ANTIPLASMODIAL AND ANALGESIC ACTIVITIES OF ETHANOL STEM BARK EXTRACT OF *CATUNAREGAM NILOTICA* (Stapf.)

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DECLARATION

I hereby declare that this work is the product of my research efforts undertaken under the supervision of Prof. (Mrs.) O.A. Salawu and has not been presented anywhere for the award of a degree or certificate. All sources have been duly acknowledged.

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CERTIFICATION

This is to certify that the research work for this dissertation and the subsequent write-up Antiplasmodial and analgesic studies of ethanol stem bark extract of *Catunaregam nilotica* (Stapf.) (Fatima Nasidi Abubakar SPS/13MPC/00023) were carried out under my supervision. .

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ABSTRACT

Malaria, a major public health problem in the world, is responsible for death of millions particularly in Sub-Saharan Africa. Today, the control of malaria has become gradually more complex due to the spread of drug-resistant parasites. Medicinal plants are the major source of effective antimalarials. The aim of the study was to evaluate the antiplasmodial, analgesic and antipyretic activities of ethanol stem bark extract of *Catunaregam nilotica* (ESCN). Oral acute toxicity study of ESCN was conducted using Lorke's method while its antiplasmodial activity was evaluated against chloroquine-sensitive *Plasmodium berghei-berghei* in early, repository and established infection models in mice. Analgesic activity of ESCN was evaluated in acetic acid-induced abdominal writhing and hot plate-induced pain models in mice and formalin-induced hind-paw licking model in rats. The antipyretic activity was evaluated using yeast-induced pyrexia model in rats. The oral LD_{50} was found to be greater than 5,000 mg/kg. The extract at oral doses of 250, 500 and 1,000 mg/kg produced significant ($p<0.05$) and dose-dependent decrease in level of parasitaemia in early (suppressive), repository (prophylactic) and established (curative) infections in the mice. All doses of ESCN significantly and dose-dependently prolonged the survival time of the infected mice in all the three tests compared to the control. The extract produced significant ($p<0.05$) and dose-dependent analgesic activity in acetic acid-induced writhing, hot plate and formalin hind paw tests in the mice and rats. The extract produced significant ($p<0.05$) reduction of rectal temperature in the extract-treated rats only at the highest dose (1,000 mg/kg) used. The *C. nilotica* stem bark extract is practically non-toxic acutely and possesses significant antiplasmodial, analgesic and antipyretic activities which could represent a lead for the development of a new antimalarial drug.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Malaria is an infection caused by the parasite of the genus plasmodium; four distinct species of plasmodium have been identified, namely: *P. Vivax, P. falciparum, P. ovale* and *P. malariae*. *P. falciparum* causes the most serious form of the disease and is responsible for over ninety five percent (95%) of infections in sub-Saharan Africa. *P. knowlesi* is a fifth species recently documented to cause human infections and is presently restricted to countries of Southeast Asia (Lee *et al.,* 2011). The parasites are transmitted through the bite of an infected anopheles mosquito and the infection is said to be endemic in tropical countries in the world. According to Chukwuocha *et al.* (2009), malaria is among the common causes of childhood mortality in Nigeria. It is estimated that fifty percent (50%) of the population have at least one episode of malaria each year, while children under five years of age have, on the average 2-4 attacks in a year.

Malaria is today a disease of poverty and underdeveloped countries, but it remains an important health problem globally. In the last decade, the prevalence of malaria has been escalating at an alarming rate, especially in Africa. An estimated three hundred to five hundred million cases each year cause 1.5 to 2.7 million deaths, more than 90% in children below 5 years of age in Africa (Ayoola *et al.,* 2008).

Herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. The first documented records of herbal medicinal use date back 5,000 years (Garodia *et al.,* 2007) in China. Similarly, India's Ayurvedic medicine tradition is thought to be more than 5,000 years old and herbal medicines remain an essential component of its practice (Walker, 2002).

Today, the populations of some countries still depend on herbal medicines to address healthcare needs (Robinson and Zhang, 2011).

Additionally, older adult populations are more likely to use both conventional drug therapy and herbal medicines (Barnes and Bloom, 2008). This population is also more likely to have a higher incidence of chronic diseases, which often require the use of increasingly complex conventional drug therapy with the potential for herb-disease and herb-drug interactions (Loya *et al.,* 2009). In many countries including the U.S., herbal medicines are not regulated as extensively as conventional drug therapy (Rivera *et al.,* 2002). Also, globalization has greatly increased accessibility of herbal medicines from all parts of the world to any single consumer (Kennedy *et al.,* 2008).

Plants have always been considered to be a possible alternative and a rich source of new drugs with most of the conventional antimalarial drugs such as quinine and artemisinin either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates (Zirihi *et al.,* 2005).

Problems associated with antimalarial therapy include drug resistance, prevalence of sub-standard and counterfeit drugs in the market and inaccessibility of essential drugs. This may be due to inadequate production and/or affordability of conventional medicines in many tropical countries leading to dependence on traditional medical remedies mainly from plants (Garodia *et al.,* 2007). New drugs or drug combinations are thus urgently required for treatment of malaria (Mann *et al.,* 2003). Preferably, the new drugs should have novel modes of action or be chemically different from the drugs in current use (Builders *et al.,* 2011).

In ethnomedicine, the same plants and/or related species are used for the treatment of related ailments within the same region or across different regions of the world. For instance, while *Maytenus senegalensis* is used in many African regions for the treatment of various ailments including chest pains, rheumatism, snakebites and malaria, decoctions of plants of the same

genus are used in South America as anti-inflammatory and analgesic remedies (Edith *et al.,* 2006). This is possible because both the inflammatory pain and immunosuppression are manifestations of malaria. Some plants may thus lack direct antiplasmodial activity but may possess antipyretic, analgesic and immune stimulatory effects (Muregi *et al.,* 2003). Only a limited number of new antimalarial drugs are currently at an advanced stage of clinical development. Identification of sesquiterpene lactone, artemisinin (quighaosu) renewed interest in plant products research. An attractive option for resource-poor nations is the exploitation of the possible therapeutic effects of local herbs (Guede *et al.,* 2005; Idowu *et al.,* 2010).

1.2 STATEMENT OF RESEARCH PROBLEMS

Malaria remains one of the most important diseases of the developing world. It was reported by Sudhanshu *et al.,* (2003) that nearly half of the world population is at risk with mortality rates being extremely high among children below five (5) years of age. In 2013, an estimated 198 million cases of malaria occurred worldwide and 500,000 people died (CDC, 2015). Approximately 50% of malaria cases in the world occur in Africa affecting mostly children under five (5) years and pregnant women in their first trimester (WHO, 2014). The prevalence in Africa has been escalating at an alarming rate in the last decade (Nchinda, 2006). This dramatic increase in the prevalence of malaria is due to the increasing resistance of mosquito vectors to insecticides and the resistance of the parasites, mainly *Plasmodium falciparum*, to available modern drugs (Kalra *et al.,* 2005).

In Nigeria, malaria is the most common cause of hospital attendance in all age groups with clinical symptoms of fever, chills, body pain and anaemia. Severe form of the disease may lead on to delirium, metabolic acidosis, cerebral malaria, multi-organ system failure, coma and death. Over 100 million people are at risk of malaria every year and about fifty percent

(50%) of adults in Nigeria experience at least one episode yearly while the under five (5) years children have up to 2-4 attacks annually (FMH, 2005). Children and pregnant women are more adversely affected with at least thirty percent (30%) and eleven percent (11%) mortality respectively, as a result of malaria (FMH, 2006). Malaria is associated with high socio-economic burden. Every year, Nigeria loses over 132 billion Naira from malaria related cost of treatment and transport, and absenteeism from work, school and farms (FMH, 2005). The disease thus imposes a heavy cost not only on a country but also on its rates of economic growth.

1.3 JUSTIFICATION

The human burden caused by malaria infection is enormous. Malaria has been treated with quinine, chloroquine, mefloquine and artemisinin, among other drugs. However, the protozoans have developed resistance against many of the current treatment regimens (White, 2004). In the quest to identify new antimalarial chemotherapeutic agents that are more effective, safe, available and cheap, many research groups have resorted to plant sources (Chin *et al.,* 2006).

Plant-derived compounds have always played a major role in drug discovery including that of antimalarial (Wells, 2011). The African continent is biodiversed in plant materials endowed with natural products (NPs) with intriguing chemical structures and promising biological activities (Anthony *et al.,* 2012).

People in the rural areas of Rano Local Government Area of Kano State, Nigeria use the readily available stem bark decoction of *Catunaregam nilotica* in the management of malaria and pain conditions, but its safety and efficacy has not been proven scientifically. The plant

may well prove to be a source of new antimalarial drug in view of the success of the two important chemotherapeutic agents, Quinine and Artemisinin (Paul *et al.,* 2011).

This research, therefore seeks to evaluate the antiplasmodial, analgesic and antipyretic activities of ethanol stem bark extract of *Catunaregam nilotica.*

1.4 RESEARCH HYPOTHESIS

The ethanol stem bark extract of *Catunaregam nilotica* does not possess antiplasmodial and analgesic activities.

1.5 AIM AND OBJECTIVES

1.5.1 Aim of the study

To evaluate the ethanol stem bark extract of *Catunaregam nilotica* for antiplasmodial and analgesic activities in mice and rats.

1.5.2 Objectives

The specific objectives were to:

i. Determine the acute toxicity profile of the plant extract
ii. Determine the preliminary phytochemical constituents of the plant
iii. Evaluate the antiplasmodial activity of the plant extract using curative, suppressive and prophylactic tests in *Plasmodium berghei-berghei* infected mice

iv. Carry out analgesic studies on the plant extract using acetic acid-induced writhing, hot plate-induced pain models in mice and formalin-induced hind-paw licking pain model in rats.

v. Evaluate the antipyretic activity of the extract using brewer's yeast induced-pyrexia model in rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 PREVALENCE AND INCIDENCE OF MALARIA

Malaria parasites belong to the genus *Plasmodium* (phylum *Apicomplexa*). In humans, malaria is caused by *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi* (Nadjm and Behrens, 2012). Among those, *P. falciparum* is the most common species identified (~75%) followed by *P. vivax* (~20%). Although *P. falciparum* traditionally accounts for the majority of deaths, evidence suggests that *P. vivax* malaria is associated with potentially life-threatening conditions about as often as with a diagnosis of *P. falciparum* infection (Fairhurst and Wellems, 2010). *P. vivax* is proportionally more common outside Africa. There have been documented human infections with several species of *Plasmodium* from higher apes; however, except for *P. Knowlesi*, a zoonotic species that causes malaria in macaques, these are mostly of limited public health importance (Greenwood *et al*., 2005).

Malaria, a life threatening disease caused by these parasites, is transmitted to humans through the bites of infected mosquitoes (WHO, 2013). Malaria still ravages much of Africa despite attempts to find lasting solution to the dreadful and deadly parasitic disease. It is largely endemic in the tropical countries such as Latin America and Africa where more than 40% of the world's population is residing (Francesco *et al.,* 2012). Globally, 300-500 million people are believed to contact the disease annually resulting in about 1.2-2.7 million deaths (Sachs and Malaney, 2002). An estimated 90% of malaria infections occur in sub-Saharan Africa with Nigeria accounting for a quarter of all malaria cases in the world (WHO, 2010). Africans and people living in stable transmission zones develop partial immunity to malaria infection and this is lost when the individual's exposure to the sporozoite inoculation stops as a result of prolonged stay in a non-endemic region or zone (Kurtis *et al.,* 2001). Socio-

economic factors of underdevelopment particularly poverty, ignorance and inadequacy of both health structures and health care delivery as well as unavailability of effective drugs at the locations where they are needed in the tropics also influence the severity, prevalence and management of malaria (Chukwuani,1999).

Factors that account for upsurge of malaria include drug resistance, failed malaria control programme, inadequate public health facilities and poor living standards. The effects of weather and climate on malaria transmission have attracted particular attention in recent years due to the parasites' sensitivity to changes in climatic and environmental conditions. Major areas of human health concern of climate change include heat waves, increase in sealands, flooding, drought and malnutrition. Climate change and the resultant increases in temperature (global warming) during the twentieth century has allowed the introduction of malaria into higher altitude areas (such as Kenya, Colombia and Ethiopia) previously too cold for the disease to thrive, thus, putting millions of people at risk for the disease (Worrall, 2010).

2.1.2 Causative Agent and Vector of Malaria

P. falciparum is responsible for over ninety percent (90%) of malaria infections in man (Fairhurst and Wellems, 2010). It is virulent and deadly causing severe or complicated malaria and possessing an inherent capacity to develop resistance to antimalarial drugs (Worrall *et al.,* 2005). *P. malariae* is responsible for only five percent (5%) of human malaria and usually occurs as mixed infections with *P. falciparum.* Plasmodia infection is transmitted into the blood by the bite of malaria carrier vector, the female *Anopheles* mosquitoes which are more frequent in tropical and sub-tropical than in temperate regions. The vectors are most active at night during which they move indoors in search of blood meal for pregnancy (Sachs and Malaney, 2002). The factors that favour rapid multiplication of the vector include: water

and humidity greater than sixty percent (>60%), generous rainfall, ambient temperature of 20-30°C and topography of less than 2,000 m elevation above sea level (WHO, 2007). The female *Anopheles* mosquito has an average life span of 2 to 6 weeks under favourable climatic conditions (WHO, 2013).

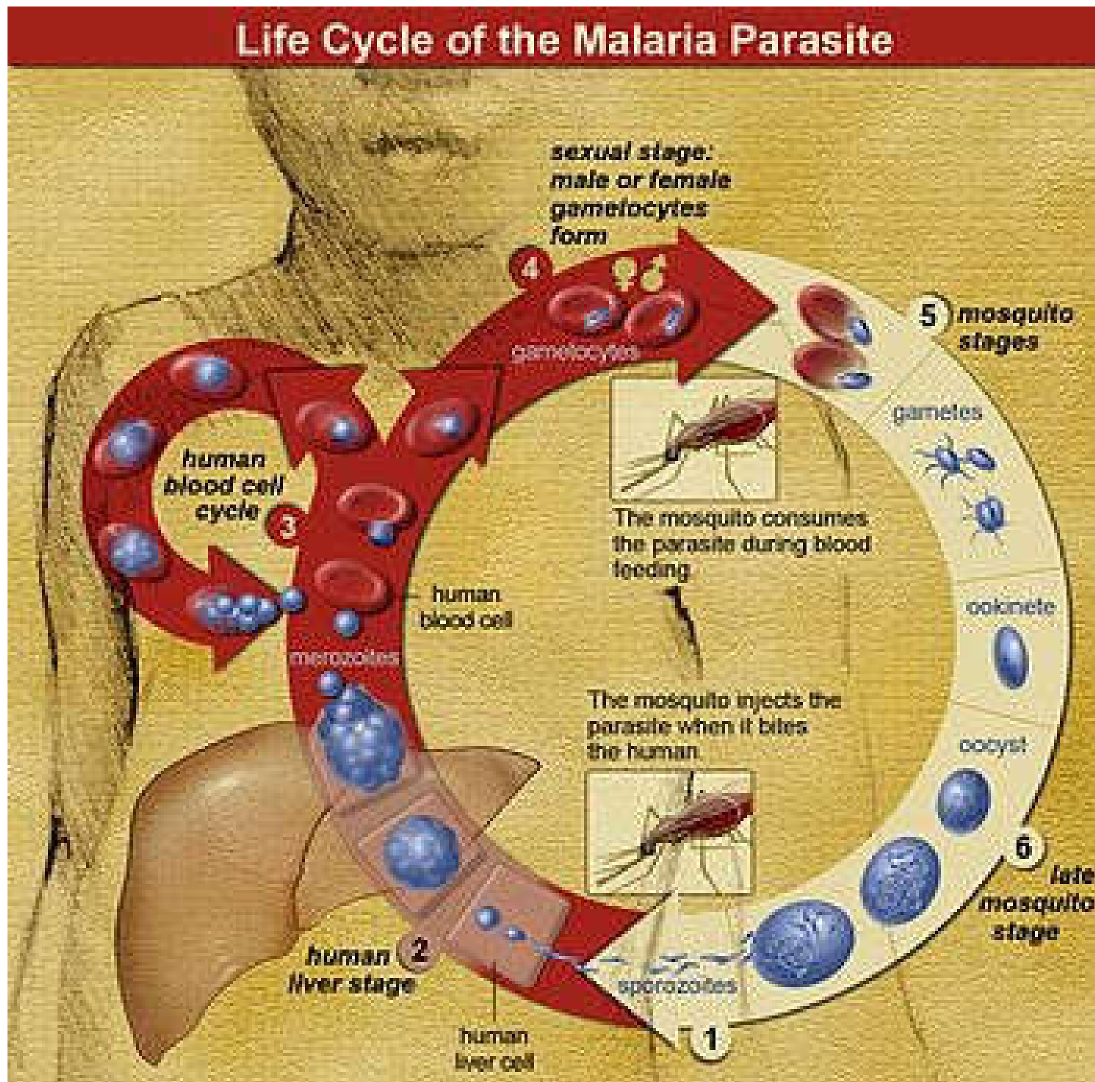
Transmission and life cycle of malaria parasite

In the life cycle of *Plasmodium*, a female *Anopheles* mosquito (the definitive host) transmits a motile infective form (called the sporozoite) to a vertebrate host such as a human (the secondary host), thus acting as a transmission vector. A sporozoite travels through the blood vessels to liver cells (hepatocytes), where it reproduces asexually (tissue schizogony), producing thousands of merozoites. These infect new red blood cells and initiate a series of asexual multiplication cycles (blood schizogony) that produce 8 to 24 new infective merozoites, at which point the cells burst and the infective cycle begins anew (Riley and Stewart, 2013).

Other merozoites develop into immature gametocytes, which are the precursors of male and female gametes. When a fertilised mosquito bites an infected person, gametocytes are taken up with the blood and mature in the mosquito's gut. The male and female gametocytes fuse and form an ookinete (a fertilized, motile zygote). The Ookinetes develop into new sporozoites that migrate to the insect's salivary glands, ready to infect a new vertebrate host. The sporozoites are injected into the skin from the saliva, when the mosquito takes a subsequent blood meal (Layne, 2007).

Only female mosquitoes feed on blood while male mosquitoes that feed on plant nectar, do not transmit the disease. The females of the *Anopheles* genus of mosquito usually start

searching for a meal at dusk and continue throughout the night. Malaria parasites can also be transmitted by blood transfusion, though rarely (Gething *et al.,* 2011).



The life cycle of malaria parasite (Tilley *et al.,* 2011).

Pathophysiology of malaria

Malaria infection develops via two phases: the first phase takes place in the liver (exo-erythrocytic phase) while the other one involves red blood cells or erythrocytes (erythrocytic phase). When an infected mosquito pierces a person's skin to take a blood meal, sporozoites in the mosquito's saliva enter the bloodstream and migrate to the liver where they infect hepatocytes, multiplying asexually and asymptomatically for a period of 8–30 days (Greenwood and Mutabingwa, 2002).

After a potential dormant period in the liver, these organisms differentiate to yield thousands of merozoites, which following rupture of their host cells, escape from the liver undetected by wrapping itself in the cell membrane of the infected host liver cell, into the blood and infect red blood cells to begin the erythrocytic stage of the life cycle (Gollin and Zimmermann, 2007).

Within the red blood cells, the parasites multiply further, again asexually (merozoites), periodically breaking out of their host cells to invade fresh red blood cells. Several such amplification cycles occur producing the classical descriptions of waves of fever (WHO, 2010).

Some *P. vivax* sporozoites do not immediately develop into exo-erythrocytic-phase merozoites, but instead produce hypnozoites that remain dormant for periods ranging from several months (7–10 months is typical) to several years. After a period of dormancy, they reactivate and produce merozoites. Hypnozoites are responsible for long incubation and late relapses in *P. vivax* infections (Taylor *et al.,* 2012).

The parasite is relatively protected from attack by the body's immune system because for most of its human life cycle it resides within the liver and blood cells and is relatively

invisible to immune surveillance. However, circulating infected blood cells are destroyed in the spleen. To avoid this fate, the *P. falciparum* parasite displays adhesive proteins on the surface of the infected blood cells, causing the blood cells to stick to the walls of small blood vessels, thereby sequestering the parasite from passage through the general circulation and the spleen. The blockade of the microvasculature causes symptoms such as fatigue, lack of energy and lack of characteristic fever as seen in placental malaria. Sequestered red blood cells can cross the blood–brain barrier and cause cerebral malaria (Olupot *et al.,* 2013).

Recurrent malaria

Symptoms of malaria can recur after varying symptom-free periods depending upon the cause. Recurrence can be classified as recrudescence, relapse, or re-infection. Recrudescence is when symptoms return within two weeks of treatment after a symptom-free period. It is caused by parasites surviving in the blood as a result of inadequate or ineffective treatment (Cox, 2002). Relapse occurs when symptoms reappear after the parasites have been eliminated from blood but persist as dormant hypnozoites in liver cells. Relapse usually occurs between 8–24 weeks and is commonly seen with *P. vivax* and *P. ovale* infections. *P. vivax* malaria cases in temperate areas often involve overwintering by hypnozoites, with relapses beginning the year after the mosquito bite (Prugnolle *et al.,* 2011). Reinfection means the parasite that caused the past infection is eliminated from the body but a new parasite is introduced. Partial immunity develops upon frequent exposure to infections (Feachem *et al.,* 2010).

Genetic resistance

According to a 2005 review, the high levels of mortality and morbidity caused by malaria—especially the *P. falciparum* species have placed the greatest selective pressure on the human genome in recent history. Several genetic factors including sickle cell trait, thalassaemia traits, glucose-6-phosphate dehydrogenase deficiency and the absence of Duffy antigens on red blood cells are responsible for the resistance (Guerra *et al.,* 2007).

