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Original Research Article

Hypoglycemic Activity of the Extract and Fractions of *Anthocleista vogelii* (Planch) Stem Bark

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Abstract

Purpose: *To investigate the hypoglycemic effect of the methanol extract and fractions of Anthocleista vogelii stem bark.*
Methods: *The methanol extract of A. vogelii stem bark (ME) was subjected to gradient chromatographic separation using four solvents - chloroform, ethyl acetate, acetone and water - to afford the respective fractions - CF, EF, AF and WF. ME was administered orally to normoglycemic rats at 200 and 400 mg/kg and fasting blood glucose (FBG) monitored for 6 h. Alloxan-induced diabetic rats were also treated orally with ME and the various fractions (each at 200 mg/kg and 400 mg/kg), with glibenclamide (0.2 mg/kg) and normal saline (2 ml/kg) serving as standard and control, respectively. ME and the fractions were also subjected to phytochemical analysis following standard procedures.*
Results: *The extract possessed comparable hypoglycemic effect to glibenclamide in healthy rats. The extract and its fractions also exhibited significant ($p < 0.05$) antidiabetic effect. ME, CF, EF, AF and WF each at 400 mg/kg, produced maximum reduction (64.10, 38.53, 36.50, 60.77 and 12.79 %, respectively) in FBG of the animals after 6 h, compared to 53.77 % for glibenclamide. Presence of alkaloids, carbohydrates, reducing sugars, saponins, flavonoids, glycosides, steroids, terpenoids, tannins, proteins, fats and oils were observed in ME, EF and AF. Alkaloids, flavonoids, steroids, terpenoids, fats and oil were also detected in CF while WF showed the presence of carbohydrates, glycosides, saponins and proteins.*
Conclusion: *This study establishes the antidiabetic activity of the stem bark of A. vogelii. The acetone fraction is the most active antidiabetic fraction.*

Keywords: *Anthocleista vogelii, Antidiabetic, Hyperglycemia, Hypoglycemia, Phytochemical analysis*

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INTRODUCTION

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 [1]. It is estimated that 25 % of the world population is affected by this disease [2]. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations [3]. For instance, the available synthetic antidiabetic agents produce serious side effects like hypoglycemia and hepatorenal disturbances [4]. Medicinal plants play a great role in the traditional management of the disease due to their relative safety and low cost. Scientific investigation into some of these medicinal plants shows that they increase insulin secretion,

enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from intestine and glucose production from liver [5]. However, very few of these medicinal plants have received scientific scrutiny despite the World Health Organization (WHO) recommendations. In our search for more potent and safer antidiabetic principles, we are currently investigating the stem bark of *Anthocleista vogelii* for its hypoglycemic potentials.

Anthocleista vogelii Planch (family Gentianaceae) is a plant that is common in tropical Africa, Cameroon, Sudan, and Sierra Leone. It is also found in Northern, Western and Eastern Nigeria particularly in swampy areas near streams and closed forests [6]. The combination of the stem bark and the leaves is used as anti-inflammatory and antidiabetic agents and also in the treatment of wounds [7]. Abu *et al* [8] has demonstrated the hypoglycemic activity of the root extract of the plant in both normal and hyperglycemic rabbits. The α-amylase inhibitory effect of the *A. vogelii* was recently demonstrated in an in vitro model which shows the hypoglycemic potential of the plant [9]. Xanthone derivatives and steroidal compounds have been isolated from the stem bark of the plant [10]. These constituents were evaluated for their antimicrobial potentials but none could be traced to the hypoglycemic effect of the plant.

In the present study, therefore, the objective was to investigate the hypoglycemic activity of the stem bark of *A. vogelii* in rats to provide a scientific support to the folkloric claims of the antidiabetic activity of the plant part.

EXPERIMENTAL

Chemicals

Solvents used for the extraction and fractionation were methanol, chloroform, ethyl acetate (Sigma Chemicals, USA) and distilled water. The following reagents were freshly prepared from the analytical grade chemicals purchased locally from JoeChem Ent., Nsukka, Nigeria and used for the phytochemical tests: Fehling's solution A and B, Dragendorff's reagent, Wagner's reagent, Mayer's reagent, picric acid. Other chemicals used include alloxan monohydrate (Sigma, USA), glibenclamide (NGC, Nigeria), silica gel (100-400 mesh, Burgoyne Burbidges & Co., India).

