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***In vivo* ANTIVENOM POTENTIAL OF METHANOLIC EXTRACTS OF
PHYTOLACCA DODECANDRA AND VERNONIA AMYGDALINA**

BY

SSEBUGUZI PHILLIP KIKAWA

22/U/3828/PS

2200703828

BLT III

**A RESEARCH REPORT SUBMITTED TO THE COLLEGE OF VETERINARY
MEDICINE, ANIMAL RESOURCES AND BIOSECURITY, DEPARTMENT OF
LIVESTOCK AND INDUSTRIAL RESOURCES IN PARTIAL FULFILLMENT
FOR THE AWARD OF THE DEGREE IN BACHELOR OF BIOMEDICAL
LABORATORY TECHNOLOGY**

AUGUST, 2026

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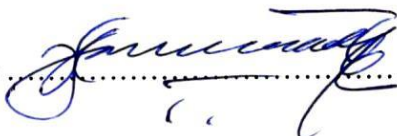
Signature:  Date: 14.08.26.

APPROVAL

This research report has been prepared and submitted under the supervision and guidance of my supervisor.

Dr. John Kateregga (PhD)

Department of Veterinary Pharmacy, Clinics & Comparative Medicine (PCM),
School of Veterinary Medicine and Animal Resources (SVAR),
College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB),
Makerere University, P.O Box 7062, Kampala, Uganda.

Signature:  Date: 14/08/2026

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Name: SSEBUGUZI PHILLIP KIKATAWA

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Tel No.: 0752434906 E-Mail: ssebuguziphillip@gmail.com

Signature: [Signature] Date: 12.08.2026

Supervisor's endorsement: [Signature]

DEDICATION

I dedicate this research proposal to my loving parents, Mr. Ssebuguzi Cranmer and Mrs Hanifa Nayiga S.S who have selflessly supported me throughout this education journey. To the Almighty God who has guided me through every step in life and finally, my siblings, friends, and mentors

ACKNOWLEDGEMENT

I would like to thank God who has granted me the knowledge to reach this far in life. With sincere gratitude, I thank my supervisor, Dr. John Kateregga and Mr. Mukwaya Joel for their guidance, time, and assistance in writing this proposal.

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LIST OF ABBREVIATIONS

%:	Percentage
μL:	microliters
ANOVA :	Analysis of Variance
BLT:	Bachelor of Biomedical Laboratory Technology
CNS :	Central Nervous System
COVAB :	College of Veterinary Medicine, Animal Resources and Biosecurity
g:	grams
h :	hours
IM :	Intramuscular
kg :	kilograms
mg :	milligrams
mg/kg :	milligrams per kilogram
mg/mL :	milligrams per milliliter
mL :	milliliters
n:	sample size
NaOH :	Sodium Hydroxide
PCM :	Veterinary Pharmacy, Clinics & Comparative Medicine
PD :	Phytolacca dodecandra
SD:	Standard Deviation
SVAR :	School of Veterinary Medicine and Animal Resources
VA :	Vernonia amygdalina
WHO :	World Health Organization

ABSTRACT

The venom from various snake species contains complex bioactive molecules that cause many severe local and systemic effects, potentially leading to death and this poses a significant public health concern. Current antivenoms, though effective, are often costly and inaccessible in high-risk areas. Traditional medicinal plants such as *Phytolacca dodecandra* and *Vernonia amygdalina* have been used for centuries in sub-Saharan Africa to treat snakebites, but their antivenom potential has not been thoroughly investigated. This study is aimed at evaluating the antivenom properties of methanolic extracts from these plants and identifying their phytochemical constituents.

Experimental studies involved the use of 20 Wister Albino rats that were divided into 5 groups of 4 each ie the normal control, the negative control, *Vernonia amygdalina* treatment, *Phytolacca dodecandra* treatment and the combination group. The effect of the methanolic extracts as antivenoms on the animals was measured using time taken to die and the observed clinical signs in every group such as paralysis, irritability, gasping and polyuria.

The methanolic extracts from the plants showed good antivenom properties that were dose dependant and showed better results when used at high dosage of 400mg/kg for individual plant extracts. The plant extracts also showed synergism portrayed by the combination of these plant extracts showing better antivenom properties even at lower dosages.

There was antivenom potential demonstrated by the methanolic plant extracts in the animal models as they were able to prolong the time taken to die for the animals administered with the venom and the extracts. The plants also contained phytochemicals such as alkaloids, saponins, phenols, tannins and flavonoids that contribute to their antivenom properties.

CHAPTER ONE

INTRODUCTION

1.1 Background

Snake bites and venom are a significant public health concern worldwide, particularly in tropical and subtropical regions. Every year, millions of people are bitten by snakes, resulting in a considerable number of deaths and disabilities. (WHO, 2020; Gutiérrez et al., 2017) Venomous snakes are found on every continent except Antarctica, and they belong to various families, including Elapidae (cobras, coral snakes, and taipans), Viperidae (vipers), and Crotalidae (rattlesnakes, copperheads, and cottonmouths) (Warrell, 2010). The venom of these snakes is a complex mixture of bioactive molecules, including proteins and peptides, which are designed to immobilize and kill prey (Calvete, 2017). The venom of snakes can cause a wide range of systemic and local effects, including pain, swelling, bruising, and blistering (Chippaux, 2017). In severe cases, snake venom can cause respiratory failure, cardiac arrest, and renal failure, leading to death (Maduwage & Isbister, 2014).

