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# *In vitro* neutralization of *Naja nigricollis* venom by stem-bark extracts of *Commiphora africana* A. Rich. (Burseraceae)

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***Abstract*:** *Commiphora africana is a plant used as herbal remedy against snakebite in Nigeria, Central Africa and South Africa. This study was aimed at evaluating the plant's effect against Naja nigricollis venom. Crude methanol extract (CME) and n-butanol fraction (NBF) of the extract were used. Mixtures of CME and minimum lethal dose ($LD_{99}$) of N. nigricollis venom were pre-incubated at $37^0C$ for 15 minutes. Twenty mice were allocated into four equal groups. Group I was given $LD_{99}$ of the venom only. Mice in groups II, III and IV were respectively given 200, 300 and 400 mg/kg of the extract incubated each with $LD_{99}$ (9.70 mg/kg) of the venom. The same procedure was adopted using NBF. However, NBF was evaluated at 200, 400 and 600 mg/kg. All administrations were intraperitoneal. Animals were observed over 24 hours. All animals died in group I. For the CME treated groups, one death was recorded in group II while mice in groups III and IV all survived. The NBF at 200, 400 and 600 mg/kg offered 20, 60 and 80% survival, respectively. The extracts manifested a dose-dependent in vitro neutralization of N. nigricollis venom. This may explain the basis for using this plant to treat snakebite.*

***Key words*:** *Commipora africana, Extract, venom, in vitro, neutralization*

## I. Introduction

Snakebite envenoming is a common global health problem of high magnitude and complexity that is somewhat neglected, with frequently devastating consequences [1, 2]. Snake bite is a common occurrence in Nigeria as it is in many other parts of Africa and the tropics. It is an environmental and occupational disease that affects mostly people in rural communities of developing countries [3, 4]. Snakebite in Africa causes thousands of deaths annually and considerable permanent physical disability. The incidence of snake bite in rural West Africa was estimated to be as high as 174 per 100,000 population, with an 11–17% mortality rate [5, 6, 4]. Nigeria is reported to have one fifth of all West African region snake bite cases, with 174 cases in every 100,000 hospital admissions [7]. A more recent study estimated that over 314,000 bites, 7,300 deaths and nearly 6,000 amputations occur from snakebites annually in sub-Saharan Africa [8]. Snake bite in animals constitutes an important medical and economic problem resulting in loses due to mortality and cost of treatment which is mostly unsuccessful. There is paucity of data on the epidemiology of snake bite in animals in Nigeria. Envenomation of domestic animals by snakes occurs frequently in certain geographic areas. However, reports describing clinical signs, clinicopathologic abnormalities, therapeutic approaches, and outcomes are sparse [9]. Current management of snake bite involves the use of anti-snake venom serum (AVS), which is the specific therapy. The use of AVS is not without some limitations, which may be high cost, unavailability, storage problems and hypersensitivity reactions [10]. Moreover, AVS does not completely reverse the effects of the venom, especially local tissue injury [11].

*Commiphora africana* (A. Rich) Engl. Syn. *Heudelotia africana* (Family Burseraceae) is a shrub with short lateral branches, sharply pointed at the apex, bearing leaves in small clusters below the tip [12]. The plant is well suited to dry areas and is often grown as a hedge in Northern Nigeria [13]. Different parts of *C. africana* are used medicinally in several West African countries [14]. A macerate of crushed leaves of the plant in oil is drunk in Cote d'Ivoire and in Burkina Faso as a sedative and soporific [15]. The gum is widely used to prepare antiseptic washes and baths for skin infections, sores and leprosy. The bark of *C. africana* is chewed with natron and applied to scorpion stings in Nigeria while inflammation of the eyes is treated by placing the head over a steaming pot containing the bark in it [16]. In Central Africa, the bark mixed with salt is applied to snake bite site [17, 18]. Research has established that the plant has analgesic and anti-inflammatory [19], anti-diabetic [20], antimicrobial [21], and sleep inducing [22] activities.

