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Original Research Article

Evaluation of Antisnake Venom Activities of Some Medicinal Plants Using Albino Rats

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Abstract

Snakebite is a neglected public health problem in most of the countries in Africa. Its means of treatment is parenteral administration of serum-based antivenins. But due to the limitations of these antivenins, snakebite victims mostly depend on herbal antidotes. Hence, this research was designed to screen the antisnake venom activity of some medicinal plants on mixed venoms of *Naja nigricollis*, *Echis ocellatus* and *Bitis arietans* using Albino rats' model. Information on the medicinal plants used for the treatment of snakebite envenomation in Zuru Local Government Area was obtained through ethnobotanical survey. Four (4) most cited plants; *Parkia biglobosa* stem-bark, *Calotropis procera* root, *Sterculia setigera* stem-bark and *Bauhinia rufescens* seed were selected and screened against the venom-induced lethal effect on albino rats. Albino rats of both sexes were randomly divided into six (6) groups of five (5) rats each and used for the antivenom screening of each plant methanol extract. Group 1 received distilled water. Groups 2, 3 and 4 received 0.8mg/kg b. wt. of the venom mixture, but 3 and 4 were treated with 200 and 400 mg/kg b. wt. of an extract respectively. Group 5 received 400mg/kg b. wt. of an extract only. Finally, group 6 received 0.8mg/kg b. wt. of the venom mixture and treated with standard antivenin. The plants' extracts exhibited antivenom activities with varying degrees of efficacy. At 400mg/kg b. wt. of extract, the recorded mean animal survival times were 18.52 ± 1.50h, 14.04 ± 4.79h, 18.66 ± 4.32h and 5.96 ± 2.79h for *C. procera* root, *P. biglobosa* stem-bark, *B. rufescens* seed and *S. setigera* stem-bark extracts respectively. Thus, *B. rufescens* methanol seed extract was the most potent antivenom agent. These findings suggest that, the selected plants have potent antivenom activity on the selected snake venoms and can serve as lead for the development of safe, readily available and affordable antivenoms that can substitute the use of serum-based antivenins.

Keywords: Medicinal Plants, Snakebite, Venom, Envenomation, Antivenom.

INTRODUCTION

Snakebite envenomation remains a common health problem in almost all rural areas of the tropics. Unfortunately, in most rural areas, modern health facilities are either very poor or completely absent [1]. In places where such facilities exist, delicate medicines such as serum-based antivenins (that require refrigeration) are not available due to erratic power supply. Considering the urgency required in administering antivenom after snakebite (that is before venom toxins overwhelm the body), transporting victims to far away city hospitals, where antivenins might be found, is in many cases fruitless. Furthermore, the conventional antivenins are expensive and thus not affordable to most poor victims. Additionally, the antivenins can cause adverse reactions, such as anaphylactic shock, pyrogen reaction and serum sickness [2]. They do not provide enough protection against venom induced toxicities, such as, necrosis, haemorrhage, cytotoxicity, nephrotoxicity, etc. [3].

Although no known plant-based antivenom is available in Nigerian markets yet, it is a common phenomenon to see some people simultaneously playing with different kinds of poisonous snakes in public places and during festivities. These people claim to have effective cure and themselves are immune to snakebites. They serve as the first points of call when snakebites occur in their communities and there are many instances where their medication evidently cured what could have been a fatal envenomation and all this 'power' comes from plants. This observation gives hope that if these plants are properly investigated there is a high possibility of coming up with effective and wide-spectrum antivenom at least against the common poisonous snakes found in Northern parts of Nigeria.

MATERIALS AND METHODS

Area of Study/Sampling

The appropriate plants were identified from snake-charmers and hunters of the *Lelna* tribe, Zuru, Kebbi State, Nigeria. Zuru is the headquarters of Zuru Emirate as well as Zuru Local Government Area of Kebbi State, Nigeria. It has an area of 653 km^2 and geographical coordinates are 11^o 25' 49" North, 5^o 14' 15" East. The main language spoken by the *Lelna* tribe is *C'lele* [4]. Acquaintances and informants were used to gain access to the snake-charmers and hunters. Information on the method of preparation, administration, dosages, etc. was also obtained. Zuru is the home to the *Lelna* tribe who are well-known for their successful use of plants in almost all their medications including prevention and treatment of snakebites.

