Trop J Nat Prod Res, July 2020; 4(7):286-291 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phospholipase A_2 from *Naja nigricollis* and Inhibitory Activity of Extracts from *Commiphora africana* (A. Rich.) Engl. (Burseraceae)

Abdulmumin Z. Abubakar*, Abdulrahman Adamu, Umar F. Shehu, Zainab Mohammed, Garba Ibrahim, Mujtaba S. Abubakar

Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria.

ARTICLE INFO

Article history:
Received 24 June 2020
Revised 13 July 2020
Accepted 22 July 2020
Published online 27 July 2020

ABSTRACT

Snakebite envenomation is a neglected global health problem responsible for substantial mortality, disability and psychological morbidity especially in rural tropical and subtropical regions of the world. Phospholipase A_2 (PLA_{2s}) enzymes constitute a major toxic venom component especially the venoms from elapids. *Naja nigricollis* is of the family Elapidae. *Commiphora africana* is a species of the family Burseraceae which is traditionally used in many parts of Africa for the treatment of number of ailments including snake envenomation, cancer and some inflammatory diseases. The present study was designed to determine the hydrolytic actions of PLA_2 present in the *N. nigricollis* venom in an egg yolk suspension mixture and to access the PLA_2 inhibitory effects of extracts of *C. africana* by acidimetric assay method. The snake venom PLA_2 was found to release free fatty acids from the egg yolk suspension in a dose-dependent manner. The venom concentration of 0.1 mg/mL was found to liberate 13.3 µmol of free fatty acids with a decline of pH of 0.1 which may be attributable to the phospholipase A_2 in the venom. Higher liberations of 133 µmol levels of free fatty acids was achieved with a decrease of pH of 1.0 at concentrations of 4.0 mg/mL of the venom. The PLA_2 inhibitory activity observed from the extracts and fractions also showed concentration-dependent inhibition as depicted by the IC_{50} values. These results may provide some scientific basis for the usefulness of the plant in the traditional management of snakebite envenomation across Africa.

Keywords: Phospholipases A_2, *Commiphora africana*, *Naja nigricollis*, inhibition.

Introduction

Snakebite envenoming is a potentially life-threatening disease that typically results from the injection of a mixture of different toxins following the bite of a venomous snake. It is a neglected public health issue in many tropical and subtropical countries with about 5.4 million snake bites occurring each year, resulting in 1.8 to 2.7 million cases of envenoming around the world leading to over 140,000 deaths and around three times as many amputations and other permanent disabilities each year with most occurring in Africa, Asia and Latin America.[1] Thus, snake envenomation has been included in the list of Neglected Tropical Diseases (NTDs) and formally listed as a highest priority neglected tropical disease.[1] The highest incidence of snakebite in Africa occurs in the West African savanna region. In this region, saw-scaled or carpet vipers, spitting cobras and puff adders are common causes of serious envenoming.[2,3] *Naja nigricollis* or the black-necked spitting cobra is a widely distributed venomous snake species of the family Elapidae.[4] It is one of the most important snakes associated with envenoming in Nigeria.[5] Snake venoms are complicated mixtures generally consisting of phospholipase A_{2s}, metalloproteases, C-lectins, serine proteases, L-amino acid oxidases, disintegrins and a few other compounds in different proportions.[6] The potent elapid venoms generally exhibit significantly higher concentrations of phospholipase A_2s and lower concentrations of snake venom metalloproteases.[7] Snake venom phospholipase A_2s ($svPLA_2$s) are found to exist in almost all kinds of snake families that were studied with the highest amounts been found in venomous families such as Elapidae, Viperidae and Hydrophidae, while the lowest was observed in Colubridae which is usually nonvenomous.[8] Studies by several researchers also indicates that PLA_2s are the predominant and fatal toxins in most snake venoms.[9-12] Along with their catalytic activity, $svPLA_2$s elicit a wide variety of pharmacological effects that play a pivotal role in envenomation damage.[11] Hence, neutralization of the $svPLA_2$s could weaken or inhibit toxic damage. *Commiphora africana* is a species of the family Burseraceae. It is traditionally used for the treatment of a number of ailments including the management of snake envenomation, typhoid, wound healing, pain, dysentery, heart burn, ulcer and malaria.[13-17] The stem bark of *Commiphora africana* is chewed with natron and sometimes mixed with shea butter and applied to scorpion stings and snakebite sites in Nigeria while inflammation of the eyes is treated by placing the head over a steaming pot containing the stem bark.[14] In Central Africa, the bark mixed with salt is applied to snake bite site.[18] Previous phytochemical and biological investigations on *Commiphora africana* extracts reported the presence of some of the phytoconstituents such as flavonoids, tannins, triterpenes, saponins,[14, 15] and antiradical scavenging, antimicrobial, anthelmintic, antiplasmodial, antitumoral and antivenom activities.[18-22] Currently, qualitative antivenin is the only specific treatment towards envenomation and to effectively prevent or reverse most of the venomous effects of snake bites. Due to difficulties in accessing effective antivenins as a result of shortages occasioned by lack of cold chain storage or high cost in addition to common clinical side-effects of current antivenin, there has been concerted efforts to develop antidotes from herbal extracts, marine compounds, mammalian and snake serum, modified chemical molecules and peptides.[10,11] The

