

## Antidiabetic properties of *Solanum villosum* and *Solanum nigrum* var *sarrachoides* in a streptozotocin-induced diabetic mice model

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### ABSTRACT

**Background:** Diabetes mellitus (DM) poses immense challenge to the health of people worldwide. Current therapies are limited by cost and adverse effects. *Solanum nigrum*, a complex of many species in the family *Solanaceae* has been recorded to be used by many communities in the management of DM. The aim of this study was to evaluate the phytochemical, antidiabetic efficacy and safety of two species, namely; *Solanum villosum* and *S. nigrum* var *sarrachoides* using streptozotocin-induced diabetic mice model.

**Methods:** Qualitative assessment for phytochemical constituents was carried out. Acute toxicity was conducted based on 'Organisation of Economic Cooperation and Development' 2001 guidelines. Diabetes was induced by injection of streptozotocin at a dose of 200 mg/kg body weight intraperitoneal after the mice fasted for 8 hours. Aqueous extracts were administered orally using an oral gavage at doses of 150, and 300 mg/kg body weight for each plant daily and monitored weekly for 28 days.

**Results:** Both plants contain vital phytochemicals. Flavonoids, alkaloids, tannins, saponins, phenols, and glycosides were present in both plants. However, phytosterols and coumarins were absent in *S. villosum*. Additionally, both plants did not show toxicity. Both plants showed efficacy with *S. nigrum* var *sarrachoides* being more potent at both doses.

**Conclusions:** The study validates the use of these plants by herbalists and recommends further studies on them with the aim of elucidating the active compounds that can be used as novel therapies for diabetes. Additionally, the study recommends the evaluation of other species in this complex for antidiabetic properties.

**Keywords:** *S. villosum*, *S. nigrum* var *sarrachoides*, Antidiabetes, Phytochemicals

### INTRODUCTION

The term diabetes mellitus describes a non-communicable metabolic disorder of multiple aetiologies.<sup>1,2</sup> It is characterized by chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.<sup>3</sup> The sustained hyperglycemia causes both short-term and long-term pathophysiological defects.<sup>4</sup> Cases of DM have been on

the rise both in the developed and the developing countries.<sup>5</sup> Once termed as a disease of the rich, DM has now transcended that definition to be among the commonest non-communicable diseases globally.<sup>2</sup> The International Diabetes Federation (2015) projected that the number of diabetic people in the world will rise to 642 million by the year 2040. Insulin which is vital in the management of DM has been limiting for many patients especially in low income setups.<sup>6</sup> Due to this, there has been increased reliance on herbal remedies many of

which scientific validation is not available.<sup>7</sup> African nightshade (ANS) *Solanum nigrum* L. which is a complex of several species has been recorded to possess antidiabetic properties.<sup>8,9</sup> This study aimed at assessing phytochemical composition, safety and antidiabetic properties of aqueous extracts of two species of the *S. nigrum* L. complex; *S. villosum* and *S. nigrum* var *sarracoides* in a mice model.

## METHODS

### Growing of the ANS genotypes

*S. villosum* and *S. nigrum* var *sarracoides* genotypes used were grown in Kalro Muguga experimental site during the long rains season. The area normally receives bimodal mean rainfall of 900 mm to 1000 mm annually with long rains of 550 mm falling in mid-March to June and the short rains of 400 mm falling in mid-October to December.



Figure 1: *S. nigrum* var *sarracoides*.



Figure 2: *S. villosum*.

### Harvesting, identification, and preparation of plant samples

Whole plant samples were handpicked from the field and placed in a sisal basket for transportation to the lab. The plant samples were identified at the University of Nairobi Herbarium in the Department of Botany. Leaves were sorted, washed and air dried in the shade for 10 days.

Once dried, the sample was weighed and ground into a fine powder ready for extraction process.

### Aqueous extracts preparation

The extract was prepared by decoction method.<sup>10</sup> This was made by adding 100 grams of powder leaves in one litre of distilled water for 30 minutes in a hot plate. The decoction was then filtered using Whatman filter paper (No.2), and the filtrate centrifuged at 5000 rpm for 10 minutes to collect the supernatant. The extract was then freeze-dried (Christ Beta 336, Martin Christ Freeze dryers, Osterode Germany). The freeze-dried samples were placed in airtight containers, labelled and stored for further analysis.

