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Adebayo Gbolade
 Phytotherapy Research Group,
 Department of Pharmacognosy,
 College of Pharmacy, Igbinedion
 University Okada, Edo State,
 Nigeria.

Oluwasegun Adedokun
 Phytotherapy Research Group,
 Department of Pharmacognosy,
 College of Pharmacy, Igbinedion
 University Okada, Edo State,
 Nigeria.

Elizabeth Igbinomwanhia
 Phytotherapy Research Group,
 Department of Pharmacognosy,
 College of Pharmacy, Igbinedion
 University Okada, Edo State,
 Nigeria.

Moshhood Alli
 Phytotherapy Research Group,
 Department of Pharmacognosy,
 College of Pharmacy, Igbinedion
 University Okada, Edo State,
 Nigeria.

Lethality of *Bryophyllum pinnatum* (Lam.) Oken (Crassulaceae) Leaf Methanol Extract and Fractions against *Naja nigricollis* and *Bitis arietans* Venoms

Adebayo Gbolade, Oluwasegun Adedokun, Elizabeth Igbinomwanhia and Moshhood Alli

Abstract

Bryophyllum pinnatum (Lam.) Oken (Crassulaceae), a traditional anti-hypertensive, anti-inflammatory, anti-fever and anti-cancer remedy is also popular in Indian traditional herbal remedies for snake bite treatment. Studies evaluating the anti-venom activity of this plant are scarce. This study aims to evaluate the inhibitory effects of leaf methanol extract and solvent fractions of *B. pinnatum* against local effects induced by *Bitis arietans* and *Naja nigricollis* snake venoms. Inhibition of adenosine diphosphate-induced platelet aggregation, antioxidant, total phenol content and *in vivo* and *in vitro* effects on toxicity induced by the two venoms were evaluated. With *Bitis arietans* *in vitro* assay model, methanol extract, dichloromethane (DCM) and aqueous fractions gave concentration-dependent inhibition of platelet aggregation at 100 - 500 µg/ml which ranked as: methanol extract > aqueous fraction > DCM fraction at 500 µg/ml. The aqueous fraction with maximal inhibition of 31.5% appeared to be the most potent inhibitor of *Naja nigricollis* induced platelet aggregation. Concentration-dependent DPPH free radical scavenging activity which were comparatively less than the reference antioxidant, ascorbic acid was observed at tested concentrations of 20 - 100 µg/ml. There appears to be a correlation between the total phenolic contents of this plant and its radical scavenging activity with the polar aqueous fraction being the richest (31.3 mg gallic acid equivalent/g). Venom neutralization was remarkable in a dose-dependent manner at 24h and 48h (75% protection for DCM fraction, and 100% for aqueous fraction at 100 mg/kg) against *Bitis arietans*-LD₉₉ envenomed mice. Order of antivenin potency is: DCM fraction > aqueous fraction = methanol extract on *Naja nigricollis* at 24h. Rats envenomed with LD₅₀ of snake venoms displayed complete protection of *Naja nigricollis*-treated rats only with methanol extract and DCM fraction in 48h, and of *Bitis arietans*-envenomed animals with all tested doses of methanol extract at 24h and the aqueous fraction in 48h. In conclusion, the results indicate the potential antiophidic activity of *B. pinnatum* leaf particularly against local effects induced by *Bitis arietans* thereby justifying its folkloric usage.

Keywords: *Bryophyllum pinnatum*, *Naja nigricollis* venom, *Bitis arietans* venom, platelet aggregation, antioxidant activity, phenolic content

Introduction

Snake bite is an occupational hazard in tropical and sub-tropical countries like Nigeria. The WHO reports that 174 snake bites per 100,000 population occur in Nigeria yearly and that the saw-scaled or carpet viper is responsible for 90% of bites ^[1]. Four families of venomous snakes found in Nigeria include Viperidae, Elapidae, Colubridae and Actaspididae, but three species, carpet viper (*Echis ocellatus*), black-necked spitting cobra (*Naja nigricollis*) and puff adder (*Bitis arietans*) belonging to the first two families are the most important snakes associated with envenoming in Nigeria ^[2, 3]. Snakebite envenomation is a worldwide problem, which is a major cause of mortality and morbidity in developing countries and still remains a neglected public health problem.

In Nigeria, the Benue valley is predominant for most of these tragedies where prevalence of 497 per 100,000 persons per year and mortality rate of 12.2% are reported, with *Echis ocellatus* accounting for about 66% ^[2, 3]. Males and farmers were reported to be more vulnerable to snake bites especially in the rainy season in Gombe ^[4] and Benue communities ^[3] of Nigeria. Recently, studies of snake bites among hospitalized Nigerian children in Benin City in the South ^[5] and Sokoto in the North West ^[6] with low prevalence rates have been published. Taraba state in Nigeria has been reported to have one of the high annual incidence (40.4% bites per 100,000) in the country ^[7].

