three fractions (methanol, hexane and chlorofom) andmethanol extract of A. djalonensis and its three fractions (methanol, hexane and chlorofom) separately at a dose of 1 g/Kg body weightsuspended in 1.5 ml distilled water. Glibenclamide (2.5 mg/Kg) was used as reference drug. Significant reductions in blood glucose level wereobserved in both acute and chronic studies. In the

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acute study,the methanol extracts of A. vogelli and A. djalonensis leaves significantly ($p<0.05$) reduced the fasting blood glucose levelsby 15.71% and 13.00% respectively. More so, in the chronic study, theleaves methanol extract ofA. vogelli, its chlorofom fraction andtheleaves methanol extract of A. djalonensis significantly ($p<0.05$) reduced the fasting blood glucose levels by 60.56%, 74.34% and 72.26% respectively. The blood glucose lowering effects of the leaves methanol extract of A. vogelli, its chlorofom fraction andtheleaves methanol extract of A. djalonensiswere stronger than that of glibenclamide used as a positive control which reduced the fasting blood glucose level by 45.41% in the chronic study. The findings of this experimental study indicate that A. vogelli and A. djalonensis leaves possess antidiabetic properties and that lend pharmacological support to folkloric use of these plants in the control of diabetes in some parts of Nigeria.

Keywords: *Anthocleista vogelli*; *Anthocleista djalonensis;* Antihyperglycemic activity;Alloxan-induced diabetic ratsand Glibenclamide

Introduction

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of population all over the world (Burke *et al.*, 2003). Its worldwide frequency is expected to continue to grow by 6% per annum, potentially reaching 200-300 million cases in 2010 (Kahn and Shecher, 19990; Moller, 2001), this is because sulfonylureas and other antidiabetes therapies have limited efficacy and various side effects and thus searching for new classes of compounds is essential to overcome these problems (Banskota *et al.*, 2004).

The vegetation and floral biodiversity of Africa provides African traditional health practitioners with an impressive pool of "natural pharmacy" from which plants are selected as remedies and/or ingredients to prepare herbal medicines for plethora of human and veterinary disorder (Ojewole, 2006). Since time immemorial, patients with non-insulin dependent diabetes mellitus have been treated orally with a variety of plant extracts (Roa, 2006). *Anthocleistadjalonensis* and *Anthocleista vogelli* (Gentianaceae), commonly known as candelabrum or cabbage tree are two of such

plants. *A. djalonensis* and *A. vogelli* are commonly found in West Africa secondary forest and forest outliers respectively (Keay, 1989). Herbalist claim a high percentage of "cure" in their patients treated with *A. djalonensis* (Onocha *et al.*, 1995). Abo *et al.*, (2000) also attach the same use to *A. vogelli* in an ethnobotanical survey of antidiabetic plants in some local government in Ogun State, Nigeria. *A. vogelli* is also used locally for the treatment of snake and scorpion bites (Keay, 1989). *A. djalonensis* is known for its antipyretic, analgesic and purgarive actions (Oliver- Bever, 1986).Because of the uses of these plants in traditional medicine as antidiabetic agents, attempts were made in this present study for systematic evaluation of their crude extracts and fractions for antidiabetic activity.

Materials and methods

Plant material

Anthocleista vogelli and *A. djalonensis* leaves were collected fresh from Ilisan, Ogun state, Nigeria. The plants were identified and authenticated at the Forestry Herbarium Ibadan, Nigeria. Voucher specimens (FHI 107911 and FHI 107912) were deposited in this herbarium. The leaves were shade- dried at 25ÚC and ground with an electrical mill. The powdered leaves were extracted immediately.

Preparation of the plants extract and fraction

Methanol extract

A suspension of 300g of the dried leaves was macerated in 600 ml of methanol for 72 hours at room temperature. The residue was removed by filtration and the solvent removed from the extract at low temperature (< 40ÚC), under reduced pressure.

Hexane fraction of the extract

A suspension of extract (40g) was prepared in 25% methanol and poured into a separating funnel. This was first partitioned with equal volume of hexane twice. The hexane fractions were collected and combined. The mother liquor was reserved for subsequent partition while thesolvent was removed from the hexane fractions at low temperature (< 40ÚC) under reduced pressure.

Chloroform and methanol fractions of the extract

The mother liquor reservedwas repartitioned with an equal volume of chloroform twice in a separating funnel. The chloroform fraction was removed and the methanol fraction separated. Solvent was removed from the fractions at low temperature (< 40ÚC) under reduced pressure.

Aliquot portions of the crude extracts and fractions were weighed and dissolved separately in distilled water for use on each day of this experiment (Ojewole, 2006).