### Phytochemical analysis

The dried plant powder was screened for alkaloids, flavonoids, coumarins, tannins, phenols saponins, and glycosides using standard procedures according to Trease and Evans (2000).

### Ethical approval

Ethical approval was obtained from the Biosafety, animal use and care committee of the Faculty of Veterinary Medicine, University of Nairobi.

### Animals

Mice were sourced from the Department of Public health, Pharmacology, and Toxicology, University of Nairobi. They were housed in plastic and acrylic cages in groups of six and maintained in standard laboratory conditions (temperature of between 20-25°C, humidity about 60% and a 12/12 light day cycle) and acclimated for 1 week in the laboratory. They were fed on mice chow (Sigma feeds) and water *ad libitum*. The mice were randomly assigned into experimental and control groups (n=6) as indicated below.

Table 1: Groups of animals.

Group	Description	Treatment
1	Normal control	Vehicle (normal saline)
2	Diabetic (untreated)	Vehicle (normal saline)
3	Diabetic treated	Glibenclamide (5 mg/kg bwt)
4	Diabetic treated	AESNS (150 mg/kg bwt)
5	Diabetic treated	AESNS (300 mg/kg bwt)
6	Diabetic treated	AESS (150 mg/kg bwt)
7	Diabetic treated	AESS (300 mg/kg bwt)

AESS- Aqueous extracts of *S. villosum*; AESNS- Aqueous extracts of *S. nigrum* var *sarracoides*.

### Acute toxicity study

Acute oral test was carried out based on OECD guidelines 423.<sup>11</sup> Female white albino mice 6-8 weeks old

were used. The extracts were dissolved in normal saline as a vehicle and administered orally, at doses not exceeding the volume of 0.1 ml. Starting dose was 300 mg/kg body wt; if no deaths occurred at 300 mg/kg body wt, the test would be repeated at 2000 mg/body wt. The animals continuously separately once during the first 30 minutes, periodically for the first 24 hours, and daily thereafter for a maximum of 14 days. Toxicity signs and the time they occurred and disappeared were noted. Based on the outcome of the acute toxicity test, LD<sub>50</sub> (24 h) was estimated.<sup>11</sup> At the close of the experiment, the animals were sacrificed under anaesthesia.

### Induction of diabetes

Streptozotocin-induced diabetes mice mode was used. Mice were fasted for 8 hours before intraperitoneal injection with Streptozotocin (STZ, Sigma Aldrich, USA) single dose of 200 mg/kg body weight. Streptozotocin was reconstituted using sterile normal saline (0.9% sodium chloride) at a concentration of 20 mg/ml. A drop of blood was collected from the tail vein after overnight fast using glucometer (Softstyle®, Chemlabs, Kenya). Mice with fasting blood glucose levels above 250 mg/dl were considered diabetic.

### Determination of blood glucose levels

Blood was obtained from a prick on the lateral tail vein and blood glucose determined using a glucometer (Softstyle®, Chemlabs, Kenya). Glucose readings were expressed in g/dl. Fasting blood glucose level was determined on day 7, 14, 21 and day 28.

### Biochemical parameters

Blood was collected by cardiac puncture after euthanasia in serum tubes. A clinical biochemical analyzer (Humalyzer 2000, Wiesbaden, Germany) was used to analyze serum samples for liver enzymes alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase (ALT, AST and ALP) using commercial reagent kits (Human Diagnostics, Wiesbaden, Germany) according to the manufacturer's instructions.

### Data analysis

Data was subject to analysis of variance (ANOVA) using statistical analysis system SAS version 9.2. Significance level was set at p<0.05.

## RESULTS

### Phytochemical analysis

Characterization of constituent compounds of aqueous extracts of *S. villosum* and *S. nigrum var sarrachoides* leaves showed that they contain bioactive plant compounds which belong to various multi-component classes of; alkaloids, flavonoids, tannins, saponins,

phenols, phytosterols, coumarins and glycosides. However, phytosterols and coumarins were absent in *S. villosum* extract (Table 2).

