EVALUATION OF THE EFFICACY OF THREE PLANT EXTRACTS AGAINST VENOMS OF FIVE SNAKE SPECIES IN NORTHERN NIGERIA

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DECLARATION

I declare that the work in this thesis entitled **"Evaluation of the efficacy of three plant extracts against venoms of five snake species in Northern Nigeria"** has been performed by me in the Department of Veterinary Public Health and Preventive Medicine. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

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CERTIFICATION

The thesis entitled **"Evaluation of the efficacy of three plant extracts against venoms of five snake species in Northern Nigeria"** by **YUNUSA Yahaya** meets the regulations governing the award of Doctor of philosophy degree in Veterinary Public Health and Preventive Medicine of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Africa is blessed with abundance of natural resources including both fauna and flora. Nigeria has a lot of medicinal plants that are not yet exploited and some are traditionally known to be used in the prevention and or treatment of snake bites. Screening some of these plants scientifically will prove the traditional claims by the snake charmers and herbalists may lead to the production of single polyvalent antidote for the prevention and treatment of snake bites. The objectives of the study were to: Select, collect and identify plants with potential antivenom properties and five species of poisonous snakes; Determine the phytochemical constituents of the three plants; Carry out acute toxicological studies of the selected plant extracts; Determine the LD_{50} of venoms of different snake species and the plant extracts; Determine the antivenom potentials of the different fractions of the plant extracts; Carry out interactive studies with various plant extract fractions on the venom *in vivo*; Determine the protective effects of three plant extracts against the haemorrhagic effect of snake venoms in rats; Carry out studies on the effects of various plant extracts in neutralizing snake venoms on 1kb plasmid DNA migration on agarose gel electrophoresis. Results of the phytochemical analysis of the crude extracts of the three plants (*Olax manni, Urginea altissima* and *Xeromphis nilotica*) showed the presence of carbohydrates, glycosides, steroids/triterpens, cardiac glycosides, saponins, glycosides and alkaloids. In addition, Extract of *Urginea altissima* contained free anthracenes. However, the ferric chloride test failed to detect glycosides in all the extracts, while Bontrager's test and modified Bontrager's test did not detect free anthracenes and combined anthracenes respectively in the extracts of *Olax manni* and *Xeromphis nilotica.* Similarly, Mayer's, Dragendoff's and Wagner's tests failed to detect alkaloids in *Olax manni,* and *Xeromphis nilotica.* The *in vivo* studies revealed that the

aqueous fractions of the 3 plants (A_4, B_4 and C_4) have the highest activity against the snake venoms when administered 30 minutes before, after and when venom and extracts were administered, which showed that the three plants have both curative, prophylactic and neutralization effects against snake venom. Plant B (*Urginea altissima*) had the highest (100 %) activity with all the rats treated with its aqueous extract surviving envenomation. The prophylactic method of inoculation was found to be the most effective. Toxicity studies showed that the LD_{50} of the venom of *N. haje, N. katiensis,* N. *nigricollis, E. ocellatus and B. arietans* in Wistar rats were found to be 0.22, 0.55, 0.87, 1.24 and 1.80 mg/kg, respectively. *Bitis arietans* has the highest LD_{50} followed by *Echis ocellatus* while *Naja haje* was having the lowest LD_{50} follwed by *Naja katiensis,* found to have the lowest LD_{50} Toxicity. Anticoagulant activity of the snake venom was measured by the time taken for blood to clot. The clotting time for *Naja nigricollis, Naja haje* and *Naja katiensis* were found to be 132, 128 and 115 minutes, respectively. However, the venoms of *Bitis arietans* and *Echis ocellatus* did not clot during the period of the experiment. Studies on haemorrhagic effect of venoms in rats indicated that aqueous fraction of *Urginia altissima* showed antivenom activity against the venoms of *Naja nigricollis, Naja haje, Naja Katiensis, Bitis arietans and Echis ocellatus* there were no patches observed. The aqueous extract fraction of *Olax manni* showed protective effect against venoms of *Naja nigricollis, Naja haje, Naja katiensis, Bitis arietans and Echis ocellatus* while aqueous fraction of *Xeromphis nilotica* also had protective effect against the venom of *Naja nigricollis, Naja haje, Naja katiensis* and *Bitis arietans*. However, there were some patches of blood in the skin of rats inoculated with venom of *Echis ocellatus.* The results established the aqueous fraction of the three plants the neutralization effects of venom. The interactive study of the aqueous fraction of *Urgines altissima,*

venom and the plasmid DNA measured by migration on agarose gelelectrophoresis showed that aqueous fractions had high antivenom activity against the venoms *of Naja nigricollis, Naja haje, Naja katiensis, Bitis arietans and Echis ocellatus.*1 kb DNA ladder and the plasmid DNA migrated very well on agarose gel electrophoresis. The mixture of plasmid DNA with separate venom of the snakes, showed no migration of the DNA on agarose gel electrophoresis. The plasmid DNA incubated with the aqueous fraction of plant B showed clear migration of the DNA on agarose gel electrophoresis. In conclusion the extract of three plants demonstrated curative, prophylactic and detoxifying effects against the venoms of the five venomous species of snakes.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATION

ABUCAUC- Ahmadu Bello University Committee for Animal Use and Care

Ach - Acethylcholine

ADP - Adenosine di-phosphate

ALT - Alanine amino transferase

AST - Aspartate amino transferase

ATPase - Adenosine tri-phospatase

Ba - *Bitis arietans*

BJC – Bothrojaracin

$CaCl_2$ - Calcium Chloride

DNA - Deoxyribonucleic acid

DTT - Dithiothreitol

EDTA - Ethylenediaminetetraacetic acid

Eo -*Ehis oscellatus*

ECM - Extracellular matrix

FIV - Feline immunodeficiency virus

g - Gram

H_2O_2 - Hydrogen peroxide

HIV - Human immunodeficiency virus

HCl - Hydrochloric acid

HSI - Hunnane Society International

Im – Intramuscular

Ip – Intraperitoneal

Iv - Intravenous

Kb – Kilo bite

KDa - Kilo Dalton

KOH - Potassium hydroxide

LAAO - L-amino acid oxidases

LD_{50} - Medium lethal Dose

Mg/kg -Milligram per kilogram

mtDNA - Mitochondrial Deoxyeibonucleic acid

nAChR - Nicotinic acetylcholine receptor

NaCl - Sodium chloride

N. atra - Naja atra

N. flava - Naja flava

NH_4OH - Ammonium sulphate

N. haje* - *Naja haje

N. katiensis* - *Naja katiensis

N. nigricollis* - *Naja nigricollis

N. nivea* - *Naja nivea

NTDs -Neglected Tropical Diseases

^oC - Degree celcious

^oK - Degree Kelvin

% - Percentage

Om -*Olax manni*

PAP - Protease-activated receptors

pDNA - Plasmid deoxyribonucleic acid

PLA_2- PhospholipaseA_2

PLS - Phospholipids

TBE -Tris borrate Ethylenediaminetetraacetic acid

TLEs - Thrombin-like enzyme

Ua - *Urginea altissima*

V - Venom

Xn -*Xeromphis nilotica*

WHO - World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Snake venoms are complex mixtures of biologically active proteins and peptides. Many of the venoms affect haemostasis by activating or inhibiting coagulant factors or platelets, or by disrupting endothelium (Chippaux, Williams, and White, 1991). Based on sequence, these snake venom components have been classified into various families, such as serine proteases, metalloproteinases, C-type lectins, disintegrins and phospholipases (Sanchez *et al*., 2000). The various members of a particular family act selectively on different blood coagulation factors, blood cells or tissues. For almost every factor involved in coagulation or fibrinolysis there is a venom protein that can activate or inactivate it (Du *et al*., 2002; Rosing and Tans, 1992; Sanchez *et al.*, 2000).

Variation in venom composition among venomous snakes is a well- known and well-documented phenomenon (Hoso *et al*., 2007; Mounier *et al*., 2001; Shimokawa *et al*., 1997). The existence of these variations has serious implications, not only in clinical medicine and the treatment of snake-bite patients, but also in toxicology and in research. Venom variation can occur at very low taxonomic levels, and very closely related and similar species or subspecies may have radically different venom composition. An incomplete understanding of the systematics of a group of venomous snakes can therefore have a number of effects on the treatment of snake-bite patients and toxicological research. These include inefficient antivenoms (Warrell, 1986, 1989; Warrell, 1996; Warrell and

Arnett, 1976) unexpected symptoms (Gillissen *et al.*, 1994) and difficulties in replicating experimental results (Ohkura *et al*., 1988; Namiranian and Hider, 1992)

Many of the most medically important venomous snakes are part of complex groups of often very similar species. The systematics of many such complexes, and the definition and identification of the individual species, is the subject of considerable debate. Venom composition can vary widely even between closely related species; as a result, a sound and explicit taxonomic framework is of great importance for toxinological and clinical research and the production and use of antivenoms (Andrews, *et al*., 2001; Fry, 2005; Kuch *et al*., 2006; Stromblad and Cheresh, 1996; Wuster and McCarthy, 1996).

Snakevenoms are a complex mixture of components which have a diverse array of actions both on prey and human victims. These components are biologically highly active proteins, whose primary function is to kill or immobilize the prey and also to assist in its digestion of that prey and different species of snakes have its own particular requirements for the action of venom onthe type of prey it seeks (Bryan *et al*., 2009; Sanchez, 2007; Tan and Ponnudurai, 1992; Wüster *et al*., 2008).

This requirement of specialization in venom composition may occour independent of morphological variation. To achieve the end result of prey acquistion and/or digestion, the venoms may contain a mixture of the following active components: neurotoxic (pre/post synaptic), cardiotoxic, myolytic, coagulant, haemostatic (activating/inhibiting), haemorrhagic and possibly directly nephro- or hepatotoxic components. Snake bite is an important issue in developing countries especially the tropics and subtropics causing both morbidity and mortality and also leaving health care impacts especially in countries of

Africa and Asia (Isbell, 2006; Theakston *et al*., 2003), though there has been underreporting of deaths and snake bite cases in under developed countries (Warrell, 1995; Warrell and Arnett, 1976). The spitting cobra- *Naja nigricollis,* African puff adder- *Bitis arietans* and the carpet *viper-Echis ocellatus* are responsible for 95% snake bite cases in Nigeria (Habib, Gebi, and Onyemelukwe, 2001). The venom of the cobras is mainly neurotoxic which cleave the beta chains of fibrinogen; while that of vipers are myotoxic and cleave the beta and gamma chains offibrinogen thus exhibiting different fibrinogenolytic activity making the vipers more fibrinogenolytic (Ajagun, 2010; Habib, 2013; Malhotra and Thorpe, 2004; Pook *et al.*, 2009; Wuster and Broadley, 2007).

Snake envenomation has been enlisted in the 2009 list of Neglected Tropical Diseases (NTDs) (Gutiérrez *et al*., 2013). Venomous or poisonous snakes use their fangs located in the mouth to either kill or immobilize their prey using venom which is modified saliva to achieve this. There are six families of venomous snakes. Four families of venomous snakes are found in Nigeria-Viperidae, Elapidae, Colubridae and Actraspididae,but three species i.e., 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 (Habib *et al.*, 2001). *Echis ocellatus* accounts for the highest percentage of bites in Kaltungo, Gombe state. Bites occur more often while victims are farming, herding or taking walks in bush paths (Warrell, 1995; Abbas *et al*., 2009).

Snake bites can be deadly, but the venoms also contain components of medical and biotechnological value. The proteomic characterization of snake venom proteomes, snake venomics, has thus a number of potential benefits for basic research, clinical diagnosis, and

development of new research tools and drugs of potential clinical use. Snake venomics is also relevant for a deep understanding of the evolution and the biological effects of the venoms, and to generate immunization protocols to elicit toxin-specific antibodies with greater specificity and effectiveness than conventional systems.The diffusion of toxins from the site of a bite into the circulation is essential for successful envenomation. Degradation of hyaluronic acid in the extracellular matrix (ECM) by venom hyaluronidase is a key factor in this diffusion. Hyaluronidase not only increases the potency of other toxins but also damages the local tissue. In spite of its important role, little attention has been paid to this enzyme. Hyaluronidase exists in various isoforms and generates a wide range of hyaluronic acid degradation products. This suggests that beyond its role as a spreading factor, venom hyaluronidase deserves to be explored as a possible therapeutic target for inhibiting the systemic distribution of venom and also for minimizing local tissue destruction at the site of the bite (Girish and Kemparaju, 2006; Lu *et al*., 2004).

1.2 Statement of the Research Problem

There are many species of snakes in Nigeria found in a wide range of habitat from terrestrial, arboreal to aquatic habitats preferring sparsely populated areas, such as food storage facilities, unkempt bushes, holes, crevices, and areas where prey is readily or likely to be available for them (Altimari, 1998; McDiarmid *et al*., 1999; Schmidt and Inger, 1957).

Snake envenomation is still a health problem worldwide especially in many tropical countries. It has been estimated that global mortality from snake envenomation is up to 100, 000 people per year (Chippaux, 1998; Kasturiratne *et al*., 2008). *Naja nigricollis*,

Echis ocellatus and *Bitis arietans* are responsible for 95% snake bite cases in Nigeria. Inaccessibility and the cost of antivenom limits the supply to where the therapy is strongly useful and till date there is no general single antidote for the curative and or prevention of snakebites. Data on animal's envemation globally is scarce. From hospital medical records Iran recorded 4.4% animal envenomation.

Medicinal plants have been used as folk medicine for treatment of snake bites, reliance on medicinal plants is primarily due to their effectiveness, cultural preferences, inexpensive nature, non-poisonous nature and dependence on neighboring forests (Andrews *et al*., 2004; Du *et al*., 2001; Gupta and Peshin, 2012).

Globally, traditional healers are using herbal medicine to cure and prevent snake envenomations. Nigeria has many medicinal plants that are yet to be exploited and some are known traditionally to be used in the prevention and treatment of snake bite. However, there is the need, to scientifically evaluate the efficacy of these traditional herbal drugs against snake venoms. Studies on the pharmacological effect of biological plants against snake bite are few.

1.3 Justification

Snakebite is of public health, medical and economic importance in developing countries where agriculture, forestry, hunting and fishing are the main occupationsof the populace. The environments where these activities are carried out harbour venomous snakes. The rampant cases of snake bites in Nigeria has led to the death or deformities of many victims especially the peasant farmers, nomads as well as their animals and these cause a lot of

economic consequences most especially reduced yield in farm produce and meat production (Bilgrami *et al*., 2004; Maita *et al*., 2003).

In several cases of snake envenomation, survivors are left with chronic functional disability from necrotic effects of venom, requiring amputations, chronic ulceration, chronic renal failure and neurological squealae from intra-cranial haemorrhages and thromboses (Theakston *et al*., 2001).

Screening some of these plants scientifically will prove the claim by snake charmers and herbalists and may lead to production of antidote for treatment of snakebites. In such screening, emphasis should be on proper design of both *in vivo* and *in vitro* studies, so that they relate exactly to the clinical situations (Borges *et al*., 2000; Golubkov *et al*., 2003; Koh *et al*., 2006; Samy *et al*., 2006; Santos *et al.*, 2000). This study, however, will assess some plants that are found locally in order to explore their potential use for the production of antidotes for the treatment of snake bites. Also the molecular interaction of these plants with snake venom and plasmid deoxyribonuleic acid (DNA) will be exploited to further understanding of the efficacy of these plants against snake venoms.

1.4 Aim of the Study

The aim of this study was to determine the antivenom properties of selected indigenous plants (*Olax manni, Urginea altissima* and *Xeromphis nilotica*) and their activities against five species of venomous snakes: *Naja nigricollis; Naja haje; Naja katiensis Bitis arietans* and *Echis oacellatus.* We selected the three plants based on the pilot studies conducted with 10 species of plants: *Annona senegalensis; Aspilia Africana*; *Hibiscus scabdariffa; Luffa*

aegyptiaca, Nicotiana rustica, Olax manni, Parkia biglobosa, Tamarindus indica, Urginea altissima and *Xeromphis nilotica.*

1.5 Objectives of the Study

The objectives of the study were to:

1. Select, collect and identify plants with potential antivenom properties and five species of poisonous snakes.
2. Determine the phytochemical constituents of the three plants
3. Carry out acute toxicological studies of the selected plant extracts.
4. Determine the LD_{50} of venoms of different snake species and the plant extracts.
5. Determine the antivenom potentials of the different fractions of the plant extracts.
6. Carry out interactive studies with various plant extract fractions on the venom *in vivo*.
7. Determine the protective effects of three plant extracts against the haemorrhagic effect of snake venoms in rats.
8. Carry out studies on the effects of various plant extracts in neutralizing snake venoms on 1kb plasmid DNA migration on agarose gel electrophoresis.

1.6 Hypotheses

- Ho: The extracts of *Olax manni, Urginea altissima,* and *Xeromphis nilotica* have no antivenom properties.
- Ho: the extracts of *Olax manni, Urginea altissima* and *Xeromphis nilotica* have no protective effect against the haemorrhagic effects snake venoms *in vivo.*
- Ho: the extracts of *Olax manni, Urginea altissima* and *Xeromphis nilotica* have no effect on snake venom plasmid deoxyribonucleic acid (pDNA) migration on agarose gel electrophoresis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Background

2.1.1 Taxonomy

Snakes are classified in the phylum *Chordata*, subphylum *Vertebrata*, class *Reptilia*, order *Squamata*, suborder *Serpentes*. The classification of snakes is strongly linked to and dependent on their evolution. There are 19 snake families with 417 genera and approximately 2900 species (Arni and Ward, 1996; Bergmeier *et al*., 2001; Ditmars, 1933; Mattison, 2007; Schmidt and Inger, 1957; Zhou, Dangelmaier, and Smith, 1996).

Cobras are among the venomous snakes of which there is high public awareness. Within the genus *Naja*, the most extensively revised taxa are the Asian representatives of the genus, where successive revisions have raised the number of recognised species from one to eleven (Slowinski and Wuster, 2000; Wuster and Thorpe, 1991; Wüster, Thorpe *et al*., 1995). And the African spitting cobras, in which the number of recognised species has risen from one to five (Broadley, 1968; Broadley and Wuster, 1974; Roman, 1968, 1969; Wuster and Broadley, 2003).

Snakes are very specialized and most modern reptiles, first appearing in fossil record during the time of the Dinosaurs (Cheresh, 1998). They evolved from the ground dwelling or burrowing lizards that exploited the survival advantages to be found in a cyclical legless body. They gave up external ears and developed clear scales to shield their ever open eyes from damage and dust (Endo and Tamiya, 1991; Fan and Cardoso, 1995; Juan, Calvet, and Libia, 2007; Mattison, 2007; Murari *et al*., 2005; Rosing *et al*., 2001).

Snakes have also evolved a host of instinctive behaviours that enabled them to find and catch prey, hide from predators, reproduce and survive in a great variety of climates: tunneling beneath dirt and sand, swimming in aquatic environments, climbing (arboreal) trees and crawling on the land (Dong–Zong *et al*., 1997). For these reasons snakes have become an integral part of the ecosystem throughout the world.

Snakes possess elongated internal organs, specialized muscles and resilient, scaled skins of varied pattern and colour that provides them with camouflage and some limited protection from predators (Booth *et al*., 2011; Greene, 1997). They have developed the ability to move without legs over and through all types of terrain, vegetation and water. They capture and feed on their prey without the aid of appendages, as well as to periodical shed of an old skin (Mattison, 2007; Roman, 1968).

2.1.2 Morphology

Snakes do not have legs, ears, or eyelids and their bodies are greatly elongated and mostly cylindrical. Often the sex organs of a snake may protrude from the anal plate area and are confused for legs. Snakes are ectotherms, regulating their body temperature by taking heat from or giving off heat to the environment (Dörmann *et al*., 2001). Because their body temperature is affected by environmental temperatures and varies with surrounding conditions, snakes become inactive during very hot seasons (aestivation) and very cold seasons (hibernation) (Girish *et al*., 2004). Some snakes may go for several weeks without eating because of frequent periods of inactivity. Snakes rely on behaviour to regulate their body temperature, during the hot part of the day, snakes move to shady areas, and on cool days they sun themselves on rocks, roads or in warm open areas (Gutiérrez *et al*., 2006).

Though most or all snakes are thought to be dangerous, poisonous and regarded as threats hence the negativistic attitude to them from most people, the majority of snakes are non-poisonous. Venomous snakes are fewer than non-poisonous snakes (Kalafatis *et al*., 2003). Venomous snakes have some basic morphological distinguishing features that differentiate them from non-poisonous snakes. These include – a triangular shaped head, elliptical pupils of the eyes, the tip of the tail may have two sets of scales and the underside scales go all the way across in a single row from the anal plate while non-poisonous snakes have two rows of scales from the vent to the end of the tail (Boycott and Haacke, 1979; Calvete *et al*., 2007).

Although conventional morphological approaches have contributed considerably to the resolution of the systematics of these complexes (Broadley, 1968; Broadley and Wuster, 1974) more advanced approaches such as multivariate morphometrics (Broadley, 1968; Broadley & Wuster, 1974) and their combined use with mtDNA (Broadley and Wuster, 2004; Slowinski and Keogh, 2000; Wuster and Broadley, 2003; Wüster *et al*., 1995) have been especially valuable in unravelling the systematics of groups with more subtle patterns of morphological variation.

Morphological differences between populations may be due either to natural selection, independent of phylogenetic affinities, or to phylogenesis (Thorpe *et al*., 1994; Thorpe *et al.*, 1995), and the pattern of variation alone cannot differentiate between these two hypotheses. On the other hand, the presence of multiple mtDNA haplotype clades does not necessarily indicate the presence of multiple species (Puorto *et al*., 2001), and may even mask patterns of gene flow and incipient speciation (Ogden and Thorpe, 2002; Thorpe and Richard, 2001). Congruence between molecular and morphological markers indicates that

the observed morphological differences are indeed a result of the populations being different evolutionary lineages, and that the mtDNA haplotype clades correspond to separate organismal lineages.

The nomenclatural history of the African spitting cobra parallels that of other taxa that fell victim to the phenomenon of the "inertial species concept" (Good, 1994; Rollin and Kesel, 1990): a plethora of forms was described in the 19th and early 20th century, often as full species, followed by the lumping of all African spitting cobras into the single species *N. nigricollis*, with most authors following the taxonomy. The first description of an African spitting cobra was by Andrew Smith, who described *Naja nigra* in 1838, but this name is preoccupied by *Naja nigra* Gray, a synonym of *N. atra* of China. (Smith *et al*., 1838) subsequently illustrated this snake, known as a 'spitter', under the name "*N. haje* Var. C", but Boulenger nevertheless included it in the synonymy of *N. flava* Merrem (*N. nivea*).

The taxonomy of the African spitting cobras has fluctuated greatly since the description of the Black-necked spitting cobra *Naja nigricollis* from Ghana. *Naja mossambica* was described from Tete and Sena by Peters (1854). Günther (1894) described *Naja nigricollis* var. *crawshayi* on the basis of a dry skin from Lake Mweru, while (Bocage and Barboza (1895) described from Angola the varieties *occidentalis, melanoleuca* (preoccupied by *N. melanoleuca* Hallowell) and *fasciata* (preoccupied by *N. fasciata* Laurenti, a synonym of *Naja naja*). The largest extant snakes live in the tropics of South America and Southeast Asia where high temperatures facilitate the evolution of large body sizes among air-breathing animals whose body temperatures are dependant on ambient environmental temperatures (poikilothermy). Very little is known about ancient tropical terrestrial ecosystems, limiting our understanding of the evolution of giant snakes and their

relationship to climate in the past. The maximum size of poikilothermic animals at a given temperature is limited by metabolic rate, and a snake of this size would require a minimum mean annual temperature of 30–34 °C to survive. This estimate is consistent with hypotheses of hot Palaeocene neotropics with high concentrations of atmospheric CO_2 based on climate models. Comparison of palaeotemperature estimates from the equator to those from South American mid-latitudes indicates a relatively steep temperature gradient during the early Palaeogene greenhouse, similar to that of today. Depositional environments and faunal composition of the Cerrejón Formation indicate an anaconda-like ecology for the giant snake, and an earliest Cenozoic origin of neotropical vertebrate faunas (Puorto *et al*., 2001). (Boulenger, 1896), in the third volume of his *Catalogue of Snakes*, included var. *crawshayi* under the *forma typica* and listed a new variety *pallida* as well as Peters' *Naja mossambica* under *N. nigricollis*, thus establishing the basis for the assumption of monospecificity for the African spitting cobras that was to become the generally accepted arrangement for more than seventy years. Additional forms were described as varieties or subspecies of *N. nigricollis* during the first half of the 20th century. The form *katiensis* was described as a variety of *N. nigricollis* from Kati, Mali, by (Angel, 1922), but gene flow and incipient speciation (Ogden and Thorpe, 2002; Thorpe and Richard, 2001).

2.2 Biology of Snakes

2.2.1 Body size of snakes

The largest extant snakes are the reticulated python, which measures about 6.95 m (22.8 ft) long (Head *et al*., 2009), and the anaconda, which measures about 5.21 m (17.1 ft) long and is considered the heaviest snake on Earth and weighing up to 97.5 kg (215 lb) (Rivas, 2000). The smallest extant snake is *Leptotyphlops carlae* (Barbados threadsnake), with a length of about 10.4 cm (4.1 in) (Fry, 2005). Most snakes are fairly small animals, approximately 1 m (3.3 ft) in length (Boback and Guyer, 2003; Branch, 1979; Hamako *et al*., 1998).

2.2.2 Perception

Pit vipers, pythons, and some boas have infrared-sensitive receptors in the deep grooves of their snout, which allow them to detect the radiated heat of warm-blooded prey. In pit vipers, the grooves are located between the nostril and the eye in a large "pit" on each side of the head. Other infrared-sensitive snakes have multiple, smaller labial pits lining the upper lip, just below the nostrils (Rao, and Kini, 2004).

Snakes use smell to track their prey. They smell by using their forked tongues to collect airborne particles, then passing them to the vomero-nasal organ or Jacobson's organ in the mouth for examination. The fork in the tongue gives snakes a sort of directional sense of smell and taste simultaneously. They keep their tongues constantly in motion, sampling particles from the air, ground, and water, analyzing the chemicals found, and determining the presence of prey or predators in the local environment. In water-dwelling snakes, such as the anaconda, the tongue functions efficiently under water. The underside is very

sensitive to vibration. This allows snakes to be able to sense approaching animals by detecting faint vibrations in the ground (Chatterjee *et al*., 2004; Rao *et al*., 2004).

Snake vision varies greatly, from only being able to distinguish light from dark to keen eyesight, but the main trend is that their vision is adequate although not sharp, and allows them to track movements (Harvey *et al*., 1998; Roman, 1969; Schlöndorff and Blobel, 1999; Smith *et al*., 1838; Tseng *et al*., 2004; Walter *et al*., 1999). Generally, vision is best in arboreal snakes and weakest in burrowing snakes. Some snakes, such as the Asian vine snake (genus *Ahaetulla*), have binocular vision, with both eyes capable of focusing on the same point. Most snakes focus by moving the lens back and forth in relation to the retina, while in the other amniote groups, the lens is stretched. Many nocturnal snakes have slit pupils while diurnal snakes have round pupils (Harvey *et al*., 1998; Roman, 1969; Schlöndorff and Blobel, 1999; Smith, 1838; Tseng *et al*., 2004; Walter *et al*., 1999).

The shedding of scales is called ecdysis (or in normal usage, molting or sloughing). In the case of snakes, the complete outer layer of skin is shed in one layer. Snake scales are not discrete, but extensions of the epidermis—hence they are not shed separately but as a complete outer layer during each molt, akin to a sock being turned inside out (Pardal *et al*., 2004; Roman, 1968; Shirwaikar, Malini, and Kumari, 2003).

The shape and number of scales on the head, back, and belly are often characteristic and used for taxonomic purposes. Scales are named mainly according to their positions on the body. In "advanced" (*Caenophidian*) snakes, the broad belly scales and rows of dorsal scales correspond to the vertebrae, allowing scientists to count the vertebrae without dissection (Lewis, 2006).

2.2.3 Reproduction

Pathenogenesis is a natural form of reproduction in which growth and development of embryos occur without fertilization. *Agkistrodon contortrix* (copperhead) and *Agkistrodon piscivorus* (cotton mouth) can reproduce by facultative parthenogenesis. That is, they are capable of switching from a sexual mode of reproduction to an asexual mode (Booth *et al*., 2012). The type of parthenogenesis that likely occurs isautomixis with terminal fusion, a process in which two terminal products from the same meiosis fuse to form a diploid zygote. This process leads to genome wide homozygosity, expression of deleterious recessive alleles and often to developmental abnormalities. Both captive-born and wild-born *A. contortrix* and *A. piscivorus* appear to be capable of this form of parthenogenesis (Booth *et al*., 2012).

Reproduction in squamate reptiles is almost exclusively sexual. Males ordinarily have a ZZ pair of sex determining chromosomes, and females a ZW pair. However, the Colombian Rainbow boa, *Epicrates maurus* can also reproduce by facultative parthenogenesis resulting in production of WW female progeny (Booth *et al*., 2011; Chioato and Ward, 2003). The WW females are likely produced by terminal automixis.

2.2.4 Winter dormancy

In colderregions of the world especially during winters, snakes become inactive and unlike hibernation, in which mammals are actually asleep, brumating reptiles are awake but inactive. Individual snakes may brumate in burrows, under rock piles, or inside fallen trees, or snakes may aggregate in large numbers (Booth *et al*., 2012).

2.2.5 Feeding

All snakes are strictly carnivorous, eating small animals including lizards, frogs, other snakes, small mammals, birds, eggs, fish, snails or insects (Barlow, Pook, Harrison, and Wüster, 2009; Harborne and Williams, 2000; Mehrtens, 1987; Sanchez, 2007). Because snakes cannot bite or tear their food to pieces, they must swallow prey whole. The body size of a snake has a major influence on its eating habits. Smaller snakes eat smaller prey. Juvenile pythons might start out feeding on lizards or mice and graduate to small deer or antelope (Tattersall *et al*., 2004; Head *et al*., 2009).

The snake's jaw is a complex structure. Contrary to the popular belief that snakes can dislocate their jaws, snakes have a very flexible lower jaw, the two halves of which are not rigidly attached, and numerous other joints in their skull, allowing them to open their mouths wide enough to swallow their prey whole, even if it is larger in diameter than the snake itself (Behler and King, 1979). For example, the African egg-eating snake has flexible jaws adapted for eating eggs much larger than the diameter of its head (Mehrtens, 1987). This snake has no teeth, but does have bony protrusions on the inside edge of its spine, which it uses to break shells when it eats eggs (Mehrtens, 2001).

Although, the majority of snakes eat a variety of prey animals, there is some specialization by some species. King cobras and the Australian bandy-bandy consume other snakes. Pareas iwesakii and other snail-eating colubrids of subfamily Pareatinae have more teeth on the right side of their mouths than on the left, as the shells of their prey usually spiral clockwise (Mehrtens, 2001; Hoso *et al*., 2007).

Some snakes have a venomous bite, which they use to kill their prey before eating It (Mehrtens, 1987; Hoso *et al*., 2007). Other snakes kill their prey by constriction (Mehrtens, 1987). Still others swallow their prey whole and alive (Mehrtens, 1987; Sanchez, 2007). After eating, snakes become dormant while the process of digestion takes place (Havesteen, 1990; Stephen *et al*., 1995; Sharma *et al*., 2004). Digestion is an intense activity, especially after consumption of large prey. In species that feed only sporadically, the entire intestine enters a reduced state between meals to conserve energy.

The digestive system is then 'up-regulated' to full capacity within 48 hours of prey consumption. Being ectothermic ("cold-blooded"), the surrounding temperature plays a large role in snake digestion. The ideal temperature for snakes to digest is 30 °C (86 °F). So much metabolic energy is involved in a snake's digestion that in the Mexican rattlesnake (*Crotalus durissus*), surface body temperature increases by as much as 1.2 °C (2.2 °F) during the digestive process (Tattersall *et al*., 2004). Therefore, a snake disturbed after having eaten recently will often regurgitate its prey to be able to escape the perceived threat. When undisturbed, the digestive process is highly efficient, with the snake's digestive enzymes dissolving and absorbing everything but the prey's hair (or feathers) and claws, which are excreted along with waste (Stephen *et al*., 1995a; Leon *et al*., 1997; Tattersall *et al*., 2004; HomeoVision, 2011).

2.3 Geographical Distribution of Venomous Snakes in Nigeria

Snakes are an important component of the natural ecosystem of the environment and are commonly encountered in rural and urban areas of savannah zone (Paramonte, 2007). Live snakes are in great demand and are purchased forvarious purposes like venom extraction, as

food, ornamental decoation, guards and display. The snakes are even exported to foreign countries for exhibition shows, zoological gardens and manufacture of leather goods. Some families also depend for their livelihood on exhibiting snakes and they are known as snake charmers. In some cultures, snakes are worshipped and offered milk etc (Paramonte, 2007).

Several studies have reported variations in species of snakes found in the ecological zones of the Sahel desert, Sudan, Guinea savannah and rain forest. However, little information is available on different snake species found within the narrow belt-line between the southern and northern guinea savannah zone of Nigeria.

Four main families of snakes (Viperidae, Elapidae, Colubridae and Actraspididae) have been identified to be responsible for snake bites in Nigeria, with three species from the first two families - carpet viper (*Echis ocellatus*), black-necked spitting cobra (*Naja nigricollis*) and puff adder (*Bitis arietans*) being the most common culprits for envenomation in Nigeria (Habib *et al.*, 2001). *E. ocellatus* has been reported to be responsible for the majority of envenomation in the savannah region of Nigeria (Habib *et al*., 2001; Abubakar *et al*., 2010).

The saw-scaled or carpet viper (*Echis ocellatus*) is responsible for 90% of bites and 60% of deaths. In the Benue Valley of north-eastern Nigeria, the incidence of snake bite was found to be 497 per 100 000 population per year with a mortality of 12.2% (Warrell and Arnett, 1976). Most bites and deaths were attributed to saw-scaled vipers (*Echis ocellatus*) and its distribution ranges throughout Nigeria in its natural habitat of forest edges and savannah, up to 3,000 feet above sea level. The carpet viper's breeding season coincides with heavy rainfall during the annual monsoon season, driving it to seek shelter in higher, drier habitats

to lay eggs (Ward *et al*., 1996; Venkateswarlu *et al*., 2002; Lee *et al.*, 2003; Kanaji *et al*., 2003; Kini, 2005; Habib *et al*., 2008).

A community survey of snake bite by the black-necked spitting cobra (*Naja nigricollis*) in Malumfashi, northern Nigeria, found that in a population of 43 500 there were 15–20 bites/100 000/year (Pugh *et al*., 1980). *N. nigricollis* usually inhabits savannah and semi-desert regions of Africa. However, they can be found at altitudes up to 1,800 metres (5,900 ft), as well as in tropical and subtropical regions of central Africa in moist savannah and cleared former forest regions, particularly near rivers and streams (WCH, 2011). Adaptable snakes, *Naja nigricollis* occurs in south-eastern Nigeria where their habitat has been transformed from rainforest to man-made farmlands, plantations, suburban areas, and a few fragmented forests. This species of cobra has found advantages in the drastic changes which have taken place in Nigeria's rainforests. A study by (Luiselli, 2001) suggests this snake now forages in much drier microhabitats. The range of *Naja nigricollis* is currently expanding from the south-eastern regions of Nigeria to the more desert and arid conditions in the central part of the nation (Abriol, 2011). They also live in coastal scrubs and dry grasslands. Like other cobra species, they may find abandoned termite mounds or rodent holes to hide or cool off. However, tree trunks seem to be their favourite hiding places. They are excellent tree climbers, thus can be arboreal at times. Because they are so common across Africa, they are encountered in villages or small towns where they may come in direct contact with people (Teng *et al*., 1984; Kamiguti *et al*., 1996; Abriol, 2011; Kini, 2003; Chung *et al*., 2004).

(Adeiza and Minka, 2013) in a survey of snake species in the Northern and Southern Guinea Savannah reported a total of nine snake species identified from the two ecological

zones. Resultsof this study showed that of the 100 samples of snakes captured, 58% of the snakes belonging to the genus *Naja*. Of this number 86.2% of the *Naja* spp. snakes were captured from the ecological zone of northern guinea savannah. The most common species of *Naja* captured was *Naja nigricollis* (black necked spitting cobra) (30%), followed by *Naja mossambica* (12%), *Naja haje* (10%) and *Naja katiensis* (6%). Similarly, 20% of *Naja nigricollis* were captured around homes (Adeiza and Minka, 2013).

Bitis arietans (puff adder), *Caucus rhomobeatus* and *Dispholdus typus* species were found basically in the ecological zone of southern guinea savannah and were the most abundant snakes found in this area (Adeiza and Minka, 2013). These species of snakes have been reported to flourish in the forest areas, but also do migrate to the southern savannah zone (Hughes, 1983), but rarely found in northern guinea savannah zone. In general, the species found in the savannah are almost totally different from those found in rain forest zones. However, within the guinea savannah some species found in the northern are also found in the southern guinea savannah, especially at the very belt-line that divides northern guinea form the southern guinea savannah (Laurent, 1955; Laing *et al*., 1995).

2.4 Snake Venom Composition and Pathology

Snake venoms are complex mixtures of predominantly proteinaceous and peptidyl toxins. In addition, small organic substances, such as citrate, nucleosides and acetylcholine may also be present (Leon *et al*., 1999; Aird, 2002; Smalligan *et al*., 2004; M´enez *et al*., 2006; Silva *et al*., 2007; WHO, 2007; Bos *et al*., 2009).

Historically, venoms have been differentiated from poisons by the route of entry into a recipient organism: venoms are injected or introduced into a wound produced by the delivering organism, and poisons are ingested (accidentally or intentionally) by the recipient organism. The term venom typically is applied to simple or complex secretions (usually containing multiple toxins) produced in a specialized gland in an animal and which causes deleterious effects and/or death when injected into a recipient organism. A toxin, on the other hand, is a biologically produced unique molecular entity, which can damage or kill an organism through its action on specific tissues. But even in the scientific literature, one still occasionally encounters the description of venom as a "neurotoxin" or a "haemotoxin", particularly in reference to the venoms of front-fanged snakes are encountered (families Atractaspididae, Elapidae and Viperidae). The term “haemotoxin” is really a misnomer, because there are no venom toxins, though the dominant pharmacological effect of venom may be described superficially as "neurotoxic" or “tissue-damaging”, no snake venom described to date contains only a single molecular or pharmacologically-active component. (Markland, 1998; McDiarmid *et al*., 1999; Lu *et al*., 2005; Nair *et al*., 2007; Sant’Ana *et al*., 2008; Harrison *et al*., 2009).

Snake venom is a mixture of toxins and different enzymes, (phospholipase A2, phosphodiesterases, hyaluronidases, amino acid oxidases, ATPases, proteases e.t.c.) used for purposes like increasing the prey's uptake of toxins to disarm it and in defence against danger. Some snake vemoms are specific to the tissues (mycotoxic), heart (cardiotoxic) or nervous system (neurotoxic) depending on the snake species. The venoms are composed of various collections of polypeptides or proteins. These polypeptides or proteins are basically divided into two - either enzymes or non-enzymatic polypeptides (Matsui *et al*., 2000; Zhang *et al*., 2003; Radis-Baptista *et al*., 2006). However, it should be noted that the proteins of snake venoms belong to a small number of superfamilies of proteins. The members in a single family show remarkable similarities in their primary, secondary and tertiary structures (Nathan *et al*., 1982; Yeh *et al*., 2000; Rr *et al*., 2004). At times, however, they differ from each other in their biological targeting and hence their pharmacological effects. Thus, each family of protein toxins is similar molecularly, but exhibits multiple functions. These structure-function relationships and the mechanisms of action of snake venom proteins are intriguing and posing exciting challenges to scientists (Pirkle, 1998; Rao *et al*., 2003; Lewis, 2006).

The effects or actions of these toxins are mostly by a means of degradation of cells, tissues or intercellular bonds or by a competitive inhibition, blocking transmission of acetylcholine. Other venom components have been identified including carbohydrates, lipids, nucleoside and some metalloproteases ranging from components such as magnesium, calcium, and zinc as the most prevalent metals found in the venom (Tokunaga *et al*., 1988; Abubakar *et al*., 2010; Williams *et al*., 2011; Lewis, 2006).

Based on sequence, these snake venom components have been classified into various families, such as serine proteases, metalloproteinases, C-type lectins, disintegrins and phospholipases (Sanchez *et al*., 2000). The various members of a particular family act selectively on different blood coagulation factors, blood cells or tissues. For almost every factor involved in coagulation or fibrinolysis there is a venom protein that can activate or inactivate it (Rosing and Tans, 1992; Sanchez *et al.*, 2000; Ahmed *et al*., 2008).

Venom proteins affect platelet function by binding or degrading vWF or platelet receptors, activating protease-activated receptors or modulating ADP release and thromboxane A2 formation. Some venom enzymes cleave key basement membrane components and directly affect capillary blood vessels to cause hemorrhaging. L-Amino acid oxidases activate platelets via H_2O_2 production. It is generally believed that the over-all action of snake venoms is caused by the combined effect of a number of different proteins (both enzymatic and none-nzymatic) found in the venoms (Chippaux, Williams, and White, 1991).