Experimental Animals

Adult Wister albino rats of both sexes aged 2-3 months and weighing between 200 – 250 g were used for the experiments. They were purchased from National Veterinary Research Institute, Vom, Nigeria and kept under standard laboratory conditions (22–24^oC; 12:12 h dark/light cycle). The animals were allowed free access to both food (commercial rodents pellets) and water *ad libitum* [5], they were allowed to acclimatize for 2 weeks. Weight of each rat was taken before the commencement of each experiment. All animal experiments were conducted in accordance with the guidelines for the use and care of experimental animals [6].

Standard Snake Venom Antiserum (Antivenin)

The lyophilized polyvalent snake venom antiserum (Batch No.: 01AS83659, Man. Date: March, 2018, Exp. Date: February, 2021) was used as standard to compare with the efficacy of the various plants extracts. It was produced by a standard pharmaceutical company (VINS Bioproducts Limited, Andhra Pradesh, India).

Collection of Snakes

The snake species; *Naja nigricollis* (black-necked spitting cobra), *Echis ocellatus* (carpet viper) and *Bitis arietans* (puff adder) were captured and housed in wooden cages with the help of snake charmers. After collection, they were duly identified by a Zoologist at the Department of Animal and Environmental Biology, Kebbi State University of Science and Technology, Aliero, Nigeria.

Milking of Venom

The venoms were collected between 5.00 pm to 6.30 pm, in a low light condition at ambient temperature according to the method of Goswami *et al*., [7] by using a short-acting general anesthesia; halothane (Piramal Healthcare Limited, U.K.). The glands below the eyes of each snake were compressed to release the stored venom into a clean and sterilized container.

Preparation of Venom

After milking, the venoms were mixed and lyophilized using a freeze-dryer (Millrock Technology, USA) and kept in a refrigerator (HR135A, Haier-Thermocool, Lagos, Nigeria) in a light resistant and air-tight container. The lyophilized venom mixture was reconstituted in 0.9% saline (regarded as the venom) and used for the study. Before use, it was kept at 4°C. The venom concentration was expressed in terms of dry weight (mg/ml) [8].

Ethnobotanical Survey

Ethnobotanical data were collected through personal interviews with eleven (11) snake-charmers for the names (in Hausa Language) of the medicinal plants, parts used, method of preparation, dose and the mode of administration. The data collected were used to select four (4) most cited plants; *Parkia biglobosa* stem-bark, *Calotropis procera* root, *Sterculia setigera* stem-bark and *Bauhinia rufescens* seed for the antivenom screening in this study.

Collection of the Selected Medicinal Plants

The parts of the selected plants were collected on Saturday, 07^{th} September, 2019 from Zuru town, Kebbi State, Nigeria. They were authenticated at the herbarium of the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero, Nigeria.

Preparation of the Plants' Methanol Extracts

The plant extracts were prepared according to the method of Dupont *et al*., [9]. The collected plants parts were washed with clean water and air-dried under shade, pulverized using pestle and mortar. Five hundred grammes (500 g) of each powdered plant material was weighed and soaked in 1.5 L of 95% methanol. The mixture was then kept at room temperature for 48 h and filtered twice; initially with a muslin cloth and later with a Whatman filter paper No.1. The filtrates were evaporated to dryness at 40^oC using rotary evaporator. The residues were used for the antivenom activity screening.

Screening of the Extracts for Antivenom Activity

For each of the four (4) plant extracts, thirty (30) rats were randomly distributed into six (6) groups of five (5) rats each.

Group 1: received only distilled water and served as normal control.

Group 2: were injected intraperitoneally (i.p.) only with 0.8 mg/kg b. wt. of the venom and served as venom control.

Group 3 and 4: served as treatment groups, they were injected (i.p.) with 0.8 mg/kg b. wt. of the venom, after 30 min they were treated orally with a plant extract at doses of 200 and 400 mg/kg b. wt. respectively.