**Table 2: Phytochemical composition.**

Phytochemical	<i>S. villosum</i>	<i>S. nigrum var sarrachoides</i>
Flavonoids	+ve	+ve
Alkaloids	+ve	+ve
Tannins	+ve	+ve
Saponins	+ve	+ve
Phenols	+ve	+ve
Phytosterols	-ve	+ve
Courmarins	-ve	+ve
Glycosides	+ve	+ve

### Acute toxicity

Aqueous extracts of both *S. villosum* and *S. nigrum var sarrachoides* did not cause any mortality even at the highest dose of 2000 mg/kg body weight. Equally, there was no discernible change in the demeanor and behaviour of the animals during the observation period. Additionally, there was no colour change on the coat of the test animals even on day 14. Both plants were therefore placed at category 5 based on OECD (2001).

### Effects of *S. villosum* and *S. nigrum var sarrachoides* on body weight in mice

The percentage changes are presented in Table 1 below. Notably, all diabetic mice showed significant loss of body weight (p<0.05) when compared to the non-diabetic mice. *S. villosum* at 150 mg produced weight loss that was not significant different compared to diabetic control group. However, at 300 mg significant weight recovery was achieved. *S.nigrum var sarrachoides* at both 150 mg and 300 mg produced significant weight recovery (Table 3).

**Table 3: Percentage change in body weights.**

Treatment	Change in body weight
Normal mice	15.4% (gain)
Diabetic non-treated	39.4% (loss)
Glibenclamide 5 mg/kg body weight	15.2 (gain)
AESV 150 mg/kg body weight	29.6 (loss)
AESV 300 mg/kg body weight	21.5 (loss)
AESS 150 mg/ kg body weight	20.6 (loss)
AESS 300 mg/ kg bodyweight	15.4 (loss)

### Effect of *S. villosum* aqueous leaf extracts on the fasting blood glucose levels in mice

At the start of the experiment (day 0), all the diabetic mice groups had significantly (p<0.05) higher blood

glucose levels as compared to the non-diabetic mice (Table 4). Administering 300 mg/kg body weight of *S. villosum* aqueous leaves extract produced significant ( $p<0.05$ ) reduction in fasting blood glucose level as compared to the untreated diabetic group by day 14 (Table 4). This, however, was significantly ( $p<0.05$ ) higher when compared to the standard drug glibenclamide at 5mg/kg body weight (Table 4). Notably, at 150 mg/kg body weight, there was no significant difference ( $p<0.05$ ) when compared to the untreated diabetic group by day 14. On day 21, with a dosage of 300 mg /kg body weight, there was a significant ( $p<0.05$ ) reduction in fasting blood glucose levels of the mice when compared to the diabetic mice on day 21 of the study. However, at 150 mg/kg bodyweight, there was no significant reduction in blood glucose between the treated and untreated diabetic mice on day 21 (Table 4). After 28 days of administration with

*S. villosum* extracts (150 mg and 300 mg), the mice showed significant ( $p<0.05$ ) reduction in levels of fasting blood glucose when compared to the diabetic untreated.

#### **Effect of *S. nigrum var sarrachoides* aqueous leaf extracts on the fasting blood glucose levels in mice**

*S. nigrum var sarrachoides* extract at both doses caused a significant ( $p<0.05$ ) reduction in blood glucose level as compared to the untreated diabetic group (Table 4). This was however significantly ( $p<0.05$ ) higher compared to mice treated with glibenclamide (5 mg/kg body weight) on day 14. There was no significant ( $p<0.05$ ) difference in the levels of fasting blood glucose between mice treated with the extract at a dose of 300 mg per kg body weight and those treated with glibenclamide by day 28 (Table 4).