*Corresponding author. E mail: azabubakar01@gmail.com; azabubakar@abu.edu.ng
Tel: +2348065904149

Citation: Abubakar AZ, Adamu A, Shehu UF, Mohammed Z, Ibrahim G, Abubakar MS. Phospholipase A_2 from *Naja nigricollis* and Inhibitory Activity of Extracts from *Commiphora africana* (A. Rich.) Engl. (Burseraceae). Trop J Nat Prod Res. 2020; 4(7):286-291. doi.org/10.26538/tjnpr/v4i7.6

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

snake venom PLA_2s have been identified as one of the most widely used target for antidote screening and researches have shown that both natural and synthetic $svPLA_2$ inhibitors are able to attenuate the morbidity and mortality of snakebite envenomation.[12] Thus, the search for complementary therapies to treat snakebites is relevant and medicinal plants could be a rich source of natural inhibitors and pharmacologically active compounds with ability to neutralize a broad spectrum of venomous toxins. *Commiphora africana* has been a useful medicinal plant in the management of snake envenomation across Africa and thus the desire to carry out this study.

Materials and Methods

Plant Identification

The herbarium specimen of the plant sample was identified and authenticated by cross matching with an existing specimen voucher number no. 2848 as *Commiphora africana* by Taxonomist Sanusi Namadi of the herbarium unit of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria - Nigeria.

Collection and preparation of plant material

The leaf and stem bark of *Commiphora africana* were collected around the vicinity of Ahmadu Bello University Dam, Samaru campus, Zaria in the month of September, 2017. The collected samples were washed with distilled water, air dried under shade for 10 days, after which they were pulverized with the aid of a milling machine, sieved and packaged into tightly closed plastic containers and kept at room temperature until required for use. They were subsequently referred to as the leaf and stem bark powdered plant materials, respectively.

Extraction and Fractionation

The extraction was carried out according to the modified method of Su *et al*.[23] using cold maceration technique. The dried powdered leaf and stem bark of *Commiphora africana* (50 g each) were extracted with 80% aqueous ethanol (100 mL) using a mechanical shaker for 12 hours and the mixture filtered using Whatman No.1 filter paper. The filtrates were concentrated using rotatory evaporator and completely dried on a water bath to obtain residues which were transferred into a glass container, labeled as *Commiphora africana* leaf extract (CALE) and *Commiphora africana* stem bark extract (CASE), respectively. The glass containers were kept in desiccators at room temperature prior to use.