Corresponding Author:

Adebayo Gbolade
 Phytotherapy Research Group,
 Department of Pharmacognosy,
 College of Pharmacy, Igbinedion
 University Okada, Edo State,
 Nigeria.

Reviews on anti-snake venom plants in India [8-10], Kenya [11] and Colombia [12] have been published. In Nigeria however, ethnomedicinal surveys are available for snake bites treatment in the north viz Gombe [4] and Taraba state [7]. Importation of anti-snake venoms has been hampered by the economic downturn bedevilling the many African countries. There is therefore need to tackle this major public health problem in rural communities by searching for more effective alternative means which is believably inherent in the nations' rich biodiversity.

The air plant, *Bryophyllum pinnatum* (Lam.) Oken (Crassulaceae), growing widely is a succulent perennial plant up to 1m tall with fleshy leaves and cylindrical stem, and reddish coloured young stem and flowers [13]. The plant, which is a rich source of bufadienolide cardiac glycosides, is traditionally employed to treat hypertension, while the roasted plant is used against inflammation and cancer, infusion is a popular remedy for fevers and leaf decoction as a folkloric antidote for snake bites [13]. It is popular in the Indian traditional herbal remedies for snake bite treatment among the Nath people of Assam in the north East [14], West Bengal [15] and in Koraput district of Odisha [9]. Aside from the report of Fernandes *et al.* [16] on the phospholipase activity of *Kalanchoe pinnata* (syn. *B. pinnatum*) hydroethanolic leaf extract on *Bitis jararaca* snake venom, and the comprehensive review of its ethno pharmacology, phytochemistry and pharmacology by Fernandes *et al.* [13], no further information was available in the literature on the antiophidian potential of this plant.

We therefore investigated *B. pinnatum* leaf to expand the database of potent plant remedies for treating snake bites which is prevalent in some parts of Nigeria and tropical Africa.

2. Materials and Methods

2.1 Collection and Preparation of Plant Extracts

Fresh leaves of *B. pinnatum* were collected in November 2018 from the Jericho forest area in Ibadan North-east Local Government in Oyo State. The plant was authenticated (voucher no. IUO/16/116) at Department of Pharmacognosy Herbarium, Igbinedion University, Okada. The leaves were shade-dried at room temperature, pulverized using the locally fabricated milling machine and stored in a well closed container at ambient temperature.

2.2 Extraction of plant material and fractionation

Dried and ground plant material (600g) was extracted to exhaustion with methanol in a Soxhlet apparatus. The crude extract was concentrated *in vacuo* and weighed (52.5%). An appropriate amount of the crude methanol extract was fractionated with dichloromethane (DCM) in a separatory funnel to yield DCM fraction (15.34%) and aqueous fraction (14.2%). Both fractions, together with the crude extract were refrigerated (4°C) until needed.

2.3 Phytochemical screening

Preliminary phytochemical analysis of the extracts was performed determine their chemical constituents using standard methods described by Evans [17].

2.4 Chemicals

Chemicals such as 2,2-Diphenyl-1-picrylhydrazyl (DPPH), quercetin, gallic acid, were purchased from Sigma Chemical Co. Other chemicals such as normal saline (Fidson, Nigeria). All the other solvents (e.g methanol and dichloromethane)

used for extraction and phytochemical investigation were of analytical grade and sourced from Pharmatrends Nigeria Ltd, Benin-City, Nigeria) while chemicals used for phytochemical screening were appropriately sourced.

2.5 Determination of DPPH Antioxidant Activity

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma -Aldrich) free radical scavenging assay method described by Villano *et al.* [18] with some modifications. Accordingly, DPPH (0.02 g) was dissolved in 500 mL methanol (Analar grade). Each tested agent (crude methanol extract, DCM and aqueous fractions, 0.01 g) was dissolved in 2 mL dimethyl sulfoxide (DMSO) and 8 mL water, which served as the stock. 0.2 mL of the stock solution was mixed with 19.8 mL water and used as the final sample solution. Different quantities of methanolic solution of DPPH (0.8, 0.6, 0.4 and 0.2 mL) were added to different test tubes and different concentrations of the final solution for each tested agent (20, 40, 60, 80, 100 µg/mL) were added respectively to each test tube. Then, 2 mL DPPH solution were added to each test tube and incubated in the dark for an hour before measuring the absorbance spectrophotometrically at 517 nm (UV/VIS Spectrophotometer, UV 752 (D), PEC Medical, USA). A negative control was prepared containing the same amount of methanol and DPPH, while ascorbic acid in methanol served as positive control. Percentage inhibition of DPPH radical was determined using the formula:

$$\% \text{ inhibition} = [(Ac - As) / Ac] \times 100$$

Where Ac is the absorbance of the control and as is the absorbance of the extract.