*Naja nigricollis* or the black-necked spitting cobra is a venomous snake species belonging to the family Elapidae, which is the most venomous and widely distributed snake family [23]. It is one of the most important snakes associated with envenoming in Nigeria [4]. Snake venom is a complex mixture of several different

compounds consisting of proteins, peptides, enzymes, cytotoxins, neurotoxins, coagulants and anti-coagulants. The enzymes include phosphodiesterases, phospholipase A2, anti-cholinesterases, serine proteinases, metalloproteinases, hyaluronidase, oxidases and proteases and ATPase [2, 24, 25]. In addition, small organic substances such as citrate, nucleosides and acetylcholine may also be present [26]

## II. Materials and Methods

### 2.1 Plant collection and identification

The stem-bark of *C. africana* was collected in the month of March 2012 in the main campus of the Ahmadu Bello University (ABU) Zaria. The plant was identified and authenticated by Mr. Umar Galla, of the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, where a voucher specimen no. 900300 was deposited for reference purposes.

### 2.2 Plant preparation and extraction

The stem-bark of *C. africana* was cleaned, air dried under shade and ground into powder. The powder (~500 g) was macerated in 98% methanol (ratio 1:3, w/v) at room temperature for 48 hrs. The entire process was repeated twice. The methanol extracts were pooled together and filtered using a filter paper (Whatmann size no.1). The filtrate was evaporated to dryness in a water bath at 40ºC. The dried extract obtained was weighed and kept in airtight bottle in a refrigerator at 4$^{o}$C.

### 2.3 Fractionation of extract

The crude methanol extract of *C. africana* was dissolved in 500 ml water and then poured into a separatory 1 litre funnel. An equal volume of *n*-hexane was gently poured into the funnel, closed and shaken carefully. The pressure that builds up within the flask was released by inverting and opening the tap at the base of the funnel. The process was repeated several times. The resulting suspension was allowed to stand for about 15 minutes and the lower aqueous layer was decanted and collected in a container. The remaining *n*-hexane fraction was also collected in a separate container. The aqueous methanol fraction was partitioned serially between ethyl acetate and *n*-butanol in a similar fashion. The fractions obtained were concentrated to dryness under a fast moving stream of air.

### 2.4 Phytochemical screening

Phytochemical screening of stem-bark crude methanol extract of *C. africana* as well as its fractions was carried out to identify the chemical constituents using standard analytical techniques as described by Evans [27]. The screening involves the detection of presence or absence of secondary metabolites such as alkaloids, flavonoids, tannins, anthraquinones, saponins, terpenoids, steroids, glycosides and reducing sugars.

### 2.5 Experimental animals

Young adult male Swiss albino mice weighing between 18 and 22g were used. The animals were obtained from the National Institute for Trypanosomiasis Research (N.I.T.R), Kaduna and were housed in the Animal Room, Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria. The mice were maintained on commercial poultry grower feed, maize bran and groundnut cake compounded in a ratio of 4:2:1 and water was provided *ad libitum.* The animals were kept in plastic cages at room temperature throughout the study, and were acclimatized for two weeks prior to their use in the experiment.

### 2.6 Venom collection and preparation

*Naja nigricollis* venom was obtained by milking from black-necked spitting cobra (*Naja nigricollis*) which was kept and maintained at the Serpentarium, Department of Veterinary Pharmacology and Toxicology, using the milking method of Markfarlane [28]. The venom was collected with the help of a skilled snake handler. The pooled venom was thereafter placed in a dessicator containing activated silica and allowed to dry at room temperature. The crystallized venom was subsequently transferred into a refrigerator and stored at -18$^{o}$C.

### 2.7 Toxicity Studies

#### 2.7.1 Acute Toxicity Test on Extract

The determination of the median lethal dose ($LD_{50}$) of the stem-bark methanol extract of *C. africana* was carried out in mice by the method described by Lorke [29] (1983). In the initial phase, 9 mice were randomly divided into three groups of three mice each. Groups I, II and III were given 10, 100 and 1000 mg/kg, respectively of the extract intraperitoneally. In the second phase of the toxicity study, three mice were divided at random into three groups of one mouse each. Mouse I, II and III were given 1600, 2900 and 5000 mg/kg of the extract, respectively. The mice were observed over a period of 24 hours for signs of toxicity and mortality. The median lethal dose ($LD_{50}$) was calculated as the geometric mean of doses that produced 0% and 100% mortality.

