EVALUATION OF MOLLUSCICIDAL ACTIVITIES OF CRUDE AND ALKALOIDAL FRACTION OF ANOGEISSUS LEIOCARPUS (AFRICAN BITCH) AND PSEUDOCEDRELA KOTSCHYI (DRY ZONE CEDAR) ON VECTOR OF SCHISTOSOMIASIS

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MTech/SLS/ 2017/7198

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ABSTRACT

Over the years, the use of plant-based molluscicides has received substantial consideration in the satisfaction for cost-effective alternatives to chemotherapy and synthetic molluscicides in schistosomiasis control. This study was aimed at evaluating the molluscicidal potentials of crude and Alkaloidal fractions of *Anogeissus leiocarpus* and *Pseudocedrela kostchyi* against vector of schistosomiasis. Both aqueous and methanol crude extracts of the plants were tested for the presence of bioactive metabolites. Ten snails each was exposed to different concentrations (10 mg/L, 20 mg/L, 40 mg/L, 80 mg/L, 160 mg/L, 200 mg/L and 300 mg/L) of aqueous, methanol and alkaloid extracts of *Anogeissus leiocarpus* and *Pseudocedrela kostchyi* for the interval of 40 minutes, 24 hours and 72 hours respectively. Fifty other snails were also exposed to 200 mL of distilled water for 24 hours as control. The results indicated the presence of phytochemicals such as flavonoid, Tannis, Saponins, Alkaloids and terpenoids in both aqueous and methanol extract of both plants. Steroid was only present in methanol extract. Cardia glycoside was absent in aqueous extract of the extracts. The results showed dose dependent activities with highest dose having a significant ($P<0.05$) highest snail mortality. The two plants extracts showed appreciable molluscicidal efficacies against the two exposed snail species with LC_{50} of 157.29, 88.56, and 240.03mg/L for aqueous, methanol and Alkaloidal fraction of *A. leiocarpus* against *B. pfeifferi* and LC_{50} of 128.73, 78.07 and 344.02mg/L for *P. koschyi* respectively. There was a significant decrease in the level of Alamine transaminase (ALT) and Aspartate triaminase (AST) in the tissue of the exposed snail compared to the control. Based on the findings of this study, there is need to examine the Histoachitecture of the exposed snail in other to ascertain the observed mechanism of action found in the alteration of the tissue enzymes.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Schistosomiasis (also known as Bilharzia) is a debilitating disease caused by species of parasitic worms or helminths of the genus *Schistosoma*, which are also known as blood flukes (Kimeu *et al.,* 2015). Globally, Schistosomiasis is the second most prevalent parasitic disease in Africa ranked only after malaria regarding the number of infected people and those at risk (Grimes *et al.,* 2014). Bilharzia is of great public health and social economic importance in the developing world. WHO, (2015) reports estimate that 500-600 million people in 74 tropical and sub-tropical countries are at risk of Schistosomiasis. Over 200 million people in these countries are infected, 85% of whom live in sub-Saharan Africa where *S. haematobium, S. intercalatum S.* and *S. mansoni* are endemic. Nigeria is said to be highly prevalence with an estimated 101 million at risk and 26 million people infected (WHO, 2015).

Schistosomiasis is caused by digenetic trematodes belonging to phylum Platyhelminthes, super family Schistosomatoida, and genus Schistosoma. It is usually attributed to three species, subdivided into intestinal *Schistosoma mansoni* and *Schistosoma japonicum* or urinary *Schistosoma haematobium* types, according to the site preferred by the adult worms. In Nigeria, the two main species of Schistosomiasis reported are *Schistosoma mansoni* and S. *haematobium* whose intermediate hosts are fresh water snails, *Biomphalaria pffeiferi*, and *Bulinus trancatus* respectively. Two host have been attributed to the life cycle of schistosome, namely; a definitive host (man) where parasite undergoes sexual reproduction, with a single intermediate snail host, where there are number of asexual reproduction stage risk (Grimes *et al.,* 2014). In

humans, these blood flukes reside in the mesenteric and vesicle venules. They have a life span of many years and produce large numbers of eggs daily, which must traverse the gut and bladder tissues on their way to the lumens of the excretory organs. Many of the eggs remain in the host tissues, inducing immunologically mediated granulomatous inflammation and fibrosis. Heavy worm burdens may produce hepatosplenic disease in *Schistosoma mansoni* and *S. japonica* and urinary tract disease in *S. haematobia*. Since both the schistosomes and the eggs utilize host metabolites, and because the host responses to the parasite are affected by its nutritional status, malnutrition strongly affects both the parasite and the complex host-parasite relationship (Victor, 2014).

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 employing praziquantel**.** Selective molluscicide treatment in snail-infested bodies of water at main human contact points is the preferred way to approach controlling snail populations (Mwonga *et al.,* 2015)**.** Three drugs have been used for schistosomiasis treatment. These are Praziquantel (effective in the treatment of all forms of schistosomiasis, with hardly any side effects), Oxamniquine (used to treat intestinal schistosomiasis), and Metrifonate (effective for the treatment of urinary schistosomiasis (CDC,2011). Praziquantel has been found to be more effective in treating *S. haematobium* infections compared to metrifonate and more effective in treating *S. mansoni* when compared with oxamniquine because it is effective when treating advanced hepatosplenic schistosomiasis, with few side effects. Praziquantel is currently the drug of choice for treatment of any kind of schistosomiasis. The main limitation is the cost which restricts its use in many developing countries. Unfortunately, the long-term worldwide application of Praziquantel coupled with the recent discovery of Praziquantel-tolerant schistosomes has generated concern over the development of

drugs-resistant *Schistosoma* strains. A large number of plant products, which possess molluscicidal activity, have been identified and used for treatment of schistosomes (Mwonga *et al.,* 2015).

Members of the kingdom Plantae are used medicinally in different countries and are sources of many potent and powerful drugs. Among several factors contributing towards the potential use of phyto-medicine are safety, lack of adverse reactions and minimal side effects which have been mostly found to particularly influence the use of such medicines in developed countries (Renckens and Dorlo, 2013) as most of the developing countries have adopted traditional medical practice as integral part of their culture. Similarly, herbal preparations represent one of the most important traditional medicine therapies and are still the backbone of majority of the world populations, mainly in the developing countries, for primary health care (Mukhtar *et al.,* 2017).

In Nigeria, traditional herbal medicine, being a vital element in the cultural inheritance, remains the main source for a huge majority of people in treating various diseases and ailments. *Anogeissus leiocarpus* is a tree widely distributed in northern Nigeria. The bark and seed of the tree is used for the treatment and prevention of worm infestation in equine species (Ahmad, 2014). *Pseudocedrela kotschyi* commonly called dry-zone cedar belongs to the family Meliaceae which grows in the savannah zone in tropical Africa from Nigeria. The bark decoction or macerations of the plant are applied externally to ulcers, sores, rheumatism, leprosy and itches. Internally they are used to treat fever, stomach-ache, diarrhea and dysentery and it is reported to possess anthelmintic effects on *Ascaris suum.* It is against this background that this study was designed to elucidate the bioactivities of the extract of *Anogeissus leiocarpus* and *Psedocedrela kostchyi* against vector and parasite of Schistosomiasis.

1.2 Statement of the Research Problem

In order to achieve effective Schistosomiasis control, there is need for intervention programs that are effective, reliable and sustainable. In addition, the control method(s) should have minimal environmental impact. Currently, control of Schistosomiasis relies heavily on diagnosis and mass chemotherapy principally using the drug, praziquantel (PZQ) (Doenhoff, *et al*., 2009). However, the prospect of relying on a single drug for a disease affecting 200 million people is an alarming situation (CDC, 2011). There is a lot of concern due to loss of Praziquantel efficacy and its inaccessibility and high cost requiring hard currency which set back helminthes control efforts. In addition, use of molluscicides in the control of Intermediate host snails is important in integrated Schistosomiasis control. Despite the great deal of attention directed to the control of aquatic molluscs using saponins- containing materials, no investigation is known to previously have been made to use these compounds to control *Biomphalaria sp*. This is so despite the significant problems posed by the snails in Schistosomiasis transmission. Synthetic molluscicides have shown a number of drawbacks including high cost, altering of the structure of the environment by acting as biocides and destroying flora and fauna of the ecosystem (Mwonga *et al.,* 2015).

The near exclusive use of praziquantel (PZQ) for treatment of human Schistosomiasis has raised concerns about the possible emergence of drug-resistant schistosomes. Further, some molluscicides like copper sulphate and sodium pentachlorophenate are general biocides and have been shown' to destroy most aquatic organisms. They have high mammalian toxicity, and the durability of copper may result in a build-up of copper salts in the water after prolonged treatment. Also, Sodium pentachlorophenate is highly irritating (Grimes *et al.,* 2014) and inhalation of the powder produces acute symptoms. Sprayers continually using this substance suffer from lacrimation and

chronic coughs. Therefore, this research is aimed at evaluating native plants for potential active substances against vector and the parasite.

1.3 Justification of the Study

Schistosomiasis is a debilitating disease with 500-600 million people in 74 tropical and subtropical countries being at risk. Development of antischistosomal resistance in helminthes reported in a number of countries (WHO, 2015) gives a clear indication that control programs based exclusively on their use are not sustainable. The molluscicidal and antischistosomal agents used conventionally to control this disease have many setbacks besides being very expensive. Many plants have been investigated for molluscicidal and antischistosomal activities and have been found to exhibit some activity. Herbal medicines are cheap, more readily available to people and are less toxic, although a lot still needs to be done to confirm this (Mukhtar *et al.*, 2017).

Continuous research for new drugs with high activity and reduced adverse effects is very important, especially considering that in Nigeria parasitic diseases still constitute a serious public health problem, in spite of the aggressive control over the years. Screening the biodiversity of the Nigerian plants can reveal new phyto-therapeutic drugs. The biodiversity existing in the Nigeria flora is a potential source of many new bioactive molecules.

1.4 Aim of the Study

This study is aimed at evaluating the molluscicidal potentials of crude and Alkaloidal fractions of *Anogeissus leiocarpus* and *Pseudocedrela kostchyi* against *Biomphalaria pfeifferi* and *Bulinus globossus* as the vectors of schistosomiasis.

1.5 Objectives of the Study

The objectives of the study are to determine the:

i. phytochemical constituents of the crude methanol extract of crude and Alkaloidal fraction of *Anogeissus leiocarpus* and *Psedocedrela kostchyi.*

ii. molluscicidal activities of the crude extract and Alkaloidal fraction of *Anogeissus leiocarpus* and *Psedocedrela kostchyi.*

iii. medial (LC_{50}) and upper (LC_{90}) lethal concentrations of the extraction host of Schistosomiasis

iv. effect of the extracts on biochemical variables host of Schistosomiasis

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diseases Burden of Schistosomiasis

Schistosomiasis also known as snail fever and bilharzia is a disease caused by parasitic flatworms called schistosomes Global Borden of Disease (GBD, 2015). The urinary tract or the intestines may be infected disease that is basically caused by a parasitic worm. It is an acute and chronic parasitic disease caused by blood flukes (trematode worms) of the genus Schistosoma. Estimates show that at least 220.8 million people required preventive treatment in 2017. Preventive treatment, which should be repeated over a number of years, will reduce and prevent morbidity. Schistosomiasis transmission has been reported from 78 countries. However, preventive chemotherapy for Schistosomiasis, where people and communities are targeted for large-scale treatment, is only required in 52 endemic countries with moderate-to-high transmission Global Health Estimate (GHE, 2016).