The severity of the symptoms depends on various factors, including the species of the snake, the amount of venom injected, and the location of the bite (Alirol et al., 2010). In many parts of the world, especially in rural areas, snake bites are often untreated or inadequately treated, leading to high morbidity and mortality rates (Harrison et al., 2009). However, with prompt medical attention, including the administration of antivenom, the prognosis for snake bite victims can be significantly improved (Chippaux & Goyffon, 2016).

Antivenom, also known as antivenin or antivenene, is a type of medicine that is used to treat snake bites. It is made by milking the venom from snakes and then using it to immunize animals, such as horses or sheep (Theakston & Warrell, 2000). The antibodies produced by these animals are then harvested and used to create antivenom. Antivenom is highly effective in neutralizing snake venom and preventing its toxic effects (Fry B.G., 2005; Laustsen et al., 2016). However, plants have been used for centuries in traditional medicine to treat various ailments, including snakebites (Warrell & Arnett, 1976). Several plant species have been identified as potential antivenom agents, exhibiting inhibitory

effects against snake venom toxins (Gomes et al., 2007). These plants contain bioactive compounds, such as alkaloids, glycosides, and terpenoids, which have been shown to neutralize snake venom (Kumar, S., & Kumar, V., 2018; Oliveira et al., 2018). Some of these plants include *Aloe vera*, *Mimosa pudica*, *Ocimum sanctum* (Kumar, S., & Kumar, V., 2018). *Phytolacca dodecandra* and *Vernonia amygdalina* have also been used in some parts of sub-Saharan Africa as antivenom plants (Oduola et al., 2014; Ijeh & Ejike, 2011).

In conclusion, snake bites and venom are a significant public health concern that requires prompt medical attention. With the administration of antivenom and supportive care, the prognosis for snake bite victims can be significantly improved (WHO, 2020; Gutiérrez et al., 2017).

1.2 Problem Statement

Envenomation from snake bites remains a significant challenge to the public health sector especially in the rural and tropical areas where there's less access and shortage of antivenoms. This inaccessibility and shortage are usually because of the high cost of manufacturing the current available antivenoms which in turn makes the final products expensive making it hard for people in rural areas to attain. This then leaves such people with a challenge to face adverse effects from the envenomation, such as death.

Phytolacca dodecandra and *Vernonia amygdalina* are medicinal plants traditionally used in various regions for their therapeutic properties that include anti-inflammatory and detoxifying effects. However, their potential as antivenom agents has not been investigated thoroughly in vivo. The methanolic extracts of these plants may contain bioactive compounds capable of neutralizing snake venom toxins and hence providing a natural remedy to curb the effects of envenomation. This study therefore seeks to investigate the anti-venom qualities of *Vernonia amygdalina* and *Phytolacca dodecandra* that could be easier to obtain to save situations where individuals are victims of envenomation.

1.3 Objectives

1.3.2 General Objective

To determine the antivenom potential of the methanolic extracts of *Phytolacca dodecandra* and *Vernonia amygdalina* and their phytochemical constituents.

1.3.3 Specific Objectives

- i. To determine the phytochemicals, present in the methanolic extracts of *Vernonia amygdalina* and *Phytolacca dodecandra*.
- ii. To evaluate the antivenom properties of the methanolic extracts of *Phytolacca dodecandra* and *Vernonia amygdalina* in animal models.

1.4 Research Questions

1. What are the present phytochemicals in *Vernonia amygdalina* and *Phytolacca dodecandra*?
2. How effective are the extracts from the plants in curing venom bites and the degree of damage caused by venom introduction in an organism?

1.5 Significance of study

The importance of the study is that it would contribute to the discovery of natural anti-venom compounds from the aqueous plants which can be used later for the development of more anti-venom therapies that would be much more readily available especially to people in the areas where they are most susceptible to the bites from organisms that release venom. The study also provided more information about the phytochemicals present in *Vernonia amygdalina* and *Phytolacca Dodecandra*.

1.6 Justification of the study

It is reported that there is an increased number of people that are injured and some die from bites from venomous organisms. *Phytolacca dodecandra* and *Vernonia amygdalina* have been used by some communities in Sub-Saharan Africa as antivenins but there hadn't been any scientific validation, and this therefore has made it important for an intervention to carry out research on these plants (World Health Organisation 2023).

CHAPTER TWO

LITERATURE REVIEW

2.1 The Venomous Snakes

Snake venom is a complex mixture of bioactive molecules, including proteins and peptides that are designed to immobilize and kill prey. The venom of snakes can cause a wide range of systemic and local effects, including pain, swelling, bruising, and blistering (Warrell, 2010). The composition of snake venom varies greatly between species, and even within the same species, venom composition can vary depending on factors such as the snake's diet, age, and geographic location (Fry, 2005). Snake venom contains a variety of enzymes, including phospholipases, proteases, and hyaluronidases, which break down tissues and cause cell death (Mebs, 2002). Some of the most venomous snakes in the world include the inland taipan (*Oxyuranus microlepidotus*), the eastern brown snake (*Pseudonaja textilis*), and the black mamba (*Dendroaspis polylepis*) (Sutherland, 1983). These snakes are found in various parts of the world, including Australia, Africa, and Asia.

