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Evaluation of the Anti-snake Venom Activity of Leaf Extract of *Sansevieria liberica* ger.& labr (Agavaceae.) in Mice

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Abstract: The prevalence of snake bite and death from snake bite envenomation is becoming a serious public health problem globally. The cost, non availability and the adverse reactions associated with antisnake venom serum has favoured the use of medicinal herbs by traditional healers in the treatment of snakebites. In this study we evaluated the antisnake venom activity of the leaves of *Sansevieria liberica* against *Naja naja nigricollis* venom in mice. *S. liberica* is very popular among the traditional healers in south east Nigeria for the treatment of snake bites. The ground fresh leaves were extracted with ethanol and a portion of the ethanol extract was fractionated with n-hexane, ethyl acetate and butanol to afford the respective solvent fractions. The extract was subjected to acute toxicity (LD_{50}) and phytochemical testing. The LD_{50} of the venom was similarly determined, The antisnake venom activity of the extract/fraction was determined against the LD_{50} (353.5 ug/kg) and double the LD_{50} (707 ug/kg) of the venom. The effect of the extract on bleeding and clotting time of the venom-intoxicated mice was investigated. Also studied was the effect of the extract on acetylcholine-induced contraction of the isolated frog rectus abdominus. The extract/fractions significantly protected the mice from *Naja naja nigricollis* venom-induced mortality in mice. The bleeding and clotting time of the venom-intoxicated rats were significantly ($p < 0.05$) decreased by the extract/fractions. The acetylcholine-induced contraction of frog rectus abdominus was significantly inhibited by the extract. These results suggest that the leaves of *S. liberica* exhibit antisnake venom properties that could be harnessed in treating patients with snakebite envenomation.

Keywords: *Sansevieria liberica*, *Naja naja nigricollis*, Bleeding Time, Clotting Time, Frog Rectus Abdominus

Introduction

Envenomation resulting from snake bites is an important public health problem in rural areas of tropical and sub- tropical countries of Africa, Asia, Oceania and Latin America (Chippaux 1998). It is a major socio-medical problem of south east Asia and sub-sahara African countries. About 216 species of snake occur in India alone, and about 25 % of which is poisonous (Bawaskar, 2004). Poisonous snake species like *Echis carinatus, Naja naja, Daboia russelli, Bungarus caeruleus* and *Ophiophagus hannah* account for the majority of the bites and mortality (Gomes et al, 2010). The exact epidemiology as well as number of deaths from snake bites is difficult to obtain. This is partly due to the fact that most of the snake bites occur in the rural areas, and also the dependence on traditional healers.
Snake venom is a very complex poison comprising a mixture of enzymatic and non-enzymatic toxic compounds and other non-toxic proteins, carbohydrates and metals. The poison components can incorporate proteases, nucleases, phosphodiesterases and other substances which alter cell functions and physiological processes (Sajon et al, 2017). The venom toxins are mainly neurotoxins, cytotoxins, myotoxins and cardiotoxins which evoke the variety of adverse reactions and death associated with snake venoms.

The treatment of snake bite varies as the bite and snake species. Antivenom immunotherapy remains the specific treatment against snake bite. In most poor resource countries where incidentally, snake bites are frequent, antivenoms are expensive and limited in supply. The mode of administration, usually parentrally, is another limitation. Most antivenoms are not broad spectrum, and their effectiveness may dependent on the type of snake involved and the time lag before administration. Antivenom therapy is associated with a number of side effects like anaphylactic shock, pyrogen reaction and serum sickness (Maya Devis et al, 2002).

The plant kingdom offers alternative option for the management of snake bites. Over the years many attempts have been made for the development of

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snake venom antidotes from plants sources. In most rural areas, traditional healers are the first port of call in cases of snake bite, and some of these cases were successfully treated with folk medicines, especially medicinal plants. Several medicinal plants, which appear in old drug recipes or which have been passed on by oral tradition, are believed to be snakebite antidotes and are recommended for the treatment of snake bite (Alam and Gomes, 2003; dos Santos Gomes et al, 2010; Kaushik et al, 2013). A number of medicinal plants including Jatropha species (Gomes et al, 2016; Felix-Silva et al, 2018), *Asystasia gangetica* (Enenebeaku et al, 2018), *Parkia biglobosa* (Asuzu and Harvey, 2003), *Aloysia citriodora* (Caceres et al, 2017), *Sapindus saponaria* (da Silva et al, 2012), *Albizia lebbeek* (Amog et al, 2016), *Carissa spinarum* (Janardhan et al, 2015), *Piper longum* (Shenoy et al, 2013) and *Crinum jagus* (Zadani et al, 2018) have been reported to possess antivenom properties.

