EVALUATION OF THE ANTIMALARIAL POTENCY OF THE CRUDE METHANOL EXTRACT OF *ANOGEISSUS LEIOCARPUS* AND *CURCUMA LONGA LINN* IN *PLASMODIUM BERGHEI* EXPERIMENTALLY INFECTED MICE

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ABSTRACT

Malaria control remained public health challenge for developing countries as a result of resistance of the parasite and absence of licensed vaccine which has led to aggressive search for natural based and eco-friendly alternative. This study therefore elucidated the *in vivo* antimalarial potency of crude and alkaloidal fractions of *Curcuma longa* and *Anogeissus leiocarpus* in *Plasmodium berghei* infected mice. The plants' crude extract was analyzed qualitatively and quantitatively for the presence and concentrations of secondary metabolites, standard laboratory procedures. The *in vivo* anti-malarial efficacy of the plants' crude extract and alkaloidal fraction was assessed using the four days test for anti-malarial activity against a chloroquine sensitive *P. berghei* NK65 strain in Swiss albino mice. Acute oral toxicity for the determination of the plants' medial lethal dose (LD_{50}) in mice was also determined. The result of the acute oral toxicity revealed that both plants are safe for oral administration at the tested concentrations with LD_{50}, extrapolated to be greater than 5000 mg/kg body weight. Bio-active metabolite of public health significance including; flavonoids, alkaloids, tannins and cardiac glycosides were detected in the crude methanol extract of both plants. In-vivo antiplasmodial bioassay of the crude extract increased with an increase in extract concentration and days of administration. Parasitaemia was significantly ($P<0.05$) highest in the group treated with 400 mg/kg for both plant extracts. However, the highest paratiaemia efficacy was recorded in group treated with 200 mg/kg b. wt *C. longa* compared to other treatments and the control. The extracts also prevented weight loss and elongates the survival time of the *P. berghei*-infected group treated with the plant extract compared with the infected untreated group. Findings from this study show that *C. longa* and *A. leiocarpus* are promising candidates in the development of an outstanding anti-malarial drug.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes. Malaria in humans is caused by five Plasmodium parasites: *Plasmodium falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi* (Isacchi *et al*., 2012). The distribution of humanpathogenic *Plasmodium* species shows preponderance of *P. falciparum* in tropical Africa, while *P. vivax* prevails over *P. falciparum* in South America (Adewoye, 2010). Endemic *P. vivax* is transmitted throughout the tropics in much the same geographical pattern as *P. falciparum* (Soforowa*.,* 2006). Among these species, *P. falciparum* remain the chief lethal etiological agent of human malaria and it is becoming resistant to standard antimalarial drugs in almost all parts of endemic areas especially in the tropics and sub-tropics. In 2018, *P. falciparum* accounted for 99.7% of estimated malaria cases in the WHO African Region 50% of cases in the WHO South-East Asia Region, 71% of cases in the Eastern Mediterranean and 65% in the Western Pacific.

Malaria is not only among the most prevalent tropical diseases worldwide, but it is a major threat to the public health and economic development of many nations (Khin *et al.,* 2017). In 2010, there were an estimated 219 million cases of malaria and 660 000 malaria deaths globally. Of these, 80% of cases and 90% of deaths are estimated to occur in the African Region (WHO, 2017). Approximately, 86% of malaria deaths globally were of children under 5 years of age (WHO, 2012), and this indicates every 30 seconds a child dies from malaria (Khin *et al*., 2017).

According to the latest *World malaria report*, released on 30 November 2020, there were 229 million cases of malaria in 2019 compared to 228 million cases in 2018. The estimated number of malaria deaths stood at 409 000 in 2019, compared with 411 000 deaths in 2018. The WHO African Region continues to carry a disproportionately high share of the global malaria burden. In 2019, the region was home to 94% of all malaria cases and deaths. In 2019, 6 countries accounted for approximately half of all malaria deaths worldwide: Nigeria (23%), the Democratic Republic of the Congo (11%), United Republic of Tanzania (5%), Burkina Faso (4%), Mozambique (4%) and Niger (4% each). Children under 5 years of age are the most vulnerable group affected by malaria; in 2019 they accounted for 67% (274 000) of all malaria deaths worldwide.