The impact of sickle cell trait on malaria immunity illustrates some evolutionary trade-offs that have occurred because of endemic malaria. Sickle cell trait causes a change in the haemoglobin molecule in the blood. Normally, red blood cells have a very flexible, biconcave shape that allows them to move through narrow capillaries; however, when the modified haemoglobin S molecules are exposed to low amounts of oxygen, or crowd together due to dehydration, they can stick together forming strands that cause the cell to sickle or distort into a curved shape. In these strands, the molecule is not as effective in taking or releasing oxygen, and the cell is not flexible enough to circulate freely. In the early stages of malaria, the parasite can cause infected red cells to sickle, and so they are removed from circulation sooner. This reduces the frequency with which malaria parasites complete their life cycle in the cell. Individuals who are homozygous (with two copies of the abnormal haemoglobin beta allele) have sickle-cell anaemia, while those who are heterozygous (with one abnormal allele and one normal allele) experience resistance to malaria without severe anaemia (White, 2004).

2.2 CLASSIFICATION OF MALARIA

Malaria is classified into either "severe" or "uncomplicated" by the World Health Organization (WHO, 2014).

2.2.1 <u>Uncomplicated Malaria</u>

The classical malaria attack lasts 6-10 hours (CDC, 2004) and consists of:

i. cold stage (sensation of cold, shivering)
ii. hot stage (fever, headaches, vomiting, seizures in young children)
iii. sweat stage (sweating, tiredness).

Generally, early signs of malaria are fever and chills accompanied by tachycardia, sweats, headaches, nausea, vomiting, frequent urination and 'flu-like' symptoms. (Etkin, 2003; CDC, 2004). Physical findings may include elevated temperature, perspiration, weakness and enlarged spleen. In *P. falciparum* malaria, additional findings may include mild jaundice, enlargement of the liver and increased respiratory rate. Interfebrile episodes are characterised by leucopaenia and thrombocytopaenia.

2.2.2 <u>Severe Malaria</u>

Severe malaria occurs when *P. falciparum* infections are complicated by serious organ failure or abnormalities in the patient's blood or metabolism (CDC, 2004). The manifestations include the following:

i. cerebral malaria with abnormal behaviour, impairment of consciousness, seizures, coma or other neurologic abnormalities.
ii. severe anaemia due to haemolysis
iii. haemoglobinuria, pulmonary oedema or acute respiratory distress syndrome, which may occur even after the parasite counts have decreased in response to treatment
iv. abnormalities in blood coagulation and thrombocytopenia
v. cardiovascular collapse and shock
vi. kidney and other organ dysfunction, including hepatosplenomegaly

vii. hyperparasitaemia where more than five percent (5%) of the red blood cells are infected by malaria parasites

viii. metabolic acidosis, often in association with hypoglycaemia

Where malaria is endemic, children younger than 5 years bear the burden of morbidity and mortality, while older children and adults may develop an "immunity tolerance", a protection against super-infection (Taylor- Robinson, 2002).

2.3 CONTROL AND PREVENTION OF MALARIA

The scourge of malaria is increasingly becoming more demanding and challenging not only to the medical professionals, health services, scientific and research world, but also to the government (Ferri, 2009). The main global control strategies or approaches used in tackling malaria include:

i. early diagnosis and prompt effective treatment of cases
ii. chemoprophylaxis and chemotherapy in susceptible groups
iii. vector control
iv. reduction of man-vector contact
v. surveillance, information, education and communication
vi. vaccine development and research (Ferri, 2009).

Chemoprophylactic drugs do not prevent the attack of malaria, but reduce the risk of the severity and fatality. Chemoprophylactic treatment is needful for individuals in the high-risk group (children, pregnant women, non-immune immigrants or travellers) (Korenromp *et al.,* 2005). However, individuals residing in endemic areas do not require antimalarial chemoprophylaxis because they tend to acquire partial or natural immunity from frequent or

repeated exposures to the infection (Bhatt *et al.,* 2015). Prevention is aimed at keeping the parasite completely away from the host so as to reduce morbidity and intensity of transmission. According to Lim *et al.,* (2009), reduction in human –mosquito contact consists the use of appropriate prophylactic measures such as mosquito repellants, insecticide treated nets, pyrethrum house spraying and anti-mosquito fumigants. Other measures are practice of good sanitation, adequate hygiene and adequate participation in public health education. Surveillance is also necessary as it helps to promptly alert the authority of any risk of return of transmission.

2.4 MALARIA CHEMOTHERAPY

The essence of malaria chemotherapy is to prevent mortality, reduce morbidity and socio-economic loss in man. Rational malaria chemotherapy consists of timely supportive treatment (symptomatic care) often in the form of:

i. ensuring adequate hydration of the patient
ii. prompt reduction of fever with antipyretics
iii. reduction of headaches, body and joint pains using analgesics
iv. prevention of vomiting with antiemetic
v. adequate feeding of the patient to maintain caloric requirements and
vi. prompt specific treatment of both the underlying disease complications and the malarial parasite (Sallares, 2002).

Malaria treatment varies with the clinical spectrum (Sallares, 2002), but most malaria parasites are sensitive to the widely used antimalarials irrespective of the geographical origin of the parasite or patient (WHO, 2014).

Artemisinin combination therapy (ACT) is defined as the simultaneous administration of two or more blood schizonticidal antimalarial drugs (co-formulated or co-administered) with independent modes of action and different biochemical targets in the parasite (Bhatt *et al.,* 2015). A relatively weak antimalarial is sometimes combined with a more active one as separate products given together or sequentially to enhance the action of the more active product. In some cases, an effective rapidly – acting antimalarial with a short duration of action is given with an equally effective more slowly acting product with a long duration of action in order to reduce duration of admininstation of the short-acting drug or to prevent recrudescence which commonly occurs when the short acting drug is given alone e.g artemether and sulphadoxine-pyrimethamine (Worrall *et al.,* 2005). Combination therapy is one method of overcoming the global challenge of drug-resistant *P. falciparum*. The Nigerian National Antimalarial Treatment Policy on uncomplicated malaria adopted the use of Artemether-Lumefantrine (At - Lu) and Artesunate-Amodiaquine (As-Am) as first line drugs in line with the WHO recommendation in 2005 (Sutherland *et al.,* 2010).

2.4.1 Antimalarial Drugs

Antimalarial drugs designed to prevent or cure malaria may be used for some or all of the following (Bhatt *et al.,* 2015):

i. treatment of malaria in individuals with suspected or confirmed infection (Curative treatment)
ii. prevention of malarial infection in individuals visiting a malaria-endemic region who have no immunity (prophylactic treatment)
iii. routine intermittent treatment of certain groups in endemic regions (Intermittent preventive therapy)

Prompt parasitological confirmation by microscopy, or alternatively by rapid diagnostic tests, is recommended in all patients suspected of malaria before treatment is started. Treatment solely on the basis of clinical suspicion should only be considered when a parasitological diagnosis is not accessible (Sutherland *et al.,* 2010).

2.4.2 Classification of Antimalarial Drugs

Antimalarial drugs can be classified according to the stage of the parasite that they affect and the clinical indication for their use or based on their chemical structure (Peter, *et al.,* 2012)

Classification based on antimalarial activity (White, 2008; Peter *et al.,* 2012).

i. TISSUE SCHIZONTICIDES FOR CAUSAL PROPHYLAXIS: These drugs act on the primary tissue forms of the plasmodia which grow within the liver and invade the erythrocytes. By blocking the erythrocytic stage, further development of the infection can be theoretically prevented. Drugs in this group include proguanil, pyrimethamine and primaquine. However, since it is impossible to predict the infection before clinical symptoms begins this mode of therapy is more theoretical than practical.

ii. TISSUE SCHIZONTICIDES: These drugs act on the hypnozoites of *P. vivax* and *P. ovale* that cause relapse of symptoms on reactivation in the liver. Primaquine is the prototype drug and pyrimethamine also has such activity. They thus produce radical cure.

iii. ERYTHROCYTIC SCHIZONTICIDES: These drugs act on the blood forms of the parasite and thereby terminate clinical attacks of malaria. They produce clinical cure. These agents include chloroquine, quinine, mefloquine, artemisinin etc. Other less effective blood schizonticides are the antifolate (pyrimethamine, sulphadoxine) and

the antibiotics (tetracycline, clindamycin) which are commonly used in conjunction with their more rapidly acting counterpart.

iv. GAMETOCIDES: These agents act against sexual erythrocyte form (gametocytes) of plasmodium, thereby preventing transmission of malaria parasites to mosquitoes. Chloroquine and quinine have gametocidal activity against *P. vivax* and *P. malariae*, while primaquine and artemisinin have gametocidal activity against all plasmodia species.

v. SPOROZONTICIDES: Drugs in this class prevent the development of oocytes and sporozoites in infected mosquitoes. Primaquine and chloroguanide have this action.

Classification based on chemical structure (White, 2010)

i. 4- AMINOQUINOLINES: This class consists of drugs such as chloroquine and amodiaquine used for prophylactic, suppressive and curative treatment.

ii. ARYL AMINO ALCOHOLS: This class consists of quinine, quinidine (cinchona alkaloids), mefloquine and halofantrine. The drugs are also used for suppressive, prophylactic and curative treatment.

iii. 8-AMINOQUINOLINES : Examples of these drugs include Primaquine, Tafenoquine, Balaquine

iv. NAPHTHOQUINONES: Atovaquone, a potent prophylactic and suppressive drug belongs to this class.

v. FOLATE SYNTHESIS INHIBITORS: These are of two types. Type 1, are the competitive inhibitors of dihydrofolate synthetase (sulphones, sulphonamides) and type 2 inhibits dihydrofolate reductase (include biguanides like proguanil and

chloroproguanil and diaminopyrimidine like pyrimethamine). The drugs are mainly used for curative treatment.

vi. SESQUITERPENE LACTONE DERIVATIVES: These consist of artemisinin (Qinghaosu) derivatives – artemether, arteether and artesunate artelinic acid used in curative therapy.

vii. ANTIMICROBIALS: This class consists of tetracycline, doxycyline, clindamycin and azithromycin used also as curative agents.

2.4.3 Chloroquine

Chloroquine is the prototype antimalarial drug, most widely used to treat all types of malarial infections. It is also the cheapest, time tested and safe antimalarial agent (Molina and Kimberley, 2012).

It is a 4-aminoquinoline compound with a complicated and still unclear mechanism of action. Being alkaline, the drug reaches high concentration within the food vacuoles of the parasite and raises its pH. It is found to induce rapid clumping of the pigment. Chloroquine inhibits the parasitic enzyme heme polymerase that converts the toxic heme into non-toxic hemazoin, thereby resulting in the accumulation of toxic heme within the parasite. It may also interfere with the biosynthesis of nucleic acids. Other mechanisms suggested include formation of chloroquine-heme complex and intercalation of the drug with the parasitic DNA (Martin *et al.,* 2009).

About ninety percent (90%) of the drug is absorbed from G.I.T after oral administration and rapidly absorbed from intramuscular and subcutaneous sites. It has a large distribution volume due to extensive sequestration in tissues of liver, spleen, kidney and lungs, hence the need for a larger loading dose. Therapeutic blood levels persist for 6-10 days and elimination

half-life is 1-2 months. Half of the drug is excreted unchanged by the kidneys while the remaining is converted to active metabolites in the liver (Alcantara *et al.,* 2013).

Chloroquine is highly effective against erythrocytic forms of *P. vivax*, *P. ovale*, *P. malariae,* sensitive strains of *P. falciparum* and gametocytes *of P. vivax.* It rapidly controls acute attack of malaria with most patients becoming febrile within 24-48 hours. It is safer and more effective than quinine for sensitive cases (Chen *et al.,* 2011).

Chloroquine is a relatively safe antimalarial. At therapeutic doses, it can cause dizziness, headache, diplopia, disturbed visual accomodation, dysphagia, nausea, malaise, and pruritus of palms, soles and scalp. It can also cause visual hallucinations, confusion and occasionally frank psychosis. These side effects do not warrant stoppage of treatment. It can also exacerbate epilepsy. When used at prophylactic dose of 300 mg of the base/ week, it can cause retinal toxicity after 3-6 years (after 50-100 g of chloroquine). Intramuscular injections of chloroquine can cause hypotension and cardiac arrest, particularly in children (Kurup *et al.,* 2010).

It should also be used with caution in patients with hepatic disease (as it is distributed widely in the liver and converted to active metabolites there), severe gastrointestinal, neurological or blood disorders. It should not be co-administered with gold salts and phenylbutazone, because all the three can cause dermatitis. Chloroquine may interfere with the antibody response to human diploid cell rabies vaccine (Wesche *et al.,* 2000).

Chloroquine is available as chloroquine phosphate tablets; each 250-mg tablet containing 150 mg of the base. Chloroquine hydrochloride injection contains 40 mg of the base per ml. Orally, chloroquine is given as 10 mg/kg stat, then three doses of 5 mg/kg, over 36-48 hours. Parenrerally, intravenous infusion of 10 mg/kg in isotonic fluid over 8 hours followed by 15 mg/kg over 24 hours is administered. In intramuscular or subcutaneous injections, 3.5 mg of

base/kg every 6 hours or 2.5 mg of base/kg every 4 hours is given (Intramuscular injection can cause fatal hypotension, especially in children) (Cabello *et al.,* 2011).

2.4.4 Quinine

Quinine is the major alkaloid of cinchona bark (known as 'Fever Bark'), a tree found in South America. Calancha, an Augustinian monk of Lima, first wrote about the curative properties of cinchona powder in "fevers and tertians" as early as 1633. In 1820, Pelletier and Caventou isolated quinine and cinchonine from cinchona. Even today, quinine is obtained entirely from natural sources due to difficulties in synthesising the complex molecule (Esu *et al.,* 2014).

Quinine acts as a blood schizonticide with similar mechanism of action as chloroquine. It is less effective and more toxic than chloroquine. It however has a special place in the management of severe *falciparum* malaria in areas with known resistance to chloroquine (Valdir, 2012).

Quinine is readily absorbed when given orally or intramuscularly. Peak plasma concentrations are achieved within 1-3 hours after oral dose and plasma half-life is about 11 hours. In acute malaria, the volume of distribution of quinine contracts, clearance is reduced and the elimination half-life increases in proportion to the severity of the illness. Therefore, maintenance dose of the drug may have to be reduced if the treatment is continued for more than 48 hours. The drug is extensively metabolised in the liver and only 10% is excreted unchanged in the urine. There is no cumulative toxicity on continued administration (Henry and Sanjeev, 2011).

Cinchonism (a typical syndrome of quinine side effects) can be mild in usual therapeutic dosage or severe in larger doses. Mild cinchonism consists ringing in the ears, headache, nausea and disturbed vision. Functional impairment of the eighth nerve results in tinnitus, decreased auditory acuity and vertigo. Visual symptoms consist of blurred vision, disturbed colour perception, photophobia, diplopia, night blindness and rarely, even blindness. These changes are due to direct neurotoxicity, although vascular changes may contribute to the problem (Dorndorp *et al.,* 2005).

Gastrointestinal symptoms like nausea, vomiting, abdominal pain and diarrhoea may be seen. Rashes, sweating, angioedema can occur. Excitement, confusion, delirium are also seen in some patients. Coma, respiratory arrest, hypotension and death can occur with over dosage. Quinine can also cause renal failure (Sutherland *et al.,* 2010). Massive haemolysis and haemoglobinuria can occur, especially in pregnancy or on repeated use. Hypoprothrombinemia, agranulocytosis are also reported. Quinine has little effect on the heart in therapeutic doses and hence regular cardiac monitoring is not needed. However it can cause hypotension in the event of overdose (Reyburn *et al.,* 2009).

Quinine stimulates insulin secretion and can cause hypoglycaemia in therapeutic doses. This can be severe in patients with severe infection and in pregnancy. Hypoglycaemia in malaria may go unnoticed and could even cause death. Therefore, it is advisable to monitor blood glucose levels at least once in 4-6 hours while quinine is administered, especially in severe infection and in pregnancy. Quinine-induced hypoglycaemia can recur even after administration of 25% or 50% dextrose. In such situations, maintenance with a 10% dextrose infusion is advisable. Resistant hypoglycaemia due to quinine can be managed with Injection of octreotide, 50 microgram subcutaneously, every 6 to 8 hours (Achan *et al.,* 2009).

Hypersensitivity in the form of rashes, angioedema, visual and auditory symptoms are indications for stopping the treatment. It is contraindicated in patients with tinnitus and optic neuritis. It should also be used with caution in patients with atrial fibrillation. Haemolysis is indication for immediately stopping the drug. It is also contraindicated in patients suffering from myasthenia gravis (Reyburn *et al.,* 2009).

2.4.5 Halofantrine

Halofantrine was developed in the 1960s by the Walter Reed Army Institute of Research. It is a phenanthrene methanol structurally related to quinine. Its mechanism of action may be similar to that of chloroquine, quinine and mefloquine; by forming toxic complexes with ferritoporphyrin IX that damage the membrane of the parasite. This synthetic antimalarial is effective against multi drug-resistant parasites including mefloquine resistant *P. falciparum* malaria (De-villiers *et al.,* 2008).

Its bioavailability is low and variable (may be doubled if taken with a fatty meal). The peak plasma concentration is achieved in 4-8 hours after oral dose. The elimination half-life is 1-3 days for the parent drug and 3-7 days for the active metabolite (Friedman and Caflisch, 2009).

Side effects of halofantrine include abdominal pain, diarrhoea and arrhythmias that could be fatal. It is contraindicated in patients with congenital, electrolyte disorders and myocardial disease. However, it appears less toxic than quinine and mefloquine. It is also contraindicated in pregnancy, lactation and infants (Friedman and Caflisch, 2009). Halofantrine is no longer used in the treatment of chloroquine-resistant and multi-drug resistant, uncomplicated *P. falciparum* malaria.

2.4.6 Mefloquine

Mefloquine was developed during the Vietnam war, as a result of research into newer antimalarials, to protect the American soldiers from the multi drug resistant *falciparum* malaria (González *et al.,* 2014).

Its mechanism of action is unknown but like quinine, it produces swelling of the *P. falciparum* food vacuoles. It may act by forming toxic complexes with free heme, damaging membranes and interacting with other plasmodial components. It is effective against the blood forms of *falciparum* malaria, including the chloroquine resistant types (Schlagenhauf *et al.,* 2010).

Mefloquine is well absorbed after oral administration, extensively bound to plasma proteins and concentrated in various tissues. Its elimination half-life is about 2-3 weeks due to continuous circulation through the enterohepatic and enterogastric systems. It is mainly excreted in faeces (Gofton *et al.,* 2010).

It is generally well tolerated in therapeutic doses up to 1500 mg. Nausea, vomiting, abdominal pain and dizziness can occur in doses exceeding 1 g. Less frequently, it can cause nightmares, sleeping disturbances, dizziness, ataxia, sinus bradycardia, sinus arrhythmia, postural hypotension, and an 'acute brain syndrome' consisting of fatigue, asthenia, seizures and psychosis. Mefloquine should be used with caution in patients with heart block, patients taking beta blockers, patients with history of epilepsy and psychiatric disease (McArdle *et al.,* 2005).

2.4.7 Atovaquone

This is a synthetic hydroxynaphthoquinone that was developed in the early 1980s and found to be useful against the Plasmodia (as well as Toxoplasma and Pneumocystis carinii). It is a

highly lipophilic molecule that supposedly interferes with the mitochondrial electron transport and thereby ATP and pyrimidine biosynthesis (Dohn *et al.,* 2004). It is found to target cytochrome b complex and disrupt the membrane potential in plasmodia. Its bio-availability after oral administration is poor and may be increased by a fatty meal. It has a long half-life of 2-3 days and undergoes enterohepatic circulation. It is available as 750 mg tablets. It may cause rashes, fever, vomiting, diarrhoea and head ache. Safety in pregnancy, lactation, children and elderly is yet to be established (Hughes *et al.,* 2013).

A fixed dose combination of atovaquone and proguanil hydrochloride (Malarone™) is now approved for both treatment and prophylaxis of malaria. It is available as 250 mg atovaquone + 100 mg proguanil per tablet for adults and 62.5 mg atovaquone + 25 mg proguanil per tablet for children (Djurković *et al.,* 2002).

It has been shown to be highly efficacious in the treatment of uncomplicated malaria caused by *plasmodium falciparum*, including malaria that has been acquired in areas with chloroquine-resistant or multidrug-resistant strains (Färnert *et al.,* 2003).

2.4.8 Chloroguanide (Proguanil)

Proguanil, a biguanide derivative was developed by British antimalarial research in 1945. It is converted to an active metabolite called cycloguanil pamoate which binds to and inhibits parasitic dihydrofolate reductase enzyme and inhibit folic acid metabolism. It has causal prophylactic and suppressive activity against *P. falciparum* and cures the acute infection. It is also effective in suppressing the clinical attacks of *vivax* malaria (Sutherland *et al.,* 2008).

Chloroguanide is slowly but adequately absorbed from the gastrointestinal tract. Peak plasma levels are attained within 5 hours and elimination half-time is about 16-20 hours.

Chloroguanide is available as tablets, each containing 100mg of the drug. The dose for prophylaxis is 100-200 mg daily. Chloroguanide along with chloroquine are used as an effective prophylaxis against *P. falciparum* malaria (Payen *et al.,* 2008).

At the prophylactic doses, it produces occasional nausea and diarrhoea. It is otherwise a safe drug and can be used in pregnancy (Thapar *et al.,* 2003).

2.4.9 Sulphadoxine and Pyrimethamine

Pyrimethamine and sulphadoxine are very useful adjuncts in the treatment of uncomplicated, chloroquine resistant *P. falciparum* malaria. It is now used in combination with artesunate for the treatment of *P. falciparum* malaria. It is also used in intermittent treatment in pregnancy (Matondo *et al.,* 2014).