The stem bark extract (CASE) (10 g) was properly dissolved in distilled water (50 mL) to obtain a suspension which was filtered using the Whatman No.1 filter paper, the filtrate was subsequently fractionated according to the modified method of Woo *et al*.[24] by partitioning with ethyl acetate and n-butanol solvents which were subsequently concentrated using rotatory evaporator and completely dried on a water bath to obtain residues which were transferred into a glass container, labeled as ethyl acetate fraction (EAF), saponin-rich fraction (SRF), flavonoid-rich fraction (FRF) and residual aqueous fraction (RAF), respectively (Figure 1).

Source of snake venom

The profiled freeze-dried venom of *Naja nigricollis* was obtained from the laboratory of Prof. M. S. Abubakar of the Venom, Anti-Venom and Natural Toxins Research Centre, Ahmadu Bello University, Zaria, Nigeria.

Determination of the hydrolytic activity of the phospholipase A_2 enzyme in N. nigricollis venom

Phospholipase A_2s are enzymes found to catalyze the hydrolysis of fatty ester in the 2-acyl ester bond in phosphoglycerides leading to the release of free fatty acids. The acidimetric assay for PLA_2 enzymes was conducted as described by Yap *et al*.[25] An egg yolk suspension mixture (15 mL) was prepared by mixing three equal parts consisting of one-part chicken egg yolk, one part 18 mM calcium chloride and one part 8.1 mM sodium deoxycholate. The pH of the egg yolk suspension was adjusted to 8.0 with 1 M sodium hydroxide and stirred for 10 minutes to ensure homogenous suspension. Thereafter, 0.1 mL of *N. nigricollis* venom (1 mg/mL) was added to the egg yolk mixture to initiate the process of hydrolysis. A decrease in pH of the suspension was noted after two minutes with the help of a pH meter. The above procedure was repeated for (0.5, 1.0, 2.0 and 4 mg/mL) venom concentrations. The normal saline was used as control. A decline of 1 pH unit from (8.0 to 7.0) corresponds to 133 µmoles of fatty acid release.[26] Enzyme activity was expressed as µmoles of fatty acid released/minute. The procedure was done in triplicates.

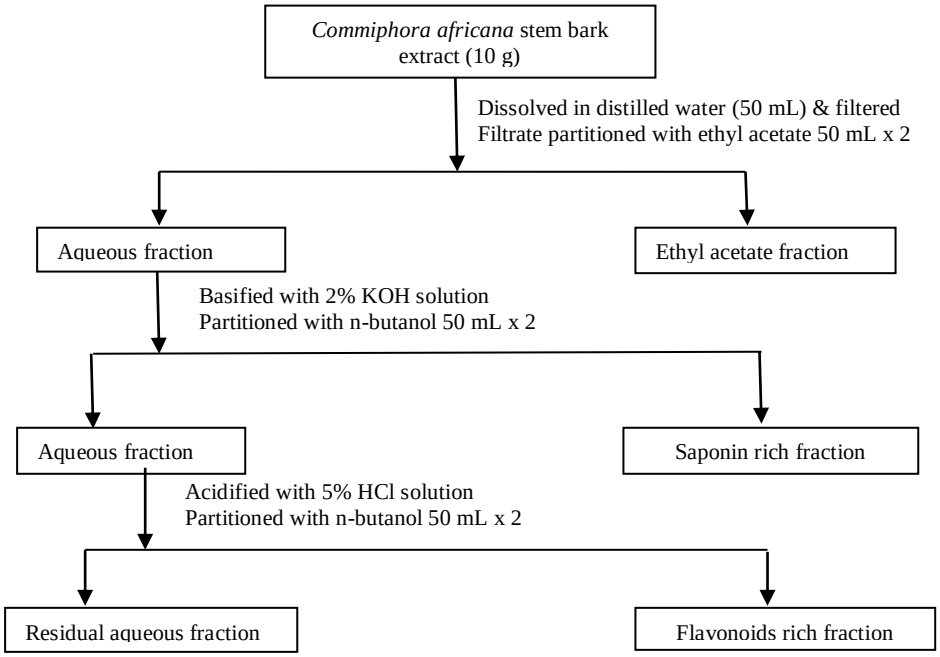

Figure 1: Schematic chart for the fractionation of *C. africana* stem bark extract using a modified method of Woo *et al*.[24]