The worm, or fluke, has several different species. It affects the intestines and the urinary track preferentially, but because it lives in the blood vessels, it can harm other systems in the body too. Bilharzia mostly known as Schistosomiasis described as both an acute and chronic disease, the symptoms appear as the body reacts to the parasite's presence, but complications can persist long-term. The disease can affect different parts of the body, including the lungs, the nervous system, and the brain. The area of damage will depend on the species of parasite. Schistosomiasis is not usually fatal immediately, but it is a chronic illness that can seriously damage the internal organs. In children, it can lead to reduced growth and problems with cognitive

development. Some types of Schistosomiasis can affect birds and mammals, such as water buffalo World Health Organization (WHO, 2015).

2.2 **Transmission of Schistosomiasis**

According to (WHO, 2015) the infection starts when a person comes into direct contact with fresh water where certain types of water snail carry the worm. The parasites enter the body when a person is swimming, washing, or paddling in contaminated water, they can also become infected by drinking the water or eating food that a person has washed in untreated water and the infective form of the fluke is known as cercariae (CDC, 2019). The cercariae emerge from the snails, pass through a person's skin when they are in the water, and develop into adult worms that live in the individual's blood. Depending on the type of worm, bilharzia can affect: the intestines, the urinary system, increasing the risk of bladder cancer, the liver, the spleen, the lungs, the spinal cord and the brain.

The infection cycle of the parasite begins when the worm's eggs enter fresh water through the feces and urine of humans who already have the infection. The eggs hatch in the water, releasing tiny larvae, and the larvae reproduce inside the water snails. After they have infected water snails, the worm's cercariae, are released (CDC, 2019). The cercariae can survive for up to 48 hours. The cercariae can penetrate human skin and enter the bloodstream. There, they travel through the blood vessels of the lungs and liver, and then to the veins around the bowel and bladder. After some weeks, the worms are mature. They mate and start producing eggs. These eggs pass through the walls of the bladder, the intestine, or both. Eventually, they leave the body through

urine or feces. At this point, the cycle starts again. A person with schistosomiasis cannot pass it on to another person. Humans only become infected through contaminated water where the snails are living.

More than 200 million people have bilharzia worldwide, according to (CDC, 2019), the places where the parasite mostly occurs include: Africa, including Egypt and the Nile Valley, South America and parts of the Caribbean, Southeast Asia and Yemen, in the Middle East.

Bilharzia can affect people of any age in an affected area, but those who are most at risk include: Children, people who swim, work, or have other contacts with freshwater rivers, canals, lakes, and streams (WHO, 2013).

2.3 Life Cycle of Schistosomiasis

Schistosoma eggs are excreted from the human host into a fresh water environment through urine or feces. Once an egg comes in contact with fresh water, it hatches and releases a miracidium, a free-living and ciliated form, which remains infective for 6–12 hour. The miracidium swims by ciliary movement toward the snail intermediate host and penetrates its soft tissue. The *Schistosoma* species are transmitted by different fresh water snails that serve as their intermediate hosts: Biomphalaria, Bulinus, and Oncomelania for *S. mansoni, S. haematobium*, and *S. japonicum*, respectively (Gryseels and Strickland, 2013).

The miracidium that penetrated the snail loses its cilia and develops into a mother sporocyst, which multiplies asexually to produce daughter sporocysts. These migrate to and develop in the hepatic and gonadal tissue of the snail. Within 2–4 weeks, these daughter sporocysts metamorphose into cercariae. Under the stimulation of light,

hundreds of free-swimming, fork-tailed cercariae leave the snail intermediate host (Gryseels, 2012). They swim through the water until they come into contact with human skin or the skin of other mammalian hosts, in the case of *S. japonicum. S. japonicum* can infect more than 40 mammals that can serve as reservoir hosts. The cercariae penetrate the skin by mechanical activity and via proteolytic enzymes. Upon skin penetration, the cercariae lose their tail and become schistosomules which pass through the epidermis and dermis before exiting via the blood or lymphatic vessels. There seems to be a difference in the way different schistosome species migrate through the skin (Gryseels, 2012).

Within 2 hours of exposure, more than half of the *S. japonicum* schistosomula are found in the dermis, and as early as this, some are already in the dermal blood vessels. The pace of movement of *S. mansoni* and *S. haematobium* through the human skin layers (Figure 2.1) is slower than that of S. japonicum, it takes about 48 hours for the majority of *S. mansoni* and *S. haematobium* Schistosomula to reach the dermis and 72 h for these species to be found in the vicinity of the dermal blood vessels. The Schistosomules leave the dermis via the venous or lymphatic vessels and migrate to the heart and lungs (CDC, 2019).

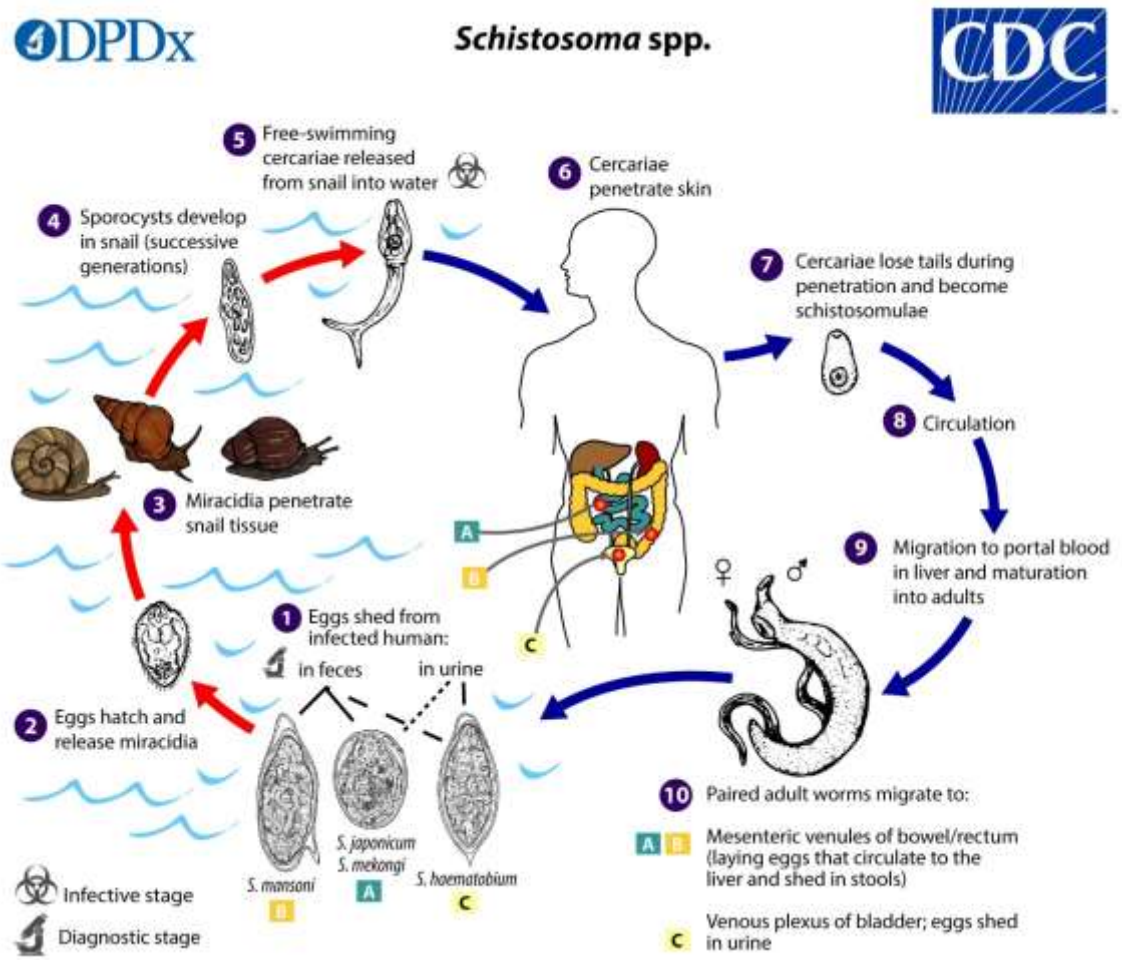

Figure 2.1: Life Cycle of Schistosomiasis

Source: CDC (2019)

Schistosomules then migrate to the portal or vestibule circulation where they mature into adult worms. Adult *S. japonicum, S. mekongi*, and *S. intercalatum* worms stay in the portal and mesenteric vessels while *S. haematobium* worms live in the vesical plexus. Mating of the adult worms also occurs in these locations. During mating, the male adult Schistosome embraces the female worm into its gynecophoric canal. Four to 6 weeks after the cercaria has penetrated the human skin, the female adult worm starts producing eggs, except for *S. haematobium* worms, which take about 60–63 days (or 9 weeks) before oviposition takes place. These adult female worms continue

producing throughout their lifetime. The number of eggs produced per day ranges from about 300 eggs for *S. mansoni* and *S. haematobium* to as many as 3,000 eggs for *S. japonicum.* About half the number of eggs produced are excreted with feces or urine while the rest stay in the tissues (Figure 2.1).

2.4 Signs and Symptoms of Schistosomiasis

Many individuals do not experience symptoms. If symptoms do appear, they usually take 4–6 weeks from the time of infection. The first symptom of the disease may be a general feeling of illness. Within 12 hours of infection, an individual may complain of a tingling sensation or light rash, commonly referred to as "swimmer's itch", due to irritation at the point of entrance. The rash that may develop can mimic scabies and other types of rashes. Other symptoms can occur 2–10 weeks later and can include fever, aching, a cough, diarrhea, chills, or gland enlargement. These symptoms can also be related to avian Schistosomiasis, which does not cause any further symptoms in humans. The manifestations of schistosomal infection vary over time as the cercariae, and later adult worms and their eggs, migrate through the body. If eggs migrate to the brain or spinal cord, seizures, paralysis, or spinal-cord inflammation are possible (Grimes *et al.,* 2014).

According to WHO (2013), symptoms of Schistosomiasis are caused by the body's reaction to the worms' eggs. Intestinal Schistosomiasis can result in abdominal pain, diarrhoea, and blood in the stool. Liver enlargement is common in advanced cases, and is frequently associated with an accumulation of fluid in the peritoneal cavity and hypertension of the abdominal blood vessels. In such cases there may also be enlargement of the spleen.

The classic sign of urogenital Schistosomiasis is haematuria (blood in urine). Fibrosis of the bladder and ureter, and kidney damage are sometimes diagnosed in advanced cases. Bladder cancer is another possible complication in the later stages. In women, urogenital schistosomiasis may present with genital lesions, vaginal bleeding, pain during sexual intercourse, and nodules in the vulva. In men, urogenital schistosomiasis can induce pathology of the seminal vesicles, prostate, and other organs. This disease may also have other long-term irreversible consequences, including infertility (WHO, 2013).The impact of an infection depends on the type of worm and the stage of infection. Symptoms occur when the body reacts to the worm's eggs.

