EVALUATION OF BIOACTIVE METABOLITE CONTENTS, MOSQUITO LARVICIDAL AND ANTI-PLASMODIAL ACTIVITIES OF EXTRACTS OF THE SPIDER Neoscona adianta

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ABSTRACT

In spite of the extensive control efforts, over the years, mosquito still transmit serious human diseases, cause millions of deaths every year and the development of resistance to chemical insecticides resulting in rebounding vectorial capacity. This situation is aggravated with the re-emergence of drug resistance of mosquito borne diseases especially malaria. The current study was therefore designed to assess the mosquito larvicidal potency and antiplasmodial efficacy of the crude and fractions of the extract of the spider (Neoscona adianta). The zoochemical components of the crude and fractions of the spider extracts were assessed following standard procedures. The larvicidal potency of the crude and fractions of the spider was carried out according to World Health Organization (WHO) standard protocol with slight modifications. Graded concentrations (ranging from 0.2 to 2.0 mg/k) of the crude and fractions of the spider extracts were tested against 25 batches of healthy 4th instar larvae of *Culex* mosquito species, and larval mortality was recorded after 24 hours exposure period. Acute oral toxicity of the crude extract was carried out to establish the oral safe dose. The antiplasmodial activates of the crude extract and fractions were bio-assayed against established infection in chloroquine-sensitive *Plasmodium berghei* infected mice. The results indicated the presence of zoochemicals including flavonoid, tannins, saponnins, alkaloids steroids total phenol and terpeniods in the crude extract and fractions of the spider. The results of the larvicidal bio-assay revealed that both crude and fractions showed a dose and concentration-dependent larvicidal potency. Larvicidal activities was significantly higher (P<0.05) in the ethylacetate and n-henane fractions than in the crude methanol extract. Similarly, only the ethylacetate and n-hexane recorded 100% mortality for the highest concentration tested (2.0 mg/h). The best larvicidal activity was found in the nhexane fraction with an LC50 of 0.46mh/L, followed by ethylacetate with LC50 of 0.94mg/L. The results of the acute oral toxicity revealed that the spider extract is safe for oral administration with an LC50 greater than 5000mg/kg body weight. The crude and fractions of the spider extract showed a dose dependent antiplasmodial activities with peak activity recorded of the group of mice treated with 600mg/kg b.wt crude extract. The crude and fractions did not ameliorate fall in PCV but promoted body weight change and elongated the survival time. The results support the medicinal use of spider extracts in folkloric medicine and suggest that this spider contained bio-active compounds that could be developed as potent antimalarial drug as well as potent bio-pesticide agent against mosquito vector.

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CHAPTER ONE

1.0

INTRODUCTION

1.1 Background to the Study

Malaria is a mosquito-borne infectious disease of humans and other animals caused by eukaryotic protists of the genus *Plasmodium*. In humans, the disease is transmitted by the female mosquito of the genus *Anopheles*. The *Plasmodium* species that cause malaria in human include *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and the zoonotic is *P. knowlesi* (mainly monkey parasite, similar to *P. malariae* and confirmed by PCR) (WHO, 2018).

Malaria is a potentially deadly disease characterized by cyclical bouts of fever with muscle stiffness, shaking and sweating (WHO, 2015; Macleod (1998) also stated that malaria is a parasitic infection transmitted to humans through the bites of an infected female Anopheles mosquito. The name "malaria" is derived from the Italian words *Mal* (bad) and *aria* (air). It arose originally because the citizens of Rome thought that the disease was contracted by breathing the bad air of the Pontine Marshes (Greenwood and Mutabingwa, 2002). (Hornby, 2007) defined malaria (ague, marsh fever, periodic fever, paludism) as an infectious disease due to the presence of parasitic Protozoa of the genus *Plasmodium (P. falciparum, P. malariae, P. ovale* or *P. vivax*) within the red blood cells. The disease is confined to tropical and subtropical areas.

Malaria has a worldwide distribution, affecting people of all ages, with an enormous burden amounting to 300-500 million clinical cases per year (Lucas and Gills, 2003; Williams *et al.*, 2016). Globally ten new cases of malaria occur every second, which is a major public health problem in the tropics where about 40% of the world population lives. It is responsible for more than a million deaths each year, of which 90% occur in sub-Saharan Africa (WHO, 2009). Malaria is caused by four different protozoa in the *plasmodium* genus: either *Plasmodium vivax*, which is more prevalent in low endemic areas, *P. ovale*, *P.* *malaria*, and *P. falciparum*, the most dangerous of the four. The *P. falciparum* has a life cycle in the mosquito vector and also in the human host. The *Anopheles gambiae* mosquito is the vector responsible for the transmission of malaria.

The prevalence of malaria is dependent on the abundance of the female anopheles species, the propensity of the mosquito to bite, the rate at which it bites, its longevity and the rate of development of the *plasmodium* parasite inside the mosquito. When the female mosquito bites and sucks the blood of a person infected with malaria parasites she becomes infected; she then transmits the parasites to the next human host she bites. Malaria incubates in the human host for about eight to ten days. (WHO, 2020).The spread of malaria needs conditions favorable to the survival of the mosquito and the *plasmodium* parasite. Temperatures of approximately 70 - 90 degrees Fahrenheit and a relative humidity of at least 60 percent are most conducive for the mosquito (WHO, 2009).

Nigeria is at an alarming pace of malaria diseases, been the most populous country in Africa. The success of its malaria control programs will have a significant impact on the overall control of malaria in the region. Because a large proportion of the population in Nigeria's rural areas lives in poverty, a control plan focused on those areas will be effective. Also, there are factors that are responsible for the increase in the resurgence of malaria that must be addressed in malaria transmission and control. These factors include the large scale resettlement of people usually associated with ecological changes and conflicts, increasing urbanization disproportionate to the infrastructure, drug resistant malaria, insecticide resistant mosquitoes, inadequate vector control operations and public health practices (Onah *et al.*, 2017).

The year 2000 went down in history as the year in which the most influential alliance (till date) in efforts to eradicate malaria converged in Abuja, Nigeria. That was the Roll Back

Malaria (RBM) Partnership, and the targets set have come to be known as the "Abuja Targets". One of the goals set by the RBM Partnership was that by 2010, 80% of patients with malaria would be diagnosed and treated with effective antimalarial medicines (RBM, 2005). Over 1 decade later, malaria remains a public health concern in the world's poorest countries, Nigeria chief among them. As at 2010, deaths from malaria in Nigeria were the highest recorded worldwide (Onah *et al.*, 2017). In 2005, artemisinin-based combination therapies (ACTs) were adopted as the first-line treatment for uncomplicated malaria in Nigeria (FMOH, 2005).

This is a strange phenomenon since so much effort has been geared towards eradicating this dreaded disease in Nigeria. Hence the need to critically investigate the reasons or challenges confronting eradication efforts of Malaria in Nigeria. Conventional antimalarial drugs have been the mainstay of clinical management, both for prophylaxis and treatment. Artemisin based combination drugs are the frontline for treatment currently with artemisininlumefantrine being the first line and artemisininpiperaquine being second line oral treatment. Parenteral artesunate is initially instituted with intravenous quinine being the last line of defence for severe malaria. Previously used drugs such as sulphadoxinepyrimethamine (SP), mefloquine, chloroquine, primaquine, amodiaquine have been limited due to development of resistance. Emergence of multidrug resistant strains which has accompanied each new class of antimalarial drugs may be viewed as one of the most significant threats to the health of tropical populations. While it is widely agreed that a new approach to prevention and treatment is needed, solutions have targeted more of development of new drug classes'. With renewed interest and funding, there are over 15 new antimalarials in various development stages. The main concern is that they act at known targets and therefore may be subject to common resistance mechanisms. New drugs for new plasmodia targets are needed.

Animal venoms are valuable sources of novel pharmacological tools whose specific actions are useful for characterizing their receptors. Hundreds of toxins from snakes, scorpions, spiders and marine invertebrates with a range of pharmacological activities have all been characterized (Lucas *et al*, 2003; Park, 2002; Hill *et al.*, 2006).

Spider venoms contain a wide spectrum of biologically active substances, which selectively target a variety of vital physiological functions in both insects and mammals (FMoH, 2005). The spider toxins are "short" polypeptides with molecular mass of 3-8 kDa and a structure that is held together by several disulfide bonds. There are two main groups of these peptides, the neurotoxins that target neurone receptors, neurone ions channels or presynaptic proteins involved in neurotransmitter release, and the non-neurotoxic peptides, such as necrotic peptides and antimicrobial peptides (for a review, see (WHO,2005)). Recent studies have characterized the venoms of the genuses *Brachypelma*, *Pterinochilus* and *Theraphosa* (Bawah and Binka, 2005 ;). Several peptides that inhibit atrial fibrillation (United Nations Country team, 2004), block the Kv2 and Kv4 subfamilies of voltage-dependent potassium channels (Lawn *et al.*, 2005), or multiple sodium channels (Adam *et al.*, 2005), and protongated sodium channels (Steketee *et al.*, 2001) have been recently isolated. Although spider toxins bind to their receptors with high affinity, specificity, and selectivity, little is known about them and little work has been done to develop these toxins for therapeutic use.

Spider venoms are mixtures of biologically active peptides, proteins, glycoproteins, and small organic molecules which interact with cellular and molecular targets to trigger severe, sometimes fatal effects. However, the spider venom could be particularly interesting for the treatment of general diseases as a scaffold for toxin-based drug research. Several venombased drugs or venom-derived molecules have found extensive use as tools for therapies. For instance, "Captopril", a competitive inhibitor of angiotensin-converting enzyme, is broadly used and well-established antihypertensive drug developed from a polypeptide toxin isolated from the venom of *Bothrops jararaca*; "Conotoxin" from the sea cone snail *Conus magus* used as an analgesic for severe chronic pain and "exendins"; and, recently, proteins obtained from the saliva of the Gila monster *Heloderma suspectum* benefited the treatment of type II diabetes (Lucas, 2003; Park, 2002; Hill *et al.*, 2006).

1.2 Statement of the Research Problem

Malaria remains one of the most serious world health problems and the major cause of cause of morbidity and mortality in the endemic areas (WHO, 2018). Despite all effort to curb the prevalence of malaria, the disease continues to spread even across some areas where it had been previously eradicated (WHO, 2018). *Plasmodium falciparum*, the most lethal etiological agent for human malaria, is becoming increasingly resistant to standard antimalarial drugs in almost all parts of endemic areas especially Africa (WHO, 2020).

1.3 Justification of the Study

Malaria is one of the most important parasitic diseases in the world. It remains a major public health problem in Africa responsible

1.4 Aim and Objectives of the Study

The aim of the study is to evaluate the antimalarial and mosquito larvicidal activities of the extracts of spider

The objectives of the study are to determine the:

i. entomochemical constituents of the extract of spider.

ii acute oral toxicity (LD50) of the crude extract of spider.

iii. antiplasmodial potency of the crude and solvent fractions of spider in *Plasmodium berghei*-infected mice.

iv. effect of the spider crude and fractionated extracts on body weight change and haematological parameters of *Plasmodium berghei*-infected mice.

v. larvicidal activities of the crude and fractionated extracts of spider.

vi. LD50 and LD90 of the crude and fractionated extracts of spider.

vii. structure of the bioactive compound in the extract or fraction with best for the annual death of over one million children below the age of five years (White, 2010). *Plasmodium falciparum* is becoming increasingly resistant to standard antimalarial drugs which necessitate a continuous effort to search for new drugs, particularly with novel modes of action. "Bioactivities".