Determination of the phospholipase A_2 inhibitory activity of the extracts and fractions of C. africana

The inhibitory assay was carried out according to the method of Asad *et al*.[27] with little modification. One milliliter of *N. nigricollis* venom (0.1 mg/mL) was incubated with (1 mL) each of various concentrations (0.1, 0.25, 0.50 and 1mg/mL) of the extracts and fractions of *C. africana* for thirty minutes at 37°C. Thereafter, 0.1 mL of the preincubated mixture was added to each egg yolk mixture (15 mL) to initiate hydrolysis and normal saline was used as control. A decrease in pH of the suspension was noted after two minutes using a pH meter. From the hydrolytic activity studies of the phospholipase A_2, it was observed that 0.1 mL of *N. nigricollis* venom (1 mg/mL) was able to lower the pH of the egg yolk mixture from 8.0 to 7.5 and thus was adopted as reference. Results are indicated as inhibition percentages, where 100% is the activity induced by the crude venom alone and the IC_{50} was determined from the linear portion of the response dose curves. All tests and readings were carried out in triplicates.

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of the three analytical triplicates by using Microsoft Excel version 2007 and comparisons between groups was carried out using the Student's t-test. Level of significance was set at $P \leq 0.05$.

Results and Discussion

Extractive profiles of the extracts and fractions of Commiphora africana

The extractive profile of 50 g each of *C. africana* powdered leaf and stem bark yielded 14 g of *Commiphora africana* leaf extract (CALE) and 15 g of *Commiphora africana* stem bark extract (CASE), while the fractionated portions of the stem bark extract yielded 1.0 g of ethyl acetate fraction, 0. 2 g of saponin-rich fraction and 0.7 g of flavonoid-rich fractions.

Hydrolytic activity of the phospholipases A_2 in N. nigricollis venom

Snake venom (0.1 mg) was found to liberate 13.3 µmol of free fatty acids. However, on increasing the quantity of venom (0.5, 1.0 and 2.0 µg) larger amounts of fatty acids (39.9, 66.5 and 93.1 µmol/min) were liberated, respectively. One unit drop in pH from 8 to 7 was observed at concentration of 4.0 mg of *N. nigricollis* venom (Table 1). The hydrolytic activity of snake venom PLA_2s at the 2-acyl ester bond in phospholipids or lecithin in the egg yolk mixture lead to the release of free fatty acids which can be measured in terms of pH changes of between pH 8.0 to 7.0. The assay is adjudged to be highly reproducible and as sensitive as most established assays for phospholipase A.[26] Snake venom PLA_2s, in particular, play a critical role in early morbidity and mortality from snakebite, causing death by paralysis as well as destruction of tissues and derangement of homeostatic mechanisms critical for regulation of coagulation and oxygen transport.[25] They also play a very important role in neurotoxicity and myotoxicity which are of major clinical relevance in snake envenomation.[26, 28] The phospholipases from venoms of elapid family for example *N. nigricollis* exert pronounced neurotoxic effect resulting to flaccid paralysis as a major clinical manifestation.[29] The enzymatic activity of PLA_2 was observed to positively correlate with increased quantity of venom (Table 1). PLA_2 can also interact with other components in the cells particularly the lipids.[28] Both specific and nonspecific PLA_2 develop protein - phospholipids interaction with covalent, non-covalent or disulfide interactions leading to hydrolysis of phospholipids and release of free fatty acids.[30]

Inhibitory properties of C. africana leaf and stem bark extracts against N. nigricollis phospholipases A_2

