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ANTIDIABETIC ACTIVITY OF ISOLATES FROM THE LEAVES OF *SECAMONE AFZELII* RHOME (ASCLEPIADACEAE)

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ABSTRACT

Secamone afzelii Rhome is used in ethnomedicine for diabetes and other disease conditions. This study was aimed at evaluating the antidiabetic activity of the aqueous ethanol extract and its isolates. Chromatographic techniques were employed in the isolation. Doses of the extract ranging from 100 to 500 mg/kg were evaluated in normoglycaemic rats. The dose of 500 mg/kg (crude extract and solvent fractions), 100 mg/kg (VLC fractions) and 50 mg/kg (isolated compounds) were evaluated in Streptozotocin-induced diabetic rats. Glibenclamide 1.25 mg/kg was used as the reference standard. The various doses of the crude extract produced significant ($p < 0.05$) reduction in blood glucose levels in normoglycaemic and streptozotocin-induced diabetic rats. These doses were more effective than glibenclamide in normoglycaemic rats. Solvent partitioning, Vacuum Liquid Chromatography and Column Chromatographic analysis of extract of *S. afzelii* resulted in the isolation of two compounds which produced significant hypoglycaemic effects. *S. afzelii* and its isolated constituents possess antidiabetic properties in rats and are good candidates for further investigations.

KEYWORDS: **Antidiabetic, Asclepiadaceae, Chromatography, *Secamone afzelii***

INTRODUCTION

Secamone afzelii Rhoem (Asclepiadaceae) known as arilu, ailu or alu (Yoruba), utunta (Ibo) and Ewuonkwonegie (Bini) [1], is a familiar creeping woody climber found on fences and on trees and grows to a very long length.

Extracts of *S. afzelii* are widely used in traditional medicine practices for stomach problems, diabetes, colic, dysentery and also for kidney problems [1]. Phytochemical screening of the leaves revealed the presence of flavonoids [2]. The antioxidant activity of the methanol extract has been associated with its α-tocopherol constituent [3]. The plant also possesses antibacterial, antifungal and anti-inflammatory properties [4].

Diabetes mellitus is a growing public health concern worldwide. It is characterized by a group of metabolic disorders causing severe and costly complications. Cardiovascular diseases, retinopathy, neuropathy and nephropathy are some of the complications associated with Type 2 diabetes [5]. The enormous cost and associated side effects of modern medicines for the treatment of diabetes indicates that alternative strategies are required for better management [6]. Traditional plant medicines including preparations from *S. afzelii* are used throughout the world in the management of diabetes. Studies on hypoglycaemic activities of plants have identified compounds like alkaloids [7], tannins [8], polypeptides [9] amongst others to be responsible for reported activity. The present study was undertaken with the aim of isolating the active constituent(s) responsible for the observed antidiabetic activity of *S. afzelii*.

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MATERIALS AND METHODS

Preparation of plant extract

The leaves of *Secamone afzelii* Rhoem (Asclepiadaceae) were collected in Ugbowo area of Benin City, Edo State, Nigeria. The plant was authenticated by the curator at the Forest Research Institute of Nigeria (FRIN), Ibadan where a voucher specimen with the number FIH 107158 was deposited. The fresh leaves were air–dried for 72 h and powdered using an electric mill.

Animals

Male Wistar rats (207. 00 ± 7. 94g) were obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. All the animals were kept under standard environmental conditions and were handled according to international protocol for use of animals in experiments [10]. They were fed with standard pellets and tap water *ad libitum*. Ethical approval for the study was obtained from the College of Medicine, University of Benin Ethics Committee (ADM/F. 22A/Vol. viii/349).

Extraction and Isolation

The dried leaves of *S. afzelii* (2 kg) were extracted with MeOH-H_2O (80: 20). Evaporating the solvent yielded an extract which was subsequently resuspended in water and successively partitioned into n-hexane (3 X 2L), Chloroform (3 X 2L) and n-BuOH (3 X 2L). The n-BuOH solvent fraction (360 g), being the most active was subjected to Vacuum Liquid Chromatography with $CHCl_3$: n-BuOH (1:0, 4:1, 3:2, 2:3, 1:4 and 0:1, each 2L). Fractions with similar R_f values were bulked together to give three (3) sub-fractions, namely SAB-F1, SAB-F2 and SAB F-3. The most promising fraction SAB-F3 (196 g) was subjected to column chromatography (10 X 120 cm) on silica gel with a chloroform-ethylacetate-methanol gradient elution system. Compounds with similar R_f values were bulked together to give two (2) isolates, E and F.

