EVALUATION OF COMBINED MOLLUSCICIDAL PLANT EXTRACTS FOR CONTROL OF THE *BIOMPHALARIA* SNAIL (*Biomphalaria pfeifferi)*

BY

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MTech/SLS/2018/7758

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NOVEMBER, 2021

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSIY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN APPLIED HYDROBIOLOGY OF THE FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA NIGER STATE.

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ABSTRACT

The need to search for better and ecofriendly alternative molluscicide, as a result of high cost of synthetic molluscicide, their environmental toxicity to non target organism and their chemical residue in the environments, propelled this study to elucidate the mono and combined molluscicidal efficacy of *Vernonia amydalina, Calotropis procera* and *Amaranthus hybridus* ethanolic extracts. The phytochemical constituents of the single and combined plant extract was determined using standard procedure. The molluscicidal bioassay against *Biomphalaria pfeifferi* using graded concentrations of the extract ranging from 50 to 400 mg/l was performed following standard protocol. Mortality was recorded after 24, 48, and 72 hrs exposure period. The effect of the plants on the snail architecture was determined histopathologically. The lethal doses was estimated using probit linear regression analysis. The result indicated that the three plants possess bioactive secondary metabolite including flavonoids, alkaloids, tannins and saponins. *V. amygdalina* has more phytochemical constituent than other plants. Molluscicidal activities increases with increase in extract concentrations and exposure period. Amongst the plants, *Vernonia amygdalina* showed the highest molluscicidal activities with LC_{50} of 183.33 mg/L, after 24 hrs exposure period. However the combined extract of *V. amydalina* and *C. procera* was most potent with an LC_{50} (53.65 mg/L) value better than the application of only *V. amygdalina* with LC_{50} of 183.33 mg/L, after 24hrs which show synergestic effect. The histopathological results showed that the extract caused mild and severe cytoplasmic fragmentation and vacuolization in the snail digestive and excretory cells, which is more pronounced in the combined extracts. Findings of this study suggest the combinatorial application of *V. amydalina* and *C. procera* extracts as an alternative agents in the control of snail intermediate host of schistosomiasis.

TABLE OF CONTENTS

CHAPTER THREE

CHAPTER FOUR

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Schistosomiasis is a neglected tropical disease that affects more than 250 million people in tropical and subtropical regions of the world, with Sub-Saharan African accounting for approximately 90 % of worldwide cases World Health Organization, WHO (2017). Estimates shows that at least 236.6 million people required preventive treatment in 2019, (WHO, 2021). It is very important to note that the transmission cycle of the *Schistosoma* species, requires a specific fresh water snail as the intermediate host (Zhou et *al*., 2008). The snails do not transmit the disease from one host to another but are indispensable for the development of the parasite (Molgaard *et al.,* 1999). Each schistosome species uses a different snails species as its intermediate host, hence the availability of a suitable snail host determines the endemicity of a particular species of *Schistosoma,* Snails of the genus *Biomphalaria* and *Bulinus* acts on the intermediate most of *Schistosoma mansoni* and *Schistosoma haematobium* respectively.

At least four approaches to controlling infection have been tried at the community level, these are Control of Snails, public health education, Sanitation and community based chemotherapy using niclosamide (Jocelyn, 2004). Selective Molluscicide treatment in snail infested bodies of water at human contact point is the preferred ways to approach controlling the snail population (Jocelyn, 2004).

The use of Molluscicide to eradicate the snail vector is considered the method of choice to eliminate Schistosomiasis (WHO, 1985) as several strategies have been used to control snail population such as physical (McCullough, 1986), chemical (Masola *et al.,* 2003) and biological method (Kloos *et al.,* 2001; Rashed, 2002). Chemical control by

Molluscicide is performed by using the different compound (Tantawy, 2006; Essawy *et al.,* 2009; Krisstoff *et al.,* 2010*).*

Currently there is an increase attention for the use of new plant Molluscicides that are highly effective, rapidly biodegradable less expensive and probably easily applicable using simple techniques than synthetic Molluscicides. One of the new trends in the biological control of vectors of disease is testing the toxicity of plant extracts as alternative to chemical Molluscicides as synthetic Molluscicides formulations based on niclosamide and metaldehyde application is widely practice for effective snail control. But however it has a negative impact on the environment and has a high cost of application and has hence stimulated interest in the search of plant derived Molluscicides which are target specific and environmentally and toxicologically safe (Li *et al.,* 2012). Molluscicidal properties have been reported in more than 1400 species of plants (Teixeira *et al., 2012*). In the recent past most research effort usually are focused on individual plant extracts for their Molluscicidal potency against the *Bulinus* and *Biomphalaria* snails and other freshwater snails. Plants such as *Euphorbia, Polygonum sp, Zizyphus, Allium, Azadirachata* sp, *Vernonia* sp, *Garcinia*, *Amaranthus* sp, *Calotropis procera* and many other plant has being effective against the snail, however, the majority of individual plant was not proven to be entirely effective in field condition, therefore to leverage the cumulative benefits of synergistically acting phytochemical mixtures, a selective combination of potent molluscicidal compound from various plant extracts might be effective against these snails.

1.2 Statement of the Research Problem

The persistence of Schistosomiasis in developing countries is strongly associated with the presence of the fresh water snail, and these snail control may be attained through

chemical, environmental and biological methods (McCulluogh,1986) recently the most dependable method of effectively controlling snails is through short term application of synthetic molluscicide formulation based on niclosamide and metaldehyde (McCulluogh, 1986). But this molluscicide are expensive and its negative impact on the community and its environmental effects i.e. its toxicity to fish and other aquatic organisms have stimulated the interest scientist to search for alternative molluscicides of plant origin, in the control of human Schistosomiasis (Oguche & Olofintoye, 2018).

1.3 Aim and Objectives of the Study

To evaluate the molluscicidal activity and histopathalogical effect of individual, binary and tri-herbal extracts on the *Biomphalaria pfeifferi* snail which is achieved through the following objectives:

The objectives of the study are to:

i. identify the bioactive compounds in the extracts that are responsible for the molluscicidal potency
ii. determine molluscicidal activity of individual crude extracts of *Calotropis procera, Vernonia amygdalina,* and *Amaranthus hybridus* on the *Biomphalaria* snail at different concentrations (50,100, 200, 300, 400 mg/L).
iii. determine molluscicidal activity of binary combinations of plant extract on the *Biomphalaria* snail at different concentration (10,30,50, 150, 250, 350 mg/L).
iv. determine molluscicidal activity of tri-herbal combinations of plant extracts on the *Biomphalaria* snail at different concentrations (10,30,50, 150, 250, 350 mg/L).
v. determine the effect of the combined plants extract on the histopathological parameters of the snails.

1.4 Justification for the Study

The search of alternative molluscicide particularly of plant origin is imperative as the control of snails is an important part of the integrated approach to combat the disease in endemic areas. Molluscicidal properties have been reported in more than 1400 species was be (Teixeira *et al.,* 2012). Plant molluscicides have received considerable attention in search of cheaper, effective, and environmentally friendly alternatives to existing synthetic molluscicide (Ojewole, 2004).

In the recent past most research efforts are focused on individual plants extracts for their molluscicidal potency against the snail host, however, the vast majorly of individual plants extracts has not proven to be entirely effective. Therefore to leverage the cumulative benefit of synergistically acting phytochemical mixtures and a selective combination of potent molluscicidal compound from various plant extracts might be effective against the Biomphalaria and *Bulinus* snail.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Schistosomiasis

Schistosomiasis (*Bilharziasis* or Snail fever) is a disease caused by infection with fresh water parasitic flat worms called schistosome (WHO, 2018). It is an acute and chronic inflammatory disorder initiated by infection with some species of blood fluke (trematode worms) of the genus *Schistosoma* (WHO, 2018).

Schistosomiasis is a disease of the poor who live in conditions that favor transmission. It is also an insidious disease, poorly recognized at early ages and disabling to men and women during their most productive years (WHO, 2007). Rarely fatal, but strongly linked to diarrhea, pain, fatigue, hemoglobin deficit, under nutrition, and reduced exercise tolerance; Schistosomiasis' effects are not negligible for those who are infected and live in endemic areas where recurring infections are possible (Biu *et al*., 2009). This disease is caused by schistosomes, which are parasitic trematode worms. Five species infect humans: *Schistosoma mansoni, Schistosoma japonicum, Schistosoma mekongi, Schistosoma intercalatum, and Schistosoma haematobium*.

Schistosoma infections occur through direct contact with fresh water that harbors free-swimming larval forms of the parasite. Free-swimming larvas, known as cercariae, are able to penetrate human skin to cause infection (WHO, 2018).

Species of *Schistosoma* that infect humans: *Schistosoma masoni* and *Schistosoma intercalatum* cause intestinal Schistosomiasis; *Schistosoma haematobium* causes urinary Schistosomiasis and *Schistosoma japonicum* and *Schistosoma mekongi* cause Asian intestinal Schistosomiasis and also Avian Schistosomiasis causes swimmer's itch and clam digger itch.(Wang *et al*., 2012).

2.2 Snail (The Intermediate host)

About 350 species of snails of medical and veterinary importance have been estimated, out of this are three genera which serves as intermediate host of human *Schistosoma* parasites, they include: *Biomphalaria, Bulinus* and *Oncomelania*. The species involved can be identified by shape of their shells. They can be grouped into two based on their habitat: Aquatic snails; they include *Biomphalaria* and *Bulinus*; Amphibious snails: *Oncomelania*.

In Africa and also America, snails of genus *Biomphalaria* (Figure 2.1) serve as intermediate host of *S. mansoni*. While snail of genus *Bulinus* serve as the intermediate host of *S. haematobium* in Africa and eastern Mediterranean, as well as of *S. intercalatum* in Africa. In south-east Asia, *Oncomelania* serve as the intermediate host of *S. japonicum*, and *Tricula* as the intermediate host of *S. mekongi* (Wang *et al*., 2012).

2.2.1 Scientific classification of *Biomphalaria* species

Kingdom: Animalia

Phylum: Mollusca

Subclass: Heterobranchia

Super order: Hygrophila

Super family: Lymnaeoidea

Family: Planorbidae

Genus: *Biomphalaria*

Species: *Biomphalaria pfeifferi*

Figure 2.1 *Biomphalaria* species

Source: Field photograph

2.3 Epidemiology

Schistosomiasis is an important cause of disease in many parts of the world, most commonly in places with poor sanitation. School-age children who live in these areas are often most at risk because they tend to spend time swimming or bathing in water containing infectious cercariae (Martin, 2019).

The disease is prevalent in tropical and subtropical areas, (Figure, 2.2) in Africa, the Caribbean, Eastern South America, Southeast Asia, and in the Middle East estimate show that at least 229 million people required preventive treatment in 2018 (WHO, 2018). Its transmission has been from 78 countries but endemic in 52 countries with moderate-to-high transmission (WHO, 2018).

Among human parasite diseases, Schistosomiasis ranks second behind malaria in terms of socio-economic and public health importance in tropical and subtropical areas (Waknine-Grinberg *et al*., 2010).

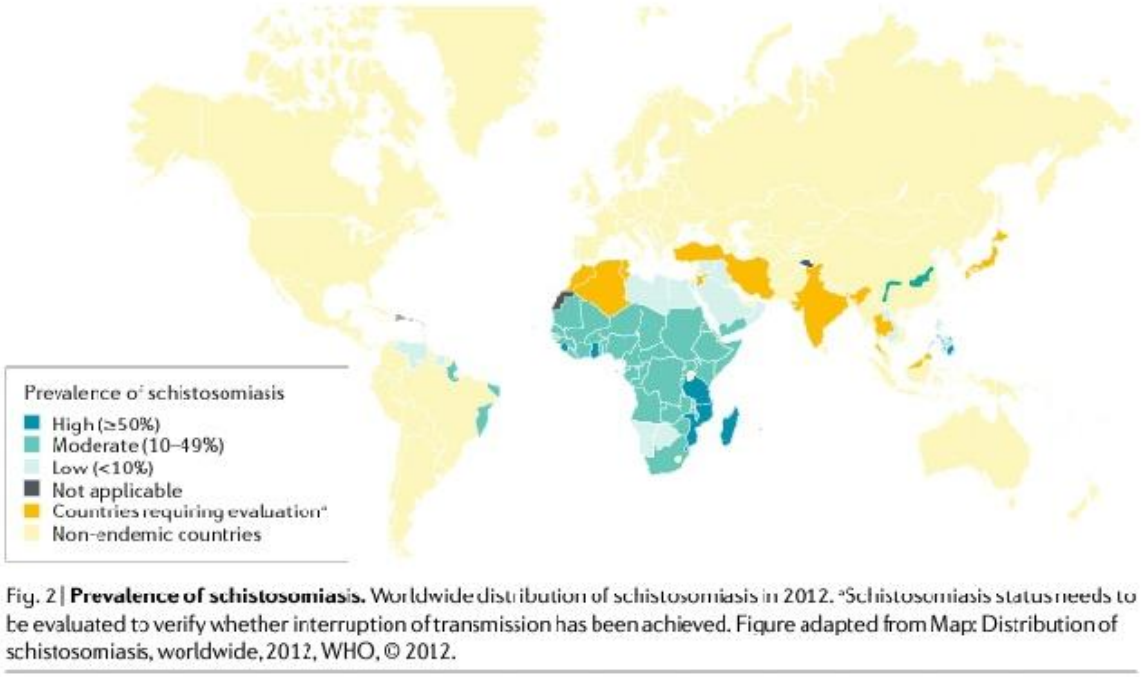

Fig. 2 | **Prevalence of schistosomiasis.** Worldwide distribution of schistosomiasis in 2012. *Schistosomiasis status needs to be evaluated to verify whether interruption of transmission has been achieved. Figure adapted from Map: Distribution of schistosomiasis, worldwide, 2012, WHO, © 2012.

Figure 2.2: Distribution of Schistosomiasis in the world (Africa).
Source: (WHO, 2012)

The five *Schistosoma* spieces that can infect humans have different geographical distributions. *Schistosoma haematobium* has been in 54 countries and is the most common species, occurring in sub- Saharan Africa and the Middle East (WHO, 2018). A few countries have eradicated the disease, and many more are working towards doing same. The world health organization is promoting such efforts. Urbanization, pollution and or consequent destruction of snail habitat has reduced exposure, with a subsequent decrease in new cases. The most common way of getting Schistosomiasis in developing countries is by wading or swimming in lakes, ponds and other bodies of water that are infested with the snails of *Biomphalaria, Bulinus* or *Oncomelania* genera) that are the natural reservoir of the *Schistosom*a pathogen (Martin, 2019).

2.4 Life Cycle of Schistosomiasis

The schistosome life cycle occurs in 2 hosts (Figure, 2.3), snails and mammals. Either asexual or sexual reproduction occurs, depending on the type of host. Asexual

reproduction occurs in fresh water snails. In the snail, this begins with the development of miracidia into a sporocyst. Sporocyst multiplies and grows into *cercariae*. In the mammalian host, parasites grow to become mature, mate and produce eggs. Mammalian hosts include humans, mice and dogs (Martin, 2019).

2.4.1 Snail host

Mammal host release worm eggs into the external environment through feces or urine. In fresh water, these eggs form miracidia. Which hatch and infect snails. *S. haematobium* infects snails of the genus *Bulius, S. japonicum* infect snails of the genus *Neutricula. S. masoni* infects snails of the genus *Biomphalaria*.

After infiltration, the miracidium removes the ciliated plates, develops into a mother sporocyst, and then produces daughter sporocysts. Daughter sporocyst produces either *cercaria* (cercariogenous sporocyst) or more daughter sporocyst can also experience a re-differentiation into new daughter sporocyst. Snails can shed hundreds of *cercariae* daily; about 200 for *S. haematobium*, 15 to 160 for *S. japonicum*, and 250 to 600 for *S. masoni* (Martin, 2019).

2.4.2 Mammalian hosts

Cercariae enter the human skin and shed their forked tail, forming schistosomula. The schistosomula migrates throughout the body's tissues through blood circulation, grows into schistosomes and adult worms. These worms each have a ZZ chromosome pair in males and ZW chromosome pair in females.

Adult worms in humans exist in various locations specific to each species. *S. haematobium* exist in the bladder and ureters, but it can also exist in the rectal venules. *S. japonicum* exist more frequently in the small intestine. *S. mansoni* worms can exist in either large or small intestine and they are able to transfer between those sites. Water containing cercariae can cause human Schistosomiasis (WHO, 2018).

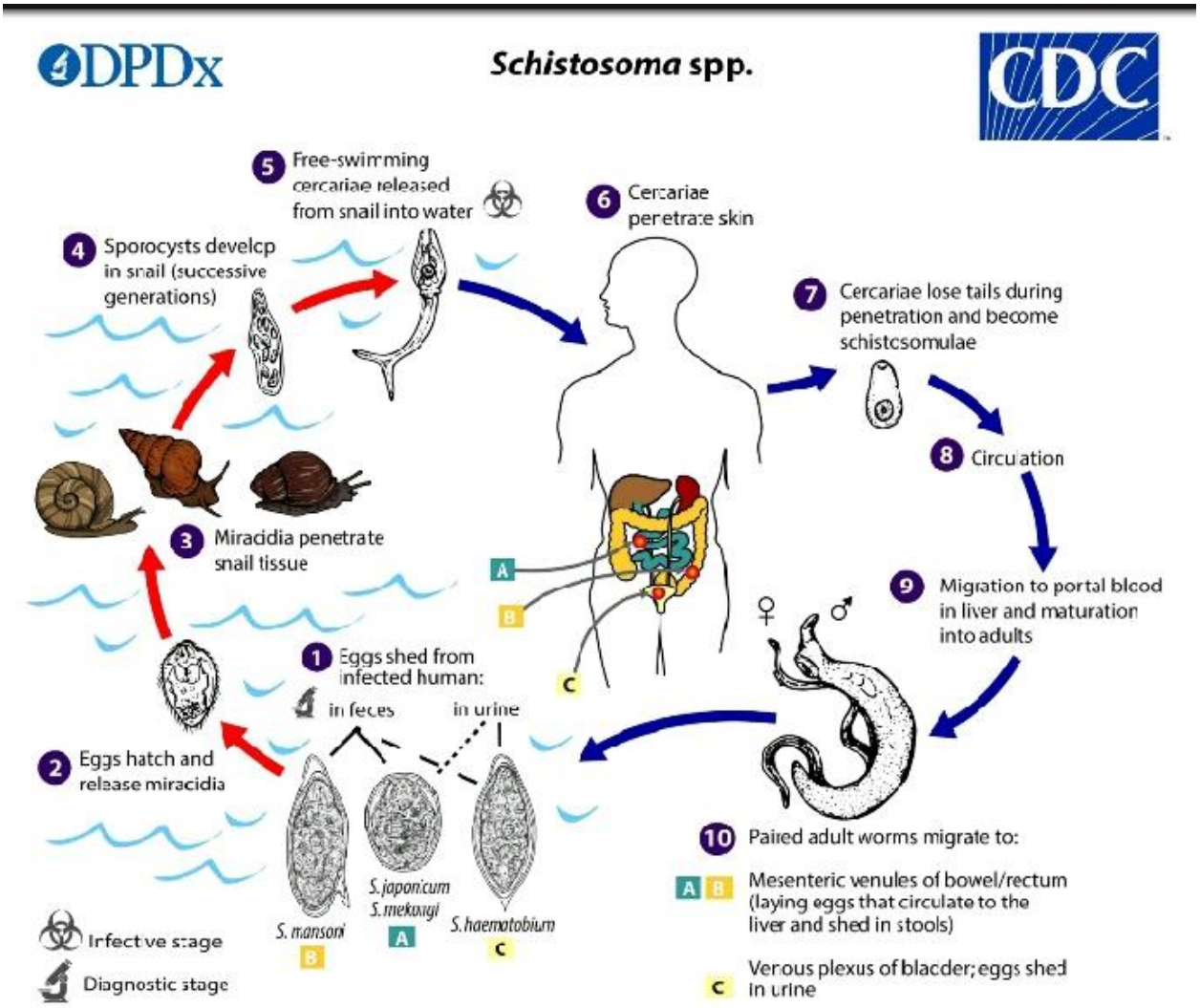

Figure 2.3: Life cycle of Schistosome
Source: (Carter center, 2010)

2.5 Signs and Symptoms of Schistosomiasis

Schistosomiasis is caused by trematode parasites of one of five species: *Schistosoma mansoni, S. haematobium, S. japonicum, S. intercalatum,* or *S. mekongi*. Symptoms of Schistosomiasis are caused by the body's reaction to the worms' eggs (WHO, 2018). It spreads via skin contact with fresh water containing infectious larvae. After 1–8 weeks of exposure, symptoms such as dermatitis, fatigue and fever arise. Long term infection, if left untreated, leads to anemia and eventually to liver fibrosis and hydronephrosis (Salvana and King, 2008). This disease is a particular threat as it readily spreads through travelers from Africa and can become epidemic with the availability of fresh water bodies (WHO, 2018).