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Malaria Disease

2.1.1 Etiology of malaria

Malaria is caused by *plasmodium* parasites which are protozoa and it is transmitted by the female mosquito of the genus Anopheles in humans. The Plasmodium species in human include P. falciparum, P. vivax, P. malariae, P. ovale and the zoonotic species is P. knowlesi. P. knowlesi, mainly a monkey parasite, is similar to P. malariae and is confirmed by PCR (WHO, 2010). Of the five *Plasmodium* species infecting humans, *P. falciparum* is responsible for a high proportion of the morbidity and nearly all the mortality. Investigation of the Plasmodium species present in an infection is necessary because, firstly P. falciparum and P. knowlesi infections cause rapidly deteriorating severe illness or death and prompt commencement of treatment is crucial. The other species are less likely to cause severe malaria. Secondly, P. vivax and P. ovale infections also require treatment for the dormant hypnozoite liver forms that can cause infection relapse. P. vivax causes more gradual and predictable progressing disease. Finally, the resistance patterns for *P. falciparum* and *P.* vivax vary in various regions (CDC, 2011). P. falciparum is widespread with highest density being in tropical regions of Africa, Asia and South America. P. vivax is widespread but chloroquine resistant strains are mostly occur in Papua New Guinea and Indonesia. P. vivax infections occurring in Papua New Guinea or Indonesia should initially be treated with a regimen recommended for chloroquine-resistant P. vivax infections which includes quinine sulphate plus doxycycline or tetracycline, or, Atovaquone-proguanil, or mefloquine. These three treatment options are equally recommended. Chloroquine is effective for P. ovale infections.

2.1.2 Life cycle and transmission of *Plasmodium* stages in man

Sporozoites are inoculated in humans for the asexual phase by female anopheles mosquitoes upon feeding on human blood meal, which they require to nourish eggs. They are injected during probing before aspirating blood, and enter circulation directly or through lymph channels (approx. 20%). They invade human liver cells, being cleared from the blood stream within 45 minutes, differentiate and multiply to release tens of thousands of merozoites (WHO, 2018). Only few cells are infected and this stage is asymptomatic. This lasts 5.5 and 15 days for P. falciparum and P. malariae respectively. In the case of P. ovale and P. vivax some intra hepatic ones do not develop and rest as inert hypnozoites to awaken and cause relapse weeks to months later. The parasites migrate to the blood and infect erythrocytes with individual merozoites undergoing further multiplication to produce 12-16 merozoites within schizonts. The length of this erythrocytic stage depends on the species and is usually 48hours for P. falciparum, P. vivax, and P. ovale, 24hr for P. knowlesi and 72 hours for P. malariae. These then burst out infecting more red cells, at usually 10-fold per cycle, leading to fever and damage of vital organs (WHO, 2018). Only a subpopulation of cells is infected mainly as a result of their age. P. (vivax and falciparum) prefer younger cells, with 13% and 40% uncomplicated malaria cell invasion respectively, while P. malariae prefer older erythrocytes. However P. falciparum infections in South East Asia have shown unrestricted cell invasion. The erythrocytes also release gametocytes which get ingested by feeding mosquitoes thus completing the lifecycle, while ingested sporozoites do not survive and get digested in the mosquito (White, 2010). Stages in the Vector. The sexual (sporogony) intravector phase starts with ingestion of gametocytes by the feeding mosquito. The male and female gametocyte ration is about 1:4, with each male having 8 microgamete and female as a macrogamete (WHO, 2018). One of each sex is needed for successful infection to occur. In the mosquito's midgut, the male gametocytes undergo rapid nuclear division, to produce 8 flagellated micro gametes for fusion and meiosis with the female macrogametes and form zygotes, which become diploid and then develop to the rapidly moving Ookinete. These transverse the mid gut wall and form Oocysts, each of which differentiates into thousands of Sporozoites which migrate to the mosquito"s salivary glands. Sporogonytakes 8-35 days depending on temperature and mosquito species (White, 2010). The lifecycle is as shown in Figure 2.1.



Figure 2.1: Lifecycle of the *Plasmodium* Indicating the Development Stages in Man and Vector (WHO, 2010).

Malaria can also be spread by inoculation of blood from an infected person. In this form, asexual forms are directly inoculated into the host's blood, by-passing the pre-erythrocytic hepatic development of the parasite (Jain and De Fillips, 2002). Hence, this malaria type

has a shorter incubation period. The most efficient malaria vector is the African *Anopheles gambiae*. This can be attributed, partly, to its relatively long life, strong anthropophily and endophily (the tendency to target humans for blood meal and the tendency to enter and rest inside of houses, respectively). Other species are *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles funestus*, all found in Africa and are some of the sixty anopheline mosquitoes able to transmit in humans.

2.1.3 Epidemiology of malaria

Malaria is the world's most widespread infection and there were 216 million episodes in 2010. Compared to a century earlier, the area of malaria risk has reduced from 53% to 27% of the Earth's land surface and the number of countries exposed to some level of malaria risk has fallen from 140 to 106. Africa bore more than 81% of the infected cases and 91% of the deaths in 2010, with sub -Sahara Africa affected the most, (WHO, 2011). It is the fifth leading cause of death worldwide with almost half the world's population at risk. Malaria is a classic disease with high morbidity, affecting productivity and has been estimated to cost 1.3% of GDP in endemic countries, like in Asia, Latin America, and Africa (WHO 2010). In Africa alone, an economic burden exceeding US\$ 12 billion of lost GDP is encountered annually. However, there has been approximately 17% global fall in malaria incidences between 2000 and 2010 however (WHO, 2011). In Kenya the disease is endemic in the lowlands, particularly the coastal strip and Lake Victoria basin where transmission is sufficiently intense. Both incidence and prevalence of infection reach more than 90% of the population within 10 - 12 weeks after the beginning of the rainy season in Kenya (MoPHs, 2010).

2.1.4 Clinical epidemiology of malaria

Severe malaria is rare in the first six months of life but with high mortality if it does. This is due to passive transfer of maternal immunity and their higher haemoglobin F which retards parasite development. In young children progression can be rapid with seizures suggesting that sequestration causes significant damage even in conscious patients. A history of less than a day's illness is common in these cases of cerebral malaria. In holoendemic areas, although infants are repeatedly inoculated, severe malaria is rare for them with the clinical impact being anaemia. Indigenous adults do not develop severe malaria unless they leave the transmission area. Immunity is boosted continually and malaria awareness leads to early interventions, preventing high parasitaemia. Most infections are asymptomatic. In less intense or unstable transmission areas young children, besides infants, are also affected by severe malaria with cerebral malaria also dominating. In low transmission areas seasonal infections are common, symptomatic infections occur at any age with cerebral malaria mainly manifesting for severe cases. Epidemics are associated with migration of new host, introduction of new vectors or change in their habitats (White, 2010).

2.1.5 Mixed infections

Infection with *P. falciparum* mostly suppresses simultaneous *P. vivax* infection although the reverse is sometimes observed. In Thailand about 30% of *P. falciparum* infected patients show symptomatic *P. vivax* infection in 2 months without exposure to new malaria infections, and the reverse in 8% of patients. In low transmission areas co-infection with the two species lowers severe malaria risk four-fold, but in high transmission areas higher morbidity is seen (White, 2010).

2.1.6 Diagnosis and clinical symptoms

The severity of the disease varies from mild asymptotic infection to the critical disease which causes death. Common symptoms include periodic flu-like symptoms such as fever, chills, sweating, muscle aches and headaches. Other symptoms include nausea, vomiting, coughing, diarrhea, abdominal pain, myalgia (limbs and back), loss of appetite, orthostatic hypotension, jaundice, anaemia, liver or kidney failure, enlarged liver and spleen and convulsions. Severe malaria is regarded as a multisystem disorder and includes cerebral malaria and severe malarial anaemia. Severe malaria delirium, metabolic acidosis and multiorgan dysfunction which may occur, if untreated could result in coma and eventually death.

Cyclical symptoms such as fever, seizures, chills and anaemia are said to correspond with the erythrocytic stage of the *Plasmodium* life cycle during which merozoites are released into the bloodstream. Together with this release, there is also a deposit of parasitic waste products and debris, which is believed to give rise to the malarial paroxysm, i.e. the sequenced events of shaking chills, fever and sweating. Symptoms for complicated or severe malaria includes seizures, altered state of consciousness (or coma), excessive sleepiness, prostration (feelings of helplessness), respiratory distress, inability to ingest any fluids, bleeding problems, jaundice, the absence of urine and the dark coloration of urine if there is any.

Splenomegaly and anaemia are major/principal clinical symptoms of malaria. The latter is caused by the destruction of RBCs and the simultaneous loss of haemoglobin or by the removal of the infected erythrocytes as an immune response. Anaemia may also be caused by the ceased production of RBCs due to the bone marrow suppression. Complications in malaria infections are mainly a cause of blocked capillaries. The obstruction of these capillaries is as a result of infected RBCs and or the parasitic RBC debris. The serious

complications that can result from this obstruction includes glomerulonephritis, nephrotic syndrome and renal failure, which are all renal complications that may be life-threatening. Cerebral malaria infections involve severe headaches, cortical blindness, stroke and death (White, 2010).

2.2 Pathophysiology of Malaria

Malaria pathophysiology is from erythrocyte destruction, release of plasmodium and erythrocyte material into circulation and the host's response to these P. falciparum infected cells sequester in microvasculature of vital organs, interfering with perfusion and tissue metabolism. Erythrocytes with mature forms are sequestered by attaching to vascular endothelium, mainly venules, and thus disappear from circulation. As haematocrit rises blood viscosity rises and also the ease of cell adherence to endothelium. Viscosity may also affect secondary events like cardiac workload hence blood pressure. Cells with ring stages are also sequestered, although to a lesser extent, mainly in the spleen and placenta. Infected cells also undergo resetting by adhering to uninfected ones, with up to five times force as compared to normal adherence, hence clumping and reducing blood flow with subsequent events such as raised cardiac workload. They also aggregate around platelets and obstruct vessels. Infected cells are also deformed and in P. falciparum become spherical and rigid from the biconcave shape. These are removed at the spleen as they are not easily filtered. Sequestration may be a mechanism to avoid removal at the spleen as they cluster and hold back from getting to the spleen. It is opposite for P. vivax where cells become larger and more deformable. In both cases the cells are compromised in their ability to transport oxygen. Mild generalized permeability of systemic vessels also occurs, and may marginally contribute to oedema especially in cerebral malaria cases. Other effects are coma, renal failure, pulmonary oedema, fluid and electrolyte changes, anaemia, hypoglycaemia, blackwater fever, coagulopathy, gastrointestinal dysfunction, end-organ dysfunction(liver,

spleen, placenta, brain, heart, lungs, kidneys and bone marrow), and secondary bacterial infections. Only the first one third of stages is diagnosed well by microscopy as plasmodia are already sequestered in the second two thirds of the asexual lifecycle. This can misguide clinicians and contribute to the outcome of the patient. The presence of more mature parasites on a blood film, even with a lower parasitaemia, might suggests worse prognosis for a patient than early stage ring forms. The mature forms may be the remnants of much greater sequestration. In this case two patients with the same peripheral parasitaemia may have a hundred fold differences in number of parasites (White, 2010).

2.3 Treatment of Malaria

Treatment of Malaria Fevers have always haunted mankind and several ingenious remedies were tried to combat the fevers. In the ancient times, limb blood-letting, emesis, amputation and skull operations were tried in the treatment of malarial fever. In England, opium from locally grown poppies and opium-laced beer were tried. Even the help of astrology was sought as the periodicity of malarial fevers suggested a connection with astronomical phenomena. Malaria is a focal disease which differs in its characteristics among regions (WHO, 2010), mainly depending on climatic conditions, hence varying malarial control strategies are applicable. Prevention is based on awareness by risk recognition, avoiding mosquito bites, compliance with appropriate chemoprophylaxis, and diagnosing quickly with prompt treatment.