Antidiabetic test

Diabetes was induced in rats fasted for 16 h by intraperitoneal injection of 55 mg/kg of freshly prepared Streptozotocin (STZ) in 0. 1M acetate buffer, pH 4.5 after baseline blood glucose were estimated [11]. To overcome the hypoglycemia which occurs during the first 24 h following STZ administration, diabetic rats were orally given 5 % glucose solution. After 5 days, rats with blood glucose levels of 250 mg/dl and above were considered to be diabetic and selected for the study.

Oral doses of 100, 200, 300, 400 and 500 mg/kg of the extract, 100 mg/kg of VLC Fractions, 50 mg/kg of isolated compounds and 1.25 mg/kg glibenclamide prepared in 1 % aqueous Tween 80 were administered respectively to the groups of diabetic rats. Animals in the control group received only the vehicle (5 ml/kg) [12 and 13]. The blood glucose levels were determined in the time intervals of 1, 4, 8 and 12 h for normoglycemic rats and at 0. 5, 1, 2, 4, 6, 8 and 12 h for STZ-induced diabetic rats. In all cases, blood was obtained from the tail tips of the rats.

Estimation of blood glucose level

Blood glucose concentration (mg/dl) was determined using a glucometer (AAU-CHEK, ROCHE, UK), the principle of which is based on the glucose oxidase method [14 & 15].

The percentage glycaemic change at any time point was calculated using the formula:

$$\% \text{ Glycemic change} = \frac{\text{FBG} - \text{GC}}{\text{FBG}} \times \frac{100}{1}$$

Where GC is the glucose concentration at different time points and FBG is the fasting blood glucose concentration representing baseline value.

Statistical analysis

Data are expressed as mean ± SEM and "n" represents the number of rats used. The differences between the means were analyzed using one way analysis of variance (ANOVA). Values of $P < 0.05$ were taken to imply statistical significance between compared data.

RESULTS

Hypoglycemic effects

Administration of the crude extract of *S. afzelii* on normoglycaemic wistar rats produced significant hypoglycaemic effects after 1 h at tested doses (Table 1). In all cases, except at 500 mg/kg, percentage blood glucose reduction began at 1 h, maximum at the 8 h and reduced at the 12 h. The percentage reductions produced by the various concentrations were significant when compared with 1 % Tween 80 and glibenclamide. The highest percentage reduction of 44.90 ± 1.61 % was recorded by 500 mg/kg at the 12 h.

Table 1: The hypoglycaemic effects of *S. afzelii* extract on normoglycaemic rats

Experimental Group	Dose (mg/kg)	Percentage blood glucose reduction (mg/dl) (mean±SEM)			
		1 h	4 h	8 h	12 h
1 % Tween 80*	-	2.46±1.42	4.40±4.43	9.49±5.48	9.40±3.53
Glibenclamide**	1.25	6.82±0.95	13.68±0.07	26.55±3.59	19.67±0.59
S. afzelii	100	10.69±0.68	27.96±1.48	37.01±2.12	36.85±0.72
S. afzelii	200	15.77±0.85	28.94±0.21	39.48±0.11	31.49±0.38
S. afzelii	300	13.84±0.12	29.19±0.64	40.75±0.79	37.12±2.19
S. afzelii	400	15.02±0.29	30.90±1.08	41.77±1.77	39.08±1.65
S. afzelii	500	17.34±1.13	31.39±2.14	42.81±1.62	44.90±1.61

Blood glucose reduction by the various doses of *S. afzelii* was significant all through the period of assay $p < 0.05$ compared to glibenclamide and $p < 0.02$ compared to distilled water. n = 4 per group