Group 5: received only a plant extract at a dose of 400 mg/kg b. wt. orally and served as extract control.

Group 6: were injected (i.p.) with 0.8 mg/kg b. wt. of the venom, after 30 min, they were treated intravenously (i.v.) with standard conventional serum antivenin at a dose of 1ml/0.6mg venom and served as standard control.

All the groups received same volume of preparations. In all the groups, the duration of animal survival and number of rats that survived were recorded for 24 h [10].

DATA ANALYSIS

The data generated from the study are presented as mean ± SEM and subjected to one way analysis of variance (ANOVA) and statistical difference between the means were separated using New Duncan's Multiple Range Test at $P<0.05$ with the aid of a statistical package (IBM SPSS Statistics 20)

RESULTS

Ethnobotanical Survey for the Antivenom Medicinal Plants

Eleven (11) medicinal plants were surveyed. The medium of preparation of each was either in water or milk, and the mode of administration was oral. Four (4) plants' materials with highest number of citations namely; *Parkia biglobosa* stem-bark (9), *Calotropis procera* root (9), *Sterculia setigera* stem-bark (8) and *Bauhinia rufescens* seed (8) were selected. The medicinal plants used in Zuru Emirate for the treatment of snakebite envenomation are presented in Table 1.

Antisnake Venom Activities of the Selected Medicinal Plants

The venom mixture at the dose of 0.8 mg/kg b. wt. produced 100% mortality in the albino rats. The mean survival times (MSTs) of the animals administered with the snake venom without any treatment were less than 1 h (Tables 2-5).

Table 2 presents the Antisnake venom activity of *C. procera* root methanol extract. There was significant increase ($P<0.05$) in the MST with increase in the dose of the extract. It shows that, the doses 200 and 400 mg/kg b. wt. of the extract produced MSTs of 11.25 ± 1.54 and 18.52 ± 1.50 h respectively. But none of these doses was able to protect the animals from death as 100% mortality was recorded for each dose (Table 2).

The antisnake venom activity of *P. biglobosa* stem-bark methanol extract is presented in Table 3. There was a significant ($P<0.05$) increase in the MST (12.29 ± 4.55 h) upon administration of 200 mg/kg b. wt. compared to the venom control group. But, there was no significant difference between the MSTs (12.29 ± 4.55 and 14.04 ± 4.79 h) at 200 and 400 mg/kg b. wt. doses of the *P. biglobosa* stem-bark extract respectively. In all these doses administered, none protected the animals from death (Table 3).

The methanol extract of *B. rufescens* seed significantly increased mean survival time (MST) at the dose of 200 mg/kg b. wt., but could not protect the animals from death at that dose (Table 4). The dose of the plant extract when doubled to 400 mg/kg b. wt. was found to be more effective against the venom, presenting MST of 18.66 ± 4.32 h. At this dosage, the percentage of animal survival was significantly ($P<0.05$) increased to 20% compared to the animal group treated with the standard antivenin with 100% survival rate (Table 4).

The antisnake venom activity of *S. setigera* stem-bark methanol extract is presented in Table 5. In all the doses administered, none protected the animals from death. Thus, at 400 mg/kg b. wt. extract dose, a significant ($P<0.05$) increase in animal MST of 5.96 ± 2.79 h was observed (Table 5). At 200 mg/kg b. wt. of the extract, there was no significant ($P>0.05$) increase in the MST when compared to that of the venom control group.

Table 6 presents comparative analysis of the MSTs for selected plants. All the animals in the extract control group (group 6) for each plant presented 100% survival and with no sign of toxicity. At 200 mg/kg b. wt., the animals administered with *P. biglobosa* stem-bark extract presented the longest MST of 12.29 ± 4.55 h. *C. procera* and *B. rufescens* follow *P. biglobosa* in terms of the potency at this particular dose (200 mg/kg b. wt.) with 11.25 ± 1.54 and 8.19 ± 5.33 h respectively. The animals with least MST were those treated with *S. setigera* stem-bark extract with 3.98 ± 0.54 h (Table 6). At 400 mg/kg b. wt., of plant extract, animals treated with *B. rufescens* seed extract have the longest MST of 18.66 ± 4.32 h compared to those of the animals treated with the other plants. Eventually, animals treated with 400 mg/kg b. wt. of *S. setigera* have the least MST of 5.96 ± 2.79 h.