1.2 Statement of the Research Problem

Resistance to antimalarial drugs is a challenge in malaria control in most parts of the world. Since early 60's, the sensitivity of the parasites to chloroquine, the best and most widely used drug for treating malaria, has been on the decline. The loss of effectiveness of chemotherapy constitutes the greatest threat to the control of malaria. The development of drug resistance to the potent and affordable drugs, lack of concerted vector control strategy, and absence of licensed vaccine have left many developing countries without sustainable options for malaria control, and chemotherapy remains the only prompt and effective option. This necessitates a continuous effort to search for new drugs, particularly with novelmodes of action (Okeola *et al*., 2010). Therefore, to effectively manage malaria, new knowledge, products and tools are urgently needed and particularly new drugs (Shuaibu *et al.,* 2008).

1.3 Justification for the Study

New antimalarial drugs were discovered in an effort to tackle this problem, but unfortunately the drugs are either expensive or have undesirable side effects. The development of drug resistance to potent and affordable drugs, and absence of licensed vaccine have left many developing countries without sustainable options for malaria control, and chemotherapy remains, the only effective option (Borimas and Nicholas, 2016). Therefore, this necessitates a continuous effort to search for new drugs, particularly with novel modes of action. *Anogeissus leiocarpus*, belonging to the family Combretaceae, has been reported to have antiparasitic activity (Gansane *et al.,* 2010). Although the antimalarial activities of *A. leiocarpus* and *C. longa* have been well established and used by the traditional healers, the therapeutic potentials of the plants have not been scientifically evaluated.

Besides, plants used in the treatment of disease are said to contain active compounds called phytochemicals some of which are responsible for the numerous bioactivities of the plant. There is considerable interest by phytochemist to identify the therapeutic agent contained in this plant in order to establish the basis for their uses in traditional medical practice. These compounds form the basis of the pharmacological effects of such plants (Ahmed, 2014). Numerous plants containing a wide variety of phytochemicals as their bioactive components have shown anti-plasmodial, anti-inflammatory, analgelsic and anti-nociceptic potentials (Alshawsh *et al.,* 2007). Thus, necessitating interest in biotechnology, as most of the drug industries depend in part on plants for the production of pharmaceutical compounds.

1.4 Aim and Objectives of the Study

The aim of this study is to evaluate the antiplasmodial activity of *A. leiocarpus* and *C. longa* in *P. berghei* infected mice.

The objectives of the study were to:

I. determine the metabolites in the crude extract of *A. leiocarpus* and *C. longa* crude oral toxicity of the crude extract of *A. leiocarpus* and *C. longa.*

II. determine the antiplasmodial potency of the crude extract and alkaloidal fraction of *A. leiocarpus* and *C. longa* in *Plasmodium berghei* infected mice

III. determine the anti-plasmodial effect of the combined extract of *A. leiocarpus* and *C.longa*.

IV. determine the effect of the extract on body weight, packed cell volume and hematological parameters of *Plasmodium berghei* infected mice.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malaria

Malaria is parasitic disease caused by five *Plasmodium* parasites: *vivax*, *falciparum*, *malariae*, *Knowlesi* and *ovale* . More than half of the world's population are at risk of malaria leading to a global tally of 212 million new cases and 429, 000 death each year (Okeola *et al*., 2010; WHO, 2017). Sub-Saharan Africa accounts for over 90% of the malaria cases and deaths predominantly in children of age below five years and pregnant women (El Ghazali *et al*., 2003). Poor rural dwellers in in tropical and subtropical areas are highly vulnerable to this attacked owing to the favourable and ideal climatic condition for the reproduction and development of vectors and parasites. In addition, drug resistance is one of the major challenges facing malarial control program worldwide (Umar *et al.,* 2012)

2.1.1 Transmission of malaria

A person gets malaria when bitten by an infected female mosquito. This occurs mainly between dusk and dawn. Tere are other rare mechanisms for the transmission of malaria which include congenitally acquired diseases, blood transfusion, sharing of contaminated needles and organ transplantation (Umar *et al.,* 2012)

2.1.2 Symptoms of malaria

The malaria destroys red blood cells causing anaemia. It also adheres to cells in certain tissues and organs. They affect single or multiple organs with different levels of severity and which can be determined as neurologic, renal dysfunction, hematologic, cardiovascular and respiratory dysfunction as well as hepatic and metabolic dysfunctions. There is also a significant decrease in the level of hemoglobin and

significant increase in the levels of SGOT, SGPT, ALP, bilirubin, creatinine and urea. (Batawila *et al.,* 2005). The common symptoms of malaria are sometimes similar to those of many other infectious diseases caused by bacteria, viruses or parasites. They include fever, chills, head ache, sweats, fatigue, nausea, vomiting, multi organ failure system which may progress to coma and death (Tiwari *et al. ,*2012) .Other symptoms could be dry (nonproductive) cough, muscle or back pain and enlarged spleen. (Okpekon, 2004).