Plant materials

The plant material (stem bark) was collected from the forests in Calabar, Nigeria during the month of May. The plant was identified and authenticated by Mr. A. Ozioko, a taxonomist with the Bioresource Development and Conservative Centre (BDCC), Nsukka. The voucher specimen (INTERCEDD/30) has been deposited in our research laboratory for further reference. Fresh stem bark was collected in bulk, washed, shade dried and pulverized in a grinder to obtain coarse powder.

Preparation of extract and fractions

The powdered plant material (3000 g) was extracted by macerating it in methanol (95 %, 12 L) for 72 h at room temperature (30 ± 2 ^{o}C) with constant stirring. After filtration through a cotton wool plug, the filtrate (liquid extract) was concentrated in vacuum (40 ^{o}C) using a rotary evaporator (Buchi Rotavator-R, China) to afford the methanol extract. 150 g of dried ME was triturated with 300 g of silica gel in a mortar and then transferred into an air-tight bottle and partitioned successively with four solvents according to increasing order of polarity starting with chloroform, ethyl acetate, acetone to water. The collected liquid samples were concentrated to afford the corresponding fractions which were labeled CF, EF, AF and WF respectively.

Animals

Adult Wistar albino rats (150 - 200 g) of either sex were used for the hypoglycemic evaluation. The animals were procured from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept in standard polypropylene cages at room temperature (30 ± 2 ºC) and at 60 - 65 % relative humidity during the experimental work with 12 h day: 12 h night cycle. They were fed with normal laboratory diet (Vital Feeds Nig., Ltd.) and allowed to drink water *ad libitum*. The animals were allowed to acclimatize for seven days before being used for the studies. The experimental protocols was in accordance with the guidelines of the Ethics Committee of the University of Nigeria as registered by the National Health Research Ethics Committee of Nigeria (as per the approved ref: NHREC/05/01/2008B). The animal care and handling was in line with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) [11].

Evaluation of methanol extract in normal rats

The animals were fasted for 12 h but were allowed free access to water before and throughout the duration of the experiment. At the

end of the fasting period, taken as zero time (0 h), blood was withdrawn from the tip of the tail of each rat under mild anesthesia and the fasting blood glucose (FBG) was estimated with a blood glucometer (Accu-Check, Roche, Germany) [12]. The normal rats were then divided into four groups of four animals each. Group-I served as the negative control and received only vehicle (2 ml/kg of normal saline) through oral route. Group-II received glibenclamide (0.2 mg/kg, p.o.) [13]. Group-III and IV received ME (p.o.) at two doses of 200 and 400 mg/kg respectively. Blood glucose concentration was measured after 1, 3 and 6 h of administration of single dose of each of the treatments.

Determination of extract activity in Alloxan-induced hyperglycaemic rats

The animals were kept fasting for 12 h with water *ad libitum* and injected intraperitoneally at dose of 150 mg/kg of alloxan monohydrate in normal saline [12]. After one hour, the animals were provided with standard laboratory diet *ad libitum*. The FBG was checked before and 48 h after alloxan injection by withdrawing blood from the tip of the tail of each rat under mild anesthesia [14]. The FBG was measured as described above. Animals were considered diabetic when the FBG was raised beyond 200 mg/dl. The animals were segregated into twelve groups of four animals in each. Group-I served as control and received vehicle (normal saline, 2 ml/kg, p.o.). Group-II received glibenclamide (0.2 mg/kg, p.o.) [13]. Group III and IV received ME while group-V to XII received the respective fractions (CF, EF, AF and WF) each at doses of 200 and 400 mg/kg (p.o). As described above, FBG was monitored up to the 6th h post administration of various treatments.

Phytochemical screening

Standard screening test of the extract and fractions of the plant material was carried out for various plant constituents [15]. They were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins using standard procedures

Statistical analysis

The data obtained was subjected to one way analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's post hoc test. A $p < 0.05$ was considered to be statistically significant. Data were analyzed with SPSS 16.0 software.