2.4.1 Acute stage of schistosomiasis

A fever and a rash are symptoms of bilharzia, it can take between 14 and 84 days to appear, according to the WHO, (2014). Approximately 3 to 8 weeks after infection, the person may experience: a rash, Fever, Headache, Body aches, or Myalgia (Muscular pain) and Breathing difficulties.

2.4.2 Chronic stage of schistosomiasis

In long-established disease, adult worms lay eggs that can cause inflammatory reactions. The eggs secrete proteolytic enzymes that help them migrate to the bladder and intestines to be shed. The enzymes also cause an eosinophilic inflammatory reaction when eggs get trapped in tissues or embolize to the liver, spleen, lungs, or brain. The long-term manifestations are dependent on the species of schistosome, as the adult worms of different species migrate to different areas (Grimes *et al.,* 2014). Many infections are mildly symptomatic, with anemia and malnutrition being common in endemic areas. Many people do not show symptoms at the early stage, but they

may develop symptoms as the disease progresses. These later symptoms again depend on the type of parasite (WHO, 2014). If the parasites affect the liver or intestines, symptoms may include diarrhea and constipation, blood in the feces, intestinal ulcers ,liver fibrosis ,portal hypertension, or high blood pressure around the digestive system.

If the parasites affect the urinary system, there may also be blood in the urine, painful micturition and higher risk of bladder cancer. Over time, anemia can develop in rare cases, the parasite may affect the central nervous system. Children may have stunted growth and a reduced capability to acquire knowledge (WHO, 2014).

2.5 Diagnosis of Schistosomiasis

2.5.1 Identification of eggs in stools

Diagnosis of infection is confirmed by the identification of eggs in stools. Eggs of S. mansoni are about 140 by 60 µm in size and have a lateral spine. The diagnosis is improved through the use of the Kato technique, a semi-quantitative stool examination technique. Other methods that can be used are enzyme-linked immunosorbent assay, circumoval precipitation test, and alkaline phosphatase immunoassay (Stothard *et al.*, 2013).

Microscopic identification of eggs in stool or urine is the most practical method for diagnosis. Stool examination should be performed when infection with *S. mansoni or S. japonicum* is suspected, and urine examination should be performed if *S. haematobium* is suspected. Eggs can be present in the stool in infections with all *Schistosoma* species. The examination can be performed on a simple smear (1 to 2 mg of fecal material). Because eggs may be passed intermittently or in small numbers, their detection is enhanced by repeated examinations or concentration procedures, or both. In addition, for field surveys and investigational purposes, the egg output can be quantified by using

the Kato-Katz technique (20 to 50 mg of fecal material) or the Ritchie technique. Eggs can be found in the urine in infections with *S. haematobium* (recommended time for collection: between noon and 3 PM) and with *S. japonicum*. Quantification is possible by using filtration through a nucleopore filter membrane of a standard volume of urine followed by egg counts on the membrane. Tissue biopsy (rectal biopsy for all species and biopsy of the bladder for *S. haematobium*) may demonstrate eggs when stool or urine examinations are negative (CDC, 2016).

2.5.2 Antibody detection of schistosomal infection

Antibody detection can be useful to indicate schistosome infection in people who have traveled to areas where Schistosomiasis is common and in whom eggs cannot be demonstrated in fecal or urine specimens. Test sensitivity and specificity vary widely among the many tests reported for the serologic diagnosis of Schistosomiasis and are dependent on both the type of antigen preparations used (crude, purified, adult worm, egg, cercarial) and the test procedure (CDC, 2016).

At the U.S. Centers for Disease Control and Prevention, a combination of tests with purified adult worm antigens is used for antibody detection. All serum specimens are tested by FAST-ELISA using *S. mansoni* adult microsomal antigen. A positive reaction (greater than 9 units/µl serum) indicates infection with *Schistosoma* species. Sensitivity for *S. mansoni* infection is 95 % to 99 % , for *S. haematobium* infection, and less than 50% for *S. japonicum* infection. Specificity of this assay for detecting Schistosome infection is 99 %. Because test sensitivity with the FAST-ELISA is reduced for species other than *S. mansoni*, immunoblots of the species appropriate to the patient's travel history are also tested to ensure detection of *S. haematobium* and *S. japonicum* infections. Immunoblots with adult worm microsomal antigens are species-specific, so a positive reaction indicates the infecting species. The presence of antibody is indicative

only of schistosome infection at some time and cannot be correlated with clinical status, worm burden, egg production, or prognosis. Where a person has traveled can help determine which *Schistosoma* species to test for by immunoblot (Stothard *et al.,* 2013).

2.5.3 Treatment of schistosomiasis

Two drugs, Praziquantel and Oxamniquine, are available for the treatment of Schistosomiasis. They are considered equivalent in relation to efficacy against Schistosomiasis and safety (Coulibaly *et al.,* 2012). Because of Praziquantel's lower cost per treatment, and Oxaminiquine's lack of efficacy against the urogenital form of the disease caused by S. haematobium, in general Praziquantel is considered the first option for treatment. The treatment objective is to cure the disease and to prevent the evolution of the acute to the chronic form of the disease. All cases of suspected Schistosomiasis should be treated regardless of presentation because the adult parasite can live in the host for years (Danso *et al.,* 2013).

Schistosomiasis is treatable by taking by mouth a single dose of the drug Praziquantel annually. World health Organization (WHO, 2014) has developed guidelines for community treatment based on the impact the disease has on children in villages in which it is common: When a village reports more than 50 % of children have blood in their urine, everyone in the village receives treatment. When 20 to 50 % of children have bloody urine, only school-age children are treated. When fewer than 20 % of children have symptoms, mass treatment is not implemented. Other possible treatments include a combination of praziquantel with metrifonate, artesunate, or mefloquine. A Cochrane review found tentative evidence that when used alone, metrifonate was as effective as praziquantel (Kramer *et al.,* 2014).

Another agent, mefloquine, which has previously been used to treat and prevent malaria, was recognized in 2008–2009 to be effective against Schistosoma (Xiao, 2013).

2.5.4 Prevention and control of schistosomiasis

The basic means of preventing *Schistosoma* infection is avoiding contact with fresh water infested with Schistosome parasites. Swimming, wading, or any other aquatic activities in these bodies of water exposes the skin to possible penetration by the cercariae. In cases when there is brief accidental contact with infected water, vigorous towel drying is advised to help prevent the cercariae from penetrating the skin. In using water from these fresh water sources for drinking or bathing, water must be brought to the boil for at least 1 minute to kill the parasite that may be present in the water (CDC, 2014). Allowing the water to stand for 24 hours or more before using it may also help in preventing infection. Fine-mesh filters may also be used to filter the cercariae possibly contained in the water. Insect repellants such as DEET (N, N-Diethyl-meta-toluamide) may be applied topically to prevent cercariae from penetrating the skin, but this is not a very reliable measure (CDC, 2014).

Contact with infected water cannot always be avoided, especially by people in endemic areas whose occupation (such as fishing, rice farming) or day-to-day activity exposes them to these waters.it was recommended that preventive chemotherapy as a strategy for morbidity control will help lessen the occurrence, extent, and severity of the consequences of infection (WHO, 2014). Although preventive chemotherapy cannot prevent reinfection, it may cause a reduction in the production of eggs, which in turn prevents the morbidity caused by deposition of the egg in the human tissue (Stothard *et al.*, 2013). Preventive chemotherapy is targeted toward school-aged children in endemic areas because they are known to have a high risk of infection. In areas where the

prevalence of infection is at least 10 %, preventive treatment should also be given to those who are at high risk of infection because of their occupation, such as fishermen, farmers, and irrigation workers, as well as women who may be exposed to infected waters when performing their domestic chores. Pregnant and lactating women in these areas should also be included in preventive chemotherapy campaigns because they are considered at high risk of developing morbidity due to Schistosomiasis infection (WHO, 2014).

In countries where Schistosomiasis causes significant disease, control efforts usually focus on: Reducing the number of infections in people and/or eliminating the snails that are required to maintain the parasite's life cycle. For all species that cause Schistosomiasis, improved sanitation could reduce or eliminate transmission of this disease. In some areas with lower transmission levels, elimination of Schistosomiasis is considered a "winnable battle" by public health officials. Control measures can include mass drug treatment of entire communities and targeted treatment of school-age children. Some of the problems with control of Schistosomiasis include: Chemicals used to eliminate snails in freshwater sources may harm other species of animals in the water and, if treatment is not sustained, the snails may return to those sites afterwards. For certain species of the parasite, such as *S. japonicum*, animals such as cows or water buffalo can also be infected. Runoff from pastures (if livestock are infected) can contaminate freshwater sources (CDC, 2014).

2.6 Description and Classification of *Biomphalaria pfeifferi* Host (snails)

Biomphalaria pfeifferi is a species of air-breathing freshwater snail, an aquatic pulmonate gastropod mollusk in the family Planorbidae, and the ram's horn snails. This snail is a medically important pest, because of transferring the disease schistosomiasis.

Biomphalaria pfeifferi is an African species. It has recently expanded its native range (Ibikounlé *et al.,* 2012). It is widely distributed in Western Africa, Senegal and South Africa.

Kingdom: Animalia

Phylum: Mollusca

Class: Gastropoda

(unranked): clade Heterobranchia

clade Euthyneura

clade Panpulmonata

clade Hygrophila

Superfamily: Planorboidea

Family: Planorbidae

Subfamily: Planorbinae

Tribe: Biomphalariini

Genus: *Biomphalaria*

Species: *B. pfeifferi*

Biomphalaria pfeifferi is one of 18 *Biomphalaria* species known to transmit S. mansoni. *Biomphalaria pfeifferi* has a broad geographic distribution in sub-Saharan Africa where the majority of cases of *S. mansoni* occurs and exhibits a high degree of susceptibility to *S. mansoni*. For instance*, B. pfeifferi* typically shows high infection rates (50 %)

following exposure to *S. mansoni* from locations throughout Africa, but even to isolates originating from the Americas. For these reasons, it can be argued that *B. pfeifferi* is the world's most important intermediate host for *S. mansoni*. Understanding the role of *B. pfeifferi* in human schistosomiasis transmission becomes more critical because expanding agriculture and water development schemes and climate change threaten to alter the geographic range of both this snail species and of *S. mansoni* as well (Lu *et al.,* 2016).

2.7 Description and Classification of *Bulinus globossus* Host (snails)

Bulinus globosus is a species of a tropical freshwater snail with a sinistral shell, an aquatic gastropod mollusk in the family Planorbidae, the ramshorn snails and their allies. *Bulinous globosus* is an intermediary host of *Schistosoma haematobium,* along with *Bulinus truncatus.* This species occupies different kinds of environments in Africa, which are generally subjected to large variations in water availability. The mating system of this species is outcrossing, although selfing has been suggested for one population (Pedersen *et al.,* 2014).

Earlier studies observed that *B. globosus,* the intermediate host snail of *S. haematobium,* had high thermal tolerance and adapted in areas with fluctuating temperature. This snail has also been observed to have marked increase in survival time at temperatures as high as 34.0 °C while its optimal temperature for fecundity and growth has been shown to be 25.0 °C. Furthermore, this temperature coincides with the optimal temperature range (22 – 27 °C) for disease transmission. This suggests that the anticipated rise in temperature due to climate change may have

serious implications on snail population size and schistosomiasis spread patterns (Pedersen *et al.,* 2014).