In the eastern part of Nigeria, the leaves of *Sansevieria liberica* ger.& labr is a common folk remedy for the treatment of snake bite, irrespective of the species of snake involved. Traditional healers in the area have attested to the usefulness and effectiveness of *S. liberica* leaves in the treatment of snake bite.

Sansevieria liberica belongs to the family Agavaceae. It is one of the bowstring hemp species and a pretty plant with bright green leaves growing 45 to 100 cm tall, with a smooth texture and light grey tip (Evans, 2005). The leaves are typically arranged in a rosette (Chahinian, 2005). It is known as "Moda" (Hausa), "Ebubagu" (Igbo) and "Ijo-ikoko" in (Yoruba) tribes of Nigeria. The common English name is Bow string hemp. The medicinal uses of *S. liberica* depend on the region. In the Northern part of Nigeria, it is used in traditional medicine to treat menorrhagia and menstrual pains, and to normalise abnormal menstrual period (Sambo and Ali, 2008). In the Western and Eastern parts of Nigeria it is used to treat diarrhoea, abdominal pains, gonorrhea, eczema, pile, snake bite, impotence, asthma, and high blood pressure (Odugbemi, 2008).

The anti-inflammatory (Ratheesh, 2007; Eze et al, 2011), anti-anaemic (Ikewuchi et al, 2010), sedative and anti-convulsant activities (Adeyemi et al, 2007) of the leaves and roots have been reported Also reported are the antidiarrhoeal (Adeyemi, et al, 2009), antihypertensive (Ikewuchi et al, 2011), analgesic (Umukoro et al, 2008), in vitro antitrypanosomal, antileishmanial and antiplasmodial (Bero et al, 2009, 2011), diuretic (Omodamiro and Jimoh, 2017) and anti-oxidant (Ikewuchi et al, 2013) activities.

To the best of our knowledge, there is no scientific report on the anti-snake venom potentials of *S. liberica*. The aim of this study was to evaluate the anti-snake venom activity of the leaves of the plant against *Naja n.nigricollis* venom in rodents.

Materials And Methods

Collection and authentication of plant materials

Fresh leaves of *S. liberica* were harvested from Ekwulobia in Aguata Local Government Area of Anambra State Nigeria and were identified Mr. Patrick Ugwuozo of the Department of Botany, Nnamdi Azikiwe University, Awka Anambra State, Nigeria. Voucher specimen No. NAU 456 was deposited at the Herbarium of the Department of Pharmacognosy, Nnamdi Azikiwe University, Awka, Nigeria.

Preparation of plant materials

The methods used traditionally to prepare the plant materials which include powdering of the plant material followed by solvent (aqueous or organic) extraction was adopted. The fresh leaves were washed clean, cut into smaller pieces and pounded with a mortar and pestle. About 400 g of ground wet leaves was macerated with 2.5 litres of ethanol for 48 hours. The resulting solution was filtered using muslin cloth and concentrated under vacuum using rotary evaporator. The extract was further concentrated to dryness using water bath at 50 ^{o} C to yield 24.53g (6.13%) of crude extract. Using solid-liquid method of fractionation on a silica gel the crude extract was partitioned with n-hexane, ethyl acetate and butanol to obtain n-hexane (HF), ethyl acetate (EAF) and butanol (BF) fractions respectively in order of increasing polarity.

Snake venom

Freeze-dried venom of *Naja. n. nigricollis* was obtained from the Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Animals

Albino mice of either sex (21-25 g) were used for the study. They were obtained from the Animal House of the Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Awka, Nigeria. The animals were housed in standard laboratory cages and conditions of 12 h light/dark cycle, at room temperature. Rodent feed (Guinea Feeds Nigeria Ltd) and clean water were provided *ad libitum*. All animal experiments was conducted in strict compliance with NIH guide for care and use of laboratory animals (National Institute of health (NIH) (2011) Pub No: 85-23).

Phytochemical screening

Qualitative phytochemical analysis of the extract was carried out to identify the presence of secondary metabolites using standard methods (Harborne, 1984).

Acute toxicity studies
Acute toxicities (LD_{50}) of the extract (oral) and the snake venom (intraperitoneal) were determined using Lorke's method (Lorke, 1983).