Several plant species have been identified as having anti-snake venom properties. For example, the extract of *Aloe vera* has been shown to inhibit the haemorrhagic activity of *Bothrops jararaca* venom (Tan et al., 2015). Similarly, the latex of *Euphorbia tirucalli* has been traditionally used to treat snakebites in some parts of Africa (Abreu et al., 2015). *Phytolacca dodecandra* and *Vernonia amygdalina* have been studied for their potential as snake antivenins. *Phytolacca dodecandra* has been traditionally used in Ethiopian medicine to treat various ailments, including snakebites (National Library of Medicine.,2023). *Vernonia amygdalina*, on the other hand, has been studied for its anti-venom properties against certain snake species. Research has shown that extracts from the plant's leaves can inhibit the activity of snake venom. While these findings are promising, more research is needed to fully explore the potential of *Phytolacca dodecandra* and *Vernonia amygdalina* as snake antivenins.

2.2 The mechanism of action of venom

It involves the following steps in the body of an organism. First, venom injection, where the venomous organism bites or stings another animal and introduces venom into its body system. This is then followed by distribution of the venom and its absorption in the body

of an organism. The distribution depends on the properties of the specific venom from a specific organism. The venom then binds to receptors on the surface of cells triggering effects in the body of a person or an animal. Some of these effects are irreversible and may require amputation to save one. Physiological processes such as neurotransmission, muscle contraction, blood coagulation and inflammation. This then followed by immune responses that include activating various immune system cells and this in turn can lead to inflammation, tissue damage and complications. Neurotoxins in venom affect the nervous system while hemotoxins affect the blood and cardiovascular system. Cytotoxins affects cells and tissues and finally myotoxins affects muscles (National Institutes for Medicine. 2020).

2.3 Risk Factors of exposure to venom

There are several risk factors that determine how people encounter venomous organisms which include some of the following. People living in rural areas stand a higher risk of encountering venomous organisms as they are closer to the natural habitats of these organisms such as bushes. Agricultural activities where farmers and herders may be at a risk of snakebites scorpion stings and other venomous organisms due to working in proximity with the natural habitats of the organisms is also a risk factor. Failure to wear protective gear for example gumboots for farmers also increases the risk of venomous animals easily biting (National Institutes for Health,2020).

2.4 Toxic effects caused by venom from snake bites, first aid their treatment and management

The general first aid for treating of venomous organisms' bites such as snake bites involves firstly keeping the patients calm as panicking could lead to one having a faster heart rate and thus causing the venom to spread faster (WHO, 2020; Gold et al., 2002). Removing any constricting items and loosening any tight clothing or jewellery (Chippaux, 2017). Keeping the affected limb lower than the heart level can also help prevent the venom from spreading faster (Kularatne, 2003). It is also advised not to attempt sucking out the venom as this can cause more harm (Warrell, 2010) and applying heat or ice as first aid should also be avoided as this can worsen effects of the venom (Alirol et al., 2010). For snake bites it is advised to identify the snake, if possible, to make the treatment easier and then this should be followed by application of an antivenom (Theakston & Warrell, 2000). Monitoring of

vital signs is also important such as patients' pulse, blood pressure and respiratory rate (Maduwage & Isbister, 2014). Pain management can be done through administering of pain relief medicines (Harrison et al., 2009). Antihistamines can also be given to manage allergic reactions (Gutiérrez et al., 2017). Wounds developed from bites should be taken care of for example by cleaning them and dressing them.

2.4.1 Haemolysis

This is a toxic effect caused by snake venom. The venom usually contains proteins and enzymes for example: Phospholipase A2 which directly cleaves the phospholipid bilayer and the hydrophobic three finger toxins which can bind to the cell membrane and promote cell lysis (Fry, 2005). Severe haemolysis causes haemolytic anaemia. Haemolysis can be treated through blood transfusions (Gutiérrez et al., 2017).

2.4.2 Coagulopathy

This refers to the condition where one's blood is unable to clot. The venom reduces the blood clotting factors through activating of the body's clotting system causing depletion of clotting factors. Coagulopathy therefore increases the risk of bleeding. Venom contains pre-coagulant toxins which activate prothrombin, factor V, factor X, or thrombin-like enzymes (Warrell, 2010). These effects can be managed through blood transfusions and treatment with antivenoms (Theakston & Warrell, 2000).

2.4.3 Renal failure

Renal failure is caused mainly through cytotoxic effects of damaging kidney cells. Other ways it causes this is by haemolysis caused by the venom which can damage the kidneys, hypotension and systemic myotoxicity which can lead to kidney damage (Maduwage & Isbister, 2014).

2.4.4 Tachycardia

Tachycardia refers to an abnormally elevated heartbeat and this can prevent the heart from effectively pumping blood. Venom contains bioactive components that can induce catecholamine release which in turn would lead to hypersensitivity of the sympathetic nervous system, hence causing tachycardia. It can be treated mainly through administration of antivenom (WHO, 2020).

2.4.5 Fever

Fever refers to an elevated body temperature from the norm. The introduction of snake venom into the body causes the immune system to respond and this in turn can lead to swelling and inflammation, hence causing fever. It is treated through application of antipyretics and antivenom (Harrison et al., 2009).