RESULTS

Extraction yield

The yield of ME was 12.04 % while the various fractions had a yield of 4.30, 34.97, 42.01 and 18.72 % for CF, EF, AF and WF, respectively.

Effect of methanol extract in normal rats

The effect of different doses of ME on the FBG level was assessed in normal rats at various time intervals (Table 1). The extract at 400 mg/kg produced a significant ($p < 0.05$) reduction (46.57 %) in the FBG after 6 h of treatment which was comparable to the effect of glibenclamide (36.80 %).

Table 1: Effect of ME on fasting blood glucose (FBG) level in normal rats

Group	Treatment	Fasting blood glucose (FBG) concentration (mg /dl)			
		0	1	3	6
I	[a]Normal saline (2 ml/kg)	114.75±7.85	113.75±7.77	110.75±7.54	106.25±6.69
II	Glibenclamide 0.2 mg/kg	123.50±7.42	107.75±5.96 (12.75%)	97.75±9.00 (20.85%)	78.00±8.09* (36.8%)
III	ME 200 mg/kg	118.00±10.20	112.00 ±5.79 (5.08%)	102.00±4.41 (13.56%)	96.50±3.23 (18.22%)
IV	ME 400 mg/kg	124.00±2.16	112.50±4.11 (9.27%)	86.50±6.36 (30.24%)	66.25±9.20** (46.57%)

[a]Control group; Results are expressed as mean ± SEM (n = 4); [*]p<0.05, [**]p < 0.01 as compared with control group at the same time;one-way, ANOVA followed by Dunnet's t-test); figures in parenthesis denote percentage reduction of blood glucose from 0 h

The 200 mg/kg dose of ME did not effect a significant ($p < 0.05$) blood glucose lowering within the 6 h observation period in normoglycemic animals.

Antidiabetic activity of ME and its fractions

Administration of single dose of alloxan (150 mg/kg) to the normal animals generally resulted in a 1.5 to 2.5 fold increase in the FBG thereby inducing diabetes in the animals (Table 2). Treatment of the alloxan-induced diabetic animals with the extract and its various fractions produced a decline in the FBG which was generally sustained throughout the six hours of observation (apart from that of 400 mg/kg WF). The ME produced a significant ($p < 0.05$, 0.01) dose-dependent reduction in the FBG of the animals after 1 h of treatment, an effect which persisted up to the sixth hour. Maximum reduction of 48.48 % and 64.10 % in blood glucose was produced by the 200 mg/kg and 400 mg/kg of the ME respectively after 6 h of treatment. These effects are comparable with that of 0.2 mg/kg of glibenclamide which produced maximum reduction of 53.77 % after 6 h. Among the fractions, CF and EF appear to produce similar dose-dependent reduction in FBG. CF produced significant ($p < 0.05$, 0.01) reduction (24.30 % and 38.53 % for 200 mg/kg and 400 mg/kg respectively) in the FBG after 6 h. EF similarly produced a significant ($p < 0.01$) hypoglycemic effect with reductions of 27.13 % and 36.50 % respectively for the doses after the same period. Lower dose of WF (200 mg/kg) did not produce any significant ($p < 0.05$) reduction in the blood glucose while 400 mg/kg produced maximum reduction (25.00 %) after one hour of treatment which was not sustained for six hours. AF however, effected the highest reduction in the FBG with a dose-dependent significant ($p < 0.01$) reduction of 53.89 % and 60.77 % respectively for the two doses after 6 h compared with the control. Before treatment of the diabetic animals, the mean blood glucose level was 285.50 ± 8.99 mg/dl and this was normalized after six hours to 112.00 ± 4.81 mg/dl by the 400 mg/kg dose of AF. Thus both ME and AF normalized the FBG of the alloxan-induced diabetic rats after 6 h and their effects are comparable (though slightly better) to that of glibenclamide.