Pyrimethamine inhibits the dihydrofolate reductase of plasmodia and thereby blocks the biosynthesis of purines and pyrimidines, which are essential for DNA synthesis and cell multiplication. This leads to failure of nuclear division at the time of schizont formation in erythrocytes and liver (Osher *et al.,* 2011).

Sulfadoxine inhibits the utilisation of para-aminobenzoic acid in the synthesis of dihydropteroic acid. The combination of pyrimethamine and sulfadoxine thus offers two step synergistic blockade of plasmodial division (Limpert *et al.,* 2013).

Pyrimethamine is slowly but completely absorbed after oral administration and is eliminated slowly with a plasma half-life of about 80-95 hours. Suppressive drug levels may be found in the plasma for up to 2 weeks. The drug is excreted in breast milk (Lange *et al.,* 2013).

Sulfonamides are rapidly absorbed from the gut and are bound to plasma proteins. They are metabolised in the liver and excreted in urine. They pass through the placenta freely. Sulfadoxine is a long acting sulfonamide with a half-life of 7-9 days (Limpert *et al.,* 2013).

Pyrimethamine can cause occasional skin rashes and depression of hematopoiesis. Excessive doses can produce megaloblastic anemia. Sulfonamides can cause numerous adverse effects. Agranulocytosis, aplastic anemia, hypersensitivity reactions like rashes, fixed drug eruptions, erythema multiforme of the Steven Johnson type and exfoliative dermatitis, serum sickness, liver dysfunction, anorexia, vomiting and acute hemolytic anemia can also occur (Vasudevan *et al.,* 2013). The drug is contraindicated in patients with known hypersensitivity to sulfa, infants below 2 months of age, patients with advanced renal disease and first and last trimesters of pregnancy (Matondo *et al.,* 2014).

Pyrimethamine and sulphadoxine are no longer used as single drugs, but only in combination with artesunate.

2.4.10 Artemisinin and its Derivatives

Artemisinin or Qinghaosu is the active principle of the Chinese medicinal plant, *Artemisia annua*. It has been used for treatment of fevers in China for more than 1000 years. The antimalarial value of *Artemisia annua* was first documented in Zhou Hou Bei Ji Fang (Handbook of prescriptions for emergency treatments) as early as 340 AD by Ge Hong of the Eastern Jin Dynasty (Douglas *et al.,* 2010). The active antimalarial constituent of this plant was isolated in 1971 and it was named artemisinin. A water soluble ester called artesunate and two oil soluble preparations called artemether and arteether have been developed (Rehwagen, 2006).

It is a sesquiterpene lactone with a chemically rare peroxide bridge linkage. Its antimalarial action involves the production of free radicals within the plasmodium food vacuole following cleavage of its endoperoxide bridge. It is also believed to covalently bind to and damage specific malarial proteins (Krudsood *et al.,* 2010).

It is the fastest acting antimalarial available. It has proven to be effective against all forms of muti-drug resistant *P. falciparum*. It is thus strictly controlled by WHO and every care is taken to ensure compliance, adherence and prevent other behaviours associated with the development of resistance. Onset of action is within 12 hours. Artemisinin derivatives are metabolised in the liver and excreted primarily in the bile (Dondorp *et al.,* 2005)

Artemisinin derivatives are absorbed well after intramuscular and oral administration. Toxic effects have been reported less frequently with the artemisinins than with other antimalarial agents. These include nausea, vomiting, anorexia, diarrhoea and dizziness (probably due, in many patients, to acute malaria rather than to the drugs). More serious toxic effects, including neutropenia, anemia, hemolysis, and elevated levels of liver enzymes have been noted rarely (Hou *et al.,* 2008).

The ether derivatives are more soluble in oil and are available as injections of 150 mg in 2ml for intra muscular use. Artemether is available as injection of 80 mg/ml and as capsules containing 40 mg of the drug. Artesunate is an ester derivative that is more soluble in water. The drug is available as a powder first dissolved in 1 ml of 5% sodium bicarbonate (usually provided with the vial) and shaken for 2-3 minutes then diluted with 5% dextrose or saline. Intravenous dose should be injected slowly at a rate of 3-4 ml/minute. It is also available as tablets, each containing 50 mg of the drug. There are no known interactions between artesunate and other drugs (Dondorp *et al.,* 2010).

The short half-lives of artemisinins limit the possibility of selection for resistance. Recent heavy use of artemisinins, including monotherapy, has however created selective pressure. Resistance to artesunate has been reported from Cambodia. Some parasites isolated from French Guiana and Senegal recently showed diminished *in-vitro* sensitivity to artemether and the efficacies of artemisinin-based combination agents have apparently decreased along the Thailand–Cambodia border (Krishna *et al.,* 2014). However, at present, the likelihood of true artemisinin resistance in malaria parasites is low, and this concern should not prevent the use of intravenous artesunate to treat severe malaria (Bhattarai *et al.,* 2007).

Artemisinin combination therapy (ACT)

ACT is a combination of an artemisinin derivative and another structurally unrelated and more slowly eliminated antimalarial. They are rapidly and reliably effective. Efficacy is determined by the drug partnering the artemisinin derivative, for artesunate–mefloquine, artemether–lumefantrine, and dihydroartemisin-piperaquine, this usually exceeds 95%. Artesunate–sulfadoxine–pyrimethamine and artesunate–amodiaquine are effective in some areas, but in other areas resistance to the partner precludes their use (Hou *et al.,* 2008). Most malaria endemic countries have now adopted artemisinin-based combination treatments as first-line treatment of *falciparum* malaria. Provided the partner drug is effective, ACTs ensure prompt recovery, high cure rates and are generally well tolerated (Krudsood *et al.,* 2010).

There is still uncertainty over the safety of artemisinin derivatives in the first trimester of pregnancy, when they should not be used unless there are no effective alternatives. Otherwise, except for occasional hypersensitivity reactions, the artemisinin derivatives are safe and remarkably well tolerated. The adverse effect profiles of the ACTs are determined by the partner drug (Mckee *et al.,* 2006).

2.4.11 Doxycline

Doxycline is a tetracycline compound derived from oxytetracyclines. It is one of the prevalent antimalarial drugs prescribed due to its relative effectiveness and affordability Chopra *et al.,* 2001). Tetracyclines are bacteriostatic agents, supposedly acting by inhibiting protein synthesis by binding to the 30s ribosome subunit thus preventing the 50s and 30s subunits from bonding (Connell *et al.,* 2003).

Tetracyclines are incompletely absorbed from the gut after oral administration and the absorption may be hampered by antacids containing aluminium hydroxide, calcium, magnesium, zinc salts and bismuth subsalicylate. They distribute widely in the tissues and accumulate in liver, spleen, bone marrow, bone, dentine and enamel of teeth. The drug is mainly excreted through the kidney (except minocycline) and that may be hampered in renal failure (McKee *et al.,* 2006).

Adverse effects include gastrointestinal irritation, nausea, vomiting, diarrhoea, photosensitivity, hepatotoxicity, aggravation of uremia, hypersensitivity reactions and staining of the teeth if used in young children and pregnant women (Connell *et al.,* 2003).

Doxycycline is useful in the treatment of drug resistant *P. falciparum* malaria. They act relatively slowly and hence should always be combined with a faster acting drug like quinine. They are contraindicated in children below the age of 8 years and in pregnant women because of adverse effects on bones and teeth (Chopra *et al.,* 2001).

2.4.12 Clindamycin

Clindamycin is a derivative of lincomycin with a slow action against blood schizonticides. It inhibits protein synthesis by binding to the 50s subunit of ribosomes. It is more toxic than other antibiotics and used only in cases where tetracyclines are contraindicated (for example

in children). It can be used for drug resistant malaria in conjuction with quinine at a dose of 10 mg/kg 8 hourly for 5 days. Adverse effects include nausea, vomiting, abdominal cramps and pseudomembrane colitis. In a study conducted by Brook *et al.,* (2005), a cure rate of only fifty percent (50%) was observed.

2.5 RESISTANCE

Antimalarial drug resistance has been defined as: "the ability of a parasite to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject" (WHO, 2014). The drug must gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action. In most instances, resistance refers to parasites that remain following an observed treatment thus excluding all cases where antimalarial prophylaxis has failed (Pearce *et al.,* 2009). In order for a case to be defined as resistant, the patient must have received a known and observed antimalarial therapy whilst the blood drug and metabolite concentrations are monitored concurrently. The techniques used to demonstrate this are: *in-vivo, in-vitro,* animal model testing and the most recently, molecular techniques (Cui and Su, 2009).

Drug resistant parasites are often used to explain malaria treatment failure. Drug resistance may lead to treatment failure, but treatment failure is not necessarily caused by drug resistance despite assisting with its development. Other factors that may be responsible for treatment failure include problems of non-compliance and adherence to therapy, poor drug quality, interactions with other pharmaceuticals, poor absorption, misdiagnosis and

administration of incorrect doses. Most of these factors also contribute to the development of drug resistance (Fairhurst and Wellems, 2010).

The generation of resistance can be complicated and varies between plasmodium species. It is generally accepted to be primarily initiated through spontaneous single point or multiple chromosome mutation. Mutations can be fatal for the parasite or the drug pressure will remove parasites that remain susceptible, however, some resistant parasites survive. Resistance can become firmly established within a parasite population, existing for long periods of time (White, 2004).

The first type of resistance to be acknowledged was to chloroquine in Thailand in 1957. The biological mechanism behind it was subsequently discovered to be related to the development of an efflux mechanism that expels chloroquine from the parasite before the level required to effectively inhibit the process of haem polymerization that is necessary to prevent build-up of the toxic by-products formed by haemoglobin digestion. This theory has been supported by evidence showing that resistance can be effectively reversed on the addition of substances which halt the efflux. The resistance to other quinolone antimalarials such as amodiaquine, mefloquine, halofantrine and quinine are thought to have occurred by similar mechanisms (Premji *et al.,* 2009).

Two gene mutations are thought to be responsible for resistance to antifolate combination drugs (sulphadoxine and pyrimethamine) with consequent synergistic blockade of two enzymes (dihydrofolate synthethase and dihydrofolate reductase) involved in folate synthesis. Regional variations of specific mutations give differing levels of resistance (Lim *et al.,* 2009).

Resistance to atovaquone is thought to originate from a single-point mutation in the gene coding for cytochrome-b (Greenwood *et al.,* 2005).

2.5.1 Spread of Resistance

A number of factors have been found to be responsible and/or influence the spread of resistance to antimalarial drugs. These include aspects of economics, human behaviour, pharmokinetics and the biology of vectors and parasites (Nadjm and Behrens, 2012).

The biological influences are based on the parasites ability to survive the presence of an antimalarial thus enabling the persistence of resistance and the potential for further transmission despite treatment. In normal circumstances, any parasite that persists after treatment are destroyed and removed by the host's immune system, thus any factor that acts to reduce effectiveness of the system could facilitate the development and intensification of resistance. This mechanism explains the poorer treatment response observed in immuno-compromised individuals, pregnant women and young children (D'Alessandro, 2009).

It was suggested that certain parasite and vector combinations can either enhance or inhibit the transmission of resistant parasites, causing 'pocket-like' areas of resistance (MacDonald *et al.,* 2001).

The use of antimalarials with closely related basic chemical compositions can increase the rate of resistance development. An example is the cross-resistance between the two 4-aminoquinolones (chloroquine and amodiaquine) and mefloquine conferring resistance to quinine and halofantrine (Kidgell *et al.,* 2006).

The resistance to some antimalarials among some *falciparum* parasites may be increased by a process of genetics (phenotypic) plasticity which allows the rapid development of resistance to a new antimalarial drug that has not been previously experienced (Lim *et al.,* 2009).

The pharmacokinetics of the chosen antimalarial is of important consideration. The decision to use a long half-life drug in preference to one that is metabolised quickly is complex and still remains unclear. Drugs with shorter half-life require more frequent administration to maintain the correct plasma concentrations, thus potentially presenting more problems if levels of adherence and compliance are unreliable. On the other hand, longer-lasting drugs can increase the development of resistance due to prolonged periods of low drug concentration (Koenderink *et al.,* 2010).

The pharmacokinetics of antimalarials should be considered when using combination therapy. Mismatched drug combinations, for example having an 'unprotected' period where one drug dominates can seriously increase the likelihood of selection for resistant parasites. The treatment regimen prescribed can have a substantial influence on the development of resistance. This can involve inadequate drug intake, pharmokinetic and pharmacodynamic properties of the drug or drug combination and drug interactions (Pearce *et al.,* 2009).

2.6 NATURAL PRODUCTS AND MALARIAL THERAPY

Traditional herbal medicines have been used to treat malaria for thousands of years in various parts of the world. There are records of age-long folkloric uses of plants as sources of therapeutic agents (Sofowora, 1993). There is a consensus among the scientific community that natural products have been playing a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases (Adegoju, 2008). The first antimalarial drug used in the occident was extracted from the bark of the *Cinchona* (*Rubiaceae*) species with the alkaloid quinine still largely used. Infusions of the plant bark were used to treat human malaria as early as 1632 (Samba, 2003). Quinine was isolated and characterized (Trapsida, 2003), thus becoming the oldest and most important antimalarial

drug. Another ancient medicinal plant of millennium used in the West is *Artemisia annua*, rediscovered in China in the seventies as an important source of antimalarial artemisinin (Sofowora, 1980). *Artemisinin* is currently a component of ACTs used as first line antimalarial drug in many countries.

It is estimated that over eighty percent (80%) of the world population depend on this form of health care (WHO declaration of Alma-Ata, 1978). Various concoction of plants and crude extract are effectively used for prevention and treatment of malaria and other ailments in several parts of the world (Slatter *et al.,* 1983).

The past decade has witnessed a significant increase in the global use of herbal medicines in response to the Alma-Ata declaration of 1978 for the inclusion of herbal medicines of proven efficacy and safety into the health care programme of developing countries (WHO declaration of Alma-Ata, 1978). Lapachol, a prenylated naphthoquinone from *Tabebuia* sp. (Bignoniaceae) provided the new pharmacophore that lead to the development of atovaquone (a synthetic 2-alkyl-3-hydroxy-1, 4-naphthoquinone) (Barennes *et al.,* 2006). *Dichroea febrifuga* is used against *P. Vivax* and *P. Ovale* but its liver toxicity makes it unacceptable as useful antimalarial drug (Steck, 1972).

Crude extract of *Salamun nudum (salanaceae*) has anti-falciparum activity *(*Pabon *et al.,* 2002*)* while alkaloid of *Dioncophyllum thollonii (dioncophllaceae*) was shown to be active against chloroquine-sensitive and resistant strains of *P. falciparum* (Bringmann *et al.,* 2002).

Other plants that are traditionally used for treatment of malarial include stem bark extract of *Amaranthus,* leaf extract of *Aphloia theiforms,* leaves extract of *cassia occidentalis,* whole plant extract of *Cissus quandrangulari*, shoot extract of *Cardiospermum halicacabum*, root bark extract of *Vangueria infausta* and fruits of *Tetrapleura tetraptera* (Dahanukar *et al.,* 2000; Douglas *et al.,* 2010).

2.7 PAIN

Pain is a distressing feeling often caused by intense or damaging stimuli, such as stubbing a toe, burning a finger, putting alcohol on a cut, and bumping the "funny bone". Because it is a complex, subjective phenomenon, defining pain has been a challenge. The International Association for the Study of Pain's widely used definition states that “pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Treede *et al.,* 2014).

Pain motivates the individual to withdraw from damaging situations, to protect a damaged body part while it heals and to avoid similar experiences in the future. Most pain resolves once the painful stimulus is removed and the body has healed, but it may persist despite removal of the stimulus and apparent healing of the body. Sometimes pain arises in the absence of any detectable stimulus, damage or disease. Simple pain medications are useful in twenty percent (20%) to seventy percent (70%) of cases (Breivik *et al.,* 2008).

Pain is the most common reason for physician consultation in most developed countries. It is a major symptom in many medical conditions, and can interfere with a person's quality of life and general functioning. Psychological factors such as social support, excitement or distraction can significantly affect pain's intensity or unpleasantness. In some arguments put forth in physician-assisted suicide or euthanasia debates, pain has been used as an argument to permit terminally ill patients to end their lives (Raj, 2007).

Pain is usually transitory, lasting only until the noxious stimulus is removed or the underlying damage or pathology has healed, but some painful conditions, such as rheumatoid arthritis, peripheral neuropathy, cancer and idiopathic pain may persist for years (Rajagopal, 2006).

Pain is a vital function of the nervous system in providing the body with a warning to potential or actual injury (Reddi and Curran, 2013). Pain is transmitted by nociceptors which are specialized sensory receptors responsible for detecting unpleasant stimuli and transforming them into signals which are then conducted to the central nervous system. Nociceptors are free nerve endings of primary afferent Aδ and C fibres, distributed throughout the body and can be stimulated mechanically, chemically or thermally. Mediators such as bradykinin, cytokines and prostaglandins produced during inflammatory process can stimulate nociceptors directly resulting in reduced activation threshold. Experience of pain is complex and subjective and is affected by factors such as mood, beliefs, cognition and genetics (Sinatra *et al.*, 2009).

2.7.1 Classification of Pain

Classifying pain is helpful in guiding, assessment and treatment. There are many ways to classify pain and according to Thienhaus and Cole (2002), these include:

i. Nociceptive (inflammatory) pain: This refers to pain that is caused by the stimulation of the nociceptor. It is further sub-divided into somatic and visceral pain depending on the site of origin. Somatic pain is of musculoskeletal (joint pain, myofascial pain) and cutaneous origin often localized and usually presented as aching, sharp or throbbing pain but it responds to common analgesics like ibuprofen and aspirin. Visceral pain results from activation of the thoracic, pelvic or abdominal organs. Visceral structures are highly sensitive to distension (stretch), ischemia and inflammation but relatively insensitive to other stimuli that evoke pain such as cutting or burning.
ii. Neuropathic pain: This is pain that is generated by impulse within the pathway proximal to the nociceptor (the nerve, spinal cord or the brain). Neuropathic pain is

also further subdivided into; neural injury pain, nerve compression pain and Complex Regional Pain Syndrome (CRPS). Neuropathic pain can be caused by pressure on nerve(s) presented as burning, numbness or heavy sensation. Nociceptive and neuropathic pains are not mutually exclusive; example is in a condition like sciatica.

Pain is also classified on the basis of duration of pain (Sinatra *et al*., 2009) as:

iii. Acute: Acute pain is due to traumatic tissue damage, usually limited in duration (less than two to three months) and associated with temporal reductions in intensity. It has distinct onset with short, well characterised duration and resolves with healing when there is effective therapy.

iv. Chronic: This is pain that can be defined as discomfort persisting 3-6 months beyond the expected period of healing. In some chronic pain conditions, symptoms of underlying disease states may be of more relevance than the duration of the pain. Chronic pain is characterised by multiple causes, gradual or distinct onset and may be refractory to treatment (Sinatra *et al*., 2009).

Traditionally, the distinction between acute and chronic pain has relied upon an arbitrary interval of time from onset. The two most commonly used markers being 3 months and 6 months since the onset of pain, though some theorists and researchers have placed the transition from acute to chronic pain at 12 months. Others apply acute to pain that lasts less than 30 days, chronic to pain of more than six months' duration and subacute to pain that lasts from one to six months. A popular alternative definition of chronic pain, involving no arbitrarily fixed durations, is "pain that extends beyond the expected period of healing". Chronic pain may be classified as cancer pain or else as benign (Debono *et al.,* 2013).

Inadequate treatment of pain is widespread throughout surgical wards, intensive care units, accident and emergency departments, in general practice, in the management of all forms of chronic pain including cancer pain and in end of life care (Peter, *et al.,* 2012).

The International Association for the Study of Pain advocates that the relief of pain should be recognized as a human right, chronic pain should be considered a disease in its own right and pain medicine should have the full status of a specialty. Currently, it is a specialty only in China and Australia. Elsewhere, pain medicine is a subspecialty under disciplines such as anesthesiology, physiotherapy, neurology, palliative medicine and psychiatry. In 2011, Human Rights Watch alerted that tens of millions of people worldwide are still denied access to inexpensive medications for severe pain (Peter, *et al.,* 2012).

2.8 BODY TEMPERATURE REGULATION AND CONTROL

Temperature is ultimately regulated in the hypothalamus. A trigger of the fever, called a pyrogen, causes a release of prostaglandin E_2 (PGE_2). PGE_2 acts on the hypothalamus to generate systemic response back to the rest of the body, causing heat-creating effects to match a new temperature level (Sajadi *et al.,* 2012).

In many respects, the hypothalamus works like a thermostat. When the set point is raised, the body increases its temperature through both active generation of heat and retention of heat. Peripheral vasoconstriction reduces heat loss through the skin and causes the indivisual to feel cold. If these measures are insufficient to make the blood temperature in the brain match the new set point in the hypothalamus, then shivering begins in order to use muscle movements to produce more heat. When the hypothalamic set point moves back to baseline either spontaneously or with medication, the reverse of these processes (vasodilation, end of

shivering and nonshivering heat production) and sweating are used to cool the body to the new lower setting (Johnson, 2002).

2.8.1 <u>Fever</u>

Fever, also known as pyrexia and febrile response, is defined as having a temperature above the normal range due to an increase in the body's temperature set-point. There is not a single agreed upon upper limit for normal temperature with sources using values between 37.5 and 38.3 °C (99.5 and 100.9 °F) (Garmel and Gus, 2012). Fevers do not typically go higher than 41 to 42 °C (105.8 to 107.6 °F). Fever is defined as a morning oral temperature of >37.2 °C (>98.9 °F) or an afternoon oral temperature of >37.7 °C (>99.9 °F) while the normal daily temperature variation is typically 0.5 °C (0.9 °F). The increase in set-point triggers increase muscle contraction, causes a feeling of cold, results in greater heat production and efforts to conserve heat. When the set-point temperature returns to normal the individual feels hot, becomes flushed and may begin to sweat. Rarely can a fever trigger a febrile seizure except in young children (Sullivan and Farrar, 2011).