Anti snake venom activity
The anti- snake venom activity of the crude extract and the fractions was tested in vivo against the LD_{50} (353.5 ug/kg) and double the LD_{50} (707 ug/kg) of the snake venom. The extract and fractions were administered at the dose of 125, 250 and 500 mg/kg orally. For this study, a total of 100 mice were used. The mice were divided into 4 groups (1-4). Group 1 served as the control, and received distilled water plus the two doses of the venom respectively. Groups 2-4 were used for immediate (concomitant), prophylaxis and curative treatments respectively. They were each subdivided into 6 (n=5) and treated as follows:

Group 1 (control)
1A: Distilled water (5 ml/kg) + venom (353.5 ug/kg).
1B: Distilled water (5 ml/kg) + venom (707 ug/kg).

Group 2. The venom was administered concomitantly with the extract.
2 A: Extract (125 mg/kg) + venom (353.5 ug/kg).
2 B: Extract (250 mg/kg) + venom (353.5 ug/kg).
2C: Extract (500 mg/kg) + venom (353.5 ug/kg).
2D: Extract (125 mg/kg) + venom (707 ug/kg).
2E: Extract (250 mg/kg) + venom (707 ug/kg).
2F: Extract (500 mg/kg) + venom (707 ug/kg).

Group 3. The venom was administered 1 hour after the extract (Prophylaxix)
3A: Extract (125 mg/kg) + venom (353.5 ug/kg).
3B: Extract (250 mg/kg) + venom (353.5 ug/kg).
3C: Extract (500 mg/kg) + venom (353.5 ug/kg).
3D: Extract (125 mg/kg) + venom (707 ug/kg).
3E: Extract (250 mg/kg) + venom (707 ug/kg).
3F: Extract (500 mg/kg) + venom (707 ug/kg).

Group 4. The extract was administered 30 minutes after the venom.
4A: Venom (353.5 ug/kg) + extract (125 mg/kg).
4B: Venom (353.5 ug/kg) + extract (250 mg/kg).
4C: Venom (353.5 ug/kg) + extract (500 mg/kg).
4D: Venom (707 ug/kg) + extract (125 mg/kg).
4E: Venom (707 ug/kg) + extract (250 mg/kg).
4F: Venom (707 ug/kg) + extract (500 mg/kg).

Anti snake venom activity of the fractions
The fractions (125 mg/kg p o) and the venom (707 ug/kg ip) were administered immediately (concurrently). The animals were observed for mortality for 24 hours.

Evaluation of bleeding
Twenty five (25) adult albino mice grouped into five (n=5) were used for the study. Group 1 received distilled water and severed as the control. Group 2 had the venom (707 ug/kg) alone, while groups 3-5 received the venom and the extract 125, 250 and 500 mg/kg respectively. The bleeding time was measured using the method of Mohammed et al (1969). Two hours after administering the venom (707 ug/kg), and treatment with the extract, pressure was built at the tail of the animal by stroking it with fingers. Then a sterile needle was used to puncture one of the blood vessels lying alongside of the tail. The blood that oozed out of the site was blotted gently but completely with filter paper every 15 seconds until bleeding ceases. The time of the first appearance of blood and stopping of the blood flow was taken as the bleeding time.

Evaluation of clotting time
This study involved twenty five (25) adult albino mice grouped into five (n=5). Group 1 received distilled water and severed as the control. Group 2 had the venom (707 ug/kg) alone, while groups 3-5 received the venom and the extract 125, 250 and 500 mg/kg respectively.

The clotting time was evaluated using capillary coagulation method. Non-heparinized capillary tube was used to collect blood from the retro-orbital plexus of the animal through capillary action until whole length of the tube was filled with blood. At 15 seconds interval, one half of the capillary tube was carefully broken off at one end and gently pulled apart to look for the fill in thread which is an indication of clotting. The breaking of the tube was done at 15 sec interval until fibrin thread was seen. The time it took the thread to form is taken as the clotting time (Igboechi and Anuforo, 1986).

Invitro study
Effect on frog rectus abdominus
Adult frog were killed and cut open. The rectus abdominus was obtained and mounted in an organ bath containing Frog Ringer solution of the following composition gram/litre NaCl (6.0), KCl (0.075), $CaCl_2$ (0.100), $NaHCO_3$ (0.100) and aerated with air and maintained at room temperature. The resting tension on the tissue was 1.5 g. The tissue was equilibrated for 30 minutes before graded responses to acetylcholine were obtained Responses to the sub-maximum concentration of Ach were re-established in the pressure of 10 and 20 mg of the extract after 15 minutes incubation. The responses were recorded on Unirecorder 7050 (Ugo Basile, Italy) through an isotonic Transducer, 7004 (Ugo Basile, Italy).