**Table 4: Effects of extracts on fasting blood glucose in mice.**

Description	Blood glucose level in mg/dl (Lsmean±SEM)				
	Initial	Day 7	Day 14	Day 21	Day 28
Normal	102.52±11.34 <sup>b</sup>	99.8±14.35 <sup>b</sup>	98.98±12.53 <sup>f</sup>	98.1±12.10 <sup>d</sup>	102.67±10.97 <sup>c</sup>
Diabetic (untreated)	305.58±12.35 <sup>a</sup>	323.4±12.41 <sup>a</sup>	341.75±12.34 <sup>a</sup>	381.32±13.41 <sup>a</sup>	368.23±13.37 <sup>a</sup>
Glibenclamide	337.18±11.82 <sup>a</sup>	296.08±13.21 <sup>a</sup>	191.53±13.12 <sup>cde</sup>	126.17±14.21 <sup>d</sup>	104.60±12.94 <sup>dce</sup>
AESV 150 mg/kg bwt	317.37±12.34 <sup>a</sup>	322.78±11.62 <sup>a</sup>	288.58±11.72 <sup>ab</sup>	276.63±13.13 <sup>b</sup>	272.10±11.81 <sup>b</sup>
AESV 300 mg/kg bwt	321.15±13.10 <sup>a</sup>	303.72±15.13 <sup>a</sup>	247.98±12.81 <sup>bc</sup>	214.52±12.68 <sup>c</sup>	170.18±13.14 <sup>ce</sup>
AESS 150 mg/kg bwt	305.33±12.16 <sup>a</sup>	273.25±13.26 <sup>a</sup>	230.18± 13.16 <sup>bc</sup>	203.08±12.34 <sup>c</sup>	196.32±12.61 <sup>c</sup>
AESS 300 mg/kg bwt	333.77±11.76 <sup>a</sup>	293.90±12.68 <sup>a</sup>	238.45±12.71 <sup>bd</sup>	198.13±12.21 <sup>c</sup>	162.25± 13.42 <sup>ce</sup>

Means within a column with different superscript letters denote significant differences ( $p<0.05$ ) (n=6). AESV-Aqueous extracts of *S. villosum*; AESS- Aqueous extracts of *S. nigrum var sarrachoides*.

**Table 5: Effects of *S. villosum* and *S. nigrum var sarrachoides* on liver enzymes AST, ALT and AP in mice.**

Treatment	Enzyme		
	Alanine amino transferase	Aspartate amino transferase	Alkaline phosphatase
Normal	19.6±1.40 <sup>a</sup>	12.05±0.21 <sup>a</sup>	166.55±2.47 <sup>d</sup>
Diabetic	35.40±2.27 <sup>b</sup>	48.23±1.24 <sup>b</sup>	235.63±4.12 <sup>a</sup>
Standard drug	23.5 ±1.23 <sup>c</sup>	24.37±2.12 <sup>acd</sup>	191.6±2.62 <sup>cd</sup>
AESV 150	33.00±2.21 <sup>c</sup>	30.30±2.43 <sup>c</sup>	236.25±3.18 <sup>a</sup>
AESV 300	23.58±1.13 <sup>c</sup>	29.02±2.19 <sup>c</sup>	229.20±5.73 <sup>ab</sup>
AESS 150	24.88 ±2.25 <sup>c</sup>	25.50±1.37 <sup>acd</sup>	224.58±5.47 <sup>ab</sup>
AESS 300	22.10±1.26 <sup>c</sup>	23.06±0.3 <sup>acd</sup>	204.83±3.51 <sup>bc</sup>

Means within a column with different superscript letters denote significant differences ( $p<0.05$ ) (n=6). AESV- Aqueous extracts of *S. villosum*; AESS- Aqueous extracts of *S. nigrum var sarrachoides*.

#### **Effects of *S. villosum* and *S. nigrum var sarrachoides* on liver enzymes**

##### *Alanine aminotransferase levels*

There was a significant difference in the levels of Alanine aminotransferase (ALT) between the diabetic control mice and all the other test groups. However, there was no significant ( $p<0.05$ ) difference in the levels of ALT between the mice groups treated with aqueous extracts of *S. villosum*, those treated with either glibenclamide (5

mg/kg) or aqueous extracts of *S. nigrum sub sarrachoides* (Table 5).

##### *Aspartate aminotransferase*

The levels of aspartate aminotransferase (AST) in serum of the diabetic control mice was significant ( $p<0.05$ ) compared to all the other groups (Table 5). In addition, the levels of AST in the mice that received glibenclamide (5mg/kg body wt), *S. villosum* (300 mg/kg body wt), and *S. nigrum sub sarrachoides* (300 mg/kg body wt) were

not significantly different ( $p < 0.05$ ) from the normal control group (Table 5).