2.1.3 Malaria parasites

Malaria parasites are microorganisms that belong to the genus *Plasmodium*. There are more than a hundred species of *Plasmodium* that infect many species of animals such as reptiles, birds and various mammals (CDC, 2015). Five species have long been recognize to infect humans; *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium Knowles.*

2.1.3.1 Life cycle of the *Plasmodium* parasite

Plasmodium undergo a complex life cycle alternating between vertebrate and arthropod hosts. The transmission to man starts after the inoculation of sporozoites into blood circulation by an infected mosquito. In the case of human malaria, only female mosquitoes of the genus *Anopheles* are potential vectors, since male anophelines do not feed on blood (Rotimi, 1988). Possibly guided by chemotaxis and recognizing its targets, sporozoites leave the capillary lumen and enter hepatocytes (in the case of mammalian plasmodia). At this moment, parasites undergo a drastic morphological change. They appear round or oval and contain a chromatin nucleus surrounded by a cytoplasm.

During the tissue (exo-erythrocytic) schyzogony, the nucleus divides and the cytoplasmatic mass grows. The number of nuclear divisions and their intervals vary among different species (Rotimi, 1988).

By the end of nuclear division stage, the cytoplasm segregates and merozoites are formed, consisting of a single nucleus and cytoplasm. The number of merozoites produced by one hepatic schizont is estimated to be about 2000 in *P. malariae*, 10000 in *P. vivax*, 15000 in *P. ovale* and more than 30000 in *P. falciparum*. Hepatic merozoites are then delivered into the blood circulation to infect erythrocytes that will invaginate to form the parasitophorous vacuole. The erythrocytic merozoites are ovoid or elongated structures and species-specific in size (Rotimi, 1988). Once within the parasitophorous vacuole, the parasite rapidly transforms into an immature trophozoite (ring stage).

Hemoglobin is ingested and digested to produce the typical malaria pigment (hemozoin). During this process, the parasite grows and the nuclear material of the mature trophozoite increases and undergoes several nuclear divisions to form a schizont. The mature schizont finally bursts to liberate individual erythrocytic merozoites. The latter differ from the hepatic form, mainly by the presence of malaria pigment. Erythrocytic merozoites can now infect other erythrocytes. The duration of blood schyzogony is generally a multiple of 24 h, usually 24, 48 or 72 hours and is related to the clinical manifestation of the disease (Rotimi, 1988). Upon invading a new erythrocyte the merozoites can either initiate renewed blood schyzogony or develop into a gametocyte from the ring stage trophozoite (Rotimi, 1988). Gametocytes can be ingested by the female *Anopheles* mosquito during a blood meal. The parasites' multiplication in the mosquito is known as the sporogonic cycle. Once in the mosquito's stomach, the microgametes (male gametes) penetrate the macrogametes (female gametes) generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the

intestinal wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which migrate to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the life cycle (Rotimi, 1988).