The PLA_2 inhibitory activity was observed from the leaf and stem bark extracts of *Commiphora africana* which showed concentration-dependent inhibition with the leaf extract having IC_{50} value of 576.49 µg/mL while the stem bark had IC_{50} value of 213.56 µg/mL (Table 2). The stem bark extract was observed to exhibit a more significant PLA_2 inhibition when compared to the leaf extract as depicted by its lower IC_{50} value. The half maximal inhibitory concentration (IC_{50}) represents the concentration of a substance that is required for 50% inhibition *in vitro*. The IC_{50} value is inversely proportional to inhibitory activity, thus the lower the value, the more active is the extract. There have been documented evidence of use of herbs as remedy against snakebite especially in the tropical and subtropical snake prone regions of Africa, Asia and South America.[31] These medicinal plants and other biological products having antidotal efficacy against snake envenomation have been evaluated using various models.[32-35] Most plant antivenom agents have been proposed to function by neutralizing snake venom PLA_2's toxicity.[39] The inhibitory property exhibited by the leaf and stem bark extracts of *C. africana* may be due to the presence of the active chemical constituent(s) in the extract and the relative abundance of these constituents determines the inhibitory activities of the extracts as reflected by the IC_{50} values.

Due to the superior phospholipase A_2 inhibitory activity exhibited by the stem bark extract as compared to the leaf extract and coupled with the reported traditional uses of the stem bark for the management of snake bite envenomation,[14,15] the stem bark extract was further fractionated (Figure 1).

Inhibitory activity of fractions from stem bark extract of C. africana against N. nigricollis phospholipases A_2

The fractionated portions (ethyl acetate, saponin-rich and flavonoid-rich fractions) from the stem bark extract exhibited a much higher inhibition as depicted by the IC_{50} values of 21.36, 26.47 and 17.52 µg/mL, respectively (Table 3). The flavonoid-rich fraction was found to be the most potent fraction, then the ethyl acetate fraction and the saponin-rich fractions as indicated by their lower IC_{50} values (Table 3). Polyphenols such as terpenoids, xanthenes, flavonoids, rosmarinic acid, aristolochic acid and α-tocopherol have been reported to inhibit PLA_2s from snake venoms.[36, 37] Other compounds such as ferulic acid, caffeic acid and gallic acid have shown an inhibitory ability against the activities induced by whole snake venoms.[38] Recent researches have also shown that human secretory PLA_2 inhibitors such as quercetin, bioflavonoids and other flavonoids isolated from plant extracts can also inhibit snake venom PLA_2.[25, 39] Similar results were obtained with other polyphenolic compounds (flavonoids and isoflavones) for inhibiting telomerase and aromatase.[39] *Commiphora africana* have been reported to contain some of these phytoconstituents such as flavonoids, tannins, triterpenes and saponins.[14, 15] Medicinal plant extracts as traditional antidotes have long been used in countries around the world and especially in communities where the access to effective antivenins is a challenge, thus these traditional and herbal treatments are often used as adjuvant therapies before or along with the antivenin treatment.[39] The inhibition of the venom phospholipase activity in the presence of the extract implies that the active constituent(s) or component in the extract could have an effect on the action of this enzyme and thus higher amounts or concentrations of the extract may lead to lowered enzyme activity and consequently alleviate or ameliorate the clinical symptoms related to the physiological activity of the enzyme. Although the component(s) of the extracts responsible for the observed PLA_2 inhibitory activity in this study has not yet been identified, it is likely that the extract is acting through a direct physical interaction with the venom or through some enzymatic processes. Direct physical interaction is a characteristic mode of action of many polyphenolic compounds found in plants examples of which includes tannins (epigallocatechin, epi-catechin), flavonoids, saponins and long-chain esters of some organic acids.[22,32] Medicinal plants are endowed with abundance of these constituents and therefore may have therapeutic properties in application as natural inhibitors. The inhibitory effect of the extract in this assay may be attributed to this pathway, however further work is necessary to isolate and examine such component(s) and also conduct other assay models to validate these speculations.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Effect of various concentration of *N. nigricollis* venom on the amount of free fatty acids released in terms of change in pH of egg yolk suspension.