Table 2: The hypoglycaemic effects of *S. afzelii* extract on streptozotocin-induced diabetic rats

Experimental Group	Dose (mg/kg)	Percentage blood glucose reduction (mg/dl) (mean±SEM)						
		0.5 h	1 h	2 h	4 h	6 h	8 h	12 h
1 % Tween 80*	-	-1.78±0.25	-4.36±2.52	-3.91±1.76	-6.75±4.60	-5.33±3.18	-6.34±4.19	-8.10±0.0
Glibenclamide **	1.25	10.65±3.93	14.47±5.18	19.71±6.67	35.83±2.81	37.55±2.13	38.25±2.22	34.58±4.68
S. afzelii	500	8.83±0.59	15.98±1.78	23.96±2.66	34.38±2.15	23.20±0.14	37.53±1.79	33.59±0.46

Blood glucose reduction by 500 mg/kg dose of *S. afzelii* was significant all through the period of assay $p < 0.05$ compared to distilled water. n = 4 per group.

Table 3: The hypoglycaemic effects of solvent partitioning of *S. afzelii* extract on streptozotocin-induced diabetic rats

Experimental Group	Dose (mg/kg)	Percentage blood glucose reduction (mg/dl) (mean±SEM)						
		0.5 h	1 h	2 h	4 h	6 h	8 h	12 h
1 % Tween 80*	-	-1.78±0.25	-4.36±2.52	-3.91±1.76	-6.75±4.60	-5.33±3.18	-6.34±4.19	-8.10±0.0
Glibenclamide**	1.25	10.65±3.93	14.47±5.18	19.71±6.67	35.83±2.81	37.55±2.13	38.25±2.22	34.58±4.68
Hexane	500	-0.72±1.16	-1.19±0.43	1.04±0.39	0.84±1.29	0.08±1.34	-2.48±0.39	-1.71±0.70
Chloroform	500	0.92±0.43	1.37±0.43	2.84±1.56	2.19±1.83	3.09±2.88	4.46±3.47	6.02±4.76
Butanol	500	1.63±2.19	2.35±2.01	5.48±2.15	7.62±1.97	9.27±2.25	12.65±2.47	11.85±3.20
Aqueous	500	-0.72±0.96	-1.51±1.22	-0.08±0.83	-0.14±2.72	3.00±2.00	3.55±1.35	3.16±2.65

Blood glucose reduction by solvent partitioning of *S. afzelii* was significant all through the period of assay $p < 0.05$ compared to distilled water except for the Hexane fraction. n = 4 per group

Streptozotocin-induced diabetic rats showed significant increase in the levels of blood glucose when compared to normal rats. Oral administration of the crude extracts (500 mg/kg) and solvent fractions (500 mg/kg) showed significant decrease in blood glucose levels ($p < 0.05$) when compared with distilled water (Tables 2 and 3). Hexane fractions did not show any significant difference in percentage blood glucose reduction. The combined fractions from the Vacuum Liquid Chromatography, SAB-F1, SAB-F2 and SAB-F3 at a dose level of 100 mg/kg as well as the isolated compounds, E and F at the dose of 50 mg/kg also showed significant decrease ($p < 0.05$) in blood glucose level except for SAB-F2 and E (Tables 4 and 5).

Table 4: The hypoglycaemic effects of VLC fractions of *S. afzelii* extract on streptozotocin-induced diabetic rats

Experimental Group	Dose (mg/kg)	Percentage blood glucose reduction (mg/dl) (mean±SEM)						
		0.5 h	1 h	2 h	4 h	6 h	8 h	12 h
1 % Tween 80*	-	-1.78±0.25	-4.36±2.52	-3.91±1.76	-6.75±4.60	-5.33±3.18	-6.34±4.19	-8.10±0.0
Glibenclamide**	1.25	10.65±3.93	14.47±5.18	19.71±6.67	35.83±2.81	37.55±2.13	38.25±2.22	34.58±4.68
SAB-F1	100	2.01±1.95	1.55±2.11	3.15±1.78	4.08±1.02	3.47±2.57	2.10±0.87	-0.09±1.21
SAB-F2	100	-1.66±4.21	0.20±4.89	0.46±3.19	-1.44±3.26	-0.31±3.22	-3.15±3.88	-5.61± 4.16
SAB-F3	100	1.29±0.59	1.47±0.99	4.76±1.45	3.63±0.56	3.76±1.32	2.60±1.34	3.01±1.78

lood glucose reduction by solvent partitioning of *S. afzelii* was significant all through the period of assay $P < 0.05$ compared to distilled water. n = 4 per group.