Kingdom: Animalia

Phylum: Mollusca

Class: Gastropoda

(unranked): clade Heterobranchia

clade Euthyneura

clade Panpulmonata

clade Hygrophila

Superfamily: Planorboidea

Family: Planorbidae

Subfamily: Bulininae

Tribe: Bulinini

Genus: *Bulinus*

Species: *B. globosus*

2.8 Botanical Description and Classification of *Anogeissus leiocarpus*

Anogeissus leiocarpus is a deciduous tree species that can grow up to 15–18 m of height and measure up to 1 m diameter (Ahmad, 2014). It is known as African birch in English, Marke in Hausa, Ayin in Yoruba and Atar in Igbo (Victor *et al.,* 2014). The bark greyish and scaly, branches often drooping and slender, leaves alternate, ovate–

lanceolate in shape, 2-8 cm long and 1.3-5 cm across (Ouedraogo *et al.,* 2013). The leaves are acute at the apex and attenuate at the base, pubescent beneath. Inflorescence globose heads, 2cm across, yellow; the flowers are bisexual, petals absent. Fruits are globose, cone like heads; each fruit is broadly winged, dark grey, 3cm across. It can reproduce by seeds as well as vegetative propagation (Ouedraogo *et al*., 2013).

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Myrtales

Family: Combretaceae

Genus: *Anogeissus*

Species: *A. leiocarpa*

2.8.1 Geographical distribution and habitat *A. leiocarpus*

Anogeissus leiocarpus is typical element of woodlands and savannas of the Sudanian regional center of endemism. It has large ecological distribution ranging from the boarders of Sahara up to the out layer humid tropical forests. In West Africa, from Senegal to Nigeria, Cameroon and extends to Ethiopia and East Africa. It grows in dry forests and gallery forests ((Andary *et al.,* 2005; Ouedraogo *et al*., 2013).

2.8.2 Traditional Uses *A. leiocarpus*

Many traditional uses have been reported for the plant. Rural populations of Nigeria use sticks for dental hygiene, the end of the sticks are chewed into fibrous brush which is rubbed against teeth and gum. In Sudanese traditional medicine the decoction of the barks is used against cough. Some traditional practitioners use the plant for parasitic disease such as Malaria, Trypansomiasis, Helminthiasis and dysenteric syndrome. In Togolese traditional medicine for example, it is used against fungal infections such as dermatitis and Mycosis, also the decoction of leaves is used against stomach infections. The plant is also used for the treatment of diabetic ulcers, general body pain, blood clots, asthma, coughing and tuberculosis (Victor, 2014).

2.8.3 Phytochemical constituents of *A. leiocarpus*

Already, extensive work has been done on the phytochemical components of *A. leiocarpus.* The phytochemical analysis of the extracts of *A. leiocarpus* revealed the presence of alkaloids, glycosides, phenols, steroids, tannins, anthraquinones, saponins and flavonoids. In a similar study conducted by (Dawet *et al.,* 2014), alkaloids, tannins, saponins, flavonoids and glycosides were found to be present in ethanol and aqueous stem bark extracts of *A. leiocarpus* while tannins and flavonoids were absent in the chloroform extract of the plant. Moreover, the active compounds isolated from this plant have been shown to be mainly triterpenes and ellagic acid derivatives; flavonoids and phenolic compounds like flavogallonic acid bislactone (Abedo *et al.,* 2013) For instance, anogelline and dakaline are obtained from its bark are used as cosmetics with anti-ageing properties (Abedo *et al*., 2013). These phytochemicals have been proven

beyond reasonable doubt and established to be the driving force behind pharmacological activities of many medicinal plants (Abedo *et al*., 2013).

2.9 Botanical Description and Classification of *Pseudocedrella koschyi*

Pseudocedrella koschyi is a medicinal plant belonging to the family of meliaceae and is mainly found across West and Tropical Africa. It is a tree of up to 20 m high with a wide crown fissured bark and fragrant white flowers. The plant has also been reported to be used traditionally in the treatment of dysentery in Nigeria (Dawet *et al.,* 2014).

Pseudocedrella koschyi is a small, deciduous tree with an oblong to pyramid-shaped, usually dense crown; it can grow up to 12 m tall with occasional specimens to 20 m. The straight and cylindrical bole, which can be branchless for up to 7.5 m. is up to 70 cm in diameter. In natural stands, the bole is often low-branching and stunted because of fire damage, and this has great influence on the timber quality and quantity.

The tree provides a popular traditional medicine and a valuable timber. It is harvested from the wild, mainly for local use and is also occasionally planted as an ornamental shade tree and roadside tree (Dawet *et al.,* 2014).

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Sapindales

Family: Meliaceae

Genus: *Pseudocedrela*

Species: *kotschyi*

2.9.1 Phytochemical constituents of *Pseudocedrela kotschyi*

Pseudocedrella koschyi contains limonoids, including pseudrelones. Bark extracts showed in-vitro antibacterial activity, and anti-ulcer activity in rats. In tests the roots demonstrated broad-spectrum antimicrobial activity. Dichloromethane extracts of the root showed antileishmanial activity. The extracts and the isolated compounds 7-deacetylgedunin and 7-deacetyl-7-oxogedunin exhibited in-vitro activity against Leishmania donovani, *Trypanosoma brucei rhodesiense, Trypanosoma cruzi* and *Plasmodium falciparum,* with low cytotoxic activity against the L-6 cell line. The limonoid 7-deacetoxy-7-hydroxy-gedunin, isolated from *P. kotschyi*, showed anti-HIV activity. Extracts of *P. kotschyi* inhibited lymphocyte proliferation and exhibited molluscicidal activity. Crude ethanol extracts of the leaves showed distinct anthelmintic activity against Haemonchus contortus, a pathogenic nematode in small ruminants. Extracts of *P. kotschyi* also showed activity against Ascaris suum. The application of root juice can lead to severe skin necrosis, and care should be taken when it is applied to e.g. ulcers (Dawet *et al.,* 2014).

The essential oils from the stem and root bark are exclusively composed of sesquiterpenoids. δ -cadinene was the main constituent (31 %) in the oil from the stem bark, cubebols in that from the root bark. The antioxidant and antiradical activities of the essential oils were found to be low (Daniel, 2018).

2.9.2 Traditional uses *P. kotschyi*

P. kotschyi has numerous uses in traditional medicine, particularly its bark, roots and leaves. Bark decoctions or macerations are applied externally to ulcers, sores, rheumatism, leprosy, syphilis, yaws, itch, caries and gingivitis. Internally they are used to treat fever, stomach-ache, diarrhoea and dysentery, and as a diuretic and aphrodisiac. Root or root bark preparations are administered as powerful diuretic, to treat asthma, fever, dysentery, oedema, to facilitate childbirth, and are applied externally to ulcers, mastitis, haemorrhoids, fractures, rheumatism, caries and gingivitis, and as an aphrodisiac. In Uganda a tea made from root powder is drunk to treat liver cirrhosis. Pounded leafy twigs are rubbed in to treat headache and rheumatism, and a leaf decoction is applied as a compress to fractures and is drunk and used in a bath against rash and oedema. Young stems and roots are commonly used as chewing sticks to keep the teeth healthy. In Nigeria the stem bark is used in mixtures to treat trypanosomiasis in livestock, and leaves are administered in veterinary medicine against intestinal worms. In Nigeria the bark is used as an ingredient of arrow poison, and in Côte d'Ivoire as a fish poison. The bark yields a brownish dye that has been used in West Africa for dyeing cloth. *P. kotschyi* is occasionally planted as an ornamental shade tree and roadside tree. In Nigeria the leaves are used as a green manure (Daniel, 2018).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Medicinal Plants

The plants *A. leiocarpus* and *P. kostchyi* were collected from their natural habitats on the basis of ethno-botanical information. They were collected with bio-conservation aspects in mind from Rijiyaddaji, Kontagora Local Government (Figure 3.1). An acknowledged authority authenticates the botanical identity of the plants with the help of senior botanist from the Department of Plant Biology, Federal University of Technology, Minna and a voucher specimen will be deposited at department for future reference. Folklore knowledge of the two plants were sourced from a traditional medical practitioner; and he provided the information on the plants collected, the part used and the precise locality where it grows, when curative potency is maximal and the mode of preparation.

3.2 Collection and Preparation of Plants Part for Extraction

The plants part collected were the leaves of *A. leiocarpus* as shown in Plate 3.1 and *P. kostchyi* as shown in Plate 3.1. They were dried at room temperature for different periods of time depending on the succulence of the plant materials. The leaves were separately ground when completely dry using an electric mill at. The powdered plant materials were kept at room temperature away from direct sunlight in closed dry plastic bags.

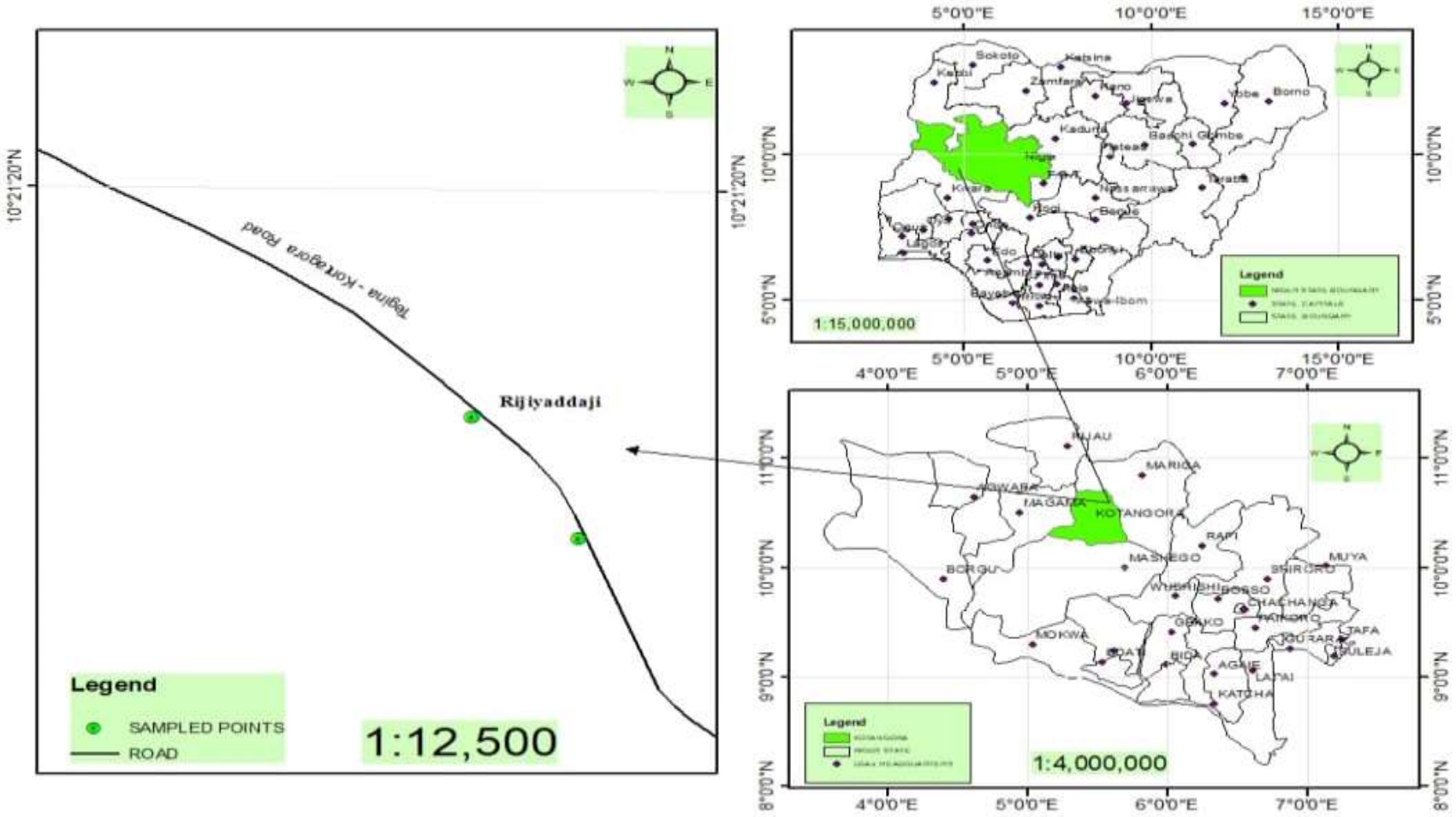

Figure 3.1 Map of Kontagora showing sampling site

Source: Remote Sensing/Geographical Information System (gis) Department, Futminna (2018)