Hence, when compared with the activity of the standard antivenin, the percentage efficacy in terms of the antivenom activities of the test plants at 400 mg/kg b. wt. of the extract, is as follows (from the most active to the one with least activity): *B. rufescens* seed (78%) > *C. procera* root (77%) > *P. biglobosa* stem-bark (59%) > *S. setigera* stem-bark (25%) respectively. Thus, *B. rufescens* seed extract was recorded as the most active at 400 mg/kg b. wt.

Table-1: Medicinal Plants Used in Zuru for the Treatment of Snakebite Envenomation

S/N	Plant Botanical Name	Name in Hausa Language	Part of the Plant Used	Number of Citations	Mode of Preparation	Mode of Administration
1	*Calotropis procera*	*Tumfafiya*	Root	9	Dried powdered sample soaked in milk.	A cup, three times a day for three days.
2	*Balanite aegyptica*	*Aduwa*	Stem-Bark	3	The dried pulverized bark is usually soaked in water.	Taken daily until relieved.
3	*Azadirachta indica*	*Dogon yaro / Darbejiya*	Leaf	7	Fresh leaf is macerated in water.	A small quantity, twice daily for three days.
4	*Sterculia setigera*	*Kukkuki*	Stem-Bark	8	Dried powdered bark is soaked in a hot water and allow to stand for 10 min.	Taken twice daily until relieved.
5	*Bauhinia rufescens*	*Matsatsagi or Jirga*	Seed	8	Soak the powdered seed in milk for 5 minutes.	Half a small cup, three times daily for three days.
6	*Parkia biglobosa*	*Dorawa*	Stem-Bark / Root	9	Boil the stem-bark or the root with potash and allowed to cool.	Two cups daily until relieved.
7	*Securidaca longepedunculata*	*Uwar magunguna*	Stem-Bark / Leaf	4	Soak the dried powdered part in diluted milk	Taken twice a day. Warm it before drinking.
8	*Catunaregam nilotica*	*Barbaji, Tsibra, Chibra*	Stem-Bark / root	5	Use either dry or fresh stem-bark / root. Boil in water.	Half a cup three times daily until relieved.
9	*Ziziphus abyssinica*	*Magarya*	Leaf	4	One spoon of the dried powdered leaf is mixed with diluted milk.	A small cup twice daily
10	*Tamarindus indica*	*Tsamiya*	Leaf	5	Boil the fresh leaf with red potash and cool.	A cup twice daily for seven days.
11	*Mangifera indica*	*Mangwaro*	Stem-Bark	3	Dried powdered bark is soaked in water mixed with honey.	Half a cup three times daily for ten days.

Table-2: Antisnake Venom Activity of Methanol Root Extract of *Calotropis procera*

Group	Treatment	Extract (mg/kg b. wt.)	Venom (mg/kg b. wt.)	Standard Antivenin (1ml/0.6mg venom)	Survival Total	% Survival	Mean Survival Time (h)
1	Control	_	_	_	5/5	100	24.00 ± 00^{c}
2	Venom Only	_	0.8	_	0/5	0	0.88 ± 0.11^{a}
3	Venom + Extract	200	0.8	_	0/5	0	11.25 ± 1.54^{b}
4	Venom + Extract	400	0.8	_	0/5	0	18.52 ± 1.50^{bc}
5	Extract Only	400	_	_	5/5	100	24.00 ± 0.00^{c}
6	Venom + Antivenin	_	0.8	1.3	5/5	100	24.00 ± 0.00^{c}

Mean survival times are presented as Mean ± SEM (n = 5).
Mean Survival Times carrying different superscripts from the venom control (group 2) are significantly ($P<0.05$) different using ANOVA and Duncan multiple range test.