A few patients may have minor skin irritation when the *cercariae* enter the skin, most people do not develop symptoms until the eggs develop (about one to two months after initial skin penetration). Then fever, chills, cough and muscle aches can begin within one to two months of infection. However, most people have no symptoms at this early phase of infection (WHO, 2018). Although, a few patient develop acute Schistosomiasis (Katayama fever) during this one to two month period and their symptoms are as follows: Fever, abdominal pain (liver/spleen area), bloody diarrhea or blood in the stool, cough, malaise, headache, rash, body aches (WHO, 2018).

The majority of the people who develop chronic Schistosomiasis have symptoms develop months or years after the initial exposure to the parasites. The following is a list of most symptoms associated with chronic Schistosomiasis such as abdominal pain, abdominal swelling (ascites), bloody diarrhea or blood in stools, blood in the urine and painful urination, shortness of breath and coughing, weakness, chest pain and palpitation, seizures, paralysis, mental status changes, lesions on the vulva or the perianal area (CDC, 2018).

2.5.1 Intestinal schistosomiasis

Intestinal Schistosomiasis may be accompanied by intermittent abdominal pain, discomfort, loss of appetite and, sometimes, bloody diarrhoea. These symptoms tend to be more pronounced with greater intensities of infection (Gray *et al*., 2011). S. intercalatum and *S. guineensis* cause generally milder intestinal manifestations than *S. mansoni*, *S. mekongi* or *S. japonicum* (Gray *et al*., 2011).

2.5.2 Hepatosplenic schistosomiasis

Periportal fibrosis is usually seen in adults and, sometimes, in adolescents living in areas with very high Schistosomiasis transmission; the severity of periportal fibrosis correlates in part with the intensity and duration of infection (Wilson, 2010). Portal branch occlusion as a result of periportal fibrosis may lead to marked portal hypertension, frequently accompanied by severe enlargement and hardening of the spleen. Of note, whereas portal hypertension is associated with periportal fibrosis in chronic hepatosplenic Schistosomiasis in older individuals (often of >15 years of age), in children with severe hepatosplenic Schistosomiasis, increased portal pressure may be evident in the absence of detectable periportal fibrosis (Wilson, 2010). In some cases, portal hypertension leads to the development of oesophageal varices, which may rupture with high risk of fatal haematemesis (vomiting of blood) (Ganapathi and Somer, 2015). Other complications include ascites (accumulation of fluid in the abdominal cavity), delayed growth and puberty and severe anaemia. However, in contrast to toxin- induced and alcohol- induced liver cirrhosis, in Schistosomiasis, even with marked periportal fibrosis and portal hypertension, no hepatocyte damage is observed and liver enzymes remain normal (Ganapathi and Somer, 2015).

2.5.3 Urogenital schistosomiasis

A common characteristic of established active *S. haematobium* infection is blood in the urine, and in endemic areas many school- aged children present with visible haematuria (Ganapathi and Somer, 2015). Children may present with dysuria with frequent urination as a direct effect of egg induced urothelial inflammatory responses (Ismail, 2014). Genital Schistosomiasis can cause pelvic discomfort and pain, abnormal vaginal discharge, itch and contact bleeding in girls and women and haematospermia and painful ejaculation in men (Kjetland, 2014). In both sexes, genital Schistosomiasis has been associated with increased risk of HIV infection (Kjetland, 2014).

2.6 Diagnosis of Schistosomiasis

The diagnosis of acute Schistosomiasis requires different diagnostic methods than those used to diagnose established active or late chronic infections (Weerakoon *et al*., 2015). In endemic areas, where individuals typically have established active or late chronic infection, diagnosis is often based on detection of schistosome eggs in faeces or urine by microscopy. However, these methods are often not sensitive enough to detect acute infections, which have low intensity, in returning travelers or recent immigrants from a Schistosomiasis endemic area. In this case, anamnestic information such as recent travels to endemic areas and exposure to fresh water bodies through recreational or other activities is important and may provide an indication of schistosomiasis (Weerakoon *et al*., 2015). Eosinophilia is a common finding in these individuals and is often a sign of helminth infection, such as Schistosomiasis (Checkley, 2010).

2.6.1 Parasitological diagnosis

The standard for diagnosing an established active infection is the detection of eggs in faeces, urine or rectal biopsy samples by microscopy (Weerakoon *et al*., 2015).

Parasitological diagnosis of Schistosomiasis in populations living in endemic areas most often relies on filtering a standardized small amount of urine or stool sample and microscopically counting all eggs in that volume (Feldmeier *et al*., 1982). The level of infection is then expressed as eggs per 10 ml of urine or eggs per gram of stool (EPG) (Feldmeier *et al*., 1993). These methods have a low sensitivity (estimated at < 50 %) (Feldmeier *et al*., 1993) and (Utzinger, 2015) and the observation of 1 egg in a slide corresponds to detection of 20–40 EPG, or 5,000–10,000 eggs per diurnal faecal portion of 250 g (in *S. mansoni* infections, 1–99 EPG are considered low-intensity infections). However, egg excretion may show a high degree of day-to-day as well as intraspecimen and diurnal variation (Feldmeier *et al*., 1983). This variability warrants collection of multiple biological samples over consecutive days or examination of a single specimen multiple times. Furthermore, as the infection reaches the late chronic stage, egg excretion can be very low or even absent despite severe clinical manifestations (WHO, 2017). For travelers or previously exposed people, this diagnostic approach is rarely sensitive enough to detect the often very low levels of infection; thus, larger amounts of urine or stool will have to be examined. Methods employed, such as formol ether concentration and sedimentation, in which a larger amount of stool is centrifugated (Weerakoon *et al*., 2015). Schistosome eggs can be detected in tissue biopsy samples and rectal snip samples, which are very sensitive even for detection of *S. haematobium* and useful in diagnosing infections in travellers. *S. haematobium* can be located in veins around the genital organs in both men and women, and eggs have been detected in semen samples and Pap smears (Weerakoon *et al*., 2015).

2.6.2 Coagulation profile

Prolonged prothrombin time indicated by an increased iNr (international normalized ratio) may be evident in established active and late chronic Schistosomiasis (WHO, 2007)

2.6.3 Urea, electrolytes and liver function

Elevated urea and creatinine levels may be evident, and hyperglobulinaemia and hypoalbuminaemia may be present in late chronic and advanced Schistosomiasis (WHO, 2012).

i. Serology may be diagnostic in patients with acute Schistosomiasis radiology

ii. Chest radiography: pulmonary infiltrates are common in acute Schistosomiasis
Abdominal ultrasonography can establish the extent of liver and spleen pathology in intestinal Schistosomiasis

iii. Pelvic ultrasonography can establish the extent of bladder, ureteral and renal pathology in urogenital Schistosomiasis (WHO, 2012)

2.7 Prevention of Schistosomiasis

Travelers to a Schistosomiasis endemic area should be aware of the risk of infection when engaging in activities involving direct contact with water (Clerinx and Van, 2011). However, for people living in rural areas where Schistosomiasis is endemic, it may be very difficult if not impossible to avoid water contact. Schistosomiasis is a poverty related disease, and preventive measures such as access to clean water and good sanitary facilities are important in reducing the risk of infection but often are not present (Grimes, 2015). Furthermore, these measures will have little effect on reducing the risk through occupational exposure, such as fishing in the rivers and lakes, and such activities are often the main or only source of income for families. *S. japonicum* infection is a zoonosis; thus, preventive measures will have to be directed towards

infections in animals as well, particularly water buffaloes, which act as major reservoirs for the parasite (Wang, 2009). Despite intensive development efforts, currently no Schistosomiasis vaccines are available (Mo and Colley, 2016).

2.8 Management and Treatment of Schistosomiasis

Management of Schistosomiasis involves the targeted treatment of patients diagnosed with Schistosomiasis in an endemic area (in a hospital or some other medical facility if available) and in returning travelers or migrants. The aim of treatment with the anti-schistosomal drug praziquantel is curative therapy, and treatment can be repeated several times until the infection is eliminated (that is, no eggs are detected upon microscopic examination of faeces or urine). The recommended dose is generally lower for *S. mansoni* and *S. haematobium* infections than for *S. japonicum* and *S. mekongi* infections; higher doses are generally split into two administrations a few hours apart (Gray *et al*., 2011).

2.9 Snail Control

Elimination of a human parasitic disease such as Schistosomiasis requires interruption of a complex pathway of transmission. In the case of *S. japonicum* associated Schistosomiasis, control is especially challenging as *S. japonicum* can persist in a range of non human mammalian hosts. Control efforts are hindered by rapid re-infection of previously treated individuals. The removal of infected snails was reducing the number of water borne infectious cercariae, thereby reducing the risk of re-infection. Snail control through the use of chemicals, such as niclosamide, released directly into water, has been implemented in many areas. However, to be most effective, chemicals should be used at least biannually, making the strategy time consuming and expensive, especially when large areas are involved (McManus *et al*., 2018). Additional concerns

are the toxicity of molluscicides to larger animals such as fish and the general pollution they cause (McManus *et al*., 2018). Other snail control approaches are environmental methods, such as digging water drain- age tunnels or ditches to flood and bury the snail habitats, and the use of biological and ecological measures, including using snail predators such as fish and crustacea or introducing schistosome resistant snail strains into the wild snail population. Despite the concerns, affordable snail control, combined with MDA using praziquantel, could be part of the optimum strategy leading to Schistosomiasis elimination (McManus *et al*., 2018).

2.10 *Vernonia amygdalina* (Bitter Leaf)

Vernonia amygdalina, a member of the daisy family, is a small shrub that grows in tropical Africa (Plate 2.2). *V. amygdalina* typically grows to a height of 2-5 m (6.6-16.4 ft). The leaves are elliptical and up to 20 cm (7.9 in) long. Its bark is rough. It is called 'bitter leaf' in English because of its bitter taste. The leaves are a staple vegetable in soups and stews of various cultures throughout equatorial Africa (Ijeh and Ejike, 2011).

2.10.1 Medical importance of *Vernonia amygdalina* (bitter leaf)

V. amygdalina has shown very high medical value and molluscicide importance, cold concoction of this plant as a treatment for malaria, intestinal parasite, diarrhea and stomach upset. For numerous African ethnic groups, a concoction of this plant is also a prescribed treatment for malaria fever, Schistosomiasis, amoebic dysentery and other intestinal parasites (Appiah, 2018).

Plate I: Leaf and stem of *Vernonia amygdalina* (bitter leaf)
Source: Field photograph (2019)

CLASSIFIACATION

Kingdom	Plantae
Phylum	Spermatophyta
Class	Dicotyledonae
Order	Asterales
Family	Asteraceae
Genus	*Vernonia*
Species	*Vernonia amygdalina*

2.11 *Calotropis procera* (Apple of Sodom)

Calotropis procera is a species of flowering plant (Plate, 2.3) in the family Apocynaceae that is to North Africa, tropical Africa, Western Asia and South Asia. The green fruit contain a toxic milky sap that is extremely bitter and turns into a gluey coating which is resistant to soap. Common names for the plant include; Apple of

Sodom, king's crown and rubber bush (Plate 2.3). The milky sap contains a complex mix of chemicals, which are steroidal components they are the cause of its toxicity .*C. procera* has shown above 50 % mortality of fresh water snail (Sayed *et al*., 2017) also in the tropics of Asia and Africa latex extract of *C. procera* are highly toxic to snails (Kushwaha, 2014).

CLASSIFICATION

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Gentianales
Family	Ascepiadaceae
Genus	*Calotropis*
Species	*Calotropis procera*

Plate II: *Calotropis procera* leaves
Source: CC0020e: Field photograph (2019)

2.12 Biology of *C. procera*

C.procera produces a simple fleshy fruit in a grey green inflated pod which contains many flat brown seeds with tufts of long, white silky hair (pappus) at each end. When

the plant is cut it produces a milky white sap (latex) which is toxic (Francis, 2003; Brandes, 2005; Orwa *et al.,* 2009). *C.procera* is a perennial plant in which flowering and fruiting occur throughout the year, with each plant producing several or numerous seeds which is dispersed by the wind. After the raining season the seedlings emerges in large numbers in which few grows into maturity (Francis, 2003).The plant is pollinated by insects especially by species such as monarch butterfly (*Danaus plexippus*) which uses the plant as a host for various stages of its lifecycle (Orwa *et al.,* 2009).

Plate III: *Calotropis procera;* flowers and fruit
Source: Field photograph (2019)

2.13 *Amaranthus hybridus* (Smooth Pig Weed)

Amaranthus hybridus, commonly called smooth pig weed, is a species of annual flowering plant. It is a weedy species found now over North America, Africa and Asia. It grows from a short taproot and can be up to 2.5 meter in height (Plate 2.5).

The seeds and cooked leaves are edible; it has been used for food and medicine by several natives American groups and in traditional African medicine (Elias *et al*., 2015). According to Kimeu *et al*. (2015) *Amaranthus hybridus* has high molluscicidal activity.

Plate IV: Leaf of *Amaranthus hybridus* (smooth pig weed)
Source: Field photograph (2019)

CLASSIFICATION

Kingdom	Plantae
Phylum	Spermatophyta
Class	Dicotyledonae
Order	Caryophyllales
Family	Amaranthaceae
Genus	Amaranthus
Species	*Amaranthus hybridus*

2.14 Mechanism of Snail Control Using Molluscicide on the Snail Tissues

Snail control by molluscicide remains one of the most effective measures of schistosomiasis control. Niclosamide has been recommended by WHO as a molluscicide since the 1960s, but there are some limitations associated with its application, such as toxicity to non-target organisms.results of different research works demonstrated that salicylanilidate(an alternative molluscicide) reduced the survival of *B.*

glabrata, *B. straminea* and *B. pfeifferi*. Importantly, salicylanilidate displayed an approximately 100-fold-decreased fish toxicity to *D. rerio* compared with that of niclosamide, indicating that salicylanilidate, to some degree, does not have the disadvantage of high fish toxicity of niclosamide and could be used as molluscicide in aquaculture areas.

Cellular enzymes play vital roles in maintaining physiological activities. Recently, several enzymes have been used as biomarkers to assess the effects of toxicants or molluscicides on aquatic animals. To elucidate the molecular mechanism associated with the toxicity of salicylanilidate to *Biomphalaria* species, after a 24-h exposure at concentration, salicylanilidate reduced the enzyme activities of NOS, LDH, and AChE and increased the activity of AKP (Li *et al*., 2012). The average change in enzymatic activity in the salicylanilidate group was similar to that of niclosamide. These results were consistent with previous reports showing that niclosamide could inhibit NOS, LDH, and AChE activities in HEK293 cells (Wang *et al.,* 2016) and *O. hupensis* (Hongjun *et al*., 2006) and a report indicating that aqueous extracts of *Croton tiglium* could significantly alter the activities of LDH, AChE, ACP and AKP in the nervous tissue and hepatopancreas of *Lymnaea acuminata* (Yadav *et al*., 2006). NOS can catalyze L-arginine and oxygen to produce the gaseous signaling molecule nitric oxide (NO). NO plays an important role in neurotransmission for snails (Nacsa *et al*., 2015) and major roles in resistance to pathogens and immune regulation in molluscs. LDH is a representative enzyme in the anaerobic glycolytic pathway and plays a crucial role in the final step of glycolysis. Glycolytic enzymes are essential for energy metabolism of the snail. Acetylcholine is a neurotransmitter in the nervous system, and AChE has a critical function in catalyzing the hydrolysis of acetylcholine to maintain the normal conduct of nerve impulses. AChE is the primary target of organophosphate

and carbamate pesticides and can be used as a potent biomarker of pesticide toxicity to snails (Essawy *et al*., 2009) accessing molluscicidal activity, the movement speed of all snails was significantly decreased after immersion with salicylanilidate and niclosamide, which may be related to the dysfunctional neurotransmission and lack of energy supply caused by decreased AChE, NOS and LDH. Therefore, physiological dysfunction caused by changes of important enzymes may be involved in the toxicity of salicylanilidate against snails.

In addition to cellular enzymes, ultrastructural features of the hepatopancreas have been widely investigated in molluscicide studies. The hepatopancreas is a pivotal detoxification organ for snail and is particularly vulnerable to injuries. The ultrastructure of the hepatopancreas was noticeably altered by salicylanilidate, including degeneration or destruction in the endoplasmic reticulum, vacuolization in the mitochondria and heterochromatin aggregation in the nucleus. The endoplasmic reticulum is closely involved in synthesis and secretion processes in the hepatopancreas. Mitochondria, a major site for sugars, amino acids and fatty acid oxidation, play crucial roles in energy supply for biological activities. Interestingly, there was little difference between the molluscicidal mechanisms of the two compounds. Salicylanilidate predominantly led to the destruction of the rough endoplasmic reticulum, and to moderate heterochromatin aggregation. In contrast, niclosamide primarily led to polymorphic alterations of the nucleus, focal lysis in the nucleolus and increased heterochromatin aggregation, as well as moderate destruction of the endoplasmic reticulum. *Biomphalaria* snails have no operculum; thus, their cephalopodia were continuously in contact with the molluscicide during the toxicity test. Therefore, gene expression profiles of several cellular enzymes in the cephalopodium and hepatopancreas were further compared using RT-PCR. Transcriptional levels of NOS,

ACP and SOD in the hepatopancreas were significantly down regulated compared with those in cephalopodium. ACP, a lysosomal enzyme, plays critical roles in catabolism, pathological necrosis, autolysis and phagocytosis (Singh and Singh, 2009). SOD can alternatively catalyze the dismutation of the superoxide radical, which has pivotal function in antioxidant defense. Therefore, the hepatopancreas may be a critical target tissue of salicylanilidate.