Similarly, the determination of the median lethal doses ($LD_{50}$) of the ethyl-acetate, *n*-butanol and aqueous-methanol fractions were done as described above.

**2.7.2 Lethality assay of the venom**

The $LD_{99}$ of *N. nigricollis* venom as indication of its lethality was determined in mice. The venom was reconstituted in normal saline and concentrations of 5, 6, 8 and 10mg/ml made. Thirty mice were randomly allocated into 5 groups of 6 mice each (n = 6). Groups I served as the control group where mice were injected with normal saline (0.2 ml each i.p). Mice in groups II, III, IV and V were given the reconstituted venom at 5, 6, 8 and 10 mg/kg, respectively through the intraperitoneal route [30] (Theakson and Reid, 1983). The time of death was recorded over a period of 24 hours from venom administration.

**2.8 Determination of the activity of fractions**

A pilot study was conducted to determine the activity of the three fractions against snake envenomation by *N. nigricollis* in mice. The *n*-hexane fraction yield was negligible that it was not considered for further evaluation. The ethyl-acetate (EA), *n*-butanol (NB) and aqueous-methanol (AM) fractions were evaluated for possible anti-venom activity. Nine mice were randomly allocated into 3 equal groups (n = 3). All the animals were administered the minimum lethal dose of the venom (9.7 mg/kg). Thereafter, mice in groups I, II and III were respectively administered 200 mg/kg each of AE, NB and AM fractions, 15 minutes later. The mice were observed for signs of toxicity and mortality over 24 hrs.

**2.9 Venom neutralizing effect of the extract**

This was evaluated adopting the method earlier described by Abubakar *et al*. [31] and Otero *et al*., [32] with slight modification. Twenty mice were randomly allocated into 4 groups of five mice each. Group I served as control where mice were given the minimum lethal dose of *N. nigricollis* venom intraperitoneally. Mice in groups II, III and IV were given the mixture of the minimum lethal dose of the venom and the extract at 200, 300 and 400 mg/kg, respectively. The venom and extract mixtures were earlier pre-incubated at $37^0$ C for 15 minutes before administration. Similarly, the *n*-butanol fraction was evaluated for *in vitro* venom neutralization ability. The NBF was selected because it demonstrated better activity in the pilot study. However, the fraction was evaluated at 200, 400 and 600 mg/kg. This was because it had higher $LD_{50}$ value. Signs of toxicity and the time of death for each mouse were noted and recorded.

## III. Results

Preliminary phytochemical screening of the stem-bark extract of *C. africana* and its *n*-butanol fraction revealed the presence of flavonoids, tannins, saponins, triterpenoids and cardiac glycosides. The crude extract, in addition contained anthraquinones. The $LD_{50}$ of the crude extract was found to be 3807.89 mg/kg. There were no deaths recorded for the *n*-butanol, ethyl-acetate and aqueous-methanol fractions up to 5000 mg/kg. The $LD_{50}$ was thus assumed to be $\geq$5000 mg/kg for each of the fractions. The results of the lethality assay in mice injected with varying doses of the venom of *N. nigricollis*, separately, within 24 hrs is summarized in Table 1. The minimum lethal dose of the venom as estimated by probit was 9.7 mg/ kg.

Activity of *n*-butanol fraction was higher when compared with ethyl-acetate and aqueous-methanol fractions as manifested by the survival of 2 mice while all 3 mice in the groups treated with EA and AM died within 24 hours. Toxic signs were alleviated in varying degrees (Table 2) and lethality of the venom inhibited by the crude extract as shown by the survival of animals in the treatment groups (except one mouse that died in group treated with the extract at 200 mg/kg). The alleviation of toxic signs was maximal at 400 mg/kg which was the highest dose evaluated. Furthermore, doses of 300 and 400 mg/kg of the CME offered protection 100% from the lethal effects of *N. nigricollis* crude venom (Table 2).