Causes of Fever

A fever can be caused by many medical conditions that include viral, bacterial and parasitic infections such as the common cold, urinary tract infections, meningitis, malaria and appendicitis among others. Non-infectious causes include vasculitis, deep vein thrombosis, side effects of medication and cancer among others.

Hyperpyrexia is a fever with an extreme elevation of body temperature greater than or equal to 41.5 °C (106.7 °F). Such a high temperature is considered a medical emergency as it may indicate a serious underlying condition or lead to significant side effects. The most common cause is an intracranial hemorrhage. Other possible causes include sepsis, Kawasaki syndrome, neuroleptic malignant syndrome, drug effects, serotonin syndrome and thyroid storm. Infections are the most common cause of fevers. However, as the temperature rises, other causes become more common. Infections commonly associated with hyperpyrexia include roseola, measles and enteroviral infections. Immediate aggressive cooling to less than 38.9 °C (102.0 °F) has been found to improve survival. Hyperpyrexia differs from hyperthermia in that in hyperpyrexia the body's temperature regulation mechanism sets the body temperature above the normal temperature, then generates heat to achieve this temperature, while in hyperthermia the body temperature rises above its set point due to an outside source (Niven *et al.,* 2013).

Hyperthermia is an example of a high temperature that is not a fever due to either too much heat production or not enough heat loss. It results from a number of causes including heatstroke, neuroleptic malignant syndrome, malignant hyperthermia, stimulants such as amphetamines and cocaine, idiosyncratic drug reactions and serotonin syndrome (Wong *et al.,* 2013). The difference between fever and hyperthermia is the underlying mechanism (Laupland, 2009).

A fever is usually accompanied by sickness behavior, which consists of lethargy, depression, anorexia, sleepiness, hyperalgesia and the inability to concentrate. Persistent fever that cannot be explained after repeated routine clinical inquiries is called fever of unknown origin (Nassisi and Oishi, 2012).

Fever is established if:

i. Temperature in the anus (rectum/rectal) is at or over 37.5–38.3 °C (99.5–100.9 °F)
ii. Temperature in the mouth (oral) is at or over 37.7 °C (99.9 °F)
iii. Temperature under the arm (axillary) or in the ear (otic) is at or over 37.2 °C (99.0 °F) (Kiekkas *et al.,* 2013).

In healthy adult men and women, the range of normal oral healthy temperatures is 33.2–38.2 °C (91.8–100.8 °F) while it is 34.4–37.8 °C (93.9–100.0 °F) rectally, 35.4–37.8 °C (95.7–100.0 °F) for tympanic membrane (the ear drum) and 35.5–37.0 °C (95.9–98.6 °F) for axillary (the armpit) (Kiekkas *et al.,* 2013).

Types of fever

The pattern of temperature changes may occasionally hint at the diagnosis. The following types of fever association are generally classic associations and overlap may occur (Schaffner, 2006):

i. Continuous fever: Temperature remains above normal throughout the day and does not fluctuate more than 1°C in 24 hours, e.g. lobar pneumonia, typhoid, meningitis, urinary tract infection, brucellosis or typhus (drops due to fever-reducing drugs are excluded). Typhoid fever may show a specific fever pattern (Wunderlich curve of typhoid fever), with a slow stepwise increase and a high plateau.
ii. Intermittent fever: The temperature elevation is present only for a certain period and later cycling back to normal. E.g. malaria, kala-azar, pyraemia or septiceamia. The following are its types:

- Quotidian fever, with a periodicity of 24 hours, typical of *Plasmodium falciparum* or *Plasmodium knowlesi* malaria.
- Tertian fever with 48-hour periodicity, typical of *Plasmodium vivax* or *Plasmodium ovale* malaria
- Quartan fever with 72-hour periodicity, typical of *Plasmodium malariae* malaria.

iii. Remittent fever: Refers to temperature that remains above normal throughout the day and fluctuates more than 2°C in 24 hours. It is usually seen in typhoid infection and infective endocarditis. It is the most common type.

iv. A neutropenic fever, also called febrile neutropenia, occurs in the absence of normal immune system function. Lack of infection-fighting neutrophils results in a bacterial infection that can spread rapidly. It is therefore, usually considered to require urgent medical attention. It is more commonly seen in people receiving immune-suppressing chemotherapy.

v. Febricula is an old term for a low-grade fever, especially if the cause is unknown. Other symptoms are present and the patient recovers fully in less than a week (Schaffner, 2006).

2.9 MANAGEMENT OF PAIN AND FEVER

The management of pain is aimed at abolishing or alleviating the perception of the stimuli. The cause and nature of pain determines the duration and type of analgesic to be employed in the management (Peter, *et al.,* 2012).

Analgesics are drugs that relieve pain by acting at some point in the pain signalling mechanisms. They are divided into two major groups. These are central nervous system (morphine and related narcotic analgesics) and peripherally acting analgesics of pain originating in the viscera or arising from severe injuries, burns or neoplasms (Nassisi and Oishi, 2012).

2.9.1 Narcotic Analgesics

The term was coined by the Greek physician Galen to refer to agents that numb or deaden, causing loss of feeling or paralysis. Narcotics are also defined as substances that bind to opioid receptors. Opioid analgesics are narcotic analgesics obtained from the opium plant. More than 20 different alkaloids are obtained from the unripe seed of opium poppy plant. The analgesic properties of opium have been known for hundreds of years (Sajadi *et al.,* 2012). The narcotics obtained from raw opium (opiates, opioids or opiate narcotics) include morphine, codeine, hydrochlorides of opium alkaloids and camphorated tincture of opium. Synthetic narcotics are analgesics with properties and actions similar to the natural opioids. Examples include; methadone, levorphanol, remifentanil and meperidine (Kiekkas *et al.,* 2013).

Narcotic analgesics act via receptors namely mu (μ), kappa (κ), sigma (σ) and delta (δ). They elicit their cellular action through reduction of neurotransmitter release by closing a voltage-gated Ca^{2+} channel on pre-synaptic neuronal terminals and/or inhibition of presynaptic neurons (hyperpolarization) by increasing K^{+} channel conductance. Putative site of action of narcotic analgesics are; nociceptive nerve endings, spinal cord, thalamus, mid-brain and medulla (Fine, 2001).

Narcotic analgesics are also classified as strong agonists (morphine and meperidine), moderate agonists (codeine and prop oxyphene), mixed agonist-antagonists (pentazocine and nalbuphine) and antagonists (naloxone and naltrexone) (Niven *et al.,* 2013).

The major use of the narcotic analgesic is to relieve or manage mild, moderate to severe pain. The ability of a narcotic analgesic to relieve pain depend on several factors such as the drug, the dose, the route of administration, the type of pain, the patient and the length of time the drug has been administered. Morphine is the most widely used opioid and an effective drug for moderately severe to severe pain. Morphine's actions, uses and ability to relieve pain are the standards to which other narcotic analgesics are often compared. Other narcotics such as meperidine and levorphanol, are effective for the treatment of moderate to severe pain. Codeine and pentazocine are used for mild to moderate pain (Schulman *et al.,* 2005).

The adverse reactions differ according to whether the narcotic analgesic acts as an agonist or as an agonist-antagonist. For agonists, the major hazards of narcotic administration are respiratory depression with a decrease in the respiratory rate and depth. The most common adverse reactions include light-headedness, dizziness, sedation, constipation, anorexia, nausea, vomiting and sweating. When these effects occur, the dose should be lowered to eliminate or decrease the intensity of the adverse reaction. Others include physical dependence, pain at injection site and local tissue irritation (Garmel and Gus, 2012).

Administration of a narcotic agonist-antagonist may result in symptoms of narcotic withdrawal in those addicted to narcotics. Other adverse reactions associated with the administration of a narcotic agonist-antagonist include sedation, nausea, vomiting, sweating, headache, vertigo, dry mouth, euphoria and dizziness (Sinatra *et al*., 2009).

All narcotic analgesics are contraindicated in patients with known hypersensitivity to the drugs. These drugs are contraindicated in patients with acute bronchial asthma, emphysema,

or upper airway obstruction and patients with head injury or increased intracranial pressure, convulsive disorders, severe renal or hepatic dysfunction and acute ulcerative colitis. Narcotic analgesics are not recommended for use during pregnancy or labour (may prolong labour or cause respiratory depression of neonate) except if the benefit to the mother outweighs the potential harm to the fetus (Treede *et al.,* 2014).

They are used cautiously in the elderly and in patients with undiagnosed abdominal pain, history of addiction to the opioids, hypoxia, supraventricular tachycardia, prostatic hypertrophy and renal or hepatic impairment. The obese must be monitored closely for respiratory depression while on narcotic analgesics (Schulman *et al.,* 2005).

The narcotic analgesics potentiate the central nervous system (CNS) depressant properties of other CNS depressants such as alcohol, antihistamines, antidepressants, sedatives, phenothiazines and monoamine oxidase inhibitors. Patients taking the agonist-antagonist narcotic analgesics may experience withdrawal symptoms if they have has been abusing or using narcotics (Garmel and Gus, 2012).

2.9.2 Non-narcotic Analgesics

Drugs belonging to this class are weaker analgesics, mainly used for treating musculo-skeletal pain. In addition, they lower the body temperature in fever (anti-pyretic action) and possess anti-inflammatory activity. Drugs in this class include acetaminophen and non-steroidal anti-inflammtory drugs (NSAIDs) (DeRuiter, 2002).

The major mechanism of action of NSAIDs is through inhibition of prostaglandin (PG) synthesis and competitive inhibition of cyclooxygenases (COX) (enzymes that catalyze synthesis of cyclic endoperoxides from arachidonic acid to form prostaglandins). There are

two major COX isoenzymes identified (COX-1 and COX-2), most NSAIDs inhibit these enzymes non-selectively. The inhibitions by NSAIDs block the formation of mediators of pain and inflammation (Nassisi and Oishi, 2012).

Most NSAIDs are weak acids, with a pKa of 3–5. They are well absorbed from the stomach and intestinal mucosa. They are highly protein-bound in plasma (typically >95%) usually to albumin, so that their volume of distribution typically approximates to plasma volume. Most NSAIDs are metabolised in the liver by oxidation and conjugated to inactive metabolites excreted in the urine, though some drugs are partially excreted in bile. Metabolism may be abnormal in certain disease states and accumulation may occur even with normal dosage (Sinatra *et al.*, 2009).

NSAIDs are classified as (Sajadi *et al.,* 2012):

i. Salicylates; this class have potent anti-inflammatory activity with mild analgesics and anti-pyretic activities. Toxicities include gastro-intestinal irritation, hypersensitivity reactions, inhibition of platelets aggregation and ototoxicity. Examples are acetylsalicylic acid and diflunisal.
ii. Propionic acid derivatives; these are also strong organic acids (pKa= 3-5). They are structurally derived from arylacetic acids. They can enter foetal circulation and breast milk and should be avoided in pregnancy and during lactation. Examples are ibuprofen, fenoprofen, and caprofen.
iii. Aryl and heteroarylacetic acids are also derived from acetic acid, but in this case the substituent at the position-2 of the heterocycle or related carbon cycle. They can also be sub-divided into indoles, pyrroles and oxazoles. Examples are indomethacin and etodolac.

iv. Anthranilates primarily have anti-inflammatory activity, it is an N-aryl substituted derivative of anthranilic acid and also used as mild analgesics. Examples are mefenamic acid and diclofenac.
v. Oxicams are characterised by the 4-hroxybenzothiazine heterocycle. Examples are piroxicam and meloxicam.
vi. Phenylpyrazolones are characterised by 1-aryl-3, 5-pyrazolidinedione structure. They also possess mild uricosuric activity. Examples are phenylbutazone and oxyphenylbutazone.
vii. Cyclooxygenase enzyme-2 (COX-2) selective inhibitors are diaryl-5-membered heterocycles. They act selectively on COX-2 but not cyclooxtgenase-1 (COX-1). Examples are celecoxib, valdecoxib and rofecoxib.
viii. Anilides are simple acetamides of aniline; they are somehow different from other NSAIDs in their mechanism of action. They possess analgesic and anti-pyretic but weak anti-inflammatory effect. The mechanism of action is not fully determined. Examples are paracetamol and phenacetin.

NSAIDs are generally used for the symptomatic relief of the following conditions: pyrexia, osteoarthritis, rheumatoid arthritis, mild-to-moderate pain due to inflammation and tissue injury, low back pain, ankylosing spondylitis, psoriatic arthritis, reactive arthritis, tennis elbow, headache, migraine, acute gout, dysmenorrhoea, metastatic bone pain, postoperative pain, muscle stiffness and pain due to Parkinson's disease (Niven *et al.,* 2013).

Common adverse drug reactions (ADRs) of NSAIDs include: raised liver enzymes, headache and dizziness. Rare ADRs include: hyperkalaemia, confusion, bronchospasm, rash, rapid and severe swelling of the face and/or body. Ibuprofen may also rarely cause irritable bowel

syndrome symptoms. NSAIDs are also implicated in some cases of Stevens–Johnson syndrome (Schulman *et al.,* 2005).

NSAIDs should usually be avoided by people with the following conditions: peptic ulcer or stomach bleeding, uncontrolled hypertension, kidney disease, inflammatory bowel disease (Crohn's disease or ulcerative colitis), undergoing coronary artery bypass surgery, in third trimester of pregnancy, undergone gastric bypass surgery and history of allergic or allergic-type NSAID hypersensitivity reactions, such as aspirin-induced asthma (DeRuiter, 2002).

2.10 *CATUNAREGAM NILOTICA*

Catunaregam nilotica is a wild fruit belonging to the family Rubiaceae*, genus Catunaregam Wolf* with synonyms including *Randia nilotica* Stapf, *Canthopsis* Miq, *Narega* Raf*, Lachnosiphonium* Hochst *Tiruvengadum* and *Xeromphis nilotica* (Stapf) Keay (Steentoft, 1998). It grows as a medium height shrub (usually less than 3 m) with grey drupes, stiff spines and deciduous leaves clustered below the spines. It is widespread in Central and East Africa, as well as in Cameroon and Nigeria (Farid *et al.,* 2002). In Nigeria, the Hausa tribe call it '*kwanarya'*, while it is called '*gi'al-goti'* in Ful-fulde. The plant in Niger republic is called '*lilo'* by the Songhai tribe (Burkill, 2000) while the Sudanese call it '*kerkir'* (Ismail and Adam, 2015) and it is known as '*maggaare'* in India (Singh and Ali, 2012).

Plate I :- *Catunaregam nilotica* plant in its natural habitat in Rano L.G.A., Kano, Nigeria.

2.10.1 Ethnomedicinal Uses of *Catunaregam nilotica*

Catunaregam nilotica has a broad range of applications in the indigenous medical system (Farid *et al.,* 2002). The fruits are traditionally used in Niger republic as emetics as well as remedy for diarrhoea and dysentery in India (Singh and Ali, 2012). In india, the roots are frequently prescribed as paste on the forehead for headaches (Singh and Ali, 2012) while the decoction of its seeds are traditionally used for headaches and the stem bark in treating joints and muscle affections (Srivastava *et al.,* 2012). Its roots and fruits are used in Sudan to lighten and consequently remove the scars of pimples while some maids use same as purgative (Ismail and Adam, 2015). Accorging to Neuwinger (1996), the roots are used for treatment of gonorrhoea, diarrhoea, colic fever, phlegmatic swelling locally, as genital stimulants and also in treatment of venereal diseases in Niger. The bark is used externally for

rheumatism and bruises, internally for fever and insomnia while the stem bark is used for the treatment of malaria in Nigeria (Burkill, 2000).

2.10.2 Previous Scientific Studies on *Catunaregam nilotica*

Methanol extracts of phenolic compounds from *Catunaregam nilotica* leaves, barks and seed cake where shown to contain natural antioxidants (Mariod *et al.,* 2012). Haemolytic activity was found in two molluscicidal saponins isolated from fruits of *Catunaregam nilotica* (Lemmich *et al.,* 1995). Mariod *et al.* (2012) reported that the seed kernels of *C. nilotica* have high economic potential due to high protein and oil contents. In earlier studies, Mariod *et al.* (2010), extracted *C. nilotica* oil using two different methods and reported very high oil content (40.0%) with linoleic oleic, palmitic and stearic acids as the major fatty acids and high amount of tocopherol (110.5 mg/100 g oil). According to Runyoro *et al.* (2006), the root bark extract of *Catunaregam nilotica* possesses anti-candida activity.

2.11 THEORETICAL FRAMEWORK

In recent times, focus on plant research has increased all over the world, with more than thirteen thousand plants studied between 1996 and 2000 (Danhanukar *et al.,* 2000). There is sufficient evidence of the immense potential of medicinal plants used in various traditional systems.

2.11.1 Assays for antimalarial activity

Rodent malaria parasites such as *Plasmodium berghei-berghei, P. yoelii, P. chabaudi* and *P. vinckei* are used extensively as models of human malaria (Phillipson, 1991). *Plasmodium berghei-berghei* an *in-vivo* model discovered in 1948 at Kreyberg in Congo is a unicellular parasite (protozoan) and one of the many species of malaria parasites that infect mammals other than humans. This parasite readily infects laboratory mice and rats and proved to be valuable for estimation of malarial activity in chemotherapeutic research programmes (Frank *et al.,* 2010).

The *in-vivo* investigation is carried out in three (3) stages using adult male albino mice infected with chloroquine-sensitive *Plasmodium berghei-berghei* parasite, namely:

i. Curative (established infection) test: - This procedure evaluates the efficacy of the plant extract as a therapeutic agent. The mice are infected and left for seventy two hours (72 hrs) before treatment with plant extract and standard drugs for five days. Smears are made from tail blood of each mouse after each treatment to monitor the parasitaemia level. The drug is said to have a curative effect if the treated animals survive at least twice as long as the control (14 days or longer). Untreated controls usually die between 6-7 days (Ryley and Peters, 1970).

ii. Suppressive (early infection) test: - In this procedure, treatment with the extract is started immediately after the mice have been inoculated for four days. Smears are made from tail blood of each mouse on the fifth day and the parasitaemia level examined microscopically. The drug is said to have suppressive effect when there is a decrease in parasitaemia level in test groups compared to the untreated control groups (Peter, 1980).

iii. Prophylactic test: - This test assesses the efficacy of the extract as prophylactic agent (causal prophylaxis). The mice are treated with the extract for five days before being inoculated with the parasite. Smears are made from tail blood of each mouse seventy two hours (72 hrs) after inoculation and level of parasitemia examined under the microscope. Significant decrease in parasitaemia level in drug-treated groups compared to untreated control groups suggests that the test compound has a prolonged action/prophylactic effect in preventing the pre-erythrocytic forms of malaria parasites (Peters, 1995).

2.11.2 Analgesic Assays

Valid and predictive preclinical animal models used for the identification and development of new pharmacotherapies for treating human chronic pain syndromes include:

Chemically- induced pain

Chemical stimulation involves the administration of algogenic agents representing a slow, progressive and reversible form of stimulation.

Acetic Acid-induced Writhing Test (Koster *et al*., 1959)

This test is used to induce pain of peripheral origin by injecting acetic acid, an irritant principle. Behavioural manifestations of abdominal writhing include an arching of back, extension of limbs and contraction of abdominal musculature. Analgesic activity of the test compound is inferred from decrease in frequency of writhings (Sigmund and Dannerman, 2011).

Formalin-Induced Hind-Paw Licking Pain Test (Dubuisson and Dennis, 1977)

Formalin test dissociates between inflammatory (peripheral) and non-infammatory (neurogenic) pain according to their site and mechanism of action. 2.5% formalin is administered subcutaneously (sc) into the sub-plantar surface of the left hind paw of rats to induce pain and the analgesic effect of the extract is determined in two (2) phases as described by Dubuisson and Dennis (1977) and modified by Tjolsen *et al.* (1992). The first phase which normally peaks 5 minutes after formalin injection represents the non-inflammatory (neurogenic) response while the second phase that peaks 15-30 minutes after formalin injection represents the inflammatory (peripheral) responses (Lee *et al.,* 2012).

Thermally- induced pain

Heat is a suitable stimulus for activating cutaneous receptors.

Hot Plate Test (Woolfe and Macdonald, 2004)

This test is used for measuring supraspinal nociceptive integrated responses. It involves evaluation of thermal pain reflexes due to footpad contact with a heated surface. Two behavioural components in terms of reaction time measured are paw-licking and jumping. Licking is a rapid response that is a direct indicator of nociceptive threshold while jumping represents a more elaborate response with latency and encompasses emotional component of pain. Significant increase in reaction time (the period between the time when the animal is placed on the heated surface and the manifestation of these behaviours by the test compound) indicates central analgesic activity (Sorbera *et al*., 2012).

2.11.3 Antipyretic Assay

Brewer's yeast test (Al-Ghamdi, 2001)

This test is a reproducible method for assaying compounds with antipyretic activity in rodents. Brewer's yeast produces hyperpyrexia by increasing synthesis of tumour necrotic factor (TNF) and prostaglandins, specifically PGE_2. Significant decrease in temperature in brewer's yeast-induced hyperpyrexia in rats by test compound indicates antipyretic activity (Ratitid *et al.,* 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Experimental Animals

Apparently healthy adult Swiss albino mice (*Mus musculus*) (18-35 g) and healthy adult Wistar rats (*Rattus noevegicus*) (125-250 g) of either sex were obtained from college of Medicine, University of Lagos animal house and National Institute for Pharmaceutical Research and Development (NIPRD) animal facility centre (AFC), Idu, Abuja.

They were housed in cages in a well-ventilated room under standard condition of temperature and light in the animal house of National Institute for Pharmaceutical Research and development (NIPRD) Idu, Abuja. They were observed for seven (7) days prior to the study, fed on commercial Pfizer grower's mash prepared in pellets and given water *ad libitum* except when fasting was necessary in the course of the study. All experiments were performed in accordance with the "principles of laboratory animal care" (NIH publication, 1985) and NIPRD standard operating procedures.