2.4.6 Edema

This refers to swelling of tissue due to fluid retention. Venom damages the lymphatic system which is responsible for absorbing venom toxins from tissues. It also damages the vascular endothelium which can lead to early edema. It is treated through elevation of affected limb and administration of antivenom (Chippaux, 2017).

2.4.7 Necrosis

Necrosis refers to cell death and tissue damage. Venom contains myotoxins which damage muscle fibres. They disrupt the sarcolemma, which is the membrane that surrounds muscle cells. This then causes a calcium influx that causes the muscle cells to hyper contract and thus leading to muscle damage (Calvete, 2017). It is treated through administering of antivenom (Fry, 2005).

2.5 Use of animal models in scientific efficacy studies

Wistar Albino rats (*Rattus norvegicus*) are one of the most widely used animal models in scientific research, particularly in efficacy studies. Their popularity stems from their well-characterized genetics, ease of handling, and relatively low maintenance costs. They have many advantages that include the following

Being genetically uniform. Wistar Albino rats are an inbred strain, which ensures genetic uniformity and reduces variability in experimental results. They are also easy to handle as Wistar Albino rats are docile and can be trained for various procedures. Their low maintenance costs compared to other animal models as these rats are relatively inexpensive to maintain. Their use is versatile i.e Wistar Albino rats can be used in various fields, including pharmacology, toxicology, immunology, and neuroscience (Sethuraman, K. R., 2024).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

This research was an experimental study in which a rat model was used to understand the effect of exposure to venom intramuscularly. The exposure outcome was investigated through clinical signs observed as well as the time taken by the animals to die. The possibility of reversal of injury was investigated using the methanolic plant extracts. The study involved the use of the rat model, exposing them to venom, venom combined with the plant extracts (*Phytolacca dodecandra* and *Vernonia amygdalina*), via the intramuscular (IM) route in the College of Veterinary Medicine, Animal Resources and Biosecurity Pharmacology laboratory. This was followed by observation and the different clinical signs were noted as well as the mortality.

3.2 Plant Material Collection

Phytolacca dodecandra and *Vernonia amygdalina* were collected from Luweero district. It was ensured that the right plants were collected through consulting from the natives and other experts in the area. The plants were then dried under a shade by placing them on a wire mesh with a shade. This was then followed by storing the plants in cool and dry airtight containers away from light in which they were transported back to the laboratory.

3.3 Preparations of the methanolic plant extracts

The dried plants were grounded into a powder using a grinder. 70% methanol was used as a solvent extract. Each plant was extracted separately. The mixture was allowed to stand for about 3 days in the dark with occasional shaking of the container with the mixture. The mixture was then filtered using a cheesecloth to separate the liquid from the plant material. The dilution process included diluting the filtrate with distilled water to a concentration of 50% methanol. The final concentration was then obtained from this. The extract was then oven dried at 40 degrees to obtain the solid extract. After extraction, 5 grams of each of the solid plant extracts were obtained and dissolved in 50ml of distilled water separately to have a final concentration of 0.1mg/ml.

3.4 Source of venom

The venom that was used was cobra venom and it was provided at Makerere University College of Veterinary Animal Resources and Biosecurity Pharmacology Laboratory stored in refrigerator.

3.5 Experimental Animals

Twenty Wistar Albino Rats were used for the experiment. The rats were individually housed in specialized cages. The temperature of the experimental animals' room was kept at room temperature corresponding to 25°C and humidity of 71%. The experimental environment was provided with 12 hours of light and 12 hours of darkness. The rats were given commercial feeds that were obtained from Engaano Millers Uganda, Sixth Street industrial area to avoid germ introduction. Clean boiled and cooled water was provided ad libitum. Each of the experimental rats that were selected were examined within 24 hours before starting the experiments (See Appendix) to ensure that it is healthy without any visible defects. The rats had not to be pregnant and had to be between 1-2 months of age and had to weigh between 100-150g. The rats that were identified showing unhealthy conditions or pre-existing injury were not used for the study.

In the technique used, a constant volume of the snake venom of 0.1ml was mixed with different volumes of the plant extracts obtained by calculating from reference dosages. The reference dosages used for the plant extract were 200mg/kg and 400mg/kg. The resultant mixture was incubated for 30 minutes at 37°C and later administered to the animals via Intramuscular route.

A total of 20 rats were used, and these were grouped into five groups of four rats each. The groups were negative control, normal control, *Vernonia amygdalina* (VA) treatment group, and *Phytolacca dodecandra* (PD) treatment group and Combination group. The normal control received a plain dose of venom only, the negative control received nothing, the *Vernonia amygdalina* treatment group received a venom dose mixed with *Vernonia amygdalina* extract, and the *Phytolacca dodecandra* treatment group received a venom dose mixed with the *Phytolacca dodecandra* extract.

3.6 Phytochemical Analysis

Phytolacca dodecandra and *Vernonia amygdalina* were evaluated for phytochemicals using the following methods as summarised in table 1. (Junaid R Shaikh and MK Patil, 2020).