**Table 1: The survivability of mice treated with different doses of *N. nigricollis* venom**

| Group | Dose (mg/kg) | Ln Dose | No. of animals | No. of animals Dead | No. of Survived | % Mortality |
|---|---|---|---|---|---|---|
| 1 | 5 | 1.16 | 6 | 2 | 4 | 33.33 |
| 2 | 6 | 1.79 | 6 | 3 | 3 | 50.00 |
| 3 | 8 | 2.08 | 6 | 5 | 1 | 83.33 |
| 4 | 10 | 2.30 | 6 | 6 | 0 | 100.00 |

Computation of the Minimum lethal dose by probit analysis

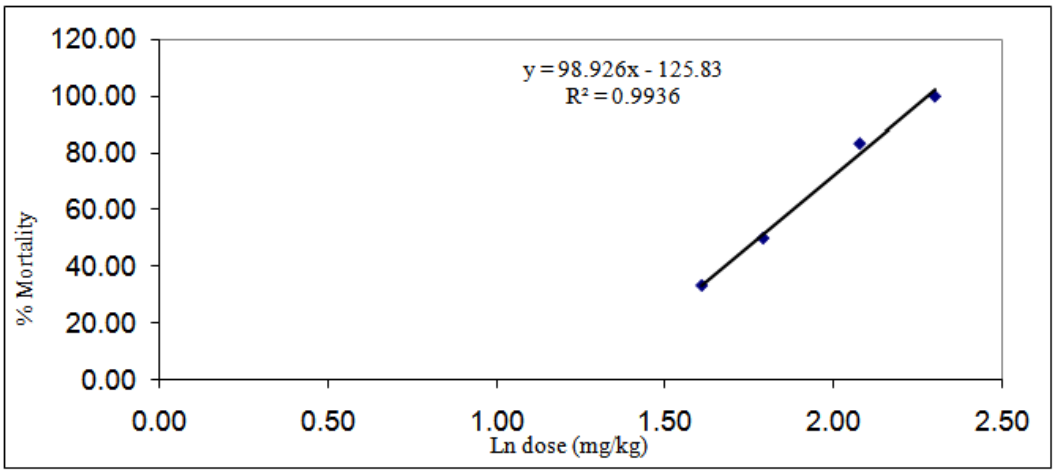


**Figure 1: Semi-logarithmic dose-mortality plot for the venom *N. nigricollis* in mice.**

The $LD_{99}$ was deduced from the equation of the plot Y= 98.92x – 125.8 as 9.70mg/kg

**Table 2: Effect of administration of pre-incubated mixture of *N. nigricollis* venom and crude methanol extract of *C. africana* in mice**

| Group | Treatment | No. of mice dead/No. of mice used | Percentage survival | Signs of Toxicity |
|---|---|---|---|---|
| 1 | $LD_{99}$ only | 5/5 | 0 | +++ |
| 2 | $LD_{99}$ + T1 | 1/5 | 80 | + |
| 3 | $LD_{99}$ + T2 | 0/5 | 100 | - |
| 4 | $LD_{99}$ + T3 | 0/5 | 100 | - |

$LD_{99}$; Min. Lethal Dose, T1; 200mg/Kg, T2; 300mg/Kg, T3; 400mg/kg, + Decreased activity; ++, mild respiratory impairment; +++, severe respiratory depression, convulsions, prostration and death.

**Table 3: Effect of administration of pre-incubated mixture of *N. nigricollis* venom and *n*-butanol fraction of *C. africana* in mice**

| Group | Treatment | No. of mice dead/No. of mice used | Percentage survival | Signs of Toxicity |
|---|---|---|---|---|
| 1 | $LD_{99}$ only | 5/5 | 0 | +++ |
| 2 | $LD_{99}$ + T1 | 4/5 | 20 | ++ |
| 3 | $LD_{99}$ + T2 | 2/5 | 60 | ++ |
| 4 | $LD_{99}$ + T3 | 1/5 | 80 | + |

$LD_{99}$; Min. Lethal Dose, T1; 200mg/Kg, T2; 400mg/Kg, T3; 600mg/kg, + Decreased activity; ++, mild respiratory impairment; +++, severe respiratory depression, convulsions, prostration and death.