3.1.2 Collection and Identification of Plant Material

The fresh stem bark of *Catunaregam nilotica* was collected from Rano Local Government Area of Kano state, Nigeria between July-August, 2015 by Muhammad Langyam. The whole plant was identified and authenticated by Baha'uddeen Said Adam of Department of Botany herbarium, Bayero University, Kano (BUK). A voucher specimen (BUKHAN 0287) was deposited in the herbarium for future reference.

3.1.3 Drugs and Chemicals

The drugs and chemicals used in the study included methanol (BDH chemicals, England), Tween 80, absolute methanol and glacial acetic acid (Lobechemi, India), Giemsa stain (Hightech Health Care, India), normal saline (Fresenius kabi AG, Germany), pentazocine, chloroquine phosphate tablet, ketoprofen and pyrimethamine (Glaxo Smith), Wagners, Mayer's reagents and Brewer's yeast (Fisher scientific, UK), ferric chloride (Fisher scientific company, USA), dilute ammonia and hydrochloric acid (Riedal de Haen/Germany), chloroform (Finkem Laboratory Reagent, India) and sulphuric acid (Farmitalia carlo erba/Italy).

3.1.4 Equipment and Apparatus

Equipment used included mortar and pestle, percolator, laboratory glass wares, wash bottle, dropping pipette, plastic water bottle, microscope glass slides with cover slip, glass staining jar, digital thermometer, digital weighing machine, tally counter, stop watch, microscope, slide box, 1 ml and 5 ml syringes and needles.

3.1.5 Plasmodium Parasite

The chloroquine-sensitive *Plasmodium berghei-berghei* was obtained from National Institute of Medical Research (NIMR), Lagos, Nigeria and maintained in mice by continuous intraperitoneal injection every 4 days in fresh mice (Adzu and Salawu, 2009).

3.2 METHODS

3.2.1 Preparation of the Ethanolic Stem Bark Extract

The stem bark of the *Catunaregam nilotica* plant was pruned while fresh and air dried at room temperature for one month. The dried stem bark was reduced to a fine powder using wooden mortar and pestle. Five hundred and six grams (506.0 g) of the powdered stem bark was extracted with four litres (4.0 L) of 70% v/v ethanol using percolation method for two weeks. The extract obtained was concentrated using rotary evaporator and evaporated to dryness in a waterbath at 45^0C. The resulting extract was brownish-orange in colour, weighed, stored in air-tight container and incubated prior to use. Percentage yield was calculated as follows:

$$\text{Yield} = \frac{\text{weight of the extract (g)}}{\text{weight of the ground plant material (g)}} \times 100$$

3.2.2 Preliminary Phytochemical Studies

The preliminary phytochemical analysis was carried out on the ethanol stem bark extract of *Catunaregam nilotica* at the Department of Biochemistry, Bayero University, Kano to identify the presence or absence of organic metabolites according to methods described by Trease and Evans (2009) and Sofowora (1993).

Test for alkaloids (dragendorff's test)

0.5 ml of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid (HCI) on water bath and later filtered. The filtrate was divided to 3 portions of 1 ml each. A few drops of Dragendorff's reagent was added to first 1 ml of the filtrate and orange red precipitate observed confirmed the presence of alkaloids.

Test for anthraquinones (borntrage's test)

The plant extract at a quantity of 0.5 g was shaken with 1 ml of benzene and filtered. The filtrate was mixed with 5 ml of 10% ammonia solution. The mixture was shaken and the presence of a red colour in the ammonia (lower) phase indicated the presence of free hydroxyl anthraquinones.

For combined anthraquinones, 0.5 g of the plant extract was boiled with 10 ml aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of benzene. The benzene layer was separated and to half its own volume and 10% ammonia solution was added. A red colouration in the ammonia phase (lower layer) indicated the presence of combined anthraquinones.

Test for cardiac glycosides (keller-killani's test)

The extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution, followed by addition of 1 ml concentrated sulphuric acid at an angle of 45 degrees. A reddish brown ring at the interface indicated the presence of a desoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for flavonoids (shinoda's test)

Little portion of the extract was dissolved in ethanol, warmed over water bath and filtered. Four to five pieces of magnesium turning were added to the filtrate, followed by addition of few drops of concentrated hydrochloric acid (HCI). The presence of pink colouration is an indication of the presence of flavonoids.

Test for saponins (frothing test)

Little portion of the extract dissolved in 10 ml of water was shaken gently for 30 seconds in a test tube and warmed on water bath. Formation of frothing persistently for 30 minutes indicates the presence of saponins.

Test for tannins (lead sub-acetate test)

A small quantity of the extract was boiled with 10 ml of water, cooled and filtered. The filtrate was used for the following test. Addition of few drops of lead sub-acetate solution to 1 ml of the filtrate will produce a white precipitate indicative of the presence of tannins.

Test for phlobatannins

Deposition of a red precipitate when an aqueous extract portion of the plant was boiled with 1% aqueous hydrochloric acid was taken as an evidence of the presence of phlobatannins.

Test for terpenoids and steroids (liebermann-burchard test)

A little of the extract was shaken with chloroform ($CHCI_3$) followed by addition of 1ml of acetic anhydride and conc. Sulphuric acid (H_2SO_4). A greenish-brown ring colour at upper layer was formed, an indication of the presence of steroids and violet colour for terpenes.

Determination of volatile oil

To 2 ml of *C. nilotica* extract, 0.1 ml of dilute NaOH was added and then 1ml of HCl and the solution shaken. Formation of white precipitate indicates the presence of volatile oil.

3.2.3 Acute Toxicity Study (Median Lethal Dose Determination)

Acute toxicity study was conducted in two phases using Lorke's method (1983). In the first phase, 9 mice were divided into three groups of three mice each and after overnight fasting they were treated with the extract at doses of 10, 100 and 1000 mg/kg orally via intragastric cannula. The mice were observed closely for the first four hours and then 24 hours after treatment for symptoms of toxicity (such as restlessness, abdominal writhing, decreased physical activity and respiratory distress) and death.

In the second phase, based on the result of the first phase, 3 mice were also divided into three groups of one mouse each and were treated with the extract at doses of 1600, 2900 and 5000 mg/kg orally, after overnight fasting. The mice were observed closely for the first four hours and then 24 hours later for signs of toxicity and death as in the first phase.

The median lethal dose (LD_{50}) was calculated as the geometric mean of the lowest dose that caused death and the highest dose at which all the mice survived. This is expressed mathematically as:

$$LD_{50} = \sqrt{A \times B}$$

Where A = minimum lethal dose

B = maximum tolerated dose

3.2.4 Experimental Design

All rats and mice used for the anti-plasmodial, analgesic and anti-pyretic studies were randomely divided into five (5) groups of six (6) animals each as follows:

- Group I Negative control (normal saline)
- Group II 250 mg/kg of plant extract
- Group III 500 mg/kg of plant extract
- Group IV 1,000 mg/kg of plant extract
- Groups V Standard drug

The doses were determined from results of acute toxicity studies.

3.2.5 Anti-plasmodial Study

The study was conducted using chloroquine-sensitive *Plasmodium berghei-berghei* parasite from a donor mouse in three (3*) in-vivo* (suppressive, curative and prophylactic) models of anti-plasmodial screening of medicinal plants in mice.

Parasite inoculation

A donor mouse infected with *Plasmodium berghei-berghei* parasite (parasitemia of about 20-30%) was decapitated and blood collected through the jugular vein into a heparinised bottle. The inoculum was prepared by determining both the percentage parasitaemia and the red blood cell (RBC) count of the donor mouse and diluting the blood with isotonic saline in such

a way that 0.2 ml of the blood contained approximately 1.0 X 10^7 infected RBCs. Inoculum in volumes of 0.2 ml was administered intraperitoneally to infect each mouse (Peters, 1965).

Antiplasmodial activity against established infection (Curative test)

Evaluation of the curative potential of the extract against established infection was carried out as described by Ryley and Peters (1970). On the first day (D_1), adult mice were inoculated and left untreated for 72 hrs (D_1-D_4) for infection to be established. On day 4, all inoculated mice were then randomized into 6 groups of 6 mice each and treated with normal saline (10 ml/kg), graded doses (250, 500 and 1,000 mg/kg body weight) of the extract and two standard drugs, chloroquine and artesunate (5 mg/kg body weight each) orally for 5 days (D_4-D_8). On each day of treatment, each mouse was tail-bled, thin blood film prepared, stained with Giemsa stain and parasitaemia levels determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields. The mice were thereafter monitored and the mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post-inoculation) in each group over a period of 28 days (Saidu *et al.,* 2000; Adzu and Salawu, 2009). The average parasitaemia level was calculated as:

$$[(A-B)/A]x100$$

where

A is the average parasitaemia level in the negative control group and B is the average parasitaemia level in the test group.

Antiplasmodial activity against early infection (suppressive test)

The 4-day suppressive test of the extract against *Plasmodium berghei-berghei* infection in mice as described by Peter (1980) was employed. On the first day (D_1), adult mice were inoculated with *Plasmodium berghei-berghei* and thereafter randomly divided into their respective groups as described in the curative test. On the same day (D_1), treatment was started and continued daily. Twenty four hours (24 hrs) after administration of the last dose (D_5), the mice were tail-bled and thin film prepared, fixed in absolute methanol and stained with 3% Giemsa solution at pH 7.2. The parasitaemia level was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of microscope.

Repository (prophylactic) test

The prophylactic activity of the extract was studied using the procedure described by Peters (1970). Adult mice were randomized into 6 groups of 6 mice each and treated as described in experimental design under grouping and dosing orally for five (5) days (D_1-D_5). The standard drugs used were 1.2 mg/kg of pyrimethamine and 5 mg/kg chloroquine. On the sixth day (D_6), all the mice were inoculated with *Plasmodium berghei-berghei*. Smears were made from tail blood of each mouse 72 hours after inoculation and the parasitaemia level was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of microscope (Abatan and Makinde, 1986).

3.2.6 Analgesic Studies

Acetic acid-induced writhing test in mice

The test was carried out as described by Koster *et al.* (1959). Thirty mice were randomized into 5 groups of 6 mice each and treated with normal saline (10 ml/kg), graded doses (250, 500 and 1,000 mg/kg body weight) of the extract and standard drug (10 mg ketoprofen/kg body weight) orally. After 1 hour of treatment, 0.6% aqueous solution of acetic acid (10 ml/kg per body weight) was administered to each mouse intraperitoneally. The number of abdominal writhing movements was counted after 5 minutes for 30 minutes. Analgesia was expressed as the reduction in the number of abdominal constrictions between control and extract-treated mice. The percentage inhibition was then calculated in relation to the control.

$$\text{Percentage (\%) inhibition} = \frac{\text{number of constrictions in control} - \text{number of constrictions in test}}{\text{Number of constrictions in control}} \times 100$$

Hot plate-induced pain in mice (Turner *et al.,* 1963)

The hot plate test was performed according to the method adopted by Vongtau *et al.* (2004). A 600 ml beaker of fifty centimetre (50 cm) diameter was placed on a hot plate (heated surface) that was maintained at 55 ± 1^0C. Each mouse was then placed in the beaker to test their pain threshold such that only mice that responded to the pain by either paw licking or jumping within 2 seconds after placement were selected for the study. Thirty selected mice were then divided into 5 groups of 6 mice each and pre-treated with normal saline (10 ml/kg), graded doses (250, 500 and 1,000 mg/kg body weight) of the extract and morphine (5 mg/kg body weight) orally. The reaction time between placement and hind paw lifting/licking or jumping in the mice was measured one hour after extract or drug administration and

subsequently at 30 minutes intervals for a total of 120 minutes. A 15 seconds cut-off exposure time was used to prevent tissue damage.

Formalin-induced hind-paw licking in rats

The method described by Dubuisson and Dennis (1977) and modified by Tjolsen *et al.* (1992) was adopted for this test. Rats were fasted for 24 hours before the commencement of the experiment, but allowed access to water *ad libitum*. Rats in group 1 (negative control) received 10 ml/kg normal saline, groups II–IV received graded doses (250, 500 and 1,000 mg/kg body weight) of the extract while the positive control received 5 mg/kg b.w of morphine orally as in the study design. One hour after extract and drug administration, 0.2 ml of 3% formalin was injected subcutaneously into the sub-plantar surface of the left hind paw of each rat. Each rat was placed in transparent observation chamber and maximum pain responses produced were scored every 1 minute for the first 5 minutes after formalin injection (first phase) and every 5 minutes between 15-30 minutes after formalin injection (second phase). The severity of pain response was recorded for each rat based on the following scale:

Score	Observed response
0	rat walked or stood firmly on the injected paw
1	partial elevation or light weight bearing of the injected paw on the floor
2	elevation of the injected paw without contact with the floor
3	shaking or licking or biting of the injected paw

3. 2. 7 Antipyretic Studies

Brewer's yeast-induced pyrexia in rats (Al-Ghamdi, 2001)

The estimation of antipyretic efficacy of ethanol extract was carried out using Brewer's yeast induced pyrexia method in rats. The normal rectal temperature of each rat was initially measured using a digital IMCORP Telethermometer coated with lubricant. Fever was induced in the rats by subcutaneously injecting 10 ml/kg b.w. of 20% w/v suspension of Brewer's yeast in normal saline. Rectal temperature of the rats were taken and only rats whose rectal temperature increased by at least 1.0°C after 18 h of yeast injection were included in the study. These rats were randomly divided into five groups containing six rats in each group. The control group (I) rats were orally administered 10 ml/kg saline while the standard group (V) rats were given 10 mg/kg aspirin and groups II–IV were given 250, 500 and 1,000 mg/kg of ethanol extract of *CN* respectively. The rectal temperature of each rat was recorded at time intervals of 30 minutes for 120 minutes after drug and extract administration.

3.2.8 Data Analysis

Results of the studies were expressed as mean plus or minus standard error of mean (M±SEM) using one-way analysis of variance (ANOVA) followed by Dunnete's post hoc test were used for comparison among groups. Statistical significance was set at $p<0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 PLANT PREPARATION AND EXTRACTION

The *Catunaregam nilotica* (*CN*) stem bark powder was dark green in colour. The ethanol stem bark extract yielded a water-soluble sticky brownish-orange substance that weighed 56.8 g. The calculated percentage yield was 11.23%.

4.2 ACUTE TOXICITY STUDIES

No deaths occurred during the observation period in both phases. Some symptoms that included initial restlessness with writhing abdominal movement that was followed by a decrease in physical activity (sedation) associated with mild respiratory distress were noted in both mice and rats 1 hour following administration of the extract. These, however resolved by 3 hours after administration. Similar signs of toxicity (decreased physical activity and respiratory distress) manifested in all the groups and doses used in the second phase which were also reversed within the first 4 hours of extract administration. Therefore, the oral LD_{50} was deduced to be greater than 5,000 mg/kg.

4.3 PRELIMINARY PHYTOCHEMICAL SCREENING

The results obtained from the phytochemical screening of ethanol stem bark extract of *CN* showed the presence of alkaloids, tannins, saponins, terpenoids, flavonoids, steroids and cardiac glycosides while phlabotannins, volatile oils and anthraquinones were absent (Table 4.1).

Table 4.1: Phytochemical Constituents of Ethanolic Extract of *C. nilotica*

Constituents	Inference
Glycosides	+
Flavonoids	+
Saponins	+
Alkaloids	+
Anthraquinones	–
Phlabotanins	–
Volatile oil	–
Tannins	+
Steroids	+
Terpenoids	+

+ Present, - Absent

4.4 *IN -VIVO* ANTI-PLASMODIUM STUDIES

4.4.1 Curative Test

There was a dose-dependent reduction in the level of parasitemia in the extract-treated groups unlike in the saline control group in which there was consistent increase in the blood parasite level. The mean survival time also increased dose-dependently. Mortality was recorded in the control group on eighth day and by the twelveth day, all mice in the group died (mean survival time of 10 days). On the other hand, the extract treated groups survived beyond 21 days. Chloroquine and artesunate treated groups survived the 28 days duration of observation (Table 4.2).

TABLE 4.2: Curative Effect of Ethanol Stem Bark Extract of *C.nilotica* in Mice Infected with *Plasmodium Berghei-berghei.*

	Mean parasitaemia level (% Clearance)					
Treatment (mg/kg)	D_4	D_5	D_6	D_7	D_8	Survival Time (Days)
N/S 10ml/kg	16.05±0.36	27.87 ± 2.97	38.87 ± 2.72	39.93±1.78	42.20 ± 3.24	10.00±1.19
ESCN (250)	14.56±0.29 (9.0%)	13.44±2.53* (52%)	11.34±1.16** (71%)	09.92±1.73** (75%)	09.06±0.97** (79%)	22.70±1.90
ESCN (500)	14.44±0.42 (10.0%)	12.00±0.28* (57%)	10.06±0.38** (73%)	08.74±0.24** (78%)	07.88±0.26** (81%)	24.30±1.80
ESCN (1,000)	12.73±0.53* (21%)	10.43±0.18* (63%)	10.08±0.29** (74%)	08.83±0.19** (78%)	06.79±0.20** (84%)	26.20±1.30
CQ (5)	11.80±1.49* (26%)	10.17±0.56* (64%)	08.08±1.67** (79%)	05.08±1.20** (87%)	04.44±2.07** (89%)	28.00±0.00
ART (5)	12.26±0.53* (24%)	11.20±0.82* (60%)	09.06±1.40** (77%)	07.97±1.68** (80%)	05.90±0.41** (86%)	28.00±0.00

Data were expressed as M±SEM. * and ** = significantly different from control at $p<0.005$ and 0.001 respectively using ANOVA and Dunnete's post hoc test. ESCN=Ethanol stem bark extract of *Catunaregam nilotica,* CQ= Chloroquine, ART= Artesunate, n= 6, D_4, D_5, D_6, D_7, D_8 indicate days 4,5,6,7 and 8 respectively.

4.4.2 Suppressive Test

The ethanol stem bark extract of *C. nilotica* demonstrated dose-dependent schizonticidal activity at the doses used in this study. The extract at 1,000 mg/kg caused 80% chemosuppression as against 79% and 67% chemosuppression induced by 500 and 250 mg/kg respectively. The standard drugs chloroquine and artesunate caused 97% and 90% chemosuppression respectively (Table 4.3).

TABLE 4.3: Suppressive Effect of Ethanol Stem Bark Extract of *C.nilotica* in Mice Infected with *Plasmodium Berghei-berghei*

Treatment (mg/kg)	Parasitaemia count/cell	% Chemosuppression
N/SALINE 10 ml/kg	24.80 ± 1.48	0
ESCN (250)	08.15 ± 1.80*	67
ESCN (500)	05.10 ± 3.00*	79
ESCN (1,000)	04.90 ± 2.50*	80
CHLOROQUINE (5)	0.86 ± 1.60*	97
ARTESUNATE (5)	02.60 ± 1.30*	90

Data were expressed as M±SEM. * =significantly different from control at p <0.001 using ANOVA and Dunnete's post hoc test. ESCN = Ethanol Stem Bark Extract of *Caunaregam nilotica*, n = 6.

4.4.3 Prophylactic Test

ESCN produced significant ($p<0.005$ and 0.001) and dose-dependent reduction in parasitaemia levels of 27%, 48% and 60% at 250, 500 and 1,000 mg/kg respectively. Chloroquine and pyrimethamine treated groups caused 90% and 88% reduction in parasitaemia level respectively in the infected mice (Table 4.4).

TABLE 4.4: Prophylactic Effect of Ethanol Stem Bark Extract of *C.nilotica* in Mice Infected with *Plasmodium Berghei-berghei*

Treatment (mg/kg)	Parasiteamia count/cell	% Prophylaxis
N/SALINE 10 ml/kg	25.01 ± 1.58	0
ESCN (250)	18.15 ± 1.60*	27
ESCN (500)	13.10 ± 2.00**	48
ESCN (1,000)	09.90 ± 2.50**	60
CHLOROQUINE (5)	02.60 ± 0.46**	90
PYRIMETHAMINE (1.2)	03.08 ± 1.30**	88

Data were expressed as M±SEM ESCN = Ethanol Stem Bark Extract of *Caunaregam nilotica*, n = 6, * and ** = significantly different from control at $p<0.005$ and 0.001 respectively using ANOVA and Dunnete's post hoc test.

4.5 ANALGESIC STUDIES

4.5.1 Acetic Acid-Induced Writhing Test

The extract significantly ($p<0.001$) decreased the number of acetic acid-induced writhes in mice in a dose-related manner. The extract provided analgesia over the monitoring period of the study and the effect increased with time. Ketoprofen also exhibited significant ($p<0.001$) and inhibition of acetic acid-induced writhes in the mice (Table 4.5).

TABLE 4.5: Effect of Ethanol Stem Bark Extract Of *C.nilotica* on Acetic Acid-Induced Writhes in Mice.

Treatment (mg/kg)	No. of Writhings	% Inhibition
N/SALINE 10 ml/kg	34.7 ± 6.4	0.00
ESCN (250)	17.4 ± 1.17**	49.86
ESCN (500)	15.2 ± 1.20**	56.20
ESCN (1,000)	10.0 ±1.20**	71.18
KETOPROFEN (10)	8.0 ± 1.60**	76.95

Data were expressed as M±SEM. * significantly different from control at $p < 0.001$ using ANOVA and Dunnete's post hoc test. ESCN = Ethanol Stem Bark Extract of *Caunaregam nilotica*, n = 6.

4.5.2 Hot Plate Induced Pain in Mice

Oral doses of *C. nilotica* stem bark ethanol extract produced significant ($p<0.05$, 0.005 and 0.001) increase in reaction time compared to control group. There was also significant ($p<0.005$ and 0.001) increase in reaction response to morphine when compared to control treated group (Table 4.6).

TABLE 4.6: Effect of Ethanol Stem Bark Extract of *C.nilotica* on Hot Plate-Induced Pain in Mice.