2.6 Estimation of Total Phenolic Content

The Folin-Ciocalteu method described by Baba and Malik [19] with some modifications was employed to determine the total phenolic content. Each test sample and 0.01 g gallic acid were dissolved in 100 mL water. A typical mixture comprised 1mL sample, 7.5% sodium carbonate (2 mL) and Folin-Ciocalteu reagent (2 mL) incubated at 40°C for 45 min. Absorbance of the reaction mixture was determined by UV spectrophotometer at 765nm. The standard mixture was prepared with 20 - 100 µg/mL concentrations of gallic acid and same amount of sodium carbonate and Folin-Ciocalteu reagent. Total phenolic content was extrapolated from the calibration curve of the gallic acid standard, and the data were expressed as milligram gallic acid equivalent per gram of tested agent.

2.7 Inhibition of Adenosine Diphosphate (ADP)-Induced Platelet Aggregation

The ability of *B. pinnatum* to inhibit ADP-induced platelet aggregation was determined according to the method of Ahmed *et al.* [20]. Platelet-rich plasma was obtained by centrifugation of citrated human blood at 190 \times g for 15 minutes. Accordingly, 0.02 g each tested agent was dissolved in 2 mL DMSO and made up to 15 mL by adding 13 mL water. Snake venom (0.004 g) was dissolved in 20 mL normal saline and 0.24 g adenosine diphosphate (ADP) was dissolved in 50 mL phosphate buffered saline. The reaction mixture contained different concentrations of each tested agent (100, 250 and 500 µg/mL), venom solution 0.5 mL (200 µg/mL) and 0.5 mL platelet-rich plasma. The reaction mixtures were maintained at 37°C and kept for 2 min with constant stirring,

0.5 mL ADP solution was added, incubated for 4 min and absorbance was measured at 414nm (UV/VIS Spectrophotometer, UV 752 (D), PEC Medical, USA). Two standard mixtures were prepared with 0.5 mL ADP, 0.5 mL plasma and 0.5 mL normal saline in one test tube, and 0.5 mL ADP, 0.5 mL venom and 0.5 mL plasma in the other. Adenosine diphosphate-induced platelet aggregation was calculated from the formula:

$$\% \text{ ADP induced platelet aggregation} = \{[(T_1-T_2) - (T_1-T_3)] / (T_1-T_2)\} \times 100$$

Where T_1 = ADP+ Platelet, T_2 = ADP+ venom+ Platelet and T_3 = ADP+ Platelet + Plant extract.

2.8 Animals

Swiss albino mice (24 - 36 g) and Wister rats (150 - 180 g) of either sex were used. The animals were supplied by the Department of Pharmacology, IUO, kept in separate cages in the animal house at 28 - 35°C under artificial (12h light /12h dark) lighting system, and maintained on grower mash feed (Eastern Premier Feed Mills Ltd, Nigeria) and water *ad libitum*. The animals were fasted for 12h before the bioassay. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee. The guide for the care and use of laboratory animals, 1996 of the Institute of Laboratory Animal Research Commission on life Science, National Research Council was duly followed.

2.9 Snake venoms and Anti-snake venom

Lyophilized snake venoms of puff adder, *Bitis arietans* (LD₉₉ 0.98 mg/kg in mice) and the black-necked spitting cobra, *Naja nigricollis* (LD₉₉ 1.025 mg/kg in mice) were procured from Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna state, Nigeria in March 2019 and preserved at 4°C until needed. A stock solution (20 mg/mL) of each venom in 0.9% normal saline was prepared. The positive control, Snake Venom Antiserum African^R (MFD Nov 2014, EXP Dec 2019) was sourced from Bharat Serums & Vaccines Ltd, India.

2.10 Neutralization of the lethal venom effect of *Naja nigricollis* and *Bitis arietans* in mice

In vivo neutralization test was performed as previously described by Ahmed *et al.* [20]. Mice fasted overnight were divided into 5 groups of four mice each. Each group was envenomed with I.P. administration of LD₉₉ dose (1.025 mg/kg) of *Naja nigricollis* venom followed by oral administration of different doses (50, 100, 200 mg/kg) of crude methanol extract after 15min to groups I-III. The experiment was repeated with a single dose, 100 mg/kg each of DCM and aqueous fractions to groups IV and V, respectively. The positive control (polyvalent ASV) at dose of 0.2 mg/kg to group VI and negative control (0.1 mL 0.9% normal saline) to group VII mice. Animals were kept in the animal house and observed for signs of venom lethality, % mortality and % protection at 24h and 48h. The experiment was repeated with *Bitis arietans* venom at LD₉₉ 0.981 mg/kg.