Phytochemical analysis

Phytochemical analysis (Table 3) revealed that all the tested phytoconstituents, including alkaloids, carbohydrates, reducing sugars, saponins, flavonoids, glycosides, steroids, terpenoids, tannins, proteins, fats and oils were present in varying degrees in ME, EF and AF. Alkaloids, flavonoids, steroids, terpenoids, fats and oil were also found in the CF while WF showed presence of carbohydrates, glycosides, saponins and proteins.

DISCUSSION

The effect of the extract on normoglycemic animals suggests that the stem back of *A. vogelii* has a mild lowering effect on normal glucose levels. This effect was comparable to that of glibenclamide, an insulin secretagogue, which also lowers blood glucose in normal animals. Provided the β-cells are fully functional, sulphonylureas, such as glibenclamide, can cause hypoglycemia since insulin release is initiated even when glucose concentrations are below the normal threshold for glucose-stimulated insulin release (approximately 5 mmol/L or 90 mg/dl) [16].

In the antidiabetic study, a single dose of alloxan (150 mg/kg) induced diabetes in the animals by elevating the FBG above 200 mg/dl. Alloxan, a beta cytotoxin, induces diabetes in a wide variety of animal species by damaging the insulin secreting pancreatic beta cells resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues [17] and this leads to various metabolic aberrations in the animals such as increased blood glucose. The significant lowering of the FBG in both normal and alloxan - induced diabetic experimental animals by the extract and fractions especially AF, is an indication of the hypoglycemic activity of the plant stem back. The results of the antidiabetic studies suggest that the extract and all its fractions possess varying degree of hypoglycemic potency since all produced at least 25 % reduction in the FBG of the animals. Kahn and Shechter [18] have suggested that a 25 % reduction in blood glucose levels is considered a significant hypoglycemic effect. The results have shown that the plant extract (ME) possesses antidiabetic effect in experimental animals and further purification of the extract yielded the acetone fraction which has comparable antidiabetic potency. Though the detailed mechanism of action of the test samples has not been investigated, they possibly act by potentiating the insulin effect either by increase in pancreatic secretion of insulin from beta cells of islets of Langerhans or by increase in peripheral glucose uptake. They could be acting similar to glibenclamide which secretes insulin

Table 2: Effect of the ME and its fractions on fasting blood glucose (FBG) level in alloxan-induced diabetic rats

Group	Treatment	Dose	Fasting blood glucose (FBG) (mg/dl)				
			Basal FBG	0	1	3	6
I	[a]Normal saline	2 ml/kg	94±2.43	273.75 ±9.56	270.25 ±9.48	265.25 ±8.59	255.25 ±6.17
II	Glibencl-amide	0.2 mg/kg	106.25±2.98	275.25 ±11.76	256.25 ±10.75 (6.90%)	180.25 ±11.16** (34.51%)	127.25 ±9.20** (53.77%)
III	ME	200 mg/kg	120.00±1.76	288.75 ±5.44	212.50 ±5.98* (26.41%)	181.00 ±4.30** (37.32%)	148.75 ±5.44** (48.48%)
IV	ME	400 mg/kg	102.58±3.82	279.25 ±7.69	165.25 14.34** (40.82%)	134.25 ±3.66** (51.92%)	100.25 ±2.74*** (64.10%)
III	CF	200 mg/kg	123.75±1.80	268.50 ±45.44	246.50 ±35.57 (8.19%)	231.50 ±29.42 (13.78%)	203.25 ±21.50* (24.30%)
IV	CF	400 mg/kg	122.75±1.67	271.25 ±26.89	238.25 ±26.75 (12.17%)	197.00 ±27.54** (27.37%)	166.75 ±10.76** (38.53%)
V	EF	200 mg/kg	116.50±3.86	264.50 ±9.58	263.50 ±9.53 (0.38%)	258.25 ±11.58 (2.36%)	192.75 ±10.10** (27.13%)
VI	EF	400 mg/kg	116.75±4.78	268.50 ±11.93	264.50 ±17.97 (1.49%)	259.50 ±13.24 (3.35%)	170.50 ±11.42** (36.50%)
VII	AF	200 mg/kg	114.76±7.65	283.00 ±7.95	244.50 ±5.84 (13.60%)	181.50 ±5.69** (35.87%)	130.50 ±10.10** (53.89%)
VIII	AF	400 mg/kg	100.75±3.01	285.50 ±8.99	208.25 ±8.13** (27.06%)	165.00 ±5.31** (42.21%)	112.00 ±4.81** (60.77%)
IX	WF	200 mg/kg	97.00±0.23	271.45 ±7.58	256.47 ±6.34 (5.52%)	234.71 ±12.21 (13.53%)	220.29 ±5.13* (18.85%)
X	WF	400 mg/kg	88.25±4.97	290.00 ±4.57	217.50 ±3.67** (25.00%)	230.50 ±9.34* (20.52%)	252.92 ±7.78 (12.79%)