Different doses of NBF of *C. africana* when incubated with the $LD_{99}$ of *N. nigricollis* venom and administered to mice produced a dose dependent inhibition of the lethal and toxic effects as depicted in Table 3 above.

## IV. Discussion

Phytochemical constituents detected in the crude methanol extract in this study were similar to those reported earlier by Goji *et al*. [20] and Ezekiel *et al*. [19, 22]. Based on the result of the acute toxicity, the crude extract could be described as being relatively non-toxic having an $LD_{50}$ of 3807.89 mg/kg while the butanol fraction with an $LD_{50} \geq 5000$ mg/kg is relatively safe.

The $LD_{99}$ for *N. nigricollis* venom obtained in this study (9.7 mg/kg) was higher than that reported earlier. An $LD_{99}$ of 6 mg/kg was earlier reported in Nsuka, Nigeria [33] while 9.55 mg/kg was reported here in Zaria, earlier [31]. Difference in the minimum lethal doses might be explained by variability in venom composition. A number of factors such as geographic location, season, age, and diet have been shown to influence conspecific venom variability [2, 34, 35].

The remission of toxic signs or and survival of laboratory animals experimentally challenged with pre-incubated mixture containing the $LD_{99}$ and the extract was used to deduce antivenom activity by the plant. The results of this study indicate that the stem-bark crude methanol extract of *C. africana* exhibits a dose dependent *in vitro* detoxifying action against the crude venom of *Naja nigricollis*. The NBF also showed dose dependent *in vitro* venom neutralizing ability. However, the effect of the NBF was lesser compared with the CME.

Many plants have been reported to have anti-snake venom effect. Several investigators have reported on the effectiveness of herbal antidotes against snake envenomation [11]. Certain naturally occurring substances

such as sitosterol, pentacyclic terpenes, nitro compounds (aristolochic acid), cinnamic acid derivatives, curcuminoids, polyphenolic compounds, and flavonoids are known compounds possessing protein-binding and enzyme-inhibiting properties [36].Tannins are known to unspecifically inactivate proteins [37, 38]. The extracts of *C. africana* were shown to contain tannins, flavonoids and other secondary metabolites similar to those earlier reported with activity against snake venom constituents. These compounds possibly act synergistically to neutralize snake venoms *in vitro.* This was evident from the higher neutralization ability demonstrated by the crude extract on the venom when compared with the *n*-butanol fraction.

The findings of this work are consistent with the reports of other authors [11, 31, 33, 37, 38] and have indicated that the extract of *C. africana* contains compounds which can neutralize *N. nigricollis* venom *in vitro*. Results obtained from this study may explain the basis for using this plant in traditional medicine to treat snake envenomation in Nigeria and other parts of Africa.

## V. Conclusion

It can be concluded from the study that the extract of *C. africana* contains phytoconstituents capable of neutralizing *N. nigricollis* crude venom *in vitro* and that the extract has potential for use as a remedy against *N. nigricollis* envenomation. In view of these findings, further elaborative studies should be conducted to identify, isolate and characterize the specific constituent(s) responsible for venom neutralization by the extract. The mechanism of action of the isolated compound(s) should be elucidated. The effect of the extract should also be further evaluated *in vivo*.

## References


[1]. J.M. Gutierrez, D. Williams, H.W. Fan and D.A. Warrell, Snake bite envenoming from a global perspective: Towards an integrated approach. *Toxicon, 56*, 2010, 1223-1235.

[2]. D.A. Warrell, Snake bite, *Lancet, 375*, 2010, 77-88.

[3]. World Health Organization, Rabies and Envenomings. A Neglected Public Health Issue. World Health Organization, Geneva, 2007

[4]. A.G. Habib, Public health aspects of snakebite care in West Africa: perspectives from Nigeria. *Journal of Venomous Animals and Toxins Including Tropical Diseases, 19*, 2013, 27.