Plate I, *A. Leiocarpus* leaves
Source: Field photograph 2018

Plate II, *P. Koschyi* leaves
Source: Field photograph 2018

3.3 Extraction Procedure of the Medicinal Plants

3.3.1 Aqueous extraction of the plants

Plant extraction was carried as per method described by (Kofi-Tsekpo *et al.*,1985). A hundred grammes (100 g) of each powdered plant material was boiled in one litre of distilled water for 2 h. After boiling, the solution was allowed to cool. The extract was then decanted into a 1-litre clean dry conical flask and filtered through a No. 1 Whatman filter paper under vacuum pump into a 500 mL clean dry conical flask. Decantation and filtration processes was repeated until the sample became clear. The filtrate was freeze-dried, weighed and stored in an airtight container at room temperature ready for bioassay.

3.3.2 Crude methanol extraction of the plants

The plant leaves were dried under room temperature and ground with an electronic blending machine or pestle and mortar. Pulverized plant sample (40 g) was extracted in the cold for 72 h using 70 % methanol. The extract was filtered with Whatman filter paper (No. 1) and solvent removed under reduced pressure in a rotary evaporator. Green coloured paste was obtained, weighed and stored in a refrigerator at 4 ^{o}C until required.

3.3.3 Alkaloids extraction of the plants

The extraction of the alkaloid was done using the continuous extraction method using the Soxhlet apparatus. Four hundred grams (400 g) of powdered plant was weighed and packed in a cheese cloth bag which served as an extraction thimble. The thimble was then placed into a suitable jar with cover. The sample was moistened with sufficient amount of 95 % ethanol. This was made alkaline with sufficient quantity of ammonia T.S. and mixed thoroughly. The sample in the thimble was macerated overnight, and then placed in the Soxhlet extractor on the next day. Sufficient amount of 95 % ethanol was placed in the solvent flask (4.8 liters). The sample was extracted for about 3 – 4 h.

The ethanol extract was filtered and will be concentrated in a Soxhlet distilling apparatus at 60 ^{o}C. The crude alkaloid extract was further treated with 1.0 N hydrochloric acid. This was filtered and the filtrate was collected. The filtrate was alkalinified with ammonia T.S. and placed in a separatory funnel. Measured quantities of chloroform was added into the separatory funnel, mixed and shaken for about five times and allowed to separate into two layers. The lower layer of chloroform contained the alkaloids and the upper layer the aqueous portion. The upper layer was extracted until the last chloroform extract was found negative to Dragendorff's reagent. The combined chloroform extract was concentrated in Soxhlet distilling apparatus at 60 ^{o}C and evaporated in water bath maintained at that temperature until semi-dry. The residue was weighed and percentage yield was calculated using the formula:

$$\% \textbf{ yield} = \frac{\textbf{Weight of the alkaloidal residue (g) x 100}}{\textbf{Weight of powdered sample (g)}}$$

3.4 Phytochemical Analysis

Plant are the richest resources of drugs of traditional system of medicine, modern medicine, neutraceuticals, food supplement, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs (Hammer *et al.,* 1999). Medicinal plants have produce some very effective treatment for malaria as in the case of artemisinin, few attempt have been made to evaluate antischistosomal activity of such natural plant (Molgaard *et al,*. 2001)

3.4.1 Phytochemical analysis of the crude plant extract

3.4.1.1 Glycoside content

A portion of the extract (0.5 g) was mixed with 2 ml of glacial acetate and 1 drop of ferric chloride solution, after which 1ml of concentrated sulphuric acid were added. The reaction was observed for a brown ring formation (Sofowora, 1996).

3.4.1.2 Flavonoids content

Few amount of the extract (1 g) was heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Yellow coloration was observed that indicated the presence of Flavonoids (Sofowora, 1993 ; Chang *et al*,. 2002 ;).

3.4.1.3 Tannins content

A portion of the extract (0.5 g) was boiled in 20 ml of distilled water in a test tube and filtered. 0.1 % ferric chloride ($FeCl_3$) solution was added to the filtrate. The appearance of brownish green or a blue-black colouration indicates the presence of tannins in the test samples (Harborne, 1973).

3.4.1.4 Saponins content

Exactly (2.0 g) of the plant extract 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 shaken vigorously to form a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion characteristic of saponins (Sauza *et al*., 2014).

3.4.1.5 Alkaloids content

A portion of the extract (0.5 g) was stirred with 5 cm^3 of 1 % aqueous HCl on a steam bath. Few drops of picric acid solution were 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 (Harborne, 1973).

3.4.1.6 Anthraquinone content

A little portion of the extract (0.5 g) was added to 2 ml of 10 % hydrochloric acid in a

test tube and boiled for about two minutes. Chloroform (5 ml) was added to the test tube and shaked twice, the chloroform layer was pipetted out and then equal volume of 10 % ammonia (5 ml) was added. A brown colour was formed in upper layer which indicated absence of anthraquinones (Sofowora, 2008).

3.4.1.7 Phenol content

A portion (0.5 g) of plant (*Anogeissus leiocarpus*) extract was stirred with 10 mls of distilled water and then filtered. Few drops of 50 % ferric chloride reagent were added to the filtrate. Blue-black or blue-green coloration or precipitation was taken as an indication of the presence of phenols (Trease and Evans, 1989; Singleton *et, al.,* 1999).

3.5 Collection, Identification and Screening of Laboratory Animals

Young adult of *Biomphalaria pfeifferi* and *Bulinus globosus* were collected using standard method from the littoral zone of Bosso dam and Pastor River as well in Minna, Niger State, Nigeria (Figure 3.2) using scoop net. They were properly washed packed in a container and brought to the Animal Biology Laboratory for identification and screening (Plate 3.3).

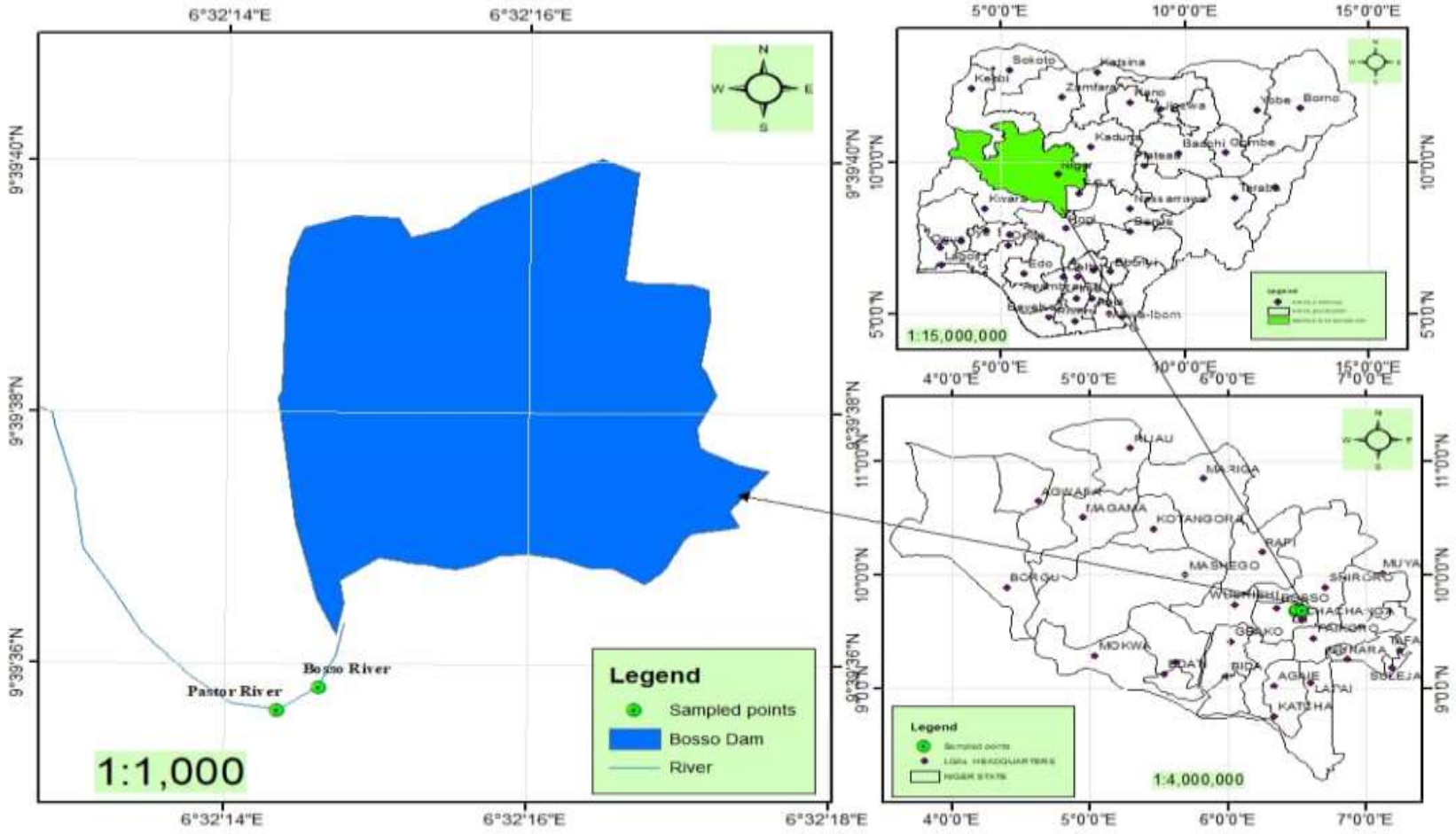

Figure 3.2 Map of Bosso Dam showing sampling sites
Source: Remote Sensing/Geographical Information System (GIS) Department, Futminna (2018)