Metaldehyde and niclosamide also affected the behavior and reproductive capacity of snails, the snails exposed to these molluscicide exhibit symptoms of poisoning such as slow movement, water leaving behavior, excretion of mucus and lack of retraction of foot. And in high concentrations of the molluscicide no sign of movement and excessive mucus secretion is witnessed (Wang *et al.,* 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Preparation of Plant Materials

The plants were selected on the basis of ethnobotanical information and recognized molluscicidal activity on *Bulinus sp* and *Biomphalaria sp* as in previous studies (Oguche & Olofuntoye., 2018)**.** They were collected with bio-conservation in mind from Chanchaga Local Government Area (LGA). *Calotropis procera* and *Amaranthus hybridus* were collected from the field while *V.amygdalina* was collected from home garden all within Chanchaga Local Government Area, Niger State. The plants were properly identified with the help of a senior botanist at the Department of Plant Biology, Federal University of Technology, Minna with reference to the standard flora. Voucher specimens were deposited at the Department for future reference. The collected plants parts to be used were the leaves and stems. The plants collected were air-dried at room temperature between 25 -30 ^{O}C for three weeks and from direct sunlight in order to prevent the ultra-violet rays of the sun from destroying the chemical content of the plants. The dried plant were then pulverized with the aid of the mortar and pestle into fine powdery form and stored until extraction for the experiment. The details of plant materials to be evaluated for their molluscicidal properties are given below in Table 3.1.

Table 3.1 Plant materials to be evaluated for molluscicidal properties.

No.	Scientific Name	Family	Parts Used
1	*Calotropis procera*	Ascepiadaceae	Leaves
2.	*Vernonia amygdalina*	Asteraceae	Leaves and stem
3	*Amaranthus hybridus*	Amaranthaceae	Leaves and stem

3.2 Collection of Snails

Snails were collected from Tudun Fulani Gabas Lake, Bosso Local Government Area, Niger State, Nigeria. The collection was be done between 8:00 am and 12:00 noon in order to avoid coming in contact with the cercaria which are usually shed from infected snails during the day when temperature rises slightly above 28 ^{O}C. Scoop net made of wire mess size of 1-2 mm in diameter were used to scoop the snail from the water, and then they were transferred to a container. The collected snails were then transported to the Animal Biology Laboratory of the University for Identification and onward studies. In the laboratory, the snails collected were identified using the snails' identification key to properly note the species to work with. The snails be used in the study are *Biomphalaria pfeifferi.*

3.3 Maintenance of Snails in the Laboratory

Collected snails were placed in beakers half filled with dechlorinated water, the beakers were exposed to daylight for one hour to allow emergence of cercariae, ones that shed cercariae are considered infected, all uninfected are maintained in separate plastic containers. The identified *Biomphalaria species* were maintained in ten different troughs of about 10 litre-15 litre capacity, with each trough containing at least ten (10) snails to 5 litres of dechlorinated and well aerated water. The snails were fed with fresh

Lactuva sativa (lettuce) and the aquaria was be maintained by changing the water three times in a week or when necessary and left to acclimatize for at least 4 weeks.

3.4 Preparation of Plant Extracts

The 100 g of dried and coarse plant material was macerated in 1000 ml of 95 % ethanol at room Temperature for three days. The 95 % ethanol was used to maintain the homogeneity in the individual plant extracts and to enhance the compatibility among the plant extracts for development of poly herbal formulations. The crude extract was separated by filtering under vacuum using Whitman filter paper). The retentate was then added to 1000 ml of fresh 95 % of ethanol, macerated for three days further at room temperature, and filtered. Both the filtrates were pooled together and concentrated to dryness using a rotary evaporator at 60 ^{O}C until the solvent was completely evaporated. The ethanolic extract of individual plants obtained were labeled and stored at 5 ^{O}C until evaluation of molluscicidal activities and analysis of phytochemicals.

Phytochemical Screening of extract

Qualitative and quantitative tests were carried out on the powdered sample of the dried and roasted seed sample using standard procedures described by (Harborne, 1973; 1976), AOAC, (1984), (Trease and Evans, 1989; Sofowara, 1993).

3.5 Qualitative Phytochemical Screening

3.5.1 Test for flavonoids

A portion of powdered plant in each case was heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Yellow coloration was observed thus indicating the presence of flavonoids (Sofowara, 1993; Harborne, 1973).

3.5.2 Test for tannins

To 0.5 g of the dried powdered sample was boiled in 20 ml distilled water in a test tube and filtered. 0.1 % ferric chloride ($FeCl_3$) solution was added to the filtered. The appearance of brownish green or a blue-black colouration indicates the presence of tannins in the test sample (Sofowara, 1993; Harborne, 1973).

3.5.3 Test for saponins

To 2.0 g of the powdered sample was boiled in 20 ml of distilled water in a test tube in boiling water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with drop of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of saponins (Harborne, 1973).

3.5.4 Test for alkaloids

To 0.5 of extract was stirred with 5 cm^3 of 1 % aqueous HCI on a steam bath, few drops of picric acid solution was added to 2 cm^3 of the extract. The formation of a reddish brown precipitate was taken as a preliminary evidence for the presence of alkaloids (Trease and Evans, 1989; Harborne, 1976).

3.5.5 Test for steroids

To 0.5 g of ethanolic extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids (Sofowara, 1993).

3.5.6 Test phlobatannins

Aqueous fraction of the extract of each sample was boiled with 1 % aqueous hydrochloric acid; the formation of red precipitate thus indicated the presence of phlobatanins. (Sofowara, 1993; Harborne, 1973)

3.5.7 Test for cardiac glycosides

To 5 ml of each plant extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution, followed by the addition of 1ml concentrated sulphuric acid. Brown ring formed at the interface indicates deoxysugar characteristics of cardenloides. A violet ring may appear beneath the brown ring, while in the acetic acid, layer; a greenish ring may also form just gradually throughout the thin layer (Harbone, 1973; 1976).

3.5.8 Test for anthraquinones (Borntrager's test)

To 0.5 g of plant extract was shaken with 5 ml chloroform, the chloroform layer was filtered and 0.5 cm^3 of 10 % ammonia was added to the filtrate. The mixture was shaken thoroughly, the formation of a pink/violet or red, yellow colour in the ammonical phase indicates the presence of anthraquinones (Harborne, 1973; 1976).

3.5.9 Test for reducing Sugar (Benedict test).

To 0.5 g of the plant extract was mixed thoroughly with 3 cm^3 distill water and filtered, 3 drops of the filtrate was added to 3cm^3 Benedict reagents and placed in a boiling water bath for 5mins. The formation of a brick red precipitate indicates reducing sugar (Harborne, 1973; 1976).

3.6 Quantitative Phytochemical Screening of Extract

3.6.1 Determination of total phenol

To 2 g of the sample were defatted with 100 ml of diethyl other using a soxhlet apparatus for 2 hr. the fat free sample was boiled with 50 ml of petroleum ether for the extraction of the phenolic component for 15 minutes, to 5 ml of the extract was pipeted into a 50 ml flask, then 10 ml of distilled water was added. To 2 ml of ammonium hydroxide solution and 5ml of concentrated amylalcohol were also added. The samples

were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm. Tannic acid was used to establish the calibration curve

3.6.2 Determination of total flavonoid

Total flavonoid was determined using aluminum chloride colorimetric method .Quercetin was used to establish the calibration curve. Exactly 0.5 ml of the diluted sample was added into test tube containing 1.5 ml of methanol. 0.1 ml of 10 % $AlCl_3$ solution and 0.1 ml sodium acetate ($NaCH_3COO^-$) were added, followed by 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The amount of 10 % $AlCl_3$ was substituted by the same amount of distilled water in blank.

3.6.3 Determination of total akaloids

To 0.5 g of the sample was dissolved in 96 % ethanol -20 % H_2SO_4 (1:1). 1 ml of the filtrate was added to 5 ml of 60 % tetraoxosulphate (VI), and allowed to stand for 5 min. Then; 5 ml of 0.5 % formaldehyde was added and allowed to stand for 3 h. The reading was taken at absorbance of 565 nm. The extinction coefficient (E296, ethanol {ETOH} = 15136 $M^{-1}cm^{-1}$) of vincristine was used as reference alkaloid (Harborne, 1976)

3.6.4 Determination of saponins

To 0.5 g of the sample was added to 20 ml of 1NHCl and was boiled for 4 h. After cooling it was filtered and 50 ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. To 5 ml of acetone ethanol was added to the residue, to 0.4 ml of each was taken into 3 different test tubes. Exactly 6 ml of ferrous sulphate reagent was added into them followed by 2 ml of concentrated H_2SO_4. It was thoroughly mixed after 10 min and the absorbance was taken at 490 nm. Standard saponin was used to establish the calibration curve.

3.6.5 Determination of tannin

To 0.2 g of sample was measured into a 50 ml beaker, to 20 ml of 50 % methanol was added and covered with para film and placed in a water bath at 77-80 ^{o}C for 1 hr. it was shaken thoroughly to ensure a uniform mixture. The extract was quantitavely filtered using a double layered whatman No.41 filter paper into a 100 ml volumetric flask, 20 ml water added, 2.5 ml Folin-Denis reagent and 10 ml of Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water mixed well and allowed to stand for 20 min for the development of a bluish-green colour. The absorbance of the tannic acid standard solutions as well as samples were read after colour development on a UV-spectrophotometer model 752 at a wavelength of 760 nm (AOAC, 1984).

3.6.6 Phytic acid content

The phytic acid content was determined using a modified indirect colorimetric method. The method depends on an iron phosphorus ratio of 4:6 and is based on the ability of standard ferric chloride to precipitate phytate in dilute HCI extract of the sample, to 5 g of the sample was extracted with 20 ml of 3 % trichloroacetic acid and filtered. 5 ml of the filtrate was used for the analysis; the phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding 5 ml of IM NaOH. The precipitate was dissolved with hot 3.2 M HNO_3 and the absorbance and immediately at 480 nm. Preparation of standard curve for phytic acid was done as follows: standard curve of different Fe $(NO_3)_3$ concentrations was plotted against the corresponding absorbance of spectrophotometer to calculate the ferric iron concentration. The phytate phosphorus was calculated from the concentration of ferric iron assuming 4:6 iron: phosphorus molar ratio.

3.6.7 Determination of cyanide

Cyanide content was determination by alkaline picrate method, to 5 g of powdered sample was dissolved in 50 ml of distilled water in a cooked conical flask and the extraction was allowed to stand over-night, filtered. To 1 ml of sample filtered was mixed with 4 ml alkaline picrate in a corked test tube and incubated in a water bath for 5 mins. After colour development (reddish brown colour) the absorbance was read at 490 nm, the absorbance of the blank containing 1 ml distilled water and 4 ml alkaline picrate solution was also recorded. The cyanide content was extrapolated from cyanide standard curve prepared from different concentration of KCN solution containing 5-50 μg cyanide in a 500 1 conical flask followed by addition of 25 ml of I NHCI.

3.6.8 Determination of oxalate

To 2 g of the sample flour was suspended in 190 ml of distilled water in 250 ml volumetric flask, 10 ml of 6M HCI was added and the suspension digested at 100 ^{O}C for 1hr, cooled, then made to the mark before filtration. Duplicate portion of 125 of the filtrate were measured into beakers and 4 drops of methyl red indicator added. This is followed by the addition of cone. NH_4OH solution drop wise until the test solution changes from salmon pink colour to a faint yellow colour (pH 4-4.5). Each portion is then heated to 90 ^{O}C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate is again heated to 90 ^{O}C and 10 ml of 5 % $CaC1_2$ solution added while being stirred constantly. After heating, it was cooled and left overnight at 5 ^{O}C. The solution was then centrifuged at 2500 rpm for 5 mins, the supernatant decanted and the precipitate completely dissolved in 10 ml of 20 % (v/v) H_2SO_4 solution. The total filtrate resulting from the digestion was made up to 300 ml aliquots of 125ml of the filtrate was heated until near boiling and then titrated against 0.05 M standardized $KMnO_4$ solution to a faint pink colour which persisted for 30 s.

3.7 Evaluation of the Molluscicidal Potency of Plant Extracts

Evaluation of molluscicidal activity of plant extracts against the *Biomphalaria* snails were performed as per the World Health Organization (WHO) guidelines (WHO, 2017). The different concentrations of individual, binary, and poly plant extracts were prepared and tested against the snails. A stock solution of individual crude plant extract was prepared by dissolving 1 g of each extract in 1000 ml of distilled water to make it a 0.1 % concentration which constitutes 1000 mg/L. From this stock solution, a 0.01 % (100 mg/L) test solution was be made by tenfold dilution with dechlorinated tap water. Similarly, the required concentration (200 mg/L to 400 mg/L) was being prepared from the 0.1 % stock solution.

3.8 Preparation of Binary, Tri and Poly Herbal Combinations of Plant Extracts

The following three different binary combinations of plant extracts was evaluated (Table 2). The different concentrations of binary plant extracts at the ratio of 1:1 v/v was be prepared from 0.1 % stock solutions of individual extracts with appropriate dilution with dechlorinated tap water.

Table 3.2: Binary combination of plant extracts.

Number of Combinations	Plant	Plant
1	*Calotropis procera*	*Amaranthus hybridus*
2	*Calotropis procera*	*Vernonia amygdalina*
3	*Vernonia amygdalina*	*Amaranthus hybridus*

Combinations of tri-herbal extract at 1:1:1 ratio (v/v) was prepared by combining individual plant extract test solutions for the evaluation against *Biomphalaria* snail

(Table 3). Different concentrations of tri-herbal extract were prepared from 0.1% stock solution of individual extract with appropriate dilution with dechlorinated water.

Table 3.3: Tri-herbal combination of plant extracts.

No.	Plant	Plant	Plant
1.	***Calotropis procera***	***Amaranthus hybridus***	***Vernonia amygdalina***

3.9 Preparation of Control Test Solution

Control experiments was performed with dechlorinated tap water alone (negative control) and Copper sulphate (Positive control).

3.10 Evaluation of Molluscicidal Potency of Plant Extracts

Groups of 10 uninfected *Biomphalaria* snails was placed in plastic trays with 1000 ml of different concentrations of individual, binary, and poly herbal extracts test solutions separately. Three different concentrations of each test solution of the plant extracts were tested, each with three replicates of 10 snails. Control experiment was being performed with dechlorinated water alone (negative control) and copper sulphate (positive control). The plastic tray was individually covered with a fine plastic mesh to prevent the snails from crawling out. A snail exposed to different concentrations of the plant extract was being left for observation for 24 hours and 48 h. After 48 hours, the plant extract suspension was decanted the snails were rinsed twice with dechlorinated water and transferred to a new container filled with dechlorinated water and observed for another 24 hours, which would serve as the recovery period, following which the mortality rates was determined. The percentage of mortality was calculated against the concentrations used. The LC50 and LC_{90} values of the tested plant extract were determined according to Litchfield and Wilcoxon (1949). The snails were not fed during the exposure and

recovery periods. Upon observation, the dead snails were removed from the containers. Snails were considered dead if they did not move and either had retracts well into their shells or was be hanging out of their shells. The death of each snail was be further ascertained by the complete opening of operculum and if the head did not respond when pricked with a sharp needle. The mortality of snails was recorded for both exposure periods and the recovery period.

3.11 Histopathalogical Procedures

After 24, 48 and 72 hrs of exposure, 36 snails from different groups were randomly chosen for histological and ultrastructural studies. Shells of snails were removed by making a cut round the whorls in a continuous manner starting at the aperture opening using a bone scissors and the broken fragments of the shell were carefully removed. Dissection was carried out under Zeiss binocular microscope. For histological studies, digestive glands from animals of different experimental groups were rapidly excised and immediately dropped in either 10% formalin or Bouin's solutions. Graded dehydration of the tissue was done by 70-100% alcohol in subsequent steps. Methyl benzoate was used as a clearing agent. Tissues were embedded in paraffin (58.6 °C) and sections were cut by a rotatory microtome (5 μm) and stained with hematoxylin and eosin or with Mallory's triple stain. Cell size as well as nuclear diameter were measured with a calibrated ocular scale (10x), using a Zeiss microscope (40x) objective and a microcytometer (American Optical). For electron microscopical studies, small pieces of fresh specimens of digestive gland were removed from animals. The tissues were fixed by immersing them immediately in formalin-glutaraldehyde fixative ($_4F_1G$) in phosphate buffer solution (pH 7.2) at 4°C for 3 h. Specimens were then postfixed in 2% OsO_4 in the same buffer at 4°C for 2 h. Samples were washed in the buffer and dehydrated at 4°C through a graded series of ethanol. Specimens were embedded in

Epon-araldite mixture in labeled beam capsules. Semithin sections (1 μm thick) were stained with toluidine blue. Ultrathin sections (60-70 nm thick) were picked upon 200 mesh copper grids and double stained with uranyl acetate for ½ h and lead citrate for 20-30 min (Reynolds, 1963). Specimens were examined under Jeol 100 CX TEM.

3.12 Data Analysis

Data obtained from the mortality of snails exposed to different concentrations of the selected plant extracts was analyzed using a one-way analysis of variance (ANOVA) and mean comparisons performed by Duncan's multiple comparison tests using SPSS) at the 5 % significant level.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Phytochemical composition of the individual plant extract

The results of the phytochemical screening of the selected plant extracts, namely *Vernonia amygdalina, Calotropis procera,* and *Amaranthus hybridus,* is detailed in Table 4.1. Tannins and Saponins were present in the three plant ethanol extracts. While flavonoids was present in both *C. procera* and *V. amygdalin.* Oxalate was present in both *C. procera* and *A. hybridus. Alkaloid was present in both C. procera* and *V. amygdalina.*

4.1.2 The phytochemical composition of the combined plant extracts

The phytochemical composition of the combined plant extracts are contained in Table 4.2. The CA showed high concentration of saponins and phenols than other combined extract. Tannins is present in both CV and VA Saponins and alkaloid was absent in CV combined.

4.1.3 The quantitative phytochemical composition and concentrations of the combined plant extracts.

A quantitative phytochemical content of the combined plant extracts is presented in Table 4.3. While Tannins ranges from 0.58 g/100g in CV to 0.16 g/100 g in VA, CA recorded the highest concentration of saponnins (0.97 g/100 g).