Table-3: Antisnake Venom Activity of Methanol Stem-Bark Extract of *Parkia biglobosa*

Group	Treatment	Extract (mg/kg b. wt.)	Venom (mg/kg b. wt.)	Standard Antivenin (1ml/0.6mg venom)	Survival Total	% Survival	Mean Survival Time (h)
1	Control	_	_	_	5/5	100	24.00 ± 0.00^{c}
2	Venom Only	_	0.8	_	0/5	0	0.95 ± 0.27^{a}
3	Venom + Extract	200	0.8	_	0/5	0	12.29 ± 4.55^{b}
4	Venom + Extract	400	0.8	_	0/5	0	14.04 ± 4.79^{b}
5	Extract Only	400	_	_	5/5	100	24.00 ± 0.00^{c}
6	Venom + Antivenin	_	0.8	1.3	4/5	80	23.54 ± 0.74^{c}

Mean survival times are presented as Mean ± SEM (n = 5).
Mean Survival Times carrying different superscripts from the venom control (group 2) are significantly (P<0.05) different using ANOVA and Duncan multiple range test.

Table-4: Antisnake Venom Activity of Methanol Seed Extract of *Bauhinia rufescens*

Group	Treatment	Extract (mg/kg b. wt.)	Venom (mg/kg b. wt.)	Standard Antivenin (1ml/0.6mg venom)	Survival Total	% Survival	Mean Survival Time (h)
1	Control	_	_	_	5/5	100	24.00 ± 0.00^{c}
2	Venom Only	_	0.8	_	0/5	0	0.89 ± 0.23^{a}
3	Venom + Extract	200	0.8	_	0/5	0	8.19 ± 5.33^{b}
4	Venom + Extract	400	0.8	_	1/5	20	18.66 ± 4.32^{bc}
5	Extract Only	400	_	_	5/5	100	24.00 ± 0.00^{c}
6	Venom + Antivenin	_	0.8	1.3	5/5	100	24.00 ± 0.00^{c}

Mean survival times are presented as Mean ± SEM (n = 5).
Mean Survival Times carrying different superscripts from the venom control (group 2) are significantly (P<0.05) different using ANOVA and Duncan multiple range test.

Table-5: Antisnake Venom Activity of Methanol Stem-Bark Extract of *Sterculia setigera*

Group	Treatment	Extract (mg/kg b. wt.)	Venom (mg/kg b. wt.)	Standard Antivenin (1ml/0.6mg venom)	Survival Total	% Survival	Mean Survival Time (h)
1	Control	_	_	_	5/5	100	24.00 ± 0.00^{c}
2	Venom Only	_	0.8	_	0/5	0	0.93 ± 0.19^{a}
3	Venom + Extract	200	0.8	_	0/5	0	3.98 ± 0.52^{a}
4	Venom + Extract	400	0.8	_	0/5	0	5.96 ± 2.79^{b}
5	Extract Only	400	_	_	5/5	100	24.00 ± 0.00^{c}
6	Venom + Antivenin	_	0.8	1.3	5/5	100	24.00 ± 0.00^{c}

Mean survival times are presented as Mean ± SEM (n = 5).
Mean Survival Times carrying different superscripts from the venom control (group 2) are significantly (P<0.05) different using ANOVA and Duncan multiple range test.

Table-6: Comparative Analysis of Mean Survival Times of the Animals Treated with Selected Plants

Group	Treatment	Extract (mg/kg b. wt.)	Mean Survival Time (h)			
			C. procera Root	*P. biglobosa* Stem-Bark	*B. rufescens* Seed	*S. setigera* Stem-Bark
1	Control	-	24.00 ± 0.00^{a}	24.00 ± 0.00^{a}	24.00 ± 0.00^{a}	24.00 ± 0.00^{a}
2	Venom Only	-	0.88 ± 0.11^{a}	0.95 ± 0.27^{a}	0.89 ± 0.23^{a}	0.93 ± 0.19^{a}
3	Venom + Extract	200	11.25 ± 1.54^{b}	12.29 ± 4.55^{b}	8.19 ± 5.33^{b}	3.98 ± 0.54^{a}
4	Venom + Extract	400	18.52 ± 1.50^{bc}	14.04 ± 4.79^{b}	18.66 ± 4.32^{bc}	5.96 ± 2.79^{a}
5	Extract Only	400	24.00 ± 0.00^{a}	24.00 ± 0.00^{a}	24.00 ± 0.00^{a}	24.00 ± 0.00^{a}
6	Venom + Antivenin	_	24.00 ± 0.00^{a}	23.54 ± 0.74^{a}	24.00 ± 0.00^{a}	24.00 ± 0.00^{a}