[5]. A.G. Habib, U.I. Gebi and G.C. Onyemelukwe, Snake bite in Nigeria. *African Journal of Medical Sciences, 30*, 2001, 171–178.

[6]. L.E. Visser, S. Kyei-Faried, D.W. Belcher, D.W. Geelhoed, J. Schagen van Leeuwen and J. van Roosmalen, Failure of a new antivenom to treat *Echis ocellatus* snake bite in rural Ghana: the importance of quality surveillance, *Transactions of the Royal Society for Tropical Medicine and Hygiene, 102*, 2008, 445–450.

[7]. A. Nasidi, Snakebite as a serious Public Health problem for Nigeria and Africa, WHO Consultative Meeting on Rabies and Envenomings: A Neglected Public Health Issue, Geneva, Switzerland, 2007

[8]. J.P. Chippaux, Estimate of the burden of snakebites in sub-Saharan Africa: a meta-analytic approach, *Toxicon, 57(4),* 2011, 586–599.

[9]. A. Goddard, J.P. Schoeman, A.L. Leisewitz, S.S. Nagel and I. Aroch, Clinicopathologic abnormalities associated with snake envenomation in domestic animals. *Veterinary Clinical Pathology, 40(3)*, 2012, 282-292.

[10]. N.A. Pereira, R.J.S Jaccoud, W.B. Mors, *Triaga Brasilica*: renewed interest in a seventeenth-century panacea. *Toxicon, 34,* 1995, 511–516.

[11]. A. Gomes, R. Das, S. Sarkhel, R. Mishra, S. Mukherjee, S. Bhattacharya and A. Gomes, Herbs and herbal constituents active against snake bite, *Indian Journal of Experimental Biology, 48*, 2010, 865-878.

[12]. T.H. Arnold and S. Dewet, *Plants of Southern Africa: Names and Distribution.* Memoirs of the Botanical Survey Africa, 1993, 62

[13]. H.M. Burkill, *The Useful Plants of West Tropical Africa* 2nd ed. Royal Botanic Gardens, Kew, 1985, 304-306.

[14]. D.K. Abbiw, *Useful plants of Ghana* Published by Intermediate Technology Publication Ltd. by S R P, Exeter U K. 1990, 232 – 241.

[15]. A.H. Adebayor, R. Aliyu and K. Gasting, The effect of *Commiphora africana* (Burseraceae) on serum lipid profile in rats, *International Journal of Pharmacology, 2(6)*, 2006, 618-622.

[16]. J.M. Dalziel, *The useful Plants of West Tropical Africa,* Crown Agents for the Colonies. London, 1937.

[17]. J.M. Watt and M.G. Breyer-Brandwijk, *The medicinal and poisonous plants of Southern and Eastern Africa*, 2nd ed. Edinburgh & London: Livingston, 1962.

[18]. M.P. Paraskeva, S.F. van Vuuren, R.L. van Zyl, H. Davids and A.M. Viljoen, The *in vitro* biological activity of selected South African *Commiphora species*, *Journal of Ethnopharmacology, 119 (3)*, 2008, 673-679.

[19]. I. Ezekiel, M.A. Mabrouk and J.O. Ayo, Study of the effect of hydro-ethanolic extract of *Commiphora africana* (Stem-bark) on inflammation and pain in rodents, *Asian Journal of Medical Sciences, 2(3)*, 2010, 81-84.

[20]. A.D.T. Goji, A.A.U. Dikko, A.G. Bakari, A. Mohammed and Y. Tanko, Evaluation of the effect of aqueous-ethanolic stem bark extract of *Commiphora africana* on blood glucose levels of alloxan induced diabetic Wistar rats, *Asian Journal of Medical Sciences, 1(2)*, 2009, 18-21.

[21]. J.S. Akor and T.S. Anjorin, Phytochemical and antimicrobial studies of *Commiphora africana* (A. Rich) Engl. root extracts, *Journal of Medicinal Plants Research, 3(5)*, 2009, 334-337.