Snakes of the family Viperidae (vipers and pitvipers) produce a complex mixture of a large number of distinct proteins 1, 2 in paired specialized venom glands located ventral and posterior to the eyes. C3 inspite of the fact that viperid venoms may contain well over 100 protein components,4 venom proteins belong to only a few major protein families, including enzymes (serine proteinases, Zn2C-metalloproteases, L-amino acid oxidase, group II PLA2) and proteins without enzymatic activity.

Snake bite results in subcutaneous/intramuscular injection of venom into the prey or human victims resulting in complicated pathology, comprising both local and systemic effects. Systemic toxicity may include pre or post-synaptic neurotoxicity, myotoxicity, pro-or

anticoagulant activities and hypotensive or rarely, hypertensive effects. The local effects include oedema, haemorrhage, dermonecrosis and myonecrosis (Chippaux *et al*., 1991; Tu, 1996; Kini, 1997; Cher *et al*., 2005). The extent of systemic effects depends on the concentrations of systemic toxins injected and on the rate at which these toxins diffuse into the circulation from the site of the bite (Petras *et al*., 2011; Gutiérrez, 2002; Cher *et al*., 2005).

Basically, toxin diffusion depends upon the extent of local tissue destruction. Locally-acting enzymes/toxins include myotoxins (enzymic/non-enzymic), zinc containing haemorrhagic metalloproteases. These degrade proteins and glycosaminoglycans of the extracellular matrix (ECM) and connective tissue surrounding blood vessels and capillaries. The loss in structural integrity facilitates toxin diffusion. However, these degradative enzymes vary in relative abundance in different venoms. Cobra venoms are rich in myotoxic phospholipase A2 while viper venoms are rich in haemorrhagic metalloproteases. In contrast, venom hyaluronidase is essentially ubiquitous in its distribution, although it does exhibit individual quantitative variation (Girish *et al*., 2002; Yingprasertchai *et al*., 2003; Girish and Kemparaju, 2005).

2.4.1 Snake venom variation

The composition of snake venom differs from one species to another. There is also variation within a single species depending on age, season and temperature. It is a complex mixture of enzymes, toxins and all sorts of smaller molecules. The most important components are the substances with a cytotoxic effect, the neurotoxins and the coagulants.

Some toxins have multiple effects (Polgár *et al*., 1997; Fujii *et al*., 2003; Cher *et al*., 2005; Hedges, 2008).

2.4.2 Snake venoms and haemostasis

Snake venoms affect haemostasis by activating or inhibiting coagulant factors or platelets, or by disrupting endothelium (Meier and Stocker, 1991). The various members of a particular family act selectively on different blood coagulation factors, blood cells or tissues. For almost every factor involved in coagulation or fibrinolysis there is a venom protein that can activate or inactivate it. Venom proteins affect platelet function by binding or degrading VWF or platelet receptors, activating protease-activated receptors or modulating ADP release and thromboxane A2 formation. Some venom enzymes cleave key basement membrane components and directly affect capillary blood vessels to cause hemorrhaging. L-amino acid oxidases activate platelets via H_2O_2 production (Marsh, 1994; Leon *et al*., 1997; Markland, 1998; Braud et al. 2000; Izidoro and Rodrigues, 2003; Morita, 2004; Shirwaikar *et al*., 2004; Shirwaikar *et al*., 2004; José María *et al*., 2005; Girish and Kemparaju, 2006).

2.4.2.1 Venom proteins acting on blood coagulation factors

Venom proteins affecting coagulation factors may be classified as acting on coagulant factors including FV activators, FX activators, prothrombin activators and thrombin-like enzymes (TLEs); anticoagulant factors including FIX/X binding proteins, protein C activators, thrombin inhibitors and phospholipase A2; and those acting on fibrinolysis, including fibrinolytic enzymes and plasminogen activator. FV activator FV is a multi-functional 330 kDa glycoprotein, with an important role in both procoagulation and

anticoagulation (McNamee, 2001; Sells *et al*., 2001; Sutherland and Tibballs, 2001; Mann and Kalafatis, 2003).

Thrombin activates FV by cleaving at 709, 1018 and 1545 to form FVa, a heterodimer consisting of a 105 kDa heavy chain and a 72/74 kDa light chain doublet. FVa acts as cofactor in FXa catalysed prothrombin activation and it enhances thrombin generation >1000-fold. Its cofactor activity is down-regulated by activated protein C and it also acts as cofactor in activated protein C-mediated FVIIIi nactivation (Leon *et al*., 2000; Winkel *et al*., 2001; Mann and Kalafatis, 2003; Marcinkiewicz *et al*., 2003; Kuruppu *et al*., 2008).

2.4.2.2 FX activator

FX activators have been isolated from many Viperidae and Crotalidae venoms as well as from a few Elapid species. FX activators are either metalloproteinases or serine proteases (Rosing and Tans, 1992). The most important FX activator is RVV-X found in *V. russelli*. RVV-X is a disulfide-linked three-chain metalloproteinase with a 58 kDa heavy chain and 19.4 and 16.4 kDa light chains. The heavy chain of RVV-X has metalloproteinase, disintegrin-like and cysteine-rich domains. The light chains resemble the C-type lectins (Nunez, 1992). It is proposed that RVV-X recognizes and binds to the Gla domain of FX via the two C-type lectin chains of RVV-X and the heavy chain of RVV-X cleaves the target Arg52-Ile53 bond in the heavy chain of FX. Metalloproteinase FX activators from other venoms have similar structures to RVV-X and probably similar catalytic mechanisms. RVV-X also activates FIX by cleaving a single peptide bond without affecting molecular mass (Currie, 2004; Isbister *et al*., 2008; Isbister, 2009; Lindquist *et al*., 1978; St Pierre *et al*., 2005).

2.4.2.3 Prothrombin activator

A large number of snake venoms contain prothrombin activators, which convert prothrombin into meizothrombin or thrombin (Rosing and Tans, 1992; Matsushita and Okabe, 2001; Isbister *et al*., 2007). Based on their structures, functional characteristics and cofactor requirements, they are classified into four groups. Group A prothrombin activators are metalloproteinases and activate prothrombin efficiently without cofactors, such as Ca^{2+}, phospholipids (PLs) or FVa. Group B prothrombin activators are Ca^{2+}-dependent. They contain two subunits linked non-covalently: a metalloproteinase and a C-type lectin-like disulfide-linked dimer. Group C prothrombin activators are serine proteases found in Australian elapids requiring Ca^{2+} and negatively charged PLs, but not FVa for maximal activity. Proexosite-1 is a cofactordependent recognition site for them as well as for prothrombin. Oscutarin from *Oxyuranus scutellatus* also activates FVII. Group D prothrombin activators are serine proteases and are strongly dependent on Ca^{2+}, negatively charged PL and FVa (Hughes and Barry, 1969; Guitterez, 1995; Yeh *et al*., 2001; Itano and Kimata, 2002; Laurich *et al*., 2004; Adamia *et al*., 2005; Punde, 2005).

2.4.2.4 Thrombin-like enzymes (TLEs)

Snake venom TLEs are widely distributed in several pit viper genera (*Agkistrodon, Bothrops, Lachesis* and *Trimeresurus*), as well as in some true vipers (*Bitis* and *Cerastes*) and the colubrid, *Dispholidus typus* (Pirkle, 1998) Sequences of >30 TLE have been determined, with a high level of similarity. The catalytic domain, including the catalytic triad residues (His57, Asp102 and Ser 195) and the S1 (Asp189) and S2 (Gly216) specificity sites, is the main conserved region. However, they show less similarity with thrombin (Mackessy, 2002; Castro *et al*., 2004; George and Stern, 2004). Like thrombin,

TLEs specifically catalyse limited cleavage of fibrinogen and lead to clotting of fibrinogen. Based on the rates of release of fibrinopeptides A and B, TLEs can be classified into three groups, venombin A, venombin B and venombin AB (Markland, 1998; Jorge and Aird, 2001). They also show some species specificity in efficiency of fibrinogen conversion. TLEs are inhibited by most serine protease inhibitors, but most are unaffected by thrombin inhibitors like antithrombin III and hirudin. Unlike thrombin, most TLEs do not activate FXIII and protease-activated receptors (PAR) on platelets. Consequently, the fibrin formed by TLEs is easily removed from the circulation allowing their clinical use as defibrinogenating agents.

2.4.2.5 FIX/X inhibitors

Many anti-coagulant C-type lectin-like proteins, interacting with FIX and/or FX, have been isolated from various snake species (Morita, 2004; Mirtschin et al., 2006) Based on their ligand recognition differences, these proteins can be classified as: blood coagulation FIX/FXbinding proteins (FIX/X-BPs), interacting with FIX or FX in a 1:1molar ratio; FIX-binding proteins (FIX-BPs), which do not interact with FX; FX-binding proteins (FX-BPs), binding predominantly to FX. They are heterodimers with highly similar structures. For example, habu FIX/X-BP and habu FIX-BP, from the same species, share an identical b subunit and their subunits differ by only 19 amino acid residues in a total of 129 amino acids (Alam and Gomes, 2003; Girish *et al*., 2004; Castoe *et al.*, 2005; Castoe and Parkinson, 2006).

The gamma-carboxyglutamic acid (Gla) domain of coagulation factors is responsible for Ca^{2+}-dependent PL membrane binding. Snake venom FIX/X binding proteins bind

specifically to the Gla domain of FX or FIX. The two subunits of habu FIX-BP are tightly associated, and form a concave surface by loop-swapping, i.e. the ligand binding site. The crystal structure of FX-BP complexed with the Gla domain peptide of FX showed that two patches of the Gla domain essential for membrane binding are buried in the complex formation leading to the anti-coagulation effect (Mizuno *et al*., 2001). The crystal structure of $Mg^{2+}$and Ca^{2+}bound Gla domain of FIX complexed with habu FIX BP showed that Mg^{2+} ionsare required for native conformation and *in vivo* function of Gla domain of FIX (Shikamoto, Morita, Fujimoto, and Mizuno, 2003; Gutiérrez and Ownby, 2003).

2.4.2.6 Protein C activators

Protein C is a vitamin K-dependent, two-chain zymogen activated by thrombin. Activated protein C degrades FVa and FVIIIa and is therefore an anticoagulant. Most protein C activators were purified from *Agkistrodon* venoms. Others come from *Bothrops, Trimeresurus,* or *Cerastes* venoms. Most venom protein C activators have sequences highly similar to other venom serine proteases. Unlike thrombin-catalysed protein C activation, requiring thrombomodin as a cofactor, venom activators directly convert protein C into the active form. The fast-acting protein C activator Protein from *Agkistrodon contortrix contortrix* venom is widely used to diagnose protein C pathway disorders (Burbrink *et al*., 2000; Bee *et al*., 2001; Bolger *et al*., 2001; Gempeler-Messina *et al*., 2001).

2.4.2.7 Thrombin inhibitors

Bothrojaracin (BJC) is a 27 kDa C-type lectin-like thrombin inhibitor from *Bothrops jararaca* venom composed of 13 and 15 kDa subunits linked by disulfide bridges (Zingali *et al*., 1993). BJC is highly resistant to urea or DTT, requiring both agents to denature it

fully. BJC has two independent mechanisms for anticoagulation; it binds strongly to exosites I and II to form a noncovalent equimolar complex, and inhibits thrombin-induced platelet aggregation and secretion but does not interact with the catalytic site of thrombin. It prolongs fibrinogen-clotting time by inhibiting competitively the binding of thrombin to fibrinogen and it inhibits thrombin binding to thrombomodulin and decreases the rate of protein C activation (Arocas *et al*., 1996). Secondly, it inhibits prothrombin activation by interacting with proexosite I. In the absence of PLs, BJC strongly inhibits the zymogen activation by FXa in the presence but not in the absence of FVa. Proexosite I blockage decreases cleavage of prothrombin by FXa-FVa complex or prothrombinase complex (Zingali *et al*. 2001). BJC-like proteins are present in other Bothrops species and *Lachesis muta* venom, such as bothroalternin, a less potent human thrombin inhibitor, from *Bothrops alternatus* venom (Castro *et al*., 1999; Clemetson *et al*., 2002; Park and Green, 1997 ;Wuster *et al*., 1997).

2.4.2.8 Fibrinolytic enzymes

Fibrin(ogen)olytic activity has been described in the venoms of members of the *Crotalidae, Viperidae* and *Elapidae* families (Markland, 1991) These fibrinolytic enzymes are divided into metalloproteinases and serine proteases (Matsui *et al*., 2000). Most of the first group of enzymes were characterized as zinc metalloproteinases and degraded the Aa-chain of fibrinogen preferentially. The second group is serine proteases and most have specific activity toward the Bb-chain of fibrinogen. However, there are exceptions to this generalization, and specificity for the a- or b-chains is not absolute, as there is substantial degradation of the alternate chain with time. Most of the metalloproteinases are fibrinolytic and many of the serine proteinases are both fibrinogenolytic and fibrinolytic (Braud *et al*.,

2000; Saha *et al*., 2006; Gutiérrez *et al*., 2009). Fibrin (ogen)olytic metalloproteinase enzymes cleave amino-terminal to hydrophobic amino acids, while serine fibrin(ogen)lytic enzymes cleave carboxy-terminal to basic amino acids.

Members of the metalloproteinases family are metal ion containing proteinases (mainly zinc), having a common methionine turn below and carboxy-terminal to a helical segment containing two of the three histidine residues involved in the zinc-binding site (Jia *et al*. 1996). The fibrin(ogen)olytic serine proteases share extensive sequence homology with other venom serine proteases but subtle differences determine their substrate specificities (Braud *et al*., 2000; Marrakchi *et al*., 1997; Wu *et al*., 2001; Yasuo *et al*., 2003).

2.4.2.9 Plasminogen activator

A direct acting plasminogen activator in snake venom was described from *Trimeresurus stejnegeri* venom (Zhang *et al*., 1995). Plasminogen activators were also reported from *Lachesis muta muta* and *Agkistrodon halys* venoms (Sanchez *et al*., 2000). TSV-PA is a 33 kDa single chain serine protease that activates plasminogen by cleaving the Arg561-Val562 bond like the physiological activators u-PA and t-PA. However, unlike t-PA, fibrin fragments do not stimulate activation by TSV-PA. TSV-PA did not active FX or protein C, and it did not degrade fibrin or fibrinogen in the absence of plasminogen.

The DNA sequence of TSV-PA shares more than 60% similarity with other snake venom serine proteases, but only _20% with t-PA or u-PA. It contains only the catalytic domain of the mammalian plasminogen activators. Furthermore, TSV-PA lacks the sequence responsible for the interaction of t-PA (KHRR) and u-PA (RRHR) with plasminogen activator inhibitor type 1 (Zhang *et al*., 1995). The three-dimensional structure of TSV-PA

shows the overall polypeptide fold of trypsin-like serine proteases and is the first snake venom serine protease structure (Parry *et al*., 1998). Site-directed mutagenesis showed that the sequence DDE96–98 contributed to its plasminogen activation activity. The TSV-PA variant F193G is eight- to ninefold more active toward plasminogen and becomes sensitive to bovine pancreatic trypsin inhibitor (Braud *et al*., 2002).

2.4.2.10 Metalloproteinases

Crotalidae and Viperidae venoms contain many metalloproteinases,which act synergistically to degrade the blood vesselextracellular matrix (ECM) and thus affect hemostasis (Hati *et al*., 1999). Based on the domain structure, the venom metalloproteinases are classified into four major groups (Hite *et al*., 1994). The P-I class has a single metalloproteinase domain. The P-II class has a metalloproteinase domain and a disintegrin domain that are separated post-translationally. The P-III class has metalloproteinase, disintegrin-like (containing a disulfide-linked XXCD, mostly SECD, in place of RGD), and cysteine-rich domains. The P-IV class contains an additional disulfide-linked C-type lectin-like domain, compared with the P-III class. The hemorrhagic metalloproteinases are stored as inactive zymogens and activated by a cysteine switch-like mechanism (Grams *et al*., 1993; Stock *et al*., 2007; Visser *et al*., 2008).

Hemorrhagic enzymes cleave key peptide bonds of basement membrane components, affecting the interactions with endothelial cells. These cells then undergo morphological and functional alterations *in vivo*, associated with hemodynamic factors such as shear stress. Eventually, gaps form between endothelial cells allowing extravasation (Leon *et al*., 2000). Disintegrin-like and cysteine-rich domains of PI-III class hemorrhagic toxins may

make them more active than PI-I or PI-II by directing the enzyme to critical locations in capillary blood vessels and modulating the catalytic specificity of the enzyme domain on ECM substrates.

2.4.2.11Nucleotidases

Nucleotidase activity is widely distributed in many viper and pit viper venoms (Tan & Ponnudurai, 1992). *Trimeresurus gramineus* venom contains a 74 kDa thermostable, single chain 5¢-nucleotidase. Activity was inhibited by EDTA but supported by Zn^{2+} or Co^{2+}. In rabbit platelet-rich plasma, 5¢-nucleotidase completely inhibited platelet aggregation induced by ADP, sodium arachadonate or collagen, most likely by ADP and possibly by generation of adenosine (Ouyang and Huang, 1983).

2.4.2.12 L-Amino acid oxidases

Venom L-amino acid oxidases (LAAOs) are homodimeric flavoenzymes, which catalyse the oxidative deamination of an L-amino acid substrate to aa-keto acid along withammonia and hydrogen peroxide. They are widely distributed in *Viperidae, Crotalidae* and *Elapidae* (Du and Clemetson, 2002). Each subunit has three domains: an FAD-binding domain, a substrate-binding domain and a helical domain (Pawelek *et al*., 2000). The reported effects of LAAOs on platelet function are quite controversial. LAAO from *Echis colorata* inhibits ADP-induced platelet aggregation (Nathan *et al*., 1982). *Agkistrodon halys blomhoffii* and *Naja naja kaouthia* LAAOs, inhibits agonist- or shear stress-induced platelet aggregation (Takatsuka *et al*., 2001; Sakurai *et al*., 2001). The authors suggested that the interaction between activated platelet integrin GPIIb/IIIa and fibrinogen was inhibited by the continuous generation of H_2O_2. LAAOs from other snakes have been reported to have

totally the opposite effect on platelets. LAAOs from *Eristocophis macmahoni, O. hannah*, *B. alternates* and *Trimeresurus jerdonii* induce human platelet aggregation through formation of H_2O_2 (Du and Clemetson, 2002; Lu *et al*., 2002; Stabeli *et al*., 2004). It is still not clear how H_2O_2 functions in LAAOs-induced platelet aggregation. It is also possible that LAAOs activate platelets in a receptor dependent way as LAAO from *A. halys* showed various binding and cytotoxic effects on different cell lines (Zhang *et al*., 2004).

2.5 Snake Venom and Necrosis

Enzymes, which help the snake to digest its prey, are often cytotoxic for man. Proteolytic enzymes have a trypsin-like activity. Hyaluronidase splits acidic mucopolysaccharides and promotes the distribution of venom in the extracellular matrix of connective tissue. Snake venom often contains various phospholipases A_2. These are esterolytic enzymes which break down membrane phospholipids such as lecithin (phosphatidylcholine) into fatty acids and lysolecithin. This causes cellular membrane damage. In human beings, all these enzymes cause oedema, blister formation and local tissue necrosis (Ownby *et al*., 1993; Cominetti *et al*., 2004). The main clinical features of *E. ocellatus* envenoming are systemic hemorrhage, incoagulable blood, shock, local swelling, bleeding and, occasionally, necrosis. Bites may be complicated by amputation, blindness, disability, disfigurement, mutilation, tissue destruction and psychological consequences (Habib, 2013).

2.6 Snake Venom and Paralysis

With regard to function, the neurotoxins of some *elapids* can be compared with curare or with the autoantibodies in myasthenia gravis. The neurotoxins block the stimulus transmission from nerve cell to muscle and cause paralysis. The venom does not penetrate

the blood-brain barrier. Some venom (cobra, mamba, death adder, *Laticauda*, krait alpha-bungarotoxin) work on the nicotinic acetylcholine receptor present on muscle (neuromuscular junction) (Fatima and Fatah, 2014).The postsynaptic effects are reversible with antivenom and neostigmine. Other types of venom work on the presynaptic nerve terminal, e.g. beta-bungarotoxin) and in such cases neostigmine will not be effective. Pre-synaptic neurotoxins inhibit the fusion of the vesicles containing acetylcholine, with the nerve's membrane of the neuromuscular junction. Appropriate treatments of snake bite victims need a complete understanding of the pharmacological roles of the different venom components. Appropriate treatments of snake bite victims need a complete understanding of the pharmacological roles of the different venom components (Fatima and Fatah, 2014).

Curare is a complex alkaloid which is derived from South American plants such as *Chondodendron tomentosum* (tubocurarine) and *Strychnos toxifera*. It acts on the postsynaptic acetylcholine-receptor of the neuromuscular junction and causes paralysis. The mechanism is comparable to *elapid* venom (Theakston and Warrell, 2000; Anai *et al*., 2002; Biondo *et al*., 2003; Borges *et al*., 2005; Williams *et al*., 2010).

2.7 Neurotoxins

The snake α-neurotoxins are the major lethal components of *Elapidae* (including, for example, cobras, kraits, sea kraits, and true sea snakes venoms (Slowinski and Keogh, 2000) and they play a fundamental role in the snake feeding mechanism and defensive strategy.The common target of a-neurotoxins is the muscle type nicotinic acetylcholine receptor (nAChR), a ligand-gated ion channel on the postsynaptic fold of the neuromuscular junction with the subunit stochiometry of a2bcd (Karlin, 2002). Upon binding to the nAChR, α-neurotoxin prevents the binding of the natural ligand

acetylcholine (ACh) and the subsequent ACh induced ion flow, resulting in a neuromuscular inhibition of the target species.

Snake α-neurotoxins of *Elapidae* venoms are grouped into two structural classes, short-chain contain 60-62 amino acid residues and four disulfide bridges and the long-chain α-neutotoxins consist of 66–74 amino acid residues and usually have a fifth disulfide bridge in addition to the four disulfide bridges present in their short-chain counterparts. While these two classes share many chemical and biological characteristics, there are also distinct dissimilarities between them, including their binding site on the nicotinic acetylcholine receptor (nAChR), specificity among species of Chordata, and the associated pharmacological effects. In comparison with short-chain α-neurotoxins, long-chain a-neurotoxins have further sequence characteristics of distinct deletions and insertions of amino acids, as well as the presence of an extra C-terminal segment of five to nine amino acid residues (Endo and Tamiya, 1991). The binding site of short-chain α-neurotoxins on the nAChR is less well characterized compared to long-chain a-neurotoxins (Weber and Changeux, 1974; Ariaratnam *et al*., 2009).

In general, it is regarded that short-chain and long-chain α-neurotoxins share a similar overall topology in their interaction with the nAChR (Teixeira-Clerc *et al*., 2002). Short-chain and long-chain α-neurotoxins have many common characteristics, including molecular structure, pharmacological action, and coexistence in conspecific venoms. Based on these similarities it is possible that the mechanism that protects a snake from the action of long-chain a-neurotoxins also protects against short chain α-neurotoxins. Furthermore, there are no data available to date describing the molecular mechanisms of natural resistance against short-chain α-neurotoxins or addressing natural resistance to sea snake

venom at the molecular level (Audebert *et al*. 1994; Zoltan *et al*., 2004; Tanos *et al*., 2008).

Venomous snakes are resistant to the components of conspecific venom. In the case of Elapidae snakes, the resistance against conspecific long-chain a-neurotoxin α-BTX has been shown to be mediated by a unique N-glycosylation of the nAChR ligand binding domain (Takacs *et al*., 2001). If this glycosylation is removed, the nAChR will be inhibited by conspecific long-chain α-neurotoxin, revealing that the binding site of α-BTX is evolutionarily conserved on the nAChR per se (Takacs *et al*., 2001). The presence of a unique glycosylation in the cobra, *Naja* spp., nAChR ligand binding domain at position 189 inhibits the effect of the short-chain snake a-neurotoxin, ETX-a. Removal of the glycosylation that is flanked by the native Elapidae ligand binding domain sequence will convert the nAChR to be ETX-a sensitive, nearly to the value observed in mouse nAChR. (Zoltan *et al*., 2004).

2.8 Action of Snake Venoms on Macromolecules

The biomolecules that constitute snake venoms have various effects on biological macromolecules. Snake venoms contain phospholipase (PLAs) as mentioned earlier. Phospholipases A2 (PLA2) of snake venoms are of primary concern. PLA2s comprise a very large superfamily of enzymes composed of 16 recognised groups within six major types (Harris and Scott-Davey, 2013). These major types include the secreted PLA2s (sPLA2), the cytosolic PLA2s (cPLA2), the calcium independent PLA2s (iPLA2) the platelet activating factor (PAF) acetyl hydrolase/oxidised lipid lipoprotein associated PLA2 (LpPLA2s), the adipose PLA2s (AdPLA2s) and the lysosomal PLA2s (LPLA2s). The

hydrolysis of glycerophospholipids by PLA2s results in the release of fatty acid and the production of the relevant lysophospholipid. The enzymes are found in virtually all forms oflife from bacteria to invertebrates, vertebrates and plants (Harris and Scott-Davey, 2013). They play a major role in the regulation of phospholipid turnover, membrane fluidity and trafficking, cell maturation and maintenance, apoptosis, and the production of the eicosanoids, leukotrienes and prostaglandins.The sPLA2s of snake venoms were the first phospholipases to be formally characterized (Warrell, 2004; Warrell, 2006; Harris and Scott-Davey, 2013). They are found in two major groups of snake—the Elapids and sea snakes (New World snakes of principally of SE Asia, Australia and parts of the America) and the vipers and crotalids (principally of the America, and Eurasia). The Elapids and sea snakes inoculate venom via short, fixed fangs of between 1 and 5 mm in length and include major species of clinical interest such as tiger snakes (genus *Notechis*) and taipans (genus *Oxyuranus*) of Australia and Papua New Guinea, the kraits (genus *Bungarus*) of SE Asia and sea snakes (Brunda and Sashidhar, 2007; Chappuis *et al*., 2007; Ariaratnam *et al.*, 2008; Canale *et al*., 2009).

Cobra snake venom reduces significantly tissue nucleic acid levels in human breast cancer, these have been attributed to the presence of nucleotidase (Tan and Ponnudurai, 1992). Other species that have nucleotidases include many viper and pit viper venoms. (Tan and Ponnudurai, 1992). *Trimeresurus gramineus* venom contains a 74 kDa thermostable, single chain 5¢-nucleotidase. These effects of nucleotidases have been employed in development of anti-viral agents. Four cytotoxins isolated from *Naja nigrocollis* snake venom showed that cells infected by a virus were susceptible to the cytotoxic action of the venom. L-amino acid oxidases (LAAO), flavoenzymes obtained from the venom of *Bothrops jararaca*, were

also found to show antiviral activity against the dengue virus. Immunokine, an oxidized derivative of the α-toxin extracted from *Naja siamensis* snake venom has shown to inhibit the infection of lymphocytes by HIV and feline immunodeficiency virus (FIV) through the chemokine receptors CCR5 and CXCR4.

A group of investigators in Asia reported a remarkable similarity between the 164-174 sequence of the short segment of gp120 of HIV-1 and 30-40 amino acid residues of the long-chain neurotoxins in the venom of snakes such as *Naja siamensis* and *Bungarus multicinctus*.Therefore, both are able to compete for the same receptor or HIV binding site. Metalloproteinase inhibitors present in snake venom may prevent the production of new viruses by inhibiting protease enzymes. Protease inhibitors commonly block the protease enzyme and prevent the cell from producing new viral particles (Silva *et al*., 2007)

2.9 Snake Envenomation in Animals

Fatal snake bites are more common in dogs than in other domestic animals. Because of the relatively small size of some dogs in proportion to the amount of venom injected, the bite of even a small snake may be fatal. In dogs and cats, mortality is generally higher in bites to the thorax or abdomen than bites to the head or extremities. Because of their larger sizes, horses and cattle seldom die as a direct result of snake bite, but deaths may follow bites on the muzzle, head, or neck when dyspnea results from excessive swelling. Serious secondary damage sometimes occurs; livestock bitten near the coronary band may slough a hoof (Sharon, 2006).

Typical viperids bites are characterized by severe local tissue damage that spreads from the bite site. The tissue becomes markedly discoloured within a few minutes, and dark, bloody fluid may ooze from the fang wounds if not prevented by swelling. The epidermis may

slough when the overlying hair is clipped or parted. Hair may hide the typical fang marks. Sometimes, only one fang mark or multiple punctures are present. Neurologic signs, including muscle fasciculations, are possible if neurotoxic elapids venom is involved. In elapid snake bites, pain and swelling are minimal, and systemic neurologic signs predominate. Signs of coral snake envenomation include tetraparesis, ptyalism, tachypnea, shallow/abdominal breathing, depressed gag reflex, ataxia, muscle fasciculation, decreased spinal reflexes, and quiet mentation. Australian elapid bite victims may show collapse, vomiting, ptyalism, tremors, tachypnea, urinary and/or fecal incontinence, tetraparesis, hemolysis, coagulopathy, rhabdomyolysis, swelling at the bite site, renal failure, and/or delayed immune-mediated hemolytic anemia (red-bellied black snake) (Sharon, 2006).

2.10 Median Lethal Dose (Ld50)

In 1927, J.W. Trevan attempted to find a way to estimate the relative poisoning potency of drugs and medicines used at that time. He developed the LD_{50} test because the use of death as a "target" allows for comparisons between chemicals that poison the body in very different ways. Since Trevan's early work, other scientists have developed different approaches for more direct, faster methods of obtaining the LD_{50} (Filippi and Petretto, 2013).

LD stands for "Lethal Dose". LD_{50} is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals. The LD_{50} is one way to measure the short-term poisoning potential (acute toxicity) of a material. This type of test is also referred to as a "quantal" test because it is measures an effect that "occurs" or "does not occur".The chemical may be given to the animals by mouth (oral); by applying on the

skin (dermal); by injection at sites such as the blood veins (i.v.- intravenous), muscles (i.m. - intramuscular) or into the abdominal cavity (i.p. - intraperitoneal) (Filippi and Petretto, 2013).

Toxicologists can use many kinds of animals but most often testing is done with rats and mice. It is usually expressed as the amount of chemical administered (e.g., milligrams) per 100 grams (for smaller animals) or per kilogram (for bigger test subjects) of the body weight of the test animal. The LD_{50} can be found for any route of entry or administration but dermal (applied to the skin) and oral (given by mouth) administration methods are the most common.

The LD_{50} value obtained at the end of the experiment is identified as the LD_{50} (oral), LD_{50} (skin), LD_{50} (i.v.), etc., as appropriate. Researchers can do the test with any animal species but they use rats or mice most often. Other species include dogs, hamsters, cats, guinea-pigs, rabbits, and monkeys. In each case, the LD_{50} value is expressed as the weight of chemical administered per kilogram body weight of the animal and it states the test animal used and route of exposure or administration; e.g., LD_{50} (oral, rat) - 5 mg/kg, LD_{50} (skin, rabbit) - 5 g/kg. So, the example "LD_{50} (oral, rat) 5 mg/kg" means that 5 milligrams of that chemical for every 1 kilogram body weight of the rat, when administered in one dose by mouth, causes the death of 50% of the test group (Filippi and Petretto, 2013).

If the lethal effects from breathing a compound are to be tested, the chemical (usually a gas or vapour) is first mixed in a known concentration in a special air chamber where the test animals will be placed. This concentration is usually quoted as parts per million (ppm) or milligrams per cubic metre (mg/m^3). In these experiments, the concentration that kills 50%

of the animals is called an LC_{50} (Lethal Concentration 50) rather than an LD_{50}. When an LC_{50} value is reported, it should also state the kind of test animal studied and the duration of the exposure, e.g., LC_{50} (rat) - 1000 ppm/ 4 hour or LC_{50} (mouse) - 5mg/m^3/ 2hour

This test has many downfalls,it has been called "crude and unscientific". LD_{50} figures for a single chemical can vary enormously according to the species, strain, age, gender and even diet of the animals used in the tested. In one survey, the LD_{50} figure for the same substance tested by two different laboratories on the same species, varied by three to eleven-fold. Unfortunately, there is not a more accurate or humane test. The test has to be repeated on more groups of animals and on a variety of species, so that an average LD_{50} figure can be obtained. The LD_{50} figure that is used to measure relative toxicities of various snake venoms is conducted using mice. This means the data is not particularly accurate for estimateing how lethal a snake bite to a human would be. Using the LD50 test for estimating the danger of a particular species to humans is obvoiusly flaured. The snake currently ranking number one on that list has not been the cause of death to a human in any documented case history (Filippi and Petretto, 2013).

2.11 Elapids

2.11.1 Classification

Cobras belong to the sub-group of snakes known as Elapids; there are over 270 species of cobras and their relatives. They are classified under Kingdo, animalia; phylum, chordate; class, reptilia; order, squamata; suborder, serpents; family, elapidae and genus Naja.

2.11.2 Mechanism of action

An elapid's venom contains postsynaptic neurotoxins that spread rapidly in its victim's bloodstream, causing respiratory failure and, eventually, death. Cobra venom is an example of a molecule that prohibits the interaction of acetylcholine molecules (transmitted from nerve endings surrounding the diaphragm muscle) with the receptor sites on the diaphragm muscle. It binds to the receptor sites, blocking them from interacting with acetylcholine molecules. Even worse, the venom molecule will not immediately break down and vacate the receptor site, effectively removing the site from active duty. It has been determined that even if only 75 to 80% of the receptor sites on your diaphragm become blocked by venom, you will cease breathing. With cobra venoms, this process can take as little as 30 minutes.

The only way to counteract the effects of cobra venom (or most other poisonous snake venoms) is to inject the appropriate antivenom shortly after the bite occurs. If antivenom is unavailable, your life can still be saved by putting you on an artificial respirator until the paralysis of the diaphragm muscle wears off (Broadley and Wuster, 2004; Wuster, *et al*., 2009; Trape *et al*., 2009).

2.11.3 *Naja haje*

It is one of the largest cobra species native to Africa, second to the forest cobra (*Naja melanoleuca*). *Naja haje* was first described by Swedish zoologist Carolus Linnaeus in 1758. The generic name *naja* is a Latinisation of the Sanskrit word nāgá meaning "cobra". The specific epithet *haje* is derived from the Arabic word hayya (حية) which literally means "snake". The neurotoxic venom of *Naja haje* is one of the most potent of all the cobra

venoms; bites from this species are one of the leading causes of snake bite mortality worldwide. (Broadley and Wuster, 2004; Wuster, *et al*., 2009; Trape *et al*., 2009).

2.11.3.1 Morphology

The Egyptian cobra is one of the largest cobras of the African continent. The head is large and depressed and slightly distinct from the neck. The neck of this species has long cervical ribs capable of expanding to form a hood, like all other cobras. The snout of the Egyptian cobra is moderately broad and rounded. The eyes are quite big with round pupils. The body of the Egyptian cobra is cylindrical and stout with a long tail. The length of the Egyptian cobra is largely dependent on subspecies, geographical locale, and population. It lacks the prominent dorsal “eye spot” seen in other cobras, but the ventral surface of the neck frequently supports a distinctive dark band (Broadley and Wuster, 2004; Mastenbroek, 2013).

The average length of this species is between 1 and 2.5 metres (3.3 and 6.6 ft) in length, with a maximum length of just under 3 metres (9.8 ft). The most recognizable characteristics of this species are its head and hood. The colour is highly variable, but most specimens are some shade of brown, often with lighter or darker mottling, and often a "tear-drop" mark below the eye. Some are more copper-red or grey-brown in colour. Specimens from northwestern Africa (Morocco, Western Sahara) are almost entirely black. The ventral side is mostly a creamy white, yellow brown, greyish, blue grey, dark brown or black in colouration, often with dark spots. They are thick-bodied with rather elongate slender tails. Many of the recognized subspecies have distinctive colour patterns (Broadley and Wuster, 2004)

2.11.3.2 Scalation

Dorsal scales on the midbody 19-20, ventral scales 191-220, subcaudal scale is paired, subcaudal scales 53-65, anal plate is single, upper labials 6, upper labials to the eye 3+4, preoculairs 1, postoculairs 3 (but can also be 2), suboculairs 2–3, supralabials 7 (rarely 6 or 8), lower labials 8, temporal 1+2/1+3 varying (Trape *et al*., 2009).

2.11.3.3 Behaviour

The Egyptian cobra is a terrestrial and crepuscular or nocturnal species. It can however, be seen basking in the sun at times in the early morning. This species shows a preference for a permanent home base in abandoned animal burrows, termite mounds or rock outcrops. It is an active forager sometimes entering human habitations, especially when hunting domestic fowl. Like other cobra species, it generally attempts to escape when approached, at least for a few metres, but if threatened it assumes the typical upright posture with the hood expanded and strikes. This species prefers to eat toads, but it will prey on small mammals, birds, eggs, lizards and other snakes. This may be reflective of the fact that *Naja haje* is a true feeding generalist (consuming a wide range of vertebrate prey, including other snakes) with what is generally described as a voracious appetite.

2.11.3.4 Venom of *Naja haje*

The venom of the Egyptian cobra consists mainly of neurotoxins and cytotoxins. The average venom yield is 175 to 300 mg in a single bite, and the murine subcutaneous LD_{50} value is 1.15 mg/kg (Filippi amd Petretto, 2013).

The venom affects the nervous system, stopping the nerve signals from being transmitted to the muscles and at later stages stopping those transmitted to the heart and lungs as well, causing death due to complete respiratory failure. Envenomation causes local pain, severe swelling, bruising, blistering, necrosis and variable non-specific effects which may include headache, nausea, vomiting, abdominal pain, diarrhoea, dizziness, collapse or convulsions along with possible moderate to severe flaccid paralysis, this species does not spit venom (Mastenbroek, 2013).

2.11.3.5 Distribution

This species ranges through most of Central and Northern Africa, as well as much of the Middle East. They occur in a wide variety of habitats like, steppes, dry to moist savannas, arid semi-desert regions with some water and vegetation. This species is frequently found near water. The Egyptian cobra is commonly found in surprising numbers around rural, and sometimes even urban, human habitation. they are nocturnal by nature, into close contact with humans; they frequently enter human dwellings or are tread upon outdoors at night (Broadley and Wuster, 2004).

These snakes have a rather "charmed" position in folklore and human sociology. It is often argued that the "asp" that Cleopatra reputedly used to end her life was actually an Egyptian cobra. In modern times these snakes are a mainstay of the African and Middle Eastern snake charming industry. Like other cobras, *Naja haje* has large eyes and is very responsive to visual stimuli. A flute, scarf, or earthen jug waved in front of an Egyptian cobra will almost always evoke a fairly stereotypic defensive display in which the anterior quarter of the body is held vertically and approximately the first 20 ribs are rotated dorsally. This

dorsal rotation of the ribs markedly expands the neck region of the cobra into what is commonly called the hood, while compressing the animal dorso-ventrally (as in the specimen here). With careful stimulation, cobras can be induced to hold this defensive posture (including sham strikes) for hours.

The snake charmer's ability to handle these visually stunning animals for the entertainment of paying tourists is a significant economic aspect of many rural villages. Increasingly popular among snake keepers in the West, the Egyptian cobra is now frequently bred in captivity and readily available commercially (Filippi and Petretto, 2013).

2.11.4 *Naja katiensis*

2.11.4.1 Distribution

This species ranges from Senegal to Cameroon, with records from the Gambia, Guinea-Bissau, far northern Guinea, southern Mali, Côte d'Ivoire, northern Ghana and Togo, southwest Niger and Nigeria, Burkina Faso; Cameroon; Côte d'Ivoire; Gambia; Mali; Senegal. Records from further north in Niger (Zinder), and from Cameroon south and east of the snake's known range (Wuster and Broadley, 2007). The possibility exists that this specimen represents a record from a market, as many live snakes displayed in markets in Zinder are collected in northern Nigeria. Three specimens are also known from the Garoua region of Cameroon close to the Nigerian border. It is possible that the species ranges into southern Chad and the Central Affrican Republic (Hoser, 2009)

2.11.5 *Naja nigricollis*- Black-necked spitting

They are moderately sized snakes that can grow to a length of 1.2 to 2.2 m (3.9 to 7.2 ft) in length. Their coloration and markings can vary considerably. They prey primarily on small rodents. They possess medically significant venom, although the mortality rate for untreated bites on humans is relatively low (~ 5–10%, in endemic regions under 1%). Like other spitting cobras, they can eject venom from their fangs when threatened (one drop over 7 metres (23 ft). The neurotoxic venom irritates the skin, causing blisters and inflammation, and can cause permanent blindness if the venom makes contact with the eyes and is not washed off (Wuster and Broadley, 2007).

Intracranial (dorsal) anterior Vidian canal position, 2–3 solid maxillary teeth, six supralabials (except some *N. nubiae* and *N. pallida*) with penultimate (fifth or sixth) shield low, combination of two preoculars and two or three anterior temporals, rostral as broad as deep; internasals as long as prefrontals, dorsal scales matte or moderately shiny and fangs adapted for spitting.