Treatment (mg/kg)	Reaction time (seconds) after placement on hot plate				
	1 hr	1 hr.30 mins	2 hrs	2 hrs.30 mins	3 hrs
N/SALINE 10ml/kg	1.60 ± 0.27	1.15 ± 0.17	1.15 ± 0.06	1.09 ± 0.15	1.02 ± 0.32
ESCN (250)	1.39 ± 0.22	1.60 ± 0.29	1.73±0.48*	1.97±0.34**	1.82 ±0.21**
ESCN (500)	1.88± 0.25	1.89 ± 0.48*	2.08± 0.40**	2.22±0.34***	2.43±0.32***
ESCN (1,000)	1.80 ± 0.22	1.99 ± 0.21**	2.30±0.28***	2.43±0.40***	2.93±0.47***
MORPHINE (5)	2.44± 0.06**	2.67 ± 0.38***	2.93±0.29***	3.08±0.23***	3.59±0.27***

Data were expressed as M±SEM. Significant at *=P < 0.05, ** = P<0.005 and ***=P<0.001 when compared to control using ANOVA and Dunnete's post hoc test. ESCN = Ethanol Stem Bark Extract of *Caunaregam nilotica*, n = 6.

4.5.3 Formalin Test

The extract exhibited a dose-dependent effect on formalin-induced hind paw licking in mice. It significantly ($p<0.005$ and 0.001) inhibited formalin-induced paw licking response in rats in both phases at all doses used compared to control. The standard drug, morphine (5 mg/kg) also significantly ($p<0.001$) reduced pain response in both early and late phases compared to control (Table 4.7).

TABLE 4.7: Effect of Ethanol Stem Bark Extract of *C.nilotica* on Formalin-Induced Pain in Rats.

Treatment (mg/kg)	Early phase (% inhibition) 0-5 minutes	Late phase (% inhibition) 15-30 minutes
N/Saline 10 ml/kg	18.00 ± 0.10	25.44 ± 0.35
ESCN (250)	9.30 ± 0.36 *(48%)	12.45 ± 0.21** (51%)
ESCN (500)	4.50 ± 0.98 ** (75%)	7.44 ± 0.28** (71%)
ESCN (1,000)	2.20 ± 0.46 ** (88%)	3.65 ± 0.76** (86%)
MORPHINE (5)	2.10 ± 0.22** (88%)	3.54 ± 0.24** (86%)

Data were expressed as M±SEM. * and ** = significantly different from control at p<0.005 and <0.001 respectively using ANOVA and Dunnete's post hoc test. ESCN = Ethanol Stem Bark Extract of *Catunaregam nilotica*, n = 6.

4.6 ANTI-PYRETIC STUDY

4.6.1 Brewer's Yeast-Induced Pyrexia Test

The extract caused dose-dependent decrease in rectal temperature of hyperpyrexic rats. The effect was significant ($p<0.05$, 0.005 and 0.001) at the highest dose of 1,000mg/kg used. Acetyl salicylic acid also caused significant ($p<0.05$ and 0.001) reduction in rectal temperature of the rats (Table 4.8).

TABLE 4.8 Effect of *Catunaregam nilotica* Stem Bark Ethanol Extract on Brewer's Yeast Induced Pyrexia.

		Rectal Temperature ^{0}C after 18hrs of Yeast Injection				
Treatment (mg/kg)	BBT	0 min	30 mins	60 mins	90 mins	120 mins
N/SALINE 10ml/kg	37.64±0.10	40.93±0.11	40.48±0.17	39.21±0.14	39.13±0.16	39.09±0.15
ESCN (250)	37.21±0.20	40.43±0.19	40.11±0.17	38.93±0.14	38.73±0.16	38.69±0.11
ESCN (500)	37.39±0.40	40.61±0.14	40.02±0.17	38.81±0.09	38.68±0.18	38.61±0.13
ESCN (1,000)	37.32±0.30	40.58±0.12	39.98±0.19*	38.31±0.24**	38.29±0.18**	38.12±0.12***
ASPIRIN (10)	37.59±0.10	40.72±0.11	39.78±0.12*	38.21±0.14***	38.09±0.19***	37.93±0.16***

Data were expressed as M±SEM, *, ** and ***= significantly different from the control at $p<0.05$, 0.005 and 0.001 respectively using ANOVA and Dunnete's post hoc test. ESCN = Ethanol Stem Bark Extract of *Catunaregam nilotica,* BBT= Basal Body Temperature, n = 6.

4.7 DISCUSSION

In traditional practice, medicinal plants and herbal medicines were considered as rich sources for disease treatment worldwide. Malaria is one of the world's most deadly infectious diseases. Spread and emergence of resistance to the front line antimalarial drugs including artemisinin is the major challenge that jeopardized all recent gains in malaria control and has major implications for public health (Marfurt *et al.,* 2010; Ashley *et al*., 2014). The scientific community is now underway to combat this problem by searching for new, affordable and effective antimalarial agents from medicinal plants and other sources (Gamo, 2014).

The preliminary phytochemical screening of ethanol stem bark extract of *Catunaregam nilotica* revealed the presence of alkaloids, tannins, saponins, terpenoids, flavonoids, steroids and cardiac glycosides. These are secondary metabolites of plants with potent biological activities. As reported by Dharani *et al.* (2010), common antimalarial plants used to treat malaria in traditional medicine contain secondary metabolites such as alkaloids, terpenoids, coumarins, flavonoids, chalcones, quinones and xanthones. Madara *et al.* (2010) attributed antimalarial activity of *Piliostigma thonningii* to its alkaloidal and terpenoidal content. The conventional antimalarial drug, quinine is an alkaloid from the bark of cinchona tree. Flavonoids, alkaloids, anthraquinones and terpenes isolated from other plants species have been found to possess antimalarial activity in both *in-vitro* and *in-vivo* tests (Bickii *et al.,* 2000; Tona *et al.,* 2004). According to Philipson and Wright (1990), alkaloids and flavonoids exert their antiplasmodial effects by either elevating red blood cell oxidation or inhibiting protein synthesis.

Flavonoids, alkaloids and saponins were shown to have pronounced anti-nociceptive activity (Vongtau *et al.,* 2000; Choi *et al.,* 2005). A study by Sawant *et al.* (2004) reported that the total alkaloid fractions of *Eclipta alba* were responsible for its central and peripheral

analgesic activities while Annegowda *et al.* (2010) reported that flavonoids show analgesic activity by enhancing the endogenous serotonin level or interact with 5-HT_{2A} and 5-HT_3 receptors.

The LD_{50} of mice and rats after oral administration of 5,000 mg/kg body weight of the extract within the observed period implied that ethanol stem bark extract of *C. nilotica* was practically non-toxic (Lorke, 1983).

Several conventional antimalarial agents including chloroquine, halofantrine, mefloquine and derivatives of artemisinin were identified using rodent malaria models (Mućnoz *et al.,* 2000; David *et al*., 2004). Rodent malaria parasites are used extensively as models of human malaria. *P. berghei-berghei* preferentially invades reticulocytes and usually produce infections in mice that induce severe pathololology. It has been used as a model to study immunopathology, experimental cerebral malaria, pregnancy-associated malaria and lung pathology (Pierrot *et al.,* 2003; Pedroni *et al.,* 2006). The ethanol stem bark extract of *Catunaregam nilotica* was thus evaluated for its *in-vivo* antimalarial activity in mice infected with chloroquine sensitive *Plasmodium berghei-berghei.* Several studies have employed *P. berghei-berghei* in predicting treatment outcome of suspected antimalarial agents. Chloroquine (4-amino-quinoline with gametocidal, rapid and marked blood schizonticidal activity), pyrimethamine (folate synthesis inhibitor with tissue and blood schizonticidal activity) and artesunate (sesquiterpene lactone with potent blood schinzonticidal, gametocytocidal activity and the most effective antimalarial drug known) were thus the appropriate reference drugs for this study (Agbedahunsi, 2000; Adzu and Salawu, 2009). Substances that significantly reduce parasite level (antiplasmodial effect) in the host are considered to possess antimalarial activity (Ryley and Peters, 1970). The determination of the percentage inhibition of parasitaemia is however the most reliable parameter (Agbedahunsi, 2000).

The results obtained from the present study showed that the ethanol stem bark extract of *C. nilotica* possesses significant suppressive effect against early plasmodium infection, curative effect against established infection and prophylactic effect against residual infection in *P. berghei-berghei* infected mice.

In the established infection test, the extract produced daily dose-dependent and significant ($p<0.005$ and $p<0.001$) reduction in the mean parasitaemia compared to control. This suggests that the extract possesses high blood schizonticidal activity against *Plasmodium berghei-berghei* parasite. There was daily increase in the level of parasitemia in the group treated with normal saline throughout the period of established infection test. The extract also increased the mean survival time of the treated mice (between 22 to 26 days) while chloroquine and artesunate treated groups recorded 28 days compared to the control group of 10 days.

The 4-days suppressive test is a standard test commonly used in evaluating the antimalarial activity of candidate agents in early infections (Peters, 1965). The extract produced significant ($p<0.001$) decrease in level of parasitaemia compared to control group. The percentage chemosuppression level of the extract ranged from 67%, 79% and 80% for the 250, 500 and 1000 mg/kg doses respectively. The group treated with Artesunate produced 90% while Chloroquine-treated group produced 97% chemosuppression, a reflection of greater sensitivity of the plasmodium parasite to chloroquine.

The extract thus showed significant and similar pattern of activity (percentage reduction in parasitaemia levels) in the curative and suppressive tests which are commonly used for antimalarial drug screening.

The stem bark ethanol extract produced low to moderate prophylactic activity of 27%, 48% and 60% for 250, 500 and 1,000 mg/kg doses respectively compared with chloroquine (90%) and pyrimethamine (88%).

Analgesic models used in this study were chosen to allow for investigation of both centrally and peripherally mediated anti-nociceptive activities to determine the probable mechanism of action of extract.

The peripheral analgesic activity of the extract was evaluated using acetic acid-induced writhing test. Administration of an irritant agent such as acetic acid into the peritoneal cavity of mice triggers the release of a variety of mediators that include arachidonic acid, cyclo-oxygenase, substance P and bradykinins (Verma *et al.,* 2005). Acetic acid also produces increased level of lipooxygenase products as well as PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluids (Soroush *et al.,* 2012). These substances activate the chemo-sensitive nociceptors that play a role in the development of typical inflammatory pain response characterized by an arching of back, extension of hind limbs and contraction of abdominal musculature in the mice. These chemosensitive nociceptive receptors are sensitive to non-steroidal anti-inflammatory drugs (NSAIDS) like aspirin, indomethacin and ketoprofen, the standard drug used (Pinheiro *et al*., 2010). NSAIDs inhibit the conversion of arachidonic acid to the unstable intermediate endoperoxide, PGH_2, the common substrate for a number of different synthetases that produce many lipid mediators including the major PGs (PGE_2, PGD_2 and $PGF_2\alpha$) as well as thromboxane and prostacyclin (PGI_2) (Su *et al.,* 2011).

C. nilotica ethanol extract showed significant (p<0.001) and dose-dependent reduction in the number of abdominal constrictions induced by acetic acid compared to the control group. Therefore, it can be assumed that the pain relief effects of the extract are supported by peripheral mechanisms (Borras *et al.,* 2004) which may be linked to inhibition of cyclo-oxygenases and/or lipoxygenases.

The hot plate test is considered to be selective for evaluating centrally acting analgesics (Le Bars *et al.,* 2001). The stem bark ethanol extract of *C. nilotica* produced significant (p<0.05,

$p<0.005$ and $p<0.001$) and dose-dependent analgesic activity as revealed by the increased reaction time to thermal pain. Centrally acting analgesics act by raising pain threshold and altering the physiological response to pain. Analgesic responses of paw-licking and jumping in mice are considered to be supraspinally integrated (Gupta *et al.,* 2008). The ability of the extract to significantly and dose-dependently increase reaction time to thermally-induced pain suggests that it possesses some central analgesic activity which may be mediated via mu and to some extent kappa opiod receptors (as morphine). The most commonly used opiods for pain management act on mu opiod receptor systems (Oweyele *et al*., 2008).

The advantage of formalin test is its ability to distinguish between compounds that act through central and peripheral pain pathways (Pan *et al.,* 2010). Centrally acting agents inhibit both early and late phases almost equally while peripherally acting agents inhibit only the late phase (Toker *et al*., 2004). Subcutaneous injection of formalin causes two pain-inflicting phases. The first phase (acute) is a neurogenic response which normally peaks 5 minutes after formalin injection. It is produced by direct chemical stimulation of the active nociceptive neurons transmitted via C-fibres and can be suppressed by opioids such as pentazocine (Negus *et al.,* 2006). The inflammatory (chronic) phase peaks 15–30 minutes after formalin injection and it is produced through activation of ventral horn neurons on the surface of spinal cord mediated by cytokines such as serotonins, histamine and prostaglandins (Gupta *et al.,* 2012). The extract significantly ($p<0.005$ and $p<0.001$) and dose-dependently inhibited both the early neurogenic phase (51%, 75%, and 88%) and late inflammatory phase (48%, 71% and 86%) of pain responses. Suppression of both phases of pain by the extract provides further evidence of dual analgesic activities involving centrally and peripherally mediated pain mechanisms.

Pyrexia is a result of secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. Mediators like interleukin α, β and TNF-α

increase the synthesis of prostaglandin E_2 near pre-optic hypothalamus area thereby triggering the hypothalamus to elevate the body temperature (Pan *et al.,* 2010; Binny *et al.,* 2010). According to Akpan *et al.* (2012), yeast induced pyrexia is caused by increase in synthesis of PGE_2. Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin produce their antipyretic action through inhibition of prostaglandin synthesis within the hypothalamus. Flavonoids such as baialini and alkaloids such as boldine were reported to exert antipyretic effect by suppressing TNF-α (Chang *et al.,* 2007) or inhibiting the synthesis of PGE_2 (Backhouse *et al.,* 1994). The ethanol stem bark extract of *Catunaregam nilotica* produced significant ($p<0.05$ and $p<0.005$) antipyretic effect in yeast induced elevation of body temperature in experimental rats at the highest dose of 1,000 mg/kg used only.

The significant antipyretic activity of the extract at 1,000 mg/kg body weight may be due to reduction in prostaglandins specifically PGE_2 concentration in the hypothalamus through inhibition of cyclooxygenase pathway, similar to aspirin the standard drug used. The presence of flavonoids in the ethanol stem bark extract of *C. nilotica* may be contributory to its antipyretic activity.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 SUMMARY

The ethanol stem bark extract of *Catunaregam nilotica* contains alkaloids, tannins, saponins, terpenoids, flavonoids, steroids and cardiac glycosides. Oral acute toxicity study revealed that the median lethal dose (LD_{50}) is greater than 5000 mg/kg in both rats and mice showing that it is practically non-toxic acutely.

In the established infection test, the extract produced daily dose-dependent reduction in the mean parasitemia level with significant ($p<0.05$ and $p<0.001$)) antimalarial activity at all doses compared to control. The extract produced a significant ($p<0.001$) decrease in the level of parasitaemia in suppressive test compared to control group. The level of Chemosuppression of the extract ranged from 67%, 79% to 80% for the 250, 500 and 1000 mg/kg doses respectively. The extract however, produced low to moderate prophylactic activity of 27%, 48% and 60% chemosuppression at 250, 500 and 1,000 mg/kg doses respectively.

The extract at doses of 250, 500 and 1,000 mg/kg produced significant ($p<0.001$) and dose-dependent inhibition of acetic acid induced writhing in mice. Similarly, the extract produced significant ($p<0.05$ $p<0.005$ and $p<0.001$) and dose-dependent increase in reaction time in hot plate induced pain and exhibited significant ($p<0.005$ and $p<0.001$) anti-nociceptive activity in both phases of the formalin test. The extract significantly ($p<0.05$ and $p<0.05$) reduced brewer's yeast-induced pyrexia in rats at the highest dose of 1,000 mg/kg used.

5.2 CONCLUSION

The ethanol stem bark extract of *Catunaregam nilotica* is practically non-toxic acutely and possesses phytochemical constituents that may be responsible for the observed curative, suppressive and prophylactic antiplasmodial, the centrally and peripherally mediated analgesic and antipyretic activities. These findings suggest scientific basis for the ethnomedical use of *Catunaregam nilotica* stem bark in malaria and/or pain in Rano Local Government Area of Kano State in Northern Nigeria.

5.3 RECOMMENDATIONS

From the present study, the following are suggested for further investigation on the plant:

i. Quantitative phytochemical investigation to clearly identify the antiplasmodial, analgesic and antipyretic active components from the plant.
ii. Evaluation of the anti-inflammatory effect of the plant

REFERENCES

Abatan, M.O. and Makinde, M.J. (1986). Screening *Azadirachta indica* and *Pisum sativum* for possible antimalarial activities. *Journal of Ethnopharmacology*, 17: 85-93.

Achan, J., Tibenderana, J. and Kyabayinze, D. (2009). Effectiveness of quinine versus artemether- lumefantrine for treating uncomplicated falciparum malaria in Ugandan children: randomised trial. *Britain Medical Journal*, 338: 2763.

Adegoju, A. (2008). A rhetorical analysis of the discourse of advertising herbal medicine in Southwestern Nigeria. *Linguistic online,* 33(1):2-18.

Adzu, B. and Salawu, O.A. (2009). Screening *Diospyros mespiliformis* extract for antimalarial potency. *International Journal of Biochemical Sciences,* 3(2): 271-276.

Agbedahunsi, J.M. (2000). Screening of crude drugs for the treatment of malaria in Nigeria, Phytomedicine in malaria and sexually transmitted diseases: Challenges for the new millennium. Drug Research and Production Unit, Faculty of pharmacy, Obafemi Awolowo University, Ile Ife, Nigeria, 13-22.

Akpan, E.J., Okokon, J.E. and Etuk, I.C. (2012). Antiplasmodial and antipyretic studies on root extracts of *Anthocleista djalonensis* against *Plasmodium berghei. Asian Pacific Journal of Tropical Disease,* 2:36-42.

Alcantara, L.M., Kim, J.W., Moraes, C.B., Franco, C.H., Franzoi, K.D., Lee, Sukjun, L.H. and Ayong, L.S. (2013). Chemosensitization potential of P-glycoprotein inhibitors in malaria parasites. *Experimental Parasitology,* 134 (2): 235–43.

Al-Ghamdi, M.S. (2001). Anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa. Journal of Ethnopharmacology,* 76:45-48.

Annegowda, H.V., Mordi M.N., Ramanathan, S. and Mansor, S.M. (2010). Analgesic and antioxidant properties of ethanolic extract of *Tamarindus catappa* L. leaves. *International Journal of Pharmacology,* 6:910-5.

Anthony, M.P., Burrows, J.N., Duparc, S.J., Moehrle, J. and Wells, T.N. (2012). The global pipeline of new medicines for the control and elimination of malaria. *Malarial Journal,* 11:316.

Ashley, E., Dhorda, M., Fairhurst, R., Amaratunga, C., Lim, P. and Suon, P. (2014). Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *New England Journal Medicine,* 371: 411-23.

Ayoola, G. A., Coker, H. A., Adesogun, S. A. and Adepoju-Bello, A. A. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for Malaria therapy in south-western Nigeria. *Tropical journal of Pharmaceutical Reseach,* 7 (3):1019-1024.

Backhouse, N., Delporte, C., Givernan, M., Cassls, B.K., Valenzuela, A. and Speisky, H. (1994). Anti-inflammatory and antipyretic effects of boldine. *Inflammation Research,* 42:114-117.

Barennes, H., Balima-Koussoubé, T. Nagot, N., Charpentier, J. and Pussard, E. (2006). Safety and efficacy of rectal compared with intramuscular quinine for the early treatment of moderately severe malaria in children: randomised clinical trial. *Britain Medical Journal,* 332 (7549): 1055–57.

Barnes, P.M. and Bloom, B. (2008). Complementary and alternative medicine use among adults and children: United States, 2007. *National health statistic report,* 12: 1-24.

Bhatt, S., Weiss, D., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., Battle, K., Moyes, C., Henry, A., Eckhoff, P., Wenger, E., Briët, O., Penny, M., Smith, T., Bennett, A., Yukich, J., Eisele, T., Griffin, J., Fergus, C., Lynch, M., Lindgren, F., Cohen, J., Murray, C., Smith, D., Hay, S., Cibulskis, R. and Gething, P. (2015). The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature,* 526 (7572): 207–211.

Bhattarai, A.M., Ali, A.S., Kachur, S. P., Mårtensson, A.K., Abbas, A.K., Khatib, R.S., Al-mafazy, A.W. and Ramsan, M.G. (2007). Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS Medicine,* 4 (11): 309.

Bickii, J., Njifutie, N., Ayafor, J. F., Basco, L. K. and Ringwald, P. (2000). *In-vitro* antimalarial activity of limonoids from *Khaya grandifoliola* C.D.C. (Meliaceae). *Journal of Ethnopharmacology,* 69: 27-33.

Binny, K., Kumar, S. and Thomas, D. (2010). Anti-inflammatory and antipyretic properties of the rhizome of *Costus Speciosa (Koen.). Journal of Basic Clinical Pharmacology,* 1(3):177-181.

Borras, M., Becerra, L., Ploghaus, A., Gostic, J., DaSilva, A. and Gonzalez, R. (2004). fMRI measurement of CNS responses to naloxone infusion and subsequent mild noxious thermal stimuli in healthy volunteers. *Journal of Neurophysiology,* 91(6):2723–2733.

Breivik, J.H., Sigmund, C. and Dannerman P. (2008). Monitoring of anasthesia and analgesia in Laboratory animals, U.S.A: Academic press, (6) 83 -99.

Bringmann, G., Messert, K., Wolf, K., Muhlbacher, J., Grune, M., Brun, R. and Louis A.M. (2002). Dionocophylline E. from *Dioncophyllum thollonii*, the first 7,3-coupled dioncophllaceous napthylisoquinoline alkaloid. *Phytochemistry,* 60: 389-397.