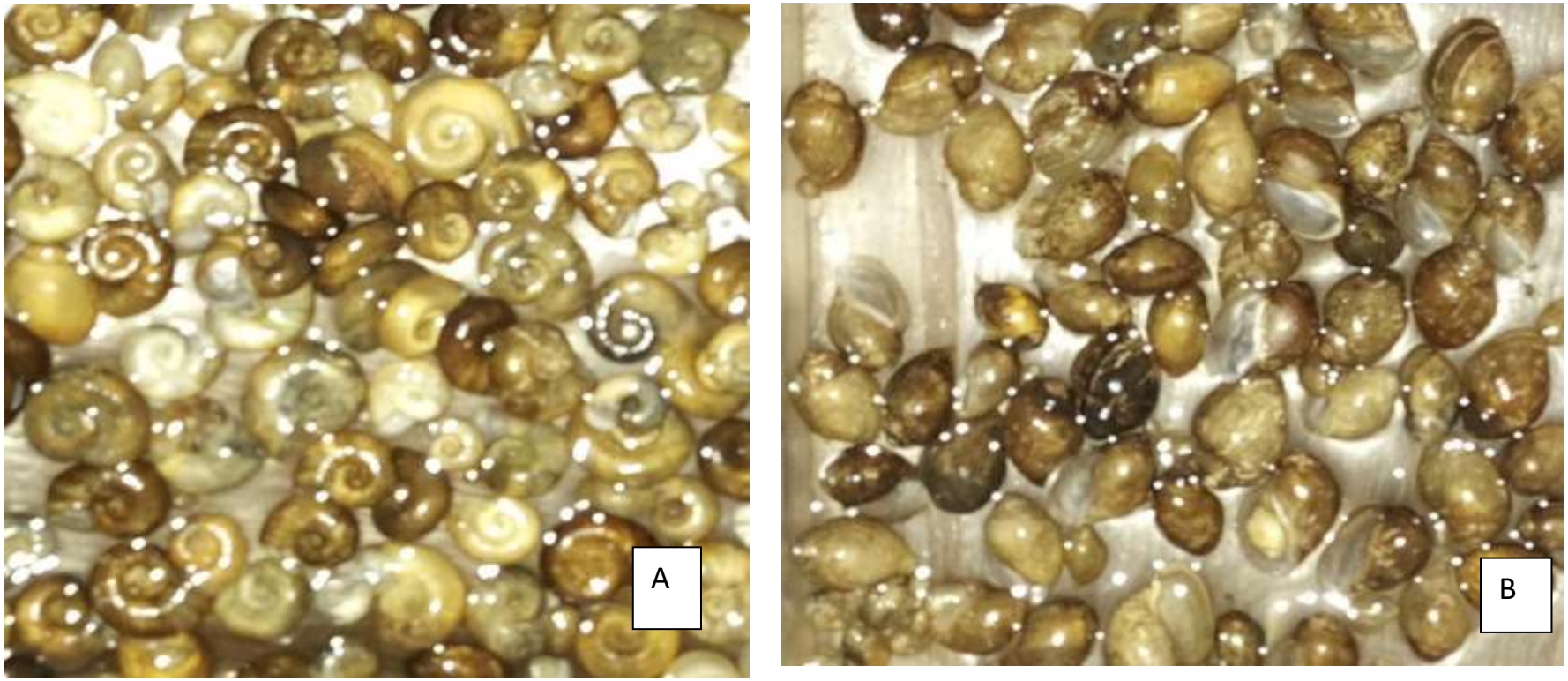

Plate III, Showing snails samples collected (A - *Biomphalaria pfeifferi* and B- *Bulinus globossus*)
Source: Field photograph 2018

3.6 Evaluation of Molluscicidal Activity on the Vector of Schistosomiasis

This objective was achieved using the method of (WHO,1965; Duncan and Sturrock, 1987). Ten snails were dropped in a clean rubber container each were exposed to each extract in triplicate of Aqueous, Methanol and Alkaloid at concentrations of 10,20,40,80,160,200 and 300 mg/L in a total volume of 200 mL distilled water and monitor for interval of 40 minutes, 24 h and 72 h. Fifty other snails were also exposed to 200 mL of distilled water for 24 h as control. After exposure, the snails were placed in fresh borehole water, provided with boiled lettuce to feed on, and allowed a recovery period of 24 h after which mortality was accessed by probing the snails with a wooden spatula to elicit typical withdrawal movements. Death was ascertained by observing the snails under a microscope for any heart activity, or mucus surrounding it. The number of dead snails was recorded.

3.7 Biochemical Analysis

Total protein was determined according to the method of Gornall *et al.*, (1949) using kits purchased from Biodiagnostic (Egypt). Total Lipid was determined according to the method of Knight *et al.*, (1972) using kits. Transaminase (AST and ALT) activities was determined in the snail haemolymph using reagent kit according to the method of Reitman and Frankel, (1957).

3.8 Data Analysis

The experimental data was analyzed using unpaired Student t-test. This test determined the significant differences between the data obtained from animal groups treated with plant extracts and control group. A value $p<0.05$ was considered significant. Statistical computer package was used.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Qualitative phytochemical compounds of aqueous and methanol extract of *A. leiocarpus* and *P. koschyi* leaves extracts

The results of the qualitative phytochemical compound of the aqueous and methanol extracts of *A. leiocarpus* and *P. koschyi* leaves extracts are presented in the table 4.1. The results indicated the presence of phytochemicals such as Flavonoid, Tannis, Saponins, Alkaloids and Terpenoids in both aqueous and methanol extract of both the plants. Steroid was only present in methanol extract. Cardia glycoside was absent in aqueous extract of the extracts.

Table 4.1 Qualitative Phytochemical components of Aqueous and Methanol Extracts of *A. Leiocarpus* and *P. Koschyi* leaves extracts

Phytochemical Constituents	*A. leiocarpus*		*P. koschyi*	
	Aqueous	**Methanol**	**Aqueous**	**Methanol**
Flavonoid	+	+	+	+
Tannis	+	+	+	+
Saponins	+	+	+	+
Alkaloid	+	+	+	+
Steroid	-	-	-	+
Phloba tannins	-	-	+	+
Cardiac glycoside	-	+	-	+
Total phenol	-	+	+	+
Terpenoids	+	+	+	+

Key

+ = Positive

- = Negative

4.1.2 Quantitative phytochemical constituents of the aqueous and methanol extracts of *A. leiocarpus* and *P. koschyi* leaf extract.

The result of the quantitative phytochemical of the aqueous and methanol extracts of both plants were presented in Table 4.2. The result indicated that Flavonoids (47.10±1.12), Saponins (11.13±0.11), are higher in methanol than in the aqueous extract of *A. leiocarpus.* However, Alkaloid contents (18.41±0.14) and total phenol (11.12±0.21) were higher in aqueous than in methanol extract. On the other hand, Flavonoids (32.23±0.23), Alkaloid (15.14±0.02) and total phenol (9.32±0.32) were higher in methanol than in the aqueous extract

Table 4.2 Qualitative Phytochemical constituent of Aqueous and methanol extracts of *A. Leiocarpus* and *P. Koschyi* leaf extract

Phytochemical Constituents	*A. leiocarpus* (mg/100g)		*P. koschyi* (mg/100g)	
	Aqueous	**Methanol**	**Aqueous**	**Methanol**
Flavonoid	41.21 ±0.12[d]	47.1 ±1.12[d]	28.12 ±0.91[e]	32.23±0.23[b]
Tannis	9.23 ±1.10[a]	12.14 ±1.14[b]	13.12±1.11[c]	10.31 ±0.07[a]
Saponins	11.13±0.11[b]	18.98 ±0.98[c]	16.38 ±0.07[d]	14.21 ±0.31[a]
Alkaloid	18.41±0.14[c]	12.12 ±0.57[b]	11.12 ±0.14[b]	15.14 ±0.018[b]
Total phenol	11.12±0.21[b]	8.98 ±0.62[a]	6.35 ±0.03[a]	9.32 ±0.32[b]

Values followed by the same superscript alphabet on the same column are not significantly different at P>0.05

Values are presented in mean standard error of two determinations

4.1.3 Molluscicidal activities of *A. leiocarpus* extract on *B. pfeifferi* and *B. globossus.*

The molluscicidal activities of *A. leiocarpus* extract on *B. pfeifferi* and *B. globossus* is presented in the Table 4.3. The extract showed significant molluscicidal activities against the tested snails with some peak activities being expressed at higher dose. At 10 mg/L extract concentration, all the snails species were resistant to this lowest concentration tested. These were no significant ($p>0.05$) difference from the result of the control. However, as the extract concentration increases from 20 mg/L to 300 mg/L snail mortality increases significantly as well. The case was not the same from the snail species in the group exposed to 20 mg/L of Alkaloid (for *B pfefferi*) and all the test group of the *B. globossus.*

For the aqueous extract, *B. globossus* were more susceptible to the extract, recording 100 % mortality than the *B. pfeifferi* that recorded 90 % snail mortality when to 300 mg/L extract concentration. On the other hand, all the snail species exposed to methanol extract were susceptible to the plant extract, especially at higher concentration of 200 and 300 mg/L. However, alkaloid fraction recorded the lowest against the snail species.

Table 4.3 Molluscicidal activities of *A. leiocarpus* extract on *B. pfeifferi* and *B. globossus*

CONCENTRATION (mg/L)	*B. pfeifferi*			*B. globossus*		
	Aqueous	**Methanol**	**Alkaloid**	**Aqueous**	**Methanol**	**Alkaloid**
10	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)
20	1.78 ± 1.6^{b} (17.80)	2.00 ± 0.00^{b} (20.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)
40	1.78 ± 0.16^{b} (17.80)	3.41 ± 0.31^{c} (34.10)	2.21 ± 0.81^{b} (22.10)	1.00 ± 0.00^{b} (10.00)	2.00 ± 2.00^{b} (20.00)	0.00 ± 0.00^{a} (0.00)
80	2.00 ± 0.00^{c} (20.00)	5.24 ± 1.12^{d} (52.40)	2.21 ± 0.81^{b} (22.10)	1.00 ± 0.00^{b} (10.00)	3.00 ± 1.28^{b} (30.00)	0.00 ± 0.00^{a} (0.00)
160	4.42 ± 0.18^{d} (44.20)	8.28 ± 0.95^{e} (82.80)	2.21 ± 0.81^{b} (22.10)	3.14 ± 0.28^{c} (31.40)	7.00 ± 2.00^{c} (70.00)	2.15 ± 0.04^{b} (21.50)
200	7.35 ± 0.58^{e} (73.50)	10.00 ± 0.00^{f} (100.00)	5.16 ± 0.76^{c} (51.60)	7.14 ± 0.13^{d} (71.40)	10.00 ± 0.00^{d} (100.00)	2.15 ± 0.04^{b} (21.50)

300	9.00 ± 0.00^{f} (90.00)	10.00 ± 0.00^{f} (100.00)	6.00 ± 0.84^{c} (60.00)	10.00 ± 0.00^{e} (100.00)	10.00 ± 0.00^{b} (100.00)	2.15 ± 0.04^{b} (21.50)

Number in parenthesis are mortality (%) of the respective doses Control (0.00)

4.1.4 Molluscicidal activities of the extract of *P.koschyi* against *B. pfeifferi* and *B. globossus* snail species.

The results of the molluscicidal efficacy of the aqueous, methanol and Alkanoid fraction of *P. koschyi* against *B. pfeifferi* and *B. globossus* snail species of Schistosomiasis is represented in the Table 4.4. The recorded data showed that the tested doses of the extracts showed a dose dependent molluscicidal efficacy against the tested snail species. There was no mortality recorded for snail species in the group exposed to 10 mg/L extract concentration and the control. Thereafter mortality increases significantly with increase in extract concentration. While highest mortality was recorded against *B. pfeifferi* at 300mg/L extract concentration for aqueous, 200 mg/L extract concentration for methanol, the snail species exposed to the Alkaloid fraction were resistant to all the extract concentration tested recording 40.00 % snail mortality at 300 mg/L extract concentration.