2.11.5.1 Distribution of Naja nigricollis

The black-necked spitting cobra is found mostly in sub-Saharan Africa. Angola, Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Democratic Republic of the Congo (Zaire, except in the center), Congo, Ethiopia, Gabon, Ghana, Guinea Bissau, Guinea, Ivory Coast, Kenya, Liberia, Mali, Niger, Nigeria, Senegal, Sierra Leone, Gambia, Mauritania, Sudan, Somalia, Togo, Uganda, Zambia, Senegal east to Kenya and south to Angola, Zambia, and Tanzania (Chirio and Ivan, 2006; Wüster *et al*., 2007).

2.12 Viparids

2.12.1 Classification

Vipers belong to the sub-group of snakes known as Viperids; they are classified under Kingdom: animalia; phylum: chordate; class: reptilia; order: squamata; suborder, serpents; family, viperidae.

2.12.2 Mechanism of action of the venom

Biochemically and pharmacologically, myotoxins isolated from snake venoms can be placed in four main groups: myotoxic phospholipases A, low molecular weight basic toxins, cardiotoxins, and hemorrhagic myotoxins. The myotoxic phospholipases A notexin, taipoxin, crotoxin, and *Bothrops asper* myotoxin induce muscle necrosis by first affecting the integrity of the plasma membrane, thereby inducing a calcium influx that culminates in cell death (Widgerow *et al*.,1994). The small basic myotoxin crotamine acts on the voltage-sensitive sodium channels of skeletal muscle sarcolemma, inducing a sodium influx which is responsible for depolarization and contraction of skeletal muscle, as well as for vacuolization of sarcoplasmic reticulum. Cardiotoxins are basic membrane-active polypeptides that disorganize the structure of membranes; the myotoxic activity of cardiotoxins results from their ability to disrupt skeletal muscle sarcolemma. Finally, two haemorrhagic toxins (haemorrhagic toxin b and viridi toxin) are myotoxic; apparently, they secondarily to ischemia which develops in muscular tissue as a consequence of the hemorrhagic action of these toxins. Puff adder envenomation causes tissue necrosis, hypotension, coagulopathy, thrombocytopenia, and spontaneous bleeding. Severe coagulopathy may occur (Branch, 2004) .

2.13 *Bitis arietans* arietans " = "striking violently" and is derived from the Latin *arieto* African puff Adder.

2.13.1 Distribution

The puff adder *Bitis arietans* is a large, and probably the most common and widespread snake in Africa (McDiarmid, 1999).This species is responsible for more snake bite fatalities than any other African snake. It is found in most of sub-Saharan Africa south to the Cape of Good Hope, including: Southern Morrocco, Senegal, Mauritania, Mali, Southern Algeria, Guinea, Sierra Leone, Coted'Ivoire, Ghana, Togo, Niger, Nigeria, Chad, Sudan, Cameroon, Csntral African Republic, Northern, Eastern and Southern Democratic Republic of the Congo, Uganda, Kenya, Somalia, Rwanda, Burundi, Tanzania, Angola, Zambia, Malawi, Mozambique, Zimbabwe, Botswana, Namibia and South Africa (McDiarmid *et al*., 1999). It also occurs on the Arabian peninsula, where it is found in southwestern Saudi Arabia and Yemen (Broadley, 1990; Broadley and Cock, 1975). It is relatively dull coloration, consisting of a pattern of dark chevrons on a lighter tan or brown background, camouflages the snake very effectively. This species is a primarily nocturnal ambush predator, preying on small mammals, birds, lizards, frogs, and toads, and while it spends most of its time on the ground, it can also swim or climb into low vegetation. It is generally slow-moving, but it can strike extremely quickly (Mallow *et al*., 2003).

Due to its wide distribution, potent venom, and highly cryptic coloration which makes it prone to being stepped on inadvertently, *Bitis arietans* is thought to kill more people than any other African snake (Mallow *et al*., 2003), accounting for nearly 32,000 deaths per year and many more disabilities (Branch, 2004; Swaroop and Grab, 1954). Its venom is highly toxic, capable of causing massive tissue necrosis, hypotension, coagulopathy,

thrombocytopenia, and spontaneous bleeding, apparently adapted to immobilize prey and begin the digestive process. However, unlike in some of its close relatives, the venom of *B. arietans* acts relatively slowly, and with proper treatment, death can be prevented in 90-95% of cases (Spawls and Branch, 1995; Mallow *et al*., 2003).

2.13.2 Morphology

This species usually reaches a maximum length of approximately 1 m, but the largest individuals may be nearly twice that long. The head has two well-marked dark bands: one on the crown and the other between the eyes. On the sides of the head, there are two oblique dark bands or bars that run from the eye to the supralabials. Below, the head is yellowish white with scattered dark blotches. Iris color ranges from gold to silver-gray. Dorsally, the ground-color varies from straw yellow, to light brown, to orange or reddish brown. The belly is yellow or white, with a few scattered dark spots. They are good swimmers and can also climb with ease; often they are found basking in low bushes.If disturbed, they will hiss loudly and continuously, adopting a tightly coiled defensive posture with the fore part of their body held in a taut "S" shape (Branch, 2004).

At the same time, they may attempt to back away from the threat towards cover. They may strike suddenly and fast, to the side as easily as forwards, before returning quickly to the defensive position, ready to strike again. During a strike, the force of the impact is so strong, and the long fangs penetrate so deeply, that prey items are often killed by the physical trauma alone. The fangs apparently can penetrate soft leather.

2.13.3 Venom of puff adder

The venom yield in a single bite ranges between 150 and 350 mg, with a maximum recorded of 750 mg. But to kill an adult human it only requires about 100 mg, and death may with occur within a day. A puff adder bite causes immediate severe pain, swelling, blistering, nausea, vomiting and later on necrosis with massive muscle and tissue damage. If not properly treated and the venom spreads it will at some point result in gangrene and secondary infections often times resulting in loss of digits and limbs.
This is due to a combination of factors, including its wide distribution, common occurrence, large size, potent venom that is produced in large amounts, long fangs, their habit of basking by footpaths and sitting quietly when approached.

The venom has cytotoxic effects and is one of the most toxic of any vipers based on LD_{50}. The LD_{50} values in mice vary: 0.4–2.0 mg/ kg IV, 0.9–3.7 mg/ kg IP, 4.4–7.7 mg/ kg SC. (Brown, 1973; Mallow *et al*., 2003) give an LD_{50} range of 1.0–7.75 mg/ kg SC. Venom yield is typically between 150–350 mg, with a maximum of 750 mg. (Brown, 1973), mentions a venom yield of 180–750 mg. About 100 mg is thought to be enough to kill a healthy adult human male, with death occurring after 25 hours.

In humans, bites from this species can produce severe local and systemic symptoms. Based on the degree and type of local effect, bites can be divided into two symptomatic categories: those with little or no surface extravasation, and those with hemorrhages evident as ecchymosis, bleeding and swelling. In both cases there is severe pain and tenderness, but in the latter there is widespread superficial or deep necrosis and compartment syndrome. Serious bites cause limbs to become immovably flexed as a result of

significant hemorrhage or coagulation in the affected muscles. Residual induration, however, is rare and usually these areas completely resolve (Mallow *et al*., 2003).

Other bite symptoms that may occur in humans include edema, which may become extensive, shock, watery blood oozing from the puncture wounds, nausea and vomiting, subcutaneous bruising, blood blisters that may form rapidly, and a painful swelling of the regional lymph nodes. Swelling usually decreases after a few days, except for the area immediately around the bite site. Hypotension, together with weakness, dizziness and periods of semi- or unconsciousness is also reported (Brown, 1973).

If not treated carefully, necrosis will spread, causing skin, subcutaneous tissue and muscle to separate from healthy tissue and eventually slough with serous exudate. The slough may be superficial or deep, sometimes down to the bone. Gangrene and secondary infections commonly occurs and can result in loss of digits and limbs.

The fatality rate highly depends on the severity of the bites and some other factors. Deaths can be exceptional and probably occur in less than 15% of all untreated cases (usually in 2–4 days from complications following blood volume deficit and a disseminated intravascular coagulopathy), although some reports show that severe envenomations have a 52% mortality rate. Most fatalities are associated with poor clinical management and neglect (Spawls *et al*., 2004).

2.14 *Echis oscellstus*- West African carpet viperocellated carpet viper

The specific name, *ocellatus*, is a reference to the distinctive series of "eye-spots" (Brown, 1973). (ocelli) which runs the length of the body (Brown, 1973; Cherlin, 1990).

2.14.1 Morphology

Small in length, cylindrical, robust bodied snake with very short tail. Can grow to a maximum total length (body + tail) is 65 cm (26 in), possibly more, while the average total length is 30–50 cm (12–20 in) (Spawls and Branch, 1995). Can grow to a maximum length of about 0.65 metres. Head is broad, flat, short snouted, pear shaped when viewed from above and distinct from neck. Canthus is indistinct. Eyes are medium in size, prominent, set near the front of the head, with vertically elliptical pupils. Head scales are keeled. Dorsal scales are dull, rough, heavily keeled, overlapping with apical pits and scales markedly serrated on lower lateral scale rows.

2.14.2 Distribution

It is found in West Africa from Mauritania, Senegal and Guinea, through, Mali, Ivory Coast, Burkina Faso, Ghana, Togo, Benin, Southern Niger, and Nigeria. It is also found in Northern Cameroon and South-western Chad (Mallow *et al*., 2003).

The type locality is described as "Haute Volta, Garango, 048 N, 033 W" (Burkina Faso) (McDiarmid *et al*., 1999a). There are also reports of single specimens found in the Bangui in the Central African Republic, and in central Sudan. It is rarely found north of the 15th parallel, after which *E. leucogaster* becomes more common. The geographic range of *E. ocellatus* extends to the coast via the Dahomey Gap (Spawls and Branch, 1995).

2.14.3 Habitat

Mainly Savannah but unlike other species in the genus is often foumd in well wooden terrain and has been found on primary rainforest fringes. Terrestrial, nocturnal and

crepuscular although sometimes found in low bushes to avoid the heat. Most active during the early hours of the evening. If disturbed it assumes an S-shaped coil position and rubs the sides of the body together making a rasping sound. Very nervous, irritable and aggressive disposition, quick to strike (and repeatedly) at the slightest provocation and does not try to escape.

2.14.4 Prey

Feeds on a wide variety of prey, including invertebrates such as scorpions and centipedes, small mammals, birds, lizards, amphibians and other snakes. Feeds on a wide variety of prey, including invertebrates such as scorpions and centipedes, small mammals, birds, lizards, amphibians and other snakes.

2.14.5 Reproduction

Sexually mature females lay between 6 and 20 eggs, usually at the end of the dry season in February to March. Hatchlings are 10–12 cm (3.9–4.7 in) in total length.

2.15 Plants Used as Anti-Venoms

2.15.1 *Olax manni*

The plant, *Olax manni* belongs to the family *Olacaceae.*It is a climbing shrub up to 2 metres height, leaves lanceolate to ovale or ecliptic up to 6 x 3 inches with 5-6 pairs of lateral looped nerves, flowers greenish – white in axillary racemes, fruits orange when ripe, Natural habitat closed forest. Distributed in the tropics especially, Nigeria Ghana and Sierra Leon (Dalziel, 1955).

Olax manni is used in the treatment of snake envenomation, yellow fever and treatment of guinea worm infection (Irvine, 1961).

2.15.2 *Urginea altissima*

Urginea altissima (*Drimia altissima)* (L.f.) BakerJ. Linn. Soc., Bot. 13: 221 (1873) Scientific Name: Drimia altissima (L.f.) Ker Gawl Bot. Mag. 27: t. 1074 (1808) Common Names include: English: African squill, Tall white squill. Africans: Maerman, Jeukbol, Maermanbol, Maermanui, Slangkop and Gadali – Hausa. Derivation of specific name: Latin *altissima*: very tall (Umberto, 2012).

Origin and Habitat: It is widely distributed in the tropics especially Nigeria, Sierra Leone, and Ghana. Southern Africa, from Senegal via East Africa (Eritrea, Ethiopia, Somalia and Djibouti) to the South Africa comprising Namibia, and Botswana. It is reported in Uganda (W. Nile District, Karamoja District, Teso District), Kenia, and Tanzania (Mwanza District, Mbeya District and Arusha District) (Baumann, 2005).

Itis a perennial bulbous plant producing a tall flowering stem followed by a rosette of leaves about 20-50 cm long. As the species name implies, this is one of the tallest among all African geophytes. The plants are often found in colonies and the large bulbs lie just below the surface of the soil. In this species, the flowering stem is exceptionally long, reaching 2 m and usually flowering before the leaves. The white flowers have a green streak down the middle of each petal and the subtended spurred bracts are clearly visible in young flowers. The species is variable, at least three forms occuring in W. Africa: large, medium and small sized plants (Umberto, 2012).

Bulb: Very large, globose to pear shaped and ca.8-12(-15) cm in diameter, often half above ground, with tough, white, overlapping fleshy scales and a brown outer tunic of deadscales.Leaves: Lanceolate, light green, glabrous, (20-)30-45(-50) cm long, (2-)5-7.5cmbroad. The inflorescence is a more or less dense, cylindrical, raceme 60-90 cm long, 3-4.5 cm in diameter with up to 700 flowers. Scape erect, usually tall and robust, sometimes 0.9-1,2 m long, up to 1 cm. in diameter light glaucous green. Pedicels spreading or patent, (8-)12-25(-30) mm long. Bracts small, lanceolate, bent up to 14 mm long and obscurely spurred below the middle (spur up to 3 mm long). Like *Drimia sanguinea* the inflorescences appear before the leaves (Kirby, 2013).

Flowers: Perianth campanulate, 18 mm long; segments (tepals) free or united for up to 2 mm, oblanceolate-oblong, 5–12 mm long, white or greenish white, with a a green or dull purple-brown keel outside, spreading. Stamens shorter than the perianth. Filaments linear to very narrowly triangular, free or basally united to tepals slightly flattened, free part 4-7 mm. Anthers oblong, 2 mm long; style 4 mm long. Ovary ovoid, 2–5 mm long; style about as long as ovary. Flowers opening in the morning, usuallyclosing by midday. Fruits (capsules): Three chambered, globose or subglobose, obtusely trigonous, sometimes with an emarginate apex, 8–15 mm long, 9-15 mm. in diameter.Young fruits green.Seeds: Semicircular 5–8 mm long glossy, black, flattened and winged. Seeds then ripen during the earlypart of the rainy season. Chromosome number: 2n = 20 (Kellerman and Coetzer, 2005).

Cultivation and Propagation: Although usually grown only by specialist bulb-grower *Drimia altissima* presents no great difficulty in cultivation. The ease of culture makes it an invaluable subject for the impassioned bulb-growers. It needs a slightly acid medium

comprising equal parts of well decomposed compost or finely milled bark, and river or silica sand (or pumice). Plant the bulbs with the neck at, or just below soil level and can remain in the same position for many years. Grows it in pots with a diameter of at least 25 cm. Give it excellent drainage (Kellerman and Coetzer, 2005).

Water: It is usually evergreen in cultivation but needs to be kept almost dry during its dormancy as the bulbs is disposed to dwindle and rot. Its winter rest must be absolute. Exposure: It is most suitably grown in semi-shaded but it will even grow and bloom in full sun.

Hardiness: They are sensitive to frost, but are said to be hardy -5° C (or even less). Garden uses: In warm and temperate climates grow it sunny courtyard gardens, in raised beds and rockeries. In countries with cold winter climates, they are best grown in containers in a cool or slightly heated greenhouse. The plants are mostly grown by specialist bulb collectors, usually as container subjects.

Pest and diseases: The bulbs and leaf bases are susceptible to attack by mealy bugs, and the leaf margins are chewed by snout beetles and slugs at night (Kellerman and Coetzer, 2005).

Traditional medicine: The drug, known in commerce as the white squill, consists of the dried sliced bulbs of *Drimia maritima* from which the membraneous outer scales have been removed. Squill has a long history of use in medicine: The earlier Greek and Egyptian physicians used various forms of squill as therapeutic agents. An oxymel of squills was part of an Arabic prescription, and a vinegar of squill was used by Dioscorides, the Greek physician. Squill occurs in two varieties, the white and the red squill. The white variety is employed in medicine, and the red squill is used mainly for the preparation of rat poison.

The African squills *Drimia indica* (Roxb.) Jessopand *Drimia altisssima* are used in folk medicine in Nigeria but are not official pharmaceutical products or articles of international trade. They have with a bitter and acrid taste and may have a Digitalis-like action (cardiotonic properties). The drug is very hygroscopic, and if powdered drug is not well stored, it turns into a solid moldy mass (Kirby, 2013). Propagation: By seed or rarely by division of bulbs. Offsets are rather slow to form (if ever), and are best separated from the mother bulb straight after flowering, just as the new leaves begin to develop. Seeds form readily and should be harvested and sown as soon as they can be easily removed from the fleshy berries. Seeds may take up to two months before the first leaf appears above ground, and a further four to five years to flower for the first time (Maurice, 2014).

2.15.3 *Xeromphis nilotica* (Stapf) Kea.y

2.15.3.1 Classification

Kingdom (Plantae); Phylum (Tracheophyta); Class (Magnoliopsida) ; Order (*Gentianales*); Family (*Rubiaceae*); Genus (*Xeromphis*). A stiff, spiny shrub, branches grey, to whitish, spines, woody up to 25 mm long, solitary and alternate above paired, leafy-short shoot, leafy-blades obovale, 10-60 x 5-20 mm, obtuse at the apex, globrous to pubescent, flowers (4) 5-6 merous, calyx lobes up to 3 mm long, corolla hairy, the tube somewhat longer than the calyx, fruits yellowish and subglobose 8-18 mm in diameter globous, often many-stemmed, with whitish bark; corolla white (Keay, 1958).

2.15.3.2 Distribution

It is found in arid types of savanna woodland, Sudan, Ethiopia and East Afica, Tanzania, Eritrea, and westwards to Nigeria and Cameroon. It is used in ethno-medicine in the treatment of snake envenomation and treatment of mental disorders (Keay, 1958).

2.15.4 Other plants

Other plants constituents have been identified to possess a neutralizing effect on snake venoms. Management of snake bites through herbal preparation is done by treating with single herbal drug or in a combination of different herbs (Abubakar *et al*., 2000; Borges *et al*., 2000; Daros *et al*., 1996; Fukuda *et al*., 2002).Recent scientific investigations have confirmed the efficacy of many of these preparations, some of which are remarkably effective (Gupta and Peshin, 2012). Traditional Indian medicinal plants such as *Excoecaria agallocha, Gloriosa superba, Nerium oleander, Sarsaparilla hemidesmus* and many others are effectively used for snake bites, however; both pre-clinical studies are required to establish the efficacy of the plants. The herbs which appear most effective as per the symptoms of snake bites arerelatively non-toxic and have substantial documented efficacy (Abubakar *et al*., 2000; Abubakar *et al*., 2008; Girish and Kemparaju, 2005; Oliveira *et al*., 2005; Soares *et al*., 2005).

(Owuor and Kisangau, 2006) in Kenya reported a comparison of plants used in snake bite treatments by two culturally distinct African groups. They observed that snake bites in rural areas are commonly treated with plant extracts (Otero *et al*., 2000); Ushanandini *et al*., 2006). Most commonly used plants include: *Steganotaenia araliacea, Combretum collinum, Solanum incanum* and 3 species of *Grewia* (*G. bicolor, G. fallax* and *G. truncata*)

reported in the Medicinal Plants of East Africa (Abubakar *et al*., 2000; Kokwaro, 1993; Habib, 2013).

In addition, species in the genera *Vernonia, Erythrina* (*E. abyssinica*) and *Sansevieria* (*S. kirkii*) were recorded. There are similar usage reports for *Annona* (leaf and bark) and among the Chewa ethnic group of Malawi, *Ensete edule* (sap from stem) used as a toothache pain reliever – alluding to pain killing properties; arterenol, a natural mediator of the autonomic nervous system, has been reported in *E. edule* (Watt and Breyer-Brandwijk, 1962).

Usage of *Allium* spp., in South America, as antivenom has been reported. *Allium cepa* contain sulfurous, volatile oils. Although, in general, the plant families *Compositae, Leguminosae* and *Solanaceae* and *Apocynaceae* are well represented in East African compendia (Kokwaro, 1993), it appears plant families or genera consistently used in snake bite treatment are difficult to establish. The plant family most used is the *Asteraceae* followed by *Annonaceae, Fabaceae, Combretaceae* and *Tiliaceae* (Owuor and Kisangau, 2006).

In Nigeria, (Abubakar *et al*., 2000), reported that the extract of the leaves of *Guiera senegalensis* was found to detoxify (*in vitro*) venom from two common northern Nigerian snake species, *Echis ocellatus* and *Naja nigricollis*, in separate experiments. They observed that there was a remarkable reduction in the mortality of albino mice after intra-peritoneal administration of reconstituted venom incubated with the extract, when compared to those challenged with the venom only.

Luffa aegyptiaca and *Nicotiana rustica* used in traditional medicine to treat snake bites exhibited inhibitory activities against *Naja nigricollis* venom protease (Ibrahim *et al*., 2011). The potency of the methanol extract of the root bark of *Annona senegalensis* was tested against cobra (*Naja nigricollis nigricollis* Wetch) venom in rats. Results indicated that the extract caused reduction in the induced hyperthermia and directly detoxified the snake venom used by 16–33%. It, however, failed to restore the biochemical functions of serum alanine amino transferase (ALT) and aspartate amino transferase (AST) of the liver. The extract exhibited a medium lethal dose (LD_{50}) of 232.7g/ml in the brine shrimp test (Adzu *et al*., 2005).

The acetone and methanolic stem bark extracts of *Balanites aegyptiaca* exhibited an antivenin activity against *Echis ocellatus* viper venom at LD_{50} (0.194 mg/ml), when administered intramuscularly to Wistar albino rats. Both extracts were found to be effective at 75 and 100 mg/ml concentrations. *Tamarindus indica* seed extract inhibited the PLA_2, protease, hyaluronidase, L-amino acid oxidase and 5'-nucleotidase enzyme activities of venom in a dose-dependent manner. The extract neutralized the degradation of the beta chain of human fibrinogen and indirect hemolysis caused by venom.

Both *Indigofera pulchra* and *Aristolochia albida* were found to neutralize the anticoagulant, hemolytic and phospholipase activity of crude venom of *Naja nigricollis* (Abubakar *et al*., 2006). Aristolochic acid has been found to form a complex with PLA2, acting as a non-competitive inhibitor of the enzyme (Viswanath *et al*., 1987; Ménez, 2003; Warrell, 2008).

The water and methanol extract of *Parkia biglobosa* stem bark significantly protected the chick biventer cervicis muscle preparation from *Naja nigricollis* venom-induced inhibition of neurally evoked twitches when it was added to the bath 3-5 minutes before or after envenomation. In astudy conducted with nineteen plant species belonging to 15 plant families used remedies for the treatment of snake bites by the Fulani herdsmen. Members of the *Asteraceae, Liliaceae, Malvaceae* and *Mimosaceae* families were used commonly while *Annona senegalensis* was the most frequent plant species used. *Annona senegalensis* was followed by *Acatia senegalis, Aspilia africana, Hibiscus scabdariffa* and *Securidaca longepedunculata;* extract of callus culture of *Sapindus saponaria* (*Sapindaceae*) (Jorge and Aird, 2001).

Seed extracts investigated for anti snake venom activity include: *Tamarindus indica* (*Leguminosae*) (Ushanandini *et al*., 2006; *Strychnus nux vomica* (Chatterjee *et al.*, 2004); *Mucuna pruriens* (*Fabaceae*) (Tan *et al*., 2009). Other part of the plant investigated for anti snake properties include: Fruits extract of (*Piper longum*) (*Piperaceae*) (Shenoy *et al*., 2013); Extract of aerial part of *Poujolzia indica* (*Utricaceae*) (Ahmad., 2010); (Ferreira *et al.*, 1992); Methanolic extract of *Argusia argentea* (*Boraginaceae*) (Aung *et al*., 2010); Butanolic extract of *Eclipta prostrate* (*Asteraceae*) (Pithayanukul *et al*., 2004); Methanolic extract of *Vitex nigundo and Emblica officinalis* (Alam and Gomes, 2003).

The stem bark extract of *Xeromphis nilotica* was found to have anti snake venom activity against the venoms of *Naja nigricollis, Naja* haje, *Naja katiences, Bitis arietans* and *Echis ocellatus* as investigated by (Ushanandini *et al*., 2009) on the stem bark extracts of *Anacardium occidentale* (*A*nacardiaceae); (Dhananjaya *et al*., 2011).

on the stem bark extract of *Parkia bigblosa* (Mimosaceae); (Asuzu and Harvey, 2003) on the stem bark of *Mangifera indica* (Anacardiaceae); (Wufen *et al*., 2007) on the stem bark of *Balanites aegyptiaca* (Balanitaceae); (Banwo *et al*., 2004), on the stem bark of *Dipteryx alata* (*Fabaceae*) and (Asuzu and Harvey, 2003) on the stem bark of *Parkia biglandulosa* (Mimosaceae).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Selection and Collection

There are many plants known to be used by herbalists in the treatment of snake bites. Three of such plants were selected based on the claim by herbalists on the use of these plants against poisonous snake bite. The plant samples were collected and identified at the Herbarium of the Department of Botany, Faculty of Life Science, Ahmadu Bello University, Zaria.

3.1.1 *Olax manni* (Plant A: Tsadar Biri- Hausa)

Olax manni (Plate I) was collected from Anchau in Kubau Local Government Area of Kaduna State. The leaves were freshly picked from the plant; shade dried and thereafter pounded to powder form for extraction.

3.1.2 *Urginea altissima* (Plant B: Gadali- Hausa)

Urginea altissima (Plate II) was collected from Ginsawa in Tofa Local Government Area of Kano State. The bulbs were removed freshly from the plant and cut to smaller sizes; shade-dried and pounded to powder form ready for extraction.

3.1.3 *Xeromphis nilotica* (Plant C: Kwanarya- Hausa)

Xeromphis nilotica (Plate III) was collected from Pala in Ikara Local Government Area of Kaduna State. The bark of the plant was scraped; shed-dried and pounded to powder form ready for extraction.

Plate I: **Picture of *Olax manni* (Tsadar Biri- Hausa), Source: Anchau in Kubau Local Government, Kaduna State**

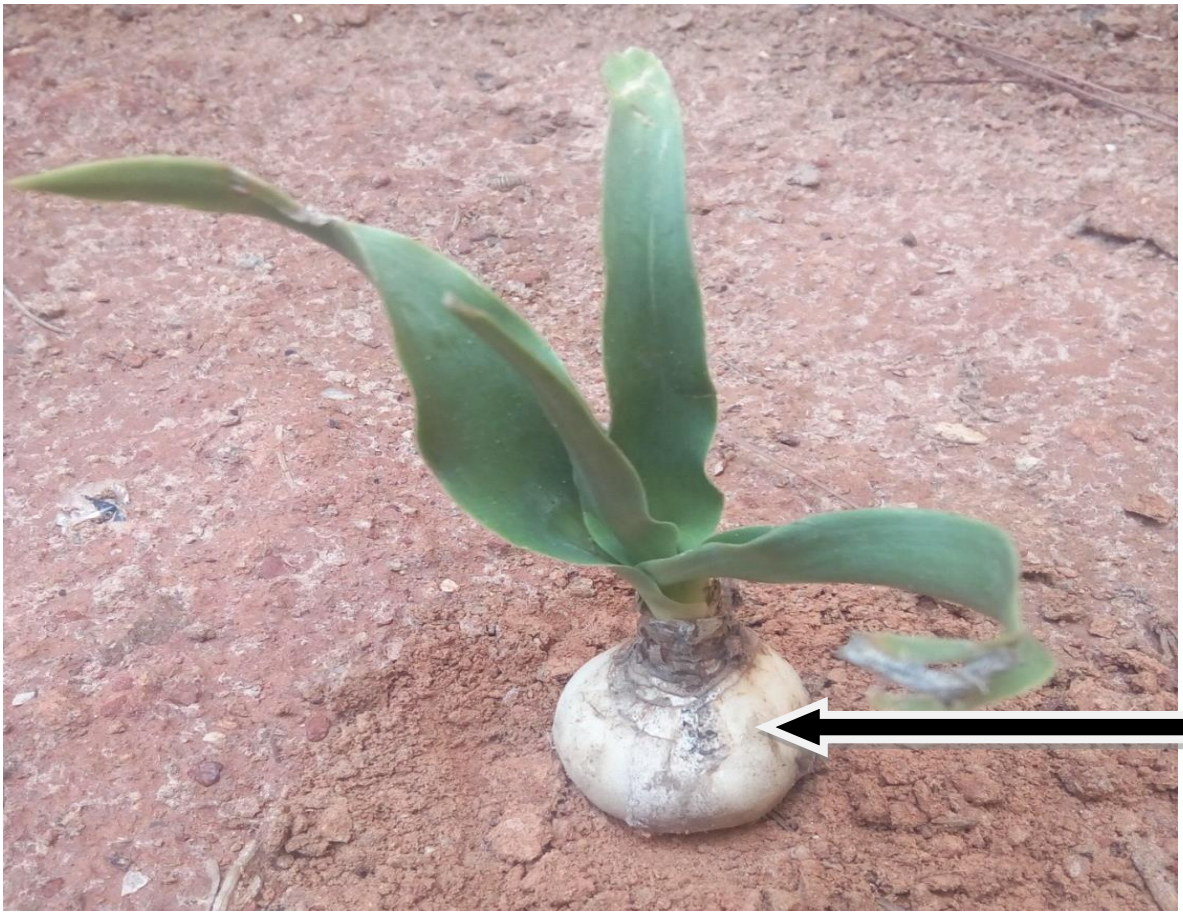


Plate II: **Picture of *Urginea altissima* (Gadali - Hausa) Source: Ginsawa in Tofa Local Government, Kano State**

Plate III: **Picture of Xeromphis *nilotica* (Kwanarya - Hausa) Source: Pala in Ikara Local Government, Kaduna State**

3.2 plant sample Extraction for phytochemical screening

The leaves of *Olax manni*, the bulbs of *Urginea altissma* and the stem bark (Sofowara, 1982) of *Xeromphis nilotica* were collected, identified, labeled, shade-dried and thereafter ground to powdered form. Five hundred grammes (500 g) of the powdered form of each plant part was weighed. Each powder was extracted by cold maceration method. The crude extracts obtained from the three species of plants were subjected to phytochemical screening using procedures described by (Sofowara, 1982).

3.3 Phytochemical Screening of the Plant Extracts

The crude extract was partitioned with di-ethyl-ether; ethyl-acetate and n-butanol. The residue left was dissolved in water and the various fractions obtained were all subjected to phytochemical screening employing the standard screening test (Sofowara, 1982).

3.3.1 Tests for carbohydrate

3.3.1.1 *Molish Test:*

One gram of the extract was put into a test tube and 3 drops of Molish reagent was added and concentrated sulphuric acid was thereafter added down the side of the test tube, to form a lower layer, a reddish coloured ring developed at the interphase indicating presence of carbohydrates.

3.3.1.2 Fehling's Test:

One gramme (1 g) of the extract was put into a test tube; 5 mililitre of an equal mixture of Fehling's solution A and B was added and boiled in a water bath for 10 minutes, brick red precipitate indicates a positive result.

3.3.2 Tests for glycosides

One gram of each extract was put in a test tube and 5 mlilitre of dilute sulphuric acid was added and boiled in a water bath for 15 minutes, thereafter cooled and neutralized with 20% Potassium hydroxide (KOH). It was then divided into two portions.

To one portion, 5 ml of a mixture of Fehling's solution A and B was added and boiled on water bath, until a brick red precipitate developed in all the samples. To the second portion 3 mililitre of ferric chloride solution was added. There was no colour change in all the samples indicating presence of glycosides

3.3.3 Bontrager'stest

This is the test for free Anthracene derivatives. One gram of the extract was put in dry test tubes, 5 mililitre of chloroform was added to each tube and then shaken for 5 minutes. They were filtered and the filtrates shaken with 10% ammonia solution. bright pink colour in the aqueous layer indicates a positive result.

3.3.4 Modified Bontrager's test

This is the test for combined Anthracene derivatives. One gram of the extracts each in a test tube was boiled with 5 mililitre of 10% hydrochloric acid (HCl) for 3 minutes; this

hydrolysed the glycosides to a glycone, which is soluble in hot water. This was then filtered and the filtrate was cooled and extracted with 5 mililitre of chloroform. The chloroform layer was pipetted off and shaken gently in a test tube with half of its volume of 10% ammonium hydroxide (NH_4OH). The lower layer became rose pink to cherry red in sample B only, indicating only B contained combined anthracene derivatives.

3.3.5 Liebermann-bucchard test

This is the test for detetion of unsaturated steroid and triterpenes. Five mililitre of acetic acid anhydride was added to 5 mililitre of the extract in a test-tube and was gently mixed. 1 mililitreof sulphuric acid was added down the side of the test tube to form a lower layer. Colour change was observed immediately and over a period of one hour from blue to blue green colour in the upper layer and pink colour in the lower layer. Indicating presence of triterpens.

3.3.6 Salkowski test for unsaturated steroid

Five drops of concentrated sulphuric acid was added to the side of the test tube containing 5 mililitreof the extract. There was red colour development over 1-hour period in the interphase of the extract and the sulphuric acid.

3.3.7 Tests for cardiac glycoside

3.3.7.1 Keller-kiliani:

A portion of the extract was dissolved in 1 mililitre glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1 mililitre of concentrated sulphuric acid was added down the side of the test tube to form a lower layer

at the bottom. There was formation of purple-brown ring at the interphase, which indicated the presence of deoxy sugars and a pale green colour in the upper acetic layer indicating the presence of cardiac glycosides.

3.3.7.2 Kedde's test:

To 1 g of the extracts, 1 ml of 2% solution of 3, 5-dinitrobenzoic acid in 95% alcohol was added. The solution was made alkaline (PH 9) with 5% sodium hydroxide; there was appearance of purple-blue colour, which indicates presence of cardiac glycoside.

3.3.8 Tests for Saponin glycosides

3.3.8.1 Frothin test:

Ten mililiters of distilled water was added to 1 g of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and observed for formation of froth that persists for a period of 5 minutes, indicating the presence of saponins.

*3.3.8.2 Haemolysis test***:**

The extract was dissolved in water and filtered through muslin filter; 2 mililitre of 18% aqueous sodium chloride (NaCl) solution was separated in to two test tubes; to one portion in a test tube, 2 ml of the filtrate was added and to the other portion, 2 mililitre of distilled water was added. With the aid of a syringe, 5 drops of sheep blood collected in EDTA bottles was added to each of the tube and then mixed by inverting the tube. The test tube was observed for 15 minutes for haemolysis in the tube containing the extract and absence

of haemolysis in the tube containing the distilled water. This showed the presence of saponins in the tube containing the blood.

3.3.9 Tests for tannins

3.3.9.1 Ferric chloride test:

To 1 gram of the extract, 5 drops of ferric chloride solution was added. A greenish-black precipitate indicates presence of tannins.

3.3.9.2 Bromine water test:

Five **(**5) drops of bromine water were added to the extracts in the test tubes. Presence of a buff coloured precipitation indicates presence of tannins.

*3.3.9.3 Lead sub-acetate test***:**

To 1gram of the extract in each of the test tube, 3 drops of lead sub-acetate solution were added. A black coloured precipitate indicates presence of tannins.

3.3.10 Flavonoid tests

3.3.10.1 Shinodatest:

One gram of the extract was dissolved in 2 ml of 50% methanol, thereafter, 2 mililitre metallic magnesium chips and 5 drops of concentrated hydrochloric acid (HCl) were added. There was development of red colouration in all the test tubes indicating presence of flavonoids.

3.3.10.2 Sodium hydroxide (NaOH) test:

Five (5) drops of NaOH were added to each of the test tube containing the extract. There was development of yellow colouration, indicating the presence of flavonoids.

3.3.10.3 Ferric chloride test:

Five **(**5) drops of ferric chloride were added to 5 mililitre of the extract in each of the test tube. Green precipitate was observed in all the test tubes, indicating the presence of flavonoids.

3.3.11 Tests for Alkaloids

3.3.11.1 Mayer's test:

Five milliliters of Meyer's reagent were added to 1 gram of the extract in all the test tubes. A cream precipitation developed in each of the test tubes which showed the presence of alkaloids.

3.3.11.2 Dragendorff's test:

Five (5) drops of this reagent were added to each of the tubes containing 1 gram of the extracts. Rose precipitate was observed in all the tubes indicating the presence of alkaloids.

3.3.11.3 Wagner's test:

Five (5) drops of this reagent were added to 1g of the extract in test tubes. A brown colour precipitate was formed indicating the presence of alkaloids.

3.4 Crude Extracts

The crude extract from each plant was fractionated using the following solvent: Di-ethyl-ether, Ethyl-acetate and n-butanol. The extracts were dissolved in water, boiled and filtered. The first solvent (di-ethyl-ether) was added and then filtered, which yielded the first fraction labeled A_1, B_1 and C_1 for each plant respectively and an aqueous portion

To the aqueous portion, ethyl acetate was added and mixed thoroughly; it was thereafter filtered and gave the second portion of the three plant fractions labeled A_2, B_2 and C_2 respectively and an aqueous portion; To the aqueous portion, n-butanol was added and then mixed thoroughly with care and then filtered and obtained the third fraction of the three plant extracts labeled A_3 B_3 C_3 respectively and an aqueous phase was collected and labeled A_4, B_4 and C_4.The fractions collected were poured into labeled evaporating dishes and the dishes were placed in hot water bath (160 ^{o}C) to facilitate drying. The dishes were then removed after the fractions had completely solidified. Each of the fractions was scraped into labeled sample beakers. The fractions were well covered with aluminum foil and kept ready for use.

3.5 Venomous Snake Captured

Venomous snakes were captured from various locations in 12 States and Capital territory: Abuja, Bauchi, Gombe, Jigawa, Kaduna, Kano, Katsina, Kebbi, Nasarawa, Niger, Plateau, Sokoto and Zamfara. A total of 605 snakes were caught alive in the jungle, bought from snake hunters, physically dug out of their holes or removed from residential areas.

The handling of the snakes was done in compliance with the standard and humane procedures of International Animal Welfare Guidelines (Rollin and Kesel, 1990). The snakes (Plate IV- VIII) were kept in safety aerated boxes and brought to the Department of Zoology, Faculty of Life Science, Ahmadu Bello University, Zaria for identification. The snakes were identified by their colour, shape, and size, and bahaviour, they were kept in the Herpetarium for periodic milking.

Plate IV: **picture of *Naja nigricollis* (Black-necked Spitting Cobra) Source: Ahmadu Bello UniversityTeaching Hospital, Shika**

Plate V: **Picture of *Naja Katiensis* (Egyptian Cobra) Source: Yantumaki, Katsina State**

Plate VI: **Picture of *Naja haje* (Mali cobra) Source close to Ahmadu Bello University, Zaria Dam**

Plate VII: **Picture of *Bitis arietans* (African fuff Adder) Source: National Animal Production Research Institute, Ahmadu Bello University, Zaria**

Plate XIII: **Picture of *Echis ocellatus* (The saw-scaled or carpet viper) Source: Kaltingo, Gombe State**

3.6 Milking of the Snakes

3.6.1 Feeding and Maintenance

Snakes were manually fed with beef meat bough from a local market, through the mouth by pushing the piece of meat gently from the mouth to just below the skull then holding the head and pushing it down the tubular body. Thereafter, they were immersed in a bucket full of water for 2 minutes. The feeding and watering was repeated every four days.

3.6.2 Milking Procedure

Milking was done prior to feeding by holding the snake on the head with one hand, with a thumb placed at the base of its skull and the index finger below the throat with care and little pressure applied to avoid strangling the animal as shown on Plate IX (Humane Society International (HSI), 2014). The head of the snake was thereafter introduced into a 100 ml beaker covered with an empty polyethylene bag tightened with a rubber band round the neck of the beaker. The snake was stimulated to bite the material, as it grabedthe polythene bag in the act of biting, it then poured out the venom into the beaker (Plate IX).

3.6.3 Venom Processing

The venom collected from each species of snake were pooled together into a labeled beaker separately and then lyophilized by putting the beaker containing the venom in a desiccator containing coarse silica gel (activated at 110^{o}C for 1hour). The desiccator was sealed and air tightened then placed in a -180^{o}C freezer for 48hrs for complete crystallization. The freeze dried venom was then stored at -4^{o}C (refrigerator)ready for use in the experiment (Humane Society International (HSI), 2014) .

Plate IX: Picture of SnakeVenom Collection Process

3.7 Experimental Animals

Wistar rats weighing 50-200 g used for the research were purchased from the Faculty of Pharmaceutical Sciences. The rats were raised at the Center for Biotechnology Research and Training Ahmadu Bello University, Zaria. The animals were allowed to acclimatize for two weeks in the laboratory prior to commencement of the experiment. The animals were housed in wired cages and fed on standard rat pellets while water was provided *ad libitum*. The experiment was conducted in accordance with the Ahmadu Bello University committee for animal use and care (ABUCAUC).