2.11 Neutralization of the lethal venom effect of *Naja nigricollis* and *Bitis arietans* in rats

In vivo neutralization test was performed as previously described by Ahmed *et al.* [20]. Rats fasted overnight were divided into 5 groups of four mice each. Each group was envenomed with i.p. administration of LD₅₀ dose (0.40 mg/kg) of *Naja nigricollis* venom followed by different doses (50, 100, 200 mg/kg) of crude methanol extract after 15min to groups I-III rats. The experiment was repeated with a single dose, 100 mg/kg each of DCM and aqueous fractions to groups IV and V respectively. The positive control (polyvalent ASV) at dose of 0.2 mg/kg to group VI and negative control (0.1 mL 0.9% normal saline) to group VII rats. Animals were kept in the animal house and observed for signs of venom lethality and mortality, % mortality and % protection at 24h and 48h. The experiment was repeated with *Bitis arietans* venom at LD₅₀ 0.325 mg/kg.

Statistical analysis

All data were presented as the Mean and n represents the number of counts from which samples were taken, and results analyzed using ANOVA (Graphpad Prism Version 6.0) and % protection were evaluated.

3. Results and Discussion

In the *Bitis arietans* *in vitro* assay model, all the three agents tested at 100-500 µg/mL gave concentration-dependent inhibition of platelet aggregation (Table 1). Maximum inhibitions were evident at the highest concentration for methanol extract (76.9%) and DCM fraction (53.3%). Ranking of platelet inhibition potential at the highest concentration is: methanol extract > aqueous fraction > DCM fraction. However, both fractions were similar in *in vitro* anti-snake venom activity. Contrariwise, platelet inhibition in the *Naja nigricollis* model was not concentration-dependent but comparable inhibitions were recorded for all tested agents at the least concentration (18 - 21.8 % inhibition). In this case, the aqueous fraction with maximal inhibition (31.5%) at the highest concentration appear to be the most potent inhibitor of *Naja nigricollis*-induced platelet aggregation. Our findings are consistent with those of other workers. Dose-dependent inhibition of platelet aggregation of *Pouzolzia indica* has also been reported [20]. Crude aqueous and ethanol extracts and fractions of *Crinum jagus* bulb was shown to give complete neutralization of hemorrhagic activity after a 24h treatment period [21]. Extract of *Pouzolzia indica* also significantly reduced viper venom-induced hemorrhagic and necrotic lesions [20]. This study offers the significance of providing some level of inhibition of snake venom toxicity, especially *Bitis arietans* after oral administration (500 mg/kg) of *B. pinnatum* crude extracts and aqueous fraction. The anti-venom activity of *B. pinnatum* leaf could be due to the presence of the bioactive components in the plant [13, 16]. This report is consistent with the publication of Fernandes *et al.* [16] on the phospholipase activity of the plant hydroethanolic leaf extract on *Bitis jararaca* snake venom. The local skin hemorrhage is an important effect of snake envenomation. Therefore, plants that can reduce the local hemorrhagic action of the venom are of great interest to treat the local effects produced by snakebite. *Bryophyllum pinnatum* leaf extracts were observed to clearly attenuate hemorrhage produced by *Bitis jararaca* venom [16].

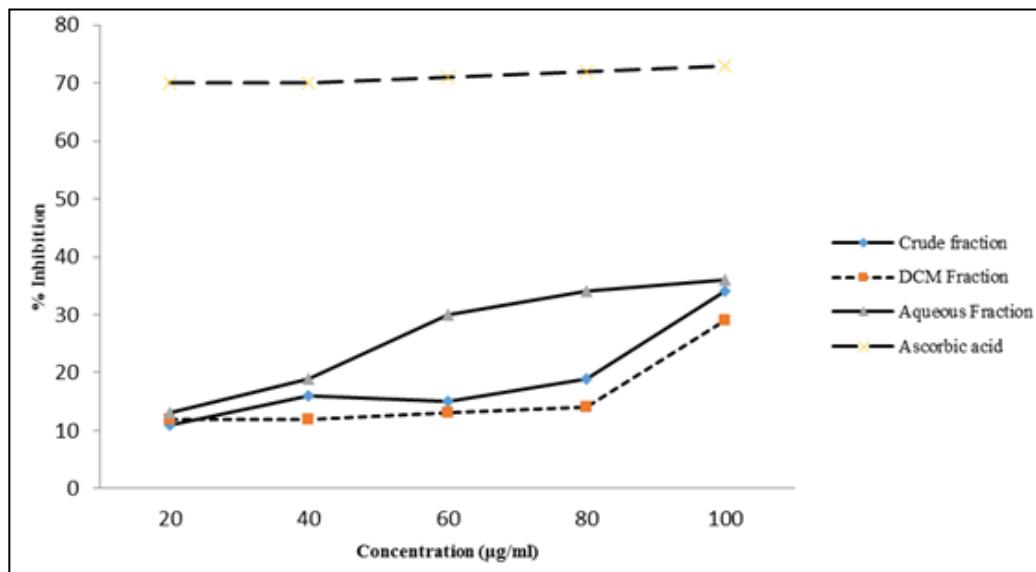
Table 1: Effect of *Bryophyllum pinnatum* on inhibition (%) of ADP-induced platelet aggregation using two snake venoms