Table 4.1: Qualitative Phytochemical Components of Ethanolic Extract of Three Plants

Phytoconstituent	*V. amygdalina*	*A. hybridus*	*C. procera*
Flavonoids	++	-	+++
Tannins	+++	+++	+
Saponins	++	++	+
Alkaloids	+	-	++
Phenols	++	-	-
Oxalate	++	+++	-
Cardiac glycosides	-	+	++

Key

+++=Highly Present ++=Moderately Present += Trace Presence -= Absent

Table 4.2 The Phytochemical Composition of the Combined Plant Extracts

Phytoconstituent	CA	CV	VA
Flavonoids	-	+	+
Tannins	+	+	+
Saponins	++	-	++
Alkaloids	-	-	+
Phenols	++	+	-
Oxalate			
Cardiac glycosides			

Key

+++=Highly Present ++=Moderately Present += Trace Presence -= Absent

CV=*C. procera+V. amydalina CA=C. procera+A.hybridus VA= V. amydalina+ A.hybridus*

Table 4.3: The Quantitative Phytochemical Concentrations of the Combined Plant Extracts

Phytoconstituent	CA (g/100g)	CV(g/100g)	VA(g/100g)
Flavonoids		0.12	0.118
Tannins	0.34	0.58	0.162
Saponins	0.97		0.310
Alkaloids		-	0.521
Phenols		0.731	-
Oxalate	0.081		-
Cardiac glycosides	-		

KEY:

CV=*C. procera+V. amydalina CA=C. procera+A.hybridus VA= V. amydalina+ A.hybridus*

4.1.4 Molluscicidal activities of *Calotropis procera* plant extract after 72 hrs exposure and recovery period

The molluscicidal activities of crude *Calotropis procera extract is* presented in Table 4.3, After 24 hrs exposure period, no mortality was recorded in the treatments except in the positive control, where 100 % mortality was recorded. Thereafter after 48 hrs exposure and 72 hrs recovery, snail mortality increases gradually with increase in extract concentration. However, while the group exposed to 400 mg/L extract concentration recorded a significant highest mortality compared to other lower concentration of the plant extract. There was no significant difference ($p<0.05$) in the snail mortality recorded after 24 hrs exposure period for group exposed to 50,100,200, and 300 mg/L extract concentration. The mortality recorded after 72 hrs exposure period was higher than at 48hrs exposure period, only 400 mg/L concentration recorded the highest percentage mortality(figure4.1) but the mortality of the positive control was significantly higher($p<0.05$) than that of 400 mg/L extract concentration,

Table 4.4 Molluscicidal *Activities* of *Calotropis procera* After 72 hrs Exposure and Recovery Perio

Sample	24 hrs	48 hrs	72 hrs
50	0.00±0.00[a]	1.00±0.41[b]	2.00±0.41[b]
100	0.00±0.00[a]	1.25±0.25[b]	2.00±0.41[b]
200	0.00±0.00[a]	1.50±0.65[b]	3.25±0.63[c]
300	0.00±0.00[a]	1.50±0.65[b]	5.00±1.58[d]
400	0.00±0.00[a]	2.75±0.75[c]	7.00±0.91e
Positive Control	10.00±0.00[b]	10.00±0.00[d]	10.00±0.00[f]
Positive Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

Values are expressed as mean ±SEM of three replicates. The values along the same row with different superscript are significantly different ($p<0.05$).

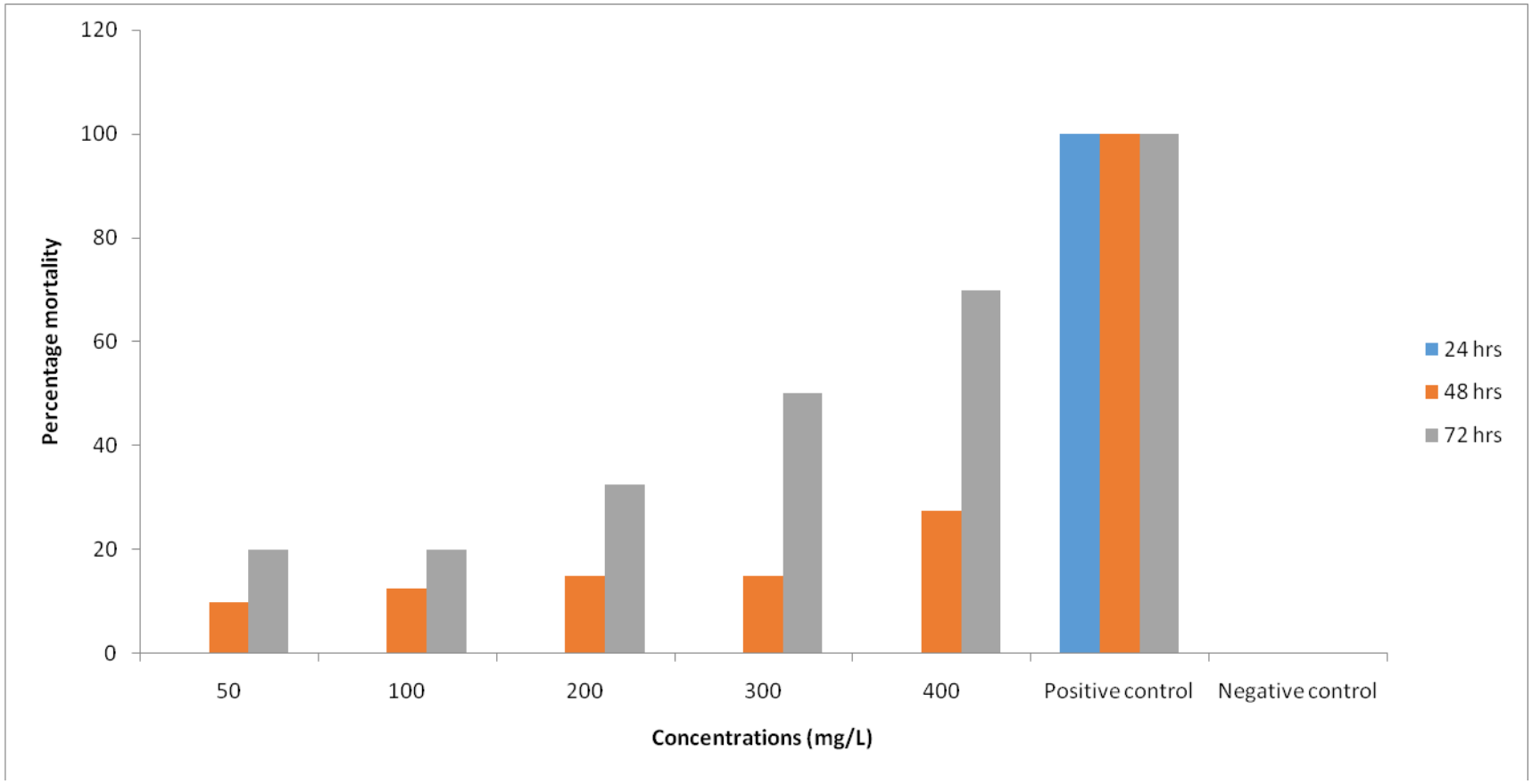

Figure 4.1: Mortality of the Molluscicidal Activities of *Calotropis procera* After 72 hrs Exposure and Recovery Period

4.1.5 Molluscicidal activities of *Vernonia amydalina* after 72 hrs exposure and recovery period.

The Results of the molluscicidal activities of the *Vernonia amygalina* extracts after 24 hrs, 48 hrs, and 72 hrs exposure and recovery period is captured in Table 4.4. On a general note the results showed that snail mortality increases with increase in extract concentration as well as time of exposure. After 24 hrs The study recorded no significant difference($P < 0.05$)in the snail mortality recorded for groups exposed to 50,100, and 200 mg/L extract concentrations it was also observed, there is no significant difference($P < 0.05$) in the mortality recorded for group exposed to 100 and 200 mg/L extract concentration, after 48 and 72 hrs exposure period . Throughout the experimental exposure period it was observed that (100 %) mortality (Figure 4.2) recorded was in the group exposed to 300 mg/L, 400 mg/L extract concentration and the positive control. No snail mortality was recorded for the negative control.

Table 4.5 Molluscicidal Activities of *Vernonia amydalina* After 72 hrs Exposure and Recovery period

Sample	24 hrs	48 hrs	72 hrs
50	2.00 ± 0.41^{b}	2.75 ± 0.75^{b}	3.00 ± 0.71^{b}
100	2.50 ± 0.50^{b}	6.00 ± 0.82^{c}	6.25 ± 0.85^{c}
200	3.00 ± 1.00^{b}	7.00 ± 0.71^{c}	7.00 ± 0.71^{c}
300	10.00 ± 0.00^{c}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
400	10.00 ± 0.00^{c}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
Positive Control	10.00 ± 0.00^{c}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
Negative Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are expressed as mean ±SEM of three replicates. The values along the same row with different superscript are significantly different ($p<0.05$).

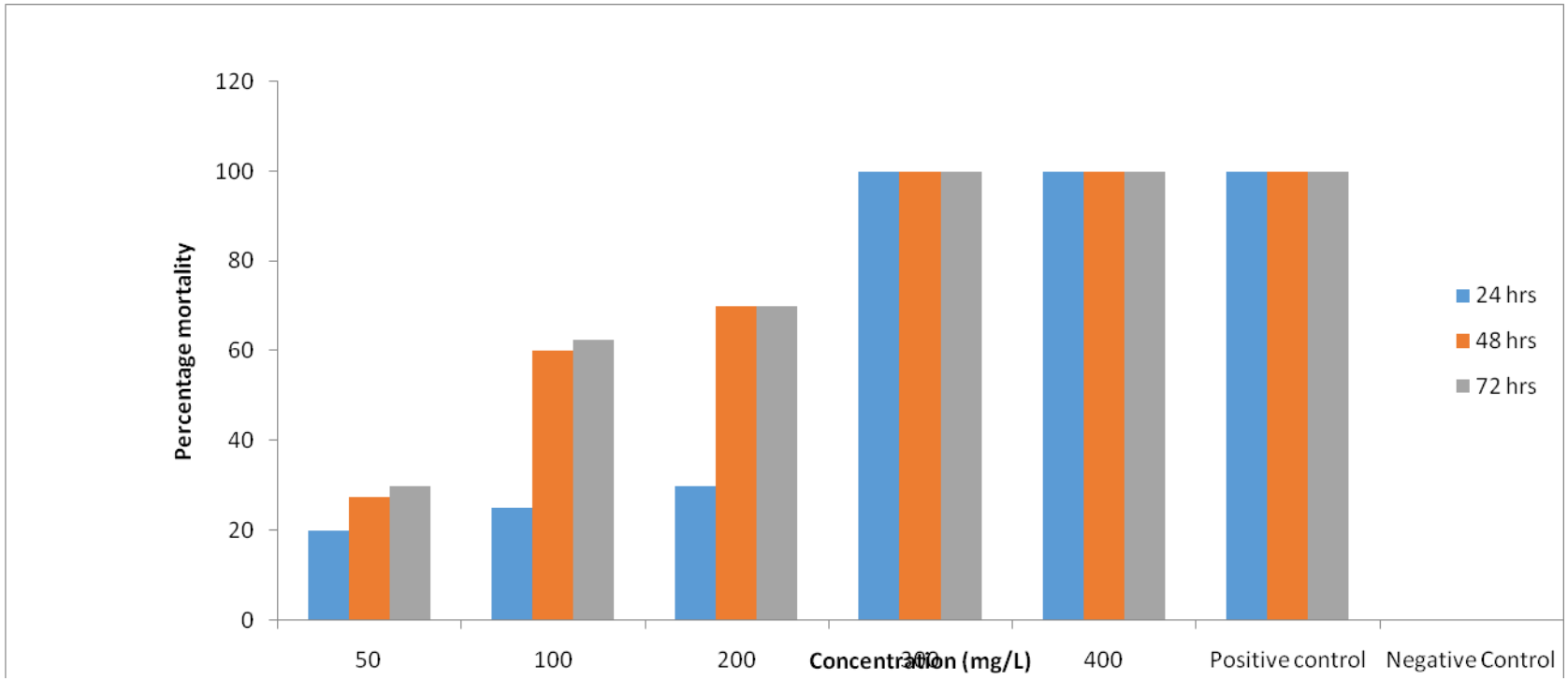

Figure 4.2: Mortality of the Molluscicidal Activities of *Vernonia amygdalina* After 72 hrs Exposure and Recovery Period

4.1.6 The molluscicidal activities of *Amaranthus hybridus*

The molluscicidal activities of of *Amaranthus hybridus* extract against *Biomphalaria pfeifferi* (intermediate host *of Schistosoma mansoni*) after 24 hrs, 48 hrs, and 72 hrs exposure period is contained Table 4.5 the molluscicidal activity increases with increase with extract concentrations and time of exposure. There was significant difference ($P < 0.05$) in the snail mortality recorded among the tested concentration. It was also observed that after 24 hrs exposure period. There is no significant difference ($P < 0.05$) in the mortality recorded for 200 and 300 mg/L extract concentration, (100 %) mortality (Figure 4.3) mortality was observed in the group exposed to 300mg/L extract concentration after 48 hrs, there was no significant difference in mortality and percentage mortality recorded for 300 (100 %) after 48hrs, 400 mg/L and the positive control (100 %). The negative control on the other hand recorded zero mortality throughout the study period.

Table 4.6: The Molluscicidal Activities of *Amaranthus hybridus*

Sample	24 hrs	48 hrs	72 hrs
50	0.00 ± 0.00^{a}	0.25 ± 0.25^{a}	0.25 ± 0.25^{a}
100	0.25 ± 0.25^{a}	1.00 ± 0.00^{b}	1.00 ± 0.00^{b}
200	1.75 ± 0.25^{b}	3.00 ± 0.00^{c}	3.00 ± 0.00^{c}
300	2.50 ± 0.50^{b}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
400	10.00 ± 0.00^{c}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
Positive Control	10.00 ± 0.00^{c}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
Positive Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are expressed as mean ±SEM of three replicates.The values along the same row with different superscript are significantly different ($p<0.05$).

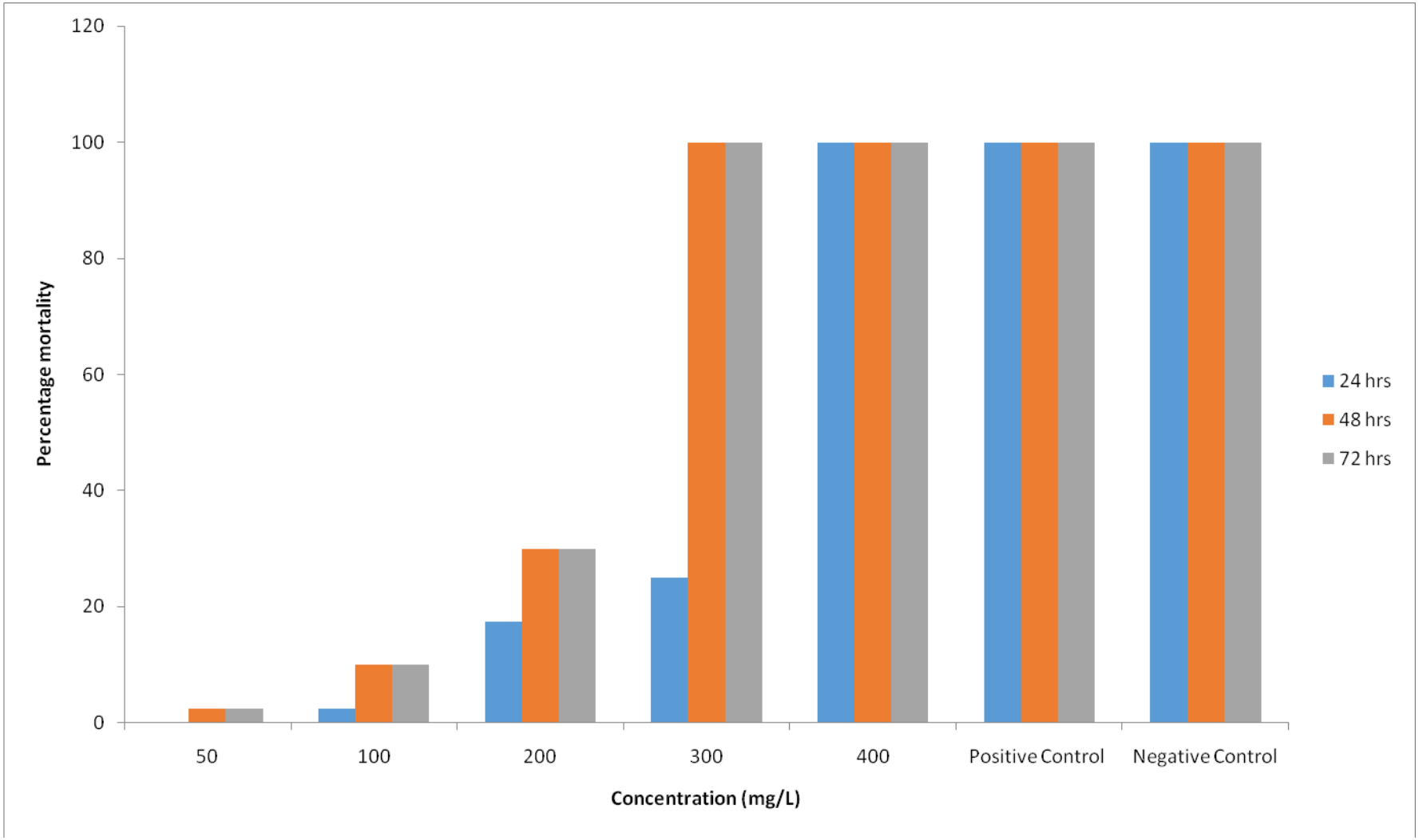

Figure 4.3: Mortality of the Molluscicidal Activities of *Amaranthus hybridus*

4.1.7 Molluscicidal activities of combined extracts of *Calotropis procera* and *Vernonia amygdalina* against *Biomphalaria pfeifferi* snails after 72hr exposure and recovery period.

The molluscicidal activities of combined extracts of *Calotropis procera* and *Vernonia amygdalina* against *Biomphalaria pfeifferi* (intermediate host of *Schistosoma mansoni*) after 24 hrs, 48 hrs and 72 hrs exposure period is contained Table 4.5, similarly the percentage mortality of the snail is as represented in figure 4.6. After 24 hrs up to 72 hrs exposure and recovery period, it was observed that snail mortality increases with increase with extract concentrations and time of exposure. There was significant difference ($P < 0.05$) in the snail mortality recorded for 10, 30, 50 mg/L extract concentration compared to negative and positive control, also more than 90 % mortality after 48hrs and 72 hrs recovery period for the group exposed to 50 mg/L extract concentration (table 4.6 and figure 4.6) complete snail mortality (100 %) mortality was observed in snail exposed to 150 and 250 mg/L extract concentration and the positive control.