The initial concentration was then serially diluted to give ranges of concentrations for the fitted LD_{50} groups of cages each containing 5 rats. They were inoculated intraperitoneally with the venoms at concentration of 0.4, 0.8, 1.2, and 1.6mg/kg body weight and the control with sterile Control. The time of death for each group was recorded and used to measure the lethal effect of each venom. The lethal dose (LD_{50}) was analyzed using the probit analysis method and probit graph plotted. The anti-log of the log concentration of the probit graph gave the LD_{50} value (Lork, 1983).

3.8 Anticoagulant Activity of Snake Venom.

Ten mililitres of bovine blood was collected in 2mililitre of 1.5% sodium citrate and centrifuged at 2500 gram for 10 minutes to obtain plasma. Sixtymicrolitres of 0.2 Molar $CaCl_2$ was added to 200 microlitres of citrated plasma. One hundred microlitres of the venom containing 500 microlitres of PBS at pH of 7.4 was added and incubated at 37^oC for 4 hours. The control was citrated plasma mixed in 60 microlitres of 0.2 Molar $CaCl_2$. The

time taken to clot in minutes was recorded and the assay stopped after 12hours (Lork, 1983; Theakston and Reid, 1983).

3.9 Haemorrhagic Study

This experiment contained six proups of three rats each. Group one: The three rats were administered with ten microliters of *Naja nigricollis* venom (10 mg/ ml) mixed with 10 microliter of aqueous extract (10 mg/ ml) of *Olax manni, Urginea altissma* and *Xeromphis nilotica* respectively and observed for 1hour, thereafter sacrificed and the skin was inspected for the presence of blood patches.

Group two: Rats were administered with ten microliters of *Naja haje* venom (10 mg/ ml) mixed with 10 microliter of aqueous extract (10 mg/ ml) of *Olax manni, Urginea altissma* and *Xeromphis nilotica* respectively and observed for 1hour, thereafter sacrificed and the skin was inspected for the presence of blood patches.

Group three: Rats were administered with ten microliters of *Naja katiensis* venom mixed with 10 microliter of aqueous extract (10 mg/ ml) of *Olax manni, Urginea altissma* and *Xeromphis nilotica* respectively and observed for 1hour, thereafter sacrificed and the skin was inspected for the presence of blood patches.

Group four: Rats were administered with ten microliters of *Bitis arietans* venom (10 mg/ ml) mixed with 10 microliter of aqueous extract (10 mg/ ml) of *Olax manni, Urginea altissma* and *Xeromphis nilotica* respectively and observed for 1hour, thereafter sacrificed and the skin was inspected for the presence of blood patches.

Group five: Rats were administered with ten microliters of *Echis ocellatus* venom (10 mg/ ml) mixed with 10 microliter of aqueous extract (10 mg/ ml) of *Olax manni, Urginea altissma* and *Xeromphis nilotica* respectively and observed for 1hour, thereafter sacrificed and the skin was inspected for the presence of blood patches.

Group six: Rats were administered with ten microliters of normal salinemixed with 10 microliter of aqueous extract (10 mg/ ml) of *Olax manni, Urginea altissma* and *Xeromphis nilotica* respectively and observed for 1hour, thereafter sacrificed and the skin was inspected for the presence of blood patches.

3.10 *In vivo* Studies

3.10.1 Experimental design

The experiment was carried out as follows: one thousand five hundred and twelve rats were used; rats were distributed into 6 groups of 7 for each fraction and for each snake species venom; venomsfrom five species of snakes was used in this study; four fractions for each of the three plant extracts was used for the experiment and three different method of administration of the venom and or extractintra-peritoneally into the rats;ten microlitres of 10 mg/ml of each snake venom was used and ten microlitres of 10 mg/ml for each fraction of the plant extracts was intra-peritoneally inoculated into the rats.

Table 3.1 Experimental Design to Determine the Antivenom Properties of Plant Extract

Groups	1	2	3	4	5	6
No. of rats	7	7	7	7	7	7
Species of snake	*Naja nigricollis*	*Naja haje*	*Naja katiensis*	*Bitis arietans*	*Echis ocellatus*	Control
Concentration of venom (mg/ml)	10	10	10	10	10	10
Concentration of Diethyl ether fraction (A_1, B_1 C_1 and aq) mg/ml	10	10	10	10	10	10
Concentration of ethyl acetate (A_2, B_2 C_2 and aq) fraction mg/ ml	10	10	10	10	10	10
Concentration of n-Butanol of A_3 B_3 C_3 and aq) fraction mg/ ml	10	10	10	10	10	10
Concentration of aqueous factions (A_4, B_4, C_4)	10	10	10	10	10	10

3.11 Determination of LD50 of the Snake Venoms in Experimental Rats

The potency of the snake venom was measured by determining the LD_{50} (lethal dose 50%) of the venom. It involves dosing several groups of animals with venom injected intraperitoneally in rats. The finishing point of the test is when half of the animals in the group have died. The amount of venom that kills half the animals gives the LD_{50} figure which indicates the standard toxicity value for each venom. Ten miligram of the lyophilized venom was dissolved in sterilized Control which served as the stock solution for the determination of the LD_{50}, which was determined as decribed by (Lork, 1983). This was first used as pilot study with five rats in each cage of seven groups. The wistar rats in the different groups were inoculated intraperitoneally with the venoms at different concentrations; 1, 2, 3, 4, 5 and 6mg/ kg body weight. The control animals were injected with sterile Control. All the rats (100%) died with the exception of the control.

3.12 Methods of Administration of venoms and or extracts into the rats

- Method 1: Inoculation of rats with venom intra-peritoneally 30 minutes before administration of the plant extracts
- Method 2: Prophylactic administrationof the plant extracts to the rats 30 minutes before inoculation of the venom.
- Method 3: Concurrent administration of the venom with the plant extracts

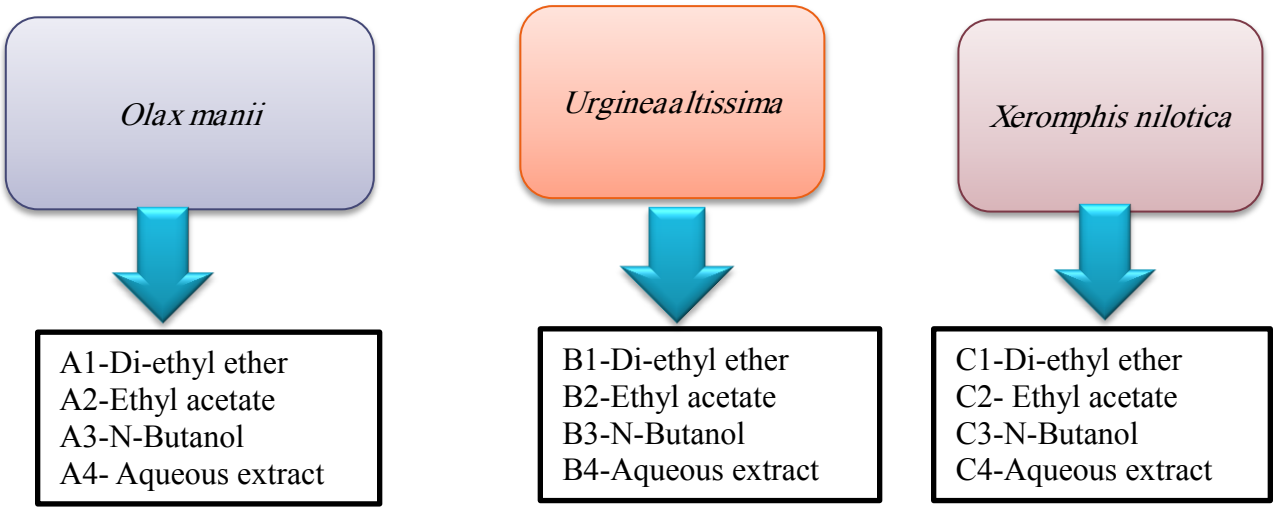


Figure 1 Fractions of the three Plants used in the study

3.13 Effect of snake venom on the migration of 1kb plasmid DNAon Agarose gelelectrophoresis

The effect of snake venom on migration of 1 kb plasmid DNA was determined using agarose gel electrophoresis. Also the protective effect of the plant extracts against this effect of snake venoms was determined as described below.

3.12.1 Protocol for agarose gel electrophoresis

1 2g of agarose powder was weighed and put into a 100 ml conical flask

2 100 ml of 1X TBE was added mixed and dissolved in a microwave oven

3 The mixture was cooled to 45^{o}C

4 Gel casting tray was assembled and the combs were positioned

5 Five (5) µl of ethidium bromide was added to the agarose and swirled gently

6 the mixture was carefully poured into the casting tray and allow to solidify

7 the combs were carefully removed from the casting tray

8 The casting tray was transferred into the gel electrophoresis tank

9 The tank was filled up to the gauge mark with 1X TBE running buffer

10 The first well labeled M was loaded with 5µl of 100 bp DNA ladder

11 Lane 1 was loaded with Plasmid DNA

12 Lane 2 was loaded with Plasmid DNA +Venom of *Naja nigricollis*

13 Lane 3 was loaded with Plasmid DNA+Venom of *Naja nigricollis*+plant extract

14 Lane 4 Plasmid DNA+ plant extract

15 Lane 5 Plasmid DNA + Venom of *Naja haje*

16 Lane 6 Plasmid DNA+Venom of *Naja haje*+plant extract

17 Lane 7 Plasmid DNA + Venom of *Naja katiensis*

18 Lane 8 D + V_3+ E(Plasmid DNA+Venom of *Naja katiensis*+plant extract

19 Lane 9 Plasmid DNA+Venom of *Bitis arietans*

20 Lane 10 Plasmid DNA+Venom of *Bitis arietans*+plant extract

21 Lane 11 Plasmid DNA+Venom of *Echis ocellatus*

22 Lane 12Plasmid *DNA*+*Echis ocellatus*+plant extract

23 Voltage was set at 75 V

24 The preparation was run for 45 minutes

25 The gel was observed in gel- documentation unit and photographed.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Screening of the Crude Extracts of *Olax manni, Urginea altissima* and *Xeromphis nilotica*

Results of the phytochemical analysis of the crude extracts of the three plants (*Olax manni, Urginea altissima* and *Xeromphis nilotica*) showed the presence of carbohydrates, glycosides, steroids/triterpens, cardiac glycosides, saponins, glycosides and alkaloids. In addition, Extract of *Urginea altissima* contained free anthracenes. However, the ferric chloride test failed to detect glycosides in all the extracts, while Bontrager's test and modified Bontrager's test did not detect free anthracenes and combined anthracenes respectively in the extracts of *Olax manni* and *Xeromphis nilotica.* Similarly, Mayer's, Dragendoff's and Wagner's tests failed to detect alkaloids in *Olax manni,* and *Xeromphis nilotica.*

Table 4.1 Inference of the phytochemical test of crude extracts of *Olax manni, Urginea altissima* and *Xeromphis nilotica*

Metabolites	Phytochemical Test	Plant species		
		O. manni	*U. altissima*	*X. nilotica*
Carbohydrates	Molish test	+	+	+
	Fehling test	+	+	+
Glycosides	Fehling test	+	+	+
	Ferric chloride test	-	-	-
Free anthracene derivatives	Bontrager's test	-	+	-
Combine anthracene derivatives	Modified bontrager's test	-	+	-
Unsaturated steroid and triterpenes	Liechermann-bucchard test	+	+	+
	Salkowski test	+	+	+
Cardiac glycoside	Keller-kiliani test	+	+	+
	Kedde's test	+	+	+
Saponin glycosides.	Frothing test	+	+	+
	Haemolysis test	+	+	+
Tannins	Ferric chloride test	+	+	+
	Bromine water test	-	-	-
	Lead sub-acetate test	+	+	+

	Shinoda test	+	+	+
Flavonoids				
	Sodium hydroxide test	+	+	+
	Ferric chloride test	+	+	+
Carbohydrates	Molish test	+	+	+
	Fehling test	+	+	+
Glycosides	Fehling test	+	+	+
	Ferric chloride test	-	-	-
Free anthracene derivatives	Bontrager's test	-	+	-
Combine anthracene derivatives	Modified bontrager's test	-	+	-
Unsaturated steroid and triterpenes	Liechermann-bucchard test	+	+	+
	Salkowski test	+	+	+
Cardiac glycoside	Keller-kiliani test	+	+	+
	Kedde's test	+	+	+
Saponin glycosides.	Frothing test	+	+	+
	Haemolysis test	+	+	+
Tannins	Ferric chloride test	+	+	+

	Bromine water test	-	-	-
	Lead sub-acetate test	+	+	+
Flavonoids	Shinida test	+	+	+
	Sodium hydroxide test	+	+	+
	Ferric chloride test	+	+	+
Alkaloids	Mayers test	-	+	-
	Dragendeff' test	-	+	-
	Wagner's test	-	+	-

Key:

+ = Positive

- = Negative

Table 4.2 Snake species collected from various locations in Nigeria

	Species of snake					
Location	*N. nigricollis*	*N. haja*	*N. katiensis*	*E. ocellatus*	*B. arietans*	ToTotal
Abuja	03	01	01	-	02	007
Bauchi	01	03	02	10	07	023
Gombe	-	-	-	58	05	063
Jigawa	03	11	12	-	01	027
Kaduna	75	16	15	13	12	131
Kano	21	11	13	10	15	070
Katsina	15	38	51	07	35	146
Kebbi	02	03	01	-	01	007
Niger	05	13	17	-	-	035
Nasarawa	06	12	09	-	-	027
Plateau	02	-	-	16	17	035
Sokoto	-	08	02	-	-	010
Zamfara	02	10	12	-	-	024
Total	135	126	135	114	095	**605**

4.2 Determination of LD50 of Snake Venoms in Wistar Rats

The LD_{50} of the venom of *N. haje, N. katiensis,* N. *nigricollis, E. ocellatus and B. arietans* in Wistar rats were found to be 0.22, 0.55, 0.87, 1.24 and 1.80 mg/kg, respectively. *Bitis arietans* has the highest LD_{50} followed by *Echis ocellatus* while *Naja haje* was having the lowest LD_{50} follwed by *Naja katiensis,* found to have the lowest LD_{50} as shown in Table 4.3

Table 4.3 Median Lethal Dose (LD_{50}) of venoms of five species of snakes in Wistar rats

Snake species	LD_{50} (mg/kg) of snake
Naja haje	0.22
Naja katiensis	0.55
Naja nigricollis	0.87
Echis oscellatus	1.24
Bitis arietans	1.80

Table 4.4 Median Lethal Dose (LD_{50}) of three species of plants in *Ratus ratus* (Wistar rats)

Plant species	LD_{50} (mg/ kg) of plant extract
Olax manni	1100
Urginea altissima	1500
Xeromphis nilotica	1000

Table 4.5 Anticoagulant activity of venom of the 5 species of snakes

Species of snake	Clotting time (minutes)
Naja nigricollis	132
Naja haje	128
Naja katiensis	115
Bitis arietans	No clot
Echis ocellatus	No clot
Citrated blood plasma (control)	No clot

4.3 Anticoagulant Activity of Snake Venoms

Results of anticoagulant activity of the snake venoms were presented in table 3 and were measured by the time taken for blood to clot. The clotting time for *Naja nigricollis, Naja haje* and *Naja katiensis* were found to be 132, 128 and 115 minutes, respectively. However, the venoms of *Bitis arietans* and *Echis ocellatus* did not clot during the period of the experiment.

4.4 The in vitro effect of snake venoms on plasmid DNA preparations in the presence of various plant extracts

The interactive study of the aqueous fraction of *Urgines altissima*, venom and the plasmid DNA measured by migration on agarose gelelectrophoresis showed that aqueous fractions had high antivenom activity against the venoms *of Naja nigricollis, Naja haje, Naja katiensis, Bitis arietans and Echis ocellatus.*1 kb DNA ladder and the plasmid DNA migrated very well on agarose gel electrophoresis. The mixture of plasmid DNA with separate venom of the snakes, showed no migration of the DNA on agarose gel electrophoresis. The plasmid DNA incubated with the aqueous fraction of plant B showed clear migration of the DNA on agarose gel electrophoresis as shown in Plate X.

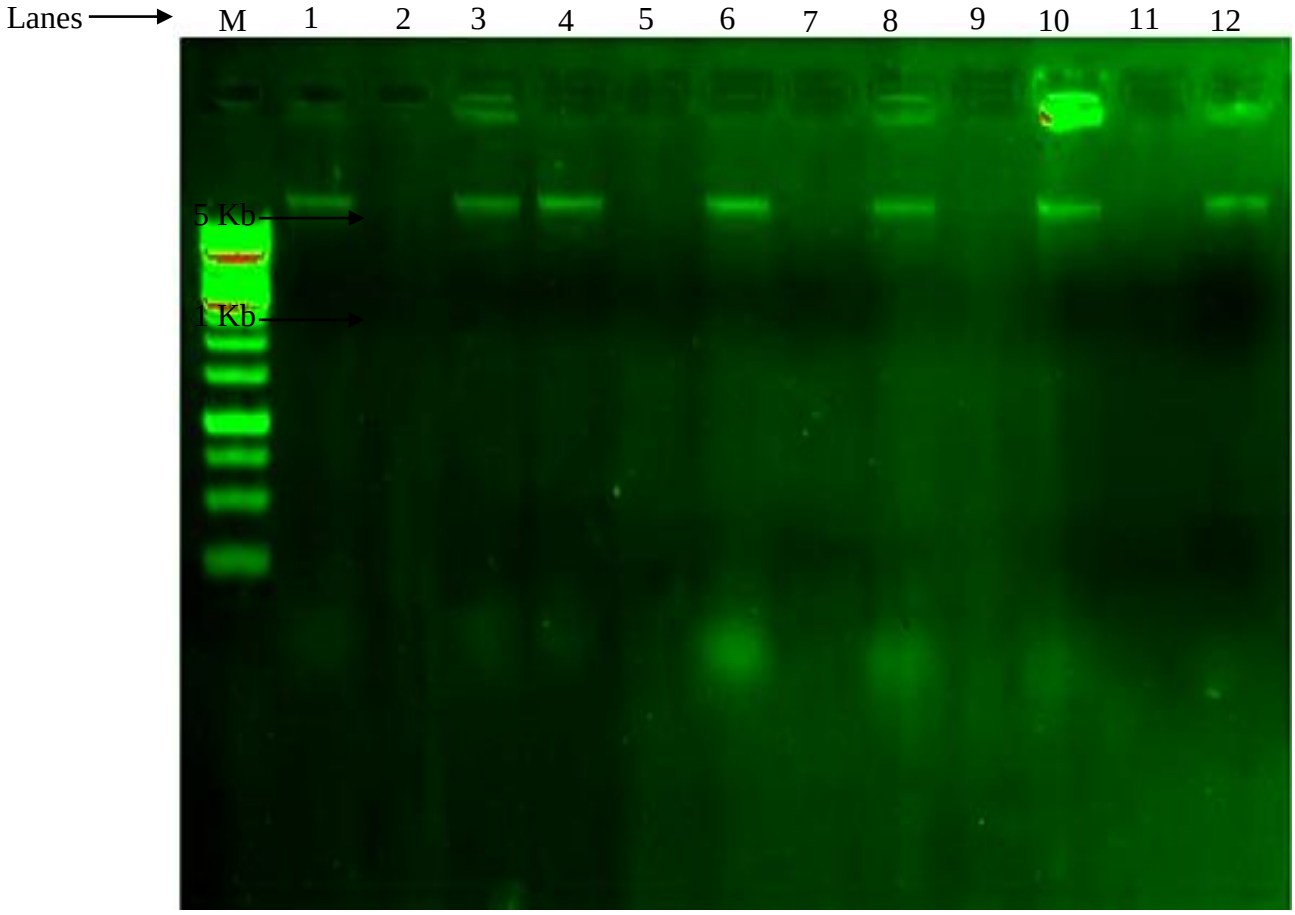


Plate X: Gel picture of agarose gel electrophoresis: DNA, Venom and Plant Extract

Key:

- Lane M 1 Kb DNA Ladder
- Lane 1 D Plasmid DNA
- Lane 2 D + V_1 (Plasmid DNA +Venom of *Naja nigricollis)*
- Lane 3 D + V_1 + E (Plasmid DNA+Venom of *Naja nigricollis*+plant extract)
- Lane 4 D + E(Plasmid DNA+ plant extract)
- Lane 5 D + V_2 (Plasmid DNA + Venom of *Naja haje*)
- Lane 6 D +V_2 + E(Plasmid DNA+Venom of *Naja haje*+ plant extract)

- Lane 7 D +V_3 (Plasmid DNA+Venom of *Naja katiensis*)
- Lane 8 D + V_3+ E(Plasmid DNA+Venom of *Naja katiensis*+ plant extract*)*
- Lane 9 D + V_4 (Plasmid DNA+Venom of *Bitis arietans)*
- Lane 10 D +V_4 + E(Plasmid DNA+Venom of *Bitis arietans*+plant extract)
- Lane 11 D + V_5 (Plasmid DNA+Venom of *Echis ocellatus*
- Lane 12 D + V_5 + E(Plasmid *DNA*+*Echis ocellatus*+plant extract)

4.6 In Vivo Studies

4.6.1 Haemorrhagic Effects of Snake Venom in the Presence of aqueous fractions of *Olax manni, Urginia* altissima and *Xeromphis nilotica* in the Skin of Rats Sacrificed 3 Hours Post Inoculation

The haemorrhagic effect of venom in rats indicated that aqueous fraction of *Urginia altissima* showed antivenom activity against the venoms of *Naja nigricollis, Naja haje, Naja katiensis, Bitis arietans and Echis ocellatus* there were no patches observed. The aqueous extract fraction of *Olax manni* showed protective effect against venoms of *Naja nigricollis, Naja haje, Naja katiensis, Bitis arietans and Echis ocellatus while* aqueous fraction of *Xeromphis nilotica* also had protective effect against the venom of *Naja nigricollis, Naja haje, Naja katiensis* and *Bitis arietans*. However, there were some patches of blood in the skin of rats inoculated with venom of *Echis ocellatus.* The results established the aqueous fraction of the three plants the neutralization effects of venom which agreed with finding of (Esmeraldino *et al*., 2005) reported that the aqueous fraction of *Croton urucurara* antagonized haemorrhagic activity of the venom of *Bothrops jarara.* Similarly, study conducted by (Mourao *et al*., 2014) reported that the leaves extract exelted

effective anti- inflammatory and anti-haemorrhagic activity against the effects induced by *Bothrops jarara* sanke venom.

4.6.1 Effect of inoculation of rats with snake venom 30 minutes before treatment with extracts of plants A, B and C

A total of 303 (60.1 %)out of 504 rats survived the inoculation of venoms before administration of the extracts 30 minutes later. Out of this number, 89 that survived were treated with plant A, 123 that survivedwere treated with plant B and 91 were treatedwith plant C extracts. The total number of rats that survived envenomation following treatment with different fractions of extract were 55 for di-ethyl-ether fractions A_1; B_1 and C_1 (16, 25 and 14, respectively); 61 for ethyl acetate fractions A_2, B_2 and C_2 (16, 27, and 18 respectively); 65 for n-butanol fractions A_3 B_3 and C_3 (18, 29, and 18 respectively) and 122 for aquoeus fractions A_4 B_4 and C_4 (39, 42, and 41, respectively).

4.6.2 Effect of treatment of rats with extracts of plants A, B and C 30 minutes before inoculation of snake venom

A total of 330 (65.5 %) out of 504 rats survived the inoculation of the snake venoms when inoculated 30 minutes after administration of the plant extracts. Out of this number, 103 (31.2. %) that survived were treated with plant A, 128 (38.8 %) that survived were treated with plant B and 99 (30 %) were treated with plant C extracts. The total number of rats that survived envenomation following treatment with different fractions of extract were 64 (19.4 %) for di- ethyl- ether fractions A_1; B_1 and C_1.18 (5.5 %), 28 (8.5 %) and 18 (5.5 %), respectively); 67 (20.3 %) for ethyl acetate fractions A_2, B_2 and C_2.21 (6.4 %), 27 (8.2 %) and 19 (5.8 %) respectively; 75 (22.7 %) for n-butanol fractions A_3 B_3 and C_3.(23 (7.0 %), 31 (9.4 %), and 21 (6.4 %) respectively) and 124 (37.6 %) for aqeuoeus fractions A_4 B_4 and C_4.(41 (12.4 %), 42 (12.7 %) and 41 (12.4 %), respectively) as shown in Table 4.5.

4.6.3 Plant extract fractions and the venom incubated together for 30 minutes before Inoculation

A total of 355 (70.4 %)out of 504 rats survived the co-administration of plant extracts and venoms. Out of this number, 113(31.8 %), 133 (37.5 %) and 109 (30.7 %) that survived were given plant A, plant B and plant C extracts, respectively. The total number of rats that survived co-administration of snake venoms with different fractions of extract were 74 (20.4 %) for diethyl ether fractions A_1; B_1 and C_1.22 (6.2 %), 29 (8.2 %) and 23 (6.5 %) respectively); 75 (21.1 %) for ethyl acetate fractions A_2, B_2 and C. 24 (6.8 %), 30 (8.5 %) and 21 (5.9 %), respectively. 80 (22.5 %) for n-butanol fractions A_3 B_3 and C_3. 25 (7.0 %, 32 (9.0 %) and 23 (6.4 %) respectively) and 126 (35.5 %) for aquoeus fractions A_4 B_4 and C_4 .42 (11.8 %; 42 (11.8 %) and 42 (11.8 %), respectively) as shown in Table 4.6.

Table 4.6: **Number and percentage of rats that survived inoculation with venom 30 minutes before treatment with the di-ethyl ether portion (A_1, B_1, and C_1) of plant extracts**

Venom of:	*O. manni* (%)	*U. altissima* (%)	*X. nilotica* %)	Total (%)
Naja nigricollis	1(14.3)	3(42.9)	1(14.3)	5(23.8)
Naja haje	2(28.6)	4(57.1)	1(14.3)	7(33.3)
Naja katiensis	2(28.6)	4(57.1)	2(28.6)	8(38.1)
Bitis arietans	3(42.9)	5(71.4)	2(28.6)	10(47.6)
Echis ocellatus	1(14.3)	2(28.6)	1(14.3)	4(19.0)
Normal saline	7(100)	7(100)	7(100)	(100)
Total (%)	16(38.01)	**25(59.52)**	14(33.33)	55(43.7)

Key

N= 7 in all treatment

Table 4.7: **Number of rats that survived inoculation with snake venoms 30 minutes after pretreatment with di-ethyl-ether portion (A_1, $B_1$and C_1) of the plants extracts**

Group of rats treated with toxins of:	*O. manni* (%)	*U. altissima* (%)	*X. nilotica* (%)	Total (%)
Naja nigricollis	4(57.1)	4(57.1)	2(28.6)	10(47.6)
Naja haje	2(28.6)	5(71.4)	2(28.6)	9(42.9)
Naja katiensis	2(28.6)	4(57.1)	3(42.9)	9(42.9)
Bitis arietans	1(14.3)	5(71.4)	3(42.9)	9(42.9)
Echis ocellatus	2(28.6)	3(42.9)	1(14.3)	6(28.6)
Normal saline	7(100)	7(100)	7(100)	21(100)
Total (%)	18(42.9)	**28(66.7)**	18(42.9)	64(50.8)

N= 7 in all treatment

Table 4.8: **Number of rats that survived after inoculation with mixture of venom and the xtracts portion of di- ethyl-ether (A_1, B_1, and C_1)**

Groups of rats treated with toxins of	*O.manni* (%)	*U. altissima* (%)	*X.nilotica* (%)	Total (%)
Naja nigricollis	3(42.9)	4(57.1)	2(28.6)	9(42.9)
Naja haje	3(42.9)	5(71.4)	4(57.1)	12(57.1)
Naja katiensis	4(57.1)	5(71.4)	4(57.1)	13(61.9)
Bitis arietans	3(42.9)	3(42.9)	4(57.1)	10(47.6)
Echis ocellatus	2(28.6)	5(71.4)	2(28.6)	9(42.9)
Normal saline	7(100)	7(100)	7(100)	21(100)
Total (%)	22(52.4)	**29(69.0)**	23(54.8)	74(58.7)

N= 7 in all treatment

Table 4.9: **Number of rats that survived inoculation with snake venoms30 minutes before treatment with ethyl acetate fraction of the plants (A_2, B_2 and C_2)**

Groups of rats treated with toxins of:	*O.manni* (%)	*U. altissima* (%)	*X. nilotica* (%)	Total (%)
Naja nigricollis	3(42.9)	4(57.1)	2(28.6)	9(42.9)
Naja haje	2(28.6)	5(71.4)	3(42.9)	10(47.6)
Naja katiensis	1(14.3)	4(57.1)	3(42.9)	8(48.1)
Bitis arietans	2(28.6)	4(57.1)	2(28.6)	8(38.1)
Echis ocellatus	1(14.3)	3(42.9)	1(14.3)	5(23.8)
Normal saline	7(100)	7(100)	7(100)	21(100)
Total (%)	16(38.1)	**27(64.3)**	18(42.9)	61(48.4)

N= 7 in all treatment

Table 4.10: **Number of rats that survived inoculation with snake venoms 30 minutes after treatment with ethyl acetate fraction of the plants (A_2 B_2 C_2)**

Groups Groups of rats treated with toxins of:	*O. manni* (%)	*U. altissima* (%)	*X. nilotica* (%)	Total (%)
Naja nigricollis	2 (28.6)	3 (42.9)	2 (28.6)	7 (33.3)
Naja haje	3(42.9)	5(71.4)	3(42.9)	11(52.4)
Naja katiensis	3(42.9)	4(57.1)	4(57.1)	11(52.4)
Bitis arietans	4(57.1)	5(71.4)	2(28.6)	11(52.4)
Echis ocellatus	2(28.6)	3(42.9)	1(14.3)	6(85.7)
Normal saline	7(100)	7(100)	7(100)	21(100)
Total (%)	21(50.0)	**27(64.3)**	19(45.2)	67(53.2)

N= 7 in all treatment

Table 4.11: Number of rats that survived inoculation of mixture of venom with ethyl acetate fraction of the plants (A_2 B_2 C_2)

Groups of rats treated with toxins of:	*O.manni* (%)	*U. altissima* (%)	*X. nilotica* (%)	Total (%)
Naja nigricollis	3(42.9)	4(57.1)	3(42.9)	10(47.6)
Naja haje	4(57.1)	5(71.4)	2(28.6)	11(52.4)
Naja katiensis	4(57.1)	4(57.1)	4(57.1)	12(57.1)
Bitis arietans	3(42.9)	5(71.4)	3(42.9)	11(52.4)
Echis ocellatus	3(42.9)	5(71.4)	2(28.6)	10(47.6)
Normal saline	7(100)	7(100)	7(100)	21(100)
Total (%)	24(57.1)	**30(71.4)**	21(50.0)	75(59.5)

N= 7 in all treatment

Table 4.12: **Number of rats that survived the inoculation with venom30 minutes before treatment with the n-butanol fraction of the plants (A_3 B_3 C_3)**

Groups of rats treated with toxins of:	*O. manni* (%)	*U. altissima* (%)	*X. nilotica (%)*	Total (%)
Naja nigricollis	2(28.6)	4(57.1)	1(14.3)	7(33.3)
Naja haje	3(42.9)	5(71.4)	3(42.9)	11(52.4)
Naja katiensis	3(42.9)	4(57.1)	2(28.6)	9(42.9)
Bitis arietans	2(28.6)	5(71.4)	4(57.1)	11(52.4)
Echis ocellatus	1(14.3)	4(57.1)	1(14.3)	6(85.7)
Normal saline	7(100)	7(100)	7(100)	21(100)
Total (%)	18(42.9)	**29(69.0)**	18(42.9)	65(51.6)

N= 7 in all treatment

Table 4.13: **Number of rats that survived the inoculation with snake venoms 30 minutes after treatment withn-butanolfraction of the plants (A_3 B_3 C_3)**

Groups of rats treated with toxins of:	*O. manni* (%)	*U. altissima* (%)	*X. nilotica* (%)	Total (%)
Naja nigricollis	3(42.9)	4(57.1)	2(28.6)	9(42.9)
Naja haje	4(57.1)	5(71.4)	3(42.9)	12(57.1)
Naja katiensis	3(42.9)	6(85.7)	4(57.1)	13(61.9)
Bitis arietans	4(57.1)	4(57.1)	3(42.9)	11(52.4)
Echis ocellatus	2(28.6)	5(71.4)	2(28.6)	9(42.9)
Normal saline	7(100)	7(100)	7(100)	21(100)
Total (%)	23(54.8)	**31(73.8)**	21(50.1)	75(59.5)

N= 7 in all treatment

Table 4.14: **Number of rats that survived after inoculation with mixture of venom and the n-butanol fraction of the plants (A_3 B_3 C_3)**

Groups of rats treated with toxins of:	*O. manni* (%)	*U. altissima* (%)	*X. nilotica* (%)	*Total* (%)
Naja nigricollis	3(42.9)	5(71.4)	3(42.9)	11(52.4)
Naja haje	4(57.1)	5(71.4)	4(57.1)	13(61.9)
Naja katiensis	5(71.4)	5(71.4)	4(57.1)	14(66.7)
Bitis arietans	4(57.1)	6(85.7)	3(42.9)	13(61.9)
Echis ocellatus	2(28.6)	4(57.1)	2(28.6)	8(38.1)
Normal saline	7(100)	7(100)	7(100)	21(100)
Total (%)	25(59.5)	32(76.2)	23(54.7)	80(63.5)

N= 7 in all treatment

Table 4.15: **Number of rats that survived the inoculation wirh venom and 30 minutes later treated with the aqueous extracts (A_4 B_4 C_4)**

Groups of rats treated with toxins of:	*O.manni* (%)	*U. altissima* (%)	*X. nilotica* (%)	Total (%)
Naja nigricollis	7(100)	7(100)	7(100)	21(100)
Naja haje	6(85.7)	7(100)	7(100)	20(95.2)
Naja katiensis	6(85.7)	7(100)	7(100)	20(95.2)
Bitis arietans	7(100)	7(100)	6(85.7)	20(95.2)
Echis ocellatus	6(85.7)	7(100)	7(100)	20(95.2)
Normal saline	7(100)	7(100)	7(100)	21(100)
Total (%)	39(92.9)	42(100)	41(97.6)	122(96.8)

N= 7 in all treatment

Table 4.16: **Number of rats that survived the inoculation with aqueous extracts (A_4 B_4 C_4) and 30 minutes later inoculated with venom**

Groups of rats treated with toxins of:	*O. manni* (%)	*U. altissima* (%)	*X. nilotica* (%)	Total (%)
Naja nigricollis	7(100)	7(100)	7(100)	21(100)
Naja haje	7(100)	7(100)	7(100)	21(100)
Naja katiensis	7(100)	7(100)	6(85.7)	20(95.2)
Bitis arietans	7(100)	7(100)	7(100)	21(100)
Echis ocellatus	6(85.7)	7(100)	7(100)	20(95.2)
Normal saline	7(100)	7(100)	7(100)	21(100)
Total (%)	41(97.6)	42(100)	41(97.6)	124(98.4)

N= 7 in all treatment

Table 4.17: **Number of rats that survived the mixture of venom and aqueous extracts (A_4 B_4 C_4) incubated for 30 minutes before inoculation**

Groups of rats teated with toxins of:	*O.manni* (%)	*U. attaltissima* (%)	*X. nilotica* (%)	Total (%)
Naja nigricollis	7(100)	7(100)	7(100)	21(100)
Naja haje	7(100)	7(100)	7(100)	21(100)
Naja katiensis	7(100)	7(100)	7(100)	21(100)
Bitis arietans	7(100)	7(100)	7(100)	21(100)
Echis ocellatus	7(100)	7(100)	7(100)	21(100)
Normal saline	7(100)	7(100)	7(100)	21(100)
Total (%)	42(100)	42(100)	42(100)	126(100)

N= 7 in all treatment

Table 4.18: Rats that survived inoculation with di-ethyl ether extracts in all the three methods of inoculation

Plant species	venom before extract	Extract before venom	Mixture of both venom and extraxt	Total (%)
Olax manni	16(38.1)	18(42.9)	22(52.4)	56(44.4)
Urginea altissma	25(59.6)	28(66.7)	29(69.0)	82(65.1)
Xeromphis nilotica	14(33.3)	18(42.9)	23(54.8)	55(43.7)
Total (%)	55(43.7)	64(50.8)	74(58.7)	193 (50.1)

Table 4.19: Rats that survived inoculation with ethyl acetate extractin all the three methods of inoculation

Plant species	venom before extract	Extract before venon	Mixture of both venom and extraxt	Total (%)
Olax manni	16(38.1)	21(50.0)	24(57.1)	61(44.4)
Urginea altissma	27(64.3)	27(64.3)	30(71.4)	84(65.1)
Xeromphis nilotica	18(42.9)	19(45.2)	21(50.0)	58(43.7)
Total (%)	61(48.4)	67(53.2)	75(59.5)	203(53.7)

Table 4.20: **Rats that survived inoculation with n-butanol extracts in all the three methods of inoculation**

Plants species	venom before extract	Extract before venon	Mixture of both venom and extraxt	Total (%)
Olax manni	18(42.9)	23(54.8)	25(57.1)	66(53.4)
Urginea altissma	29(69.0)	31(64.3)	32(73.8)	92(73.0)
Xeromphis nilotica	18(42.9)	21(45.2)	23(54.8)	62(49.2)
Total (%)	65 (51.6)	75(59.5)	80 (63.5)	220 (58.2)

Table 4.21: **Rats that survived inoculation withaqueous extractsin all the three methods of inoculation**

Plant species	Venom before extract (%)	Extract before Venon (%)	Mixture of both venom and extraxt (%)	Total (%)
Olax manni	39(92.9)	41(97.6)	42(100)	122(96.8)
Urginea altissma	42(1000)	42(100)	42(100)	126(100)
Xeromphis nilotica	41(97.6)	41(97.6)	42(100)	124(98.4)
Total (%)	122(48.4)	124(53.2)	126(59.5)	372(98.4)

CHAPTER FIVE

5.1 DICUSSION

The antivenom activity observed in the rats treated with extracts of *Olax manni, Urginea altissima* and *Xeromphis nilotica* extracts in this study could bedue to the presence of alkanoids, saponins, tannins, flavonoids, steroids and terpenoid. (Rajendran *et al*., 2010). (Lans *et al*., 2001) reported that plant alkaloids are effective against snake bites. They also reported that phenolic compounds and tannins can bind to proteins and can directly act on venom constituents. Saponins and steroids from different plants have also demonstrated antisnake venom activity in laboratory tests (Mors *et al.*, 2000). Similarly, flavonoids have been shown to possess the ability to bind to biological polymers and can inhibit the effects of toxins (Mors *et al.*, 2000) in a study which alsoreported the effectiveness of flavanoids in inhibiting the venom in *Primula denticulate*. Flavonoids also have many useful properties like anti-inflammatory, antimicrobial, enzyme inhibition, oestrogenic, antiallergic and antioxidant activity (Prabha and Savithramma, 2014) which may be useful biologically in the event of snake bites.

Furthermore, cardiac glycosides, also found in all the three plants, are known to act by inhibiting the Na^{+}/K^{+} pump. This causes an increase in the level of sodium ions in the myocytes, which then lead to a rise in the level of calcium ions whichmay be important in counteracting the haemorrhagic effects of snake venoms (Denwick, 2002).

All fractions of the three plants were found experimentally in this study to have both therapeutic and prophylactic effects against snake venom. The aqueous fractions of all the three plants were found to be more efficacious. This suggests that the bioactive compound

may be more soluble in water than the other solvents used in the study. Water is known to be a universal solvent which is mostly used to extract plant products. Many traditional snakebite healers use water for extraction (Madane *et al*., 2013).This finding contradicts the reports by other researchers who observed that most phytochemicals are more soluble in organic solvents than water (Kumar *et al*., 2009; Madane *et al*., 2013; Thite, Chavan *et al*., 2013). (Madane *et al*., 2013) observed the presence of more alkaloids, carbohydrate, glycosides, tannins, steroids, flavonoids and saponins in chloroform extracts than in aqueous extracts. Similar results were observed in case of acetone and chloroform extraction of these plants, whilein case of alcohol extraction (Kharade *et al*., 2013; *Tupe, et al*., 2015) they also observed alkaloids, carbohydrate, glycosides, tannins, steroids, flavonoids and saponins.

The extract of *Urginea altissima* was found to have the highest efficacy in all the experiments followed by those of *Olax manni* and *Xeromphis nilotica*. (Iwu, 2014) reported that *Urginea altissima* contains cardiac glycoside which can readily bind to proteins. This is useful in neutralizing proteins/enzymes present in snake venoms thus accounting for thehigh efficacy as antivenom. (James *et al*., 2013) reported that *Olax viridis* extract have a potent snake venom neutralizing capacity against *Naja katiensis* venom.