Statistical analysis
Results were presented as mean ± Standard error of mean (SEM). Data were subjected to one way analyses of variance (ANOVA) followed by Post-hoc Turkey's test for multiple comparison. Statistical package for social science (SPSS-20) was used for data analyses.
$P < 0.05$ was considered to be statistically significant.

Results

Phytochemical constituents

Qualitative phytochemical analysis revealed the presence of saponins, flavonoids and terpenoids in high amounts while alkaloids, steroids and cardiac glycosides occurred in smaller amount quantities (Table 1).

Table 1: **Phytochemistry of ethanol leaves extract of *Sansivieria liberica***

Phytochemical constituents	Amount
Alkaloids	+
Saponins	++
Tannins	-
Flavonoids	++
Steroids	+
Terpenoids	++
Cardiac Glycosides	+
Proteins	-
Carbohydrates	-

(+) present in small concentration, **(++)** present in high concentration, **(-)** absent

Acute toxicity test

The acute toxicity test indicated that the LD_{50} of the extract was above 5000 mg/kg, while that of the snake venom was 353.55ug/kg.

Effect of the extract on LD_{50} of the snake venom

On simultaneous administration of the extract with the venom, 100 % protection was afforded by 125 and 250 mg/kg of the extract (Table 2).

In prophylactic studies, all the mice survived at the doses of 125 and 250 mg/kg of the extract. However 80% protection was recorded at 500 mg/kg dose (Table 2).

When the extract was administered thirty minutes after exposure of animals to the LD_{50} dose of the snake venom, all the animals treated with 125 mg/kg of the extract were protected while 80% protection was recorded at 250 and 500 mg/kg doses (Table 2).

Table 2. Effect of the extract on the LD_{50} of the snake venom

Treatment	Dose (mg/kg)	No of animals	Mortality	% survival
Control (distilled water)	5ml/kg	5	3	40
Concomitant				
Extract	125	5	0	100
	250	5	0	100
	500	5	1	80
Prophylaxis				
Extract	125	5	0	100
	250	5	0	100
	500	5	0	100
30 minutes after administration of snake venom				
Extract	125	5	0	100
	250	5	1	80
	500	5	1	80

Effect of the extract on double LD_{50} (707 ug/kg) of the snake venom:

The effect of extract on the double LD_{50} of the venom is shown in Table 3. The extract was more effective prophylactically as all the mice survived at all the 3 doses of the extract tested,

When the extract was administered thirty minutes after exposure of the animals to twice the LD_{50} of the snake venom, the percent protection was dose dependent (Table 3).

Table 3. Effect of the extracts on double LD_{50} (707 ug/kg) of the snake venom

Treatment	Doses (mg/kg)	No of animals	No of Death	Survival	% survival
Control (distilled water)	5ml/kg	5	5	0	0
Concomitant					
Extract	125	5	0	5	60
	250	5	1	4	80
	500	5	1	4	80
Prophylaxis					
Extract	125	5	0	5	100
	250	5	0	5	100
	500	5	0	5	100
30 minutes after administration of snake venom					
Extract	125	5	3	2	40
	250	5	2	3	60
	500	5	0	5	100

Anti-snake venom effect of the fraction

The ethyl acetate fraction (125 mg/kg) protected 100% of the animals from the 707 ug/kg dose of the venom while the n-hexane and butanol fractions at the same dose afforded 60 and 40 % protection respectively. (Table 4).

Table 4. Effect of the fractions on snake venom

Treatments	Dose mg/kg	No of animals	No of death	Survival	%Protection
Control (distilled water)	5 ml/kg	5	5/5	0/5	0
n-hexane fraction	125	5	2/5	3/5	60
Ethyl acetate fraction	125	5	0/5	5/5	100
Butanol fraction	125	5	3/5	2/5	40

Effect of the extract on bleeding time.

There was a significant ($p < 0.05$) and dose-dependent reduction in the venom-induced haemorrhage in mice. (Table 5).

Table 5. Effect of the extract on bleeding time.

Treatment	Bleeding time (sec)
Control, (Distilled water 5 ml/kg)	1.1 ± 0.14
Snake venom (707 ug/kg) alone	2.2 ± 0.08
Extract 125 mg/kg	1.0 ± 0.14*
Extract 250 mg/kg	0.8 ± 0.27*
Extract 500 mg/kg	0.5 ± 0.06*

Values are presented as mean ± Standard error of mean (SEM), n =5. *P < 0.05

Effect of the extract on clotting time

The venom-induced increase in clotting time was significantly ($p < 0.05$) and dose dependently decreased by the extract (Table 6).