Values are presented as Mean ± SEM (n = 5).
Mean Survival Times carrying different superscripts (along the row) are significantly (P<0.05) different using ANOVA and Duncan multiple range test.

DISCUSSION

The plant kingdom provides an inexhaustible source of various herbal compounds with pharmacological potential [11, 12]. A plethora of medicinal plants, available locally are used widely by traditional healers in the form of plant sap, pastes, decoctions, powders and pills in the treatment of snakebite envenomation [13].

Herbal antivenoms have been reported to neutralize toxic venom constituents through several mechanisms. These include; inhibition of venom enzymes, inactivation of venom toxic proteins, antioxidant activity, adjuvant action, chelation activity and combination of these activities [14].

Various phytochemicals with protein binding properties, active against snake envenomation include flavonoids, polyphenols, saponins, tannins, terpenoids, xanthenes, quinonoids, steroids and alkaloids. These bind to toxic venom proteins thereby inactivating them [15]. They could also competitively block the target receptors.

Among the selected plant materials in this research, *B. rufescens* seed extract was identified as the most effective against the mixed venoms of *Naja nigricollis*, *Echis ocellatus* and *Bitis arietans* as increased mean survival time (MST) was recorded for each of the administered doses (Table 4). Thus, the MSTs of the treatment groups were significantly increased, and the group that received the highest dose of the plant extract (400 mg/kg b. wt.) had higher animals survival rate than those that received lower dose of 200 mg/kg b. wt., which may be due to a number of factors such as dose of administration as reported by Abubakar *et al.*, [16] and Sani *et al*., [17] who observed increase in survival time with administered plant extract in a dose-dependent manner.

The antivenom activities observed in the rats administered with the plants extracts may be attributed to the possible presence of any of phenols, steroids, glycosides, tannins, saponins or terpenoids in the plant extracts [18]. The phytochemical components of these plants materials have been established in previous studies and these studies have linked presence of the bioactive compounds to antisnake venom activities [14, 19].

The antisnake venom activities of the plants extracts in neutralizing the snake venom effects may also be attributed to the presence of phenols; as plant phenols from the aqueous extracts of *Pentace burmanica*, *Pithecellebium dulce*, *Areca catechu* and *Quercus infectoria* have been shown to block non-selectively the nicotinic acetylcholine receptor by precipitation of *Naja kaouthia* venom [20, 21]. Phenols have also been shown to possess dose-dependent inhibitory effects on phospholipase A_2, hyaluronidase, and L-amino acid oxidase of *Calloselasma rhodostoma* and *Naja naja kaouthia* venoms in *in vitro* tests [22].

Tannins from persimmon, a fruit from *Diospyrus kaki* inhibits oedema in mice induced by sea snake and also improved the survival time in mice injected with *Laticauda semifasciata* and *Trimeresurus flavoviridis* venom [23]. Therefore, prolonged mean survival time observed in the treated groups for each plant extract may be attributable to the presence of tannins in the methanol extracts.

Terpenoids, flavonoids and polyphenols possess protein binding and enzyme inhibiting properties and also inhibit snake venom phospholipase A_2 activities of both viper and cobra venom [14, 24]. A natural triterpenoid saponin extracted from the root of *Glycyrrhiza glabra* (licorice), has been characterized as a thrombin inhibitor [25]. This compound is known for its anti-inflammatory activity and it also exhibits antithrombotic properties against snake venom *in vivo* [26]. The possible presence of terpenoids, flavonoids and saponins in the extracts may also have contributed to their antisnake venom activities.