The acute toxicity studies showed that the venom of *Naja haje* had the lowest LD_{50} of 0.22 mg/kg, followed by that of *Naja katiensis* with LD_{50} of 0.55 mg/kg; *Naja nigricollis* with LD_{50} of 0.87 mg/kg; *Echis ocellatus* LD_{50} of 1.24 mg/kg and finally *Bitis arietans* with LD_{50} of 1.80 mg/kg respectively. The LD_{50} of the venoms obtained in this study were higher than that reported by (Ernst and Zug, 1996) who reported an LD_{50} of 0.23 mg/kg for *Echis ocellatus* venom; 0.14 mg/kg for *Bitis arietans* venom, 0.13 mg/kg for *Naja*

nigricollis venom and 0.19 mg/kg for *Naja haje* venom. The LD_{50} obtained for *Naja nigricollis*venom was similar to that reported by (Spawls and Branch, 1996) who reported an LD_{50} of 1.15 mg/kg. For *Naja katiensis*, the LD_{50} was lower than that reported by HomeoVision Project (HomeoVision, 2011) which was 1.75 mg/kg. These differences could be attributable to geographical origins of the snakes. Venoms of snakes are known to exhibit marked variation in their potency and the extent of induction of toxic and lethal effects due to variation of toxins which have been addressed at different levels (sex, diet, seasonal, geographical etc.), and also in terms of composition and relative abundance of toxins (Chippaux *et al*., 1991; Calvete *et al*., 2007).

Studies on clotting time showed that *Naja haje* venom produced the shortest clotting time on whole blood followed by *Naja katiensis* and *Naja nigricollis*. For *Bitis arietans* and *Echis ocellatus* venoms, no clotting was observed even after 24 hours. (Ho *et al*., 1986) developed the 20-minutes whole blood clotting test. They reported that if the blood is still in liquid state after 20 minutes, it is evidence of coagulopathy and confirms that the venom was that of a viper. Cobras or kraits do not cause anti-haemostatic symptoms. This was confirmed in the present study.

The detoxifying method of administration was found to be the best method for neutralizing venom action. However, this is not likely to occur in real life situations as victims do not know when they will be bitten by a snake as observed by (Ahmed *et al*., 2008). Therefore, curative method of administration of the plant extracts is the most practicable method in clinical situations.

Most cases of managing snke envenomation are done after the bite by snakes and it is curative, which is obtained in most pratical terms. However, individuals or animals that are potentially exposed to sudden envenomation can also be treated as a prophylactic measure against snake bitte (Ahmed *et al*., 2008).

The Study established that *Urginea altissima* and *Olax manni* aqueous fraction neutralised the haemorrhagic effects of the venoms of all the snake species except that *Xeromphis nilotica* did not neutralize the effect of *Echis oscellatus* venom.

The interaction of the snake venom with the three plants extracts showed an effect on the stability and migration of the plasmid DNA on agarose gel electrophoresis. This showed that DNA migration was inhibited by the venom which was ameliorated by the various extracts and further proved the efficacy of these plants in neutralizing the effect of the venom. (de Roodt *et al*., 2003) observed that venom from different snakes showed endo- and exo nucleolytic activity on DNA. They demonstrated DNA hydrolysis caused by venoms of 17 species of snakes by testing endonucleolytic activity by incubation of the venoms with the plasmid DNA. This effect was observed in the present study. Therefore, the plant extracts have been shown to prevent this DNA hydrolysis caused by snake venom. This suggested that the three plants have potential anti snake venom property against all the five snake species.

Snake venoms have been shown to contain necrotic phospholipases A2 (PLA2) which cause myonecrosis especially in *Naja nigricollis* venom (Ahmed *et al*., 2008; Gowda & Middlebrook, 1993). *Naja nigricollis* PLA2 was reported to be the most potent at eliciting

this effect. Cellular protein and nucleic acid syntheses have been reported to be inhibited by the venom in a time- and dose-related manner (Ahmed *et al*., 2008).

The antivenom activity observed in the rats treated with extracts of *Olax manni, Urginea altissima* and *Xeromphis nilotica* extracts in this study showed that they all have effect against the venoms of all the five snake species.

Leaves, stem bark, roots, fruits and seeds extracts of some medicinal plants used in treatment of snake envenomation have been investigated and documented by many researchers (Venkata *et al*., 2015; Kadali and Kindangi, 2015; Bhavya *et al*., 2015).

The leaves extract of *Olax manni* was found to have anti venom activities against *Naja nigricollis*, *Naja* haje, *Naja katienses*, *Bitis arietans* and *Echis ocellatus* as investigated by (Abubakar *et al*., 2000) on the leaves of *Guiera senegalensis* (Combretacceae); (Mahadeswaraswamy *et al*., 2009) on the leaves of *Vitis vinifera L. (Vitaceae)*); (Shirwaikar *et al*., 2004), on the leaves of *Acalypha indica* (Euphoebiaceae); (Momoh, 2012), on the leaves of *Bridelia fergunia* (Euphorbiaceae); (Chandra, 2009) on the leaves of *Morus alba*; (Kadiyala, 2011) on the leaves of *Andrographis paniculata* and (Juliana *et al.*, 2014) on the leaves of *Jatropha gossypiifolia* L. (*Euphobiceae*).

The bulb of *Urginea altissima* was found to hava efficacy against the snake venom of *Naja nigricollis*, *Naja haje*, *Naja katienses, Bitis arietans* and *Echis ocellatus* as reported by (Ode and Asuzu, 2006) on the Bulb extract of *Crinum jagus* (Amaryllidaceae); (Gomes, 2007) on the root extracts of *Pluchea indica* (Asteraceae); (Lobo *et al*., 2006) on the root extract of *Clerodendrum viscosum* (Verbenaceae); (Esmeraldino *et al*., 2005) on the root extract of *Croton urucurana* (Euphorbiaceae)); (Mishal, 2002) on the root extract of

Dichorostachys cinerea (Mimosaceae); (Wannang *et al*., 2005) on the root extract of *Securdaca longipedunculata* (Polygalceae); (Amagon, 2012) on the root extract of *Parinari curatellifolia* (Chrysobalanaceae); (Alam, 1994) on the root extract of *Hemidesmus indicus* (Apocyanaceae).

5.2 Scientific Contributions to Knowledge

1 Phytochemical screening of the three plant extracts showed Saponins, Flavonoids, Glycosides, Tannins, Carbohydrates and Triterpens as common phytochemicals.

2 The three selected plants were found to have both chemotherapeutic (96.2%) and prophylactic (99.1%) antivenom effects against the five species of snake venoms in experimental rats. Aqueous fraction of *Urginea altissima* was found to have the highest (100%) efficacy with all the rats surviving envenomation for all the five species of snakes.

3 The five species of snakes were ranked based on the toxicity study (LD_{50} mg/kg) *Naja haje* was found to have the most toxic venom with the lowest LD_{50} value 0.22 followed by *Naja katiensis* 0.55; *Naja nigricollis* 0.87; *Echis ocellatus* 1.24 and *Bitis arietans* 1.80.

4 This research demonstrated the effect of the venom on coagulation time in minutes for the five species of snakes. There was no clotting with venoms of *Viparids* for 1440 minutes while that of *Elapids* allowed the clotting of blood with *Naja katiensis* have the lowest clotting time of 115, *Naja haje* 128, *Naja nigricollis* 132 respectively.

5 Studies on the effect of venom on Plasmid DNA migration on agarose gel electrophoresis in the presence of aqueous fractions demonstrated the efficacy of these plants on inhibiting the action of venom on plasmid DNA.

6 Among the methods of inoculation: Administering the plant extracts first 30 minutes before venom administration was found to be the best with 100% survival rate.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The study established that extracts of *Urginea altissima, Olax manni* and *Xeromphis nilotica* were found to have curative, prophylactic and detoxifying effects against the snake venoms in experimental rats.

2. The aqueous fraction of *Urginea altissima* showed the highest (100 %) activity with all the rats surviving envonemation.

3. Among the methods used in administering the plant extracts to the rats, Detoxification method was found to be the best method because more rats survived compared to the inoculation of venom first before extract administration or byadministraring extracts before venom administration.

4. The aqueous fractions of all the 3 plants were found to have the best antivenom activity obsarved which was clearly seen from the results where almost 100 % efficacy against the snake venom examined were seen when compared to the di-ethy-ether, ethyl-acetate or n-butanol extract fractions where we have more rat motality.

5. The Study established that *Urginea altissima* and *Olax manni* aqueous fraction neutralised the haemorrhagic effects of the venoms of all the snake species except that *Xeromphis nilotica* did not neutralize the effect of *Echis oscellatus* venom.

6. Studies on the effect of venom on plasmid DNA migration in the presence of extract of *Urginea altissima, Olax manni* and *Xeromphis nilotica* demonstrated the activity of these

plants where 100 % wells incubated with mixture of plasmid, venom and the extracts migrated on garose gel electrophoresis.

7. *Naja haje* was found to have the most toxic venom with the lowest LD_{50} of 0.22 mg/kg, while *Bitis arietans* was least toxic venom with highest LD_{50} of 1.80 mg/kg.

8. Coagulopathy was observed in the viper snakes; *Bitis arietans* and *Echis ocellatus* based on the blood clotting test.

6.2 Recommendations

Based on the findings of this study, the following recommendations are proferred:

1. Further research is recommended on these potential anti-venom plants to isolate the active components of the aqueous extracts of *Urginea altissima*.
2. The synthesis of the active ingredients into simpler and accessible form that can easily be available to snake bite victims is recommended.
3. It is recommended that the plants should be taken as prophylactic medication by people who work in areas with high population of venomous snakes.
4. There is need to gather and document all plants used by traditional healers as anti venom.
5. Further screening, investigation, validation and clinical trials should be conducted on these plants.

6. Governments at all levels should encourage traditional healers to pass information on plants having the anti snake venom properties especially to the local community where snake envenomation is high.

7. It is recommended that Government should work out and have comphrehensive documentary on the different species of venomous snakes and also have a National poisons control centers.

REFERENCES

Abbas, A. D., Abubakar, A. M. and Tahir, C. (2009). Snake-bite gangrene in children: A report of two cases. *Journal of Surgical Technique and Case Report*, 1(1): 39.

Abriol, S. (2011). S pitting cobra (*Naja nigricollis*) (PDF). *Herpetology* - Dr. Dever. *Neglected Tropical Diseases,*.

Abubakar, M. S., Balogun, E., Abdurahman, E. M., Nok, A. J., Shok, M., Mohammed, A. and Garba, M. (2006). Ethnomedical Treatment of Poisonous Snake bites: Plant Extract Neutralized *Naja nigricollis*. Venom. *Pharmaceutical Biology*, 44(5): 343–348. https://doi.org/10.1080/13880200600746253

Abubakar, M. S., Suel, M. I., Pateh, . E. M., Haruna, A. K. and Jahun, B. M. (2000b). *In vitro* snake venom detoxifying action of the leaf extract. *Journal of Ethnopharmacology*, 69(3):110- 115

Abubakar, S. B., Abubakar, I. S. and Nasidi, A. (2008). Randomized double blind comparative trial of two new antivenoms for the treatment of patients envenomed by the saw-scaled or carpet viper (*Echis ocellatus*) in northern Nigeria. Global Issues in Clinical Toxinology Conference, Nigeria.

Abubakar, Abubakar, B. S., Habib, Nasidi, Durfa and Yusuf, P. O. (2010). Randomised controlled double-blind non-inferiority trial of two antivenoms for saw-scaled or carpet viper (*Echis ocellatus*) envenoming in Nigeria. *Echi Tab Study Group. Toxicon*, *4*(7): 7-10

Adamia, S., Maxwell, C. A. and Pilarski, L. M. (2005). Hyaluronan and hyaluronan synthases: potential therapeutic targets in cancer. 5: 3–14.

Adeiza, A. A. and Minka, N. S. (2013). Ecological distribution of snakes and the prevalence of parasitic infestations and bacterial isolates from snakes captured within the guinea savannah zone of Nigeria. (PDF Download Available). Retrieved 27 April 2017, from https://www.researchgate.net/publication/259366432

Adzu, B., Abubakar, M. S., Izebe, K. S., Akumka, D. D. and Gamaniel, K. S. (2005). Effect of *Annona senegalensis* root bark extracts on *Naja nigricotlis nigricotlis* venom in rats. *Journal of Ethnopharmacology*, 96(3): 507–513. https://doi.org/10.1016/j.jep.2004.09.055

Ahmad., A. (2010). Anti-snake venom activity of different extract of *Pouzolzia indica*. *International Journal of Chem Tech Research*, *2*(1): 744–751.

Ahmed, S. M., Ahmed, M., Nadeem, A., Mahajan, J., Choudhary, A. and Pal, J. (2008). Emergency treatment of a snake bite: Pearls from literature. *Journal of Emergencies, Trauma and Shock*, 1(2): 97–105.

Aird, S. D. (2002). Ophidian envenomation strategies and the role of purines. *Toxicon*, 40: 335–393.

Ajagun, E. M. (2010). Morphological and molecular characterization of cobra and viper species in selected state of Northern Nigeria. *MSc Thesis, Ahmadu BelloUniversity, Zaria*.

Alam, A. (1994). Isolation, purification and partial characterization of viper venom inhibiting factor from the root extract of the Indian medicinal plant *Sarsaparilla. Toxicon*, 32(12): 1551–1557.

Alam, M. l. and Gomes, A. (2003). Snake venom neutralization by Indian medicinal plants. *Journal of Ethnopharmacology*, 86: 75–80.

Altimari, W. (1998). Venomous Snakes: A Safety Guide for Reptile Keepers. *Herpetoogical Circular*, 24: 26–30.

Amagon, K. (2012). Anti-snake venom activity of flavonoids from the root bark extract of *Parinari curatellifolia* in mice. *Journal of Pharmaceutical Research*, 4(2): 55–58.

Anai, K., Sugiki, M., Yoshida, E. and Maruyama, M. (2002). Neutralization of snake venom hemorrhagic metalloproteinase prevents coagulopathy after subcutaneous injection of *Bothrops jararaca* venom in rats. *Toxicon*, 40: 63–68.

Andrews, R. K., Gardiner, E. E., Asazuma, N., Berlanga, O., Tulasne, D., Nieswandt, B. and Watson, S. P. (2001). A novel viper venom metalloproteinase, alborhagin, is an agonist at the platelet collagen receptor GPVI. *Journal of Biological Chemistry*, 276: 28092–28097.

Andrews, R. K., Gardiner, E. E. and Berndt, M. C. (2004). Snake venom toxins affecting platelet function. *Methods of Molecular Biology*, 273:335–348.

Angel. (1922). Amphibian Species of the World. Retrieved 29 April 2017, from http://research.amnh.org/vz/herpetology/amphibia/Bibliography/A/Angel-1922-*Bull.-Mus.-Natl.-Hist.-Nat.-Paris*

Ariaratnam, C. A., Sheriff, M. H., , Theakston, R. D. and Warrell, D. A. (2008). Distinctive epidemiologic and clinical features of common krait (*Bungarus caeruleus*) bites in Sri Lanka. *American Journal of Tropical Medicine and Hygiene*, 79: 458–462.

Ariaratnam, C. A., Sheriff, M. H. R., Theakston, R. D. G., Arambepola, C. and Warrell, D. A. (2009). Syndromic approach to treatment of snake bite in Sri Lanka based on results of a prospective national hospital-based survey of patients envenomed by identified snakes. *American Journal of Tropical Medicine and Hygiene*, 81: 725–731.

Arni, R. K. and Ward, R. J. (1996). Phospholipase A2 – a structural review. *Toxicant*, 34: 827–841.

Arocas, V., Zingali, R. B., , Guillin, M. C., Bon, C. and Jandrot-Perrus, M. (1996). Bothrojaracin: a potent two-site directed thrombin inhibitor. *Biochemistry*, 35: 9083–9089.

Asuzu, I. U. and Harvey, A. L. (2003). The anti-snake venom activities of *Parkia biglandilosa* (*Mimosaceae*) stem bark extract. *Toxicant*, 42: 763–768.

Audebert, F., Sorkine, M., Robbe-Vincent, A. and Bon, C. (1994). Viper bites in France:clinical and biological evaluation; kinetics of envenomations. *Humman Experimental Toxicology*, 13: 683–688.

Aung, H. T., Toshiaki, N. N., Durfa and Yashiaki, T. (2010). Rosamarinic Acid in Argusia Argentea Inhibits Snake-Venom-Induced Haemorrhage. *Journal of Natural Medicine*, 64: 482–486.

Banwo, G. O., Abdullahi, I. and Duguryil, M. (2004). The antimicrobial activity of the stem bark of *Parkia clappertoniana* keay family Leguminosae against selected microorganisms. 3(1):16-22.

Barlow, C., Pook, C. E., Harrison, R. A. and Wüster, W. (2009). Coevolution of diet and prey-specific venom activity supports the role of selection in snake venom evolution. In *Proceeding of Biological Sciences* 76(2): 2443–2449).

Baumann, G. (2005). *'Photographic Guide to Wildflowers of Malawi' Wildlife and Environmental Society of Malawi.* Wildlife and Environmental Society of Malawi.

Bee, A., Theakston, R. D. G., Harrison, R. A. and Carter, S. D. (2001). Novel *in vitro* assays for assessing the haemorrhagic activity of snake venoms and for demonstration of venom metalloproteinase inhibitors. *Toxicon*, 39: 1429–1434.

Behler, J. L. and King, F. (1979). The audubon society field guide to north american reptiles and amphibians. *New York.*

Bergmeier, W., Bouvard, D., Eble, J. A., Mokhtari-Nejad, R., Schulte, V., A. G. H., H. and Nieswandt, B. (2001). Rhodocytin (aggretin) activates platelets lacking α2β1 integrin, glycoprotein VI, and the ligand-binding domain of glycoprotein. *Journal of Biological Chemistry*, 25121–25126.

Bhavya, J., Vincetha, M. S., Dhananjaya, B. L. and Sunil, S. M. (2015). Anti-snake venom roperties of medicinal plants. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7: 21–26.

Bilgrami, S., Tomar, S., Yadav, S., Kaur, P., Kumar, J., Jabeen, T. and Singh, T. P. (2004). Crystal structure of schistatin, a disintegrin homodimer from saw-scaled viper (*Echis ocellatus*) at 2.5 A˚resolution. *Journal of Molecular Biology*, 341: 829–837.

Biondo, R., Pereira, A. M. S., Marcussi, S., Pereira, P. S., França, S. C. and Soare, A. M. (2003). Inhibition of enzymatic and pharmacological activities of some snake venoms and toxins by *Mandevilla velutina* (Apocynaceae) aqueous extract. *Biochimie*, 85: 1017–1025.

Boback, S. M. and Guyer, C. (2003). Empirical Evidence for an Optimal Body Size in Snakes. *Evolution*, 57(2): 345–351.

Bocage, J. V. and Barboza, D. (1895). *Herpetologie d' Angota et du Congo*. Imprimerie Nationale, Lisbon.

Bolger, M. B., Swenson, S. and Markland, F. S. (2001). Three dimensional structure of fibrolase, the fibrinolytic enzyme from Southern Copperhead Venom, modeled from the X-Ray structure of Adamalysin II and Atrolysin C. *Journal of Pharaceutical Science*, 3(2): 16.

Booth, w, Million, L., Reynolds, R. G., Burghardt, G. M., Vargo, E. L., Schal, C. and Schuett, G. W. (2011). Consecutive virgin births in the new world boid snake, the Colombian rainbow Boa, *Epicrates maurus*. *Journal of Heredity*, 102(6): 759–63.

Booth, W., Smith, C. F., Eskridge, P. H., Hoss, S. K., Mendelson, J. R. and Schuett, G. W. (2012). Facultative parthenogenesis discovered in wild vertebrates. *Biology Letters*, *8*(6): 983–5.

Borges, M. H., Soares, A. M. and Rodrigues, V. M. (2000). Effects of aqueous extract of *Casearia sylvestris* (Flacourtiaceae) on action of snake and bee venoms and on activity of phospholipases A2. *Comparative Biochemistry and Physiology*, 127: 21–30.

Bos, M. H. A., Boltz, M. and Pierre, S. (2009). Response to "Clinical relevance of brown snake (Pseudonaja spp) factor V escaping hemostatic regulation. *Blood*, 114: 2563–2564.

Boulenger. (1896). Ser. 6, 18 | Amphibian Species of the World. Retrieved 29 April 2017, from http://research.amnh.org/vz/herpetology/amphibia/Bibliography/B/Boulenger-1896-Ann.-Mag.-Nat.-Hist.-Ser.-67

Boycott, R. C. and Haacke, W. D. (1979). Note on the type-locality, distribution and juvenile colouration of *Naja nigricollis* woodi (Serpentes: Elapsidae) and an account of the colour- pattern variation in intergrade populations. *Annals of the Cape Provincial Museums (Natural History)*, 13: 31–38.

Branch, B. (2004). *Field Guide to Snakes and Other Reptiles of Southern Africa. Third Revised edition, Second impression*. Sanibel Island, Florida: Ralph Curtis Books.

Branch, W. R. (1979). The venomous snakes of South Africa part 2. *Elapidae and Hydrophidae Snakes*, 11: 199–225.

Braud, S., Bon, C. and Wisner, A. (2000). Snake venom proteins acting on hemostasis. *Biochimie*, 82: 851–859.

Braud, S., LeBonniec, B. F., Bon, C. and Wisner, A. (2002). The stratagem utilized by the plasminogen activator from the snake *Trimeresurus stejnegeri* to escape serpins. *Biochemistry*, 41: 8478–8484.

Broadley, D. G. (1968). A review of the African cobras of the genus *Naja* (Serpentes: Elapidae). *Arnoldia*, 3: 1–14.

Broadley, D. G. (1990). *FitzSimons' Snakes of Southern Africa*. Parklands South Africa: J Ball and A. D. Donker Publishers.

Broadley, D. G. and Cock, E. V. (1975). *Snakes of Rhodesia. Zimbabwe*. Zimbabwe: Longman Ltd.

Broadley, D. G. and Wuster, W. (1974). A review of the cobra of the *Naja nigricollis* complex in South- wastern African. *Cimbebasia*, (A)(2): 155–162.

Broadley, D. G. and Wuster, W. (2004). A review of the Southern African non-spitting cobra (Serpentes: Elapidae: *Naja*). *African Journal of Herpetology*, 53: 101– 122.

Brown, J. H. (1973). *Toxicology and Pharmacology of Venoms from Poisonous Snakes*. Springfield, Illinois: Charles C. Thomas.

Brunda, G. and Sashidhar, R. B. (2007). Epidemiological profile of snake-bite cases from Andhra Pradesh using immunoanalytical approach. *Indian Journal of Medical Reearch*, 125: 661–668.

Burbrink, F. T., Lawson, R. and Slowinski, J. B. (2000). Mitochondrial DNA phylogeography of the polytypic North American Rat Snake (Elaphe obsoleta): a critique of the subspecies concept. *Evolution*, 54: 2107–2118.

Calvete, J. J., Juárez, P. and Sanz, L. (2007). Snake venomics. Strategy and applications. *Journal of Mass Spectrometry*, 42(11): 1405–1414. https://doi.org/10.1002/jms.1242

Canale, E., Isbister, G. K. and Currie, B. J. (2009). Investigating pressure bandaging for snakebite in a simulated setting: Bandage type, training and the effect of transport. *Emergency Medicine Australasia*, 21(3): 184–190. https://doi.org/10.1111/j.1742-6723.2009.01180.x

Castoe, T. A. and Parkinson, C. L. (2006). Bayesian mixed models and the phylogeny of pitvipers (Viperidae: Serpentes). *Molecular Phylogenetics and Evolution*, 39(1): 91–110. https://doi.org/10.1016/j.ympev.2005.12.014

Castoe, T. C., Sasa, M. and Parkinson, C. L. (2005). Modeling nucleotide evolution at the mesoscale: The phylogeny of the Neotropical pitvipers of the Porthidium group (Viperidae: Crotalinae). *Molecular Phylogenetics and Evolution*, 37: 881–898.

Castro, H. C., Zingali, R. B., Albuquerque, M. G., Pujol-Luz, M. and Rodrigues, C. R. (2004). Snake venom thrombin-like enzymes: from reptilase to now. *Cellular and Molecular Life Sciences CMLS*, *61*(7–8), 843–856. https://doi.org/10.1007/s00018-003-3325-z

Castro, H. C., Fernandes, M. and Zingali, R. B. (1999). Identification of bothrojaracin like proteins in snake venoms from Bothrops species and *Lachesis muta*. *Toxicon*, 37: 1403–1416.

Chandra, S. K. T. (2009). Neutrilization of local and systemic toxicity of Daboia russelli venom by *Morus alba* plant leaf extract. *Phytotherapy Research*, 23(8): 1082–1087.

Chappuis, F., Sharma, S. K., Jha, N., Loutan, L. and Bovier, P. A. (2007). Protection against Snake Bites by Sleeping under a Bed Net in Southeastern Nepal. *The American Journal of Tropical Medicine and Hygiene*, 77(1): 197–199. https://doi.org/10.4269/ajtmh.2007.77.197

Chatterjee, I., Chakravarty, A. K., and Gomes, A. (2004). Antisnake venom activity of ethanolic seed extract of *Strychnos* nux vomica Linn. *IJEB* 42(05). Retrieved from http://nopr.niscair.res.in/handle/123456789/23434

Cher, C. D. N., Armugam, A., Zhu, Y. Z. and Jeyaseelan, K. (2005). Molecular basis of cardiotoxicity upon cobra envenomation. *Cellular and Molecular Life Sciences: CMLS*, 62(1): 105–118. https://doi.org/10.1007/s00018-004-4352-0

Cheresh, D. A. (1998). Death to a blood vessel, death to a tumor. *Nature Medicine*, 4(4): 395–396. https://doi.org/10.1038/nm0498-395

Cherlin, V. A. (1990). [A taxonomic revision of the snake genus *Echis* (Viperidae). II. An analysis of taxonomy and description of new forms]. *Proc. Zool. Inst. Leningrad*, 207: 193–223.

Chioato, L. and Ward, R. J. (2003). Mapping structural determinants of biological activities in snake venom phospholipases A2 by sequence analysis and site directed mutagenesis. *Toxicon: Official Journal of the International Society on Toxinology*, 42(8): 869–883. https://doi.org/10.1016/j.toxicon.2003.11.027

Chippaux, J. P. (1998). Snake-bites: appraisal of the global situation. *Bulletin of the World Health Organization*, 76(5): 515–524.

Chippaux, J. P., Williams, V. and White, J. (1991). Snake venom variability: methods of study, results and interpretation. *Toxicon: Official Journal of the International Society on Toxinology*, 29(11): 1279–1303.

Chirio, L. and Ivan, I. (2006). Biogeography of the reptiles of the Central African Republic. *African Journal of Herpetology*, 55(1): 23–59.

Chung, C. H., Wu, W. B. and Huang, T. F. (2004). Aggretin, a snake venom-derived endothelial integrin a2b1 agonist, induces angiogenesis via expression of vascular endothelial growth factor Blood. *Blood*, 103: 2105–2113.

Clemetson, K. J., Navdaev, A., Dormann, D. and Clemetson, J. M. (2002). Multifunctional snake C-type lectins affecting platelets. *Haemostasis*, 31: 148–154.

Cominetti, M. R., Terruggi, C. H. B., Ramos, O. H. P., Fox, J. W., Mariano-Oliveira, A., Freitas, M. S. D. and Selistre-de-Araujo, H. S. (2004). Alternagin-C, a Disintegrin-like Protein, Induces Vascular Endothelial Cell Growth Factor (VEGF) Expression and Endothelial Cell Proliferation in Vitro. *Journal of Biological Chemistry*, 279(18): 18247–18255. https://doi.org/10.1074/jbc.M311771200

Currie, B. J. (2004). Snake bite in tropical Australia: a prospective study in the 'Top End' of the Northern Territory. *Medical Journal of Australia*, 181(11). Retrieved from https://www.mja.com.au/journal/2004/181/11/snakebite-tropical-australia-prospective-study-top-end-northern-territory

Dalziel, J. M. (1955). The Useful Plants of West Tropical Africa. Crown Agents for Oversea Governments and Administrations, London, UK, 70–71.

Daros, M. do D., Matos, F. J. and Parente, J. P. (1996). A new triterpenoid saponin, bredemeyeroside B. from the roots of *Bredemeyera floribunda*. *Planta Medica*, 62(6): 523–527.

de Roodt, A. R., Litwin, S. and Angel, S. O. (2003). Hydrolysis of DNA by 17 snake venoms. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology: CBP*, 135(4): 469–479.

Denwick, P. M. (2002). *Natural Products: A Biosynthetic Approach. 2nd Ed*. John Wiley and Sons Ltd, England.

Dhananjaya, B. L., Zameer, F., Girish, K. S. and D'Souza, C. J. M. (2011). Anti-venom potential of aqueous extract of stem bark of Mangifera indica L. against *Daboia russellii* (Russell's viper) venom. *Indian Journal of Biochemistry & Biophysics*, 48(3): 175–183.

Ditmars, R. L. (1933). *Reptiles of the world: the crocodilians, lizards, snakes, turtles and tortoises of the Eastern and Western hemispheres*. New York: Macmillan.

Dong–Zong, H., Tain–Chen, W. J. and Fang, D. (1997). The painful experience of inappropriate therapy of snake bites: A report of two cases. *Chineese Medical. Journal*, 60: 326–330.

Dörmann, D., Clemetson, J. M., Navdaev, A., Kehrel, B. E. and Clemetson, K. J. (2001). Alboaggregin A activates platelets by a mechanism involving glycoprotein VI as well as glycoprotein Ib. *Blood*, 97(4): 929–936.

Du, X. Y., Navdaev, A., Clemetson, J. M., Magnenat, E., Wells, T. N. and Clemetson, K. J. (2001). Bilinexin, a snakeC-type lectin from *Agkistrodon bilineatus* venom agglutinates platelets via GPIb and a2b1. *Journal of Thrombosis and Haemostasis*, 86: 1277–1283.

Du, X.-Y., Clemetson, J. M., Navdaev, A., Magnenat, E. M., Wells, T. N. C. and Clemetson, K. J. (2002). Ophioluxin, a convulxin-like C-type lectin from Ophiophagus hannah (King cobra) is a powerful platelet activator via glycoprotein VI. *The Journal of Biological Chemistry*, 277(38): 35124–35132. https://doi.org/10.1074/jbc.M204372200

Du, X.-Y. and Clemetson, K. J. (2002). Snake venom L-amino acid oxidases. *Toxicon: Official Journal of the International Society on Toxinology*, 40(6): 659–665.

Endo, T. and Tamiya, N. (1991). Structure-function relationships of postsynaptic neurotoxins from snake venoms. In *Structure-function relationships of postsynaptic neurotoxins from snake venoms* (Harvey AL (ed), pp. 165–222). Oxford: Snake toxins, Pergamon Press.

Ernst, C. H. and Zug, R. (1996). Snake bites and Venom. Retrieved from www.thesnake.org

Esmeraldino, L. E., Souza, A. M. and Sampaio, S. V. (2005). Evaluation of the effect of aqueous extract of *Croton urucurana Baillon* (Euphorbiaceae) on the hemorrhagic activity induced by the venom of *Bothrops jararaca*, using new techniques to quantify hemorrhagic activity in rat skin. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, *12*(8): 570–576. https://doi.org/10.1016/j.phymed.2004.01.012

Fan, H. W. and Cardoso, J. L. (1995). Clinical Toxicology of Snake bites in South America. In *Clinical Toxicology of Snake bites in South America* (In Meier J, White J Eds, pp. 667–88). Florida CRC Press: Handbook of clinical Toxicology of Animal Venoms and Poisons.

Fatima, L. D. and Fatah, C. (2014). Pathophysiological and Pharmacological Effects of Snake Venom Components: Molecular Targets. *Journal of Clinical Toxicology*, 4(2). https://doi.org/10.4172/2161-0495.1000190

Ferreira, L. A., Henriques, O. B., Andreoni, A. A., Vital, G. R., Campos, M. M., Habermehl, G. G. and de Moraes, V. L. (1992). Antivenom and biological effects of ar-turmerone isolated from *Curcuma longa* (Zingiberaceae). *Toxicon: Official Journal of the International Society on Toxinology*, 30(10): 1211–1218.

Filippi, E. and Petretto, M. (2013). *Naja haje* (Egyptian Cobra) Diet/Ophiophagy in Herpetological, 44: 155–156.

Fry, B. G. (2005). From genome to 'venome': molecular origin and evolution of the snake venom proteome inferred from phylogenetic analysis of toxin sequences and related body proteins. *Genome Research*, 15: 403–420.

Fry, B. G., Vidal, N., van der Weerd, L., Kochva, E. and Renjifo, C. (2009). Evolution and diversification of the Toxicofera reptile venom system. *Journal of Proteomics*, 72(2): 127–136. https://doi.org/10.1016/j.jprot.2009.01.009

Fujii, Y., Okuda, D., Fujimoto, Z., Horii, K., Morita, T. and Mizuno, H. (2003). Crystal structure of trimestatin, a disintegrin containing a cell adhesion recognition motif RGD. *Journal of Molecular Biology*, 332(5): 1115–1122.

Fukuda, K., Doggett, T. A., Bankston, L. A., Cruz, M. A., Diacovo, T. G. and Liddington, R. C. (2002). Structural Basis of von Willebrand Factor Activation by the Snake Toxin Botrocetin. *Structure*, 10(7): 943–950. https://doi.org/10.1016/S0969-2126(02)00787-6

Gempeler-Messina, P. M., Volz, K., Bühler, B. and Müller, C. (2001). Protein C activators from snake venoms and their diagnostic use. *Haemostasis*, 31(3–6): 266–272. https://doi.org/48072

George, J. and Stern, R. (2004). Serum hyaluronan and hyaluronidase: very early markers of toxic liver injury. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, 348(1–2): 189–197. https://doi.org/10.1016/j.cccn.2004.05.018

Gillissen, A., Theakston, R. D., Barth, J., May, B., Krieg, M. and Warrell, D. A. (1994). Neurotoxicity, haemostatic disturbances and haemolytic anaemia after a bite by a Tunisian saw-scaled or carpet viper (Echis 'pyramidum'-complex): failure of antivenom treatment. *Toxicon: Official Journal of the International Society on Toxinology*, 32(8): 937–944.

Girish, K. S., Jagadeesha, D. K., Rajeev, K. B. and Kemparaju, K. (2002). Snake venom hyaluronidase: an evidence for isoforms and extracellular matrix degradation. *Molecular and Cellular Biochemistry*, 240(1–2): 105–110.

Girish, K. S. and Kemparaju, K. (2005). A low molecular weight isoform of hyaluronidase: purification from Indian cobra (*Naja naja*) venom and partial characterization. *Biochemistry. Biokhimiia*, 70(6): 708–712.

Girish, K. S. and Kemparaju, K. (2006). Inhibition of *Naja naja* venom hyaluronidase: role in the management of poisonous bite. *Life Sciences*, 78(13): 1433–1440. https://doi.org/10.1016/j.lfs.2005.07.015

Girish, K. S., Mohanakumari, H. P., Nagaraju, S., Vishwanath, B. S. and Kemparaju, K. (2004). Hyaluronidase and protease activities from Indian snake venoms: neutralization by *Mimosa pudica* root extract. *Fitoterapia*, 75(3–4): 378–380. https://doi.org/10.1016/j.fitote.2004.01.006

Girish, K., Shashidharamurthy, R., Gowda, T. and Kemparaju, K. (2004). Isolation and characterization of hyaluronidase a 'spreading factor' from Indian cobra (*Naja naja*) venom. *Biochimie* pp 193–202.

Golubkov, V., Hawes, D. and Markland, F. S. (2003). Anti-Angiogenic Activity of Contortrostatin, a Disintegrin from *Agkistrodon Contortrix Contortrix* Snake Venom. *Angiogenesis*, 6(3): 213–224. https://doi.org/10.1023/B:AGEN.0000021396.47009.b0

Good, D. A. (1994). Species limits in the genus *Gerrhonotus* (Squamata: Anguidae). *Herprtological Monographs*, 7: 180–202.

Gowda, T. V. and Middlebrook, J. L. (1993). Effects of myonecrotic snake venom phospholipase A2 toxins on cultured muscle cells. *Toxicon: Official Journal of the International Society on Toxinology*, 31(10): 1267–1278.

Grams, F., Huber, R., Kress, L. F., Moroder, L. and Bode, W. (1993). Activation of snake venom metalloproteinases by a cysteine switch-like mechanism. *FEBS Letters*, 335(1): 76–80.

Greene, H. W. (1997). *Snakes: The Evolution of Mystery in Nature*. Berkeley London: University of California Press Ltd.

Guitterez J M. (1995). *Handbook of Clinical Toxicology of Animal Venoms and Poisons*. Florida, CRC Press.

Günther, A. (1894). May 1, 1894. *Journal of Zoology*, 62(1): 390–455. https://doi.org/10.1111/j.1469-7998.1894.tb00577.x

Gupta, Y. K. and Peshin, S. S. (2012). Do Herbal Medicines Have Potential for Managing Snake Bite Envenomation? *Toxicology International*, 19(2): 89–99. https://doi.org/10.4103/0971-6580.97194

Gutiérrez, J. M. (2002). Understanding snake venoms: 50 years of research in Latin America. *Revsta de Biologia Tropical*, 50: 377–394.

Gutiérrez, J. M. and Ownby, C. L. (2003). Skeletal muscle degeneration induced by venom phospholipases A2: insights into the mechanisms of local and systemic myotoxicity. *Toxicon: Official Journal of the International Society on Toxinology*, 42(8): 915–931. https://doi.org/10.1016/j.toxicon.2003.11.005

Gutiérrez, J. M., Rucavado, A., Chaves, F., Díaz, C. and Escalante, T. (2009). Experimental pathology of local tissue damage induced by *Bothrops asper* snake venom. *Toxicon: Official Journal of the International Society on Toxinology*, 54(7): 958–975. https://doi.org/10.1016/j.toxicon.2009.01.038

Gutiérrez, J. M., Rucavado, A., Escalante, T. and Díaz, C. (2005). Hemorrhage induced by snake venom metalloproteinases: biochemical and biophysical mechanisms involved in microvessel damage. *Toxicon: Official Journal of the International Society on Toxinology*, 45(8): 997–1011. https://doi.org/10.1016/j.toxicon.2005.02.029

Gutiérrez, J. M., Theakston, R. D. G. and Warrell, D. A. (2006). Confronting the Neglected Problem of Snake Bite Envenoming: The Need for a Global Partnership. *PLOS Medicine*, 3(6), e150. https://doi.org/10.1371/journal.pmed.0030150

Gutiérrez, J. M., Warrell, D. A., Williams, D. J., Jensen, S., Brown, N., Calvete, J. J. and Initiative, for the G. S. (2013). The Need for Full Integration of Snake bite Envenoming within a Global Strategy to Combat the Neglected Tropical Diseases: The Way Forward. *PLOS Neglected Tropical Diseases*, 7(6): e2162. https://doi.org/10.1371/journal.pntd.0002162

Habib, A. G. (2013). Public health aspects of snake bite care in West Africa: perspectives from Nigeria. *The Journal of Venomous Animals and Toxins Including Tropical Diseases*, 19: 27. https://doi.org/10.1186/1678-9199-19-27

Habib, A. G., Abubakar, S. B., Abubakar, I. S., Larnyang, S., Durfa, N., Nasidi, A. and Warrell, D. A. (2008). Envenoming after carpet viper (*Echis ocellatus*) bite during pregnancy: timely use of effective antivenom improves maternal and foetal outcomes. *Tropical Medicine & International Health : TM & IH*, 13(9): 1172–1175. https://doi.org/10.1111/j.1365-3156.2008.02122.x

Habib, A. G., Gebi, U. I. and Onyemelukwe, G. C. (2001). Snake bite in Nigeria. *African Journal of Medicine and Medical Sciences*, 30(3): 171–178.

Hamako, J., Matsui, T., AVijay-Kumar, S., Nomura, S., Fujimura, Y., Ito, M. and Titani, K. (1998). Purification and characterization of kaouthiagin, a von Willebrand factor-binding and -cleaving metalloproteinase from *Naha kaouthia* cobra venom. *Journal of Thrombosis and Haemostasis*, 80: 499–505.

Harborne, J. B. and Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry*, 55(6): 481–504.

Harris, J. B. and Scott-Davey, T. (2013). Secreted phospholipases A2 of snake venoms: effects on the peripheral neuromuscular system with comments on the role of phospholipases A2 in disorders of the CNS and their uses in industry. *Toxins*, 5(12): 2533–2571. https://doi.org/10.3390/toxins5122533

Harrison, R. A., Hargreaves, A., Wagstaff, S. C., Faragher, B. and Lalloo, D. G. (2009). Snake envenoming: a disease of poverty. *PLoS Neglected Tropical Diseases*, 3(12): e569. https://doi.org/10.1371/journal.pntd.0000569

Harvey, A. L., Bradley, K. N., Cochran, S. A., Rowan, E. G., Pratt, J. A., Quillfeldt, J. A. and Jerusalinsky, D. A. (1998). What can toxins tell us for drug discovery? *Toxicon: Official Journal of the International Society on Toxinology*, 36(11): 1635–1640.

Hati, R., Mitra, P., Sarker, S. and Bhattacharyya, K. K. (1999). Snake venom hemorrhagins. *Critical Reviews in Toxicology*, 29(1): 1–19. https://doi.org/10.1080/10408449991349168

Havesteen, B. J. (1990). Flavonoids a class of natural products for antimicrobial agents . *Journal of EuropeanMicrobial Infectious Diseases*. 9: 455–61.