Table 4.7 Molluscicidal Activities of Combined Extracts of *Calotropis procera* and *Vernonia amygdalina* Against *Biomphalaria pfeifferi*

Sample	24 hrs	48 hrs	72 hrs
10	1.25 ± 0.25^{b}	2.50 ± 0.29^{b}	2.50 ± 0.29^{b}
30	3.00 ± 0.91^{c}	4.50 ± 1.32^{b}	7.50 ± 0.29^{c}
50	7.25 ± 0.63^{d}	9.00 ± 0.41^{c}	9.00 ± 0.41^{d}
150	10.00 ± 0.00^{e}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
250	10.00 ± 0.00^{e}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
350	10.00 ± 0.00^{e}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
Positive Control	10.00 ± 0.00^{e}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
Negative Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are expressed as mean ±SEM of three replicates.The values along the same row with different superscript are significantly different ($p<0.05$).

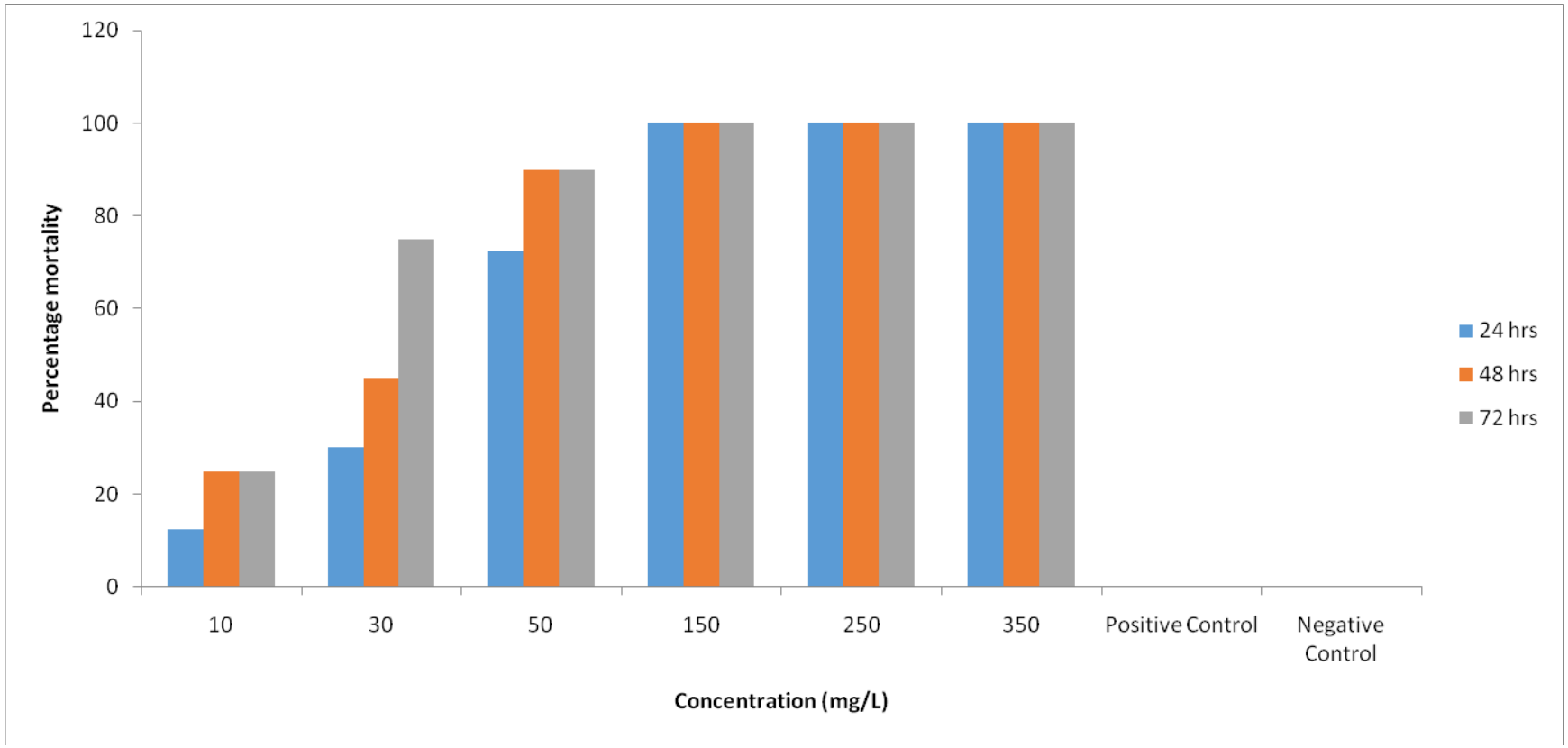

Figure 4.4: Mortality of the Molluscicidal Activities of Combined Extracts of *Calotropis procera* and *Vernonia amygdalina* Against *Biomphalaria pfeifferi* Snails

4.1.8 Molluscicidal activities of combined extracts of *Calotropis procera* and *Amaranthus hybridus* against *Biomphalaria pfeifferi* snails after 72hr exposure and recovery period.

The Results of the molluscicidal activities of the combined extracts of *Calotropis procera* and *Amaranthus hybridus* is captured in Table 4.5. The results showed that snail mortality increases with increase in extract concentration and time of exposure. The study also showed low snail mortality at 10, 30, 50, 150 and 250 mg/L extract concentration. However 100 % mortality (Figure 4.3) was recorded for the snail in the group exposed to 350 mg/L extract concentration. There was significant difference ($P<0.05$) in the snail mortality recorded for group exposed to 10 to 250 mg/L extract concentration compared with both positive and negative control, respectively.

4.1.9 Molluscicidal activities of combined extracts *Vernonia amygdalina* and *Amaranthus hybridus* against *Biomphalaria pfeifferi* snails after 72hr exposure and recovery period.

The result of the molluscicidal activites of the combined extract of *Vernonia amygdalina* and *Amaranthus hybridus* against *Biomphalaria pfeifferi* snails is represented in table4.6 and percentage mortality is captured in figure 4.4. The result showed that the combined extract caused dose and time dependent mortality against the tested snail species. the mortality caused by all the extract concentrations were significantly ($P<0.05$) higher than that of the negative control, where no mortality was recorded, throughout the study period, additionally, it was observed that group exposed to 150 and 250 mg/L extract concentration caused 100 % snail mortality (figure 4.4).this is not significantly different ($p<0.05$) from the mortality recorded for the positive control.

Table 4.8: Molluscicidal Activities of Combined Extracts of *Calotropis procera* and *Amaranthus hybridus* Against *Biomphalaria pfeifferi* Snails

Sample	24 hrs	48 hrs	72 hrs
10	0.50±0.29[a]	0.50±0.29[a]	0.50±0.29[a]
30	1.25±0.25[b]	1.75±0.48[b]	1.75±0.48[b]
50	1.50±0.29[b]	2.25±0.42[b]	2.50±0.29[b]
150	2.50±0.50[c]	4.00±0.41[c]	4.25±0.25[c]
250	3.25±0.25[c]	6.50±0.65[d]	6.75±0.63[d]
350	10.00±0.00[d]	10.00±0.00[e]	10.00±0.00e
Positive Control	10.00±0.00[d]	10.00±0.00[e]	10.00±0.00[e]
Negative Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

Values are expressed as mean ±SEM of three replicates. The values along the same row with different superscript are significantly different ($p<0.05$).

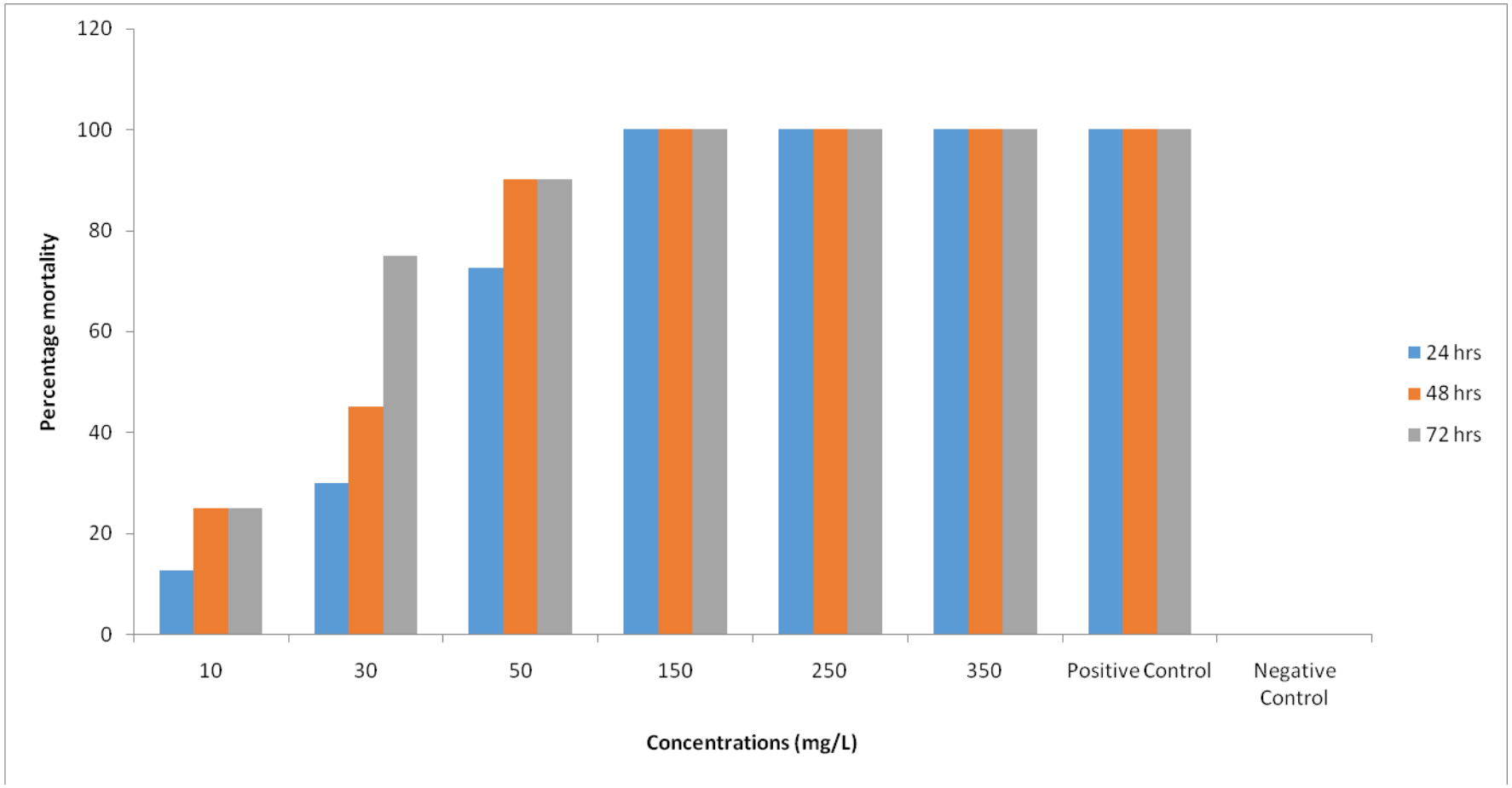

Figure 4.5: Mortality of the Molluscicidal Activities of Combined Extracts of *Calotropis procera* and *Amaranthus hybridus* Against *Biomphalaria pfeifferi* Snails After 72hr Exposure and Recovery Period

Table 4.9: Molluscicidal Activities of Combined Extracts *Vernonia amygdalina* and *Amaranthus hybridus* Against *Biomphalaria pfeifferi* Snails

Sample	24 hrs	48 hrs	72 hrs
10	0.75 ± 0.25^{b}	1.50 ± 0.29^{b}	1.50 ± 0.29^{b}
30	1.00 ± 0.00^{b}	1.75 ± 0.25^{b}	1.75 ± 0.25^{b}
50	2.00 ± 0.41^{c}	4.00 ± 0.58^{c}	4.00 ± 0.58^{c}
150	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
250	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
Positive Control	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
Negative Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are expressed as mean ±SEM of three replicates.The values along the same row with different superscript are significantly different ($p<0.05$).

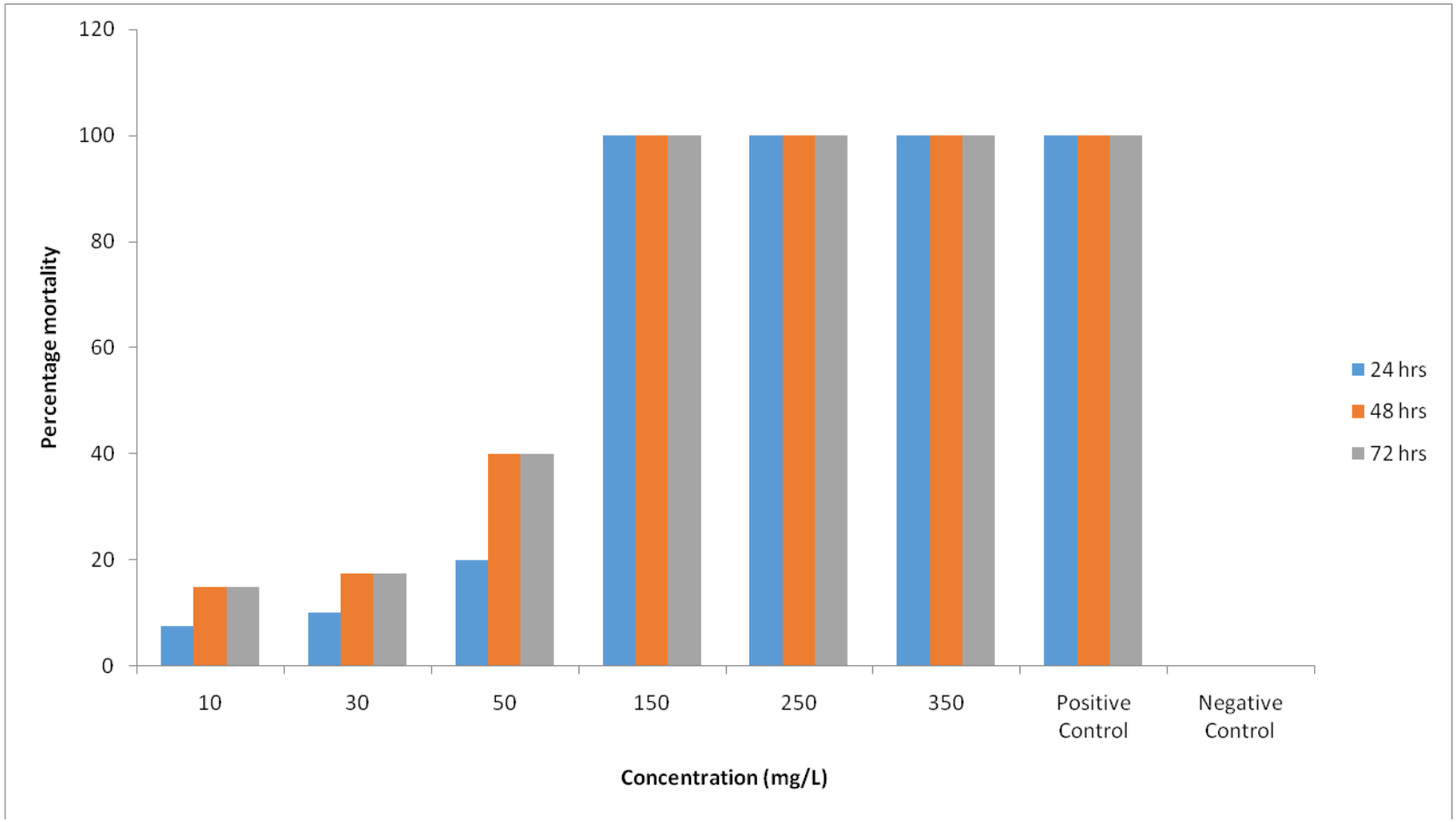

Figure 4.6: Molluscicidal activities of combined extracts *Vernonia amygdalina* and *Amaranthus hybridus* against *Biomphalaria pfeifferi* snails.

4.1.10 Molluscicidal activities of the three combined plant (*Calotropis procera, Vernonia amygdalina* and *Amaranthus hybridus*) extracts against *Biomphalaria pfeifferi* snails after 72 hr exposure and recovery period

The Results of the molluscicidal activities of the three combined plant extracts against the *Biomphalaria pfeifferi* snails after 24 hrs,48 hrs, and 72hrs exposure and recovery period is contained in table 4.7 and their respectively percentage mortality in figure 4.6,respectively,on a general note,snail mortality increases drastically with increase in extract concentration and time of exposure. There were significant differences in the recorded mortality for the different extract concentration. This observation repeated itself at 48 and 72 hrs. After 24hrs exposure period, more than 90 % (Figure 4.6) snail mortality was recorded for the group exposed to 150 mg/L extracts concentration. There was 100 % mortality recorded for the exposed snail to 250 and 350 mg/L extract concentration

Table 4.10: Molluscicidal Activities of the Three Combined Plant (*Calotropis procera, Vernonia amygdalina,* and *Amaranthus hybridus*) Extracts Against *Biomphalaria pfeifferi* Snails

Sample	24 hrs	48 hrs	72 hrs
10	1.25 ± 0.48^{b}	1.75 ± 0.48^{b}	1.75 ± 0.48^{b}
30	3.00 ± 0.91^{c}	3.75 ± 1.31^{b}	3.75 ± 1.31^{c}
50	6.75 ± 0.25^{d}	8.50 ± 0.29^{c}	9.00 ± 0.41^{d}
150	9.50 ± 0.29^{e}	10.00 ± 0.00^{d}	10.00 ± 0.00^{e}
250	10.00 ± 0.00^{e}	10.00 ± 0.00^{d}	10.00 ± 0.00^{e}
Positive control	10.00 ± 0.00^{e}	10.00 ± 0.00^{d}	10.00 ± 0.00^{e}
Negative control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are expressed as mean ±SEM of three replicates. The values along the same row with different superscript are significantly different ($p<0.05$).