Concentration of Venom (mg/mL)	Change in pH (Mean ± SD)	Fatty acid released/min (µmole)
Control (saline)	8.0 ± 0.02	0.0
0.1	7.9 ± 0.02	13.3
0.5	7.7 ± 0.03	39.9
1.0	7.5 ± 0.01	66.5
2.0	7.3 ± 0.04	93.1
4.0	7.0 ± 0.03	133.0

Conclusion

The significant phospholipase A_2 inhibitory activity exhibited by the extracts of *Commiphora africana* may suggest that the plant contain some constituents with antivenin properties with the relative variation in concentration between the leaf and stem bark. These results could provide some scientific basis for the effectiveness of the plant for the traditional management of snake envenomation in Northern Nigeria and around Africa.

Conflict of interest

The authors declare no conflicting interest

Table 2: Antidotal properties of the leaf and stem bark extracts of *C. africana* against *N. nigricollis* phospholipases A_2 in terms of an increase in pH of an egg yolk suspension.

Sample	Concentration µg/mL	pH	Percentage inhibition (%)	IC_{50} (µg/mL)	r^2
CALE	100	7.5 ± 0.03	0		
	250	7.6 ± 0.03	20		
	500	7.8 ± 0.01	60	576.49	0.9001
	1000	7.9 ± 0.03	80		
CASE	100	7.6 ± 0.04	20		
	250	7.7 ± 0.03	40	213.56	0.6846
	500	8.0 ± 0.04	100		
	1000	8.0 ± 0.01	100		
Crude venom	100	7.5 ± 0.01	reference		
Normal saline		8.0 ± 0.02	control		

CALE = *C. africana* leaf extract, CASE = *C. africana* stem bark extract. Values are expressed as mean ± SD. r^2 = regression co-efficient.

Table 3: Antidotal properties of some fractions from *Commiphora africana* stem bark extract (CASE) against *N. nigricollis* phospholipases A_2 in terms of an increase in pH of an egg yolk suspension.

Sample	Concentration µg/mL	pH	Percentage inhibition (%)	IC_{50} (µg/mL)	r^2
EAF	10	7.6 ± 0.04	20		
	25	7.7 ± 0.03	60	21.36	0.6846
	50	8.0 ± 0.04	100		
	100	8.0 ± 0.01	100		
SRF	10	7.6 ± 0.04	20		
	25	7.7 ± 0.03	60	26.47	0.6846
	50	8.0 ± 0.04	100		
	100	8.0 ± 0.04	100		
FRF	10	7.6 ± 0.04	20		
	25	7.9 ± 0.03	80	17.52	0.6284
	50	7.9 ± 0.04	80		
	100	8.0 ± 0.04	100		
Crude venom	100	7.5 ± 0.01	Reference		
Normal saline		8.0 ± 0.02	Control		

EAF = Ethyl acetate fraction, SRF = Saponin-rich fraction and FRF = Flavonoid-rich fraction. Values are expressed as mean ± SD. r^2 = regression co-efficient.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors appreciate the technical staffs of the research laboratory, Venom, Anti-Venom and Natural Toxins Research Centre and Department of Pharmacognosy and Drug Development both of Ahmadu Bello University, Zaria, Nigeria for their support in handling some of the facilities used in carrying out this research work.