Concentration ($\mu\text{g/ml}$)	<i>Bitis arietans</i>			<i>Naja nigricollis</i>		
	Crude methanol extract	Dichloromethane fraction (DCM)	Aqueous fraction	Crude methanol extract	Dichloromethane fraction (DCM)	Aqueous fraction
100	59.3 \pm 7.58	10.2 \pm 1.85	10.8 \pm 6.43	18.8 \pm 10.8	21.8 \pm 12.6	20.9 \pm 12.1
250	71.5 \pm 2.30	42.8 \pm 10.96	59.3 \pm 3.73	21.9 \pm 12.6	n. d	17.0 \pm 9.82
500	76.9 \pm 1.19	53.3 \pm 6.27	55.3 \pm 4.92	n. d	18.7 \pm 10.8	31.5 \pm 18.2

All values are expressed as Mean \pm SEM, n=3; the normal saline negative control was inactive, N.D: not determined

From Fig. 1, all tested agents showed concentration-dependent DPPH free-radical scavenging activity. Both the methanol extract and DCM fraction gave similar and comparatively low antioxidant activity compared with the reference antioxidant, ascorbic acid at tested concentrations of 20 - 100 $\mu\text{g/mL}$. However, the aqueous fraction gave the highest antioxidant activity (30 - 36% inhibition) at 60 - 100 $\mu\text{g/mL}$. It is obvious from this study that all the tested agents

were not comparable with the standard antioxidant agent, ascorbic acid. Antioxidant activity has been reported as one of the mechanisms of venom inactivation and inhibition [22]. This corroborates the higher inhibition of platelet aggregation observed for the aqueous fraction against both venoms (Table 1). Pereanez *et al.* [23] have described a correlation between antioxidant and anti-snake venom activities.

**Fig 1:** DPPH antioxidant activity of the leaf extract and fractions of *Bryophyllum pinnatum***Table 2:** Total phenolic contents in *Bryophyllum pinnatum* extract and fractions

Tested sample	Total phenolic content (mg/g plant)
Crude methanol extract	28.8 \pm 2.44
Dichloromethane fraction (DCM)	20.6 \pm 8.33
Aqueous fraction	31.3 \pm 3.93

Envenomation of mice with LD₉₉ dose of *Naja nigricollis* and *Bitis arietans* venoms followed by administration of different doses of methanol extract and fractions during the 48h incubation period resulted in varying degrees of protection (which decreased with time). Methanol extract, at all tested three doses, gave dose-dependent antivenin activity (33.3% protection) to mice against *Naja nigricollis* and *Bitis arietans* at 24h and 48h (Table 3). Similar antivenin activity, attaining a maximum of 75% protection with 200 mg/kg methanol extract was recorded at 24h and 48h with *Naja nigricollis*-treated mice. Also, *Bitis arietans*-pre-treated mice responded similarly (75 - 100% protection) to methanol extract during both incubation periods, and this indicated better antivenin activity against *Bitis arietans* snake bite. Treatment with 100 mg/kg DCM fraction gave better protection (75%) against *Naja nigricollis* bite at 24h, while the aqueous fraction offered similar antivenin activity (50% protection) in 24h and 48h. Contrariwise, both fractions offered similar protection at 24h

and 48h (75% protection for DCM fraction, and 100% for aqueous fraction) against *Bitis arietans*-treated mice, suggesting better antivenin activity for the aqueous fraction. Order of antivenin potency at 100 mg/kg is: DCM fraction > aqueous fraction = methanol extract on *Naja nigricollis* at 24h, while equal potency was demonstrated by all three tested agents at 48h. However, in the case of *Bitis arietans* venom, aqueous fraction = methanol extract > DCM fraction at 24h, and aqueous fraction > DCM fraction > methanol extract at 48h. According to this study, the more potent aqueous fraction qualifies for further phytochemical investigation to isolate the bioactive antivenin compounds.