On the other hand, the activities of the aqueous extract against *P.koschyi* was low except for 300 mg/L extract concentration that recorded 60.00 % snail mortality. Methanol extracts of showed highest potency against the exposed snails *B. pfeifferi* and *B. globossus,* recorded 100 % mortality as at 200 mg/L extract concentration. Although between 10 to 80 mg/L. Alkaloid extract administration to the snail species group, no snail mortality was recorded more than 90 % (91.40) snail mortality was recorded when exposed to the highest concentration (300 mg/L) tested.

Table 4.4 Molluscicidal activities of the extract of *P.koschyi* against *B. pfeifferi* and *B. globossus* snail species

Concentration (mg/L)	*B. pfeifferi*			*B. globossus*		
	Aqueous	**Methanol**	**Alkaloid**	**Aqueous**	**Methanol**	**Alkaloid**
10	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)
20	2.25 ± 0.12^{b} (22.50)	2.15 ± 0.15^{b} (20.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)	3.00 ± 0.00^{b} (30.00)	0.00 ± 0.00^{a} (0.00)
40	4.00 ± 0.00^{c} (40.00)	4.00 ± 0.00^{c} (40.00)	0.00 ± 0.00^{a} (0.00)	0.00 ± 0.00^{a} (0.00)	4.48 ± 0.12^{c} (44.80)	0.00 ± 0.00^{a} (0.00)
80	5.00 ± 0.00^{d} (50.00)	7.00 ± 0.00^{d} (70.00)	1.65 ± 0.04^{b} (16.50)	2.00 ± 2.00^{b} (20.00)	6.00 ± 0.00^{d} (60.00)	0.00 ± 0.00^{a} (0.00)
160	5.00 ± 0.00^{e}	9.00 ± 0.00^{e}	2.00 ± 2.00^{b}	2.00 ± 2.00^{b}	8.70 ± 0.48^{e}	3.00 ± 0.15^{b}

	(50.00)	(90.00)	(20.00)	(20.00)	(87.00)	(30.00)
200	7.23 ± 0.58^{f} (72.30)	10.00 ± 0.00^{f} (100.00)	3.41 ± 0.03^{c} (34.10)	4.40 ± 0.31^{c} (44.40)	10.00 ± 0.00^{f} (100.00)	5.00 ± 0.35^{c} (50.00)
300	9.25 ± 0.20^{g} (92.50)	10.00 ± 0.00^{f} (100.00)	4.00 ± 0.00^{d} (40.00)	6.00 ± 0.00^{d} (60.00)	10.00 ± 0.00^{f} (100.00)	9.14 ± 0.32^{d} (91.40)

Number in parenthesis are mortality (%) of the respective doses Control (0.00)

4.1.5 Effect of *A. leiocarpus* and *P. koschyi* on some body enzymes of the soft tissue of *B. pfeifferi* and *B. globossus* snail species.

The result of the effect of *A. leiocarpus* and *P. koschyi* on some body enzymes of the soft tissue of *B. pfeifferi* and *B. globossus* snail species is detailed in Table 4.5 and 4.6. The recorded data from the analysis showed that there was significant ($p<0.05$) decrease in Aspartate triaminase (AST) and Alamine transaminase (ALT) level of the extract treated groups, with higher decrease in higher dose, compared with the control groups that has higher level of Aspartate triaminase (AST) and Aspartate triaminase (AST). Also, it showed that the decrease in the level of the Aspartate triaminase (AST) was more pronounced in snail group treated with *P. koschyi.*

Table 4.5 Effect of *A. leiocarpus* and *P. koschyi* on Aspartate transaminase (AST) of the soft tissue of *B. pfeifferi* and *B. globossus* snail

Species

Extract	Concentration (mg/L)	*B. pfeifferi*			*B. globossus*		
		Aqueous	**Methanol**	**Alkaloid**	**Aqueous**	**Methanol**	**Alkaloid**
CONTROL		189.23±1.31^{e}	201.28 ± 4.14^{e}	192.13 ± 0.91^{d}	198.14±1.23^{c}	187.65±1.21^{d}	215.02 ± 1.23^{c}
A.leiocarpus	200	169.27 ±1.23^{c}	196.6 ± 1.23^{d}	192.11 ± 0.96^{d}	195.14±1.41^{a}	182.14 ±1.28^{b}	200.07 ± 1.23^{a}
	300	175.15 ±0.76^{d}	184.23 ±1.23^{c}	180.07± 0.21^{c}	198.23±2.31^{c}	187.07± 0.97^{d}	212.41± 1.41^{b}
P.koschyi	200	143.21±0.96^{b}	163.10±1.21^{b}	167.31± 0.96^{b}	197.14±2.23^{b}	184.17± 0.98^{c}	213.14± 0.96^{b}

	300	132,33± 1.21[a]	154.98± 0.97[a]	164.23 ± 0.78[a]	198.25±0.43[c]	181.23±1.24[a]	201.12± 0.97[a]

Extract	Concentration Mg/L	*B. pfeifferi*			*B. globossus*		
		Aqueous	**Methanol**	**Alkaloid**	**Aqueous**	**Methanol**	**Alkaloid**
Control		185.41±1.21^{b}	196.69 ± 0.31^{e}	189.32 ±1.21^{d}	188.65±2.39^{d}	189.63±1.21^{d}	189.31±1.23^{e}
A.leiocarpus	200	184.31± 1.23^{b}	186.32±0.23^{d}	180.11±1.31^{c}	181.23±3.14^{c}	181.10 ±1.38^{c}	171.16 ±1.21^{d}
	300	180.04±1.23^{a}	179.12 ±0.14^{b}	170.04±3.21^{a}	169.23±4.23^{a}	172.07± 2.14^{b}	167.11±1.31^{c}
P.koschyi	200	181.23±1.24^{a}	169.23±0.23^{a}	176.23±1.41^{b}	171.11±3.14^{b}	169.21±2.14^{b}	160.21±1.23^{b}

300	180.41±0.45[a]	181.41± 0.31[c]	177.37±1.24[b]	172.11±2.12[b]	161.41±0.09[a]	157.21±1.23[a]

Table 4.6 Effect of *A. leiocarpus* and *P. koschyi* on Alamine transaminase (ALT) of the soft tissue of *B. pfeifferi* and *B. globossus* snail Species

4.1.6 Effect of *A. leiocarpus* and *P. koschyi* on Alkaline Phosphate (ALP) of the soft tissue of *B. pfeifferi* and *B. globossus* snail species.

There was a significant increase in the activity of Alkaline Phosphate (ALP) level in the exposed snail species to the two extracts when compared with the control groups (Table 4.7). There was more increase in the Alkaline Phosphate (ALP) values recorded for *B. pfeifferi* groups compared with the *B. globossus.* However, in the *B. globossus* snail group treated with Aqueous, Methanol and Alkaloid of *A. leiocarpus* extract has no significant difference in mean ALP level of the exposed and the control groups.

Table 4.7 Effect of *A. leiocarpus* and *P. koschyi* on Alkaline Phosphate (ALP) of the soft tissue of *B. pfeifferi* and *B. globossus* snail species

Extract	Concentration mg/L	*B. pfeifferi*			*B. globossus*		
		Aqueous	Methanol	Alkaloid	Aqueous	Methanol	Alkaloid
Control		65.12±0.09[a]	71.02 ± 3.14[a]	71.63 ±2.35[a]	69.21±1.23[a]	68.44±1.31[a]	62.15±0.91[a]
A.leiocarpus	200	111.23±0.07[d]	92.14±1.27[b]	70.16±0.79[a]	68.21±1.41[a]	66.25±1.21[a]	62.22 ± 0.27[a]
	300	123.61±0.05[e]	109.23 ±1.14[c]	77.45±1.14[b]	69.45±6.21[a]	69.31± 0.14[a]	68.45±0.03[c]

P.koschyi	200	98.45±0.91[b]	121.32±0.21[d]	100.23±3.14[c]	71.23±1.23[b]	75.12±0.12[c]	67.23±0.91[b]
	300	101.23±0.96[c]	134.11± 2.14[e]	121.45±2.14[d]	74.15±2.14[b]	71.14±0.14[b]	66.41±0.13[b]

4.1.7 Effect of extracts of *A. leiocarpus* and *P. koschyi* on Total Protein level of *B. pfeifferi* and *B. globossus.*

The data also recorded decrease in protein level of the exposed group compared to the control groups. However, the Alkaloid fraction of *A. leiocarpus* extract showed higher level of the total protein level of the extract compared to the control group. Additionally, there was no significant difference ($p>0.05$) in the Total Protein level recorded for the group treated with 200 mg/L and 300 mg/L of the *A. leiocarpus* against both the *B. pfeifferi* *B. globossus* (Table 4.8).

Table 4.8 Effect of extracts of *A. leiocarpus* and *P. koschyi* on Total Protein level of *B. pfeifferi* and *B. globossus*

Extract	Concentration mg/L	*B. pfeifferi*			*B. globossus*		
		Aqueous	Methanol	Alkaloid	Aqueous	Methanol	Alkaloid
	Control	5.23±1.31^{c}	4.89 ± 0.03^{c}	5.12 ±0.03^{b}	4.31±0.31^{c}	5.42±0.07^{c}	4.12±0.14^{b}
A.leiocarpus	200	5.08±0.01^{c}	4.11±0.07^{c}	5.16±0.02^{b}	4.30±0.41^{c}	5.41±0.08^{c}	4.12 ±0.04^{b}
	300	3.21±0.04^{a}	2.15±0.12^{a}	5.43±0.04^{b}	3.95±0.01^{b}	3.85± 0.03^{b}	3.81±0.00^{a}
P.koschyi	200	4.32±0.10^{b}	3.89±0.03^{b}	4.92±0.31^{a}	3.95±0.21^{b}	3.21±0.02^{b}	4.12±0.03^{b}

	300	3.21±0.03[a]	3.25± 0.01[b]	5.41±0.12[b]	3.31±0.12[a]	2.86±0.07[a]	3.63±0.08[a]

4.1.8 Median (LC_{50}) and (LC_{90}) lethal concentration of the effect of *A. leiocarpus* and *P.koschyi* extract on *B. globossus* and *B. pfeifferi* after 24hrs exposed period.