Brook, I., Lewis, M.A., Sándor, G.K., Jeffcoat, M., Samaranayake, L.P. and Vera-Rojas, J. (2005). Clindamycin in dentistry: more than just effective prophylaxis for endocarditis? *Medical Pathology,* 100:550-8.

Builders, M.I., Wannang, N.N., Ajoku, G.A., Builders, P.F, Oriasdipe, A.S. and Afuiyi, J.C. (2011). Evaluation of the antimalarial potential of *Vernonia ambigua Kotschy* and *Peyr (*Asterceae). *International Journal of pharmacology,* 7 (2): 238-247.

Burkill, H.M. (2000). The useful plants of West Africa, Families M-R. Royal Botanical Gardens , Key England, 4(1): 452-453.

Cabello, C.M., Lamore, S.D., Bair, W.B., Qiao, S., Azimian, S., Lesson, J.L. and Wondrak, G.T. (2011). The redox antimalarial dihydroartemisinin targets human metastatic melanoma cells but not primary melanocytes with induction of NOXA-dependent apoptosis. *Investigation of New Drugs,* 30 (4): 1289–301.

CDC, (2004). Malaria rapid diagnostic test. Center for Disease Control (CDC). 56:686.

CDC. (2015). Malaria. Center for disease Control (CDC) http://www.cdc.gov/malaria

Chang, C.P., Huang, W.T., Cheng, B.C., Hsu, C.C. and Lin, M.T. (2007). The flavanoid baicalin protects against cerebrovascular dysfunction and brain inflammation in experimental heatstroke. *Neuropharmacology,* 52:1024-1033.

Chen, P.M., Gombart, Z.J. and Chen, J.W. (2011). Chloroquine treatment of ARPE-19 cells leads to lysosome dilation and intracellular lipid accumulation: possible implications of lysosomal dysfunction in macular degeneration. *Cell & Bioscience,* 1 (1): 10.

Chin, Y.W., Balunas, M.J., Chai, H.B. and Kinghorn, A.D. (2006). Drug discovery from natural sources. *Journal of Medicine and Food,* 8(2):E239–E253.

Choi, J., Jung, H., Lee, K. and Park, H. (2005). Anti-nociceptive and Anti- inflammatory effects of saponin and sapogenin obtained from the stem of *Akebia quinata*. *Journal of Medicine and Food,* 8 (1) 78-85.

Chopra, I., Roberts, M. and Roberts, E. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology and epidemiology of bacterial resistance. *Microbiological Molecular Biology,* 65 (2): 232–260.

Chopra, R.N., Nayar, S.L. and Chopra, I.C. (1956). Glossary of Indian medicinal plants. New Delhi: *Council of Scientific and Industrial Research,* 12:157.

Chukwuani, C.M. (1999). Socio-economic implication of multi-drug resistant malaria in the community: how prepared is Nigeria for this emerging problem. *West African journal of Medicine,* 18(4): 303-306.

Chukwuocha, U.M., Osuagwa, A. E., Dozie, I.N.S., Nwoke, B.E., Onwuliri, C.O. and Ukaga, C.N. (2009). The clinical pattern and complications of severe malaria in parts of Imo River Basin of Nigeria. *Nigerian Hospital Practice,* 3(6): 76-79.

Connell, S.R., Tracz, D.M., Nierhaus, K.H., Taylor, D.E. and Tracz, N.T. (2003). Ribosomal protection proteins and their mechanism of tetracycline resistance. *Antimicrobial Agents of Chemotherapy,* 47 (12): 3675–3681.

Cox, F. (2002). History of human parasitology. *Clinical Microbiology Reviews,* 15 (4): 595–612.

Cui, L.W. and Su, X.Z. (2009). Discovery, mechanisms of action and combination therapy of artemisinin. *Expert Reviews of Anti-Inflammation Therapy,* 7(8): 999–1013.

Dahanukar, J., Kurkarni, R. and Haussler, U. (2000). Pharmacology of medicinal plants and natural products. *Indian Journal of Pharmacology,* 32: S81- S83.

D'Alessandro, U. (2009). Existing antimalarial agents and malaria-treatment strategies. *Expert Opinion Pharmacother*erapy, 10(8): 1291–1306.

David, D. V., Aziagbo, B. N. and Ebong, O. O. (2004). Antiplasmodial Effect of the aqueous extracts of *Phyllanthus amarus* and *Schuman thonn* against *Plasmodium berghei* in Swiss albino mice. *Nigerian Journal of Physiological Sciences,* 22(2): 19-23.

Debono, T.Y., Ozek, M., Uresin, Y. and Gungor, M. (2013). Comparison of the effects of specific and non-specific inhibition of nitric oxide synthase on morphine analgesia, tolerance and dependence in mice. *Life Science,* 72(17):1943–1951.

DeRuiter, J., (2002). Non-Steroidal Anti-inflammatory Drugs (NSAIDs). *Journal of Principles of Drug Action*, 3(2): 1–26.

De-villiers, K.A., Marques, H.M. and Egan, T.J. (2008). The crystal structure of halofantrine-ferriprotoporphyrin IX and the mechanism of action of arylmethanol antimalarials. *Journal of Inorganic Chemistry,* 102 (8): 1660–7.

Dharani, N., Rukunga, G., Yenesew, A., Mbora, A., Mwaura, L. and Jamnadass R. (2010). Common antimalarial trees and shrubs of east africa. *World Agroforestry Centre,* 78(9):29-59.

Djurković, O., Milenković, V., Nikolić, A., Bobić, B. and Grujić, J. (2002). Efficacy of atovaquone combined with clindamycin against murine infection with a cystogenic (Me49) strain of Toxoplasma gondii. *Journal of Antimicrobial Chemothererapy,* 50 (6): 981–7.

Dohn, M.N., Weinberg, W.G. and Torres, R.A. (2004). Oral atovaquone compared with intravenous pentamidine for *Pneumocystis carinii* pneumonia in patients with AIDS. Atovaquone Study Group. *International Medical Journal,* 121 (3): 174–80.

Dondorp, A, Nosten, F., Stepniewska, K., Day, N. and White, N. (2005). Artesunate versus quinine for treatment of severe *falciparum* malaria: a randomised trial. *Lancet Journal,* 366 (9487): 717–25.

Dondorp, A.M., Fanello, C.I. and Hendriksen, I.C. (2010). Artesunate versus quinine in the treatment of severe *falciparum* malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet Journal,* 376 (9753): 1647–57.

Douglas, N.M., Anstey, N.M., Angus, B.J., Nosten, F. and Price, R.N. (2010). Artemisinin combination therapy for *vivax* malaria. *Lancet Infectious Disease,* 10 (6): 405–16.

Dubuisson, D. and Dennis, S.G. (1977). The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain*, 4:161–174.

Edith, A., Folade, M., Ogbole, O., Okpako, L. and Akinboye, D. (2006). *In-vivo* antimalarial and cytotoxic properties of *Annona senegalensis* extract. *African Journal of traditional, Complementary and Alernative medicine,* 3 (1): 137-141.

Esu, E., Effa, E., Opie, O., Uwaoma, A. and Meremikwu, M. (2014). Artemether for severe malaria. *The Cochrane database of systematic reviews,* 9: CD010678.

Etkin, N.L. (2003). The co-evaluation of people, plants and parasites: Biological and cultural adaptation to malaria. *Production of Nutritional society*, 62: 311-317

Fairhurst, R.M. and Wellems, T.E. (2010). "*Plasmodium* species (malaria)" In Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Philadelphia, Pennsylvania: Churchill Livingstone/Elsevier. 7(2):3437–62. ISBN 978-0-443-06839-3.

Farid, H.A.., Kunert, O., Haslinger, E. and Seger, C. (2002). Isolation and structure elucidation of iridoide and coumarin derivatives from *Xeromphis nilotica* (Rubiaceae). *Chemical Monthly,* 133: 1453-1458.

Färnert, A., Lindberg, J. and Gil, P. (2003). Evidence of *Plasmodium falciparum* malaria resistant to atovaquone and proguanil hydrochloride: case reports. *Biomedical Journal,* 326 (7390): 628–9.

Feachem, R.G., Phillips, A.A., Hwang, J., Cotter, C., Wielgosz, B., Greenwood, B.M., Sabot, O., Rodriguez, M.H., Abeyasinghe, R.R., Ghebreyesus, T.A. and Snow, R.W. (2010). Shrinking the malaria map: progress and prospects. *Lancet Journal,* 378 (9752): 1566–78.

Federal ministry of health, Nigeria (2005). National Antimalarial Treatment Policy. Abuja: *National Malaria and Vector Control Division*

Federal ministry of health, Nigeria (2006). National Antimalarial Treatment Policy. Abuja: *National Malaria and Vector Control Division*

Ferri, F.F. (2009). "Protozoal infections" In Ferri's Color Atlas and Text of Clinical Medicine. *Elsevier Health Sciences,* 332:1159. ISBN 978-1-4160-4919-7.

Fine, P.G. (2001). Opioid analgesic drugs in older people. *Clinics in Geriatric Medicine*, 17(3):479–487.

Francesco, C., Lina, R.T. and Alberto, M. (2012). Advances in the treatment of malaria. *Mediterranean Journal of Haematology and Infectious diseases,* 4(1):587-597.

Frank, F., Vinke, I. and Lips, M. (2010). Sequestration and tissue accumulation of human malaria parasites; can we learn anything from rodent models of malaria? *Plos pathogens,* 6(9): e 1001032

Friedman, R. and Caflisch, A. (2009). Discovery of plasmepsin inhibitors by fragment-based docking and consensus scoring. *Medical Chemistry,* 4 (8): 1317–26.

Gamo, F.J. (2014). Antimalarial drug resistance: new treatments options for *Plasmodium*. *Drug Discovery Technology Today,* 11: 81-88.

Garmel, F. and Gus, M. (2012). An introduction to clinical emergency medicine. Cambridge: Cambridge University Press. 2: 375. ISBN 0521747767.

Garodia, P., Ichikawa, H., Malani, N., Sethi, G. and Aggarwal, B., (2007). From ancient medicine to modern medicine: *Ayurvedic* concepts of health and their role in inflammation and cancer. *Journal Society Integrated Oncology,* 5: 25-37.

Gething P.W., Patil, A.P., Smith, D.L., Guerra, C.A., Elyazar, I.R., Johnston, G.L., Tatem, A.J. and Hay, S.I. (2011). "A new world malaria map: *Plasmodium falciparum* endemicity in 2010". *Malaria Journal.* 10 (1): 378.

Gofton, T.E., Al-Khotani, A.L., O'Farrell, L.C. and McLachlan, R.S. (2010). Mefloquine in the treatment of progressive multifocal leukoencephalopathy. *Journal of Neurosurgical Psychiatry,* 82 (4): 452–455.

Gollin, D. and Zimmermann, C. (2007). Malaria: Disease Impacts and Long-Run Income Differences Report. Institute for the Study of Labor. *Lancet Journal,* 338 (6792): 1266–78.

González, R., Hellgren, U., Greenwood, B. and Menéndez, C. (2014). Mefloquine safety and tolerability in pregnancy: a systematic literature review. *Malarial Journal,* 13: 75.

Greenwood, B. and Mutabingwa, T. (2002). "Malaria in 2002". *Nature,* 415 (6872): 670–672.

Greenwood, B.M., Bojang, K., Whitty, C.J. and Targett, G.A. (2005). "Malaria". *Lancet.* 365 (9469): 1487–98.

Greenwood, B.M., Fidock, D.A. and Kyle, D.E. (2008). Malaria: progress, perils, and prospects for eradication. *Journal of Clinical Investigation,* 118(4): 1266–1276

Guede, N.Z., Lengo, M., Frederic, G., Bernard, B. and Philippe, G. (2005). *In-vitro* antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. *Journal of Ethnopharmacology,* 98(3): 281-285.

Guerra, C.A., Hay, S.I., Lucioparedes, L.S., Gikandi, P.W., Tatem, A.J., Noor, A.M. and Snow, R.W. (2007). Assembling a global database of malaria parasite prevalence for the Malaria Atlas Project. *Malaria Journal,* 6 (1): 17.

Gupta, M., Sasmal, S., Majumdar, S. and Mukherjee, A. (2012). HPLC profiles of standard Phenolic Compounds present in medicinal plants. *International Journal of Pharmacognosy and Phytochemistry,* 4(3):162167.

Gupta, M., Shaw, B. and Mukherjee, A. (2008). Studies on antipyretic-analgesic and ulcerogenic activity of polyherbal preparation in rats and mice. *International Journal of Pharmacology,* 4(2):88-94.

Henry, M. S. and Sanjeev, K.L. (2011). Treatment and prevention of malaria : antimalarial drug chemistry, action and use: Springer Verlag. Pp. 45. ISBN 9783034604796

Hou, J., Wang, D., Zhang, R. and Wang, H. (2008). Experimental therapy of hepatoma with artemisinin and its derivatives: *in-vitro* and *in-vivo* activity, chemo sensitization and mechanisms of action. *Clinical Cancer,.* 14 (7): 5519–5530.

Hughes, W., Leoung, G. and Kramer, F. (2013). Comparison of atovaquone (566C80) with trimethoprim-sulfamethoxazole to treat *Pneumocystis carinii* pneumonia in patients with AIDS. *New England Journal of Medicine,* 328 (21): 1521–7.

Idowu, O. A., Soniran, O. T., Ajana, O. and Aworide, D. O. (2010). Ethnobotanical survey of antimalarial plants used in Ogun state, south-west Nigeria. *African Journal of Pharmacy and Pharmacology,* 4(2): 055-060

Ismail, M.I. and Adam, A.E. (2015). Checklist of plants of Rashad and Albassia localities (eastern Nuba Mountains), South Kordofan Sudan. *Journal of biodiversity data,* 11(6): 1805.

Johnson, R.W. (2002). The concept of sickness behavior: a brief chronological account of four key discoveries. *Veterinary immunology and immunopathology,* 87 (4): 443–50.

Kalra, B.S., Chawla, S., Gupta P. and Valecha N. (2005). Screening of antimalarial drugs: An overview. *Educational forum*

Kennedy, J., Wang, C.C. and Wu, C.H. (2008). Patient disclosure about herb and supplement use among adults in the US. *Evidence Based Complementary Alternative Medicine,* 5: 451-456.

Kidgell, C., Volkman, S.K. and Daily, J. (2006). A systematic map of genetic variation in *Plasmodium falciparum*. *PloS Pathology,* 2(6): 562–577.

Kiekkas, P., Aretha, D., Bakalis, N., Karpouhtsi, I., Marneras, C. and Baltopoulos, G.I. (2013). Fever effects and treatment in critical care: literature review. Australian critical care. *Official journal of the Confederation of Australian Critical Care Nurses.* 26 (3): 130–135.

Koenderink, J.B., Kavishe, R.A., Rijpma, S.R. and Russel, F.G. (2010). The ABCs of multidrug resistance in malaria. *Trends Parasitology,* 26(9): 440–446.

Korenromp, E., Williams, B., De Vlas, S., Gouws, E., Gilks, C., Ghys, P. and Nahlen, B. (2005). Malaria attributable to the HIV-1 epidemic, sub-Saharan Africa. *Emerging Infectious Diseases,* 11 (9): 1410–1419.

Koster, R., Anderson, M. and De-Beer, E. J. (1959). Acetic acid for analgesic screening, *federal proceedings*, 18: 412-417.

Krishna, S.G., Senthil, S.I., Mohamed, E.M., Cowan, M.F., Caroline, K.H., Jansen, Herwig, K.P. and Efferth, T.K. (2014). A randomised, double blind, placebo-controlled pilot study of oral artesunate therapy for colorectal cancer. *Ethnobiological Medicine,* 2: 82–90.

Krudsood, S., Looareesuwan, S. and Tangpukdee, N. (2010). New Fixed-Dose Artesunate-Mefloquine Formulation against Multidrug-Resistant *Plasmodium falciparum* in Adults: a Comparative Phase IIb Safety and Pharmacokinetic Study with Standard-Dose Nonfixed Artesunate plus Mefloquine. *Antimicrobial Agents of Chemotherapy,* 54 (9): 3730–7.

Kurtis, J.D. Mtalib, R., Onyango, F.K. and Duffy, P.E. (2001). Human resistance to *plasmodium falciparum* increases during puberty and is predicted by dihydroepiandrostene sulphate levels. *Infectious immunology,* 69(1): 123-128.

Kurup, P.E., Zhang, Y.F., Xu, J.N., Venkitaramani, D.V., Haroutunian, V.M., Greengard, P.L., Nairn, A.C. and Lombroso, P.J. (2010). Aβ-Mediated NMDA Receptor Endocytosis in Alzheimer's Disease Involves Ubiquitination of the Tyrosine Phosphatase STEP61. *Journal of Neuroscience,* 30 (17): 5948–57.

Lange, D.J., Andersen, P.M., Remanan, R., Marklund, S. and Benjamin, D. (2013). Pyrimethamine decreases levels of SOD1 in leukocytes and cerebrospinal fluid of ALS patients: a phase I pilot study. *Journal of Amyotrophic Lateral Sclerosis & Frontotemporal Degeneration,* 14 (3): 199–204.

Laupland, K.B. (2009). Fever in the critically ill medical patient. *Critical Medical Care,* 37 (7): 273–278.

Layne, R. W. (2007). Antimalarial activity of crude extracts from Brazilian plants studied *in-vivo* in *plasmodium berghei-berghei* infected mice and *in-vitro* against *Plasmodium falciparum* in culture. *Britain Journal Medical Biology,* 24: 1113-1123.

Le-bars, D., Gozariu, M. and Cadden, S.W. (2001). Animal models of nociception. *Pharmacology Reviews,* 53:597-652.

Lee, C., Jang, H., Kim S. and Cho, I. (2012). Ethyl pyruvate attenuates formalin-induced inflammatory nociception by inhibiting neuronal ERK phosphorylation. *Molecular pain,* 8:40.

Lee, K. S., Divis, P.C., Zakaria S.K., Matusop, A. and Julin, R.A. (2011). *Plasmodium knowlesi*: reservoir hosts and tracking the emergence in human and maraques. PLOS *Pathogens,* 7(4):33.

Lemmich, E., Cornett, C., Furu, P., Justian ,C.L., Knudsen, A.D., Olsen, C.E., Salih, A. and Thiilborg, S.T. (1995). Molluscicidal saponins from *Catunaregam nilotica. Journal of Phytochemistry,* 39:63-68.

Lewis, W.H. and Elwin-Lewis M.P. (1971). The treatment of malaria. *British medical Bulletin*, 1:323-328.

Lim, P., Alker, A.P. and Khim, N. (2009). Arteminisin derivatives combination therapy failure in *falciparum* malaria in Cambodia. *Malarial Journal,* 8: 11.

Limpert, A.S., Mattmann, M.E. and Cosford, N.D. (2013). Recent progress in the discovery of small molecules for the treatment of amyotrophic lateral sclerosis (ALS). *Beilstein Journal of Organic Chemistry,* 9: 717–32.

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

Loya, A.M., Gonzalez-Stuart, A. and Rivera, J.O. (2009). Prevalence of polypharmacy, polyherbacy, nutritional supplement use and potential product interactions among older adults living on the US-Mexico Border: a descriptive, questionnaire-based study. *Drugs Aging,* 25: 423-436.

MacDonald, S.M., Bhisutthibhan, J. and Shapiro, T.A. (2001). Immune mimicry in malaria: *Plasmodium falciparum* secretes a functional histamine-releasing factor homolog *in-vitro* and *in-vivo*. *National Academic Science,* 98(19):10829–10832.

Madara, N.C, Nogueira, C.R. and Lopes, Mason, L.X. (2010). Antiplasmodial natural products (Review). Molecules. *Antimicrobial agents and chemotherapy*; 16:2146-90.

Mann, A., Muhammad, G. and Abdulkadir, N.A. (2003). Medicinal and Economic plants of Nupe land. Jube-Eram and publications, Nig: Pp190

Marfurt, J., Smith, T.A., Hastings, I.M., Müller, I. and Sie A. (2010). *Plasmodium falciparum* resistance to antimalarial drugs in Papua New Guinea: evaluation of a community-based approach for the molecular monitoring of resistance. *Malarial Journal,* 9: 8

Mariod, A.A., Abdelwahab, S.I., Elkheir, S., Ahmed, J.M., Fauzi, P.N. and Chuen C.S. (2012). Antioxidant activity of different parts from *Annona squamosa*, and *Catunaregam nilotica* methanolic extract. *Acta Scientific Technology,* 11(3), 249-257

Mariod, A.A., Elkheir, S., Ahmed, Y.M. and Matthaus, B. (2010). *Annona squamosa* and *Catunaregam nilotica* seeds, the effect of the extraction method on the oil composition. *Journal of American Oil Chemical Society,* 87:763-769

Martin, R.E., Marchetti, R.V., Cowan, A.I., Howitt, S.M., Broer, S.E. and Kirk, K.D. (2009). Chloroquine Transport via the malaria parasite's chloroquine resistance transporter. *Science,* 325 (5948):16802.

Matondo, S.I., Temba, G.S. and Kavishe, A.A. (2014). High levels of sulphadoxine-pyrimethamine resistance Pfdhfr-Pfdhps quintuple mutations: a cross sectional survey of six regions in Tanzania. *Malarial Journal,* 13: 152.

McArdle, J.J., Sellin, L.C., Coakley, K.M., Potian, J.G., Quinones-Lopez, M.C., Rosenfeld, C.A., Sutatos, L.G. and Hognason, K.J (2005). Mefloquine inhibits cholinesterases at the mouse neuromuscular junction. *Neuropharmacology,* 49 (8): 1132–1139.

McKee, E.E., Ferguson, M., Bentley, A.T., and Marks, T.A. (2006). Inhibition of mammalian mitochondrial protein synthesis by oxazolidinones. *Antimicrobial Agents of Chemotherapy,* 50: 2042–2049.