Experimental animals

Male and female Swiss albino diabetic and normal rats were used for the study. They were divided randomly into sixteen groups (group I - XVI) of three each and were fed with standard pellet diet and water *ad libitum*. They were kept in clean and dry cages and maintained in a well-ventilated animal house. Rats were rendered diabetic by single intraperitoneal injections of freshly prepared aqueous solution of alloxan monohydrate at a dose of 150 mg/Kg body weight. The fasting blood glucose level was determined 72hours post-alloxan injection. Blood glucose level more than 137 mg/dl was taken as the criteria for diabetes.

Evaluation of antihyperglycemic activity

Acute study

Animals in Groups I - V were fasted for 16hrs. prior to drug administration allowing access only to water.Initially,blood samples were collected by repeated needle puncture of the tail tip vein of each rat before treatments. Thereafter, blood glucose concentrations were determined by means of Bayer's Glucometer Elite(R) and compatible blood glucose test strips.Groups I and II were given crude methanol extract of *A. vogelli* and *A. djalonensis*orally respectively at the dose of 1g/kg body weight suspended in 1.5ml distilled water. Group III received glibenclamide at a dose of 2.5mg/Kg body weight and serve as standard. Group IV served as diabetic control which received appropriate volume of distilled water orally. Group V consist of normal (nondiabetic) rats which received no treatment. Blood samples were collected from each rat at 30, 60, 90, 120 and 180

mins. after treatments and blood glucose concentrations determined.

Chronic study

Animals in Groups VI - XVI were fasted for 16hrs. prior to drug administration allowing access only to water.The zero hour blood glucose concentration of animals wasdeterminedbefore treatments. Group VI was given *A. vogelli* leaves methanol extract at a dose of 1 g/Kg body weight dissolved in 1.5 ml distilled water. Groups VII- IX received the three fractions of leaves methanol extractof*A. vogelli* viz methanol, hexane and chloroform fractions respectively at a dose of 1 g/Kg body weight in 1.5 ml distilled water. The treatment procedure used for Groups VI- IX was repeated with leaves methanol extract of*A. djalonensis* and its fractions for Groups X- XIII respectively at a dose of 1 g/Kg body weight in 1.5 ml distilled water.Group XIV received glibenclamide at a dose of 2.5 mg/Kg body weight and serve as standard. Groups XV and XVI served as diabetic control and normal rats respectively, these two groups received no treatment. All the test extracts were administered orally twice daily (at 0 hr. and 6th hr.)and this was continued for seven days while the blood glucose concentrations were determined daily at the 8th hour.

Data analysis

Data are expressed as mean ± standard error of mean (S.E.M.). Themean blood glucose concentrations were subjected to analysis of variance (ANOVA) and post-hoc comparison were done using Duncan's *t*-test. Values of *P*d" 0.05 were taken to imply statistical significance.

Results

The significant % maximal reduction of blood glucose levels for *A. vogelli* and *A. djalonensis* leaves methanol extracts was 15.78% (3hrs. and P< 0.05) and 13.00% (3hrs. and P< 0.05) respectively. Whereas, glibenclamide produced significant % maximal reduction of 25.52% (3hrs. and P< 0.05) compared to the control group. The normal rats maintained ideal blood glucose concentrations while there was a progressive increase in the blood glucose concentrations of the control group within 3 hours (Table 1).

Chronic administration of methanol extract of *A. vogelli* (MEAv),methanol extract of *A. djalonensis* (MEAd), and chloroform fraction of *A. vogelli* extract (CFAvE) to alloxan–induced diabetic rats for seven days produced significant reduction in blood glucose concentration. Significant reduction was observed from the first day bychloroform fraction of *A. vogelli* extract (CFAvE) at a dose of 1 g/Kg body weight (Table 2). At the end ofday 7methanol extract of *A. vogelli* (MEAv), chloroform fraction of *A. vogelli* extract (CFAvE)and methanol extract of *A. djalonensis* (MEAd) produced significant blood glucose reduction of 60.65% ($P < 0.05$), 73.75% ($P < 0.05$) and 55.72% ($P < 0.05$) respectively. On the other hand, glibenclamide produced significant blood glucose reduction of 45.41% ($P < 0.05$) at the end of day 7. The significant % maximal reduction of blood glucose concentrations for MEAv, CFAvE and MEAd was 60.65% (day 7, < 0.05), 74.34% (day 2, < 0.05) and 72.26% (day 3, < 0.05) respectively compared to the control group. However, glibenclamide produced significant % maximal reduction of 45.41% (day 7, < 0.05) compared to the control group. The activities of all other fractions were not significantly different ($P > 0.05$) from the control group. The normal rats maintained ideal blood glucose concentrations while there was a progressive increase in the blood glucose concentrations of the control group within 7 days (Table 2).