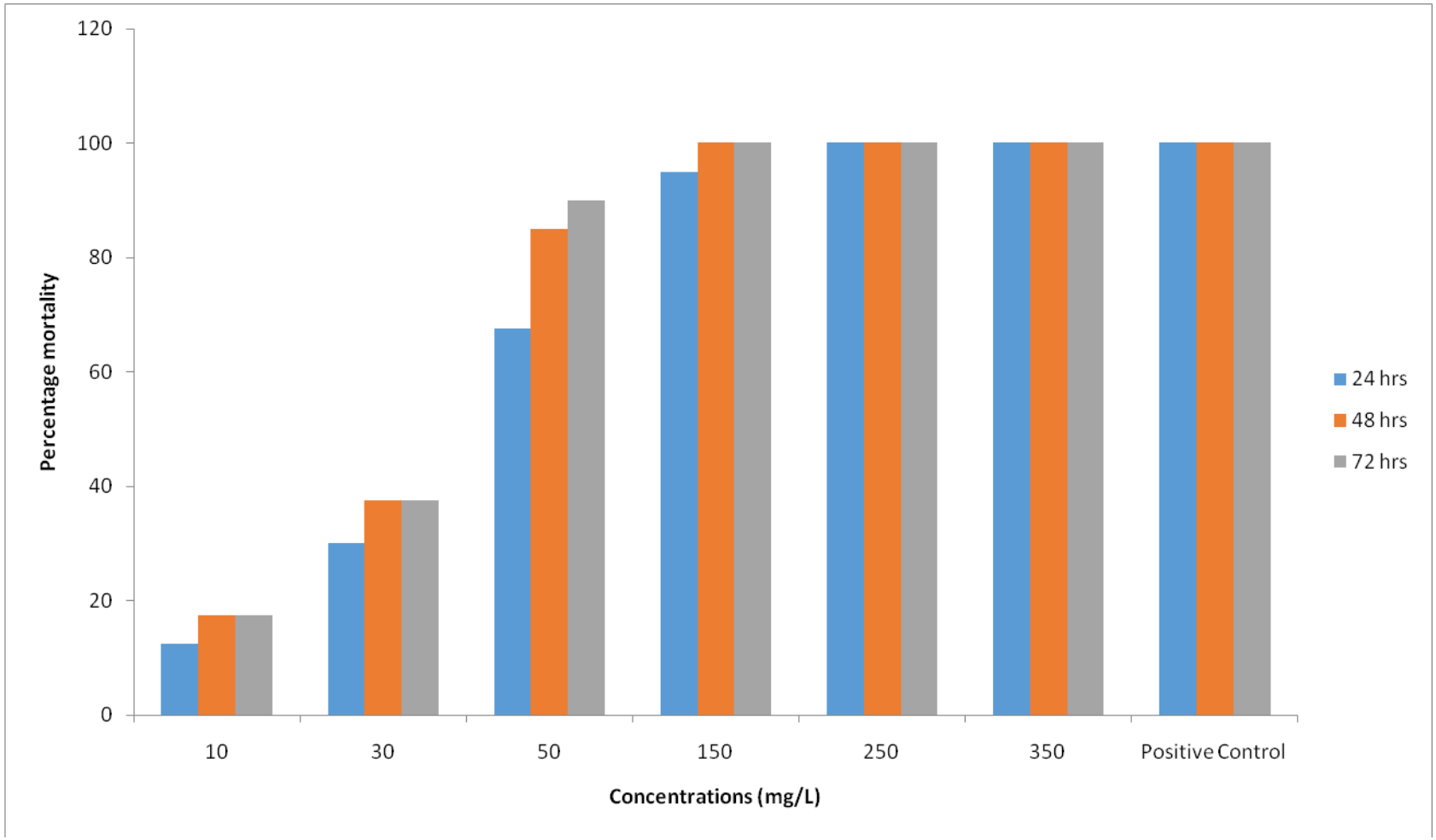

Figure 4.7: Molluscicidal Activities of the Three Combined plant (*Calotropis procera,Vernonia amygdalina,&Amaranthus hybridus*) Extracts Against *Biomphalaria pfeifferi* Snails

4.1.11 Lower (LC_{10}), media and upper lethal concentration of the molluscicidal activities of mono, binary and tri- herbal combination of plant extract.

The results of the probit regression analysis showing Lower (LC_{10}), media (LC_{50}) and upper lethal concentration of the molluscicidal activities of mono, binary and tri-combination of some plants is presented in Table 4.11. Among the single applied extract, *V. amygdalina* was the most potent with an LC_{50} of 107 mg/L after 48 hours exposure period, followed by *A. hybridus* with an LC_{50} of 201.23 mg/L. The values of the coefficient of determinations (R2) recorded indicated more than 70 % of the extract caused the recorded larval mortality. When the extract was combined binarily and in tri-combination, the CV combined was more potent than other extract combination. The recorded LC_{50} for the CV combined (28.37 mg/L), after 48 hrs exposure period was better that that of tri-combined extract.

Table 4.11: Lower (LC_{10}), Media (LC_{50}) and Upper Lethal Concentration (LC_{90}) of the Molluscicidal Activities of Mono, Binary and Tri-combinations LC: Lethal concentration

	Exposure time (hour)	LC_{10}		LC_{90}	R^2	Regression
C. procera	24					
	48	67.52	1019.9	1972.29	0.795	y = 0.042x + 7.164
	72	16.49	288.60	560.71	0.963	y = 0.147x + 7.576
V. amygdalina	24	50.00	183.33	316.67	0.777	y = 0.3x – 5
	48	-47.94	107.09	262.13	0.919	y = 0.258x + 22.37
	72	-63.21	101.875	261.99	0.904	y = 0.246x + 25.55
A. hybridus	24	151.89	529.25	906.61	0.932	y = 0.106x - 6.101
	48	96.24	201.23	306.22	0.896	y = 0.381x - 26.67
	72	91.77	195.66	299.56	0.932	y = 0.385x - 25.33
Combined efficacy						
C.procera+A.hybridus	10.00±0.00[e]	10.00±0.00[d]	10.00±0.00[d]	395.13	0.811	y = 0.229x - 0.485
	48	16.53	44.233	329.04	0.983	y = 0.256x + 5.767
	72	16.53	44.233	329.04	0.983	y = 0.256x + 5.767
C.procera+V.amygdalina	24	-15.19	53.65	122.49	0.825	y = 0.581x + 18.83
	48	-26.55	28.37	112.76	0.677	Y=0.474x + 36.55
	72	-26.55	28.37	112.76	0.677	Y=0.474x + 36.55
V.amygdalina+ C.procera	24	25.21	57.65	139	0.976	y = 0.700x - 7.650
	48	7.55	70.27	134.33	0.985	y = 0.631x + 5.237
	72	7.55	70.27	134.33	0.985	y = 0.631x + 5.237
C.procera+A.hybridus+ V.amygdalina	24	-52.15	65.84	183.83	0.775	y = 0.339x + 27.68
	48	-34.32	41.15	116.62	0.718	y = 0.530x + 28.19
	72	-37.50	38.69	114.87	0.667	y = 0.525x + 29.69

4.1.12 Histopatology analysis results

The results of the histopathology of the experimental snail species is presented in Plate I to IX. The results indicated mild cytoplasmic fragmentation and vacuolization in the excretory and digestive system, of the experimental snail as follows: V= vacuole; CF= Cytoplasmic fragmentation; TA= toxic agents; CA-350: There is mild cytoplasmic fragmentation and vacuolization in the excretory cells. CA-250: same as CA-350; CA-50: Same as CA-350; CV-350: Same as CA-350; CV-250: Same as CA-350; CV-50: Same as CA-350; VA-350: There is severe cytoplasmic fragmentation and vacuolization in the excretory cells and accumulation of the toxic agents of the extract in the cytoplasm of the digestive cells; VA-250: same as VA-350; VA-50: There is cytoplasmic fragmentation and vacuolization in the excretory cells.

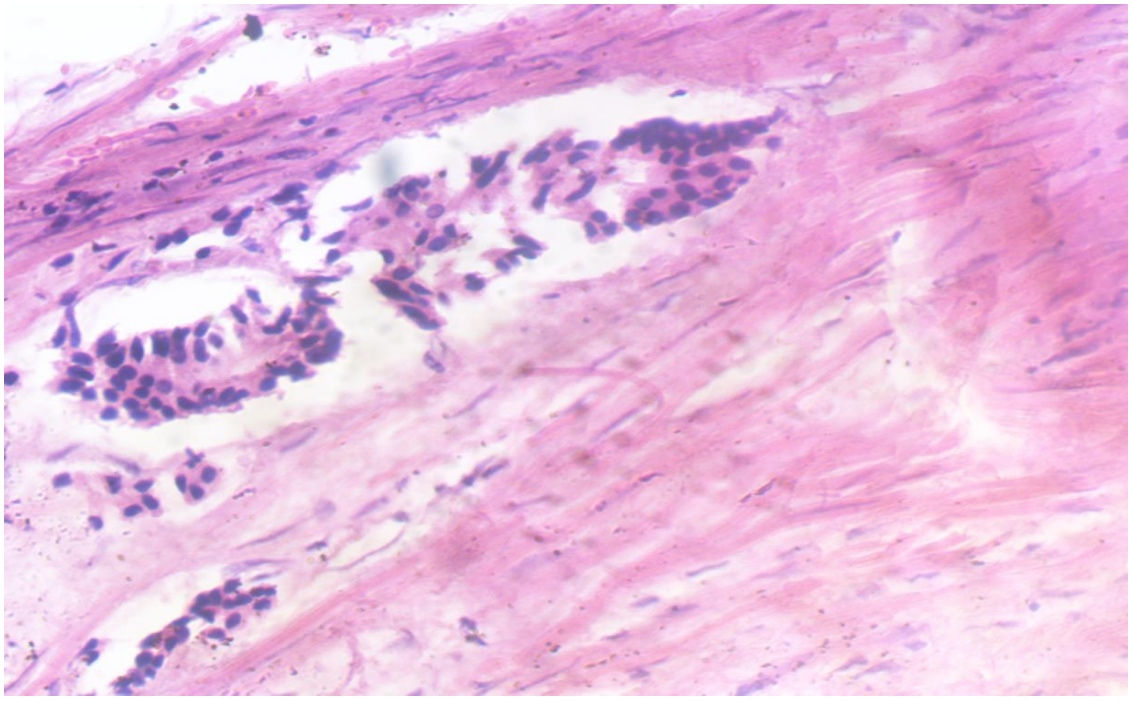

Plate V: CA-50

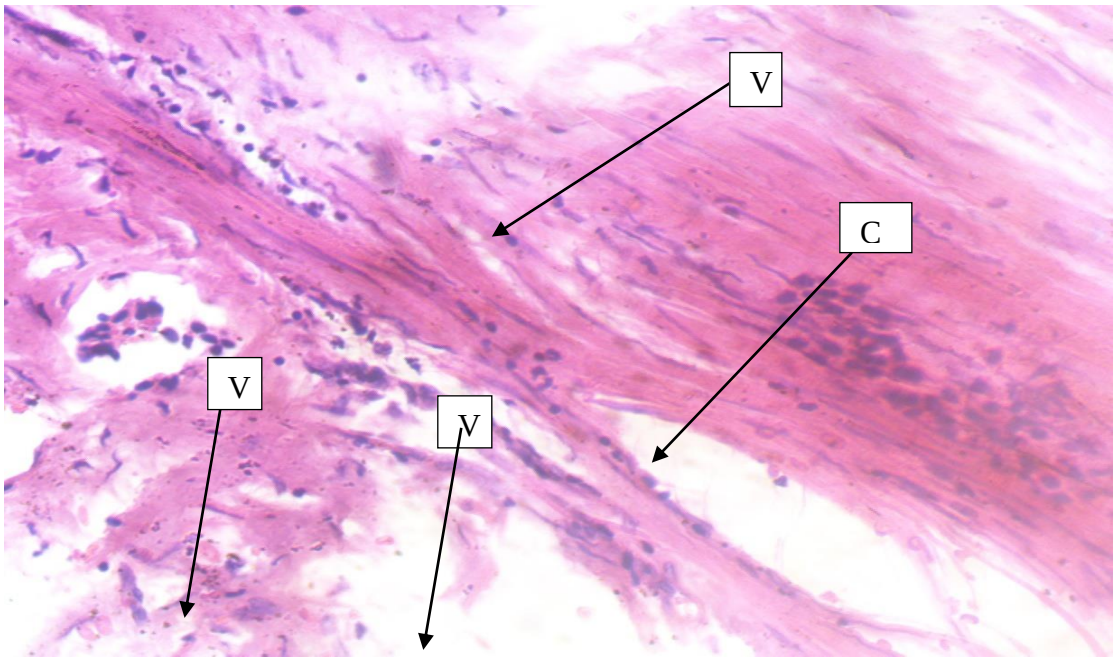

Plate VI: CA-250 Keys: V= vacuolization CF= cytoplasmic fragmentation; TA= toxic agents

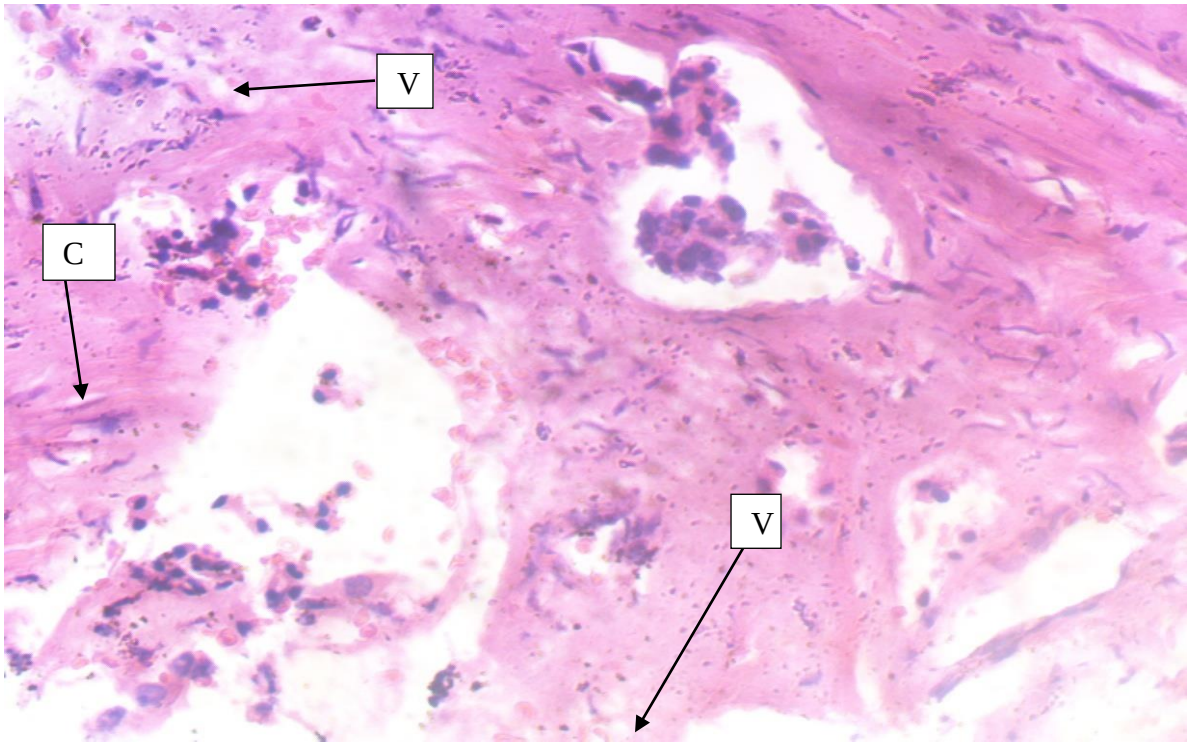

Plate VII: CA-350
Keys: V= vacuolization CF= cytoplasmic fragmentation; TA= toxic agents

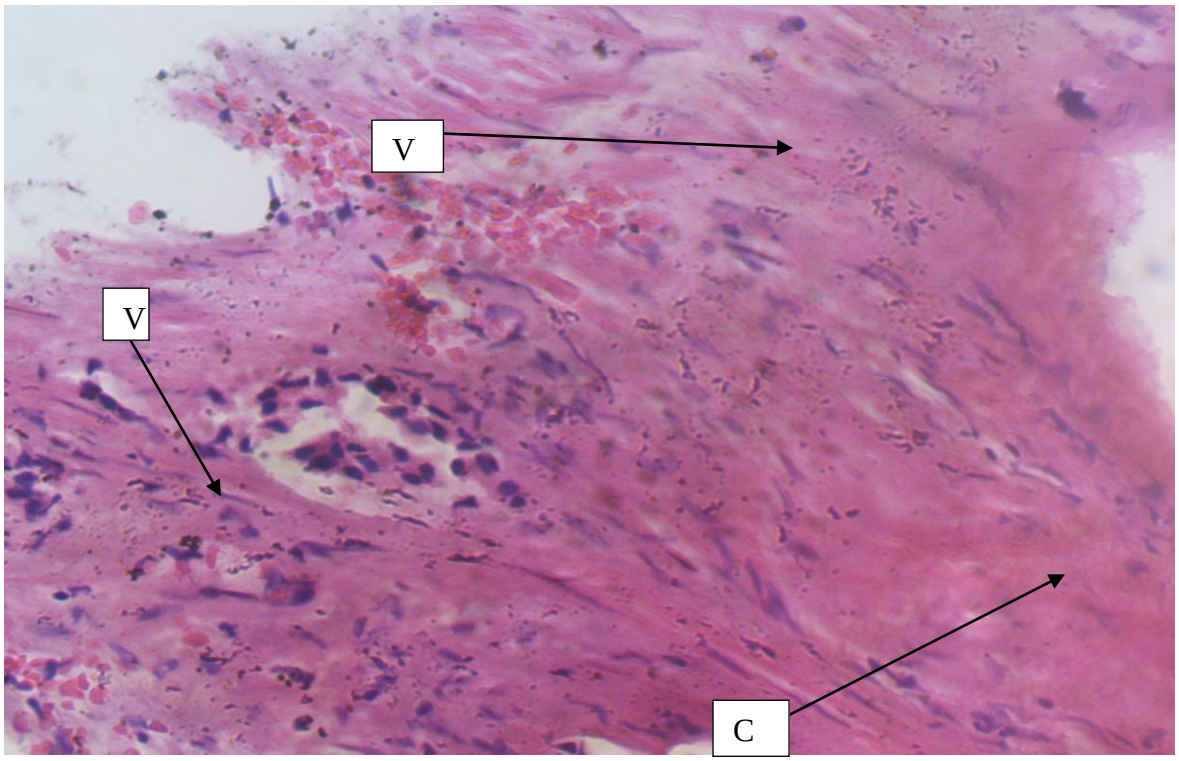

Plate VIII: CV-50
Keys: V= vacuolization CF= cytoplasmic fragmentation; TA= toxic agents