#### Alkaline phosphatase

Results show no significant ( $p < 0.05$ ) differences in the serum Alkaline phosphatase (ALP) levels between mice that were treated with aqueous extracts of *S. villosum* (both 150 mg and 300 mg / kg bodyweight), mice that received aqueous extracts of *S. nigrum sub sarrachoides* (150 mg/kg body wt) and the diabetic non treated group. *S. nigrum sub sarrachoides* (300 mg/kg bodywt) produced a reduction that was not significant ( $p < 0.05$ ) compared to the glibenclamide treated group. However, all the test groups had higher levels compared to the normal control save for the glibenclamide group.

## DISCUSSION

### Phytochemical evaluation

In the qualitative phytochemical analysis, the results show that both extracts possess important phytochemical compounds that have been shown to impart medicinal properties in plants.<sup>12,13</sup> Specific secondary metabolites that have been shown to impart antidiabetic effects include glycosides, alkaloids, terpenoids and flavonoid.<sup>14</sup> Despite their antidiabetic effects, these chemicals also possess other beneficial effects including antioxidant, anticancer, and anti-inflammatory properties.<sup>15,16</sup> In the present study, the aqueous extracts from leaves of *S. nigrum var sarrachoides* possessed all the phytochemicals tested. Comparatively, *S. villosum* extract showed the presence of all the phytochemicals with the exception of phytosterols and coumarins. The differences in the antidiabetic properties of the two extracts observed in this study can be attributed to this difference. Additionally, this finding is significant because it reinforces the need for individual species within the *S. nigrum* complex to be assessed for its anti-diabetic properties. The results agree with other researchers who have shown the presence of important phytochemicals in *S. nigrum*.<sup>12,13</sup> Further, differences in soils and climatic conditions prevalent in different regions occasions the differences in phytochemical composition.

### Acute toxicity studies

Safety of any drug is of paramount importance and can be a serious limiting factor to its utilization. OECD has developed guidelines (OECD 423) for an effective assessment of the safety of herbal remedies.<sup>11</sup> The present study revealed that both the extracts have considerably wide safety margins. The guidelines emphasize on the following as the major signs that point to toxicity; respiratory, circulatory, behavioural changes, convulsions, lethargy, diarrhoea, tremors, salivation coma or death. None of these signs were observed upon extract administration. Therefore, results from the present study

agrees with other studies reported.<sup>17</sup> The present study, therefore, adds onto the evidence already present regarding the safety of *S. nigrum* complex species. It can be concluded that both species are safe.

### Hypoglycaemic effect

In the present study, aqueous extracts of leaves from both plants were observed to possess significant hypoglycaemic effect in a dose dependent manner. These results agree with those of other workers who have reported similar effects.<sup>18-20</sup> Glucose-lowering effects of the extract are postulated to be as a result of potentiating effect of the extract on insulin. Insulin release from remnants of  $\beta$ -cells of the Islets of Langerhans in the pancreas is enhanced.<sup>21</sup> The extracts may also mediate extra-pancreatic action in glucose metabolism pathways by stimulating an increase in peripheral glucose uptake and a decrease in gluconeogenesis and increase in glycogenesis with the net effect of reducing blood glucose levels. In addition, antioxidant activities play a vital role in the amelioration of the effects.<sup>22</sup> Although all the extracts at both dosages (150 mg and 300 mg) showed a significant reduction in the fasting blood glucose, *S. nigrum var sarrachoides* exhibited a higher potency in hypoglycaemic effect. At the dosage of 150 mg/ kg bwt, it reduced the blood glucose levels significantly from day 14 compared to *S. villosum* that showed a significant reduction from day 21 at the same dosage. However, both extracts at a dosage of 300 mg reduced the fasting blood glucose level significantly. This reduction was as good as the reduction exhibited by glibenclamide. Therefore, both extracts exhibited dose-dependent effects. The above-described phenomenon can be attributed to differences in active phytochemicals and their concentrations including the peculiarity of their respective modes of action.<sup>23</sup> The secondary metabolites have different action sites in the body that can influence their action.<sup>13</sup>