[22]. I. Ezekiel, M.A. Mabrouk, J.O. Ayo, A.D.T. Goji, A.O. Okpanachi, A. Mohammed and Y. Tanko, Study of the effect of hydro-ethanolic extract of *Commiphora africana* (Stem-bark) on sleeping time and convulsion in mice, *Asian Journal of Medical Sciences, 2(3)*, 2010, 85-88.

[23]. L. Luiselli, F.M. Angelici and G.C. Akani, Comparative feeding strategies and dietary plasticity of the sympatric cobras *Naja melanoleuca* and *Naja nigricollis* in three diverging Afrotropical habitats, *Canadian Journal of Zoology, 80*, 2002, 55-63.

[24]. J.J. Calvete, L. Sanz, Y. Angulo, B. Lomonte and J.M. Gutierrez, Venoms, venomics and antivenomics, *FEBS Letter 583*, 2009, 1736-1743.

[25]. T.S. Kang, D. Georgieva, N. Genov, M.T. Murakami, M. Sinha, R.P. Kumar, P. Kaur, S. Kumar, S. Dey, S. Sharma, A. Vrielink, C. Betzel, S. Takeda, R.K. Arni, T.P. Singh and R.M. Kini, Enzymatic toxins from snake venom: structural characterization and mechanism of catalysis, *FEBS Journal, 278*, 2011, 4544-4576

[26]. S.D. Aird, Ophidian envenomation strategies and the role of purines, *Toxicon*, *40*, 2002, 335–393.
[27]. W.C. Evans, *Trease and Evans Pharmacognosy*, 16th edition, Bailliere Tindall Lodon, UK. 2009
[28]. R.G. Markfarlane, Russell's Viper Venoms, 1953–1964. *British Journal of Haematology, 13*, 1967, 437–451.
[29]. D. Lorke, A new approach to practical acute toxicity testing. *Archives of Toxicology, 53*, 1983, 275-289.
[30]. R.D.G. Theakston and H.A. Reid, The development of simple standard assay procedures for characterization of snake venoms. *Bulletin of the World Health Organization, 61*, 1983, 949–956.
[31]. M.S. Abubakar, M.I. Sule, U.U. Pateh, E.M. Abdurahman, A.K. Haruna and B.M. Jahun, *In vitro* snake venom detoxifying action of the leaf extract of *Guiera senegalensis*, *Journal of Ethnopharmacology, 69*, 2000, 253–257.
[32]. R. Otero, V. Nunez, J. Barona, R. Fonnegra, S.L. Jimenez, R.G. Osorio, M. Saldarriaga and A. Diaz, Snakebites and ethnobotany in the northwest region of Colombia. Part III: neutralisation of the haemorrhagic effect of *Bothrops atrox* venom, *Journal of Ethnopharmacology, 73*, 2000, 233–241.
[33]. O.J. Ode and I.U. Asuzu, The anti-snake venom activities of the methanolic extract of the bulb of *Crinum jagus* (Amaryllidaceae), *Toxicon*, *48*, 2006, 331-342.
[34]. J.P. chippaux, V. Williams and J. White, Snake venom variability: methods of study, results and interpretation, *Toxicon*, *29(11)*, 1991, 1279-1303.
[35]. J.C. Daltry, W. Wuster and R.S. Thorpe, Diet and snake venom evolution, *Nature*, *379*, 1996, 537-540.
[36]. S.J. Premendran, K.J. Salwe, S. Pathak, R. Brahmane and K. Manimekalai, Anti-cobra venom activity of the plant *Andrographis paniculata* and its comparison with polyvalent anti-snake venom, *Journal of Natural Science, Biology and Medicine, 2(2)*, 2011, 198-204.
[37]. T. Okonogi, Z. Hattori, A. Ogiso and S. Mitsui, Detoxification by persimon tannin of snake venom and bacterial toxins, *Toxicon*, *17(5)*, 1979, 524–527.
[38]. S.L. da Silva, A.K. Calgarotto, J.S. Chaar and S. Marangoni, Isolation and characterization of ellagic acid derivatives isolated from *Casearia sylvestris* SW aqueous extract with anti-PLA2 activity, *Toxicon*, *52*, 2008, 655-666.