Antimalarial drugs can be classified as blood schizonticidal, tissue schizonticidal, gametocidal, hypnozointicidal, or sporozointicidal. Drugs can also be classifies in 3 main groups: aryly-aminoalcohols (quinoline related) compounds (quinine, quinidine, chloroquine, amodiaquine, mefloquine, halofantrine, lumefantrine, piperaquine, pyronaridine, primaquine, tafenoquine); antifolates (pyrimethamine, proguanil, chlorproguanil, trimethoprim); artemisinin compounds (artemisinin, and

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dihydroartemisinin, artemether, artemotil, artesunate) and antibiotics (sulphonamides, sulphones, tetracyclines, clindamycin, macrolides, chloramphenical, fosmidomycin (under investigation). After rapid clinical assessment and confirmation of the diagnosis, full doses of parenteral antimalarial treatment should be started without delay with whichever effective antimalarial that is first available (WHO, 2005). Two classes of drugs are currently available for the parenteral treatment of *P. falciparum* severe malaria: the cinchona alkaloids (quinine and quinidine) and the artemisinin derivatives (artesunate, artemether and artemotil). Although there are a few areas where chloroquine is still effective, parenteral chloroquine is no longer recommended for the treatment of severe malaria because of widespread resistance (WHO, 2010)

2.4 Resistance to Antimalarial Treatment

The main concern remains that *P. falciparum* in Africa, has developed resistance to commonly prescribed drugs such as quinine, chloroquine, mefloquine, amodiaquine, primaquine, halofantrine (Halfan®), atovaquone, proguanil, sulphadoxine and dapsone (Madrid *et al.*, 2005). In Kenya, chloroquine (CQ) and sulphadoxine-pyrimethamine (SP) had been the drugs of choice, due to effectiveness and affordability. Quinine resistance in *P. falciparum* was first reported in 1910, 17 but has neither been high grade nor compromised its use). Mefloquine replaced quinine in Thailand in1984, as combination with SP in order to delay resistance, but resistance rapidly developed. Its cure rates however were raised on usage with artemisinin based therapy. Resistance to antifolates, proguanil and pyrimethamine, occurred within years of introduction and compromised their use, in both *P. falciparum* and *P. vivax*, but was not treated seriously until CQ resistance was first reported in 1957. The selection to CQ and pyrimethamine may have been due to impregnated salt as mass prophylaxis (White, 2010). CQ resistance is by efflux mechanism, before the level required to effectively inhibit the process of haempolymerization is

attained. Polymerization is necessary to prevent build-up of the toxic byproducts formed by haemoglobin digestion (Arav, 2005).

Resistance to SP, due to sequential mutation at Dihydrofolatereductase (DHFR) and dihydropteroatesynthatase (DHPS) enzymes (Hyde, 2002), led to the use of artemisinin based drugs (WHO, 2006). The first line drug for uncomplicated malaria in Kenya is artemether-lumefantrine and for severe complicated cases is quinine (WHO, 2006). Artemisinin acts rapidly on asexual blood stages particularly early forms, hence lowering the number of parasites that will mature and sequester in micro capillaries of vital organs. Also by acting on gametocytes, they limit transmission to new hosts and slow down the spread of resistant forms. This is different from CQ where drug resistant parasites have been found to have higher gametocyte density, favouring their transmission over sensitive strains (Arav, 2005). The value of drug combinations, notably those with artemisinin derivatives is widely adopted in order to improve efficacy, delay development of the disease and select the drug-resistance parasites thus prolonging their therapeutic life (Madrid *et al.*, 2005).

However, artemisinin resistance has also been reported in murine models of malaria (Arjen *et al.*, 2009). Artemisinin resistance to P. falciparum, which is now prevalent across mainland Southeast Asia, is associated with mutations in kelch13. Prolonged courses of artemisinin-based combination therapies are currently efficacious in areas where standard 3-day treatments are failing. The use of diagnostic tests prior to medication prevents resistance by avoiding unnecessary drug usage, leads to large savings and improves malaria surveillance (WHO, 2010). There is need to eliminate exposure of the parasites to sub-therapeutic drug doses in order to avoid reducing therapeutic life of the drugs. If treatments lose their effectiveness, human cost from malaria will inevitably continue to rise. Tetracyclines are active against tissue and blood forms although clinical application is

limited due to their toxicity, particularly foetal and infant, and also normal bowel flora suppression (WHO, 2010).

Simple and fast tests that detect when parasites get resistant to front line anti- malarial drugs have been developed and are an important step in monitoring. These tests are taken together and enable monitoring and countering plasmodium's ability to adapt to every new drug. They make it possible to quickly determine if a person has a form of malaria that's resistant to artemisinin, the most potent anti-malaria drug currently available. Also map the spread of resistant malaria parasites in entire communities and regions so that control of infected mosquitoes can be focused. Additionally they pinpoint artemisinin-resistant malaria parasites so scientists can identify the responsible genes and develop new drugs to get ahead of resistance. These are better than the method of administering drugs and monitoring patient's blood at six hour intervals which is cumbersome for both patients and clinicians. One way to test how a patient's parasites respond to artemisinin, the blood is taken and treated with artemisinin in a test tube for six hours, then after washing out the drug and incubating parasites for 66 hours parasitaemia is determined. A more elaborate test involves testing resistance on ring-stage parasites. Once fresh erythrocytes are infected and ring stages form they are exposed to the drug and those that get resistant can be studied to determine the mechanism involved and avail information on genetic markers which will facilitate tracking the spread of resistant parasites.

2.5 Distribution of Malaria Vector in Nigeria

Correct analysis of the distribution of specific malaria vectors is one of the prerequisites for meaningful epidemiological studies and for planning and monitoring of successful malaria control or eradication programmes (Gillies and Coetzee, 1987). Many Anopheles species has been reported in Nigeria. *An. gambiae* and *An. funestus* complexes has been reported as the two major *Anopheles* species in Southern Nigeria that are vectors of malaria with *An.*

moucheti and An. nili (Oyewole et al.,2007; Gillies and Coetzee 1987). The An. gambiae group consists of at least seven species which includes An. gambiae and An. arabiensis which are good vectors of malaria and are known to coexist in most part of West Africa (Gillies and Coetzee, 1987). In Northern Nigeria, An. gambiae was reported as the only Anopheles species in Sokoto metropolis (Abdullahi et al., 2010) also An. gambiae, An. arabiensis and An. funestus was reported as the mosquito species in Kastina metropolis, Katsina state (Bunza et al.,2010), while in Yola, the dry season mosquito collection for anophelines were; An. gambiae complex (64 %), An. funestus complex (17 %), An. pharoensis (9 %) and An. rhodesiensis (5 %) and the wet season collections were; An. gambiae complex (56 %), An. funestus complex (19.6 %), An. pharoensis (11.4 %) and An. rhodesiensis (7 %) (Umaru et al., 2006).

In a survey of mosquito in mid-western Nigeria, 3 *Anopheles* species (*An. gambiae*, *An. Pseudopunctipennis* and *An. funestus*) was reported (Okogun *et al.*, 2005). In a study at Ajumoni Estate, a peri urban area of Ogun state, Southwest, Nigeria, Anopheles mosquito constituted 18.85% of all captured mosquito (Ajayi *et al.*, 2010). Two Anopheles species; *An. gambiae* and *An. funestus* were reported in Enugu, Southeast Nigeria (Onyido *et al.*, 2009).

There are many factors that affect the control of malaria in Nigeria. This varies from region to region, depending on human knowledge, attitude and method of control. Ignorance as well as illiteracy (especially among rural dwellers) and financial impoverishment are part of these factors (Adeneye *et al.*, 2007). Studies have revealed that human knowledge, attitude and adoption of the various recommended applicable methods of personal and household protection against mosquito vary remarkably in different endemic regions of tropical countries (Govere *et al.*, 2010; Joshi and Banjara, 2008).

2.6 Challenges of Malarial Control in Nigeria

The major challenges to malaria control and prevention intervention are basically grouped into behavioural and non-behaviour factors. The behavioural factors relate to cultural practices which promote mosquito breeding and mosquito access to the people as well as failure of the risk populations to use technologies proven to be effective for the treatment, control and prevention of malaria promptly and appropriately. The main non-behavioural factors include geographical or ecological peculiarities, which also includes the tropical and subtropical condition; rainfall, high humidity and relative high temperature, the availability of mosquitoes and the presence of plasmodia (RBM, 2005). In Nigeria, some factors that are actively contributing to the resurgence of malaria include; (1) Rapid spread of resistance of malaria parasites to chloroquine and other quinolines (2) Frequent armed conflicts and civil unrest (3) High Vector abundance and transmission potential caused by climate changes as well as water development projects including dams and irrigation (4) Poverty (5) Misconceptions about Malaria (6) Counterfeit and substandard drugs and Lack of access to good health care systems (7) Low Rate of Insecticide treated Net ownership and Use.

1. Anti-malarial Drug Are Becoming Less Effective as the *Plasmodium* Parasite Develops Resistance to Common Drugs Resistance to drugs like artemisinin (a vital component of drugs used in the treatment of *P. falciparum* malaria) has been reported in a growing number of countries in Africa (WHO, 2000), pyrethroids, and the insecticides used in ITNs has been reported in 27 countries in Africa and 41 countries worldwide of becoming less effectives (Adhanom *et al.*, 2006). Unless properly managed, such resistance potentially threatens future progress in malaria control in Nigeria.

2. Displacement of a Population Due to Communal Clashes, Conflicts and Insurgency Large non-immune populations to endemic areas, resettlement of refugees to deteriorated environments that favour vector breeding (e.g., inadequate sanitation, marginal land), disruption of disease control programmes, breakdown of health systems (Garfield *et al.*, 1987; Beyrer *et al.*, 2007), and impeded access to populations for timely delivery of medical supplies (Prothero, 1994; Levy and Lidel 2002). There is virtually no city in Nigeria that is not affected by communal clashes leading to a breakdown of health systems and impedes efforts in combating malaria.

3. Favorable Climatic Condition for Vector Breeding Tropical areas such as Nigeria have the best combination of adequate rainfall, temperature and humidity allowing for breeding and survival of Anopheles mosquitoes. Temperature is an important factor which through its effect on the development of the malaria parasite and the vector greatly influences the geographical distribution of malaria transmission in general and malaria parasite species in particular. The development of P. falciparum in the female adult Anopheles requires a minimum temperature of 20°C whereas the other human malaria species can develop at temperature down to a minimum of 16°C. Higher than the minimum temperature, the development of the parasite in the vector accelerate with increasing Temperature (Gardon and Lavoipere, 1976).

4. Financial Status also Contributes to the Less Effective Control and Prevention of Malaria in Nigeria (Worral *et al.*, 2003; Breman *et al.*, 2004) At the household level, poor housing exposes people to contact with infective mosquitoes, as insecticide treated nets are unaffordable to the poorest if they must pay for them, and lack of resources prevents people from seeking timely healthcare (FMOH, 2005). Studies have revealed that a substantially higher prevalence of malaria infection occurs among the poorest population group (Bennet and Gilson, 2001), and that the poorest were most susceptible to contracting malaria (Njau *et al.*, 2006).

5. Lack of Knowledge about the Causes and Control of Malaria Misconceptions about the cause of malaria are reported in researches from all over the globe (Wakgari *et al.*, 2008). A study in Benue state, Nigeria showed that residents of both urban and rural areas still have misconceptions about the cause of malaria. Some attributed malaria to spirits/charm, poor nutrition and stress (Onah *et al.*, 2011). These are major socio-cultural setbacks in malaria treatment and control. All these contribute to the discrepancies in health seeking behavior and may cause delay in seeking appropriate treatment.

6. Availability and Access to Standard Health Care System and Drugs Lack of good roads to the health centers, poorly equipped centers, inadequate drugs for malaria treatment, substandard antimalarial medicines and as well as available ratio of patients to a doctor is alarmingly high. As a result of this, this is encouraging patients to seek treatment from unauthorized local service providers, which often lead to further complications.

7. Insecticide Treated Nets (ITNs) and Its Use Prevalence of mosquito net ownership varies greatly by residence and region. According to the 2003 Nigeria Demographic and Health Surveys (NDHS), only 12 % of households reported owning at least a net while 2 % of households report that they own an ITN (NDHS, 2003). Similarly in the 2008 (NDHS), data collected on measures to prevent malaria, shows that 17 % of household nationwide own at least a net of any type, while 8% own at least an ITN. This shows that ownership of mosquito nets is not widespread in Nigeria. Financial status, unavailability, body reaction, alternative barriers and ignorance also affects the wide spread of ITNs in Nigeria (Oyedeji *et al.*, 2009). The success of malaria control with ITNs has been bogged down by problems of delivery, distribution, usage and even acceptability of this method in Nigeria (Allessandro, 1997). Public awareness and acceptance of insecticide treated nets varies from community to community in countries where this method of malaria control has been adopted.