Relative to the positive control antivenin tested at 0.1 mL/kg, DCM fraction gave comparable activity against *Naja nigricollis* venom (75% protection) only at 24h, while the aqueous fraction gave comparable result (100% protection) only against *Bitis arietans* venom at both incubation periods.

Makhija and Khamar [28] reviewed neutralizing properties of 57 medicinal plants against different snake venoms and 72 anti-snake venom Indian traditional recipes. Furthermore, Felix-Silva [29] published a comprehensive review of medicinal plants with inhibitory potential against local effects induced by *Naja*, *Bitis* and other snakes with 203 references. Recently, Gbolade *et al.* [19] also reported neutralizing effects of *Tithonia diversifolia* on snake venoms. Our findings are in tandem with published articles.

Table 3: Effect of *Bryophyllum pinnatum* extract and fractions on lethality (LD₉₉) of venoms in mice

Tested agent/ dose	24h		48h		24h		48h		
	<i>Najanigricollis</i>				<i>Bitis arietans</i>				
	Death	Protection	Death	Protection	Death	Protection	Death	Protection	
Methanol, 50 mg/kg	3/4	1/4(25%)	3/4	1/4(25%)	1/4	3/4(75%)*	1/4	3/4(75%)*	
	100 mg/kg	2/4	2/4(50%)	2/4	2/4(50%)	0/4	4/4(100%)*	2/4	2/4(50%)
	200 mg/kg	1/4	3/4(75%)*	1/4	3/4(75%)*	0/4	4/4(100%)*	0/4	4/4(100%)*
Dichloromethane fraction (DCM, 100 mg/kg)	1/4	3/4(75%)*	2/4	2/4(50%)	1/4	3/4(75%)*	1/4	3/4(75%)*	
Aqueous fraction (100 mg/kg)	2/4	2/4(50%)	2/4	2/4(50%)	0/4	4/4(100%)*	0/4	4/4(100%)*	
Negative control (0.1mLnormal saline)	4/4	0/4(0%)	4/4	0/4(0%)	3/4	1/4(25%)	3/4	1/4(25%)	
Positive control (0.2 mg/kg)	1/4	3/4(75%)*	1/4	3/4(75%)*	0/4	4/4(100%)*	0/4	4/4(100%)*	

The values above are mean of three replicates, n=3, (x ± SEM). Values with superscript* indicate significant difference at P < 0.05 relative to that of negative control (normal saline)

Results with rats envenomed with LD₅₀ of *Naja nigricollis* and *Bitis arietans* venoms are presented in Table 4. In this present study, methanol extract and DCM fraction gave complete protection to *Naja nigricollis*-treated rats during 48h (Table 4). Aqueous fraction resulted in only 75% survival during this period. Complete protection against *Bitis arietans* envenomed animals was observed for all tested doses of methanol extract at 24h and higher doses as well as the aqueous fraction in 48h. Dichloromethane fraction gave 75% antivenin activity against *Bitis arietans* at 24h and 48h. It is noteworthy that DCM fraction was more active against *Naja nigricollis* venom and aqueous fraction against *Bitis arietans*.

Zailani *et al.* [21] have investigated *Crinum jagus* and reported 50-56% neutralization of crude extracts on carpet viper venom, but the fractions gave higher effects. Similarly, relative increases in survival of envenomed mice and rats treated with *Pouzolzia indica* has been reported [20]. There are various mechanisms by which snake venom neutralization occur such as protein precipitation, enzyme activation, chelation, adjuvant action, antioxidant and protein folding [28]. Kumar *et al.* [30] postulated an indirect antibody-mediated mechanism for neutralization effect of plant extracts in antiophidic studies.

Table 4: Effect of *Bryophyllum pinnatum* extract and fractions on lethality (LD₅₀) of venoms in rats

Tested agent/ dose	24h		48h		24h		48h		
	<i>Najanigricollis</i>				<i>Bitis arietans</i>				
	Death	Protection	Death	Protection	Death	Protection	Death	Protection	
Methanol 50 mg/kg	0/4	4/4(100%)	0/4	4/4(100%)	0/4	4/4(100%)	1/4	3/4(75%)	
	100 mg/kg	0/4	4/4(100%)	0/4	4/4(100%)	0/4	4/4(100%)	0/4	4/4(100%)
	200 mg/kg	0/4	4/4(100%)	0/4	4/4(100%)	0/4	4/4(100%)	0/4	4/4(100%)
Dichloromethane fraction (DCM, 100 mg/kg)	0/4	4/4(100%)	0/4	4/4(100%)	1/4	3/4(75%)	1/4	3/4(75%)	
Aqueous fraction (100 mg/kg)	1/4	3/4(75%)	1/4	3/4(75%)	0/4	4/4(100%)	0/4	4/4(100%)	
Negative control (0.1mLnormal saline)	1/4	3/4(75%)	1/4	3/4(75%)	4/4	0/4(0%)	4/4	0/4(0%)	
Positive control (0.2mg/kg)	N.D	N.D	N.D	N.D	0/4	4/4(100%)	0/4	4/4(100%)	