References

1. World Health Organization. Snakebite envenoming—a strategy for prevention and control. [Online]. 2019 [cited 2019 Nov 17]. Available from: https://www.who.int/snakebites/resources/who-cds-ntd-nzd-.03/en.
2. World Health Organization, Recommendations for the production, control and regulation of human plasma for fractionation.2007: Geneva. Annex 4: 189-200.
3. Chippaux JP. Estimate of the burden of snakebites in sub-Saharan Africa: A meta-analytic approach. Toxicon, 2011; 57:586–599.
4. Luiselli L, Angelici FM, Akani GC. Comparative feeding strategies and dietary plasticity of the sympatric cobras *Naja melanoleuca* and *Naja nigricollis* in three diverging Afrotropical habitats, Canad J Zool. 2002; 80:55-63.
5. Habib AG. Public health aspects of snakebite care in West Africa: perspectives from Nigeria. J Ven Anim Toxins Incl Trop Dis. 2013; 19:27-35.
6. Gutierrez JM, Williams D, Fan HW, Warrell DA. Snake bite envenoming from a global perspective: Towards an integrated approach. Toxicon, 2010; 56:1223-1235.
7. Fernandes CA, Cardoso FF, Cavalcante WG, Soares AM, Dal-Pai M, Gallacci M, Fontes MR. Structural Basis for the Inhibition of a Phospholipase A_2-Like Toxin by Caffeic and Aristolochic Acids. PLoS ONE 2015; 10:e0133370.
8. Mackessy SP. The field of reptile toxinology snakes, lizards, and their venoms, In: Handbook of Venoms and Toxins of Reptiles, S. P. Mackessy, 1st Ed., 2010. 3–19 p.
9. Calvete JJ. Proteomics in venom research: a focus on PLA_2 molecules, Acta Chim Sloven. 2011; 58(4):629–637.
10. Georgieva D, Arni RK, Betzel, C. Proteome analysis of snake venom toxins: pharmacological insights. Exp Rev Proteom. 2008; 5(6):787–797.
11. Ziganshin RH, Kovalchuk SI, Arapidi GP. Quantitative proteomic analysis of Vietnamese krait venoms: neurotoxins are the major components in *Bungarus multicinctus* and phospholipases A2 in *Bungarus fasciatus*, Toxicon, 2015; 107:197–209.
12. Gutierrez JM and Lomonte B. Phospholipases A_2: unveiling the secrets of a functionally versatile group of snake venom toxins. Toxicon, 2013; 62:27–39.
13. Hanus LO, Rezanka I, Dembitsky VM, Moussaieff A. Myrrh-commiphora chemistry. Biomed Paper 2005; 149:3-28.
14. Abubakar AZ, Adamu A, Abubakar A, Abubakar MS, Musa KY, Magaji MG. Anti-Cobra venom activities of the ethanol extract of Oleo-gum resin from *Commiphora africana* (A. Rich) Engl. (Burseraceae). J Pharmacol Trop Ther. 2014; 4(1):1-6.
15. Ezekiel I, Mabrouk MA, Ayo JO. Study of the Effect of Hydro-Ethanolic Extract of *Commiphora africana* (Stem-bark) on Inflammation and Pain in Rodents. Asian J Med Sci. 2010; 2(3):81-84.
16. Nuhu A, Danmalam UH, Ilyas N, Zakariya AM, Shehu S, Jajere UM. Evaluation of Antiulcer Activity of *Commiphora africana* (A. Rich) Engl. (Burseraceae) Stem-bark Extracts Using Ethanol Induced Ulcer Model in Albino Rats. Int J Curr Microbiol Appl Sci. 2016; 5(3):15-25.
17. Compaor´e M, Lamien-Meda A, Mogosan C, Lamien CE, Kiendrebeogo M, Vostinaru O. Antioxidant, diuretic activities and polyphenol content of *Stereospermum kunthianum* Cham. (Bignoniaceae). Nat Prod Res. 2011; 25(19):1777-1788.
18. Paraskeva MP, van Vuuren SF, van Zyl RL, Davids H, Viljoen AM. The *in vitro* biological activity of selected South African Commiphora species. J Ethnopharmacol. 2008; 119(3):673-679.
19. Gbolade AA and Adeyemi AA. Anthelmintic activities of three medicinal plants from Nigeria. Fitoter., 2008; 79(3):223-225.
20. Akor JS and Anjorin TS. Phytochemical and Antimicrobial studies of *Commiphora africana* root extracts. Int J Agric Biol. 2009; 11(6):1814-9596.
21. Ma J, Jones SH, Hecht SN. A dihydroflavonol glucoside from *Commiphora africana* that mediates DNA strand scission. J Nat Prod. 2005; 68(1):115-117.
22. Abubakar AZ, Adamu A, Abubakar MS, Mohammed Z. Anti - venom activity of the ethanol stem bark extract of *Commiphora africana* (A. Rich.) Engl. (Burseraceae). Nig J Pharm Sci. 2016; 15(2):56-63.
23. Su S, Wang T, Chen T, Duan J, Yu L, Tang Y. Cytotoxicity activity of extracts and compounds from *Commiphora myrrha* resin against human gynecologic cancer cells. J Med Plants Res. 2011; 5(8):1382-1389.
24. Woo WS, Shin KH, Kang SS. Chemistry and Pharmacology of Flavone–C-Glycoside from Ziziphus seeds. Kor J Pharmacogn, 1980; 11(3-4):141-148.
25. Yap MK, Tan NH, Fung SY. Biochemical and toxinological characterization of *Naja sumatrana* (Equatorial spitting cobra) venom. J Venom Anim Toxins Incl Trop Dis. 2011; 17:451- 459.
26. Tan NH and Tan CS. Acidimetric assay for phospholipase A2 using egg yolk suspension as substrate. Anal Biochem. 1988; 170(2):282-288.
27. Asad MHHB, Durr-E-S, Tahir Y, Ghulam M, Muhammad SH, Muhammad SH, Muhammad TN, Saira A, Shujaat AK, Izhar H. Phospholipases A2: Enzymatic assay for snake venom (*Naja Naja karachiensis*) with their neutralization by medicinal plants of Pakistan. Acta Polon Pharm Drug Res. 2014; 71(4):625 – 630.
28. Mukherjee AK, Doley R, Saikia D. Isolation of a snake venom phospholipase A2 (PLA2) inhibitor (AIPLAI) from leaves of *Azadirachta indica* (Neem): Mechanism of PLA2 inhibition by AIPLAI *in vitro* condition. Toxicon. 2008; 51:1548-1553.
29. Hart A, Scott-Davey T, Harris JB. Venom of Collett's snake blocks the binding of α-bungarotoxin to acetylcholine receptors at chick but not human neuromuscular junctions: A histochemical study. Toxicon. 2008; 52:647–650.