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Figure 2.2: Anopheles Mosquito Feeding (WHO, 2010)

2.7 Spider

Spiders are the most successful venomous animals with an estimated 100,000 extant species (Lucas, 2003). The vast majority of spiders employ a lethal cocktail to rapidly subdue their prey, which are often many times their own size. However, despite their fearsome reputation, less than a handful of these insect assassins are harmful to humans (Park, 2002; Hill *et al.*, 2006). Nevertheless, it is this small group of medically important species that first prompted scientists more than half a century ago to begin exploring the remarkable pharmacological diversity of spider venoms.

Amongst the ranks of animals that employ venom for their survival, spiders are the most successful, the most geographically widespread, and arguably consume the most diverse range of prey. Although the predominant items on a spider's dinner menu are other arthropods, larger species will readily kill and feed on small fish, reptiles, amphibians, birds, and mammals. Thus, spider venoms contain a wealth of toxins that target a diverse range of receptors, channels, and enzymes in a wide range of vertebrate and invertebrate species.

Spider venoms are complex cocktails composed of a variety of compounds, including salts, small organic molecules, peptides, and proteins (WHO, 2001; FMOH, 2005; WHO, 2005; Bawah and Binka, 2005; Alanis, 2005). However, peptides are the primary components of spider venoms, and some species produce venom containing >1000 unique peptides of mass 2–8 kDa (David, 2017). Based on the number of described spider species and a relatively conservative estimate of the complexity of their venom it has been estimated that the potential number of unique spider venom peptides could be upwards of 12 million (United Nations country team, 2004). In recent years there has been an exponential increase in the number of spider-toxin sequences being reported (WHO, 2004) due to the application of high-throughput proteomic (Lawn et al., 2005; Adam, et al., 2005) and transcriptomic (Steketee, et al., 2001; Adhanom, et al., 2006) approaches, or a combination of these methods (David, 2017). In the last 18 months alone the number of toxins in the ArachnoServer spider-toxin database (Gillies and Coetzee, 1987) has more than doubled, and is now excess of 900 (see http://www.arachnoserver.org/). Nevertheless, our knowledge of the diversity of spider-venom peptides is still rudimentary, with less than 0.01% of potential peptides having been isolated and studied.

Although only a small number of spider venom peptides have been pharmacologically characterized, the array of known biological activities is impressive (David, 2017). In addition to the well-known neurotoxic effects of spider venoms, they contain peptides with

antiarrhythmic, antimicrobial, analgesic, antiparasitic, cytolytic, haemolytic, and enzyme inhibitory activity. Furthermore, the crude venom of *Macrothel eraveni* has antitumor activity, for which the responsible component has not yet been identified (Gillies and Meillon, 2013; Abdullahi, *et al.*, 2010). Finally, larger toxins such as the latrotoxins from the infamous black widow spider (*Latrodectus mactans*) and related species induce neurotransmitter release and they have played an important role in dissecting the process of synaptic vesicle exocytosis (Bunza, *et al.*, 2010).

Since spiders employ their venom primarily to paralyse prey, it is no surprise that these venoms contain an abundance of peptides that modulate the activity of neuronal ion channels and receptors. Indeed, the majority of characterized spider-venom peptides target voltage-gated potassium (K_V) (Umaru, *et al.*, 2006) calcium (Ca_V) (Okogun, *et al.*, 2005; Ajayi *et al.*, 2010), or sodium (Na_V) (Okogun *et al.*, 2005; Onyido, *et al.*, 2009) channels. More recently, novel spider-venom peptides have been found that interact with ligand-gated channels (e.g., purinergic receptors (Adeneye, *et al.*, 2007)) and recently discovered families of channels such as acid sensing ion channels (Govere, *et al.*, 2010), mechanosensitive channels (Joshi and Banjara, 2008), and transient receptor potential channels (Oladepo *et al.*, 2010). Not only do most of these peptides have selectivity for a given channel, they can have anything from mild preference to exquisite selectivity for a given channel *subtype*. This potential for high target affinity and selectivity makes spider-venom peptides an ideal natural source for the discovery of novel therapeutic leads RBM, 2005.

Despite the advent of automation and the rise of high-throughput and high-content screening in the pharmaceutical industry there has been a sharp decline in the rate of discovery and development of novel chemical entities (WHO, 2000; Adhanom *et al.*, 2006). We recently reviewed the emerging role that venom-derived components can play in addressing this decline with an emphasis on technical advances that can aid the discovery process (Garfield *et al.*, 1987). It is worth noting that, as of 2008, two of the 20 FDA-approved peptide pharmaceuticals were derived from animal venoms (*i.e.*, ziconitide and exendin-4) (Beyrer *et al.*, 2007). In this review we specifically examine the structure, targets, and mechanisms of action of spider-venom peptides with potential therapeutic applications.

2.7.1 Diversity of Spiders

Spiders (Order Araneae) are considered the seventh largest arthropod group (David, 2007) found in all continents, with fossils dating back to about 380 million years ago and having a current diversity of over 42 751 described species placed in 3, 859 genera and 110 families (Gillies and Meillonn,2013). Spiders stand out because of their ecological importance as the dominant non-vertebrate predators in most terrestrial ecosystems (Abdullahi *et al.*, 2010). Spiders can be divided into two groups depending on how they capture their prey. Web-building spiders construct webs in undisturbed habitats to capture their prey. They live in or near their web and wait for the prey to come to them. They rely on sensing vibrations in their web to detect prey. Hunting spiders do not construct webs to capture their but, instead, they rely on speed and eyesight to chase and capture prey. These hunters may be divided into active hunters search for and chase their prey and passive hunters which lie in wait and seize their prey as it approaches.

2.7.2 Spiders as Predators of Mosquitoes

Spiders have largely been overlooked as predators of mosquitoes and its larvae in various ecosystems, yet they play an important role as stabilizing agents or regulators of insect populations in agro-forest and other terrestrial ecosystems (Umaru *et al.*, 2006). Their presence in an ecosystem may well influence the population dynamics of other arthropods present (Okogun *et al.*, 2005). Ferguson et al., (1984) (Ajayi *et al.*, 2010) and Whitmore et al., (2002) (Onyido *et al.*, 2009) reported that spiders had also been found to have great

potential to serve as biological control agent against crop pests. Several studies have shown that insect populations significantly increase when released from predation by spiders (Gillies and Meillon, 2013; Park, 2002) described the life span of most spiders ranging from 8 to 10 months and their life cycles mostly synchronises with the periods of life cycles of mosquitoes, as both of them prefer humid and warm environments. A study conducted in Bharatpur, District of Rajasthan, India on spider families that fed on mosquitoes showed the predatory potential of seven spider families on larvae and adults of mosquitoes. The spider's families included Araneidae, Salticidae, Pholcidae, Oxyopidae, Tetragnathidae, LycosidaeandPisauridae (Umaru *et al.*, 2006) (Table 2). It was observed that the families Tetragnathidae, Lycosidae, Pisauridae and Trechaleidae fed on mosquito larvae while species of spiders from Araneidae, Salticidae, Pholcidae and Oxyopidae families predated on flying mosquitoes. Their results also showed the rank wise sequence of the spider families based on the consumption of larvae and adults of mosquitoes, as follows: On the mosquitoes

larvae:Lycocidae>Tetragnathidae>Pisauridae>Salticidae>Araneidae>Oxyopidae>Salticae. On the adult mosquitoes:

Salticidae>Araneidae>Oxyopidae>Pholcidae>Lycocidae>Pisauridae>Tetragnathidae. (Adeneye, *et al.*, 2007), (Govere, *et al.*, 2010; Joshi and Banjara,2008), investigated the prey-capture behaviour of *Evarcha culicivora*, an East African mosquito-eating jumping spider, on two mosquito species, *Anopheles* gambiae sensustricto and *Culex quinquefasciatus*. When tested with live mosquitoes, small juveniles of *E. culicivora* were observed to be more effective at capturing Anopheles than *Culex*. Large juveniles of *E. culicivora* were more effective than small juveniles at capturing *Culex*, but large and small juveniles had similar success at capturing Anopheles. Moreover, they observed that *Culex* and chose sugar-fed Anopheles significantly more often than sugar-fed *Culex*, and chose sugar-fed Anopheles significantly more often than midges. Oladepo, *et al.* (2010) discovered that *E. culicivora* preyed on mosquitoes, especially females engorged after a blood meal. Generally, salticids like *E. culicivora* have unique, complex eyes and an ability to see prey in remarkably fine detail (RBM, 2005; WHO, 2000). Furthermore, (Adhanom, *et al.*, 2006), have pointed out that vision is not the only sensory modality that *E. culicivora* uses in identifying its prey but olfaction as well. These spiders use their olfaction to discern if a mosquito has just taken a blood meal based on the tilt of their abdomens, and then they pounce. This behaviour, according to (Garfield, *et al.*, 1987), is unique and no other animal targets its prey based on what that prey has eaten. Furthermore, by eating blood-filled mosquitoes, these spiders of both sexes seem to acquire a "perfume" that makes them more attractive to potential mates. That means they kill blood-carrying mosquitoes for food as well as for sex.

Role of spider venom in controlling Plasmodium Not only do spiders help reduce incidents of malaria through preying on larva and adults of mosquitoes but even their venom has also emerged as a promising important pharmacological tool against the malarial parasite (Njau *et al.*, 2006). The venom of some spiders have been observed to possess antiplasmodial activity against the intra-erythrocyte stage of *Plasmodium falciparum*. Wakgari *et al.* (2008) isolated psalmopeotoxin I (PcFK1), a 33-amino-acid residue peptide from the venom of the tarantula spider *Psalmopoeus cambridgei*. They observed that it possessed strong in vitro anti-plasmodial activity against the intra-erythrocyte stage of *Plasmodium falciparum*. What they discovered most important was that the peptide did not lyse erythrocytes and was not cytotoxic to nucleated mammalian cells and did not inhibit neuro-muscular function. However, Onah, *et al.* (2011) advise that it is not sufficient to predict the target of this peptide in the infected red blood cells, owing to the unusual biology of *P. falciparum* and lack of completely elucidated metabolism and changes in the infected red blood cells. Perhaps it would be important to carry out further investigations to help understand the unique mechanism of action of this peptide and to enhance its utility as a new antimalarial drug against the intra-erythrocyte stage of the malaria parasite.

2.7.3 Spider Toxins with Analgesic Potentials

Normal nociceptive pain is a key adaptive response that limits our exposure to potentially damaging or life-threatening events. In contrast, aberrant long-lasting pain transforms this adaptive response into a debilitating and often poorly managed disease. About 20 % of adults suffer from chronic pain, a figure that increases to 50 % for those older than 65 (Brennan and Carr, 2007). In 2007, global sales of pain medications totaled \$34 billion, highlighting the pervasive nature of this condition. Nevertheless, there are few drugs available for treatment of chronic pain, and many of these have limited efficacy and significant side-effects. Recently, a number of ion channels have been shown to be critical players in the pathophysiology of pain, and in many cases the most potent and selective blockers of these channels are spider-venom peptides. Here we review some of these peptides with promise as drug leads or as analgesics in their own right. Modulators of Acid Sensing Ion Channels. Acid sensing ion channels (ASICs) are proton-gated sodium channels that open in response to low pH. They belong to the epithelial sodium channel/degenerin (ENaC/DEG) superfamily of ion channels which have the same overall topology and selectivity for transporting sodium (Kallenberger and Schild, 2002). However, ASICs are distinguished by their restriction to chordates, their predominantly neuronal distribution, and their activation by decreases in extracellular pH (Grunder and Chen, 2010). To date, seven ASIC subunits have been identified: ASIC1a, ASIC1b, and ASIC1b2 (splice isoforms from the ASIC1 gene), ASIC2a and ASIC2b (splice isoforms of the ASIC2 gene), ASIC3 and ASIC4. Functional ASIC channels comprise either homomeric or heteromeric

trimers of these subunits. ASIC2b and ASIC4 are insensitive to protons and do not form homomeric channels, but rather are incorporated into heteromeric channels and may modify the kinetics of channel activation and inactivation. The different combinations of subunits allow the different trimeric channels to sense a wide range of extracellular pH changes.