Table 5: The hypoglycaemic effects of isolated compounds of *S. afzelii* extract on streptozotocin-induced diabetic rats

Experimental Group	Dose (mg/kg)	Percentage blood glucose reduction (mg/dl) (mean±SEM)						
		0.5 h	1 h	2 h	4 h	6 h	8 h	12 h
1 % Tween 80*	-	-1.78±0.25	-4.36±2.52	-3.91±1.76	-6.75±4.60	-5.33±3.18	-6.34±4.19	-8.10±0.0
Glibenclamide**	1.25	10.65±3.93	14.47±5.18	19.71±6.67	35.83±2.81	37.55±2.13	38.25±2.22	34.58±4.68
E	50	-0.50±0.86	-0.67±1.63	0.28±3.55	0.89±2.67	0.87±2.05	0.15±1.37	1.42±0.18
F	50	2.90±1.68	4.08±1.41	4.49±1.47	4.27±1.16	3.37±1.11	2.67±1.10	1.29±0.73

Blood glucose reduction by isolated compounds of *S. afzelii* was significant all through the period of assay $p < 0.05$ compared to distilled water. n = 4 per group.

DISCUSSION

Several investigations into the chemical and biological activities of plants have yielded compounds with properties useful for the development of modern synthetic drugs for management of several diseases including diabetes [16 and 17]. Medicinal plants of the Asclepiadaceae family have been investigated for their antidiabetic properties and their activities are generally due to various phytochemicals such as polyphenols, catechins, saponins and flavonoids [18]. Scientific studies have established the hypoglycemic effects of the aqueous and ethanol extracts of *Gongronma latifolium, Gymnema Sylvester* and *Holostemma ada* Kodien Schults, all of the family Asclepiadaceae [19, 20, 21 and 22]. The aqueous ethanol extract of the leaves of *Secamone afzelii* (Asclepiadaceae) produced significant hypoglycemic effect 1 h after administration and was maximum at 8 h. However, the responses decreased at 12 h in all groups except for *S. afzelii* (500 mg/kg). Such a phenomenon of less hypoglycemic response at higher time is not uncommon with plants possessing hypoglycemic activities and may be due to reduced concentrations of active constituents in the system.

For the study of antidiabetic agents, streptozotocin–induced hyperglycemia in rats is considered to be a good preliminary screening model and is widely used [23]. Streptozotocin (N–[methyl nitro carbamoyl]–D–glucosamine) is a potent methylating agent for DNA and acts as nitric oxide donor in pancreatic β–cells and thus β–cells are more sensitive to damage by nitric oxide and free scavenging enzymes [24]. Percentage blood glucose reduction produced by 500 mg/kg of the aqueous ethanol extract of *S. afzelii* in streptozotocin–induced diabetic rats was comparable to that produced by glibenclamide 1.25 mg/kg which produced the highest percentage blood reduction at 8 h (38.25 ± 2.22 %). This suggests a potent action in short term study and reveal a defined role in reducing blood glucose level in normal and diabetic rats.

Solvent partitioning, Vacuum Liquid Chromatography and Column Chromatographic analysis of *S. afzelii* resulted in the isolation of two compounds. The compound produced significant hypoglycaemic effects when compared to the control. The basic structure of the above two compounds are yet to be determined, though

chromatographic fingerprints indicates that they are phenolic compounds.

CONCLUSION

On the basis of the results obtained from this study, it could be said that *Secamone afzelii* and its isolated constituents possess antidiabetic properties. The results from this study thus support the claimed traditional use of *S. afzelii* in the management of diabetes.

CONFLICT OF INTEREST

Authors declared no conflict of interests in the conduct and reporting of this study

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