Molina, D. and Kimberley, C. (2012). Postmortem hydroxychloroquine concentrations in nontoxic cases. *The American Journal of Forensic Medicine and Pathology,* 33 (1): 41–2.

Mułoz, V., Sauvai, M., Bourdy, G., Callapa, J., Bergeron, S., Rojas, I., Bravo, J. A., Balderrama, L., Ortiz, B., Gimenez, A. and Deharo, E. (2000). A search for natural bioactive compounds in Bolivia through a multidisciplinary approach: Part 1. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. *Journal of ethnopharmacology,* 69:127-137.

Muregi, F.W., Chhabra, S.C., Njagi, E.N., Lang`at-Thoruwa, C.C., Njue, W.M., Orago, A.S., Omar, S.A. and Ndiege, I.O. (2003). *In-vitro* antiplasmodial activity of some plants used in Kisii, Kenya against malaria and their chloroquine potentiation effects. *Journal of Ethnopharmacology,* 84: 235-239

Nadjm, B. and Behrens, R.H. (2012). Malaria: An update for physicians. *Infectious Disease Clinics of North America,* 26 (2): 243–59.

Nassisi, D. and Oishi, M.L. (2012). Evidence-based guidelines for evaluation and antimicrobial therapy for common emergency department infections. *Emergency medicine practice,* 14 (1): 1–28.

National Institute of Health (NIH), (1985). Guide for the care and use of laboratory animals: *DHEW Publication, revised, Office of Science and Health Reports*, DRR / NIH, Bethesda, USA.

Nchinda, T.C. (2006). Ed. Malaria: A re-emerging disease in Africa. *World Health Organisation*, Geveva, Switzerland.

Negus, A.S., Hunskaar, S. and Hole K., (2006). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain,* 30: 103-114.

Neuwinger, H.D. (1996). African ethnobotany, poisons, drug chemistry, pharmacology and toxicology. London, UK. Chapman and Hall, 743-753.

Niven, D.J. Stelfox, H.T. and Laupland, K.B. (2013). Antipyretic therapy in febrile critically ill adults: A systematic review and meta-analysis. *Journal of critical care,* 28 (3): 303–310.

OECD 423 (2001). Oral Toxicity Study in Rodents. OECD guideline for the testing of chemicals. 423: 1-8.

Olupot-Olupot, P., Layman, T. and Maitland, K. (2013). Management of severe malaria: Results from recent trials. Advances in experimental medicine and biology. *Advances in Experimental Medicine and Biology,* 764: 241–50.

Osher, E., Fattal-Valevski, A., Sagie, L., Urshanski, N., Amir-Levi, Y., Katzburg, S., Peleg, L., Lerman-Sagie, T., Zimran, A., Elstein, D., Navon, R., Stern, N. and Valevski, A. (2011). Pyrimethamine increases β-hexosaminidase A activity in patients with Late onset Tay-sachs. *Molecular Genetics and Metabolism,* 102 (3): 356–63.

Owoyele, B.V., Oguntoye, S.O., Darel, K. and Alice, B. (2008). Analgesic, anti-inflammatory and antipyretic activities from flavonoid fractions of *Chromolaena odorata. Journal of critical care*, 2(9):219-225.

Pabon, A., Carmona, J., Maestre, A., Camargo, M. and Blair, S. (2002). Inhibition of *Plasmodium falciparum* by steroid from *Solanum nudum. Phytotherapy research*, 16: 59-62

Pan, M.H., Lai, C.S. and Ho, C.T. (2010). Anti-inflammatory activity of natural dietary flavonoids. *Food Function,* 1:15-31.

Paul, I.K., Bartoloni, A. and Zammarchi, L. (2011). Clinical aspects of uncomplicated and severe malaria. *Mediterranean Journal of Hematology and Infectious Diseases,* 4 (1): 22-32.

Payen, C., Monnin, L., Pulce, C. and Descotes, J. (2008). Bone marrow aplasia following acute poisoning with chloroquine-proguanil. *Clinical Toxicology,* 46 (10): 1085–7.

Pearce, R.J., Pota, H. and Evehe, M.S. (2009). Multiple origins and regional dispersal of resistant in African *Plasmodium falciparum* malaria. *PloS Medicine,* 6(4):1000055.

Pedroni, H.C., Bettoni, C.C., Spalding, S.M. and Costa, T.D. (2006). *Plasmodium berghei:* Development of an irreversible experimental malaria model in wistarrats. *Experimental Parasitology,* 113: 193-196.

Peter W. (1980). Antimalarial drug resistance. *British Medical Bulletin*, 32:187–192.

Peter, N.B., Morris, J.B. and Pankaj, S. (2012). Clinical Pharmacology. 11th edition, London; Churchill Livingstone. Pp. 227-235, 278-288

Peters, W. (1965). Drug resistance in *Plasmodium berghei*. *Experimental Parasitology*, *17*: 80-9.

Peters, W. (1995). Drug resistance in *Plasmodium berghei* chloroquine resistance. *Experimental Parasitology,* 17:80-87.

Phillipson, J. D. (1991). Assays for antimalarial and amoebicidal activities in methods in plant biochemistry. *Malarial Journal,* 6:13.

Phillipson, J.D. and Wright, C.W. (1990). Can ethnopharmacology contribute to the development of antimalarial agents? *Journal of Ethnopharmacology,* 32: 155-165

Pierrot, C., Adam, E., Laffite, S., Godin, C., Dive, D., Capron, M. and Khalife, J. (2003). Age related susceptibility and resistance to *Plasmodium berghei* in mice and rats. *Experimental Parasitology,* 104: 81-85.

Pinheiro, M.M., Bessa, S.O., Fingolo, C.E., Kuster, R.M., Matheus, M.E., Menezes, F.S. and Fernandes, P.D. (2010). Antinociceptive activity of fractions from *Couroupita guianensis* Aubl. Leaves. *Journal of Ethnopharmacology,* 127: 407–413.

Premji, Z., Umeh, R.E. and Owusu-Agyei, S. (2009). Chlorproguanil-dapsone-artesunate versus artemether-lumefantrine: a randomized, double-blind phase III trial in African children and adolescents with uncomplicated *Plasmodium falciparum* malaria". *PLoS ONE,* 4 (8): 34.

Prugnolle, F., Durand, P., Ollomo, B., Duval, L., Ariey F, Arnathau, C., Gonzalez, J.P., Leroy, E. and Renaud, F. (2011). A fresh look at the origin of *Plasmodium falciparum,* the most malignant malaria agent. *PLoS Pathogens,* 7(2):78

Raj, P.P. (2007). "Taxonomy and classification of pain" In: Kreiter, S., Beltrutti, D. and Lamberto, A. *The handbook on chronic pain.*New York: Nova science publishers Inc., USA. Pp 56.

Rajagopal, M.R. (2006). Pain - Basic Considerations. *Indian Journal of Anesthesiology*, 50:331–334.

Ratitid, W., Ruangsang, P. and Wongnawa, M. (2012). Studies of the anti-inflammatory and antipyretic activities of the Methanolic extract of *piper sarmentosum* Roxb. Leaves in rats. *Songklanakarin Journal of Science Technolology,* 29 (6): 1519-1526.

Reddi, D. and Curran, N. (2013). An introduction to pain pathways and mechanisms. Available at: http://www.ucl.ac.uk/anaesthesia /Students and Trainees /Pain Pathways Introduction.

Rehwagen, C. (2006). WHO ultimatum on artemisinin monotherapy is showing results. *Journal of Biomedicine,* 332 (7551): 1176.

Reyburn, H., Mtove, G., Hendriksen, I. and Von- Seidlein, L. (2009). Oral quinine for the treatment of uncomplicated malaria. *Britain Journal of Medicine,* 339: 2066.

Riley, E.M. and Stewart, V.A. (2013). Immune mechanisms in malaria: New insights in vaccine development. *Nature Medicine,* 19 (2): 168–78.

Rivera, J., Njifutie, N., Ayafor, J. F., Basco, L. K. and Ringwald, P. (2002). *In- vitro* antimalarial activity of limonoids from *Khaya grandifoliola* C. D. C. (Meliaceae). *Journal of Ethnopharmacology*, 69: 27-33.

Robinson, M.M. and Zhang, X. (2011). Traditional medicines: global situation, issues and challenges. *The world medicines situation,* (3):56.

Runyoro, K.B., Matee, I.N., Olipa, D.N., Cosam, C.J. and Zakaria, H.M. (2006). Screening of Tanzanian medicinal plants for anti-candida activity. *Biomedical Complementary Alternative Medicne,* 6(11).

Ryley, J.F. and Peters, W. (1970). The antimalarial activity of some quinolone esters. *American Tropical Medicine and Parasitology*, *84* : 209-22.

Sachs, J. and Malaney, D. (2002). The economic and social burden of malaria. *Nature,* 415:680-685.

Saidu, K., Onah, J., Orisadipe, A., Olusola, A., Wambebe, C. and Gamaniel, K. (2000). Antiplasmodial, analgesic and anti-inflammatory activities of the aqueous extract of the stem bark of *Erythrina senegalensis. Journal of Ethnopharmacology*, 71: 275-280.

Sajadi, M.M., Bonabi, R., Sajadi, M.R. and Mackowiak, P.A. (2012). Akhawayni and the first fever curve. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America,* 55 (7): 976–80.

Sallares, R. (2002). Malaria and Rome: A history of malaria in ancient Italy. Oxford University Press. doi:10.1093/acprof:oso/9780199248506.001.0001. ISBN 978-0-19-924850-6.

Samba, E.M. (2003). Message of the WHO regional director for Africa on the occasion of the first African Traditional Medicine Day held on August.

Sawant, M., Issac, J.C. and Narayanan, S. (2004). Analgesic studies on total alkaloids and alcoholic extracts of *Eclipta alba* (Linn). *Phytotherapy Research* 18,111–113.

Schaffner, A. (2006). Fever- useful or noxious symptom that should be treated? Therapeutische Umschau. *Review therapeutique,* 63 (3): 185–8.

Schlagenhauf, P., Adamcova, M., Regep, L., Schaerer, M. and Rhein, G. (2010). The position of mefloquine as a 21st century malaria chemoprophylaxis. *Malaria Journal,* 9: 357.

Schulman, C.I., Namias, N., Doherty, J., Manning, R.J., Li, P., Elhaddad, A., Lasko, D., Amortegui, J., Dy, C.J., Dlugasch, L., Baracco, G. and Cohn, S.M. (2005). The effect of antipyretic therapy upon outcomes in critically ill patients: a randomized, prospective study". *Surgical infections,* 6 (4): 369–75.

Sigmund, C. and Dannerman P. (2011). Monitoring of anasthesia and analgesia in Laboratory animals, U.S.A: Academic press. (6) 83 -99.

Sinatra, R.S., Nalini, V. and Whitney, C.J. (2009). Pain Physiology and Pharmacology. *Acute Pain Management*, 1:4–5.

Singh, P. and Ali, J. (2012). Ethnomedicinal plants of family *rubiaceae* of eastern U.P. *Indian Journal of Life Science,* 1(2): 83-86.

Slatter, M.J., Pettengell, J.K. and Taylor, R.K. (1983). Chloroquine-resistant malaria. *South African Journal.* 63:838.

Sofowora, A. (1980). Guidelines for research promotion and development in traditional medicine. *Nigeria Journal of Pharmacy,* 11: 117-118.

Sofowora, A. (1993). Screening plants for bioactive agents. In: Medicinal plants and traditional medicine in Africa. 2nd ed. Spectrum Books Ltd., Sunshine House, Ibadan. 81-93, 134-156.

Sorbera, F., Santagelo, A. and Crescimanno, G. (2012). The effects of diazepam on the behavioural structure of the rat's response to pain in the hot-plate test: anxiolysis vs pain modulation. *Neuropharmacology,* 63 (2): 310-321.

Soroush, S., Taherikalani, M., Asadollahi, P., Asadollahi, K., Taran, M. and Emaneini, M. (2012). *In-vitro* antimicrobial activity of *Persian shallot (Allium hirtifolium). Roum Archive of Microbiological Immunology,* 71(2):70–4.

Srivastava, A., Patel, P., Rajesh, K., Vashistha, K., Singh, A. and Puskar, K. (2012). Ethnomedicinal importance of the plants of Amarkantak region, Madhya Pradesh, India. *International Journal of Medicinal Plants,* 2(1): 53-59.

Srivastava, I.K., Rottenberg, H. and Vaidya, A.B. (2007). Atovaquone, a broad spectrum antiparasitic drug, collapses mitochondrial membrane potential in a malarial parasite. *Journal of Biological Chemistry,* 272(7): 3961–3966.

Steck, E.A. (1972). The Chemotherapy of Protozoan Diseases. Washington: *Walter Reed Army Institute of Research*, 89.

Steentoft, M. (1998). Flowering plants in West Africa. Cambridge University Press, Cambridge. 665-669.

Su, F., Nguyen, N.D., Wang, Z., Cai, Y., Rogiers, P. and Vincent, J.L. (2011). Fever control in septic shock: beneficial or harmful? *Plos Medicine,* 23 (6): 516–520.

Sudhanshu, S., Neerja, P., Jain, D.C. and Bahakuni, R.S. (2003); Anti-malarial agents from plants sources 1314 *Curriculum Science*, 35: 9.

Sullivan, J.E. and Farrar, H.C. (2011). Fever and antipyretic use in children. Section on clinical pharmacology and therapeutics; Committee on drugs. *Pediatrics,* 127 (3): 580–7.

Sutherland, C.J., Laundy, M. and Price, N. (2008). Mutations in the *Plasmodium falciparum* cytochrome b gene are associated with delayed parasite recrudescence in malaria patients treated with atovaquone-proguanill. *Malarial Journal,* 7 (1): 240.

Sutherland, C.J., Tanomsing, N. and Nolder, D. (2010). Two nonrecombining sympatric forms of the human malaria parasite *Plasmodium ovale* occur globally. *Journal of Infectious Diseases,* 201(10): 1544–1550.

Taylor, W.R., Hanson, J., Turner, G.D., White, N.J. and Dondorp, A.M. (2012). Respiratory manifestations of malaria. *Chest,* 142 (2): 492–505.

Taylor-Robinson, R.M. (2002). Association of microsatellite variations of *Plasmodium falciparum* Na^+/H^+ exchanger (*pfnhe-1*) gene with reduced *in-vitro* susceptibility to quinine: lack of confirmation in clinical isolates. Africa. *American Journal of Tropical Medicine and Hygiene,* 82(5): 782–787.

Thapar, M.G., Gupta, S., Spindler, C., Wernsdorfer, W. H. and Björkman, A. (2003). Pharmacodynamic interactions among atovaquone, proguanil and cycloguanil against *Plasmodium falciparum in-vitro*. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 97 (3): 331–337.

Thienhaus, O. and Cole, B.E. (2002). "Classification of pain" In: Weiner, R.R. *Pain management:*A practical guide for clinicians. American Academy of pain management. 6:67

Tilley, L., Dixon, M.W. and Kirk, K. (2011). The *Plasmodium falciparum*-infected red blood cell. *International Journal of Biochemistry and Cell Biology,* 43 (6): 839–42.

Tjolsen, A., Berge, O., Hunskaar, S., Rosland, J.H. and Hole, K. (1992). The formalin test: an evaluation of the method. *Pain,* 51*:* 5-17.

Toker, G., Kupeli, E., Memisoglu, M. and Yesilada, E. (2004). Flavonoids with antinociceptive and anti-inflammatory activities from the leaves of *Tilia argentea* (silver linden). *Journal of Ethnopharmacology,* 95(2- 3):393–397.

Tona, L., Cimanga, R.K., Mesia, K., Musuamba, C.T., de Bruyne, T., Apers, S., Hernans, N.,Van Miert, S., Pieters, L., Totte, J. and Vlientinck, A.J. (2004): *In-vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. *Journal of Ethnopharmacology,* 93: 27-32.

Trapsida, J. (2003). Traditional medicine our culture. *African Health Monitor*, 4(1):12

Trease, G.E. and Evans, W.C. (2009). Pharmacology 16th Ed. *Bailliere Tindall Ltd*., London, pp: 60-75.

Treede, P.W., Mathews, K., Kronen, P.W., Lascelles, D., Nolan, A., Robertson, S., Steagall, P.V.W., Wright, B. and Yamashita, K. (2014). Guidelines for recognition, assessment and treatment of pain. *Journal of Small Animal Practice,* Pp.10.

Turner, P.U., Brabb, T., Pekow, C. and Vasbinder, M.A. (1963). Administration of substances to laboratory animals: Routes of administration and factors to consider. *Journal for American Association for laboratory animal science,* 50(5):600-615.

Valdir, C.F. (2012). Plant bioactives and drug discovery : principles, practice, and perspectives. John Wiley & Sons. Pp. 2. ISBN 9780470582268.

Vasudevan, D.M., Sreekumari, S. and Vaidyanathan, K., (2013). Textbook of Biochemistry for Medical Students. JP Medical Ltd. Pp. 491. ISBN 9789350905302.

Verma, P.R., Joharapurkar, A.A., Chatpalliwar, V.A. and Asnani, A.J. (2005). Antinociceptive activity of alcoholic extract of *Hemidesmus indicus* R.Br. in mice. *Journal of Ethnopharmacology,* 102(2):298–301.

Vongtau, H.O., Abbah, J., Mosugu, O., Chindo, B.A., Ngazal, I.E., Salawu, A.O., Kwanashie, H.O. and Gamaniel, K.S. (2004). Antinociceptive profile of the methanolic extract of *Neorautanenia mitis* root in rats and mice. *Journal of Ethnopharmacology,* 92: 317-324.

Vongtau, H.O., Amos, S., Binda, L., Kopu, S., Gammaniel, K.S. and Wambebe, I. (2000). Pharmacological effects of the Aqueous extract of *Neorautaneniamitis* in rodents. *Journal of Ethnopharmacology,* 72: 207–214.

Walker, J.B., (2002). Evaluation of the ability of seven herbal resources to answer questions about herbal products asked in drug information centers. *Pharmacotherapy,* 22: 1611-1615.

Wells, T.N. (2011). Natural products as starting points for future antimalarial therapies: going back to our roots? *Malarial Journal,* 10(11):S3.

Wesche, D.L., Schuster, B.G., Wang, W.X. and Woosley, R.L. (2000). Mechanism of cardiotoxicity of halofantrine. *Clinical Pharmacology and Therapy,* 67 (5): 521–9.

White, N. J. (2010). Malaria vaccine. *Vaccine,* 28(31):4880–4894.

White, N.J. (1985). The treatment of malaria. *New England journal of medicine,* 335:800-806.

White, N.J. (2004). Antimalarial drug resistance. *Journal of Clinical Investigation,* 113 (8): 1084–92. PMID 15085184.

White, N.J. (2008). *Plasmodium knowlesi*: the fifth human malaria parasite. *Clinical Infectious Diseases,* 46(2): 172–173.

WHO, (2007). Global antimalarial drug policies database- WHO African region. September edition.

WHO, (2013). African Malaria Report. Prompt and effective Treatment. 3:31-37.

WHO, (2015). World Malaria Report 2015. World Health Organization. Geneva: 7-15.

Wong, T., Stang, A.S., Ganshorn, H., Hartling, L., Maconochie, I.K., Thomsen, A.M. and Johnson, D.W. (2013).Combined and alternating paracetamol and ibuprofen therapy for febrile children. *Paediatrics,* 41(3):23.

Woolfe, G. and MacDonald, A.D. (2004). Evaluation of the analgesic action of Pethidine Hydrochoride. *Journal of pharmacology and experimental therapeutics,* 80:300-307.

World Health Organisation (2010). Guidelines for the treatment of malaria. Geneva: World Health Organization. 2:23 ISBN 9789241547925.

World Health Organization (WHO) (1978). The promotion and development of traditional medicine. Technical Report Series, 622, WHO, Geneva.

World Health Organization. (2014). Monitoring antimalarial drug resistance. Geneva: World Health Organization, 7-15, 99-101.

Worrall, E. (2010). The development of the RTS, S. malaria vaccine candidate: challenges and lessons. *Parasite Immunology,* 31(9): 492–500.

Worrall, E., Basu, S. and Hanson, K. (2005). Is malaria a disease of poverty? A review of the literature. *Tropical Health and Medicine,* 10(10): 1047–59.

Zirihi, G.N., Mambu, L., Guede-Guina F., Bodo, B. and Grellier, P. (2005). *In-vitro* antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. *Journal of Ethnopharmacology,* (98):281-285.

ABBREVIATIONS

%	Percentages
/	Division
+	Positive
_	Negative
>	Greater than
<	Less than
=	Equal to
±	Plus-minus
×	Multiplication
µ	Micro
đ	Delta
Etc	Eccetra
▯	Kappa
-	Nil
α	Alpha
β	Beta
ACT	Artemisinin Combination Therapy
ADRs	Adverse Drug Reactions

AFC	Animal Facility Centre
ANOVA	Analysis of Variance
BUK	Bayero University Kano
CDC	Centre for Disease Control
$CHCl_3$	Chloroform
CNS	Central Nervous System
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CRCP	Complex Regional Pain Syndrome
E.g	Examples
H_2SO_4	Sulphuric Acid
HCl	Hydrochloric Acid
IP	Intra-peritoneal
IL	Interleukin
LD_{50}	Median Lethal Dose
Mg/kg	Milligram per kilogram
NaOH	Sodium Hydrooxide
NIH	National Institute on Health
NIMR	National Institute of Medical Research

NIPRD	National Institute for Pharmaceutical Research and Development
NPs	Natural Products
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
OECD	Organization for Economic Co-operation and Development
PABA	Para-aminobenzoic Acid
PG	Prostaglandins
RBCs	Red Blood Cells
Sc	Sub-cutaneous
TNF	Tumor Necrosis Factor
W/V	Weight by Volume
WHO	World Health Organization