Table 1 Effects of methanol extract of *A. vogelli* and *A. djalonensis* leaves (1g/Kg) and glibenclamide (2.5 mg/Kg) on the blood glucose concentration (mg/dL) of alloxan –induced diabetic rats for 3 hours

Treatment	n	00min	30min	60min	90min	120min	150min	180min	% Maximal reduction
Normal rats	3	56.0±3.20	55.4±1.04	54.8±1.59	57.2±1.04	56.6±2.20	56.8±2.11	59.0±1.83	Nil
Untreated diabetic rats (Control)	3	184.4 ±0.52	190.2±0.61	191.7±0.72	196.2±0.67	200.2±0.60	205.1±1.19	208.5±0.84	Nil
Methanol extract of *A. vogelii* (1g/kg)	3	183.3±0.92	185.5±0.47* (2.47%)	182.3±1.3* (4.90%)	181.6±0.70* (7.49%)	181.6±0.89* (9.29%)	179.5±1.56* (12.48%)	176.6±2.60* (15.78%)	15.78
Methanol extract of *A. djalonensis* (1g/kg)	3	185.9±0.83	184.9±1.62* (2.79%)	186.8±3.21 (2.56%)	188.0±4.56* (4.18%)	186.1±3.78* (7.04%)	182.5±1.04* (11.02%)	181.4±0.67* (13.00%)	13.00
Glibenclamide (2.5mg/kg)	3	186.2±1.27	174.6±0.34* (8.20%)	169.7±0.78* (11.48%)	168.7±0.26* (14.02%)	164.0±2.27* (18.08%)	160.4±2.78* (21.79%)	155.3±2.21* (25.52%)	25.25

Each value represents the mean ± S.E.M. from 3 rats
Figures in parenthesis indicate % reduction in blood glucose concentration
*Significantly different from the control ($p < 0.05$).

Table 2 Effects of *A. vogelli* and *A. djalonensis* leaves methanol extracts and fractions of (1g/Kg) and glibenclamide (2.5 mg/Kg) daily on the blood glucose concentration (mg/dL) of alloxan–induced diabetic rats

Treatment	n	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	% Maximal reduction
Normal rats	3	52.7± 1.74	52.7±2.34	56.7±2.38	51.4±1.97	56.0±2.37	54.7±1.73	52.7±1.74	54.7±1.73	Nil
Untreated diabetic rats (Control)	3	415.0±55.37	423.6±104.56	459.1±68.54	436.7±81.03	308.3±47.06	393.3±27.47	319.5±73.33	440.4±84.11	Nil
Methanol extract of *A. vogelii* (1g/kg)	3	354.4±33.41	229.9±57.99 (45.73%)	269.4±66.85 (41.32%)	241.7±44.48 (44.65%)	233.2±42.59 (36.05%)	208.0±45.49* (47.11%)	194.0±11.55 (39.28%)	173.3±25.25* (60.65%)	60.65
Methanol fraction of *A vogelii* extract (1g/kg)	3	400.2±29.33	320.4±64.73 (24.36%)	332.3±57.56 (27.62%)	378.5±109.02 (13.33%)	300.4±114.48 (2.56%)	327.3±129.06 (16.78)	361.6±150.50 (-13.18%)	349.1±141.34 (20.73%)	27.62
Hexane fraction of *A. vogelii* extract (1g/kg)	3	400.8±101.49 (21.27%)	333.5±73.57 (47.66%)	240.3±107.67 (38.22%)	269.8±111.58 (23.81%)	234.9±80.41 (17.77%)	323.4±113.41 (19.78%)	256.3±160.67 (29.81%)	309.1±138.75 47.34	
Chloroform fraction of *A vogelii* extract (1g/kg)	3	201.3±18.34	132.9±40.84* (68.63%)	117.8±34.65* (74.34%)	125.6±26.84* (71.23%)	114.8±30.32* (62.76%)	132.8±30.34* (66.23%)	120.0±23.42* (62.44%)	115.6±20.58* (73.75%)	74.34
Methanol extract of *A. djalonensis* (1g/kg)	3	374.2±33.39	251.6±58.03 (40.64%)	293.8±69.50 (36.01%)	259.8±40.59* (72.26%)	235.2±56.05 (23.71%)	229.9±45.49* (41.55%)	215.5±11.13 (32.55%)	195.0±25.25* (55.72%)	72.26
Methanol fraction of *A. djalonensis* extract (1g/kg)	3	272.8±14.75	406.8±78.97 (3.97%)	415.8±18.98 (9.43%)	355.1±49.49 (18.69%)	218.5±54.93 (29.13%)	520.8±62.84 (-132.42%)	424.8±76.73 (-32.96%)	529.6±32.71 (-20.25%)	29.13
Hexane fraction of *A. djalonensis* extract (1g/kg)	3	491.5±67.67	307.6±24.21 (27.38%)	324.4±47.77 (25.42%)	297.5±27.05 (31.88%)	376.7±45.94 (-22.19%)	370.7±20.56 (5.75%)	254.2±57.77 (20.44%)	301.3±32.19 (31.58%)	31.88
Chloroform fraction of *A. djalonensis* extract (1g/kg)	3	255.1±18.06	349.5±32.52 (17.49%)	490.1±69.23 (-6.75%)	394.2±19.91 (9.73%)	386.9±16.54 (-25.49%)	371.9 ± 68.19 (5.44%)	379.63±82.52 (-18.82%)	372.9±24.94 (15.32%)	17.49
Glibenclamide (2.5mg/kg)	3	341.2±33.69	354.4±14.85 (16.34%)	280.0±61.48* (39.01%)	332.6±28.70* (23.84%)	410.4±33.90 (-33.12%)	285.2±32.28* (27.49%)	216.7±21.59 (32.18%)	240.4±17.17* (45.41%)	45.41