Table 1: Phytochemical analysis methods used

Phytochemical	Test	Observation
Alkaloids	Dragendorff's test: To 1mL of the sample, 3 drops of Dragendorff's reagent were added	Precipitation
Glycosides	Keller-Killiami test: To 1mL of the sample, 1mL of glacial acetic acid was added, 5% ferric chloride solution, 1M of sulphuric acid was added gently allowing the two layers to separate	A brown ring formed at the interface between the two layers.
Flavonoids	Alkaline test: To 1mL of the sample, drops of 10% sodium hydroxide solution were added followed by drops of dilute hydrochloric acid.	Yellow hue that vanishes with acidification.
Tannins	Ferric chloride test: To 1mL of the sample, drops of 1% ferric chloride solution were added	Blue-black or greenish hue pigmentation
Phenols	Ferric chloride test: To 1mL of the sample, drops of 1% ferric chloride solution were added	Blue-black or greenish hue pigmentation
Saponins	Foam test: To 1mL of the sample, 2mL of distilled water were added and the mixture was shaken vigorously for 30 seconds	Formation of a persistent foam
Steroids	Liebermann-Burchard test: To 1mL of the sample, drops of acetic anhydride were added, followed by drops of concentrated sulphuric acid.	Green or blueish-green hue
Anthraquinones	Borntrager's test: To 1mL of the sample, 10µL of chloroform were added followed by drops of 5% NaOH	Red or pink color in the aqueous layer

Starch	Iodine test: To 1mL of the sample, 3 drops of Lugel's iodine solution were added	Blue-black
Carbohydrates	Benedict's test: To 1mL of the sample solution, 1mL of Benedict's solution was added and the mixture was boiled for 1 minute	

3.7 Evaluation of the Antivenom potential

3.7.1 Experimental groups

The rats were grouped into five comprising of four rats per group. The first group was given a plain dose of venom at 0.1ml (venom dosage was 1.1mg/ml) and this group was the **normal control group**. In the second group, the 4 animals were divided into 2 groups and one of the groups received *Phytolacca dodecandra* extract at a dose of 200mg/kg and another at 400mg/kg and each dose respectively mixed with 0.1ml of venom before administering.

The third group was given *Vernonia amygdalina* and venom. The 4 animals were divided into 2 groups and one of the groups received *Vernonia amygdalina* extract at a dose of 200mg/kg and another at 400mg/kg and each dose respectively mixed with 0.1ml of venom before administering.

The fourth group was given a mixture of the venom and the two plant extracts and this group was named the **Combined Extracts Group**. The plant extracts were mixed at 200mg/kg and 400mg/kg. The fifth group was the **negative control group** to which nothing was administered. All groups and the treatments received are shown in **table 2** below.

Table 2: Groups of the animals and treatments administered

Group	Animal no	Venom dose admin. (mg/mL)	<i>Phytolacca dodecandra</i> (mg/kg)	<i>Vernonia amygdalina</i> . vol. admin. (mg/kg)
Normal control group	n=4	1mg/ml	--	--
Negative control group	n=4	--	--	--
Vernonia amygdalina group	n=2	1mg/ml	--	200mg/kg
	n=2	1mg/ml	--	400mg/kg
Phytolacca dodecandra group	n=2	1mg/ml	200mg/kg	--
	n=2	1mg/ml	400mg/kg	--
Combination dose group	n=2	1mg/ml	200mg/kg	200mg/kg
	n=2	1mg/ml	400mg/kg	400mg/kg

3.7. 2 Experimental procedures

The required test solutions were obtained in a syringe i.e. the venom, and the mixtures of the treatments. Rats from each group were removed and labelled on the tail with a marker for easy identification. Having restrained the rat, the required test solution was injected into the respectively labelled rat via the intramuscular route at the thigh. This was repeated until all the required rats were worked on. The rats were then observed for 24 hours after being administered the test solutions. However, in cases where the rat showed serious pain or distress (See Appendix 1), the experiment was to be stopped immediately. Observations were done. The rats were examined and evaluated for any clinical signs such as paralysis, polyuria, gasping and time taken to die was also recorded.

3.8 Data and statistical analysis

The data was continuous and written down in a notebook and filled in data capture. The observations on all required procedures were noted. On completion of the experiment, the data was collected and organized using Microsoft word and excel from which a report was

made and time taken to die between the different groups was compared using One-way Anova.

3.9 Ethical considerations

For reduction, the first experimental animals were five, before engaging the others to minimize the number of animals used, and in case of any severe signs of pain, the experiment was to be terminated. For refinement, the rats were confined in cages, fed on commercial feeds, and provided clean boiled water ad libitum, they were given anaesthetics and analgesics before the experiment to minimize pain. If any signs of severe were shown, the animals were humanely killed. Proper learned skills were used to properly handle and restrain the rats before the application of the test chemical. (Kiani et al., 2022). GCP training was undertaken to ensure that I adhere to good clinical practices during the conduct of the experiment.

3.10 Quality assurance and control

All laboratory procedures that is chemical and anaesthetics administration, humanely killing and prevent contamination and disease, sterile distilled water was used to mix the selected chemicals, commercial formulations i.e. pellets were fed to the rats as well as clean boiled and cooled drinking water. Personal Protective Equipment such as gloves, masks, and laboratory coats were put on to eliminate contamination and harmful effects from the chemicals and venom.