The values above are mean of three replicates, n=3, (x ± SEM). Values with superscript* indicate significant difference at P < 0.05 relative to that of negative control (normal saline); N.D = not determined

Although snake bites are frequently treated using herbs, only few Nigerian medicinal plants have been so scientifically justified [21, 31-33]. This present investigation on *B. pinnatum* is a manifestation of growing interest in the development of plant-based antidotes for the treatment of snake bites in the rural communities in developing countries.

4. Conclusion

In summary, the results presented in this work demonstrated the potential of *B. pinnatum* leaf extract and fractions as an antiophidic agent, particularly against local effects induced by *Bitis arietans* envenomation. Our results suggest the potential of *B. pinnatum* as a source of bioactive compounds in the development of new efficient antivenoms for the treatment of ophidic accidents. The present study has confirmed the folkloric use of the plant for treating snakebite victims among the rural African population. Further study on the potent aqueous fraction could unravel more chemical antidote for snake envenoming.

Conflicts of Interest

There is no conflict of interest.

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4. References

- WHO. Snakes and snake bites Part 1: Prevention of snake bites. Pharmaceuticals Newsletter 2007; 4 (1):1-4.
- Habib AG, Gebi UI, Onyemelukwe GC. Snake bite in Nigeria, African Journal of medicine and medical sciences. 2001; 30(3):171-178.
- Malik R, Bukie J, Oigocho JJ. Assessment of snake bites in some selected communities in Benue state, Nigeria. Proceedings of 6th NSCB Biodiversity Conference, University of Uyo, Nigeria, 2018, 61-64.
- Mustapha SK. Snakebites in Gombe, Nigeria, Highland medical research journal. 2003; 1(4):22-27.
- Ndu IK, Edelu BO, Ekwochi U. Snakebites in a Nigerian children population: A 5-year review, Sahel medical journal. 2018; 21(4):204-207.
- Sani UM, Jiya NM, Ibitoye PK, Ahmad MM. Presentation and outcome of snake bite among children in