Head, J. J., Bloch, J. I., Hastings, A. K., Bourque, J. R., Cadena, E. A., Herrera, F. A. and Jaramillo, C. A. (2009). Giant boid snake from the Palaeocene neotropics reveals hotter past equatorial temperatures. *Nature*, 457(7230): 715–717. https://doi.org/10.1038/nature07671

Hedges, S. B. (2008). At the lower size limit in snakes: two new species of thread snakes (Squamata: Leptotyphlopidae: Leptotyphlops) from the Lesser Antilles" (PDF). *Zootaxa*, 1841: 1–30.

Hite, L. A., Jia, L. G., Bjarnason, J. B. and Fox, J. W. (1994). cDNA sequences for four snake venom metalloproteinases: structure, classification, and their relationship to mammalian reproductive proteins. *Archives of Biochemistry and Biophysics*, 308(1): 182–191. https://doi.org/10.1006/abbi.1994.1026

Ho, M., Warrell, M. J., Warrell, D. A., Bidwell, D. and Voller, A. (1986). A critical reappraisal of the use of enzyme-linked immunosorbent assays in the study of snake bite. *Toxicon: Official Journal of the International Society on Toxinology*, 24(3): 211–221.

HomeoVision. (2011). *Effects of venomous snakes*. Retrieved from http://www.homeovision.org

Hoser, R. (2009). A reclassification of the True Cobras; species formerly referred to the genera Naja, Boulengerina and Paranaja. *Australasian Journal of Herpetology*, 7: 1–15.

Hoso, M., Asami, T. and Hori, M. (2007). Right-handed snakes: convergent evolution of asymmetry for functional specialization. *Biology Letters*, 3(2): 169–173. https://doi.org/10.1098/rsbl.2006.0600

Hughes, B. (1983). African snake fauna. Bonner Zoologische Beitrage. 34: 311–356.

Hughes, B. and Barry, D. H. (1969). The snakes of Ghana. *Bulletin de L Institut Foundamental D' Afri Que Noire,* 31: 1004–1041.

Humane Society International (HSI). (2014). Humane Society International Annual Welfare guidelines. Retrieved from HIS.org

Ibrahim, M. A., Aliyu, A. B., Abusufiyanu, A., Bashir, M. and Sallau, A. B. (2011). Inhibition of *Naja nigricollis* (Reindhardt) venom protease activity by *Luffa egyptiaca* (Mill) and *Nicotiana rustica* (Linn) extracts. *Indian Journal of Experimental Biology*, 49: 552–554.

Irvine, F. R. (1961). *Woody plants of Ghana: with special reference to their uses*. Oxford University Press.

Isbell, L. A. (2006). Snakes as agents of evolutionary change in primate brains. *Journal of Human Evolution*, 51(1): 1–35. https://doi.org/10.1016/j.jhevol.2005.12.012

Isbister, G. K. (2009). Procoagulant snake toxins: laboratory studies, diagnosis, and understanding snake bite coagulopathy. *Seminars in Thrombosis and Hemostasis*, 35(1): 93–103. https://doi.org/10.1055/s-0029-1214152

Isbister, G. K., O'Leary, M. A., Schneider, J. J., Brown, S. G. A., Currie, B. J. and ASP Investigators. (2007). Efficacy of antivenom against the procoagulant effect of Australian brown snake (Pseudonaja sp.) venom: *in vivo* and *in vitro* studies. *Toxicon: Official Journal of the International Society on Toxinology*, 49(1): 57–67. https://doi.org/10.1016/j.toxicon.2006.09.007

Isbister, G. K., Woods, D., Alley, S., O'Leary, M. A., Seldon, M. and Lincz, L. (2008). Endogenous thrombin potential as a novel method for the characterization of procoagulant snake venoms. In: Haematology Society of Australia and New Zealand Annual Conference.

Itano, N. and Kimata, K. (2002). Mammalian hyaluronan synthases. *IUBMB Life*, 54(4): 195–199. https://doi.org/10.1080/15216540214929

Iwu, M. M. (2014). *Handbook of African Medicinal Plants, Second Edition*. CRC Press.

Izidoro, L. F. and Rodrigues, V. M. (2003). Neutralization of some hematological and hemostatic alterations induced by neuwiedase, a metalloproteinase isolated from Bothropsneuwiedipauloensis snake venom, by the aqueous extract from *Casearia mariquitensis* (Flacourtiaceae). *Biochimie*, 85: 669–675.

James, O., Godwin, E. U. and Agah, O. D. (2013). Anti-venom studies on *Olax viridis* and *Syzygium guineense* extracts. *American Journal of Pharmacology and Toxicology*, 8(1): 1–8. https://doi.org/10.3844/ajptsp.2013.1.8

Jia, L. G., Shimokawa, K., Bjarnason, J. B. and Fox, J. W. (1996). Snake venom metalloproteinases: structure, function and relationship to the ADAMs family of proteins. *Toxicon: Official Journal of the International Society on Toxinology*, 34(11–12): 1269–1276.

Jorge da Silva N Jr. and Aird S D. (2001). Prey specificity, comparative lethality and compositional differences of coral snake venoms. Comparative Biochemistry, Physiology Part C Toxicolology Pharmacology, 128: 425–456.

Juan, J., Calvet, P. J. and Libia, S. (2007). Snake venomics. Strategy and applications. *Journal of Mass Spectrometry*, 42: 1405–1414.

Juliana, F. S., Thiago, S., Yamara, A. S. M., Barbara, C., Rafael, B. G. C., Arnabio, A. S. J. and Matheus, F. F. P. (2014). Aqueous extract of *Jatropha gossypiifolia* L. (Euphobiaceae) inhibit enzymatic and biological action of *Bothrops jararaca* snake venom. *PLoS ONE*, 9(8).

Kadali, V. N. and Kindangi, K. R. (2015). Ethno-medicinal plants used by the traditional healers of West Godavari District, Andhra Pradesh, India. *Journal of Pharmacognosy and Phytochemistry*, 3(6): 117–118.

Kadiyala, G. (2011). The neutralization effect of methanolic extract of *Andrographis paniculata* on Indian cobra *Naja naja* snake venom. *Journal of Pharmacy Research*, 4(4): 1110–1012.

Kalafatis, M., Beck, D. O. and Mann, K. G. (2003). Structural requirements for expression of factor Va activity. *Journal of BiologicalChemistry*, 278: 50–61.

Kamiguti, A. S., Hay, C. R. and Zuzel, M. (1996). Inhibition of collagen-induced platelet aggregation as the result of cleavage of a2b1-integrin by the snake venom metalloproteinase jararhagin. , 320: 635–641. *Journal of Biochemisry*, 320: 635–641.

Kanaji, S., Kanaji, T., Furihata, K., Kato, K., Ware, J. L. and Kunicki, T. J. (2003). Convulxin binds to native, human glycoprotein Iba. *Journal of Biological Chemistry*, 278: 39452–39460.

Karlin A. (2002). Emerging structure of the nicotinic acetylcholine receptors. *Nature Reviews Neuroscience*, 3: 102–114.

Kasturiratne, A., Wickremasinghe, A. R. and Silva, N. (2008). The global burden of Snake bite: a literature analysis and modelling based on regional estimates of envenoming and deaths. *PLOS Medicine*, 5: 218.

Keay, R. W. J. (1958). Randia and Gardenia in West Africa. *Bulletin Du Jardin Botanique de l'État a Bruxelles*, 28(1): 15–72. https://doi.org/10.2307/3667018

Kellerman, T. S. J. and Coetzer, A. W. (2005). 'Plant poisonings and mycotoxicoses of livestock in southern Africa'. *Oxford University Press*.

Kharade, A. S., Jadhav, S. S., Jadhav, S. N., Thite, S. V. and Aparadh, V. T. (2013). Phytochemical Investigation in Commelinea bengalensis and Cyanotiscristata. *International Research Journal of Pharmacology and Applied Science*, 3(1): 46–48.

Kini, R. M. (1997). Venom Phospholipase A2 Enzymes: Structure, Function and Mechanism. *Journal, Wiley: New York*.

Kini, R. M. (2003). Excitement ahead: structure, function and mechanism of snake venom phospholipase A2 enzymes. *Toxicon*, 42: 827–40.

Kini, R. M. (2005). Structure-function relationships and mechanism of anticoagulant Phospholipase A2 enzymes from snake venoms. *Toxicon*, 45: 1147–1161.

Kirby, G. (2013). *'Wild Flowers of Southeast Botswana'*. Cape Town South Africa: Struik Nature.

Koh, D. C. I., Armugam, A. and Jeyaseelan, K. (2006). Snake venom components and their applications in biomedicine. *Cellular and Molecular Life Sciences*, 63: 3030.

Kokwaro, J. O. (1993). *Medicinal plants of East Africa*. Kenya literature bureau.

Kuch, U., Müller, J., Mödden, C. and Mebs, D. (2006). Snake fangs from the Lower Miocene of Germany: evolutionary stability of perfect weapons. *Naturwissenschaften*, 93: 84–87.

Kumar, A., Ilavarasn, R., Jayachandran, T., Decaraman, M., Aravindhan, P., Padmanaban, N. and Krishnan, M. R. V. (2009). Phytochemical investigation on a tropical plant. *Pakistan Journal of Nutrition*, 8: 83–85.

Kuruppu, S., Smith, A. I., Isbister, G. K. and Hodgson, W. C. (2008). Neurotoxins from Australo-Papuan elapids: a biochemical and pharmacological perspective. *Critical Reviews in Toxicology*, 38: 73–86.

Laing, G. D., Kamiguti, A. S., Wilkinson, M. C., Lowe, G. M. and Theakston, R. D. (1995). Characterisation of a purified phospholipase A2 from the venom of the Papuan black snake (*Pseudechis papuanus*). *Biochimica et Biophysica Acta*, 1250: 137–143.

Lans, C., Harper, T., Georges, K., Bridgewater, E. B. M. C. and Compliment, J. (2001). Alternative Medicine, 1: 10.

Laurent, R. F. (1955). Diagnoses preliminaires de quliques serpents venimeux. *Revue de Zoologie et Botanique Africaine*, 51: 127–139.

Laurich, C., Wheeler, M. A., Iida, J., Neudauer, C. L., McCarthy, J. B. and Bullard, K. M. (2004). Hyaluronan mediates adhesion of metastatic colon carcinoma cells. *Journal of Surgical Research*, 122: 70–74.

Lee, W. H., Clemetson, K. J. and Du X, Y. L. Q. M. (2003). A novel snake C-type lectin-like protein from Trimeresurus stejnegeri venom is a potent platelet agonist acting specifically via GPVI. *Journal of Thrombosis and Haemostasis*, (90): 662–671.

Leon, G., Abubakar, B. S., Alape, A., Rojas, G. and and Gutierrez, J. M. (1999). Comparative study on the ability of IgG and F(ab)2 antivenoms to neutralize lethal and myotoxic effects induced by *Micrurus nigrocinctus* (coral snake) venom. *American Journal of Tropical Medicine and Hygiene*, 61: 266–271.

Leon, G., Rojas, G., Lomonte, B. and Gutierrez, J. M. (1997). ImmunoglobulinG and F(ab)2 polyvalent antivenoms do not differ in their ability to neutralize hemorrhage, edema and myonecrosis induced by *Bothrops asper* (terciopelo) snake venom. *Toxicon*, 35: 1627–1637.

Leon G, Valverde J M, Rojas G, Lomonte B. and Gutierrez J M. (2000). Comparative study on the ability of IgG and Fab sheep antivenoms to neutralize local hemorrhage, edema and myonecrosis induced by *Bothrops asper* (terciopelo) snake venom. *Toxicon*, 38: 233–244.

Lewis, R. (2006). *Protein Scaffolds in Snake Venoms. p49. In: 15th World Congress on Animal, Plant and Microbial Toxins*. Book of Abstracts.

Lindquist, P. A., Fujikawa, K. and Davie, E. W. (1978). Activation of bovine factor IX (Christmas factor) by factor XIa (activated plasma thromboplastin antecedent) and a protease from Russell's viper venom. *Journal of Biological Chemistry*, 253: 1902–1909.

Lobo, R., Punitha, I. S. R., Rajendran, K. and Shirwaikar, A. (2006). Preliminary study on the anti-snake venom activity of alcoholic root extract of *Cleradendrum viscosum* (vent) in *Naja naja* venom Natural product of Science, 12(3): 153–156.

Lork, D. (1983). A new approach to practical acute toxicity testing. Archives of Toxicology. *Archives of Toxicology*, 54: 275–287.

Lu, Q., Clemetson, J. M. and Clemetson, K. J. (2005). Snake venoms and hemostasis. *Journal of Thrombosis and Haemostasis*, 3(8): 1791–1799. https://doi.org/10.1111/j.1538-7836.2005.01358.x

Lu, Q., Navdaev, A., Clemetson, J. M. and Clemetson, K. J. (2004). GPIb is involved in platelet aggregation induced by mucetin, a snake C-type lectin protein from Chinese habu (*Trimeresurus mucrosquamatus*) venom. *Journal of Thrombosis and Haemostasis*, 91: 1168–1176.

Lu, Q. M., Wei, Q., Wang, W. Y. and Xiong, Y. L. (2002). L-Amino acid oxidase from *Trimeresurus jerdonii* snake venom: purification characterization, platelet aggregation-inducing and anti-bacterial effects. *Journal of Natural Toxins*, 11: 345–352.

Luiselli, L. (2001). The ghost of a recent invasion in the reduced feeding rates of spitting cobras during the dry season in a rainforest region of tropical Africa. *Acta Oecologica*. 22: 311–314.

M´enez, A., St¨ocklin, R. and Mebs, D. (2006). Venomics'' or: the venomous systems genome project. *Toxicon*, 47: 255.

Mackessy, S. P. (2002). Biochemistry and pharmacology of colubrid snake venoms. *Journal of Toxicology Toxin*, 21: 43–83.

Madane, A. N., Kamble, S., Pati, B. and Aparadh, V. T. (2013). Assessment of solvent solubility by using phytochemical screen tests of some euphorbiaceae members (PDF Download Available). Retrieved 28 April 2017, from https://www.researchgate.net/publication/236839098_

Mahadeswaraswamy, Y. H., Devaraji, S., Kumar, M. S., Grouthan, Y. N. and Kemparaju, K. (2009). Inhibition of local effect of Indian Daboia/vipera resselli venom by the methenolic extract of grape (*Vitis vinifera* L.) seed. *Indian Journal of Biochemica Biophysica*, 46(2): 154–160.

Maita, S., Nishio, K., Nishimoto, E., Matsui, T., Shikamoto, Y., Morita, T. and Mizuno, H. (2003). Crystal structure of von Willebrand factor A1 domain complexed with snake venom, bitiscetin: insight into glycoprotein Iba binding mechanism induced by snake venom proteins. *Jounal of Biological Chemistry*, 278: 37777–37781.

Malhotra, A. and Thorpe, R. S. (2004). A phylogeny of four mitochondrial gene regions suggests a revised taxonomy for Asian pitvipers (*Trimeresurus and Ovophis*). *Molecular Phylogene Andt Evolution*, 32: 83–100.

Mallow, D., Ludwig, D. and Nilson, G. (2003). *True Vipers: Natural History and Toxinology of Old World Vipers.* Malabar Florida: Krieger Publishing Company.

Mann, K. G. and Kalafatis, M. (2003). Factor V: a combination of Dr Jekyll and Mr Hyde. *Blood*, 101: 20–30.

Marcinkiewicz, C., Weinreb, P. H., Calvete, J. J., Kisiel, D. G., Mous, S. A., Tuszynski, G. P. and Lobb, R. R. (2003). Obtustatin: a potent selective inhibitor of a1b1 integrin in vitro and angiogenesis in vivo. *Cancer Research*, 63: 2020–2023.

Markland, F. S. (1991). Inventory of a- and b-fibrinogenases from snake venoms. For the Subcommittee on Nomenclature of Exogenous Hemostatic Factors of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. *Journal of Thrombosis and Haemostasis*, 65: 438–443.

Markland, F. S. (1998). Snake venoms and the hemostatic system. *Toxicon*, 36: 1749–1800.

Marrakchi, N., Barbouche, R., Guermazi, S., Bon, C. and El Ayeb, M. (1997). Q Procoagulant and platelet-aggregating properties of cerastocytin from *Cerastes cerastes* venom. *Toxicon*, 35: 261–272.

Marsh, N. A. (1994). Snake venoms affecting the haemostatic mechanism – a consideration of their mechanisms, practical applications and biological significance. *Blood Coagulation Fibrinolysis*, 5: 399–410.

Mastenbroek, R. (2013). Captive care of the Egyptian Cobre (*Naja haje*)" (PDF). Devenomized. . *W.w.w. Devenomized. Com. Archived from the Original (PDF) on* 29 October 2013, (Retrieved 13 January 2012).

Matsui, T., Fujimura, Y. and Titani, K. (2000). Snake venom proteases affecting hemostasis and thrombosis, 1477: 146–156.

Matsushita, O. and Okabe, A. (2001). Clostridial hydrolytic enzymes degrading extracellular components. *Toxicon*, 39: 1769–1780.

Mattison, C. (2007). *The New Encyclopaedia of snakes, United Kinkdom: Octopus Publishing Group*. Ltd London.

Maurice, M. I. (2014). *Handbook of African Medicinal Plants, Second Edition*. CRC Press.

McDiarmid, R. W., Campbell, J. A. and Toure, T. (1999). *Snake Species of the World: A Taxonomic and Geographic Reference, Volume 1. Herpetologists' League*.

McNamee, D. (2001). Tackling venomous snake bites worldwide, 357: 1680.

Mehrtens, J. M. (1987). *Living Snakes of the World in Color* (1st Edition. edition). New York: Sterling Publishing.

Mehrtens, J. M. (2001). *Living Snakes of the World in Color*. New York: Sterling Publishers.

Meier, J. and Stocker, K. (1991). Effects of snake venoms on hemostasis. Critical Reviews in Toxicology. *Critical Reviews in Toxicology*, 21: 171–182.

Ménez, A. (2003). The subtle beast. Snakes, from myth to medicine. *Taylor and Francis, London*.

Mirtschin, P. J., Dunstan, N., Hough, B., Hamilton, E., Klein, S., Lucas, J. and Nias, T. (2006). Venom yields from Australian and some other species of snakes. *Cotoxicology*, 15: 531–538.

Mishal, H. B. (2002). Screening of ant-snake venom activity of *Dichrostachys cinerea* W. *Journal of Natural Remedies*, 2(1): 92–95.

Mizuno, H., Fujimoto, Z., A. G. H., H. and Morita, T. (2001). Crystal structure of an anticoagulant protein in complex with the Gla domain of factor X. In *Proceedings of the National Acaicd Science USA* (pp. 7230–7234).

Momoh, S. (2012). Anti-venom activity of ethanolic extract of *Bridelia ferruginea* leaves against *Naja nigricollis* venom. *Journal of Medical Research*, 1(5): 69–73.

Morita, T. (2004). Use of snake venom inhibitors in studies of the function and tertiary structure of coagulation factors. *International Journal of Hematology*, 79: 123–129.

Mors, W. B., Nascimento, M. C., Pereira, N. A. and Pereira, B. M. (2000). Plant natural products active against snake bite–the molecular approach. *Journal of Phytochemistry*55(6): 627–642.

Mounier, C. M., Bon, C. and Kini, R. M. (2001). Anticoagulant venom and mammalian secreted phospholipases A2: protein- versus phospholipid dependent mechanism of action. *Haemostasis*, 31: 279–287.

Mourao, V. B., Giraldi, G. M., Neves, L. M., Gaspi, F. O., Rodrigues, R. A., Alves, A. A. and Santos, G. M. (2014). Anti-haemaorrhagic effect of hydro-alcohol extract of the leaves of *Mikania glomerata* in lesions induced by *Bothrops jarara* venom in rats.

Murari, S. K. M., Frey, F. J. B., Gowda, T. V. and Vishwanath, B. S. (2005). Use of Pavo cristatus feather extract for the better management of snake bites: Neutralization of inflammatory reactions. *Journal of Ethnopharmacology*, 99: 229–237.

Nair, D. G., Fry, B. G., Alewood, P., Kumar, P. P. and Kini, R. M. (2007). Antimicrobial activity of omwaprin, a new member of the waprin family of snake venom protein. *Biochemical Journal*, 402(1): 93–104.

Namiranian, S. and Hider, R. C. (1992). Use of HPLC to demonstrate variation of venom toxin composition in the Thailand cobra venoms *Naja naja* kaouthia and *Naja naja* siamnsis. *Toxicon*, 30: 47–61.

Nathan, I., Dvilansky, A., Yirmiyahu, T., Aharon, M. and Livne, A. (1982). Impairment of platelet aggregation by *Echis colorata* venom mediated by L-amino acid oxidase or H2O2. *Journal of Thromobsis and Haemostasis*, 48: 277–282.

Nunez, V., Otero, R. and Barona, J. (2004). Neutralization of the edema-forming, defibrinating and coagulant effects of *Bothrops asper* venom by extracts of plants used by healers in Colombia. *Brazil Jounal of Medical and Biological Research*, 37: 969–977.

Ode, O. J. and Asuzu, I. U. (2006). The anti-snake venom activities of the methanolic extract of the bulb of *Crinum jagus* (Amaryllidaceae). *Toxicon: Official Journal of the International Society on Toxinology*, 48(3): 331–342. https://doi.org/10.1016/j.toxicon.2006.06.003

Ogden, R. and Thorpe, R. S. (2002). Molecular evidence for ecological speciation in tropical habitats. *Proceedings of the National Academy of Sciences of the United States of America*, 99(21): 13612–13615. https://doi.org/10.1073/pnas.212248499

Ohkura, K., Inoue, S., Ikeda, K. and Hayashi, K. (1988). Amino-acid sequences of four cytotoxins (cytotoxins I, II, III and IV) purified from the venom of the Thailand cobra, *Naja naja* siamensis. *Biochimica Et Biophysica Acta*, 954(2): 148–153.

Oliveira, C. Z., Maiorano, V. A., Marcussi, S., Sant'ana, C. D., Januário, A. H., Lourenço, M. V. and Soares, A. M. (2005). Anticoagulant and antifibrinogenolytic properties of the aqueous extract from *Bauhinia forficata* against snake venoms. *Journal of Ethnopharmacology*, 98(1–2): 213–216. https://doi.org/10.1016/j.jep.2004.12.028

Otero, C., Saavedra, L., Silva de Ruiz, C., Wilde, O., Holgado, A. r. and Nader-Macías, M. (2000). Vaginal bacterial microflora modifications during the growth of healthy cows. *Letters in Applied Microbiology*, 31(3): 251–254. https://doi.org/10.1046/j.1365-2672.2000.00809.x

Ouyang, C. and Huang, T. F. (1983). Inhibition of platelet aggregation by 5'-nucleotidase purified from *Trimeresurus gramineus* snake venom. *Toxicon: Official Journal of the International Society on Toxinology*, 21(4): 491–501.

Ouyang, C., Teng, C. M. and Huang, T. F. (1992). Characterization of snake venom components acting on blood coagulation and platelet function. *Toxicon: Official Journal of the International Society on Toxinology*, 30(9): 945–966.

Ownby, C. L., Fletcher, J. E. and Colberg, T. R. (1993). Cardiotoxin 1 from cobra (*Naja naja atra*) venom causes necrosis of skeletal muscle in vivo. *Toxicon: Official Journal of the International Society on Toxinology*, 31(6): 697–709.

Owuor, B. O. and Kisangau, D. P. (2006). Kenyan medicinal plants used as antivenin: a comparison of plant usage. *Journal of Ethnobiology and Ethnomedicine*, 2: 7. https://doi.org/10.1186/1746-4269-2-7

Paramonte, B. (2007). Snake bites in Nigeria. *Medical Journal of Therapy in Africa*, 1(3): 222–226.

Pardal, P. P. de O., Souza, S. M., Monteiro, M. R. de C. da C., Fan, H. W., Cardoso, J. L. C., França, F. O. S. and Warrell, D. A. (2004). Clinical trial of two antivenoms for the treatment of Bothrops and Lachesis bites in the north eastern Amazon region of Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 98(1): 28–42.

Park, D. and Green, H. W. (1997). Snakes: The evolution of mystery in Nature. *University of Califonia Press*, 351.

Parry, M. A., Jacob, U., Huber, R., Wisner, A., Bon, C. and Bode, W. (1998). The crystal structure of the novel snake venom plasminogen activator TSV-PA: a prototype structure for snake venom serine proteinases. *Structure*, 6(9): 1195–1206. https://doi.org/10.1016/S0969-2126(98)00119-1

Pawelek, P. D., Cheah, J., Coulombe, R., Macheroux, P., Ghisla, S. and Vrielink, A. (2000). The structure of L-amino acid oxidase reveals the substrate trajectory into an enantiomerically conserved active site. *The EMBO Journal*, 19(16): 4204–4215. https://doi.org/10.1093/emboj/19.16.4204

Perumal Samy, R., Pachiappan, A., Gopalakrishnakone, P., Thwin, M. M., Hian, Y. E., Chow, V. T. K. and Weng, J. T. (2006). *In vitro* antimicrobial activity of natural toxins and animal venoms tested against *Burkholderia pseudomallei*. *BMC Infectious Diseases*, 6: 100. https://doi.org/10.1186/1471-2334-6-100

Peters, W. C. H. (1854). Diagnosen neuer Batrachier, welche zusammen mit der fruher gegebenen ubersicht der Schiangen und Eidechsen mitgetheilt warden. *Bericht Uber Zur Bekanntmachung Gecignete Verhandlungen Der Koniglichen Preussischen Akademie Der Wissenschaften Zu Berlin*, 1854: 614–628.

Petras, D., Sanz, L., Segura, A., Herrera, M., Villalta, M., Solano, D. and Calvete, J. J. (2011). Snake venomics of African spitting cobras: toxin composition and assessment of congeneric cross-reactivity of the pan-African EchiTAb-Plus-ICP antivenom by antivenomics and neutralization approaches. *Journal of Proteome Research*, 10(3): 1266–1280. https://doi.org/10.1021/pr101040f

Pirkle, H. (1998). Thrombin-like enzymes from snake venoms: an updated inventory. Scientific and Standardization Committee's Registry of Exogenous Hemostatic Factors. *Thrombosis and Haemostasis*, 79(3): 675–683.

Pithayanukul, P., Laovachirasuwan, S., Bavovada, R., Pakmanee, N. and Suttisri, R. (2004). Anti-venom potential of butanolic extract of *Eclipta prostrata* against Malayan pit viper venom. *Journal of Ethnopharmacology*, 90(2–3): 347–352. https://doi.org/10.1016/j.jep.2003.10.014

Polgár, J., Clemetson, J. M., Kehrel, B. E., Wiedemann, M., Magnenat, E. M., Wells, T. N. C. and Clemetson, K. J. (1997). Platelet Activation and Signal Transduction by Convulxin, a C-type Lectin from *Crotalus durissus terrificus* (Tropical Rattlesnake) Venom via the p62/GPVI Collagen Receptor. *Journal of Biological Chemistry*, 272(21): 13576–13583. https://doi.org/10.1074/jbc.272.21.13576

Pook, C. E., Joger, U., Stümpel, N. and Wüster, W. (2009). When continents collide: phylogeny, historical biogeography and systematics of the medically important viper genus *Echis* (Squamata: Serpentes: Viperidae). *Molecular Phylogenetics and Evolution*, 53(3): 792–807. https://doi.org/10.1016/j.ympev.2009.08.002

Prabha, B. and Savithramma, N. (2014). Screening of phytochemical constituents of the leaves of *Clinacanthus siamensis* bremek and cissampelos pareira l used as antidote for snake bite in indigenous medicine. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(8): 180–182.

Pugh, R. N. H., Theakston, R. D. G., Reid, H. A. and Bhar, I. S. (1980). Malumfashi Endemic Diseases Research Project, XIII. *Annals of Tropical Medicine & Parasitology*, 74(5): 523–530. https://doi.org/10.1080/00034983.1980.11687380

Punde, D. P. (2005). Management of snake-bite in rural Maharashtra: a 10-year experience. *The National Medical Journal of India*, 18(2): 71–75.

Puorto, G., Solomon, M. G., Theakston, R. D. G., Thorpe, A., Warrel, D. A. and Wuster, W. (2001). Combining mitochondria DNA sequences and morphological data to infer species bounderies. Phylogeological of Lanceheaded pituipers in the Brazilian Atlantic forest and the station of *Bothrops pradoi* (Sequaniata: Serpentes: Viperidase). *Journal of Envolutionary Biology*. 14: 538.

Radis-Baptista, G., Moreno, F. B., Nogueira, L. and Martins, A. M. (2006). *Crotacetin, a novel snake venom C-type lectin homolog of convulxin, exhibits an unpredictable antimicrobial activity* (Vol. 44). Cell Biochemistry and Biophysis.

Rajendran, K., Shirwaikar, A., Mehta, M. and Bharathi, R. V. (2010). *In vitro* and *in vivo* anti-snake venom (*Daboia russelli*) studies on various leaf extracts of *Acalypha indica* Linn. *International Journal of Phytomedicine*, 2(3). Retrieved from http://www.arjournals.org/index.php/ijpm/article/view/111

Rao, V. S., Joseph, J. S. and Kini, R. M. (2003). Group D prothrombin activators from snake venom are structural homologues of mammalian blood coagulation factor Xa. *The Biochemical Journal*, 369(Pt 3): 635–642. https://doi.org/10.1042/BJ20020889

Rao, V. S., Swarup, S. and Manjunatha Kini, R. (2004). The catalytic subunit of pseutarin C, a group C prothrombin activator from the venom of *Pseudonaja textilis*, is structurally similar to mammalian blood coagulation factor Xa. *Thrombosis and Haemostasis*, 92(3): 509–521. https://doi.org/10.1160/TH04-03-0144

Rivas, J. A. (2000). The life history of the green anaconda (*Eunectes murinus*), with emphasis on its reproductive Biology (PDF). *(Ph.D. Thesis) University of Tennessee*.

Rollin, A. B. and Kesel, M. I. (1990). *The experimental animals in biomedical research, Vol. 1:* Inc., Boca Raton, Florida: A survey of scientific and ethical issues for investigators, CRC Press.

Roman, B. (1968). Une nouvelle epece de Naja en Haute=Volta. *Carrefour Africaine*, 286(310): 273–274.

Roman, B. (1969). Less Naja de Haute-Volta. *Revue de Zoologie et Botanique Africaine*, 82: 1–4.

Rosing, J., Govers-Riemslag, J. W., Yukelson, L. and Tans, G. (2001). Factor V activation and inactivation by venom proteases. *Haemostasis*, 31(3–6): 241–246. https://doi.org/48069

Rosing, J. and Tans, G. (1992). Structural and functional properties of snake venom prothrombin activators. *Toxicon: Official Journal of the International Society on Toxinology*, 30(12): 1515–1527.

Rr, H., Mc, S., Jh, C., Ds, L., Da, H., M, R. and Ma, M. (2004). The disintegrin echistatin stabilizes integrin alphaIIbbeta3's open conformation and promotes its oligomerization. *Journal of Molecular Biology*, 342(5): 1625–1636. https://doi.org/10.1016/j.jmb.2004.08.009

Saha, A., Gomes, A., Chakravarty, A. K., Biswas, A. K., Giri, B., Dasgupta, S. C. and Gomes, A. (2006). CNS and anticonvulsant activity of a non-protein toxin (KC-MMTx) isolated from King Cobra (*Ophiophagus hannah*) venom. *Toxicon: Official Journal of the International Society on Toxinology*, 47(3): 296–303. https://doi.org/10.1016/j.toxicon.2005.11.006

Sakurai, Y., Takatsuka, H., Yoshioka, A., Matsui, T., Suzuki, M., Titani, K. and Fujimura, Y. (2001). Inhibition of human platelet aggregation by L-amino acid oxidase purified from *Naja naja kaouthia* venom. *Toxicon: Official Journal of the International Society on Toxinology*, 39(12): 1827–1833.

Sanchez, A. (2007). 'Diapsids III: Snakes'. Father Sanchez's Web Site of West Indian Natural History. Retrieved 26 November 2007.

Sanchez, E. F., Santos, C. I., Magalhaes, A., Diniz, C. R., Figueiredo, S., Gilroy, J. and Richardson, M. (2000). Isolation of a proteinase with plasminogen-activating activity from *Lachesis muta muta* (bushmaster) snake venom. *Archives of Biochemistry and Biophysics*, 378(1): 131–141. https://doi.org/10.1006/abbi.2000.1781

Sant'Ana, C. D., Menaldo, D. L., Costa, T. R., Godoy, H., Muller, V. D. M., Aquino, V. H. and Soares, A. M. (2008). Antiviral and antiparasite properties of an L-amino acid oxidase from the snake *Bothrops jararaca*: cloning and identification of a complete cDNA sequence. *Biochemical Pharmacology*, 76(2): 279–288. https://doi.org/10.1016/j.bcp.2008.05.003

Santos, B. F., Serrano, S. M., Kuliopulos, A. and Niewiarowski, S. (2000). Interaction of viper venom serine peptidases with thrombin receptors on human platelets. *FEBS Letters*, 477(3): 199–202.

Schlöndorff, J. and Blobel, C. P. (1999). Metalloprotease-disintegrins: modular proteins capable of promoting cell-cell interactions and triggering signals by protein-ectodomain shedding. *Journal of Cell Science*, 112 (Pt 21): 3603–3617.

Schmidt, K. P. and Inger, R. F. (1957). *Living Reptiles of the World*. Hanover House.

Sells, P. G., Laing, G. D., & Theakston, R. D. (2001). An *in vivo* but insensate model for the evaluation of antivenoms (ED_{50}) using fertile hens' eggs. *Toxicon*, 39: 665–668.

Sharma, S. K., Chappuis, F., Jha, N., Bovier, P. A., Loutan, L. and Koirala, S. (2004). Impact of snake bites and determinants of fatal outcomes in southeastern Nepal. *The American Journal of Tropical Medicine and Hygiene*, 71(2): 234–238.

Sharon, M. G. (2006). *Overview of snake bite: Epidemiology of animal bites and stings in Khuzestan*. Iran.

Shenoy, P. A., Nipate, S. S., Sonpetkar, J. M., Salvi, N. C., Waghmare, A. B. and Chaudhari, P. D. (2013). Anti-snake venom activities of ethanolic extract of fruits of *Piper longum L*. (Piperaceae) against Russell's viper venom: characterization of piperine as active principle. *Journal of Ethnopharmacology*, 147(2): 373–382. https://doi.org/10.1016/j.jep.2013.03.022

Shikamoto, Y., Morita, T., Fujimoto, Z. and Mizuno, H. (2003). Crystal structure of Mg^{2+}- and Ca^{2+}-bound Gla domain of factor IX complexed with binding protein. *The Journal of Biological Chemistry*, 278(26): 24090–24094. https://doi.org/10.1074/jbc.M300650200

Shimokawa, K., Shannon, J. D., Jia, L. G. and Fox, J. W. (1997). Sequence and biological activity of catrocollastatin-C: a disintegrin-like/cysteine-rich two-domain protein from *Crotalus atrox* venom. *Archives of Biochemistry and Biophysics*, 343(1): 35–43.

Shirwaikar, A., Malini, S. and Kumari, S. C. (2003). Protective effect of *Pongamia pinnata* flowers against cisplatin and gentamicin induced nephrotoxicity in rats. *Indian Journal of Experimental Biology*, 41(1): 58–62.

Shirwaikar, A., Rajendran, K., Bodla, R. and Kumar, C. D. (2004). Neutralization potential of *Viper russelli russelli* (Russell's viper) venom by ethanol leaf extract of *Acalypha indica*. *Journal of Ethnopharmacology*, 94(2–3): 267–273. https://doi.org/10.1016/j.jep.2004.05.010

Silva, J. O. da, Fernandes, R. S., Ticli, F. K., Oliveira, C. Z., Mazzi, M. V. and Franco, J. J. (2007). Triterpenoid a saponins, new metalloprotease snake venom inhibitors isolated from *pentaclethra macroloba*. *Toxicon*, 50(2): 283–91.

Slowinski, J. B. and Keogh, J. S. (2000). Phylogenetic relationships of elapid snakes based on cytochrome b mtDNA sequences. *Molecular Phylogenetics and Evolution*, 15(1): 157–164. https://doi.org/10.1006/mpev.1999.0725

Smalligan, R., Cole, J., Brito, N., Laing, G. D., Mertz, B. L., Manock, S. and Theakston, R. D. G. (2004). Crotaline snake bite in the Ecuadorian Amazon: randomised double blind comparative trial of three South American polyspecific antivenoms. *BMJ (Clinical Research Ed.)*, 329(7475): 1129. https://doi.org/10.1136/bmj.329.7475.1129

Smith, A. (1838). contributions to south African Zoology Magazine of Natural History, 2(30): 92–94.

Smith, A., Curtis, C. M., Ford, G. H., Macleay, W. S., Wagstaff, C. E., Africa, C. of G. H. A. for E. C. and Stewart and Murray. (1838). *Illustrations of the zoology of South Africa, consisting chiefly of figures and descriptions of the objects of natural history collected during an expedition into the interior of South Africa, in the years 1834, 1835, and 1836; fitted out by 'The Cape of Good Hope association for exploring Central Africa': together with a summary of African zoology, and an inquiry into the geographical ranges of species in that quarter of the globe. London*: Smith, Elder and Co.,. Retrieved from http://www.biodiversitylibrary.org/bibliography/10416

Soares, A. M., Ticli, F. K., Marcussi, S., Lourenço, M. V., Januário, A. H., Sampaio, S. V. and Pereira, P. S. (2005). Medicinal plants with inhibitory properties against snake venoms. *Current Medicinal Chemistry*, 12(22): 2625–2641.

Sofowara, E. A. (1982). Medicinal plants and Traditional Medicine in Africa: 256.

Spawls, S. and Branch, B. (1995). *Dangerous Snakes of Africa: Natural History - Species Directory - Venoms and Snake bite* (Revised edition). Sanibel Island, FL: Ralph Curtis Pub.

Spawls, S. and Branch, B. (1996). *The Dangerous Snakes of Africa: Natural History: Venoms and Snake bite*. Retrieved from www.jstor.org/doi/xml/10.2307/1447547

Spawls, S., Howell, K., Drewes, R. and Ashe, J. (2004). *A Field Guide to the Reptiles of East Africa: Kenya, Tanzania, Uganda, Rwanda and Burundi*. London: Christopher Helm Publishers Ltd.

Stabeli, R. G., Marcussi, S., Carlos, G. B. and Pietro, R. C. (2004). Platelet aggregation and antibacterial effects of an L-amino acid oxidase purified from *Bothrops alternatus* snake venom. *Bioorganic and Medicinal Chemistry*, 12: 2881–2886.

St Pierre, L., Masci, P. P., Filippovich, I., Sorokina, N., Marsh, N., Miller, D. J. and Lavin, M. F. (2005). Comparative analysis of prothrombin activators from the venom of Australian elapids. *Molecular Biology and Evolution*, 22(9): 1853–1864. https://doi.org/10.1093/molbev/msi181

Stock, R. P., Massougbodji, A., Alagón, A. and Chippaux, J. P. (2007). Bringing antivenoms to Sub-Saharan Africa. *Nature Biotechnology*, 25(2): 173–177. https://doi.org/10.1038/nbt0207-173

Stromblad, S. and Cheresh, D. A. (1996). Integrins, angiogenesis and vascular cell survival. *Journal of Chemical Biology*, 3: 881–5.

Sutherland, S. K. and Tibballs, J. (2001). Genus *Pseudonaja*, brown snakes. Australian animal toxins. In: The Creatures, Their Toxins and the Care of the Poisoned Patient. Melbourne, Australia. *Oxford University Press*, 108.

Swaroop, S. and Grab, B. (1954). Snake bite mortality in the world, 10: 35–76.

Takacs, Z., Wilhelmsen, K. C. and Sorota, S. (2001). Snake α-neurotoxin binding site on the Egyptian cobra (*Naja haje*) nicotinic acetylcholine receptor is conserved. *Molecular Biology and Evolution*, 18(9): 1800–1809.

Takatsuka, H., Sakurai, Y., Yoshioka, A., Kokubo, T. and Usami, Y. (2001). Molecular characterization of L-amino acid oxidase from Agkistrodon halys *blomhoffii* with special reference to platelet aggregation. *Biochimica and Biophysica Acta*, 1544: 267–277.

Takeya, H., Nishida, S., Miyata, T., Kawada, S., Saisaka, Y., Morita, T. and Iwanaga, S. (1992). Coagulation factor X activating enzyme from Russell's viper venom (RVV-X). A novel metalloproteinase with disintegrin (platelet aggregation inhibitor)-like and C-type lectin-like domains. *The Journal of Biological Chemistry*, 267(20): 14109–14117.

Tan, N. H., Fung, S. Y., Sim, S. M., Marinello, E., Guerranti, R. and Aguiyi, J. C. (2009). The protective effect of *Mucuna pruriens* seeds against snake venom poisoning. *Journal of Ethnopharmacology*, 123(2): 356–358. https://doi.org/10.1016/j.jep.2009.03.025

Tan, N. H. and Ponnudurai, G. (1992). Comparative study of the enzymatic, hemorrhagic, procoagulant and anticoagulant activities of some animal venoms. *Comparative Biochemistry and Physiology. C, Comparative Pharmacology and Toxicology*, 103(2): 299–302.