*[a]Untreated diabetic (control group); Results are expressed as Mean ±SEM (n=4). *p<0.05, **p<0.01 as compared with control group at the same hour (One way, ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose from 0 h*

from beta cells in type II. Type II diabetes is characterized by a reduced pancreatic secretion of insulin or insulin resistance or both. Since glibenclamide as well as the test plant samples was found effective in lowering the blood glucose level in the alloxan-induced diabetic rats, it is possible that alloxan did not totally destroy the pancreatic cells; this suggests a model of type II for the present study. The stem bark of *A. vogelii* could therefore be a useful agent either alone or in combination with other agents for the management of type II diabetes mellitus.

The antidiabetic effect of the plant may be due to the presence of alkaloids, flavonoids, steroids or terpens and other constituents present in the stem bark which could act synergistically or independently in lowering the blood sugar level. Since WF exhibited the least activity and it

Table 3: Phytochemical constituents of the extract and fractions of *A. vogelii* stem bark

Phytochemical	Relative abundance				
	ME	CF	EF	AF	WF
Alkaloids	+++	+	++	+++	-
Carbohydrates	++	-	++	+++	+
Reducing sugar	++	-	+	+	-
Flavonoid	++	++	++	++	-
Glycoside	++	-	++	++	+
Saponin	+++	-	++	++	++
Steroids	++	+	++	++	-
Terpenoids	++	+	++	++	-
Tannins	+	-	+	++	-
Proteins	++	-	++	+	++
Fats and oil	+++	+	++	+	-

Key: - = *absent; + = present; ++ = moderately present; +++ = highly present*

contained no alkaloids or flavonoids which were abundant in the other more potent fractions, it is speculated that flavonoids and/or alkaloids might have a major role to play in the antidiabetic effect of the plant stem bark. Some alkaloids are known hypoglycemic agents. For instance, berberine, a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids has been used successfully in experimental models of diabetes mellitus [19] and in clinical studies. Berberine was shown to possess insulin sensitizing effect [19]. Also flavonoids, being polyphenolics, are known to be hypoglycemic. The antidiabetic activity of four flavonoids - boswellic acid, ellagic acid, quercetin,and rutin - were demonstrated in rats and the proposed mechanism of action was by increasing the peripheral utilization of glucose and inhibiting the glucose transporter activity from intestine [20]. The National Institutes of Health Clinical Center is currently investigating the use of quercetin on glucose absorption in obesity, and obesity with type 2 diabetes patients on oral glucose tolerance test [20]. Previous phytochemical evaluation of *A. vogelii* stem bark extract led to the isolation of decussatin (1-hydroxy-3, 7, 8-trimethoxyxanthone), swertiaperennin (1, 8-dihydroxy-3,7-dimethoxyxanthone), 7-hydroxysitosterol and sitosterol 3-O-D-glucopyranoside which displayed antimicrobial potency [10]. These compounds are polyphenolic (the xanthone derivatives) and steroidal (the sitosterols) in nature. To establish the actual antidiabetic principle(s) however, the putative compound(s) have to be isolated and evaluated. It is therefore, suggested that further purification steps would be necessary to isolate and further evaluate the antidiabetic principles of the plant.

CONCLUSION

On the basis of the current investigation, it could be concluded that the stem bark of *A. vogelii* possesses hypoglycemic activity in both normal and alloxan-induced diabetic animals. The traditional use of the plant to treat diabetes is supported by our laboratory findings. The study has also identified the acetone fraction as the most potent antidiabetic fraction among all the fractions of the plant extract.

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