- Sokoto, North-Western Nigeria, Sahel medical journal. 2013; 16:148-153
7. Ameen NA, Salihu T, Mbaoji CO, Anoruo-Dibia CA, Adedokun RAM. Medicinal plants used to treat snake bite by Fulani herdsmen in Taraba state, Nigeria, International Journal of applied agricultural and apicultural research. 2015; 11(1 & 2):10-21.
 8. Kadali VN, Kameswara RK, Sandeep BV. Medicinal plants with anti-snake venom property - A review, the pharma innovation journal. 2015; 4(7):11-15.
 9. Kumar SS, Padhan B, Palita SK, Panda D. Plants used against snakebite by tribal people of Koraput district of Odisha, India, Journal of medicinal plants studies. 2016a; 4(6):38-42.
 10. Upasani MS, Upasani SV, Beldar VG, Beldar CG. Infrequent use of medicinal plants from India in snakebite treatment, Integrative medicine research. 2018; 7(1):9-26.
 11. Owuor BO, Kisangau DP. Kenyan medicinal plants used as antivenin: a comparison of plant usage, Journal of Ethno biology and Ethno medicine. 2006; 2:7. DOI: 10.1186/1746-4269-2-7.
 12. Vásquez J, Alarcón JC, Jiménez SL, Jaramillo GI, Gómez-Betancur IC, Rey-Suárez JP et al. Main plants used in traditional medicine for the treatment of snake bites in the regions of the Department of Antioquia, Colombia, Journal of Ethno pharmacology. 2015; 170:158-166.
 13. Fernandes JM, Cunha LM, Azevedo EP, Lourenco EMG, Fernandes-Pedrosa MF, Zucolotto SM. *Kalanchoe laciniata* and *Bryophyllum pinnatum*: an updated review about ethno pharmacology, phytochemistry, pharmacology and toxicology, Revista Brasileira de Farmacognosia. 2019; 29:529-558.
 14. Sikdar M, Dutta U. Traditional Phyto therapy among the Nath people of Assam, Studies on Ethno medicine. 2008; 2(1):39-45.
 15. Sarkhel S, Ethno botanical survey of folklore plants used in treatment of snakebite in Paschim Medinipur district, West Bengal, Asian pacific journal of tropical biomedicine. 2014; 4(5):416-420.
 16. Fernandes JM, Felix-Silva J, da Cunha LM, Gomes JA, Siqueira EM, Gimenes LP et al. Inhibitory effects of hydroethanolic leaf extracts of *Kalanchoe brasiliensis* and *Kalanchoe pinnata* (Crassulaceae) against local effects induced by *Bothrops jararaca* snake venom, PLoS ONE. 2016; 11(12):e0168658. DOI: 10.1371/journal.pone.0168658
 17. Evans WC. Trease and Evans Textbook of Pharmacognosy The Alden Press, Oxford, Great Britain, 1989, 832.
 18. Villano D, Fernandez-Pachon MS, Moya ML, Troncoso AM, Garcia-Parrill MC. Radical scavenging ability of polyphenolic compounds towards DPPH free radical Talanta, 2007; 71:230-235.
 19. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume, Journal of Taibah University of science. 2015; 9:449-454.
 20. Ahmed A, Rajendaran K, Jaiswal D, Singh HP, Mishra A, Chandra D et al. Anti-snake venom activity of different extracts of *Pouzolia indica* against Russel viper venom, International journal of chem tech research. 2010; 2:744-751.
 21. Zailani AH, Magaji PK, Sarkiyayi S, Wurochekke AU. Anti-snake venom activity of aqueous and ethanolic extracts of *Cajanus jagus* bulb, Asian Journal of research in biochemistry. 2018; 2(2):1-9.
 22. Alam MI, Gomes A. Viper venom-induced inflammation and inhibition of free radical formation by pure compound (2-hydroxy-4-methoxy benzoic acid) isolated and purified from anantamul (*Hemidesmus indicus* R BR) root extract Toxicon. 1998; 36(1):207215.
 23. Pereanez JA, Lobo-Echeverri T, Rojano B, Vargas L, Fernandez M, Gaviria CA et al. Correlation of the inhibitory activity of phospholipase A₂ snake venom and the antioxidant activity of Colombian plant extracts. Revista Brasileira de Farmacognosia. 2010; 20:6.
 24. Guimarães CLS, Moreira-Dill LS, Fernandes RS, Costa TR, Hage-Melim LI, Marcussi S et al. Biodiversity as a source of bioactive compounds against snakebites, Current medicinal chemistry. 2014; 21(25):2952-2979.
 25. Sajon SR, Sana S, Rana S. Anti-venoms for snake bite: A synthetic and traditional drugs review, Journal of Pharmacognosy and Phytochemistry. 2017; 6(3):190-197.
 26. Pareanez JA, Nunez V, Patino AC, Londono M, Quintana JC. Inhibitory effects of plant phenolic compounds on enzymatic and cytotoxic activities induced by a snake venom phospholipase A2 Pharmacology and toxicology. 2011; 18:3.
 27. Alam MI, Alam MA, Alam O, Nargotra A, Taneja SC, Koul S. Molecular modeling and snake venom phospholipase A2 inhibition by phenolic compounds: structure-activity relationship, European journal of medicinal chemistry. 2016; 114:209-219.
 28. Makhija IK, Khamar D. Anti-snake venom properties of medicinal plants, Der Pharmacia Littre. 2010, 2(5):399-411.
 29. Félix-Silva J, Silva-Junior AA, Zucolotto SM, Fernandes-Pedrosa MdeF. Medicinal plants for the treatment of local tissue damage induced by snake venoms: An overview from traditional use to pharmacological evidence, Evidence-based complementary and alternative medicine, 2017, 1-52.
 30. Kumar A, Gupta C, Nair ST, Salunke DM. MP-4 contributes to snake venom neutralization by *Mucuna pruriens* seeds through an indirect antibody-mediated mechanism, Journal of biological chemistry. 2016b; 291(21):11373-11384.
 31. Abubakar MS, Balogun E, Abdurahman EM, Nok AJ, Shok M, Mohammed A, Garba M. Ethnomedical treatment of poisonous snakebites: Plant extract neutralized *Naja nigricollis* venom pharmaceutical biology. 2006; 44(5):343-348.
 32. Ibrahim MA, Aliyu AB, Abusufiyanu A, Bashir M, Sallau AB. Inhibition of *Naja nigricollis* (Reindhardt) venom protease activity by *Luffa egyptiaca* (Mill) and *Nicotiana rustica* (Linn) extracts, Indian journal of experimental biology. 2011; 49:552-554.
 33. Gbolade AA, Ukaigwe I, Omorogbe A. Neutralization effect of *Tithonia diversifolia* (Hemsl) A Gray (Asteraceae) against *Bitis arietans* and *Naja nigricollis* venoms-induced toxicity, Ethiopian pharmaceutical journal. 2019, 35.