Tanos, P. P., Isbister, G. K., Lalloo, D. G., Kirkpatrick, C. M. J. and Duffull, S. B. (2008). A model for venom-induced consumptive coagulopathy in snake bite. *Toxicon: Official Journal of the International Society on Toxinology*, 52(7): 769–780. https://doi.org/10.1016/j.toxicon.2008.08.013

Tattersall, G. J., Milsom, W. K., Abe, A. S., Brito, S. P. and Andrade, D. V. (2004). The thermogenesis of digestion in rattlesnakes. *The Journal of Experimental Biology*, 207(Pt 4): 579–585.

Teixeira-Clerc, F., Ménez, A. and Kessler, P. (2002). How Do Short Neurotoxins Bind to a Muscular-type Nicotinic Acetylcholine Receptor? *Journal of Biological Chemistry*, 277(28): 25741–25747. https://doi.org/10.1074/jbc.M200534200

Teng, C. M., Chen, Y. H. and Ouyang, C. (1984). Biphasic effect on platelet aggregation by phospholipase a purified from Vipera russellii snake venom. *Biochimica Et Biophysica Acta*, 772(3): 393–402.

Theakston, R. D. G. and Warrell, D. A. (2000). Crisis in snake antivenom supply for Africa. *The Lancet*, 356(9247): 2104. https://doi.org/10.1016/S0140-6736(05)74319-1

Theakston, R. D. G., Warrell, D. A. and Griffiths, E. (2003). Report of a WHO workshop on the standardization and control of antivenoms. *Toxicon: Official Journal of the International Society on Toxinology*, 41(5): 541–557.

Theakston, R. D. and Reid, H. A. (1983). Development of simple standard assay procedures for the characterization of snake venom. *Bulletin of the World Health Organization*, 61(6): 949–956.

Thite, S. V., Chavan, Y. R., Aparadh, V. T. and Kore, B. A. (2013). Preliminary Phytochemical Screening of Some Medicinal Plants, 3(1): 87–90.

Thorpe, R. S., Brown, R. P., Day, M., Malhotra, A., McGregor, D. P. and Wuster, W. (1994). Testing ecological and phylogenetic hypothesis in microevolutionary study: an overview. In *Testing ecological and phylogenetic hypothesis in microevolutionary study: an overview* (In: Eggleton ,P. and Vane-Wright, R. (Eds), pp. 189–206). London: Phylogenetics and Ecology, Academic Press.

Thorpe, R. S., Malhotra, A., A. G. H., H., Daltry, J. C. and Wuster, W. (1995). Relating geographic pattern to phylogenetic processs Philosophical. *Transactions of Royal Society of London*, 349: 61–68.

Thorpe, R. S. and Richard, M. (2001). Evidence that ultraviolet markings are associated with patterns of molecular gene flow. *Proceedings of the National Academy of Sciences of the United States of America*, 98(7): 3929–3934. https://doi.org/10.1073/pnas.071576798

Tokunaga, F., Nagasawa, K., Tamura, S., Miyata, T., Iwanaga, S. and Kisiel, W. (1988). The factor V-activating enzyme (RVV-V) from Russell's viper venom. Identification of isoproteins RVV-Va, -Vb, and -Vc and their complete amino acid sequences. *Journal of Biological Chemistry*, 263: 17471–17481.

Trape, J. F. L., Broadley, G. and Wüster, W. (2009). Phylogeography and systematic revision of the Egyptian cobra (Serpentes: Elapidae: *Naja haje*) species complex, with the description of a new species from West Africa. *Zootaxa*, 2236: 1–25.

Tseng, Y.-L., Lee, C.-J. and Huang, T.-F. (2004). Effects of a snake venom metalloproteinase, triflamp, on platelet aggregation, platelet-neutrophil and neutrophil-neutrophil interactions: involvement of platelet GPIbalpha and neutrophil PSGL-1. *Thrombosis and Haemostasis*, 91(2): 315–324. https://doi.org/10.1160/TH03-07-0426

Tu, A. T. (1996). Overview of Snake Venom Chemistry. In B. R. Singh and A. T. Tu (Eds.), *Natural Toxins 2* (pp. 37–62). Springer US. Retrieved from http://link.springer.com/chapter/10.1007/978-1-4613-0361-9_3

Tupe, S. B., Patil, P. D., Thoke, R. B. and Aparadh, V. T. (2015). Phytochemical Screening in some Cucurbitaceae members. *International Research Journal of Pharmacy and Applied Science*, 3(1): 49–51.

Umberto, Q. (2012). CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology. *CRC Press*, *5*.

Ushanandini, S., Nagaraju, S., Harish Kumar, K., Vedavathi, M., Machiah, D. K., Kemparaju, K. and Girish, K. S. (2006). The anti-snake venom properties of *Tamarindus indica* (leguminosae) seed extract. *Phytotherapy Research: PTR*, 20(10): 851–858. https://doi.org/10.1002/ptr.1951

Ushanandini, S., Nagaraju, S., Nayaka, S. C., Kumar, K. H., Kemparaju, K. and Girish, K. S. (2009). The anti-ophidian properties of *Anacardium occidentale* bark extract. *Immunopharmacology and Immunotoxicology*, 31(4): 607–615. https://doi.org/10.3109/08923970902911909

Venkata, N. K., Kameswara, R. K. and Sandeep, B. V. (2015). Medicinal plants with anti-snake venom property. *The Pharmacology Innovation Journal*, 4(7): 11–15.

Venkateswarlu, D., Krishnaswamy, S., Darden, T. A. and Pedersen, L. G. (2002). Three dimensional solution structure of Tropidechis ocellatus venom extract trocarin: a structural homologue of Xa and prothrombin activator , . *Journal of Molecular Modelling*, 8: 302–13.

Visser, L. E., Kyei-Faried, S., Belcher, D. W., Geelhoed, D. W., van Leeuwen, J. S. and van Roosmalen, J. (2008). Failure of a new antivenom to treat *Echis ocellatus* snake bite in rural Ghana: the importance of quality surveillance. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 102(5): 445–450. https://doi.org/10.1016/j.trstmh.2007.11.006

Viswanath, B. S., Rao, A. G. A. and Gowda, T. V. (1987). Interaction of phospholipase A2 from *Vipera russelli* venom with aristolochic acid: a circular dichroism study. *Toxicon*, 25: 939–946.

Walter, F. G., Bilden, E. F. and Gibly, R. L. (1999). Envenomations. *Critical Care Clinics*, 15(2): 353–386, ix.

Wannang, N. N., Wudil, A. M., Dapar, M. L. and Bichi, L. A. (2005). Evaluation of anti-snake venom activity of the aqueous root extract of *Securidaca longipedunculata* in rats. *Journal of Pharmacy & Bioresources*, 2(2): 80–83. https://doi.org/10.4314/jpb.v2i2.32067

Ward, C. M., Andrews, R. K., Smith, A. I., Berndt, M. C. and Mocarhagin, A. (1996). A novel cobra venom metalloproteinase, cleaves the platelet von Willebrand factor receptor glycoprotein Iba. Identification of the sulfated tyrosine/anionic sequence Tyr276-Glu282 of glycoprotein Iba as a binding site for von Willebrand factor and a-thrombin. *Biochemistry*, 35: 4929–4938.

Warrell, D. A. (1986). Tropical snake bite: clinical studies in Southeast Asia. In *Natural toxins: Animal, Plant and Microbial* (Harris J.B (Eds.), pp. 25–30). Oxford Science Publications, Oxford.

Warrell, D. A. (1989). Snake venoms in science and clinical medicine. 1. Russell's viper: biology, venom and treatment of bites. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 83(6): 732–740.

Warrell, D. A. (1995). Clinical toxicology of snakebite in Asia. In *Handbook of clinical toxicology of animal venoms and poisons* (Meier, J. and White, J., pp. 493–594). Florida: CRC Press.

Warrell, D. A. (1996). Geographical and intraspecies variation in the clinical manifestations ofen venoming by snakes (Vol. (in press)). Presented at the Symposia of the Zoological Society of London, Oxford University Press.

Warrell, D. A. (2006). Australian toxinology in a global context. *Toxicon : Official Journal of the International Society on Toxinology*, 48(7): 718–725.

Warrell, D. A. (2008). Proatheris superciliaris: the deadly venom of a rare and elusive snake revealed. *Toxicon: Official Journal of the International Society on Toxinology*, *52(*8): 833–835. https://doi.org/10.1016/j.toxicon.2008.11.004

Warrell, D. A. and Arnett, C. (1976). The importance of bites by the saw-scaled or carpet viper (*Echis carinatus*): epidemiological studies in Nigeria and a review of the world literature. *Acta Tropica*, 33(4): 307–341.

Warrell D A. (2004). Epidemiology, clinical features and management of snake bites in Central and South America. *Cornell University Press, Ithaca*, 709–761.

Watt, J. M. and Breyer-Brandwijk, M. G. (1962). *The medicinal and poisonous plants of southern and eastern Africa: being an account of their medicinal and other uses, chemical composition, pharmacological effects and toxicology in man and animal*. E. and S. Livingstone.

WCH. (2011). *Naja nigricollis* - General Details, Taxonomy and Biology, Venom, Clinical Effects, Treatment, First Aid and Antivenoms.

Weber, M. and Changeux, J. P. (1974). Binding of *Naja nigricollis* (3H)alpha-toxin to membrane fragments from Electrophorus and Torpedo electric organs. I. Binding of the tritiated alpha-neurotoxin in the absence of effector. *Molecular Pharmacology*, 10(1): 1–14.

WHO. (2007). *Rabies and envenomings: a neglected public health issue: report of a Consultative Meeting*. London

Widgerow, A. D., Ritz, M. and Song, C. (1994). Load cycling closure of fasciotomies following puff adder bite. *European Journal of Plastic Surgery*, 17(1): 40–42. https://doi.org/10.1007/BF00176504

Williams, D., Gutiérrez, J. M., Harrison, R., Warrell, D. A., White, J., Winkel, K. D. and International Society on Toxinology. (2010). The Global Snake Bite Initiative: an antidote for snake bite. *Lancet (London, England)*, 375(9708): 89–91. https://doi.org/10.1016/S0140-6736(09)61159-4

Williams, D. J., Gutiérrez, J.-M., Calvete, J. J., Wüster, W., Ratanabanangkoon, K., Paiva, O. and Warrell, D. A. (2011). Ending the drought: new strategies for improving the flow of affordable, effective antivenoms in Asia and Africa. *Journal of Proteomics*, 74(9): 1735–1767. https://doi.org/10.1016/j.jprot.2011.05.027

Winkel, K. D., Fry, B. G., Hawdon, G. M. and Carroll, T. (2001). Black snake, brown snake or mistake? A cautionary tale. *Australian Family Physician*, 30(9): 832.

Wu, W. B., Peng, H. C. and Huang, T. F. (2001). Crotalin, a vWF and GP Ib cleaving metalloproteinase from venom of Crotalus atrox. *Thrombosis and Haemostasis*, 86(6): 1501–1511.

Wufen, B. M., Adamu, H. M., Cham, Y. A. and Kela, S. L. (2007). Preliminary studies on the anti-venin potential and phytochemical analysis of the crude extract of *Balanite agyptiaca Linn*). Delile on albino rats. *Natural Product Radiance*, 6(1): 18–21.

Wuster, W. and Broadley, D. G. (2007). Get an eyeful of this: a new species of giant spitting cobra from eastern and north-eastern Africa (Squamata: Serpentes: Elapidae: *Naja*). *Zootaxa*, 1532(1): 51–68.

Wuster, W. and Broadley, G. (2003). A new species of spitting copra from North-eastern African (Serpentes: Elapidae: *Naja*). *Journal of Zoology London*, 259: 345–359.

Wüster, W., Crookes, S., Ineich, I., Mané, Y., Pook, C. E., Trape, J.-F. and Broadley, D. G. (2007). The phylogeny of cobras inferred from mitochondrial DNA sequences: evolution of venom spitting and the phylogeography of the African spitting cobras (Serpentes: Elapidae: Naja nigricollis complex). *Molecular Phylogenetics and Evolution*, 45(2): 437–453. https://doi.org/10.1016/j.ympev.2007.07.021

Wuster, W. and McCarthy, C. J. (1996). Venomous snake systemic: Implications for Snake bite Treatment and toxinology. In *Envenoming and their treatment* (Bon and Goyffon, M. (Eds), pp. 13–23). Lyon: Foundation Merieux.

Wüster, W., Peppin, L., Pook, C. E. and Walker, D. E. (2008). A nesting of vipers: Phylogeny and historical biogeography of the Viperidae (Squamata: Serpentes). *Molecular Phylogenetics and Evolution*, 49(2): 445–459. https://doi.org/10.1016/j.ympev.2008.08.019

Wuster, W. and Thorpe, R. S. (1991). Asiatic cobras: systematic and snake bite. *Experientis*, 47: 205–209.

Wüster, W., Thorpe, R. S., J. Cox, M., Jintakune, P. and Nabhitabhata, J. (1995). Population systematics of the snake genus Naja (Reptilia: Serpentes: Elapidae) in Indochina: Multivariate morphometrics and comparative mitochondrial DNA sequencing (cytochrome oxidase I). *Journal of Evolutionary Biology*, 8(4): 493–510. https://doi.org/10.1046/j.1420-9101.1995.8040493.x

Wuster, W., Wallach, V. and Broadley, D. G. (2009). In praise of subgenera: taxonomic status of cobras of the genus *Naja Laurenti* (Serpentes: Elapidae). *Zootaxa*, 2236(1): 26–36.

Wuster, W., Warrell, D. A., Cox, M. J., Jintakune, P. and Nabhitabhata, J. (1997). Rediscription of Naj, Siamensis Laurenti 1768 (SerpentesElapidae), a widely over hooked spitting copra from southern Asia: geographic variation, medical importance and designation of neotype. *Journal of Zoology*, 243: 771–788.

Yasuo, Y., Koji, T., Hideko, A. and Takashi, M. (2003). Snake VenomVascular Endothelial Growth Factors (VEGFs) Exhibit Potent Activity through Their Specific Recognition of KDR (VEGF Receptor 2). *Journal of Biological Chemistry*, 278(52): 51985–51988.

Yeh, C. H., Peng, H. C., Yang, R. S. and Huang, T. F. (2001). Rhodostomin, a snake venom disintegrin, inhibits angiogenesis elicited by basic fibroblast growth factor and suppresses tumor growth by a selective alpha(v)beta(3) blockade of endothelial cells. *Molecular Pharmacology*, 59(5): 1333–1342.

Yeh, C.-H., Wang, W.-C., Hsieh, T.-T. and Huang, T.-F. (2000). Agkistin, a Snake Venom-derived Glycoprotein Ib Antagonist, Disrupts von Willebrand Factor-Endothelial Cell Interaction and Inhibits Angiogenesis. *Journal of Biological Chemistry*, 275(25): 18615–18618. https://doi.org/10.1074/jbc.C000234200

Yingprasertchai, S., Bunyasrisawat, S. and Ratanabanangkoon, K. (2003). Hyaluronidase inhibitors (sodium cromoglycate and sodium auro-thiomalate) reduce the local tissue damage and prolong the survival time of mice injected with Naja kaouthia and Calloselasma rhodostoma venoms. *Toxicon: Official Journal of the International Society on Toxinology*, 42(6): 635–646.

Zhang, H., Teng, M., Niu, L., Wang, Y., Wang, Y., Liu, Q. and Liu, P. (2004). Purification, partial characterization, crystallization and structural determination of AHP-LAAO, a novel l-amino-acid oxidase with cell apoptosis-inducing activity from *Agkistrodon halys pallas* venom. *Acta Crystallographica Section D: Biological Crystallography*, 60(5): 974–977. https://doi.org/10.1107/S0907444904000046

Zhang, Y., Wisner, A., Xiong, Y. and Bon, C. (1995). A novel plasminogen activator from snake venom. Purification, characterization, and molecular cloning. *The Journal of Biological Chemistry*, 270(17): 10246–10255.

Zhang, Y.-J., Wang, J.-H., Lee, W.-H., Wang, Q., Liu, H., Zheng, Y.-T. and Zhang, Y. (2003). Molecular characterization of *Trimeresurus stejnegeri* venom L-amino acid oxidase with potential anti-HIV activity. *Biochemical and Biophysical Research Communications*, 309(3): 598–604.

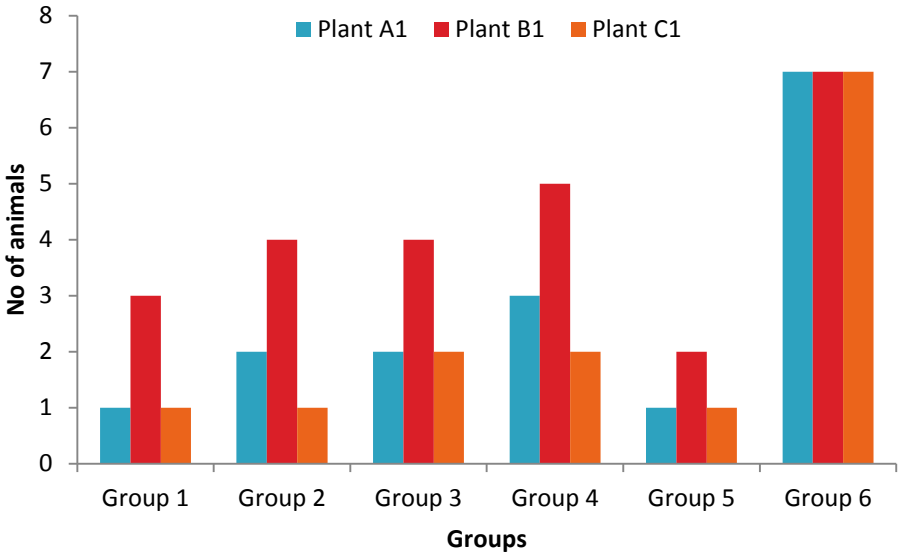
Zhou, Q., Dangelmaier, C. and Smith, J. B. (1996). The haemorrhagin catrocollastatin inhibits collagen-induced platelet aggregation by binding to collagen via its disintegrin-like domain. *Biochemical and Biophysical Research Communication*, 219: 720–726.

Zingali, R. B., Bianconi, M. L. and Monteiro, R. Q. (2001). Interaction of bothrojaracin with prothrombin. Haemostasis. *Haemostasis*, 31: 273–278.

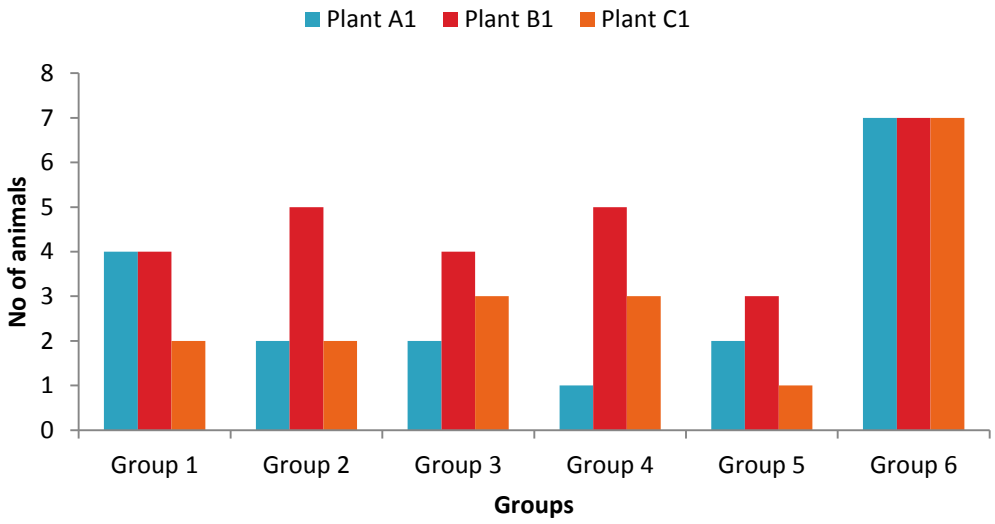
Zingali, R. B., Jandrot-Perrus, M., Guillin, M. C. and Bon, C. (1993). Bothrojaracin, a new thrombin inhibitor isolated from *Bothrops jararaca* venom: characterization and mechanism of thrombin inhibition. *Biochemistry*, 32(40): 10794–10802.

Zoltan, T., Kirk, C. and Wilhelmsen, S. S. (2004). Nicotinic Acetylcholine Receptor Exhibits Resistance to Erabu Sea Snake (Laticaudasemifasciata) Short-Chain a-Neurotoxin. *Journal of Molecular Evolution*, 58: 516–526.

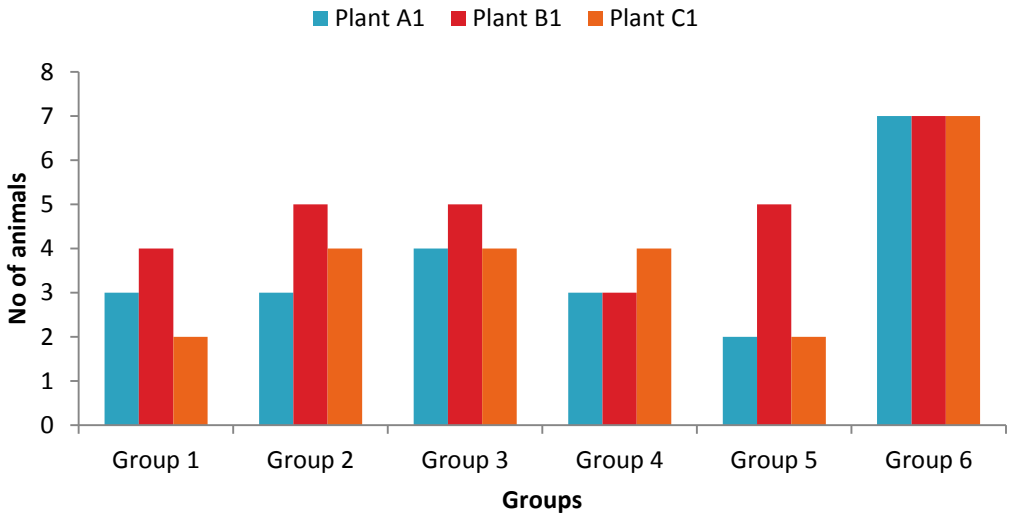
APENDIX



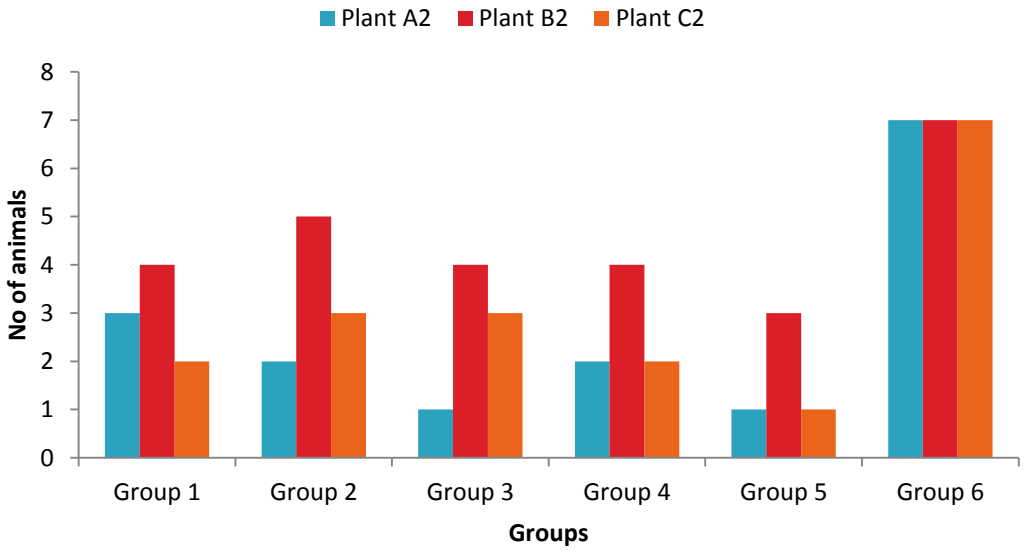
Appendix 1: Number of rats that survived the inoculation with venom 30 minutes later noculated with the first fraction (A_1, B_1, and C_1) of plant extracts.



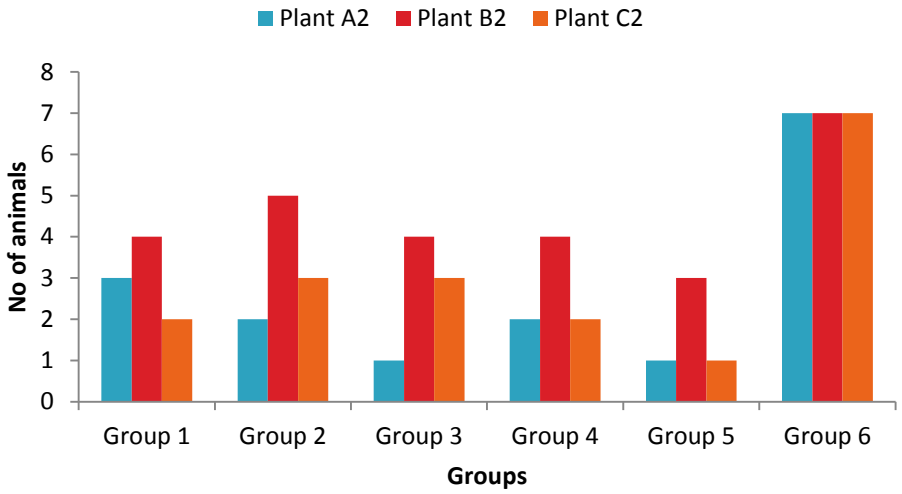
Appendix 2: Number of rats that survived the inoculation with the first fraction (A_1, B_1 and C_1) of plant extracts and 30 minutes later inoculated with venom.



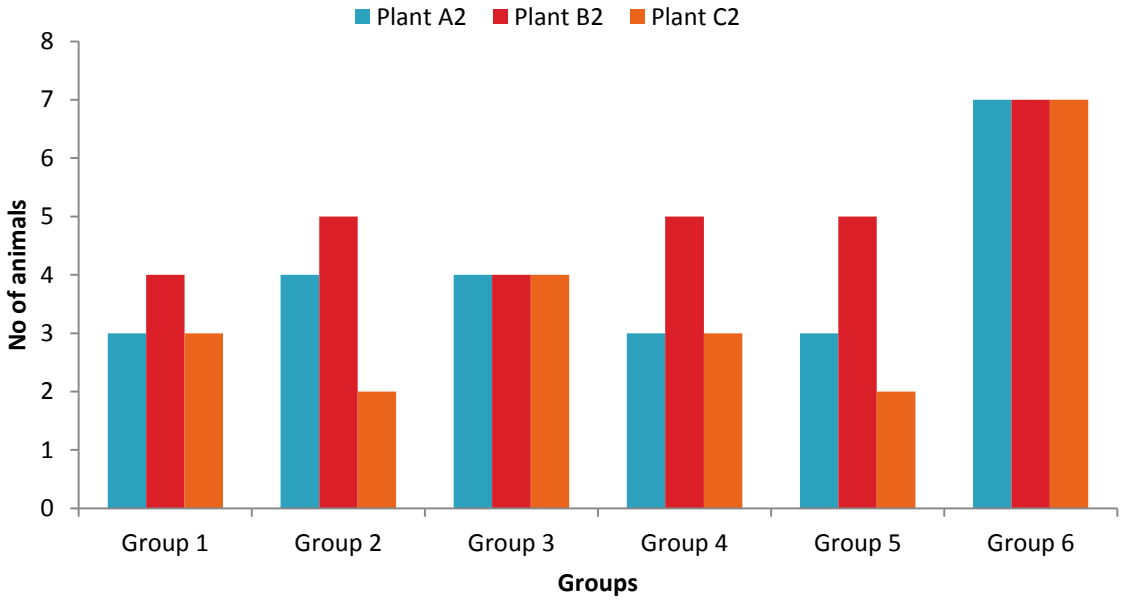
Appendix 3: Number of rats that survived after inoculation with mixture of venom and the first fraction (A_1, B_1, and C_1) of plant extracts incubated for 30 minutes.



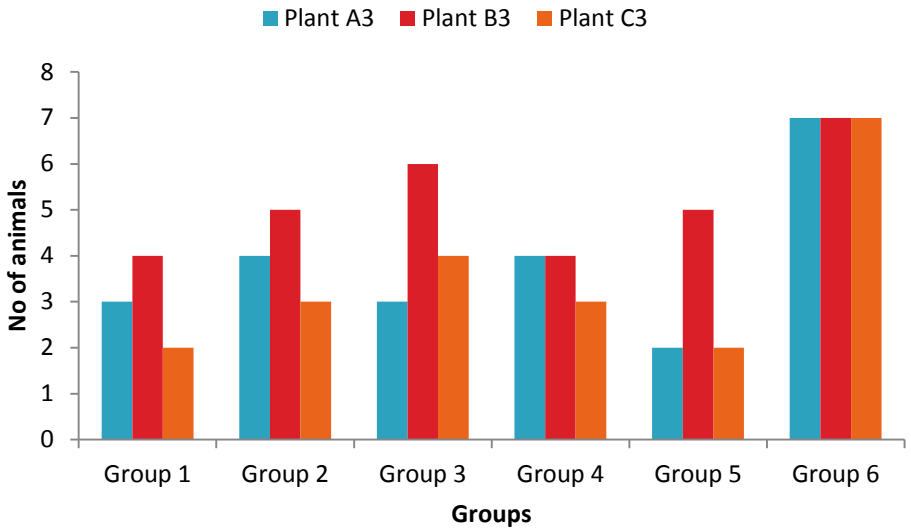
Appendix 4: Number of rats that survived the inoculation with venom 30 minutes later treated with the second fraction of plant extracts (A_2 B_2 C_2).



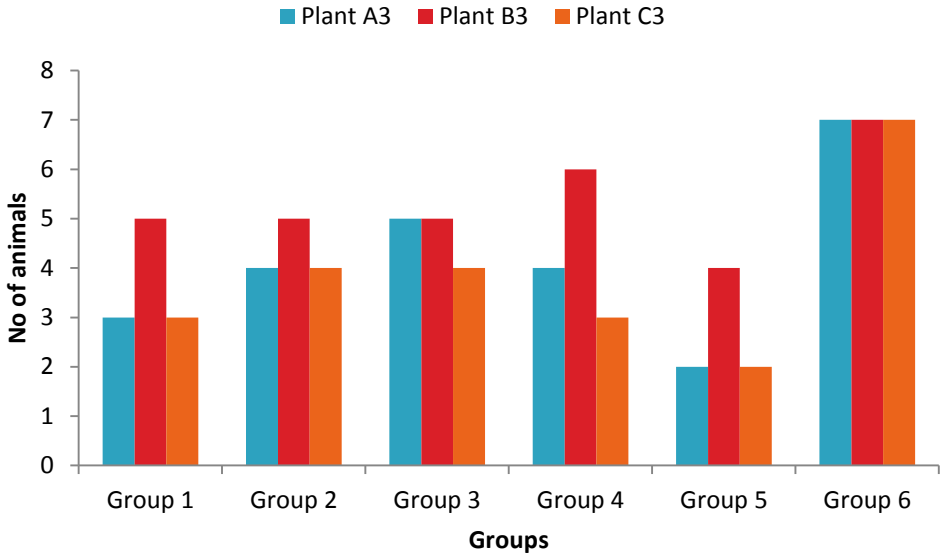
Appendix 5: Number of rats that survived the inoculation with the second fraction of plant extracts (A_2 B_2 C_2) and 30 minutes later inoculated with venom.



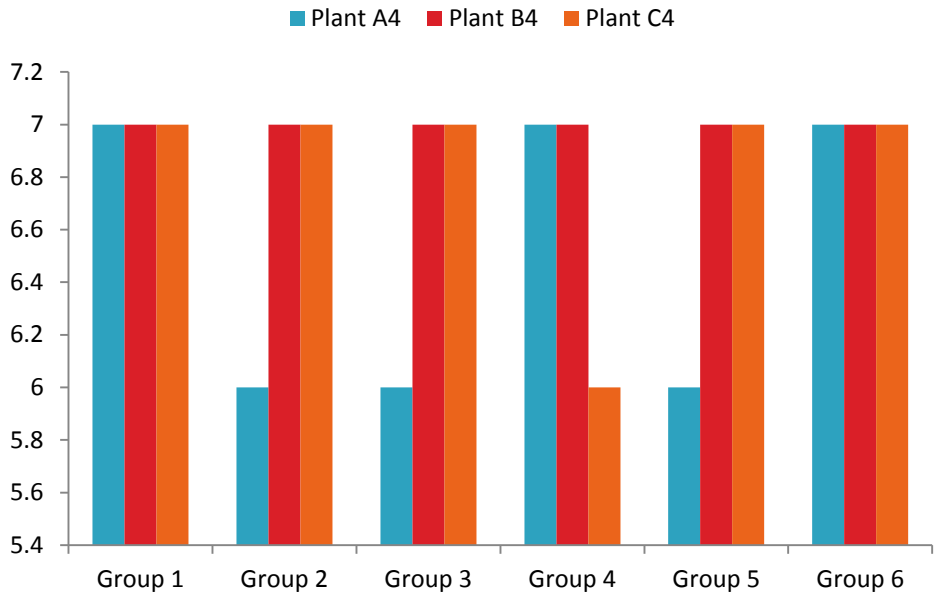
Appendix 6: Number of rats that survived after inoculation with mixture of venom and with the second fraction of plant extracts (A_2 B_2 C_2) incubated for 30 minutes.



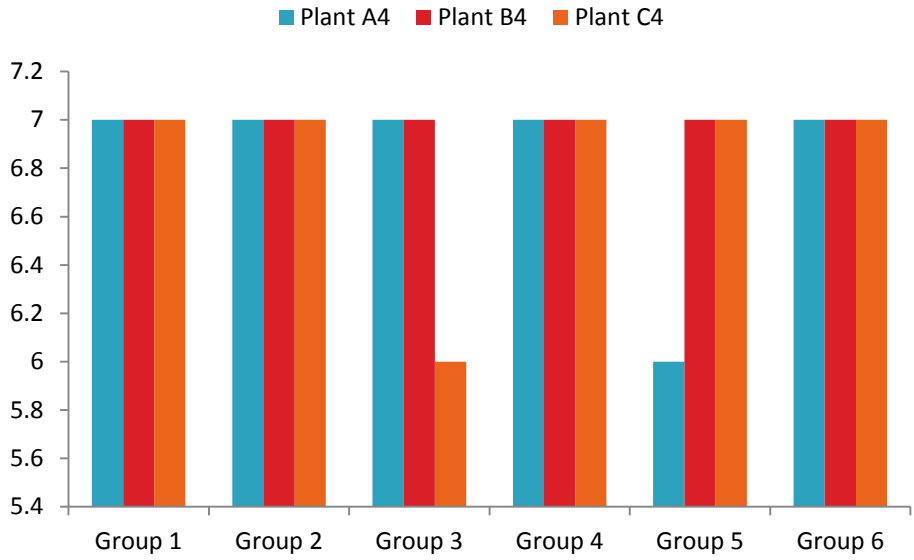
Appendix 7: Number of rats that survived the inoculation with venom 30 minutes later inoculated with the third fraction of plant extracts (A_3 B_3 C_3).



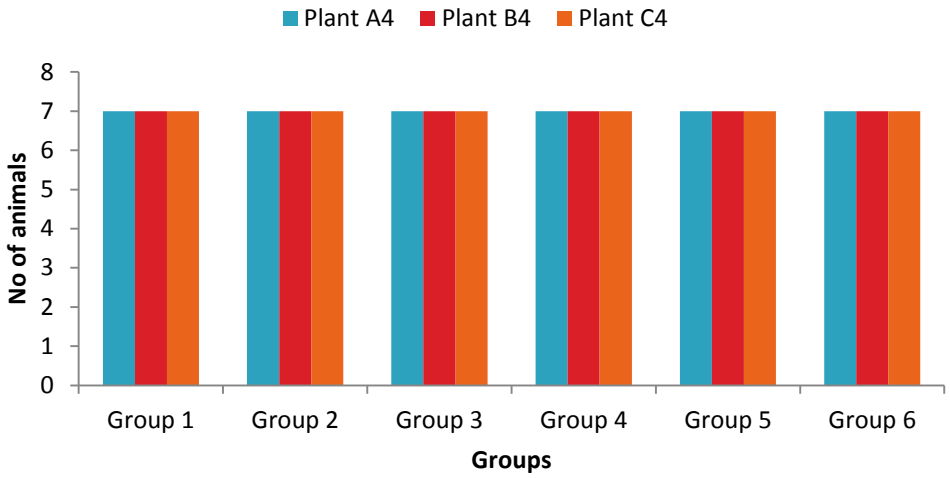
Appendix 8: Number of rats that survived the inoculation with the third fraction of plant extracts (A_3 B_3 C_3) and 30 minutes later inoculated with venom.



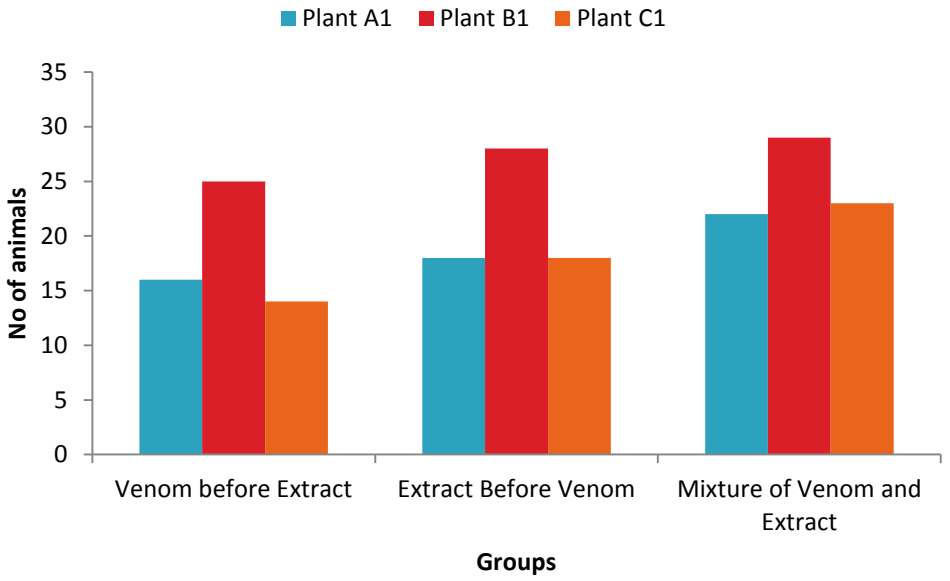
Appendix 9: Number of rats that survived after inoculation with mixture of venom and the third fraction of plant extracts (A_3 B_3 C_3) incubated for 30 minutes.



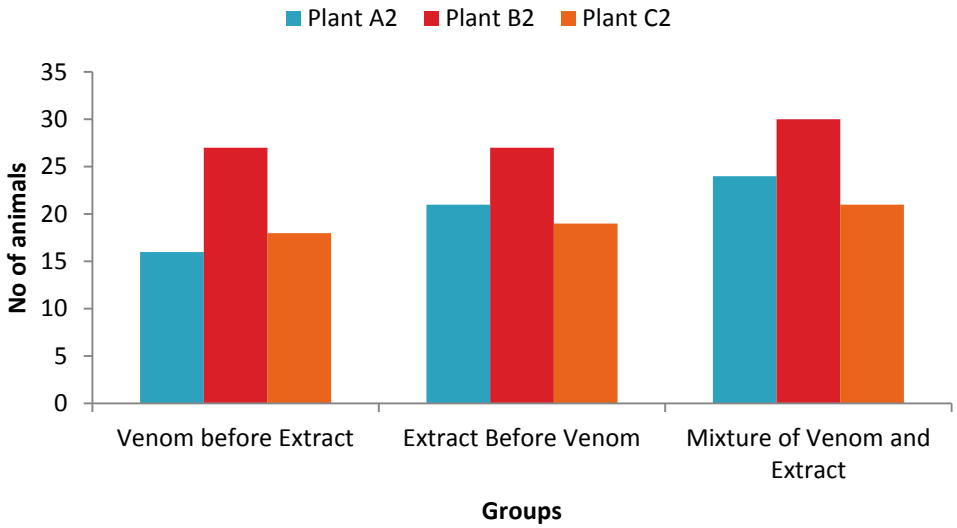
Appendix 10: Number of rats that survived the inoculation with venom 30 minutes later treated with the aqueous fraction of plant extracts (A_4 B_4 C_4).