ASIC1a is the most abundant ASIC subunit in the central nervous system (CNS) and it has the highest affinity for protons (Wemmie *et al.*, 2006). It has been implicated as a novel therapeutic target for a broad range of pathophysiological conditions including pain, ischemic stroke, depression, and autoimmune and neurodegenerative diseases such as multiple sclerosis, Huntington's Disease, and Parkinson's Disease (Wemmie *et al.*, 2006; Xiong *et al.*, 2008; Shika *et al.*, 2009). Inhibitors of ASIC1a might therefore be therapeutically valuable for some of these conditions. The only potent and specific inhibitor of ASIC1a that has been identified to date is π -theraphotoxin-Pc1a (π -TRTX-Pc1a; also known as psalmotoxin-1 (PcTx1)), a 40-residue ICK peptide isolated from the venom of the Trinidad chevron tarantula *Psalmopoeus cambridgei*. π -TRTX-Pc1a inhibits homomeric ASIC1a channels, but not other ASIC subtypes, with an IC₅₀ of 0.9 nM. π -TRTX-Pc1a was shown to be an effective analgesic, comparable to morphine, in rat models of acute pain (Mazzuca *et al.*, 2007) and peripheral administration of this peptide resulted in neuroprotection in a mouse model of ischemic stroke even when administered hours after injury (Pignataro *et al.*, 2007).

 π -TRTX-Pc1a is only effective when administered intrathecally or by intracerebroventricular injection (Mazzuca *et al.*, 2007). Thus, native π -TRTX-Pc1a is unlikely to be a clinically useful analgesic except in the most chronic pain sufferers as intrathecal administration is an invasive method of drug delivery with inherent risks (Smith *et al.*, 2008). As for Prialt®, a peptide from cone snail venom that was recently approved for the treatment of chronic pain (Kress *et al.*, 2009), intrathecal π -TRTX-Pc1a use would
likely be limited to management of severe chronic pain in patients who are intolerant or refractory to other treatments. Thus, there is much interest in developing mimetics of π -TRTX-Pc1a that might be orally active or at least deliverable via subcutaneous or intramuscular injection. Thus, several attempts have been made to model the π -TRTX-Pc1a:ASIC1a interaction (Qadri *et al.*, 2009; (Pietra, 2009) with a view to providing a template that can be used for *in silico* screening and/or rational design to develop small-molecule mimetics of π -TRTX-Pc1a. Thus, even in cases where a spider-venom peptide itself may not be a viable therapeutic, it can still be an invaluable tool for target validation and for providing a pharmacophore for rational drug design.

Voltage-gated sodium (Na_V) channels provide a current pathway for the rapid depolarization of excitable cells that is required to initiate an action potential. Functional channels are composed of a pore forming α subunit whose gating and kinetics is modified via association with one of four β subunits. The α subunits are classified into nine different subtypes, denoted Na_V1.1 to Na_V1.9 (Yu and Catterall, 2003), and they are further characterized by their sensitivity to tetrodotoxin (TTX). Na_V1.5, Na_V1.8 and Na_V1.9 are TTX-resistant whereas all other subtypes are TTX-sensitive.

Of the nine Na_V subtypes, Na_V1.3, Na_V1.7, and Na_V1.8 are involved in pain signaling (Krafte and Bannon, 2008). However, in recent years, Na_V1.7 has emerged as perhaps the best validated pain target based on several remarkable human genetic studies. Gain-of-function mutations in the gene encoding the α subunit of Na_V1.7 (*SCN9A*) underlie two painful neuropathies known as paroxysmal extreme pain disorder (PEPD) and inherited erythromelalgia (IE) (Yang *et al.*, 2004;Fertleman *et al.*, 2006), whereas loss-of-function mutations in *SCN9A* result in a *congenital indifference to all forms of pain* (Cox *et al.*, 2006; Goldberg *et al.*, 2007). Remarkably, apart from their complete inability to sense pain, partial loss of smell (hyposmia) is the only other sensory impairment in individuals with this

channelopathy (Nilson *et al.*, 2009); they have no motor or autonomic dysfunction, with normal blood pressure and temperature regulation. Nav1.7 is located at the terminal of sensory neurons, where it is ideally positioned to serve its proposed role as a threshold channel that amplifies pain signals transmitted above a certain level.

The preferential expression of Nav1.7 in peripheral sensory and sympathetic neurons makes it an ideal target for novel analgesics. Indeed, it is probable that the known analgesic effects of a number of nonspecific Nav channel blockers such as the local anaesthetic lidocaine, tricyclic antidepressants such as amitriptyline, and anticonvulsants such as carbamezepine are at least in part mediated through their effects on Nav1.7. However, the nonspecific block of Nav channels by these drugs means that they are only efficacious at or near toxic levels, with numerous CNS-related side-effects such as dizziness and ataxia (Krafte and Bannon, 2008). Thus, *subtype-specific* blockers of Nav1.7 are likely to be useful drugs for treatment of chronic pain as well as inherited neuropathies such as IE and PEPD (Krafte and Bannon, 2008). Recent studies have revealed that spider venoms may provide an excellent source of such subtype-specific blockers.

2.7.4 Antibacterial and Antifungal

The introduction of antibiotics in the 1930s and 1940s was in large part responsible for the dramatic decline in the mortality rate from communicable diseases in developed countries. However, bacteria are remarkably proficient at adapting to environmental stresses, and they have evolved at least one mechanism of resistance for all 17 classes of antibiotics that have been developed to date (Alanis, 2005). The recent widespread emergence of antibiotic resistance in clinically important bacterial pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Enterococcus faecalis*, combined with a dramatic decrease in the rate of development of new antibiotics, has led some to suggest that we may be approaching the post-antibiotic era (Alanis, 2005). While this may overstate the problem,

there is nevertheless an urgent need to develop new antimicrobials with novel mechanisms of action.

The recent successful introduction of the lipopeptide antibiotic daptomycin (Robbel and Marahiel, 2010) has rekindled interest in antimicrobial peptides (Vooturi and Firestine, 2010). To date, 40 membrane-acting antimicrobial peptides (MAMPs) have been isolated from the venom of four different families of araneomorphs, suggesting that antimicrobial activity is widespread in this infraorder of spiders. These MAMPs often have a wide range of antimicrobial activities, with some toxins active against Gram-positive and Gramnegative bacteria as well as fungal pathogens such as *Candida albicans* (Yan and Adams, 1998). Some MAMPs also have anti-trypanosomal activity (Kuhn-Nentwig *et al.*, 2002). Curiously, no MAMPs have thus far been isolated from the venom of mygalomorph spiders. It is possible that MAMPs were recruited into araneomorph venoms following the split from mygalomorphs around 280 million years ago (Ballweber *et al.*, 2002).

MAMPs differ from most other spider-venom peptides in their structure and mode of action. Rather than utilizing the ICK fold common to most spider toxins, MAMPs are α -helical amphipathic peptides that interact with and perturb cell membranes to yield their antimicrobial effects (Kuhn-Nentwig, 2003; Nonuura and Carzo, 2006; Pukala *et al.*, 2007; Dubovskii *et al.*, 2008). This mode of action can be potentially problematic from a therapeutic perspective since MAMPS that interact nonspecifically with cell membranes are cytolytic (Vorontsova *et al.*, 2010). Indeed, this property is likely to be the basis of their biological function in spider venoms. Although it has been proposed that MAMPS might protect the spider's venom apparatus against infection (Kuhn-Nentwig, 2003) their primary role is more likely to be as membrane disrupting agents that augment the activity of the disulfide-rich neurotoxic peptides by facilitating their spread (Carzo et al., 2002; Kuhn-Nentwig *et al.*, 2004). It has been shown that the cytolytic activity of at least some spider-venom MAMPs can be minimized by truncation without significantly disrupting their antimicrobial activity (Adao *et al.*, 2008). Nevertheless, the therapeutic use of these peptides is likely to be limited by their inherent susceptibility to proteolysis, which is likely to result in short gut and plasma half-lives. Whether this problem can be solved by strategies such as cyclization or grafting key sequence elements onto more stable ICK scaffolds (Henriques and Craik, 2010)] remains to be seen.

2.7.5 Antimalarial

The antimicrobial action of spider toxins is not limited to bacteria and fungi, but also extends to the malaria parasite. There were 243 million cases of malaria in 2008, resulting in a death every 35 seconds, and most of these were children under the age of five (WHO, 2009). Malaria is caused by *Plasmodium* infections spread by female anopheline mosquitoes. There are five genera of *Plasmodium* that cause malaria, with *Plasmodium falciparum* being the most virulent. Widespread resistance to chloroquine has made this drug largely ineffective for treating *Plasmodium falciparum* in high-transmission areas and few cheap alternatives are available (Enayati and Hemingway, 2010).

U₁-TRTX-Pc1a (Psalmopeotoxin I) and U₂-TRTX-Pc1a (Psalmopeotoxin II) are ICK peptides isolated from the venom of the Trinidad chevron tarantula *Psalmopoeus cambridgei* that are effective against the intra-erythrocyte stage of *Plasmodium falciparum*. Interestingly, this is the same spider from which π -TRTX-Pc1a, the most potent known blocker of ASIC1a, was isolated, indicating that a single spider can provide multiple therapeutic leads. U₁-TRTX-Pc1a and U₂-TRTX-Pc1a are unrelated peptides that comprise 33 and 28 residues, respectively. They inhibit intra-erythrocyte development of *Plasmodium falciparum* with ED₅₀ values of 1.1–1.6 µM but, unlike most MAMPS, they do not have hemolytic, antibacterial or antifungal activity. The mode of action of these

peptides is unknown. It seems unlikely that they directly target the malaria parasite since this would require the peptides to traverse both the erythrocyte membrane as well as the parasitophorous vacuolar membrane that encapsulates the parasite. One possibility is that these toxins target the new permeability pathways that are established in the erythrocyte membrane following parasite invasion (Straines *et al.*, 2005). Thus, in addition to being useful therapeutic leads, these peptide toxins might help validate a new anti-malarial drug target.

CHAPTER THREE

3.0

MATERIALS AND METHODS

3.1 Description of the Study Area

The study was within Minna, Niger State which is one of the major growing states in central Nigeria, located in latitude of 3.2° east and 8.0° longitude. Minna which is the capital of Niger state enjoys a climate typical of the middle belt zone. The rainy season lasts between 190-200 days (in May – October,) Mean annual rainfall is 133mm (52 inches) with September recording the highest amount of 300mm (11.7 inches). The mean monthly temperature is highest in March at 30.50° (85^o) and lowest in August at 22.30^o C (72^oF).

3.2 Source and Collection of Spider Specimens

The spiders were collected from their natural habitat twice a week from 7.00am -10.00am, using vegetation beating and sweep netting method as described by (Ferenc and Csaba. 2002), the specimen were preserved in 70% methanol and taken to the Department of Animal Biology Laboratory for further identification. Number of spider found in each habitat was recorded. The identification of the spider was done according to (Milind, *et al.*, 2002).



Figure 3.1: The Spider (*Neoscona adiant*) Sample (WHO, 2018)

3.3 Source, Collection and Maintenance of Mosquitoes

The *Culex spp* mosquito larvae for the study was obtained from the wild in Bosso area of Minna, Niger state, at their 1st and 2nd instar larval stage, and maintained in the Laboratory of the Department of Animal Biology, Federal University of Technology, Minna. Laboratory handling and maintenance of the mosquitoes was follow using standard procedures of $2.00 \pm 2.50^{\circ}$ C, $75.00\pm9.00\%$ relative humidity and 12:12 light: darkness photo-period (Olayemi and Ande, 2009). The 4th instar stage was used for the larvicidal bioassay.