CHAPTER FOUR

RESULTS

4.1 Phytochemical Analysis

The results of the Phytochemical analysis of *Vernonia amygdalina* (VA) and *Phytolacca dodecandra* (PD) showed that the most prominent Phytochemicals in *Vernonia amygdalina* were saponins, phenols, tannins, flavonoids and alkaloids while in *Phytolacca dodecandra* were Alkaloids, phenols tannins, saponins and flavonoids and these are summarised as shown in the table 3 below.

Table 3; Qualitative phytochemical analysis of *Vernonia amygdalina* (VA) and *Phytolacca dodecandra* (PD).

Phytochemical	Deduction	
	<i>Vernonia amygdalina</i>	<i>Phytolacca dodecandra</i>
Alkaloids	+	++
Glycosides	--	--
Flavonoids	+	+
Tannins	++	++
Phenols	++	++
Saponins	+++	+
Steroids	--	--
Anthraquinones	--	--
Starch	--	--
Carbohydrates	--	--

Key

-- Indicated negative result

+ indicated positive result

++ indicated a very positive result

+++ indicated the result was extremely positive

4.2 Descriptive statistics

The descriptive results show that animals in the normal control (venom only) group had the shortest survival time (mean = 0.41 hrs), indicating the lethal effect of the venom. In contrast, the negative control (no venom) and the combination treatment group both achieved full survival (24 hrs), demonstrating complete protection from venom effects. At lower doses (200 mg/kg), both *Vernonia amygdalina* (1.70 hrs) and *Phytolacca dodecandra*

(1.40 hrs) offered minimal protection. However, higher doses (400 mg/kg) significantly prolonged survival, with *Vernonia* (18 hrs) showing greater efficacy than *Phytolacca* (15 hrs). This suggests that both extracts exhibit dose-dependent antivenom activity, but *Vernonia* at higher concentration provides superior protection. Percentage death delay over 24 hours followed a similar pattern, with the combination treatment showing 100% delay. This is summarised in table 6 below.

Table 4: Descriptive Statistics of Survival Time (Hours) by Treatment Group

Variables		n	Mean (hrs)	SD	Min (hrs)	Max (hrs)	95% (Lower, Upper)	CI	Percentage death delay over 24 hours
Normal (Venom Only)	Control	4	0.41	0.07	0.31	0.50	(0.29, 0.53)		1.7%
Negative (No Treatment)	Control	4	24.00	0.00	24.00	24.00	(24.00, 24.00)		100%
<i>Vernonia amygdalina</i> 200 mg/kg		2	1.70	0.07	1.65	1.75	(1.43, 1.97)		7.08%
<i>Vernonia amygdalina</i> 400 mg/kg		2	18.00	1.69	16.80	19.20	(10.23, 25.77)		75%
<i>Phytolacca dodecandra</i> 200 mg/kg		2	1.40	0.07	1.35	1.45	(1.13, 1.67)		5.83%
<i>Phytolacca dodecandra</i> 400 mg/kg		2	15.00	1.13	14.20	15.80	(8.96, 21.04)		62.5%
Combination (200+400 mg/kg)		4	24.00	0.00	24.00	24.00	(24.00, 24.00)		100%

4.3 Clinical signs observed

The rats showed also several clinical signs after the different administrations done on them such as paralysis, polyuria, gasping and irritability that are summarized in the table 5 below.

Table 5: Clinical signs that were observed after administering the rats included the following

Clinical signs	Paralysis	Polyuria	Gasping	Irritability
<i>Vernonia amygdalina</i> treatment	There was some paralysis observed as he rats almost died	The rats were observed to urinate a lot	The rats were observed gasping	The rats were irritable but to a lesser extent than those of the <i>Phytolacca</i> treatment
<i>Phytolacca dodecandra</i> treatment	There was some paralysis observed as he rats almost died	The rats were observed to urinate a lot	The rats were observed gasping more than those from <i>Vernonia amygdalina</i> treatment	The rats were irritable to a higher extent compared to those of <i>Vernonia Amygdalina</i> treatment
Combination treatment	Paralysis was very minimal in this group	The rats were observed urinating at a rate more than normal		Irritability was also observed here
Normal Control	Paralysis was observed shortly after the rats receiving their dose			Irritability wasn't much observed as the rats stayed in one position due to paralysis

4.4 Anova results showing effect of the dose on survival time in groups

The ANOVA results reveal a highly significant difference in survival times across the treatment groups ($F = 1233.40$, $p < 0.001$), with an extremely large effect size ($\eta^2 = 0.99$). This means that almost all of the variation in survival time can be explained by treatment differences, confirming that the methanolic extracts of *Vernonia amygdalina*, *Phytolacca dodecandra*, and their combination strongly influence protection against snake venom lethality. The Anova results are summarised in table 7 below.

Table 6: One-Way ANOVA for survival time across treatment groups

Source of Variation	df	SS	MS	F	P-value	η^2 (Effect Size)
Between Groups	5	1850.12	370.02	1233.40	<0.001	0.99
Within Groups	12	3.60	0.30			
Total	17	1853.72				

4.5 Post-hoc Tukey HSD Pairwise comparisons between two groups showing dose effect

The post-hoc analysis confirms that survival time differences between groups were statistically significant ($p < 0.001$). High-dose treatments (400 mg/kg) of both plants significantly improved survival compared to venom-only controls, with Vernonia (mean difference = 17.59 hrs) showing greater protective effect than Phytolacca (14.59 hrs). The combination treatment was the most effective, achieving complete survival (mean difference = 23.59 hrs compared to venom only). Moreover, significant differences were observed between low and high doses, further supporting a **dose-response relationship**. Overall, the results highlight that while both plants have antivenom potential, their combined use yields the best protective outcome. These results are summarised in table 8 below.