Each value represents the mean ± S.E.M. from 3 rats.
Figures in parenthesis indicate % reduction in blood glucose concentration.
*Significantly different from the control ($p < 0.05$).

Discussion

Experimental evidence obtained in this study indicated that the chloroform fraction obtained from the methanol extract of *A. vogelli* leaves produceda consistent significant hypoglycemic effect compared to the control group in every studied point ($p < 0.05$)in the seven days antidiabetic (chronic) study. It also has the highest significant % maximal reduction of blood glucose concentration compared to all other treatments administered.It likewise justified the hypoglycemic activity observed in its source crude extract administered during the acute and chronic studies.

The significant hypoglycemic activity observed in the chloroform fraction of *A. vogelli* leaves methanol extractwas not exhibited by methanol and hexane fractions of the same extract. This observation tends to suggest that the active compound responsible for the hypoglycemic activity observed is concentrated in the chloroform fraction of *A. vogelli* leaves methanol extract. Chloroform is a moderately polar solvent. Consequently, this active compound may be a moderately polar compound. This hypothesis is in consonance with that of Roa (2006) who postulated that the chloroform extract of *Andrographispaniculata* root produced a significant hypoglycemia in alloxan-induced diabetic rat at a dose of 100 mg/Kg body weight.

Results from the chronic study showed that the hypoglycemic activities of the crude methanol extract of *A. vogelli* leaves, its chlorofom fraction andmethanol extract of *A. djalonensis* were more effective than that of glibenclamide. This observation however agrees with the work of Prince *et al.* (1997) where the hypoglycemic activity of aqueous extract of *Syzigim cumini* seeds was more effective than glibenclamide.

More so, methanol extract of *A. djalonensis* caused 72.26% significant reduction in the blood glucose level of diabetic rats on day 3. A similar observation was made by Olagunju *et al.* (1998) who reported that the leaves extract of *A. djalonensis* showed 77.71% significant reduction in the fasting blood glucose level of diabetic rats on the fifteenth day compared to the control group.

The blood glucose lowering effect of the three fractions (methanol, hexane and chloroform) of *A. djalonensis* leaves methanol extract was weaker than their source methanol extract,

indicating that there should be more synergistic effect which is common in traditional or crude drug.

Alloxan caused a massive reduction in insulin release by the â-cells of the islets of Langerhans and inducing hyperglycemia (Goldner *et al.*, 1943). In our present study we have observed thatthe methanol extract of *A. vogelli* leaves, its chloroform fraction and methanol extract of *A. djalonensis* can reverse this effect. The possible mechanism by which these test extracts bring about their hypoglycaemic activities may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from â-cells of the islets of Langerhans or itsrelease from the bound form. On the other hand, these test extracts might have probably succeeded in stimulating the body cells to absorb blood sugar in diabetic rats, which is the function of the deficient insulin. In this context a number of other plants have also been observed to have hypoglycemic and insulin-release stimulatory effect (Ojewole, 2006; Nwozo *et al.*, 2004; Roa, 2006; Sofaeng *et al.*, 2005; Jalapure *et al.*, 2004 and Banskota *et al.*, 2006).

Conclusions

Diabetes is associated with polyuria (increased urine), polyphagia (increased appetite), polydipsia (increased thirst) as well as general inactivity. These symptoms were remarkably reduced in the diabetic rats treated with *A. vogelli* and *A. djalonensis* leaves methanol extracts and fractions in our study.

Although the exact chemical compound responsible for the hypoglycemic effect of *A. vogelli* and *A. djalonensis* leaves methanol extracts and fractions still remains speculative, experimental evidence obtained in this study indicated that these plants posses antidiabetic property. This finding lends pharmacological support to the reported folkloric and anecdotal uses of these plants leaves in treatment and/or management of diabetes in some parts or Nigeria.

Further chemical and pharmacological investigations will elucidate in detail, the active principles and real mechanism of action of these plants extracts and fraction.

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