### Effect on bodyweight

Diabetes is a debilitating condition that is characterized by remarkable weight loss. The weight loss occurs as a result of aberration in the body metabolic processes.<sup>21</sup> Therefore, amelioration of weight loss is an indicator of improvement of metabolic properties. Diabetic mice showed significant loss in body weight when compared to the normal non-diabetic mice. This finding agrees with those of Wang et al, being reflective of the situation in diabetes mellitus where the patients experience progressive weight loss.<sup>5</sup> In this case, the loss in body weight after diabetes induction using STZ has been attributed to increased catabolic reactions in the body.<sup>24</sup> Diabetes mellitus occasions aberrations in glucose homeostasis and hence the body mobilizes protein sources for energy resulting in muscle wasting.<sup>25</sup> In the present study, administration of aqueous extracts of *S. villosum* at 300 mg/kg bwt and *S. nigrum var sarrachoides* at 150 mg/kg bwt and 300 mg/kg bwt produced a significant

amelioration of weight loss compared to the untreated diabetic mice. The results can be attributed to improving glycaemic control imparted by the antidiabetic elements in the plant. Many plants extracts have been shown to ameliorate body weight loss.<sup>7</sup>

*S. villosum* extract at 150 mg/kg body wt produced weight loss that was not significantly different when compared to the untreated diabetic mice. This finding can be attributed to the reduced potency of the extract for antihyperglycemic control and points to a dose-dependent activity of *S. villosum*. Other researchers have recorded a dose-dependent activity in *S. nigrum* and other herbs.<sup>20-27</sup> It is worthwhile to note that all diabetic- treated mice were unable to gain their initial weight at the end of the experiment. This phenomenon can be attributed to the severe extent of damage of  $\beta$ -cells of the Islets of Langerhans in the pancreases with only minimal residual effect functional capacity being regained.

### Liver enzymes

Transaminases are marker enzymes in the blood that are important in the manifestation liver and heart damage.<sup>28</sup> Increased levels of ALT, AST and ALP are suggestive of liver and heart damage.<sup>14,29</sup> It has been observed that administration of STZ in mice causes hepatotoxicity that causes leakage of these marker enzymes into the blood in a dose-dependent manner.<sup>29</sup> Streptozotocin at 200 mg/kg body weight in mice used in this study induced severe diabetes in mice.<sup>24</sup> The ability to ameliorate diabetic effects causes a reduction in the levels of these enzymes. In the present study, all extracts showed a significant reduction in levels of ALT compared to the untreated diabetic group. The reduction was however not sufficient to revert back the enzymes to the normal levels. Characteristically, this phenomenon can be attributed to the inability of the treatments to restore the normal state of functioning of the hepatocytes damaged by STZ. Similarly, the extract resulted in a reduction of AST levels when compared to the untreated diabetic control. The extracts were however less effective compared to glibenclamide apart from *S. nigrum var sarracoides* at 300 mg. This suggests that the latter was more effective in effect. This can be attributed to the presence of phytochemicals in increased concentration.<sup>23</sup> In the AP assay, the extracts from both plants did not produce a significant reduction in levels apart from *S. nigrum var sarracoides* which produced a reduction that was not significantly different when compared to both normal and glibenclamide treated mice. Therefore, these findings suggest increased potency. In conclusion, these results suggest that *S. villosum* and *S. nigrum var sarracoides* have protective effects on the liver and heart. Further, the results agree with the findings of other researchers.<sup>30,31</sup> This can be attributed to the presence of tannins and flavonoids.<sup>13</sup> From the study, it can be concluded that *S. nigrum var sarracoides* at 300 mg/kg body wt is more potent in reversing the liver damage in this model.

### CONCLUSION

Based on the results from this study, it can be concluded that both plants have a significant antidiabetic effect and hence their use in folklore medicine. Further, it can be concluded that both plants do not cause toxicity that can limit their use in herbal medicines. Comparatively, *S. nigrum var sarracoides* showed to be more effective in ameliorating diabetic effects.

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