Analyses revealed LC_{50} *A. leiocarpus* against *B. pfeifferi* (Aqueous 157.27, Methanol 88.56, and Alkaloid 240.03) mg/L and *B. globossus* (Aqueous 167.44, Methanol 56.73 and Alkaloidal 542.32) mg/L (Table 4.9) and LC_{50} against *B. pfeifferi* (Aqueous 128.73, Methanol 78.07 and Alkaloidal 344.02mg/L) and *B. globossus* (Aqueous 253.83, Methanol 76.89 and Alkaloidal 195.93) mg/L for *P.koschyi* (Table 4.10). This is an indication that *A. leiocarpus* has high molluscicidal potency than *P.koschyi*. The lethal concentration of the effect of *A. leiocarpus* and *P.koschyi* extracts on *B. globossus* and *B. pfeifferi* after 24 h exposed period is detailed in Table 4.9 and 4.10 respectively. The LC_{50} of *A. leiocarpus* varies from 56.73 mg/L in methanol extract against *B. globossus* to 88.86 mg/L in methanol against *B. pfeifferi*. In both snails species investigation of the coefficient of determination in all concentration was above 80 % (0.8114); this implies the recorded snail mortality was caused by the tested plant extracts.

Similarly the LC_{50} of the effect of *P.koschyi* extract against the two examined snail species varies from 76.89 mg/L (Methanol) extract against *B. globossus* to 78.07 mg/L methanol against *B. pfeiffer*. The coefficient of determination recorded that more than 86 % (0.8600) of the tested extract is responsible for the recorded mortality observed.

Table 4.9 Median (LC_{50}) and upper (LC_{90}) lethal concentration of the effect of the effect of *A.leiocarpus* extract on *B.peifferi* and *B. globossus* after 24 hours exposure period

Extraction		(LC_{50}) mg/L	(LC_{90}) mg/L	R^2	Regression Equation (RE)
	Aqueous	157.27	291.51	0.9491	Y= 0.298x + 3.129
B.pfeifferi	Methanol	88.855	173.01	0.963	Y=0.4753x + 7.7675
	Alkaloid	240.03	442.23	0.859	Y= 0.1978x + 2.5211
	Aqueous	167.44	281.31	0.9449	Y= 0.3513x – 8.8241
B.globossus	Methanol	56.73	96.73	0.9856	Y= 0.5106x – 6.7317
	Alkaloid	542.32	960.73	0.8114	Y= 0.0956 – 1.8462

Table 4.10 Median (LC_{50}) and upper (LC_{90}) lethal concentration of the effect of *P. koschyi* extract and *B. globossus* after 24 hours exposed period

	Extraction	LC_{50} (mg/L)	LC_{90} (mg/L)	R^2	Regretion equation (RE)
	Aqueous	128.73	282.58	0.8600	Y=0.2613x +16.53
B.pfeifferi	Methanol	78.07	161.18	0.8979	Y =0.4813x +12.423
	Alkaloid	344.02	611.01	0.9358	Y = 0.1498x – 1.5346
	Aqueous	253.83	441.88	0.9388	Y = 0.2127x – 3.9886
B.globossus	Methanol	76.89	166.29	0.9031	Y= 0.4474x +15.6

	Alkaloid	195.93	321.68	0.9424	Y = 0.3181x – 12.325

4.2 Discussion

The recorded data from this study indicated that both Aqueous and methanol extract of *A.leiocarpus* and methanol extract of *P.koschyi* are very rich in Flavonoid, Tannins, Saponins, Phenol and Alkaloids. Phytochemicals are secondary metabolites produced as a result of plant secondary metabolism, purposefully, to ward off infection and /or pathogens (kumar *et al.,* 2017). Angage (2013) reported that the presence of this secondary metabolites in plant produces some biological activities and it responsible for their uses as herbs in primary Health care. These components are also complicated in plant protection against infection by microorganism, predation by insects and herbivore. The differences in their concentrations observed in the current study, can be attributed to the fact that the plants are of different species and other factors including environmental factor such geographical location which may have influence on the type of soil, precipitation, light intensity and temperature (Lumpkin, 2005).

In the present study, the two plants showed appreciable molluscicidal efficacies against the two exposed snail species. There activities are concentration and extract solvent dependent. The other of their molluscicidal potency ranges from methanol to Aqueous while the least was observed in the snail species exposed to the alkaloid fraction. The molluscicidal effects as well as the varying potencies of each plant may be due to the differences in the concentration and /or type of active ingredient(s) present in each plant/extract (Abdullahi *et al.,* 2018). Both Aqueous and methanol of both plants, for instance contain appreciable amount of Saponins and tannins. These two metabolites have high solubility in water and could penetrate through the pores of thin calcite layers that enveloped the embryo. The possible mechanism of action of Saponins could be related to its potential to cause cellular lyses and complexation with steroids thereby

resulting in to biocidal action of these compound (Tunholi, *et al.,* 2011). Tannins are polyphenols containing several hydrolyses group that make possible their complexation with proteins, causing precipitation and as consequence, its inactivation. These feature could explain the possible mechanism of action of tannins (Sauza *et al*., 2014). In addition, Flavonoid act by inhibiting the detoxification system of the snail. These compounds may act individually and for synergistically compounds may act individually and /or synergistically be additive or even making easier the absorption of another compound, thus intensifying the lethal effects in the snails.

The current study Table 4.9 indicated LC_{50} of (157.27, 88.56, and 240.03) mg/L for aqueous, methanol and Alkaloidal fraction of *A. leiocarpus* against *B. pfeifferi* and LC_{50} of 128.73, 78.07 and 344.02 mg/L for *P.koschyi* (Table 4.10). This is an indication that *A. leiocarpus* has high molluscicidal potency than *P.koschyi.* The LC_{50} recorded in the current study is far better than some previously reported studies of Hitil, *et al.* (2010) recorded LC_{50} of 213.099 mg/L for Aqueous extract of *Cymbopogon nervatus* leave extraction on *B. pfeifferi.* The leaf of a particular plant, if prove effective at an appreciable concentration, should be considered a better/ best fit molluscicidal agent (Angage, 2013)

The molluscicidal potency observed in the two plant extracts, in the present investigation could be supported by the decrease in protein content and the alteration in the activities of vital enzymes in the snail tissues. This is an indication of damage to the snail tissues as well as disturbance to the physiological metabolism needed for parasite development and cercarial production (Bakry *et al.,* 2011). Furthermore, the decrease in protein content could be traced to the destruction of the snail tissue and /or impairment in protein synthesis or to the stimulation of gluconeogenesis to use protein

as a source of energy under stress condition of intoxication with eucalbanin B (Eman *et al.,* 2014).

In the current investigation, the recorded result also indicated a significant decrease in the level of Alamine transaminase (ALT) and Aspatate transaminase (AST) in the tissue of the exposed snail compared to the control. This decrease in Alamine transaminase (ALT) and Aspatate transaminase (AST) in the snail tissue may be due to their release from damaged tissue in to the haemolymph (Bakry, *et al*., 2011) on the other hand there was increase in the activities of Alkaline Phospahate (ALP). The ALP plays a critical role in shell formation (Tunholi *et al.,* 2011).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The results obtained from the study showed that both Aqueous and Methanol extract of *A.leiocarpus* and *P. koschyi* contain bioactive phytochemical including Saponin, Alkaloid, Tannins, Flavonoid and Phenol. Flavonoid was significantly higher than other phytochemicals detected in the plants. The two plants extract showed a dose-dependent molluscicidal potency against the exposed snail species. Methanol was more potent against the tested snail, but the alkaloid showed low molluscicidal potency. Exposing the snail species to the extract were observed to affect the selected enzymes. Findings from the study thus suggest the plants as promising candidate for development of a novel molluscicide in the control of Schistosomiasis.

5.2 Recommendations

Based on the findings of this study, the following recommendations are made;

i. Further bio-guided fractionation is needed to identify the active ingredient in the plant.

ii. There is need to examine the Histoachitecture of the exposed snail in other to ascertain the observed mechanism of action found in the alteration of the tissue enzymes.

iii. The extract and purify fractions can be tested against other species of medical important.

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APPENDICES

Appendix A

Plate I *A. Leiocarpus* leaves
Source: Field photograph 2018

Plate II, *Pseuodocedrella kostchyi* leaves
Source: Field Photograph 2018

Appendix C

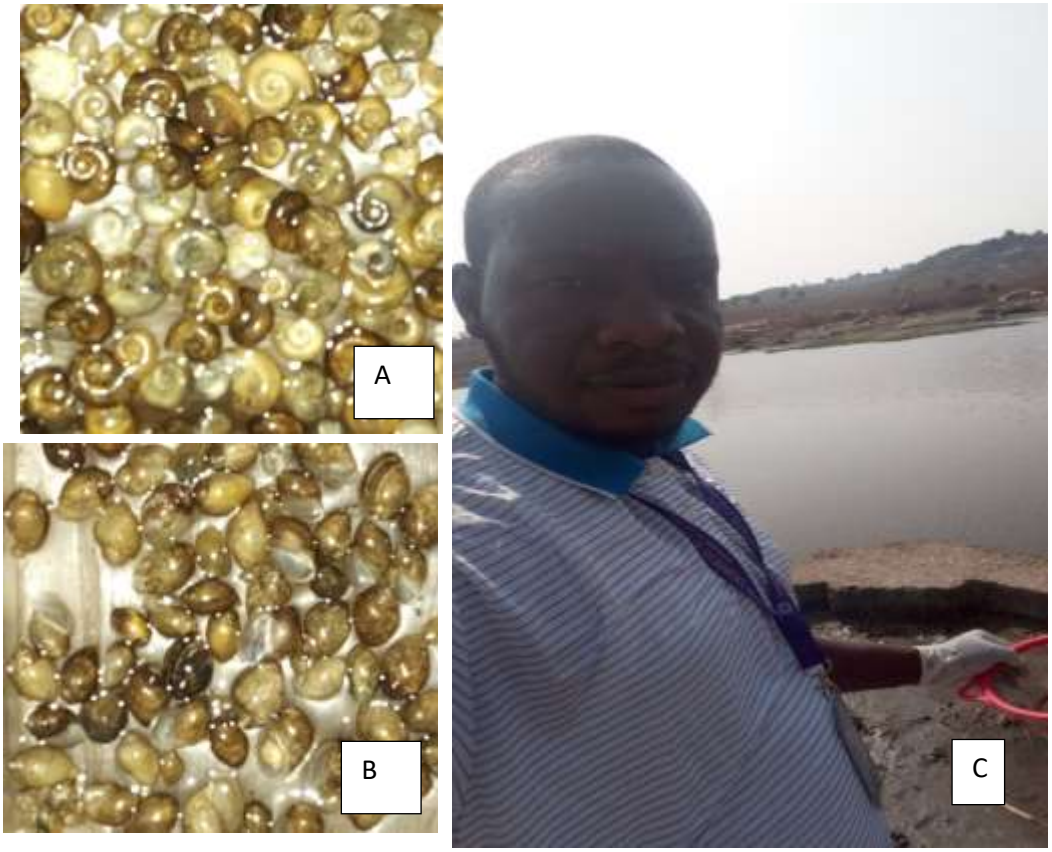

Plate III. A - *Biomphalaria pfeifferi* B- *Bulinus globossus* and C- Bosso River and snails samples collected area.
Source: Field photograph 2018