30. Kini RM. Excitement ahead: structure, function and mechanism of snake venom phospholipase A2 enzymes. Toxicon, 2003; 42 (8): 827-840.
31. Theakston RD, Warrell DA, Griffiths E. Report of a WHO workshop on the standardization and control of antivenoms. Toxicon. 2003; 41:541-557.
32. Abubakar MS, Sule IM, Pateh UU, AbdulRahman EM, Haruna AK, Jahun BM. Invitro venom detoxifying action of leaf extract of *Guiera senegalensis*. J Ethnopharmacol. 2000; 69:253-257.
33. 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. Pharm. Biol. 2006; 44:343-348.
34. Sallau AB, Njoku GC, Olabisi AR, Wurocheke AU, Abdulkadir AA, Isah S, Abubakar MS, Ibrahim S. Effect of *Guiera senegalensis* Leaf extract on some *Echis carinatus* Venom enzymes. J Med Sci. 2005; 5(4):280-283.
35. Abiodun F, Omonkhelin JO, Obasuyi O. Physiochemical, Antimicrobial and Anti-inflammatory evaluation of fixed oil from *Boa constrictor*. Acta Poloniae Pharm Drug Res. 2008; 65(4): 477- 480.
36. Asad MHHB, Murtaza G, Siraj S, Khan SA, Azhar S, Hussain MS, Ismail T, Hussain MS, Hussain I. Enlisting the scientifically unnoticed medicinal plants of Pakistan as a source of novel therapeutic agents showing anti-venom activity. Afr J Pharm Pharmacol. 2011; 5:2292-2305.
37. Ticli FK, Hage LI, Cambraia RS, Pereira PS, Magro AJ. Rosmarinic acid, a new snake venom phospholipase A_2 inhibitor from *Cordia verbenacea* (Boraginaceae): antiserum action potentiation and molecular interaction. Toxicon. 2005; 46:318-327.
38. Mors WB, Nascimento MC, Pereira BM, Pereira NA. Plant natural products active against snakebite-the molecular approach. Phytochem. 2000; 55:627-642.
39. Knudsen C and Laustsen AH. Recent Advances in Next Generation Snakebite Antivenoms. Trop Med Infect Dis. 2018; 3(2):42-55.