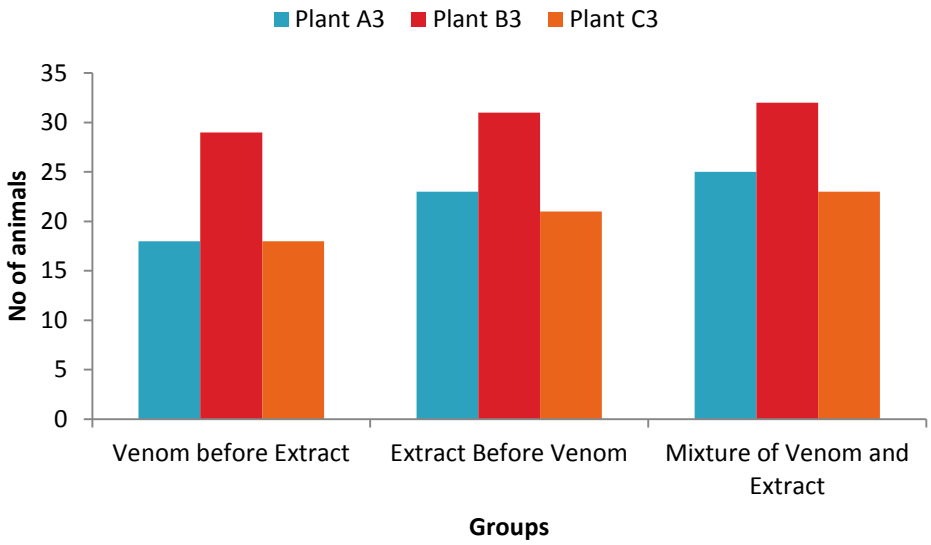
Appendix 11: Number of rats that survived the inoculation with aqueous fraction of the plant extracts (A_4 B_4 C_4) and 30 minutes later inoculated with venom



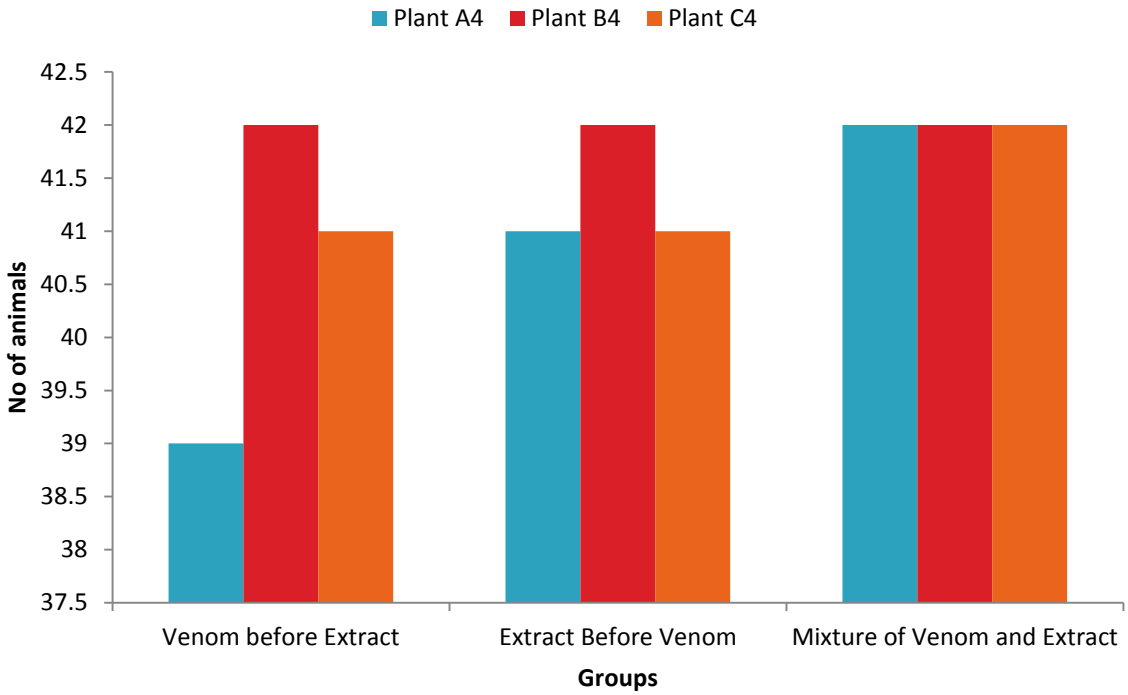
Appendix 12: Number of rats that survived after inoculation with mixture of venom and aqueous fraction of the plant extracts (A_4 B_4 C_4) incubated for 30 minutes



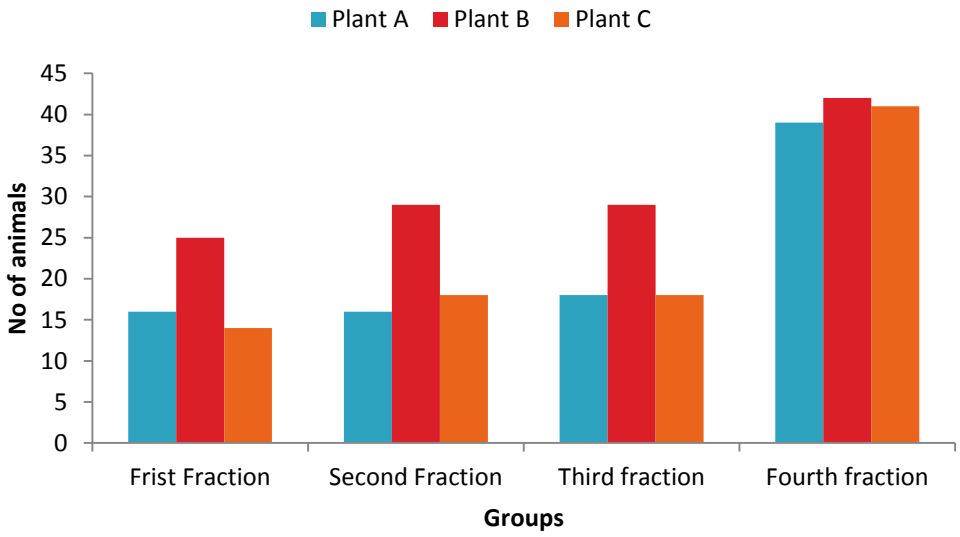
Appendix 13: The rats that survived the first fractions (A_1, B_1 and C_1)



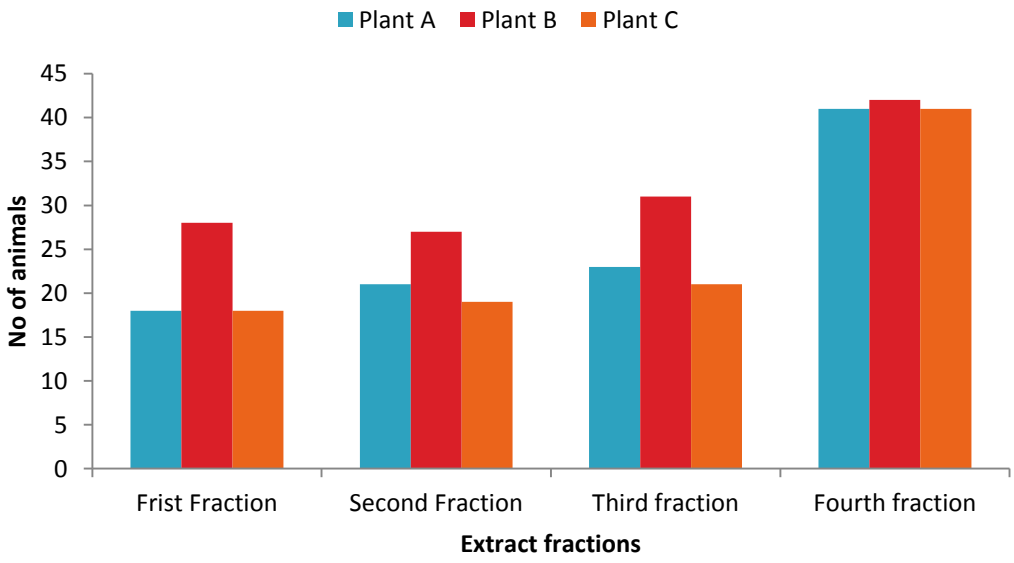
Appendix 14: The rats that survived the second fractions (A_2, B_2 and C_2)



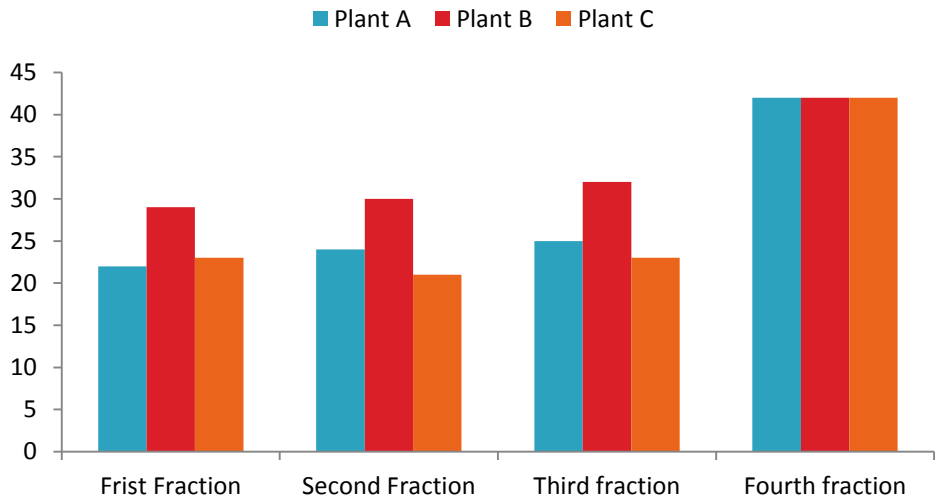
Appendix 15: The rats that survived the third fractions (A_3, B_3 and C_3).



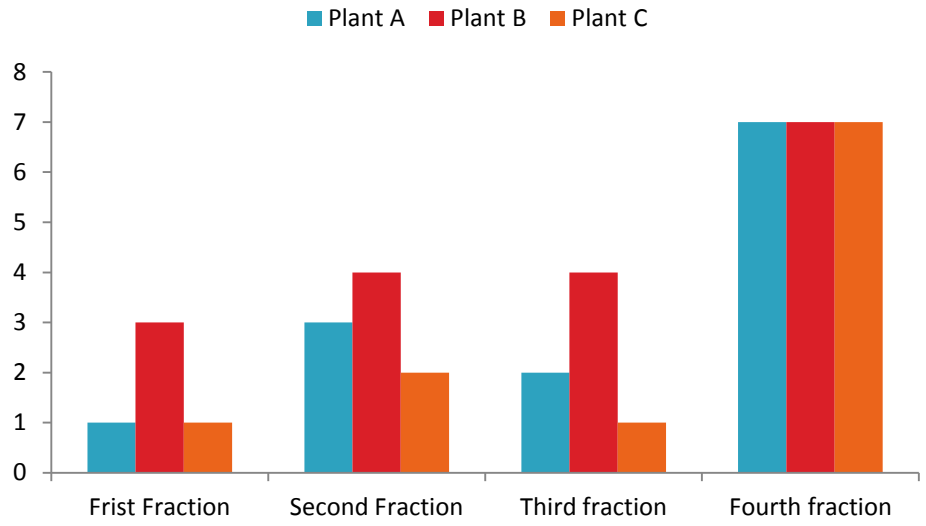
Appendix 16: The rats that survived the first fractions (A_4, B_4 and C_4)



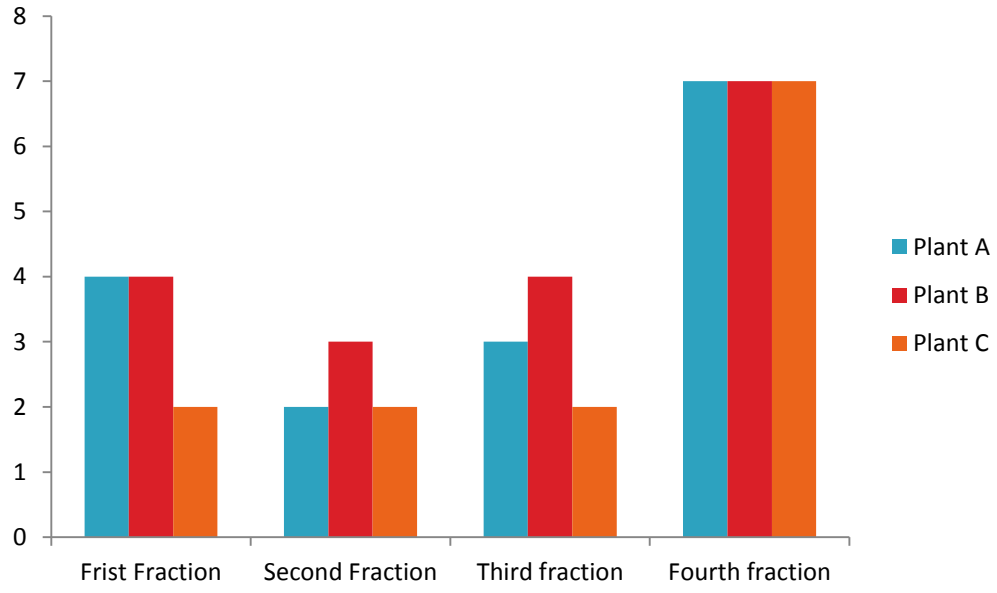
Appendix 17: The rats that survived the inoculation of venom first before administration of extract fractions



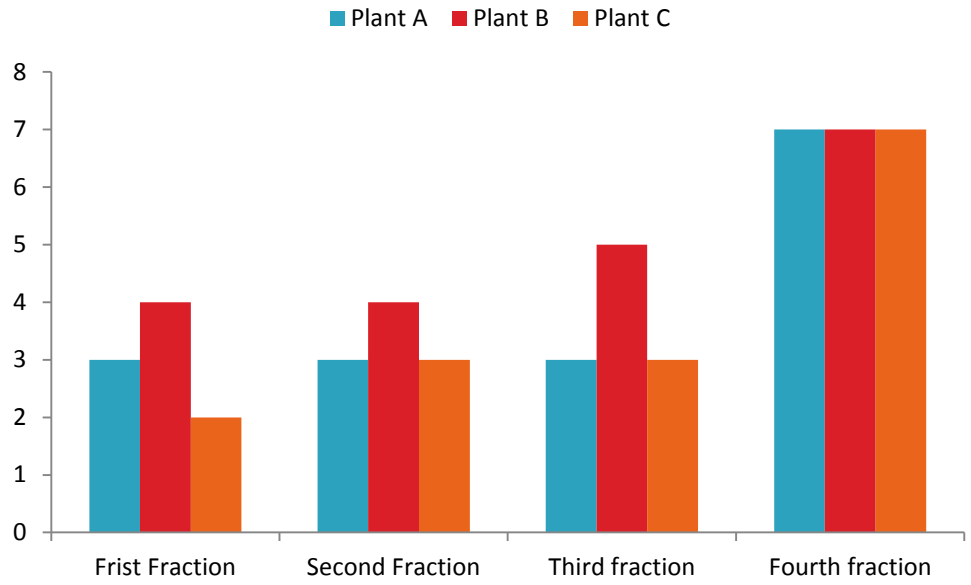
Appendix 18: The rats that survived the inoculation of extract fractions first before administration of venom



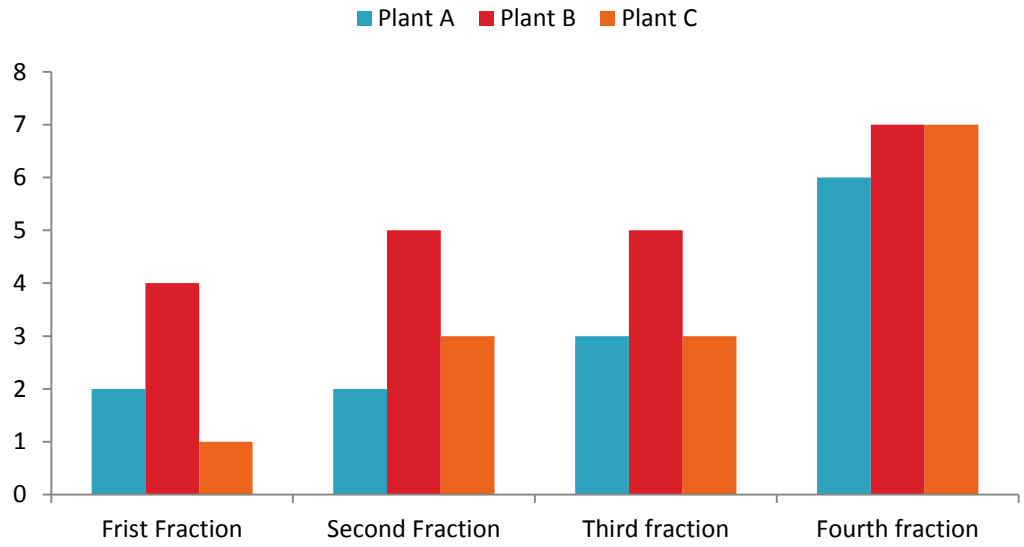
Appendix19: The rats that survived the inoculation together the mixture of extract fractions and venom.



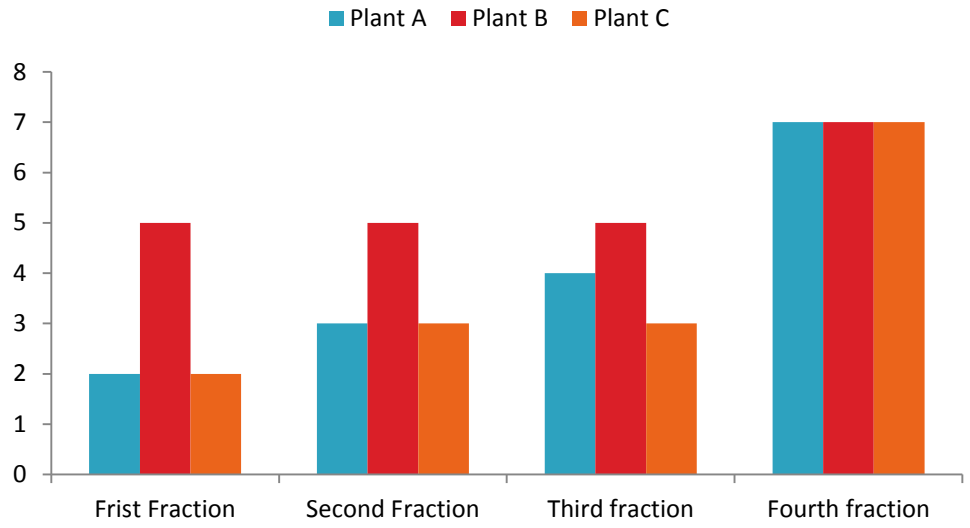
Appendix 20: The rats that survived the inoculation of *Naja nigricollis*venom before administration of fractions of the plant extracts



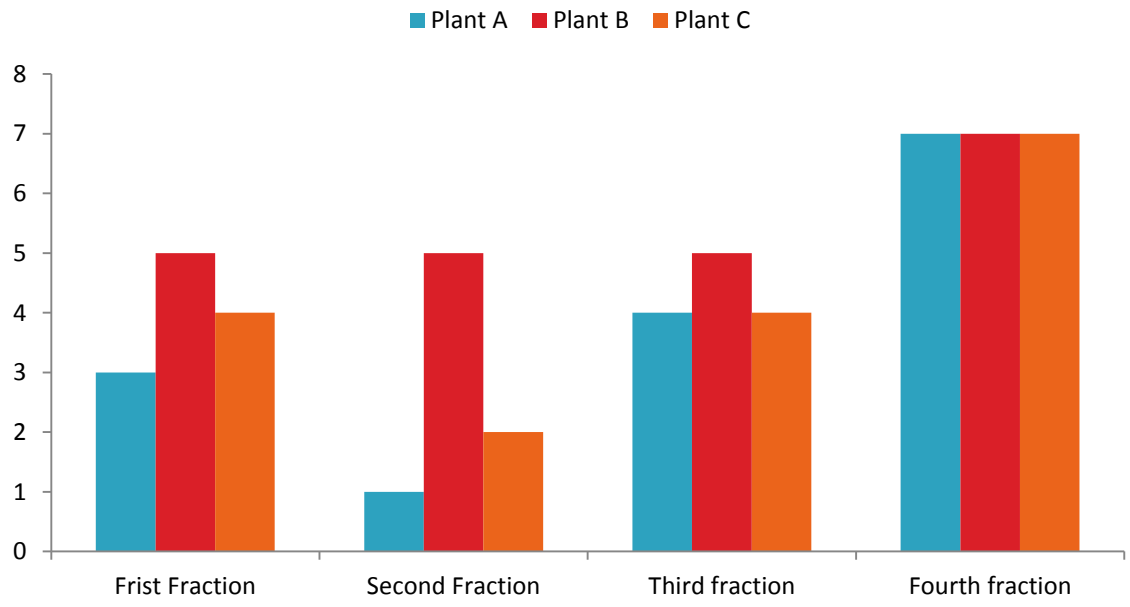
Appendix 21: The rats that survived the inoculation of fraction of the plant extracts before inoculation with *Naja nigricollis*venom.



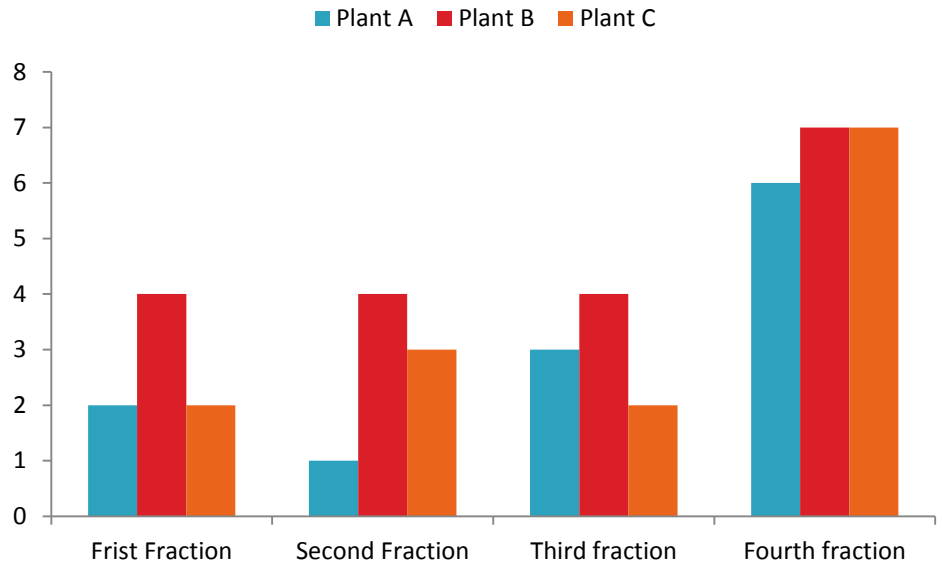
Appendix 22: The rats that survived the inoculation of the mixture of *Naja Nigricollis* venom incubated together with fractions of the plant extracts



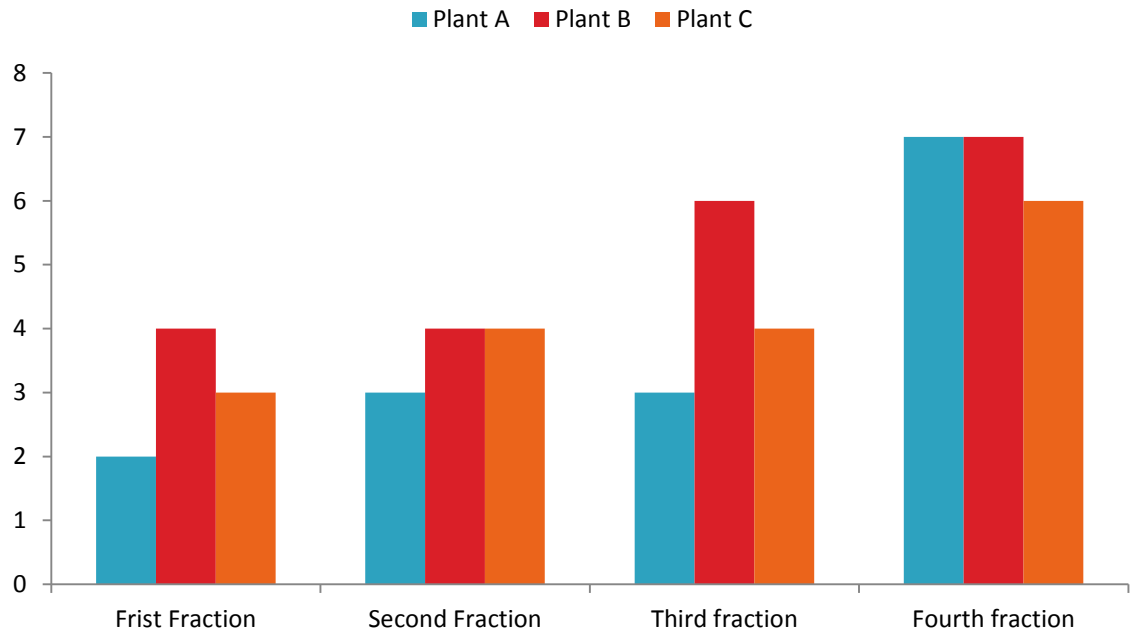
Appendix 23: The rats that survived the inoculation of *Naja haje* venom before administration of fractions of the plant extracts



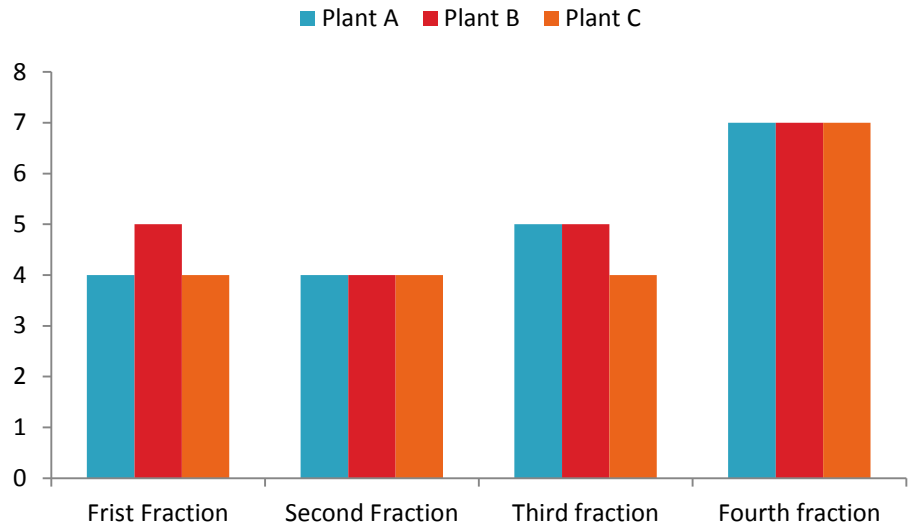
Appendix 24: The rats that survived the inoculation of fraction of extracts 30 minutes before the inoculation of Naja *haje* venom



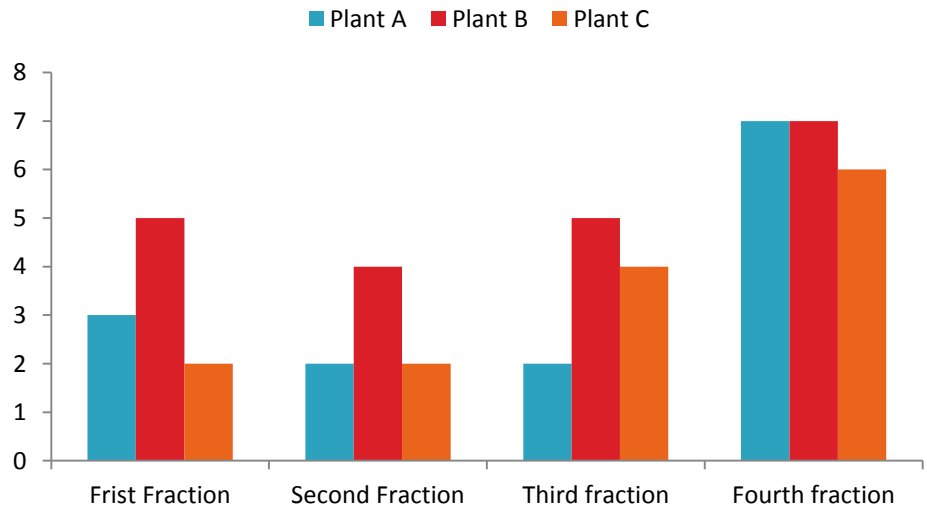
Appendix 25: The rats that survived the inoculation of *Naja nigricollis*venom together with the fractions of the plant extracts.



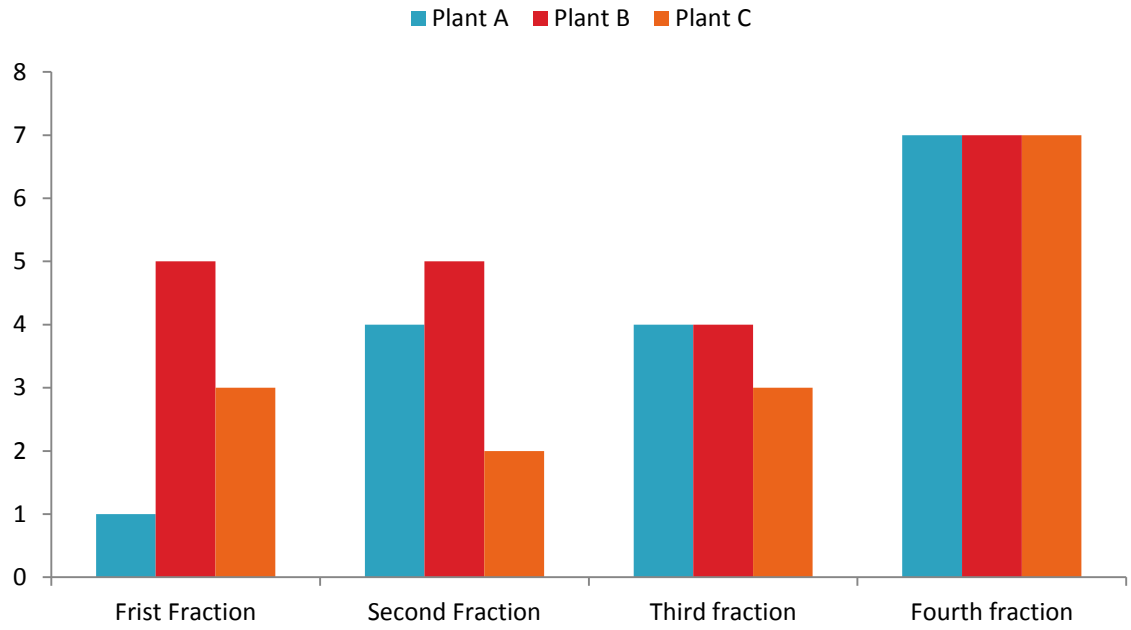
Appendix 26: The rats that survived the inoculation of *Naja katiensis* venom before administration of fractions of the plant extracts.



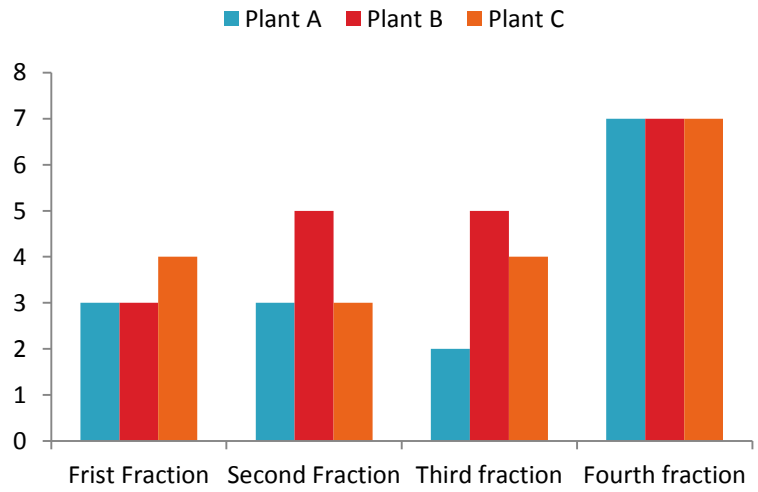
Appendix 27: The rats that survived the inoculation of plant fraction extracts before inoculation with *Naja katiensis* venom



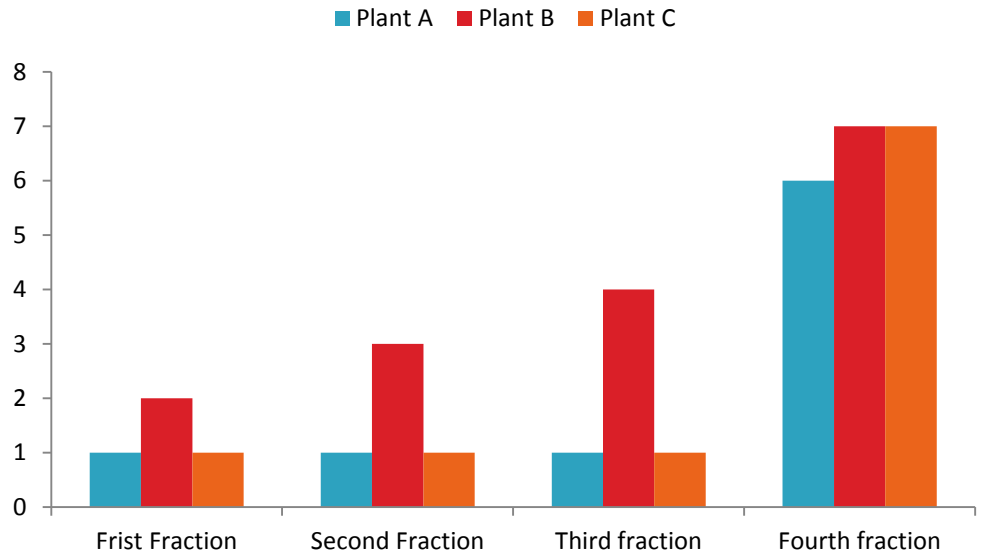
Appendix 28: The rats that survived the inoculation of *Naja katiensis* venom together With the plant extracts



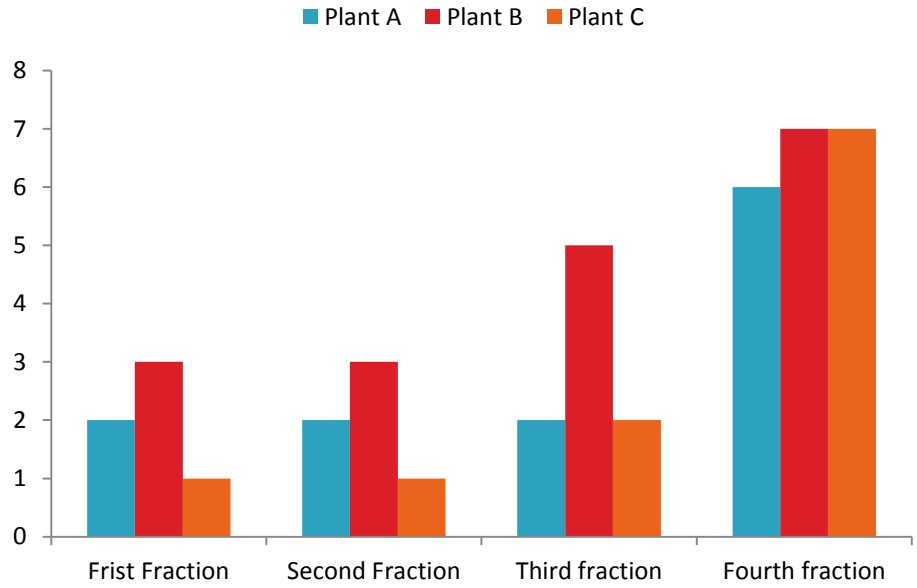
Appendix 29: The rats that survived the inoculation of *Bitis arietans* venom before administration of fractions of the plant extracts.



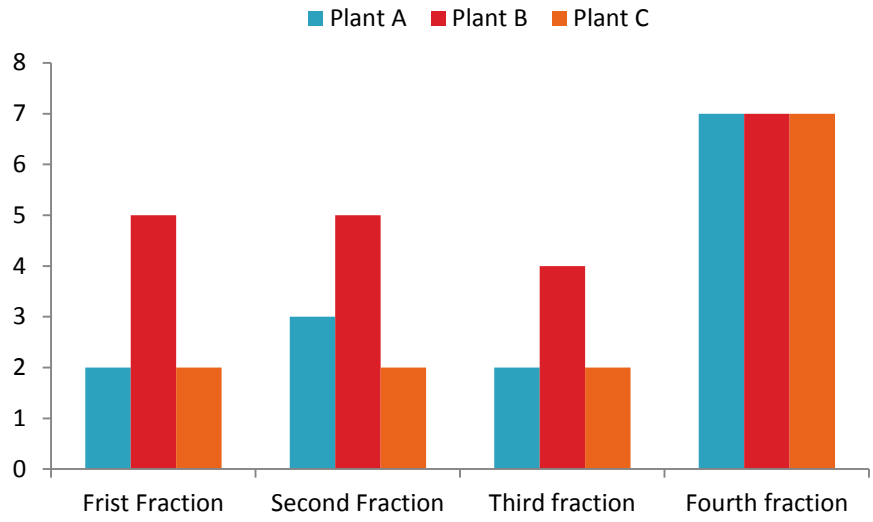
Appendix 30: The rats that survived the inoculation of plant extracts before inoculation of *Bitis arietans* venom



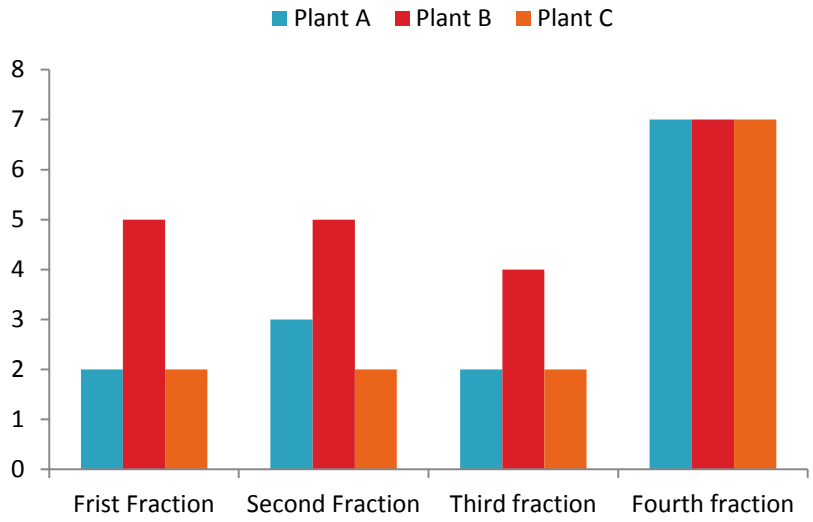
Appendix 31: The rats that survived the inoculation of *Bitis arietans* venom together with the fractions of the plant extracts



Appendix 32: The rats that survived the inoculation of *Echis ocellatus*venom before administration of fractions of the plant extracts



Appendix 33: The rats that survived the inoculation of plant extracts before inoculation of *Echis ocellatus* venom



Appendix 34: The rats that survived the inoculation of *Echis ocellatus*venom together with the fractions of plant extracts

Appendix 35: Survival rates of rats envenomated with snake venoms and administered di-ethyl- ether extracts of plants A, B and C

		Methods of Administration		
Extract 1 (di-ethyl ether)	No. of rats that survived envenomation	Method 1	Method 2	Method 3
Plant A	56	16 (28.6)	18 (32.1)	22 (39.3)
Plant B	82	25 (30.5)	28 (34.1)	29 (35.4)
Plant C	55	14 (25.5)	18 (32.7)	23 (41.8)
Total	**193**	**55 (28.5)**	**64 (33.2)**	**74 (38.3)**

Appendix 36: Survival rates of rats envenomated with snake venoms and Administered ethyl acetate extracts of plants A, B and C

		Methods of Administration		
Ethylacetate Extracts	No. of rats that survived envenomation	Method 1	Method 2	Method 3
Plant A	61	16 (26.2)	21 (34.4)	24 (39.3
Plant B	84	27 (32.1)	27 (32.1)	30 (35.7)
Plant C	58	18 (31.0)	19 (32.8)	21 (36.2)
Total	203	61 (30.0)	67 (33.0)	75 (36.9)

Appendix 37: Survival rates of rats envenomated with snake venoms and administered N- butanol extracts of plants A, B and C.

		Methods of Administration		
N-Butanol Extracts	No. of rats that survived envenomation	Method 1	Method 2	Method 3
Plant A	66	18 (27.3)	23 (34.8)	25 (37.9)
Plant B	92	29 (31.5)	31 (33.7)	32 (34.8)
Plant C	62	18 (29.0)	21 (33.9)	23 (37.1)
Total	220	65 (29.5)	75 (34.1)	80 (36.4)

Appendix 38: Survival rates of rats envenomated with snake venoms and Administered aqueous extracts of plants A, B and C

		Methods of Administration		
Aqueous extracts	No. of rats that survived envenomation	Method 1	Method 2	Method 3
Plant A	122	39 (32.0)	41 (33.6)	42 (34.4)
Plant B	126	42 (33.3)	42 (33.3)	42 (33.3)
Plant C	124	41 (33.1)	41 (33.1)	42 (33.9)
Total	372	122 (32.8)	124 (33.3)	126 (33.9)

Appendix 39: Snake Fangs

Appendix 40: Bitis arietans