Table 6: Effect of the wet extract on clotting time.

Treatment	Clotting time (sec)
Control, (Distilled water 5ml/kg)	1.31 ± 0.14
Snake venom (707 ug/kg) alone	2.60 ± 0.9
Extract 125 mg/kg	1.43 ± 0.12*
Extract 250 mg/kg	1.08 ±0.06*
Extract 500 mg/kg	0.84 ± 0.17*

Values are presented as mean ± Standard error of mean (SEM), n =5. *P < 0.05:

Effect of the extracts on acetylcholine induced contraction of the frog rectus abdominus

The sub-maximum response induced by acetylcholine was reduced to 80 and 100 % by 10 and 20 mg of the extract respectively (data not shown)

Discussion

The adverse reactions associated with antisnake serum (Morais and Massaldi, 2009) have necessitated the search for alternatives for snake bite therapy. Serious attention has been shifted to herbal remedies mainly due to the acclaimed successes by the traditional healers in the management of snake bites with herbs. In this modern time, many locals still rely on the use of medicinal plants by traditional healers for the treatment of snake bites. A good number of plants have been reported to show good potentials for the treatment of snake bites (Mors, 1991., Gomes et al., 2010), and many procedures are been used to investigate plants with antisnake bite activity (Harvey, 2003, Adazu, et al 2005, Ode and Asuzu, 2006, da Silva et al 2012, Felix-Silva et al 2014). In view of the high cost of conventional anti-venoms and the significant percentage of patients who react adversely to them (Corrigan *et al*, 1978; Boyer *et al*, 2001), a systematic investigation of plant-based remedies for snake bite is justified.

In this study we investigated the leaves of *Sansevieria liberica* for antisnake venom activity. The results showed the ethanol extract of the leaves as a good candidate for treating snake bites. The result of the phytochemical screening of the extracts indicated the presence of some secondary metabolites that have been reported to have antisnake venom actions. Selvanayagam *et al* (1996) reported that many phytochemical constituents like flavonoids, quinonoids, xanthene, terpenoids and polyphenol possess protein binding and enzyme inhibiting properties and also inhibit snake venom phospholipase A2 (PLA2) activities of both viper and cobra venom. Chatterjee *et al* (2006) reported that lupeol acetate (an alkaloid) isolated from the root extracts of Indian *Sarsaparilla hemidesmas indicus* neutralized the lethality, haemorrhagenation, edema and phospholipase A2 (PLA2) effects induced by *Doboia kauthia* venom. Furthermore, triterpenoid present in *V. negunda* and *E. officinalis* were reported to be involved in venom inactivation processes and pentacyclic triterpenes are found widely in several anti-snake venom plants such as *Aegle marmelos*, *Centipeda minima*, *Aloe vera*, *Phyllanthus niruri*, *Alstonia scholaris*, *Phyllanthus emblica*, *Elephentopus scaber*, etc. (Mors *et al*, 2000). The effective venom neutralizing effect of *Pioer longum* fruit against Russell 's viper venom was attributed to the presence of piperine (Shenoy et al, 2013). The high flavonoids and alkaloids contents of the extract may partly contribute to the anti-venom activity of the extract, since they have been reported to possess protein binding and enzyme inhibiting properties, and also inhibit snake venom phospholipase A2 (PLA2) activity (Santosh *et al*, 2004).

Most snake venoms cause local and systemic bleeding which is the consequence of the damage to blood vessel walls by venom components. Therefore the ability of the extract to prevent the venom-induced hemorrhagic activity may contribute to its anti-venom activity. Similar results have been reported for *Hemidesmus indicus* (Alam et al, 1994), *Vitex negundo* and *Emblica officinalis* (Alam et al, 2003), and *Cclipta prostrate* (Pithaynnkul et al, 2004).

The more potent anti-snake venom activity demonstrated by the ethylacetate fraction is indicative of the presence of more bioactive secondary metabolites in the fraction as ethylacetate has been reported by various authors to be an efficient solvent having the ability to scavenge both polar and nonpolar bioactive secondary metabolites especially alkaloids, flavonoids and saponins (Santosh *et al*, 2004). The ability of the extract to block acetylcholine-induced contraction of the skeletal muscle (Frog Rectus Abdominus) may be useful in ameliorating the venom-induced myotoxicity.

The results of this study indicate that the leaves of *Sansevieria liberica* contain pharmacologically active compounds that could be effective in treating patients with snakebite envenomation.

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