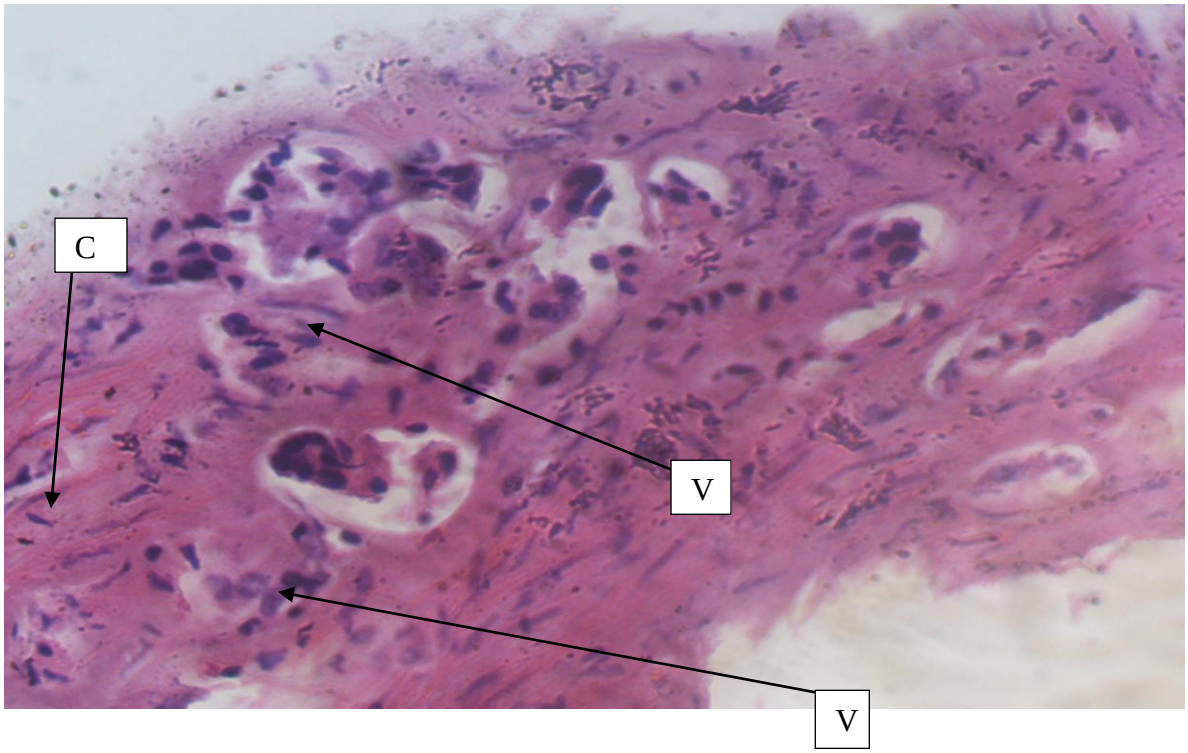

Plate IX: CV-250
Keys: V= vacuolization CF= cytoplasmic fragmentation; TA= toxic agents

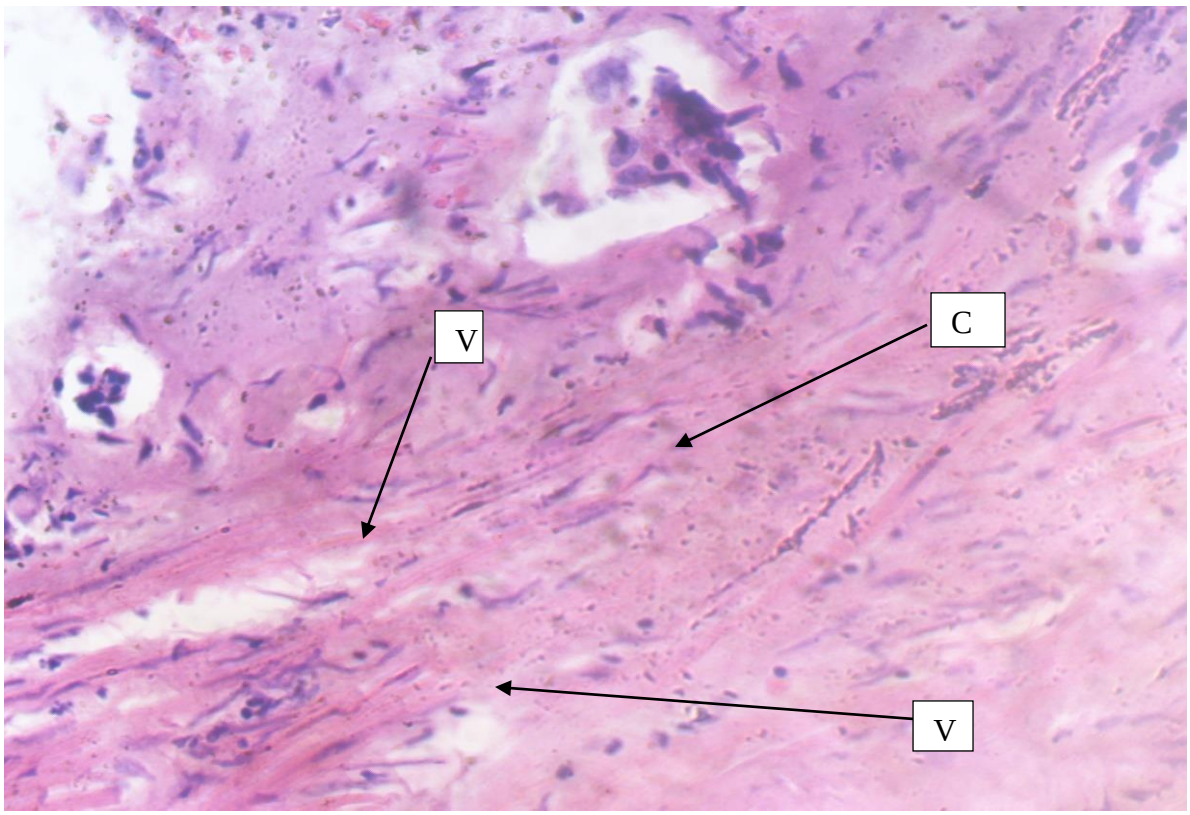

Plate X: VA-50
Keys: V= vacuolization CF= cytoplasmic fragmentation; TA= toxic agents

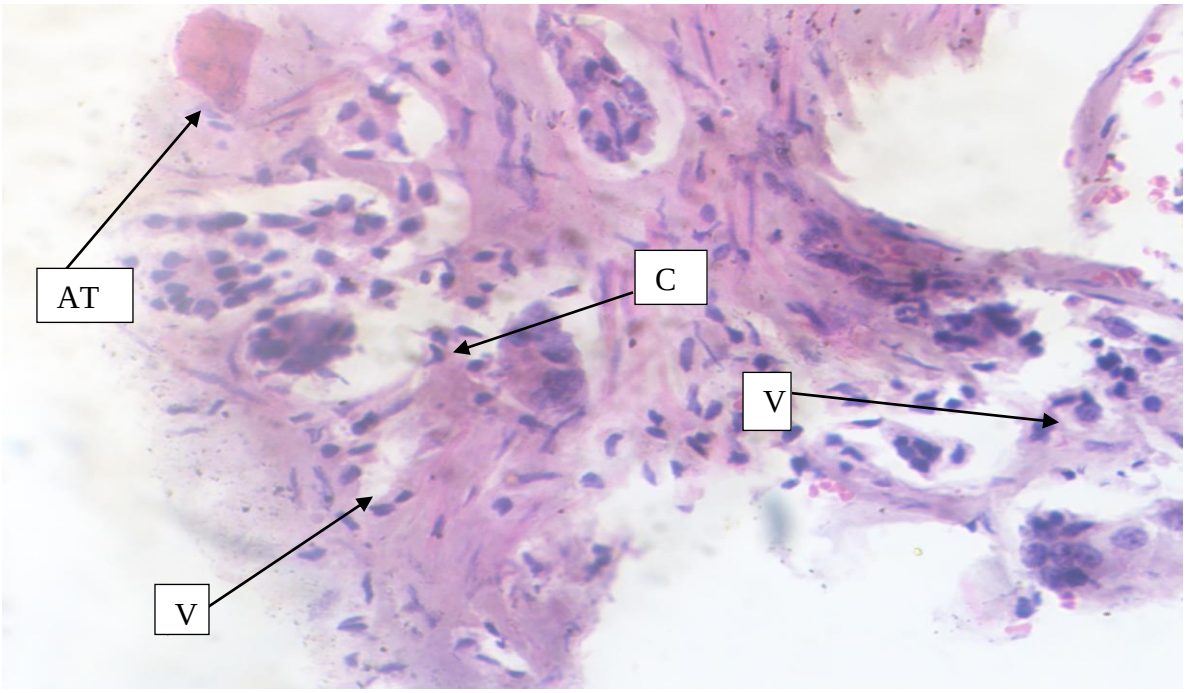

Plate XI: VA-250
Keys: V= vacuolization CF= cytoplasmic fragmentation; TA= toxic agents

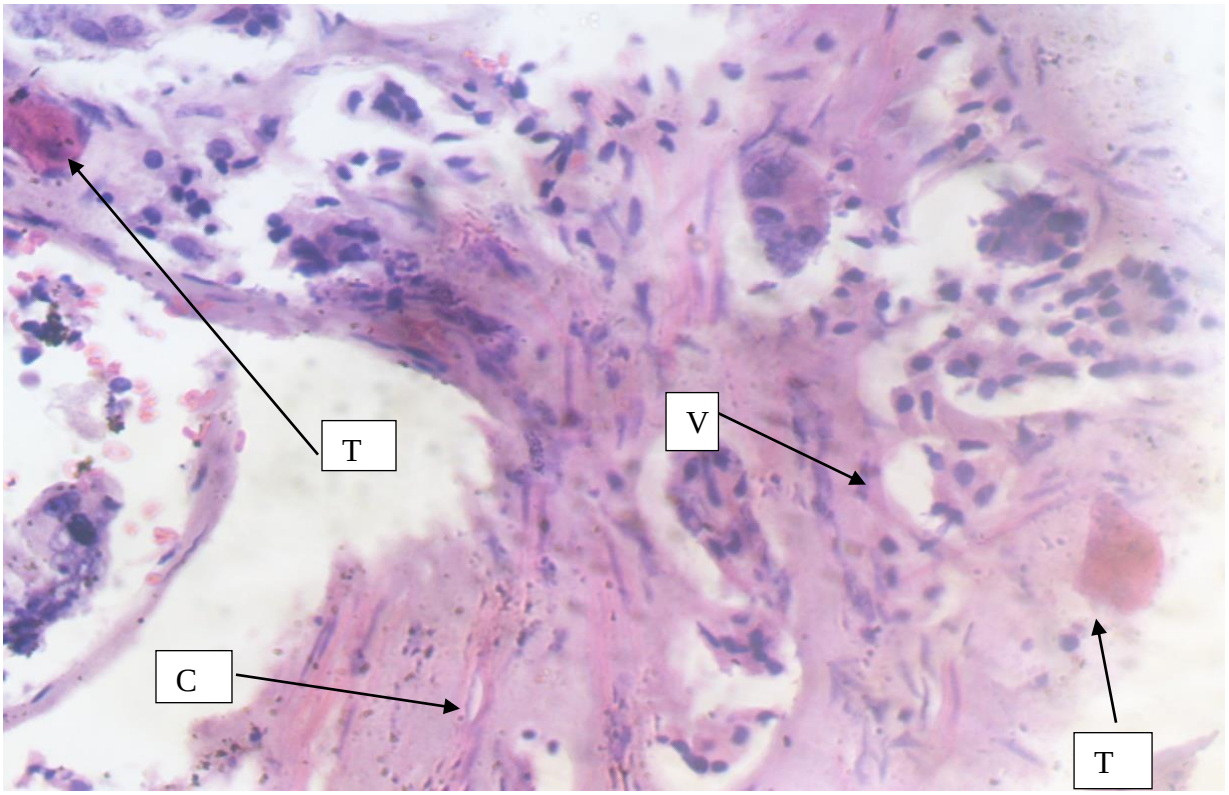

Plate XII: VA-350
Keys: V= vacuolization CF= cytoplasmic fragmentation; TA= toxic agents

4.2 Discussion

4.2.1 Phytochemical composition of the plant extracts

Findings from this study indicated that the ethanolic extracts of the three plant namely; *Vernonia amygdalina, Calotropis procera,* and *Amaranthus hybridus* contains an appreciable amount of bioactive compounds including flavonids, saponins, tannins, phenols, oxalate and cardiac glycosides. These compounds are synthesized via secondary metabolic activities by plants in order to defend themselves against infections and pathogens (Kumar *et al.,* 2017).These bioactive compounds have been established by previous researchers and experts to possess various biological properties. This activity includes anti-inflammatory, anticancer, antidiabetes, antibacterial, analgesic sand antioxidants potentials (Alara *et al*., 2017). Dhama (2018) added that the therapeutic potencies of these plants are the main reason for their herbal usage in the primary and local health care. These compounds also help the plants in developing protection against infections, insect and herbivores predation.

4.2.2 Quantitative phytochemical concentrations of the constituent

This study also shows the variations in the presence and concentrations in secondary metabolites (phytoconstituents) in the three plant extracts examined. These observation could be attributed to the facts that difference occur in the accumulation of these compounds in the plants .According to Altemime *et al*. (2017) difference occur in the solubility of phytochemicals. Lumpkin (2005) added that the differences observed in the plants extracts could be attributed to difference in plant species and other environmental variable including soil type, light intensity, temperature and geographical location.

4.2.3 Molluscicidal activity of individual plant extract

In the current investigation, it was generally observed that molluscicidal efficacy was dose and time dependent. This was observed when the plants was applied singly a because the higher the increase in extracts concentration and time of exposure, the higher the rate of snail mortality in the treated groups. These scenarios could be attributed to the increase in concentration of the bioactive compounds, which increased the toxicity potentials of the extract as well as bioaccumulation of these toxicant in the snail body as time of exposure increases. These findings is in harmony with the previous reports of Fayez *et al*. (2006) who time dependent molluscicidal potency of *Agave filitera*. Similarly Rawi *et al*. (2011) added that plant molluscicidal potency is time and concentration dependent. Amongst the three plant extracts tested, this study also observed *V. amygdalina* extract was more potent against the exposed Biomphalaria snails. These could be attributed to the fact that the preliminary screening showed that the plants possess more secondary metabolites than the other two plants screened. This could be that the phytochemicals caused synergistic effect against the snail species compared to other plant.

4.2.4 Molluscicidal activity of the combined extract

This study also observed that the three plants extracts when applied in combination caused appreciable varying molluscicidal efficacy. The mortality recorded could be attributed to the presence of phytochemicals that were identified during the experiments. This group of secondary metabolites may be responsible for the mortality of snails (Alinsub and Bagot, 2019). It has been well established that Tannin-bearing plants possesses molluscicidal potency and molluscs tends to avoid these plant in the ecosystem. (Molgard, 1986; Schaufelberger and Hostettmann, 1983). Saponins have been mentioned severally as active molluscicide (Bezeira *et al*., 2002; Musman *et al*.,

2014) with hemolytic properties. In addition presence of saponins caused deformation of a complex reaction with plasma as well as membrane cholesterols causing cell membrane damages (Moses *et al*., 2014). According to Guruswamy *et al*. (2017), cardiac glycosides decreases acetylcholinesterase (AChE) activities and impaired the hepatopancrease tissues of snails thereby causing fetal inhibition of the digestive enzymes and feeding rate. This is an indication that these bioactive metabolites while acting singly and/or synergistically could inhibit the activities of detoxification system of the snail, hence the observed mortality.

Despite the fact that the current study observed a significant difference in the mean mortality of snails treated with each plant ethanolic extracts within the 72 hrs exposure time, higher mortality was recorded in combinations of the plants extract. This contradict the previous reports of Akinpelu *et al*. (2012) who reported that single applications of plant especially in case of *Senna occidentalis* was more effective than combination of two or more plants against the tested snail species. However, the findings in this study is similar to that of Amrita and Singh (2001), who mentioned that the binary and poly combination of plants extract is more effective as molluscicidal agent. It was also observed that under the dilution effect, if the plant extract are synergistic then concentration of the extract required to obtain 90 % mortality would be reduced. In this study 150 mg/L of the combined extract caused 100% mortality while it required 300 mg/L and above concentration of the individual extract to obtain 100% mortality. A similar synergistic effect was with binary combination of *ferulic* and *Azadirachta indica* extract against Fasciola larvae in the snail *Lymnaea acuminata* which was 64.28 times more effective compared to single treatment with ferulic acid (Sunita *et al*., 2013); Rao and Singh (2014), added that the synergistic action of binary and tri-herbal combination were more potent as molluscicide than individual extract.

The synergistic action observed in this current study can hence be associated with the combined toxicity of the secondary metabolites of the individual extract. Thus the combination of the lethal actions of the active compounds of the tested plant in the current study possibly caused an increase in snail mortality.

4.2.5 Lower (LC_{10}), media and upper lethal concentration of the molluscicidal activities of mono, binary and tri- herbal combination of plant extract

The result of the probit regression analysis supported the fact that the combined extract of the current study is more potent and toxic against the *Biomphalaria pfeifferi* snails than individual extracts. The best plant combination was that of *V. amygdalina* and *C. procera* with the least of lethal concentration LC_{50} of 28.37 mg/L after 48 hrs.

4.2.6 Histopathogical analysis

The current study found out that these extract both in single, binary and tri-herbal combinations cause mild and severe cytoplasmic fragmentation and vacuolization in the snail excretory and digestive gland cells. These could be related to bioaccumulation of the toxicants in the extract and the bio-effect in the exposed snails. These observed activities of the current plant extracts when applied individually or in combinations is in harmony with the study of Hamed *et al*. (2007), who found out that the plants they tested expressed severe cytoplasmic fragmentation and vacuolization. They attributed their findings to the accumulation n of toxicants in the extract.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The tested plant in the current study contains phytochemical constituent with therapeutic properties. The ethanolic extracts of the plants when applied singly or in combination showed dose dependent molluscicidal activities. It was also recorded that snail mortality increased with increase in time of exposure. *V. amygdalina* was the most active among the three plant extract. When combined, the binary combination of *Vernonia amygdalina* and *C. procera* was the most active. The extracts were also found to cause cytoplasmic fragmentation and vacuolization in the histoachitecture of the snail tissues. The binary combination of the plants can hence be integrated into the control of *Biomphalaria pfeifferi* snail as an intermediate host of Schistosomiasis

5.2 Recommendations

The purification and characterization of bioactive compounds in the most active plant combination is hereby suggested. The effect of the extract in detoxifying snail enzymes can be established to identify the sustainability of the extract as plant molluscicide. It is recommended that of the most active plant combination should be tested in open area as ecofriendly molluscicide.

REFERENCES

Akinpelu, L., Rutz, L., Valls, M. Z. & Gabon, D. (2012). The global status of schistosomiasis and its control using different plant extracts. *Acta Tropica*, 77(1), 45-51.

Alara, O. R., Abdurahman, N. H. & Olalere, O. A. (2017c). Ethanolic Extraction of Flavonoids, Phenolics and Antioxidants from *Vernonia Amygdalina* leaf using two-level factorial design. *Journal of King Saud University Science,* 32(1)

Alinsub, C. J. & Bagot, M. (2019). In vitro molluscicidal activity of wormwood (*Artemisia dubia*) leaves against against *Oncomelania hupensis quadrasi*. *Annals of Tropical Research*, 41, 16-20.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G. & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6, 42-49.

Amrita, S. & Sing, D. K. (2001). Molluscicidal activity of the custard Apple (*Annona squamosa)* Alone and in combination with other plant derived molluscicides. *Journal of Herbs, Spices and Medicinal Plants,* 8(1), 23-29.