CONCLUSION

The limitations of serum antivenin are well known and the world is searching for an alternative for snakebite treatment. Till date no suitable alternative measures are available, except the natural herbal remedies, which are showing promising expectations. The advantages of herbal compounds are that, they are cheap, easily available, and stable at room temperature and could neutralize a wide range of venom toxins. The methanol extracts of the selected medicinal plants showed antivenom activities against the mixed venoms of *Naja nigricollis*, *Echis ocellatus* and *Bitis arietans* with varying degrees of efficacy. *B. rufescens* seed extract was found to be the most potent. Its antivenom properties have been established. This research work has provided scientific proof on the antivenom properties of the selected medicinal plants. Hence, it lends support to the traditional use of the plants in the treatment of snakebites. Finally, these findings would be of importance in the area of drug development with a view to actualizing the substitution of conventional snakebite therapeutic options with effective, cheap, accessible and less allergic plant-based compounds.

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REFERENCES

1. Sani, I., Umar, A. A., Jiga, S. A., Bello, F., Abdulhamid, A., & Fakai, I. M. (2020). Isolation Purification and Partial Characterization of Antisnake Venom Plant Peptide (BRS-P19) from Bauhinia rufescens (LAM FAM) Seed as Potential Alternative to Serum-Based Antivenin. *Journal of Biotechnology Research*, *6*(4), 18-26.
2. Morais, V. (2018). Antivenom therapy: efficacy of premedication for the prevention of adverse reactions. *J. Venom. Anim. Toxins Incl. Trop. Dis.*, 24:(7) 1-7