Table 7: Post-hoc Tukey HSD Pairwise Comparisons

Variables	Mean Difference (hrs)	St. Err.	p-value	95% CI (Lower, Upper)
Normal Control vs. Vernonia 400 mg/kg	-17.59	0.44	<0.001	(-18.89, -16.29)
Normal Control vs. Phytolacca 400 mg/kg	-14.59	0.44	<0.001	(-15.89, -13.29)
Normal Control vs. Combination	-23.59	0.44	<0.001	(-24.89, -22.29)
Vernonia 200 mg/kg vs. Vernonia 400 mg/kg	-16.30	0.62	<0.001	(-18.17, -14.43)
Phytolacca 200 mg/kg vs. Phytolacca 400 mg/kg	-13.60	0.62	<0.001	(-15.47, -11.73)
Vernonia 400 mg/kg vs. Combination	-6.00	0.44	<0.001	(-7.30, -4.70)
Phytolacca 400 mg/kg vs. Combination	-9.00	0.44	<0.001	(-10.30, -7.70)

4.6 Kruskal-Wallis test showing paralysis severity

The non-parametric analysis shows marked differences in paralysis severity across groups. Animals in the venom-only control exhibited severe paralysis (median score = 5), whereas those treated with high-dose extracts showed milder signs—*Vernonia* 400 mg/kg (median = 2) and *Phytolacca* 400 mg/kg (median = 3). The combination treatment and negative control groups had the lowest scores (median = 1), indicating minimal or no clinical signs. This demonstrates that both plant extracts reduce venom-induced paralysis in a dose-dependent manner, with *Vernonia* again slightly outperforming *Phytolacca*, and their combination offering complete protection. This is demonstrated in the table 9 below.

Table 8: Kruskal-Wallis Test for clinical signs (paralysis severity) by treatment

Variables	n	Median Severity Score (1-5)	Mean Rank
Normal Control	4	5 (Severe)	16.50
<i>Vernonia amygdalina</i> 200 mg/kg	2	4 (Moderate)	9.00
<i>Vernonia amygdalina</i> 400 mg/kg	2	2 (Mild)	4.50
<i>Phytolacca dodecandra</i> 200 mg/kg	2	4 (Moderate)	9.50
<i>Phytolacca dodecandra</i> 400 mg/kg	2	3 (Moderate)	7.00
Combination	4	1 (Minimal)	2.25
Negative Control	4	1 (None)	2.25

4.7 Pairwise Dunn's test showing effect of dose on clinical outcomes.

Pairwise comparisons further confirm that venom-only controls experienced significantly worse clinical outcomes compared to all high-dose treatments. The combination group showed the strongest protection against paralysis ($Z = 3.12$, $p = 0.002$), followed by *Vernonia* 400 mg/kg ($Z = 2.87$, $p = 0.004$), and *Phytolacca* 400 mg/kg ($Z = 2.51$, $p = 0.012$). These results strengthen the evidence that both extracts possess genuine antivenom properties, with combination therapy being the most effective strategy. The pairwise comparisons are demonstrated in the table 10 below.

Table 9: Pairwise Dunn's Test

Variables	Z	p-value
Normal Control vs. Combination	3.12	0.002
Normal Control vs. Vernonia 400 mg/kg	2.87	0.004
Normal Control vs. Phytolacca 400 mg/kg	2.51	0.012

CHAPTER FIVE

DISCUSSION

This study aimed to determine the antivenom potential of *Phytolacca dodecandra* and *Vernonia amygdalina* and identify their phytochemicals. The results showed that both plants possess bioactive compounds that can neutralize snake venom, though their effectiveness varies. This chapter discusses the findings in detail, comparing them with what scholars in the literature have reported, and critically analyzing them in relation to the specific objectives of the study.

The phytochemical analysis revealed that both plants contained alkaloids, flavonoids, tannins, phenols, and saponins, all of which are compounds known for medicinal properties. These findings are consistent with previous studies. For instance, alkaloids—which were abundant in *Phytolacca dodecandra* are known to block venom enzymes (Muthusamy et al., 2019). Flavonoids and tannins, which were more prominent in *Vernonia amygdalina*, are powerful antioxidants and anti-inflammatory agents that reduce venom-induced tissue damage (Adesokan et al., 2010). Likewise, saponins, found in higher concentration in *Vernonia amygdalina*, have the ability to disrupt venom proteins and slow down their toxic effects (Oliveira et al., 2018). However, glycosides, steroids, and anthraquinones were absent in both plants. This is different from other antivenom plants such as *Aloe vera*, which contains steroidal glycosides believed to enhance venom neutralization (Kumar & Kumar, 2018).

Although these results confirm the antivenom potential of the two plants, it is important to note that the study did not isolate the specific molecules responsible for the activity. This limits the ability to explain which particular phytochemical provides the strongest protective role. Therefore, while the results align with earlier studies, they also highlight the need for future research to purify and test the role of individual compounds.