Appiah, K. (2018). Medicinal plants used in the Ejisu-juaben Municipality, Southern Ghana: An Ethno-botanical study. *Medicine*, 6(1), 1-27.

Association of Official Analytical Chemists, AOAC (1984) Official methods of analysis of the Association of Official Analytical Chemists. 14th ed. Washington, DC.

Bezeira, J. C. B., Silva, I. A., Ferreira, H. D., Ferri, P.H. & Santos, S. C. (2002). Molluscicidal activity against *Biomphalaria glabrata* of Brazilian cerrado medicinal plants. *Fitoterapia,* 73 (5), 428-430.

Biu, H. B. & Agbadu, E. T. (2009). Prevalence of *Schistosoma haematobium* infection in school aged children of Konduga Local Government Area, Northeastern Nigeria. *International Journal of Biomedicals and Health Sciences*, 5(4), 20-28.

Brandes, D. (2005). Calotropis procera on Fuerteventura working group for vegetation ecology, Institute of Plant Biology, Technical University Braunschweig, Germany.

Carter center (2010). Citing online source Schistosomiasis control program, Available from http:www.catercenter.org/health/schistosomiasis, access date 10/2/2019.

Center for Disease control and Prevention. (2018). Parasites-Schistosomiasis. Available from http://www.cdc.gov/parasites/Schistosomiasis/.Accessed February 25, 2018.

Checkley, A. M. (2010). Eosinophilia in returning travellers and migrants from the tropics: UK recommendations for investigation and initial management. *Journal of Infectious Diseases*, 60, 1–20.

Clerinx, J. & Van Gompel, A. (2011). Schistosomiasis in travellers and migrants. *Travel Medicine and Infectious Diseases,* 9, 6–24

Dhama, K., Karthik, K., Khandia, R., Munj al, A., Tiwari, R., Rana, R., Khurana, S.K., Alagawany, M. & Joshi, S. K. (2018). Medicinal and Therapeutic Potential of Herbs and Plant Metabolites / Extracts Countering Viral Pathogens - Current Knowledge and Future Prospects. *Current Drug Metabolite,* 19 (3), 236-263.

Elias, T. S. & Dykeman, P. A. (2015). Edible Wild Plants: A North American Field Guide to Over 200 Natural Foods. New York: Sterling, 72.

Essawy, A. E., Abdelmeguide, N. E., Radwan, M. A., Hamed, S. S. & Hegazy, A. E. (2009). Neuropathological effect of carbamate Molluscicides on the land snail *Eobania vermiculata*. *Cell Biology Toxicology*, 25, 275-290.

Fayez, A. B., Bakry, K., Salwa, A. & Hamdi, H. (2006). The molluscicidal activity of some plants extract against *Biomphalaria alexandrina* snails. *Egypt Journal of Experimental Biology*, 2, 99-106.

Feldmeier, H. & Poggensee, G. (1993). Diagnostic techniques in schistosomiasis control. A review. *Acta Tropica*, 52, 205–220.

Feldmeier, H., Doehring, E. & Daffalla, A. A. (1982). Simultaneous use of a sensitive filtration technique and reagent strips in urinary schistosomiasis. *Architectural Pathology in Laboratory Medicine*, 129 (4), 502–506.

Feldmeier, H., Doehring, E. & Daffalla, A. A. (1982). Simultaneous use of a sensitive filtration technique and reagent strips in urinary schistosomiasis. *Architectural Pathology in Laboratory Medicine*, 129 (4), 502–506.

Francis, J. K. (2003). Calotropis procera U.S Department of Agriculture, Forest services. International Institute of Tropical Forestry.

Ganapathi, L. & Somers, M. A. (2015). Children with gross hematuria. *New England Journal of Medicine,* 37, 3-11.

Gray, D. J., Ross, A. G., Li, Y.S. & McManus, D. P. (2011). Diagnosis and management of schistosomiasis. *BMJ,* 342, 26-51.

Grimes, C., Croll, D., Harrison, W. E., Utzinger, J., Freeman, M. C. & Templeton, M. R. (2014). The relationship between water, sanitation and schistosomiasis: a systematic review and meta-analysis. *PLoS Neglected Tropical Diseases*, 8, 32-37

Guruswamy, P., Subhash, J. B. & Manikam, R. (2017). Development and Evaluation of Poly Herbal Molluscicidal Extracts for Control of Apple Snail (*Pomacea maculata*). *Agriculture,* 7, 22-29.

Hamed, S. S., Essawy, A. E., Abdelmeguide, N. E., Radwan, M. A. & Hegazy, A.E. (2007). Histological and ultrastructural changes induced by carbamate molluscicide Eobania vermiculata. *Journal of Biological Science,* 7, 1017-1037.

Harborne, J. B. (1973). Phytochemical methods. A guide to modern technology of Plant Analysis. Chapman and Hall. New York, 185.

Harborne, J. B. (1976). Phytochemical Methods. Chapman and Hall Ltd., London, Inc 124.

Hongjun, L. L., Jianrong, D.A., Ming, X. U. & Weiei, R. (2006). Enzyme-histochemical observation on influence of suspension concentrate of niclosamide in Oncomelania hupensis snails. *China Journal of Schistosomiasis Control*, 18(6), 427-431.

IARC (2009). Working Group on the Evaluation of Carcinogenic Risks to Humans. Biological agents. Volume 100 B. A review of human carcinogens. IARC, 100, 1–441.

Ijeh, I. I. & Ejike, C. E. (2011). "Current perspectives on the medicinal potential of Vernonia amygdalina. *Journal of Medicinal Plant Research*, 5(7), 1051–1061.

Ismail, H. (2014). Prevalence, risk factors, and clinical manifestations of schistosomiasis among school children in the White Nile River basin, Sudan. *Parasites & Vectors*, 7, 478.

Jolcelyn, D. (2004) Schistomiasis. *Medical Ecology,10,115-116.*

Kimeu, B. M., Njagi, E. W., Yole, S. D. & Ngugi, M. P. (2015). Molluscicidal effects of aqueous extracts of some selected medicinal plants in Makueni Country, Kenya. *Pharmaceutica Analytica Acta* ,6,11-17.

Kittel, B., Ruehl-Fehlert, C., Morawietz, G., & Wadsworth, P. F. (2004). Revised guides for organ sampling and trimming in rats and mice – Part 2. *Experimental Toxicology and Pathology*, 55, 433–449

Kjetland, E. F. (2014). Classification of the lesions observed in female genital schistosomiasis. *International Journal of Gynecology Obsterics*, 127, 227–228.

Kloos, H. C., De Souza, A., Gazzinell, B. S., Soares, F. & De Costa, T. (2001). The distribution of *Biomphalaria* Spp. In different habits in relation to physical, biological water contact and cognitive factors in rural areas in minas Gerian, Brazil. Oswaldo Cruz, 96, 57-66

Kristoff, G., Guerrero, N. R. U. & Cochon, A. C. (2010). Inhibition of cholinesterases and carboxylesterases of two Inveterbrate species, *Biomphalaria glabrata* and *Lumbriculus variegates* by the carbamate pesticide carbarly. *Aquatic Toxicology*, 96, 115-123.

Kumar, K. H., Hullatti, K. K.,Sharanappa, P. & Sharma, P. (2017). Computative antimicrobial activity and tlcbioautographic analysis of root and aerial parts of *Andrographis serpyllifolia*. *International Journal of Pharmaceutical Science*, 2, 52–54.

Kushwaha, V. B. & Singh, A. (2009). Toxicological evaluation of *Calotropis procera* against freshwater Snails. *Journal of Herbs, Spices and Medicinal plants*, 17, 35-46

Li, X., Deng, F., Shan, X., Pan, J., Yu., P .& Mao, Z. (2012) . Effect of the Molluscicidal agent GA-C13: A natural occurring gingkolic acid on snail mitochondria. *Pesticide Biochemistry and. Physiology*, 103, 1115-1120.

Litchfield, J. T. J. & Wilcoxon, F. (1949). A simplified method of evaluating dose effect experiments. *Journal of pharmacology and experimental therapeutics*, 96 (2), 99-113.

Lumpkin, H. M. (2005). A Comparison of Lycopene and Other Phytochemicals in Tomatoes Grown under Conventional and Organic Management Systems. Technical bulletin (Asian Vegetable Research and Development Center). *Asian Vegetable Research and Development Center*, 5, 623-628.

Martin, L. N. (2019). Schistosomiasis: Lifecycle, Diagnosis and Control. *Current Therapeutic Research*, 1, 42-49.

Masola, B., Chibi, Y. S., Naik, E. & Kandoare, M. F. (2003). Activities of glutamate dehydrogenase and alanine amino transferees in fresh water snail *Thelisoma* duryin and *Lymnaea natalensis* exposed to copper. *Biomarkers*, 8, 33-42.

Mccullough, F. S. (1986). Snail control in relation to a strategy for reduction of morbidity due to schistosomiasis control, *Bulletin of World Health Organisation*, 58, 681-689.

McManus, D. P., Dunne, D.W. & Sacko, M. (2018). Schistosomiasis. *Nature Reviews Disease Primers*, Retrieved from https://doi.org/10.1038/s41572-018-0013-8, access date 11/6/2019.

Mo, A. X. & Colley, D. G. (2016). Workshop report: schistosomiasis vaccine clinical development and product characteristics. *Vaccine*, 34, 995–1001.

Molgaard, P., Chihaka, A., Furu, P., Ndekha, A., & Nyazema N. (1999). Community based schistosomiasis control in Zimbabwe, using molluscicidal berries of *Phytoplacca dedecanda* AETFAT conference in Harare, Zimbabwe.

Molgard, P. (1986). Food plant preferences by slugs and snails; a simple method to evaluate the relative palability of the food plants. *Biochemical Systematics and Ecology*, 14 (1), 113-121.

Morawietz, G., Ruehl-Fehlert, C., Kittel, B., Bube, A., Keane, K., Halm, S., Heuser, A. & Hellman, J. (2004). Revised guides for organ sampling and trimming in rats and mice – Part 3.*Experimental Toxicology and Pathology*, 55, 433–449.

Moses, T., Papadopoulou, K. K. & Osbourn, A. (2014). Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. *Critical Reviews in Biochemistry and Molecular Biology*, 49 (6), 439-462.

Musman, M., Karina, S. & Rizki, F. (2014). Saponins extract from *Barringtonia racemosa* as molluscicide to brackishwater pond snails (*Cerithidea cingulata*). *International Journal of Applied Research and Technology*, 3 (6), 92-97.

Nacsa, K., Elekes, K. & Serfozo, Z. (2015). Ultrastructural localization of NADPH diaphorase and nitric oxide synthase in the neuropils of the snail CNS. *Micron*, 75, 58–66.

Oguche, O. & Olofintoye, L. K. (2018). Molluscicidal effects of *Vernonia amygdalina* and *Momordica charantia* linn on. Bulinus (phy) globossus. *International Journal of Multidiscipline Science and Engineering*, 9, 23-27.

Ojewole, J. A. O. (2004). Indigenous plant and Schistosomiasis control in South Africa: Molluscicidal activity of some Zulu medicinal plants. *Boletin Latinoamericano Pathology,* 15, 374-379.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Simons, A. (2009). *Calotropis procera* Agroforestry Database: A tree Reference and Selection Guide. World Agroforestry Center, Kenya

Rao, I. G. & Singh, D. K. (2001). Combinations of *Azadirachta indica* and *Cedrus deodara* oil with piperonyl butoxide, MGK-264 and *Embelia ribes* against *Lymnaea acuminata*. *Chemosphere*, 44, 1691–1695.

Rashed, A. A. (2002). Biological studies on the intermediate hosts of schistosomiasis with special emphasis or using larval Echinostomes as biocontrol agents against larval schistosomes and snails. *Journal of the Egyptian Society of Parasitology*, 32, 757-784.

Rawi, S. M., Al-Hazmi, M. & AL-Nassr, F. (2011). Comparative study of the molluscicidal activity of some plant extracts on the snail vector of *Schistosoma mansoni*, *Biomphalaria alexandrina*. *International Journal of Zoological Research*, 7, 169-189.

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology,* 17, 208-212.

Salvana, E. M. & King, C. H. (2008). Schistosomiasis in Traveler and Immigrants. *Current Infectious Disease*, 10, 42-49.

Sayed-Ahmed, M., El-Tantawy, M., Mansour, F. A. & Heba M. F. (2017). Molluscicidal potency of four plant extracts on three pulmonate snails (Gastropoda) in Egypt. *International Journal of Biological Science*, 7, 115-130.

Schaufelberger, D. & Hostettmann, K. (1983).The molluscidal activity of tannin containing plants. *Planta medica*, 48(6), 105-107. Doi: 10.1055/s-2007-969899.

Singh, A. & Agarwal, R. A. (1993). Effect of cypermethrin on lactate and succinic dehydrogenase and cytochrome oxidase of snail and fish. *Bulletin of Environmental* Contamination *and Toxicology*, 51(3), 445–452.

Singh, V. & Singh, D. K. (2009). The effect of abiotic factors on the toxicity of cypermethrin against the snail Lymnaea acuminata in the control of fascioliasis. *Journal of Helminthology*, 83(1), 39–45.

Sofowora, A. (1993). Medicinal Plants in traditional medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria. 289-300.

Sunita, K., Kumar, P., Singh, V. K. & Singh D. K. (2013). In vitro phytotherapy of vector snails by binary combination of larvicidal active components in effective control of fascioliasis. *Revista Instituto de Medicina Tropica*, 55, 303-308.

Tantawy, A. A. (2006). Molluscicidal effect of fenitrothion and anifolos on Lymnaea natalensis and Biomphalaria alexandrina snail and on the free level stages of Schistosoma mansoni. *Egyptian Society of Parasitology*, 36, 629-64.

Teixeira, T., Rosa, J. S., Ranna, N., Batista, J. & Rodrigues, A. (2012). Assessment of molluscicidal activity of essential oils from five Azorean plant Radix peregra (Muller, 1774). *Chemosphere*, 87, 1-9.

Trease, G. E. & Evans, W. E. (1989). Pharmacognosy, 11th Edn., Macmillan Publishers, London, 88.

Utzinger, J., Becker, S. L., van Lieshout, L., van Dam, G. J. & Knopp, S. (2015). New diagnostic tools in schistosomiasis. This comprehensive review of past, current and potential future diagnostic tools for schistosomiasis emphasize target product profiles that are required for different stages of control and elimination efforts. *Journal of Clinical Microbiology and Infection,* 21, 529–542.

Waknine-Grinberg, J. H. & Daniel, G. A. O. (2010). *Schistosoma mansoni* infection reduces incidence of muricine cerebral malaria. *Malaria Journal*, 9, 1-5.

Wang, L., Xing, Y., Qu, G., Dai, J. & Liang, Y. (2009). China's new strategy to block Schistosoma japonicum transmission: experiences and impact beyond schistosomiasis. *Tropical Medicine and International Health*, 14, 1475–1483

Wang, W. S., Qin, Z. Q., Zhu, D.,Wei, Y. F., Li ,S. Z. & Duan, L. P. (2016). Synthesis, bioactivity evaluation, and toxicity assessment of novel salicylanilide ester derivatives as cercaricides against *Schistosoma japonicum* and molluscicides against Oncomelania hupensis. *Antimicrobial Agents and Chemotherapy,* 60(1), 323–331.

Wang, W., Li, Y., Xing, Y., Qu ,G., Dai, J. & Liang, Y. (2012) .Immunodiagnostic efficacy of detection of *Schistosoma japonicum* human infections in China: A meta-analysis. *Asian Pacific Journal of Tropical Medicine* 5, 15–23.

Weerakoon, K. G., Robert, G. N., Cai, P. & McManus, D. P. (2015). Advances in the diagnosis of human schistosomiasis: Reviews. *Journal of Clinical Microbiology*, 28, 939–967.

Wilson, S. (2010). Health implications of chronic hepatosplenomegaly in Kenyan school- aged children chronically exposed to malarial infections and *Schistosoma mansoni. Transactions of Royal Society of Tropical Medical and Hygiene*, 104, 110–116.

Wilson, S., Vennervald, B. J. & Dunne, D. W. (2011). Chronic hepatosplenomegaly in African school children: a common but neglected morbidity associated with schistosomiasis and malaria. *PLOS Neglected Tropical Diseases*, 5, 11-49.

World Health organization (1985). The control of schistosomiasis. Technical Report 728, WHO, Geneva, Switzerland. World Health Organization (2018). Schistosomiasis. Fact sheet.

World Health Organization (2012). Meeting Report WHO Shanghai, China. Expert consultation to accelerate elimination of Asian schistosomiasis.

World Health Organization (2017). Preventive Chemotherapy data bank WHO Geneva.

World Health Organization (2018). Schistosomiasis: Fact Sheet. [(accessed on 20 February 2019) and available online:http://www.who.int/mediacentre/factsheets/fs115/en/

World Health Organization, WHO (2007). Expert Committee on the Control of Schistosomiasis Prevention and control of schistosomiasis and soil-transmitted helminthiasis: WHO Technical Report Series, 912.

World Health Organization, WHO (2017). Guidelines for laboratory and field testing of molluscicides for the control of schistosomiasis. Geneva, 92.

World Health Organization, WHO (2021). Schistosomiasis: Fact Sheet. (accessed on 9 January 2022) www.who.int/mediacentre/factsheets/fs132/en

Yadav, R. P., Singh, D., Singh, S. K. & Singh, A. (2006). Effects of extracts of the bark of the stem of *Croton tiglium* on the metabolism of the freshwater gastropod *Lymnaea acuminata. American Malacological Bulletin*, 21(1), 87–92.

Zhou, X. N. M., Yang, J., Yank, K., Wang, X. H. & Hong, Q. B. (2008). Potential Impact of Climate Change on Schistosomiasis in China. *American Journal of Tropical Medicine and Hygiene*, 78, 188-194.

APPENDIX A: Preparation of stock solution and test solutions of Different Concentrations

Extract	Weight of extract(g)	Amount of water added in ml	Stock solution in mg/l	Amount of stock solution added in ml	Amount of water added in ml	Final concentration in mg/l
Individual	1g	1000	1000	10	90	100mg/l
	1g	1000	1000	20	80	200mg/l
	1g	1000	1000	30	70	300mg/l
	1g	1000	1000	40	60	400mg/l
Binary extracts	1g	1000	1000l	2.5+2.5=5	95	50mg/l
	1g	1000	1000	7.5+7.5=15	85	150mg/l
	1g	1000	1000	12.5+12.5=25	75	250mg/l
	1g	1000	1000	17.5+17.5=35	65	350mg/l
	1g	1000	1000	1.6+1.6+1.6=5	95	50mg/l
Tri-herbal extract	1g	1000	1000	5+5+5=15	85	150mg/l
	1g	1000	1000	8.3+8.3+8.3=25	75	250
	1g	1000	1000	11.6+11.6+11.6=35	65	350