3.4 Larvicidal Bioassay

Bioassay was performed against 4th instar larvae of *Culex spp* complex mosquitoes, according to the standard method for testing the efficacy of bioinsecticides (WHO, 2005), with slight modification. Stock solution was prepared by adding 1g of the extract into 10ml solvent of the extraction. From the stock solution, 2ml was taken and dissolved in 18ml

distilled water; thereafter, graded concentrations of both the crude extract and fractions was then be prepared to obtain 0.4, 0.6, 1.0 and 1.2mg/L, for crude and 0.1, 0.2, 0.3, 0.4 and 0.5mg/L for the fractions, respectively, in the final volume of 100ml distilled water. Batches of 20 healthy 4th instar larvae of the *Culex* mosquitoes was separately exposed to each extract graded concentration assay medium. Five replicates which comprise 20 larvae each was used in the experiment. A control experiment, containing only 1ml of the solvent for each assay in 100ml of distilled water, was set up. The experiment was also repeated twice and conducted under laboratory condition at 25-30 $^{\circ}$ C and 80-90 % relative humidity. Mortality of the larvae was noted and recorded after 24 hours exposure period.

3.5 Acute Oral Toxicity Test

The acute orals toxicity of the spider crude extract was determined by evaluation, its Median Lethal Dose (LD₅₀) using Lorke's method (Lorke, 1983) with slight modification. The experiment was carried out in two phases. In Phase 1, Nine (9) mice was randomly divided into three groups consisting of three mice each. The three groups was administered orally with the spider crude methanolic extract graded concentrations of 10, 100 and 1000mg/kg body weight respectively. Similarly, in the second phase, nine mice was divided randomly into three groups with each group consisting of three mice. These groups was receive extract graded concentrations of 1000, 2900 and 5000mg/kg body weight of the extract respectively. The mice was handled according to WHO procedure (WHO, 2018).

3.6 In Vivo Anti-malarial Assay of Crude and Fractioned Extracts

Thirty (30) Swiss albino mice of known white were distributed into six groups, each group comprising five mice. The first group (normal control) was not infected with *Plasmodium berghei*, while other groups was infected intraperitioneally with an aliquot of 0.2ml of standard inoculums (1 x 10^7) *Plasmodium berghei* strain NK 65 parasitized erythrocytes. The second group was not treated (negative control). The third group was infected with the

parasite and treated with5mg/kg body weight of chloroquine (positive control), the fourth, fifth and sixth groups was infected and treated with 150, 300 and 600mg/kg body weight of n-Hexane extract of the spider venom. All treatments was orally administered once daily for five consecutive days. Blood was taken from the tail vein of the mice before treatment once daily to assess the parasitemia levels. All the fractions was subjected to bioassay as stated above using 150 and 300mg/kg body weight for each extract.

3.7 GC-MS Analysis

Gas Chromatography/Mass Spectra (GC/MS) analysis for identifying difference substances within the spider extracts was carried out on QP2010 PLUS SHIMAZU at the Biotechnology Research Laboratory in ABU, Zaria using David Methods (2017).

3.8 Determination of Packed Cell Volume (PCV)

The capillary tubes was filled with blood to about 1cm or two-third (2/3) of it length and the vacant end of each of the capillary tubes was sealed by plastic seal or sealer to protect the blood level from spilling. The tubes was placed in haematocrit centrifuge with seal side towards the periphery and then centrifuged for 5 - 6 minutes. The percentage of packed cell volume of haematocrit was read directly from haematocrit reader (Dacie, *et al.*, 1991).

3.9 Determination of Survival Time and Mean Weight

The survival time (in days) was recorded for all mice and the mean survival time of the extract groups was compared to the control treated group. Body weight of each mouse prior to inoculation, after infection and at the end of treatment was taken to see if there was body-weight change as an adverse effect of the extract treatment.

3.10 Data Analysis

Data obtained was analyzed using SPSS software (Version 23.0). Numerical data was presented as mean \pm standard error of mean. Significant differences among treatment of

larvicidal bioassay and among groups of anti-plasmodial efficacy, as well as, records of larvicidal bioassay was determined using Analysis of Variance (ANOVA) coupled with Duncan Multiple Range Tests (DMRT). For the determination of medial (LD_{50}) and upper (LC_{50}) Lethal Concentration of the Crude and fractionated extracts inducing GC/MS analysis, probity linear regression analysis was employed. P. value < 0.05 was considered statistically significant. Analysis was carried out using Microsoft Excel 2010 and statistical package for social science, 20th version.

CHAPTER FOUR

4.0

RESULTS AND DISCUSSION

4.1 Zoochemical Constituents of the Crude and Fractions of Spider

The results of the Zoochemical constituents of the crude and fraction of spider venom are presented in Table 4.1. The results indicated that both the crude and solvent fractions contain bioactive contents including flavonoid, tannins, Saponins, alkaloids, and terpenoids. Steroid was absent in n-Hexane fraction but present in crude methanol and ethylaretate. Cardiac glycosides were absent in crude methanol and n-hexane but present in ethylacetate.

Phytochemical	Crude methanol	n-Hexane	Ethylacetate
Flavonoid	+	+	+
Tannins	+	+	+
Saponnins	+	+	+
Alkaloids	+	+	+
Steroid	+	-	+
Phlobatannins	+	+	+
Cardiac glycosides	-	-	-
Total phenol	+	+	+
Terpenoids	+	+	+

 Table 4.1: The results of the Zoochemical Constituents of the Crude and Fraction of

 Spider Venom

Keys += Present -= Absent

4.1.2 Acute Oral Toxicity Profile of the Crude Methanol Extract of Spider

The result of the acute oral toxicity profile of the crude methanol extract of spider is detailed in Table 4.2. The result indicated that, in both phase 1 and 2 where the experimental mice were subjected to graded concentrations of the crude methanol extract, no sign of mortality was observed in the mice. Also no mortality was recorded up to the dose of 5000mg/kg body weight. Thus, the LD₅₀ can be extrapolated to be above 5000 mg/kg body weight.

Phase	Dosage (mg/kg b. wt)	No. of mice/group	Mortality
1	10	3	0/3
	100	3	0/3
	1000	3	0/3
2	1600	3	0/3
	2900	3	0/3
	5000	3	0/3

Table 4.2: Acute oral Toxicity Profile of the Methanol Extract of Spider

The LD50 is thus extrapolated to be above 5000mg/kg body weight.

4.1.3 Parasite count (field⁻¹) of *Plasmodium berghei*-Infected Mice Treated with Crude Methanol Extract and Solvent Fractions of Spider

The result of the parasitaemia level of *Plasmodium berghei*- infected mice treated with crude and solvent fractions of spider is presented in Table 4.3. The result indicated that the spider extract showed antiplamodial activities in a dose dependent manner. The parasite level reduces similarly with increase in day of administration, on the 7th day the group treated with the standard drug showed complete parasite clearance. This was significantly (P<0.05) between than the antiplasmodial activities of other extract concentration. It was also found that the antiplasmodial activities recorded for the group treated with the 600mg/lg b.wt (2.14 \pm 0.75) was significantly better than those recorded for other extract concentration. On the other hand, the proparasitaernia recorded for the infected untreated group increased throughout the experimental period.

Table 4.3: Parasite count (field⁻¹) of *plasmodium berghei*-Infected Mice Treated with Crude Methanol Extract and Solvent Fractions of Spider Mean Parasite Count (\overline{X} unit + SE)

Extract	Dose mg/kg	Day 3	Day 4	Day 5	Day 6	Day 7	Day 10
Crude Methanol	150	21.32±0.56 ^a	19.21±2.14 ^c	18.41±1.98 ^c	14.10 ± 2.10^{d}	13.15±1.21 ^d	14.18 ± 1.17^{f}
	300	23.14±0.48 ^a	20.21±0.19 ^c	19.14±1.31°	14.12±1.39 ^d	8.41±0.09 ^c	6.12±1.16 ^d
	600	21.89±1.86 ^a	17.14±0.07 ^b	15.15±0.01 ^b	9.12±0.76 ^b	4.31±0.81 ^b	2.14±0.75 ^b
Ethyl acetate	150	23.15±1.14 ^a	18.12±0.03°	16.31±0.76°	11.21±0.14 ^c	8.31±0.12 ^c	8.16±0.01 ^e
	300	23.21±0.69 ^a	19.11±0.16 ^c	17.12±0.17 ^c	15.29±0.31 ^d	11.31±0.31 ^d	7.21±0.13 ^d
N-hexane	150	19.14±3.11 ^a	17.13±0.16 ^b	14.13±2.01 ^b	13.21±0.31 ^d	8.31±0.31 ^c	6.21±0.34 ^d
	300	23.21±0.03 ^a	17.01±0.08 ^b	15.31±0.12 ^b	12.16±0.31 ^c	7.14±0.14 ^b	3.21±0.31 ^c
Standard		21.01±2.09 ^a	14.17±0.14 ^a	8.21±0.01 ^a	1.04±0.03 ^a	0.00±0.00 ^a	0.00 ± 0.00^{a}
Untreated		22.14±1.11 ^a	23.14±0.03 ^d	27.14±0.17 ^b	29.14±0.17 ^e	32.14±0.01 ^e	38.01±0.01 ^d

Values are expressed in mean \pm standard error of three replicates. Values followed with n=>0.005

4.1.4 Mean PCV (%) level of *Plasmodium berghei*-Infected Mice Treated with Crude Methanol and Solvent Fractions of Spider

The results of the mean PCV (%) level of *plasmodium berghei*-infected mice after treating with crude and solvent fraction of spider is contained in Table 4.4. There was no significant difference P>0.05) in the mean PCV of all the experimental mice before infection. Thereafter, 72hours after infection of experimental mice (except the mice in the normal control group) with the *plasmodium berghei* infected, there was drastic loss in the mean PCV of all the infected mice. However, after treatment with the extracts and standard drug, the mean PVC (%) also dropped slightly. However the mean PCV (%) recorded for the infected untreated group was significantly lowest <P>0.05) compared to other group.

Extract	Dose	Mg/kg	Before	72 hrs	after	7 days after treatment
treatment	b.wt		infection	infection		
Crude methanol	150		45.31 <u>+</u> 0.01 ^a	42.15 <u>+</u> 0.03 ^b		41.31 <u>+</u> 0.03 ^b
	300		44.21 <u>+</u> 1.21 ^a	41.31 <u>+</u> 0.41 ^a		40.46 <u>+</u> 0.16 ^b
	600		45.81 <u>+</u> 31 ^a	43.21 <u>+</u> 1.35 ^b		41.38 <u>+</u> 09.41 ^a
Fraction	150		44.34 <u>+</u> 0.82 ^a	43.21 <u>+</u> 1.71 ^b		43.1 <u>+</u> 1.21 ^b
N-hexane						
	300		43.69 <u>+</u> 1.13 ^a	40.14 <u>+</u> .1.31 ^a		39.21 <u>+</u> 2.01 ^b
Ethyl acetate	150		44.14 <u>+</u> 0.76 ^a	41.31 <u>+</u> 1.31 ^a		41.21 <u>+</u> 1.21 ^b
	300		43.16 <u>+</u> 1.34 ^a	40.81 <u>+</u> 2.01 ^a		40.31 <u>+</u> 0.04 ^b
Standard			44.21 <u>+</u> 1.31 ^a	40.11 <u>+</u> 1.29 ^a		40.10 ± 0.56^{b}
Untreatment			45.12 <u>+</u> 0.21 ^a	41.23 <u>+</u> 0.06 ^a		36.21 <u>+</u> 0.01 ^a
Normal			43.21 ± 0.71 ^a	43.16 <u>+</u> 1.64 ^b		43.21 <u>+</u> 1.12 ^b

Table 4.4: Mean PVC (%) level of *plasmodium berghei*-Infected Mice Treated with Crude Methanol and Solvent Fractions of Spider Mean PVC ($\overline{X} = SE\%$)

4.1.5 Weight change of *plasmodium berghei*-Infected Mice Treated with Crude and Solvent Fraction of Spider

The results of the effects of crude and solvent fraction of spider on weight change of plasmodium berghei-infected mice is presented in Table 4.5. The result indicated that before infection, there were no significant difference $\langle p \rangle 0.05$) in the weight of the experimental mice. After 72hrs of inoculation/infection, the weight of the mice dropped significantly in all infected animals in the experimental group. However, after treatment, the infected treated animals with extract and standard drug, showed weight gain when compared with the infected untreatment group that showed a significant loss in weight throughout the study period.