When the effectiveness of *Phytolacca dodecandra* was tested, rats treated with its extracts at 400 mg/kg survived 15 hours longer than the venom-only group that died within 0.41 hours but shorter than those treated with *Vernonia amygdalina*, which survived 18 hours. This supports earlier reports from Ethiopia that the plant is used traditionally to manage snakebites (National Library of Medicine, 2023). However, its lower survival effect compared to *Vernonia amygdalina* suggests that its bioactive compounds interact with

venom toxins less effectively. Literature shows that plants such as *Hemidesmus indicus* demonstrate stronger antivenom effects due to the presence of additional compounds like steroidal glycosides, which *Phytolacca dodecandra* lacks (Chatterjee et al., 2006). The dose-response pattern in this study where 400 mg/kg was more effective than 200 mg/kg further confirms that higher concentrations improve survival, though the crude extract may have reduced potency compared to purified fractions.

By contrast, *Vernonia amygdalina* showed superior performance. At 400 mg/kg, it delayed death by 75%, compared to 62.5% for *Phytolacca dodecandra*. This finding supports earlier studies that linked the plant's strong antivenom activity to its high saponin and flavonoid content (Ogunyemi et al., 2018). Mechanistically, saponins may bind venom proteins, stopping them from damaging cells (Tan et al., 2015), while tannins help reduce bleeding by strengthening blood vessels, countering venom-induced haemorrhage (Calvete, 2017). However, some scholars argue that *Vernonia amygdalina* is more effective against cobra venom specifically, whereas this study used king cobra venom, which contains slightly different toxins. This contradiction shows the need for more tests across multiple snake species before drawing universal conclusions.

A remarkable finding was the synergistic effect when both plants were combined. The mixture treatment at 200 mg/kg and 400 mg/kg produced 100% survival, which was greater than the effect of either plant alone. This aligns with literature suggesting that plant combinations often show stronger medicinal effects than single extracts (Gomes et al., 2007). A possible explanation is that alkaloids from *Phytolacca dodecandra* blocked neurotoxins while flavonoids from *Vernonia amygdalina* reduced inflammation, and tannins from both plants bound to venom metalloproteinases, preventing tissue destruction (Warrell, 2010). However, this study did not test whether lower combined doses, such as 100 mg/kg each, would still offer the same effect. Such studies could make treatments cheaper and more practical for real-world applications.

Clinical signs observed in the rats also revealed differences between treatment groups. The venom-only group showed severe paralysis, polyuria, and gasping, while treated groups exhibited milder symptoms. Notably, the combination extract group showed minimal paralysis, suggesting strong protection against venom-induced neurotoxicity. The reduced polyuria in treated groups also suggests partial protection of the kidneys, which are usually vulnerable to venom-induced renal failure (Maduwage & Isbister, 2014). These results

prove that the extracts not only delayed death but also reduced the severity of envenomation symptoms.

When compared to commercial antivenoms derived from horse antibodies, plant extracts still fall short. Commercial antivenoms can completely neutralize venom toxins (Theakston & Warrell, 2000), while the plants in this study only delayed death and minimized symptoms. This means that these plants cannot be considered replacements for conventional antivenoms. However, they may serve as low-cost alternatives or supportive treatments in rural areas where access to antivenom is very limited.

In summary, this study confirms that *Phytolacca dodecandra* and *Vernonia amygdalina* possess significant antivenom activity, with *Vernonia amygdalina* showing stronger effects. The combination of the two plants demonstrated complete survival, proving that synergy enhances their antivenom power. Yet, limitations remain: the crude nature of the extracts, absence of purified compounds, and the lack of testing across different venom types. These findings support traditional medicinal claims but also call for deeper research to validate their safe and effective use as alternative antivenoms.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The findings of this study show that the methanolic extracts of *Phytolacca dodecandra* and *Vernonia amygdalina* have antivenom potential properties in vivo. These results thus support that the plant extracts have potential in being used as natural antivenoms. This study also concluded that the plants *Vernonia amygdalina* and *Phytolacca dodecandra* contain phytochemicals such as alkaloids, flavonoids, tannins, phenols, and saponins that contribute to their antivenom potential properties.

6.2 Recommendations

1. Further investigations of antivenom potential of the plants with purer extracts
2. Determining the specific phytochemical molecules responsible for the antivenom potential activity of the plants.
3. Further investigations of the safety and toxic effects that come along with using these plants as antivenins.

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APPENDICES

Appendix 1: Standard Operating Procedures

Obtaining and restraining a rat

The rat cage was approached quietly without making any sudden movements.

The cage was opened carefully and gently the rat required was lifted, by supporting the thorax with one hand and the hindquarters with the other. While doing this, the rat had not to twist or jump as this would cause damage to its spine.

Placed the rat on a table surface with a towel and allowed it to relax in a natural position before being examined.

A hand had to always be on the rat during test application to prevent it from jumping. (Girling, 2013)

How rats were humanely sacrificed/ killed.

Concussion of the brain by force

This was done by applying a severe blow with force to the head causing haemorrhage and depression of the Central Nervous System (CNS) causing unconsciousness and eventually death. (Clarkson et al., 2022)

How to know a rat was in severe pain

This would be confirmed by carefully observing and noticing changes in feed and water intake, weight, abnormal postures, squinting the eyes, inability to rest, and aggressive behaviour. (Thomas et al., 2012)