3. Sani, I., Umar, R.A., Hassan, S.W., Faruq, U.Z., Abdulhamid, A., Bello, F. and Fakai, I.M. (2019). Major Enzymes from Snake Venoms: Mechanisms of Action and Pharmacological Applications. *Asian J. Biol. Sci., 12*(3): 396-403.
4. Dabai, T.J. (2010). Antisnake venom activity, Microbial and Phytochemical Screening of some Locally Used Medicinal Plants. M.Sc. Dissertation, Usmanu Danfodiyo University, Sokoto, Nigeria.
5. Aboubakar, O.B.F., Bella, N.M.T., Ngo lemba, T.E., Bilanda, D.C., & Dimo, T. (2012). Antihypertensive Activity of *Jateorhiza meacrantha* (Menispermaceae) Aqueous Extract on Ethanol-induced Hypertension in Wister Albino Rats. *Intern. J. Pharm. Sci*., *4*(2).
6. AUCC. (2009). Guide to the use and care of experimental animals. Animal use and Care Committee, National Veterinary Research Institute, Vom, Nigeria: vol. 1.
7. Goswami, P.K., Samant, M., & Srivastava, R.S. (2014). Snake Venom, Anti-Snake Venom and Potential of Snake Venom. *Inter. J. Pharm. Pharmec. Sci*., *6*(5): 4-7
8. Razi, M.T., Asad, M.H.H.B., Khan, T., Chaudhary, M.Z., Ansari, M.T., Arshad, M.A., & Najamus Saqib, Q. (2011). Antihaemorrhagic (Antivenom) Potentials of *Fagonia Cretica* against Pakistani Cobra Venom (*Naja Naja Karachiensis*). *Nat. Prod. Res.*, 25: 1902-1907
9. Dupont, S., Caffin, N., Bhandari, B., & Dykes, G.A. (2006). *In Vitro* Antimicrobial Activity of Australian herb extracts against food related bacteria. *Food Cont*., 17: 929-932
10. Premendran, S.J., Salwe, K.J., Pathak, S., Brahmane, R. and Manimekalai, K. (2011). Anti-cobra venom activity of plant *Andrographis paniculata* and its comparison with polyvalent antisnake venom. *J. Nat. Sci., Biol. Med., 2*(2): 198-204.
11. Gupta, Y.K., & Peshin S.S. (2012). Do herbal medicines have potential for managing snakebite envenomation? *Toxicol. Int*., 19:89-99.
12. Sani, I., Umar, R.A., Hassan, S.W., Faruq, U.Z., & Bello, F. (2020). Isolation of Antisnake Venom Agents from *Azadirachta indica* (A. Juss) Leaf Extracts. *Acad. J. Chem.*, *5*(2): 10-16.
13. Faruq, U.Z., Zuru, A.A., Abdulrahman, F.W., & Bilbis, L.S. (2002). Preliminary Screening of *Sclerocarya birrea* Bark as Snake Venom Antidote against *Naja nigricollis*. *Nig. J. Biochem. Mol. Biol.*, Vol. 17:1
14. Sani, I., Umar, R.A., Hassan, S.W., & Faruq, U.Z. (2018). Antisnake Venoms and their Mechanisms of Action: A Review. *Saudi J. Med. Pharm. Sci., 4*(5): 512-520
15. Sani, I., Umar, R.A., Hassan, S.W., Faruq, U.Z., Bello, F., & Abdulhamid, A. (2019). Protective Effect of *Azadirachta indica* Leaf Fractionated Extracts on Renal and Haematological Indices against Snake Venom Toxicity in Albino Rats. *Res. J. Med. Plants*, *13*(3): 103-108.
16. Abubakar, M.S., Sule, M.I., Pateh, U.U., Abdurahman, E.M., Haruna, A.K., & Jahun, B.M. (2000). *In vitro* snake venom detoxifying action of the leaf extract of *Guiera senegalensis*. *J. Ethnopharmacol. 69*(3): 253-257.
17. Sani I., A. Abdulhamid, F. Bello, A. Sulaiman and H. Aminu. (2020). Antisnake Venom Effect of *Diospyros mespiliformis* Stem-Bark Extract on *Naja nigricollis* Venom in Albino Rats. *Singapore J. Sci. Res.*, *10*(3): In Press.
18. Rajendran, K., Annie, S., Maneesh, M.R. and Vijaya, B. (2010). *In vitro* and *In vivo* anti snake venom (*Daboia russelli*) studies on various leaf extracts of *Acalpha indica* Linn. *Int. J. Phytomed.*, 2: 217-220.
19. Santhosh, M.S., Hemshekhar, M., Sunitha, K., Thushara, R.M., Jnaneshwari, S., Kemparaju, K., & Girish, K.S. (2013). Snake Venom Induced Local Toxicities: Plant Secondary Metabolites as an Auxiliary Therapy. *Mini-Rev. Med. Chem.*, 13: 106-123.
20. Leanpolchareanchai, J., Pithayanukul, P., & Bavovada, R. (2009). Anti-necrosis potential of polyphenols against snake venoms. *Immunopharm. Immunotoxicol., 31*(4): 556-562.
21. Lewis, R.L., & Gutmann, L. (2004). Snake venoms and the neuromuscular junction. *Semin. Neurol.* 24:175-179.
22. Akunyili, D.N., & Akubue, P.I. (1986). Schumanniofoside, the antisnake venom principle from the stem bark of *Schumanniophyton magnificum* Harms, *J. Ethnopharmacol.*, 18:176-172.
23. Okonogi, T., Hattori, Z., Ogiso, A., & Mitsui, S. (1979). Detoxification by persimmon tannin of snake venom and bacterial toxins. *Toxicon*, *17*(5): 524 – 527.
24. Alam, M.I., Auddy, B., & Gomes, A. (1994). Isolation, purification and partial characterization of viper venom inhibiting factor from the root extract of the Indian medicinal plant sarsaparilla (*Hemidesmus indicus* R. Br.). *Toxicon, 32*(12): 1551-1557.
25. Francischetti, I.M., Monteiro, R.Q., Guimaraes, J.A., Francischetti, B. (1997). Identification of gycyrrhizin as a thrombin inhibitor. *Biochem. Biophysiol. Comm.* 235: 259.
26. Assafim, M., Ferreira, M.S, Frattani, F.S, Guimaraes, J.A., Monteiro, R.Q., & Zingali, R.B. (2006). Counteracting effect of glycyrrhizin on the hemostatic abnormalities induced by *Borthrops jararaca* snake venom. *J. Pharmacol*., 148: 807.