Extract treatment	Dose Mg/kg b.wt	Before infection	72 hrs after infection	after treatment
Crude methanol	150	24.31 + 0.16 ^a	21.26 ± 0.02^{b}	23.14 + 0.76 ^b
	300	24.58 ± 0.31 ^a	22.01 +0.03 ^b	24.15 + 0.17 ^b
	600	23.13 + 0.21 ^a	$20.42{\pm}0.14^{a}$	23.16+0.45 ^b
Fraction	150	24.01 ± 0.24 a	$21.46 \pm 0.15_h$	2315 + 0.23 ^b
N- Hexane				
	300	25.12 + 0.59 ^a	21.31 +0.31 ^b	21.41 + 0.37 ^b
Ethyl acetate	150	24.01 ± 1.62 ^a	21.03 +0.01 ^b	23.16 + 1.04 ^b
	300	23.81 + 0.34 ^a	20.21 +0.03 ^a	22.37 ± 0.79^{b}
Standard		23.69 ± 0.08^{a}	20.03 +0.32 ^a	22.49. + 0.32 ^b
Urrtreatment		$24.14 \pm 1.21 \ ^a$	21.13 +0.13 ^b	18.14 ± 0.14 ^a
Normal		24.16 + 0.41 ^a	25.18 +0.04 °	25.34 ± 0.18^{b}

Table: 4.5: Weight change of *Plasmodium berghei* Infected Mice Treated with Crude and Solvent Fraction of Spider Average wt Change ($\overline{X} \pm SE$ unit)

4.1.6 Effect of Crude Methanol Extracts and Solvent Fractions of Spider on Haemotological Variables of *Plasmodium bergheri*-Infected Mice

The results of the effect of crude and fractionated extracts of spider in *Plasmodium berghei*infected mice are contained in Table 4.6. The results indicated that both the crude and fractions of the tested spider ameliorated the reduction in heamatological parameter as observed in the infected untreated group. The results also showed that there was a significant difference in the mean heamatological parameters recorded during the experiment. The group treated with 600mg/kg body weight recorded significantly higher MCV (FL), and MCHC (FL)g/dl) levels compared to the result of other concentrations and fractions.

Extract	Dose mg/kg	HB(g/dL)	MCV (FL)	MCH(Pg)	MCHC (g/dL)	RBC (x10 ¹² /L)	WBC (x10 ¹² /L	PLC (X10 ⁹ /L
Crude	150	18.42±0.12 ^e	44.01±1.21 ^c	17.14±1.01 ^b	24.01±1.29 ^a	5.67±1.01 ^a	16.31±0.43 ^a	381.16±1.21 ^b
Methanol								
	300	7.21±0.98 ^a	44.31±0.31°	18.21±0.71	24.31±0.79 ^a	6.01±0.37 ^c	15.21±1.68 ^e	307.21±0.01 ^f
	600	16.21±1.61 ^d	48.31±0.21 ^d	17.69±0.08 ^b	28.31±1.23 ^b	6.41±0.03 ^a	8.31±0.07 ^b	291.27±2.21 ^e
Ethyl acetate	150	8.36 ± 0.08^{b}	41.31 ± 0.11^{b}	15.13±1.31 ^b	31.07±0.03 ^c	5.89±1.69 ^a	11.45±1.37°	188.145 ± 1.31^{a}
	300	6.14±0.96 ^a	46.21±0.16 ^d	14.31±0.39 ^b	26.31±0.01 ^b	8.14±0.01 ^b	14.68±0.76 ^d	225.14±0.36 ^c
N-hexane	150	7.31 ± 1.62^{a}	47.01 ± 0.14^{d}	16.31±1.39 ^b	27.10±1.17 ^b	7.49±0.31 ^b	9.21±0.39 ^b	205.71 ± 1.38^{b}
	300	6.31±0.01 ^a	41.31±2.49 ^b	15.01±1.34 ^b	31.64±1.3 ^c	8.31±0.79 ^b	11.23±1.23 ^c	232.16±2.06 ^d
Standard		9.31±0.26 ^b	49.14±3.16 ^d	14.71±1.09 ^b	29.14±0.11 ^b	7.64±1.26 ^a	9.89±0.07 ^b	235.10±1.63 ^d
Untreated		6.12±1.14 ^a	23.14±1.31ª	12.01±0.01 ^a	23.15±0.13 ^a	$6.84{\pm}0.04^{a}$	4.89±0.17 ^a	198.41±1.31 ^b
Normal		12.31±0.41°	44.51±0.14 ^c	16.10±0.03 ^v	34.51±1.4 ^d	11.31±0.28 ^e	9.34±0.37 ^b	236.21±2.14 ^d

Table 4.6: Effect of Crude Methanol and Solvent Fractions of Spider on heamatological parameters of *P. berghei*-Infected Mice

Values are expressed in mean \pm standard error of three replicates Values followed with

4.1.7 Effect of Crude and Solvent Fractions of Spider on the Survival Time of *Plasmodium berghei* Infected Mice after 42 days Experimental Period

The results of the crude and fractions of spider on the survival time of *plasmodium berghei*infected mice experimental period is contained in Table 4.7 on the general note, all the dose of the crude and solvent fractions of the spider tested prolong the survival period of the experimental period. On the other hand, the infected untested mice did not survive up to 17 days experimental period. There was no significant difference in the mean survival time (days) of experimental animals treated with 300 mg/kg b.wt < 40.36±1.09), and 600 mg/kg b.wt crud extrmact (41.49±2.36); 150 mg/kg b.wt n-hexane fraction (40.35±0.01) and 150 mg/kg b.wt ethylacetate fraction (38.49±0.42), this was not significantly different from the mean survival time recorded for the group treated with 300 mg/kg 6.wt n-hexane fraction (42.31±0.67).

Extract	Dose mg/kg b. wt	Survival time	
Crude	150	35.14±0.39a	
	300	40.36±1.09c	
	600	41.49±2.36c	
Fraction			
n-Hexane	150	40.35±0.01c	
	300	42.31±0.67d	
Ethylacetate	150	38.49±0.25c	
-	300	39.21±0.44c	
Standard		42.91±0.42d	
Untreated		16 38+0 71a	

 Table 4.7: Effect of Crude and Solvent Fractions of Spider on the Survival Time of

 Plasmodium berghei
 Infected Mice after 42days Experimental Period

4.1.8 Larvicidal activities of the Crude Methanol Extract of Spider after 48hrs Exposure Period

The results of the larvicidal activities of the crude methanol extract of spider is contained in Table 4.8. On the general note, the results showed a dose-depended larvicidal activities and not the same larvicidal activities increase with increase in time. From zero to the first 30mins experimental period, no mortality was recorded in all the time exposed to the extract concentration and control. Also at concentration between 0.2 and 1.2 extract concentration no larvae mortality, however larvae mortality increase thereafter with increase in extract concentration. The highest larvae mortality was recorded at 48 hour exposure period and for 2.0mg/h extract concentration (14.12 ± 0.23) . Table 4.8: Larvicidal activities of the Crude Methanol Extract of Spider after 48hrs Exposure Period Mean Larvicidal Activities ($\overline{X}\pm S$.E unit)

					Experimental Duration					
Concentration (Mg/L)	0min	5mins	10mins	30mins	1hr	3hrs	6hrs	12hrs	24hrs	48hrs
0.2	0.00±0.00ª	0.00 ± 0.00^{a}	0.00±0.00ª	0.00±0.00ª	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	$0.00{\pm}0.00^{a}$	0.00 ± 0.00^{a}
0.4	$0.00 \pm .00^{a}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
0.8	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1.2	0.00 ± 0.00^{a}	0.40 ± 0.24^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.23 ± 0.00^{b}	1.23±0.21 ^b	1.23±0.00 ^b	2.04±0.15°
1.6	0.00 ± 0.00^{a}	0.20 ± 0.20^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	$1.00{\pm}0.71^{d}$	4.80 ± 0.58^{b}	5.40±0.71°	6.20 ± 0.58^{b}	8.14.00±0.10 ^c	10.00 ± 1.26^{d}
2.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	$3.35{\pm}1.23^{a}$	6.43±0.13°	7.12 ± 0.37^{d}	9.20±0.43 ^c	11.23 ± 0.10^{d}	14.12±0.23 ^e
Positive Control	0.00±0.00ª	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00±0.00ª	$0.00{\pm}0.00^{a}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.01±0.01 ^b
Negative Control	0.00±0.00ª	0.00±0.00 ^a	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.00±0.00ª	0.10±0.00ª	0.13±0.01ª	0.00±0.00ª	0.00±0.00ª

n=>0.005

4.1.9 Larvicidal activities of the n-hexane Fraction of Spider against *Culex* quinquefasciatus after 24 hours Exposure Period

The larvicidal activities of the n-hexane fraction of spider against *Culex quinquefasciatius* after 24 hours exposure period was detailed in Table 4.9. On the general note, the results indicated a dose-dependent and time dependent larvicidal activities against the tested 4th instar larvae of *Culex quinquefasciatus*. Also, these was a significant increase (P<0.05) in larvicidal activities with increase in extract concentration. These was no larval mortality in the first 5mins exposure period. After 12 hours exposure period, more than 90 percent larvae mortality has been recorded for the group exposed to 2.0 n-hexane fraction, of spider. However, do mortality was recorded for the positive and negative control. Also, after 24hours exposure period, complete larval group exposed to bit 1.6 and 2.0 mg/tg b.wt extract concentration.

Table 4.9: Larvicidal activities of the n-hexane Fraction of Spider against Culex quinquefasciatus after 24hrs Exposure PeriodLarvicidal activities ($\overline{X} \pm S.E$ unit)

Concentration (mg/L)	0MINS	5MINS	10MINS	30MINS	1HR	3HRS	6HRS	12HRS	24HRS
0.2	0.00 ± 0.00^{a}	1.40 ± 0.86^{b}	3.80 ± 0.74^{b}	7.20 ± 0.18^{b}	9.60±1.21 ^b				
0.4	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.80±0.37 ^b	3.00±0.32 ^c	5.40±0.81°	9.40±0.83 ^c	12.60±0.87 c
0.8	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.40 ± 0.25^{b}	1.60±0.40 ^b	6.40 ± 0.98^d	10.00±1.00 d	12.40±0.87 d	16.40±0.51 d
1.2	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	1.40±0.25 ^c	4.60±0.25 ^c	11.20±0.80 ^e	14.80±0.66 ^e	16.40±0.40 ^e	18.34±0.10 e
1.6	0.00±0.00 ^a	0.00 ± 0.00^{a}	0.20 ± 0.20^{b}	4.20±0.49 ^d	7.60 ± 0.68^{d}	12.40 ± 1.12^{f}	14.80±1.16 ^e	18.40 ± 1.17^{f}	$25.00{\pm}0.00^{\rm f}$
2.0	0.00±0.00 ^a	0.00±0.00 ^a	3.20±0.49 ^c	8.40±1.12 ^e	11.45±0.8 9 ^e	15.85±1.45 g	17.36±0.27 ^f	24.17±0.23 g	$25.00{\pm}0.00^{\rm f}$
Positive control	0.00±0.00 ^a	0.00 ± 0.00^{a}	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a				
Negative control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a				

n=>0.005

4.1.10 Larvicidal activities of the ethylacetate Fraction of Spider against 4th Instar Larval of *Culex quinquefasciatus*

The result of the larvicide activities of the ethylacetate fraction of spider venom is contained in Table 4.10. There was no mortality in the first 30mins exposure period. There after larval mortality increase significantly and time of exposure. In the first 1hour exposure period, the larval mortality recorded for the experimental group exposed to 0.4 mg/L extract concentration (1.25 ± 1.13) was not significantly different from larval mortality recorded for 0.8 mg/L extract concentration (2.00 ± 0.00) . Larval mortality was significantly highest in the group exposed to 2.0 mg/L extract Concentration.

Table 4.10: Larvicidal activities of the ethylacetate Fraction of Spider against 4th Instar of *Culex quinquefasciatus* Mean Larvicidal Activities ($\overline{X} \pm S.E$ unit)

Concentration (mg/L)	Omins	5mins	10mins	30mins	1hrs	3hrs	6hrs	12hrs	24hrs
0.2	0.00±0.00 ^a	$0.00{\pm}0.00^{a}$	0.00 ± 0.00^{a}	$0.00'0.00^{a}$	0.00'0.00 ^a	2.20±0.20 ^b	2.20±0.20 ^b	3.60±0.25 ^b	4.60±0.25 ^a
0.4	0.00±0.00 ^a	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00±0.00 ^a	1.25±1.13 ^b	$2.43{\pm}0.12^{b}$	3.20 ± 0.20^{c}	4.60 ± 0.25^{b}	$6.60{\pm}0.25^{b}$
0.8	0.00±0.00 ^a	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00±0.00 ^a	2.00 ± 0.00^{b}	4.60±0.40°	6.00 ± 0.32^{d}	8.20±0.37°	11.20±0.37°
1.2	0.00±0.00 ^a	0.00 ± 0.00^{a}	0.00±0.00 ^a	$2.30{\pm}1.47^{b}$	4.20±0.20 ^c	8.20 ± 0.20^{d}	9.60±0.40 ^e	11.79±0.37 ^d	14.71 ± 0.37^{d}
1.6	0.00±0.00 ^a	0.00±0.00 ^a	0.00 ± 0.00^{a}	1.86±1.05 ^b	5.60±0.40°	14.40±0.40 ^e	$15.89{\pm}0.25^{\rm f}$	17.12±0.45 ^e	19.12±0.45 ^e
2.0	0.00±0.00ª	$0.00{\pm}0.00^{a}$	0.00 ± 0.00^{a}	3.10±0.20 ^c	7.00 ± 0.00^d	15.65±0.01 ^e	17.43±0.16 ^g	19.98±2.56 ^e	$25.00{\pm}0.00^{\rm f}$
Negative control	0.00±0.00 ^a	0.00±0.00 ^a	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Positive Control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a					

n=>0.005



Figure 4.1: Percentage Mortality of the Crude and Fractionated of Spider

The percentage mortality of the crude and fractionated of spider on larvae mosquito is contained in figure 4.1. The results indicated variations in the percentage larval mortality with respect to the different solvent of extracts. It was found that the only n-hexane extract recorded 100% larval mortality when exposed to 1.6mg/l extract concentration. However, the larval group subjected to 2.0mg/l extract concentration also recorded 100% mortality after 20hours exposure period. On the general note, the group subject to crude methanol extract recorded the least percentage mortality after 24hours exposure period.

4.1.11 Medial (LC₅₀) and Upper (LC₉₀) Lethal Concentrations, Coefficient of Determination (r^2) and Regression Equations of the Effect of Crude and Solvent Fractions of Spider on *Culex quinquefasciatus* after 24hours Exposure Period.

The result indicated that the extract and/or fraction with highest and/or best larvicidal activity was the n-hexane fraction with LC₅₀ and LC₉₀ of 2 0.46 and 1.44 mg/L respectively. This is followed with ethylacetate (LC₅₀ = 0.94; LC₉₀ = 185 mg/L).

The coefficient of determination recorded for all the tested extract and fractions was above 0.9. This implies that more than 90 % of the tested concentrations was responsible for the observed mortality.

Table 4.11: Medial (LC50) and Upper (LC50) Lethal Concentrations, Coefficient of Determination (R²) and Regression Equations of the Effect of Crude and Solvent Fractions of Spider on *Culex quinquefasciatus* after 24hrs Exposure Period

Extract	LC50 (mg/L)	LC90 (mg/L)	R ²	Regression equation
Crude	2.14	3.14	$R^2 = 0.9365$	y = 39.91x - 35.504
n-Hexane	0.46	1.44	$R^2 = 0.9681$	y = 40.422x + 31.598
Ethylacetate	0.94	1.85	$R^2 = 0.9944$	y = 44.097x + 8.5844

4.2 Discussion

This study demonstrated that spider crude and fractions are rich in bioactive metabolits such as Saponins, Flavonoids, and Tannins. The presence of these metabolites indicate that the spider whole body contains metabolites of medicinal significance. Metabolites, Specifically, Secondary Metabolites are manufactured by organism to ward off infections and pathogens and these may vary from one organism to the other as a result of environmental factors including geographical locations, temperature and a soil type (Kumar *et al.*, 2017). The variation in these metabolites with respect to the solvent of extraction could be attributed to the polarity of each solvent (Hammani *et al.*, 2011; Olayemi *et al.*, 2017). Furthermore the presence of these metabolities in spider could be attributed to their breading and foraging behaviours on other organisms that are rich in bioactive metabolites (Robert *et al.*, 2012), according to (Ragunath *et al.*2017, defense mechanisms employed by an organism could activate the synthesis of other metabolites. One of the moral strategies towards perverting malaria transmission is through the interrupting the mosquito life cycle it the larval stage (Mbare, 2015; Ram and Jiang-Shiou 2006), mentioned that larval stage of mosquito are special target in mosquito control, at this stage the mosquito are more concentrated, with respected to population, and susceptible to insecticide.

In the present study, both the crude and solvent fraction of the spider, at almost all concentration showed a dose dependent larvicidal activities against the tested *Culex* mosquito. These observed activities could be as a result of increase in concentration of the inherent larvicidal metabolites in the spider extract which in turn cause an increase in the larvicidal potency of the extract (Kwela *et al.*, 2011). According to Wellington *et al.* (2017), one of the special characteristics of natural products is the dose-dependent potency which thus identify the spider extract as a potential insecticidal agent.

The significant larvicidal potency in the crude extract and fractions observed in the present study could be attributed to the bio-active metabolites detected in spider. These chemicals have been found and documented by previous researchers to possess mosquito larvicidal potebncy. (Ubulom *et al.*, 2013). In the report of Chowdhury *et al.* (2008), these metabolites including saponins, tannins, terpenoids, flavonoids and steroids, work by interacting with the cuticle membrane of the larva, consequently disarranging the membrane, which is the most probable reason for larval death. It is possible that these metabolites work jointly on independently contribute to producing toxic activity against the tested mosquito species (Liu *et al.*, 2014). Furthermore, spider has been characterized as one of organism with venom toxins. These spider venom toxins have been reports do show multiple biological effects, neurotoxic effects with anti-arrythmic, antimizrobical, cycbolytic and enzyme inhibitory activities (Liu *et al.*, 2014;). The toxins could be the main driver behind the larvicidal potency observed in the whole body contact of the spider venom.

In the current study, the n-hexane fraction was the most potent larvicidal when compound with the crude and ethylacetate fraction. It is possible that the active metabolite that work against the tested mosquito larvae is more concentrated in the n-hexane fraction (Olayemi *et al.*, 2013), has earlier asserted that variation in larvicidal activities of extractors is could be attributed to the different solvent of mention that variation in larval mortality could be attributed to the polarity of solvent of extraction. This corroborate the earlier report of (Ghosh *et al.*, 2012) who indicated that n-hexane may more appreciable and effective organic solvent in the extraction of larvicidal compounds from natural products

In this study the in vivo profile assay of the crude methanol extraction of spider, in mice, indicated that up to a dose of 5000 mg/kg b.wt, there was no distinct sign of toxic. This implies that the LD50 can be extrapolated to be above 5000 mg/kg b. wt. This implies that the extract of the spider while body at the tested dose are save for oral administration.

According to organization for economic congregation and development (OECD) guideline for testing the efficacy of natural chemicals (OGCD, 2008), any LD50 beyond 5000mg/k b.wt, is of no practical significance and It is the maximum allowable dose. In addition, (Marogo *et al.*, 2013), asserted that any chemical substance with LD50 estimate greater than 3000-5000 mg/kg (oral route) could be considered of low toxicity and safe.

This study showed that the tested crude and fractions of the spider venom show appreciable antiplasmodial activities against the fated strain of *Plasmodium berghei* in the experimental mice. The observed antiplasmodial activities could be attribute to the presence of bio-active metabolites identified in the crude and solvent fractions of the spider (Jeruto *et al.*, 2015). According to the report of Adewoye *et al.* (2010), saponins for instance have andiplasmodial potency as well as deformating potency in the rumen. Ajayi *et al.* (2012) mertior led that the antiplasmodial potency of saponins is as a result of its potency in

promoting feeding in animal which in turn address the problem if loss of appetite during malaria infraction. Falconoid exert antiplasmodial activity by chelating with the nuclei acid base pairing of the parasite thereby producing the antiplasmodial effects (Okokon *et al.*, 2016). Some other metabolites may exert their antiplasmodial efficacy either by causing elevation of red blood cell oxidation and/or by inhibiting protein synthesis (Al-Adhroey *et al.*, 2011).

The study also found that the spider crude and fractions showed a significant dose dependent and parasitaemia with time. This implies that the extract posse a communicative antiplsamodial effect as a result of increase in concentration of the bio-active commonness.

The crude methanol extract showed the highest antisplamodial potency than the fractions. This is in line with earlier report of Lawal *et al.* (2016), who opined that crude extract are more potent than the tested fractions. This observation could be that the bio-active metabolites are more concentrated in the crude extract and they therefore act synergistically to produce the observed antiplasmodial potency.

The current investigation found that both the crude and fractionated extract of the spider ameliorated the loss in weight causal by the innoculated parasite. According to Gonzalo (2013) body weight loss are common features of *P.berghei* infected mice and that a good anti=malaria agent is expected to prevent body weight loss in infected mice due to rise in parasitaemia. By implication, these extracts possess some bio-active compound with the potential to counter.berghei replication and probably, reduce the overall pathogenic effect of the invading parasite (Goulielmaki *et al.*, 2017). Furthermore, the current investigation recorded a significant increase in the heamatological parameters of the extract treated group, standard and normal control group compound to the reduction observed in the negative control group. This observation is an indication that the extracts possess a protective effect against the parasite induced reduction in heanmatological variables. This is in line with the assertion of Casida (2009), that higher concentration of bio-active metabolites, from natural products, possess appreciable ameliorate and/or inhibitory effect against *P. berghei* induced complications.

However the extract in both crude and fraction could not appreciably ameliorate the loss in PLV of the infected treated mice. This may be due to the fact that the extract laces bioactive metabolites that have the potential to avert anemia due to malaria infection which might be due to destruction (clearance) and/or sequestration of infected erythrocytes' (Desye *et. al.*, 2019),

The study observed that both crude and solvent fraction of the spider prolonged the survival time of the experimental mice compared with the control (infected untreated) mice that died before the 18th day of the experimental period. Perhaps, the extracts possess the ability to suppress and reduce the overall pathology effect of the parasite on the experimental mice (Bantie *et al.*, 2014).

CHAPTER FIVE

5.0

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The current study revealed that the crude and solvent fractions of whole spider body possess bio-active metabolites such as Phenol, Tannis, alkaloids and Saponnis. The crude and fractions also possessed a dose and time-dependent larvicidal activities against the tested mosquito larval. The n-hexane fraction showed the highest larvicidal potency. The active oral toxity result indicated that the spider extract is safe for oral administration with an LD_{50} extrapolated to be above 5000mg/kg b.wt. The crude and fraction showed appreciable antiplasmodial activities with the crude extract being the most active. The extracts avert the loss in weight of environmental mice due to parasite but unable to ameliorate the loss in PLV as a result of parasite invasion. However the crude and fractions of the spider extract promoted the survival time of the *plasmodium berghei*-infected mice.

5.2 **Recommendations**

There is need to isolate and characterize the active ingredient of the spider for further study to elucidate the extract mechanism and action of the spider extract. The extracts as well as its fractions should be tested on other mosquito species and their insect growth regulatory activities should be established. The *in vitro* evaluates on the spider extract against human malaria is very needed to argument and authenticate the finding of the present study.

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