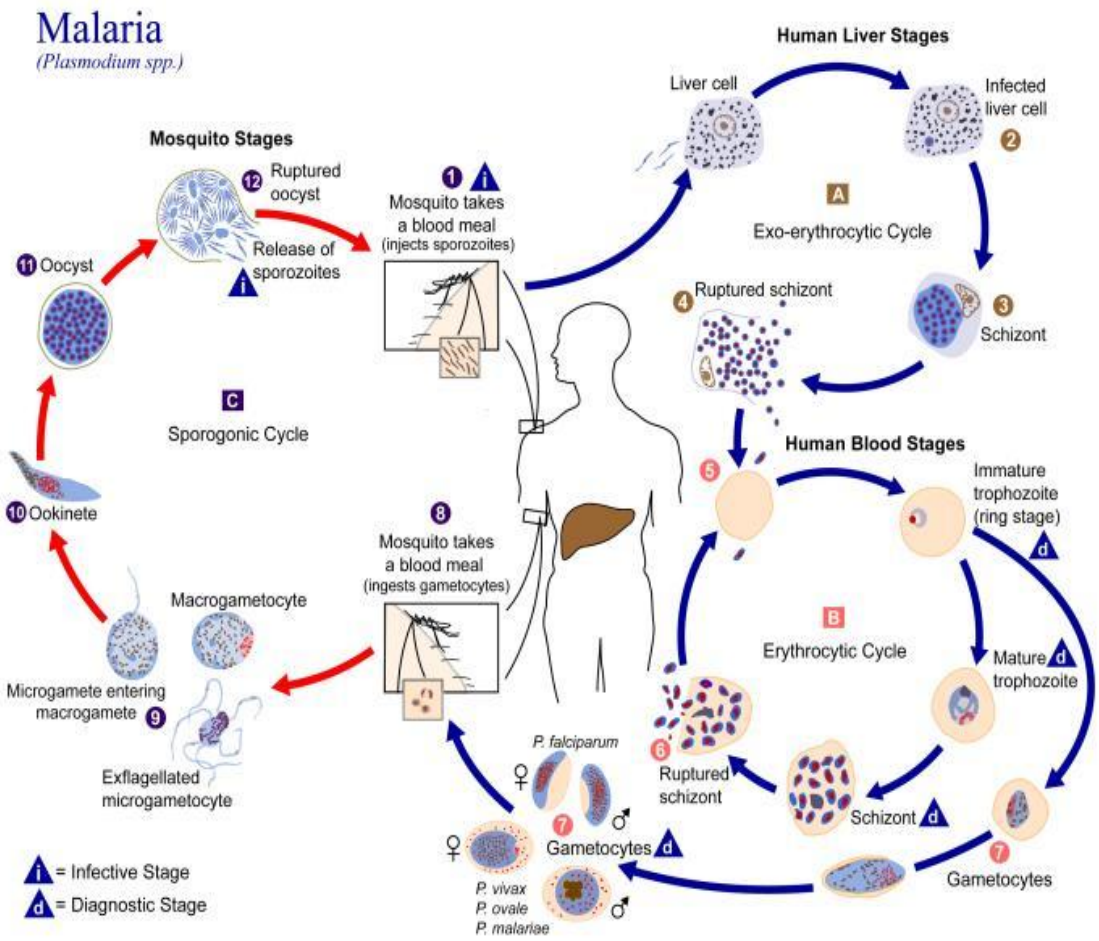

Figure 2.1: *Plasmodium* Life Cycle (Centre for Disease Control, 2015)

2.1.4 General clinical manifestations of malaria

More than 80% of non-immune patient with malaria present with fever, rigor, malaise and headache. Afebrile patients usually give a history of chills and sweat. Fever is usually irregular initially, when symptoms may be non-specific and difficult to distinguish from those caused by other infections, such as influenza, dengue and typhoid-fever. Vomiting occurs in up to 34% and diarrhea in about 16% of patients.

Classic periodic fever (every second day in tertian parasites *P. falciparum*, *P. vivax* and *P. ovale*, and every third day in quartan parasite *P. malariae*) is uncommon initially, although if present is highly suggestive of malaria. However, periodic fever is neither necessary nor sufficient for the diagnosis of malaria. Splenomegaly and splenic tenderness are the most common physical findings. Tachycardia, tachypnoea, icterus, hepatomegaly and hypotension also occur (Read and Babson, 1960). The incubation period is the time between the infective bite by the *Anopheles* mosquito and the first symptoms. Shorter periods are observed in general with *P.falciparum*, and longer with *P. malariae*. *P. vivax* and *P. ovale* can produce dormant liver stage parasites (hypnozoites), thus the liver stages may reactivate and cause disease several months after the infective mosquito bite, this is called a relapse (Rotimi, 1988).

2.1.5 Current chemotherapy in the management of malaria

Malaria control requires an integrated approach comprising prevention targeted at vector control and treatment with effective antimalarials. The successful management of malaria depends on prompt diagnosis, an accurate clinical assessment and instituting suitable chemotherapy as soon as possible. Treatment depends not only on the parasite species but also the susceptibility to antimalarial drugs, the severity of the illness, and the age and background immunity of the patient (Gillespie and Pearson, 2001). The affordable and widely available antimalarial drug chloroquine that was in the past the main treatment in malaria control is now ineffective in most *falciparum* malaria endemic areas and resistance to sulfadoxine pyrimethamine is increasing rapidly. The discovery and development of the artemisinin derivatives in China, and their evaluation in Southeast Asia and other regions, have provided a new class of highly effective antimalarials, and have already transformed the chemotherapy of malaria. Artemisinin based combination therapies (ACTs) are now generally considered as the best current

treatment for uncomplicated *falciparum* malaria. The recommended ACTs are: artemether lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, artesunate + sulfadoxine–pyrimethamine. As second-line treatment for uncomplicated *falciparum* malaria, the following combinations are used: artesunate + tetracycline, or doxycycline or clindamycin; and quinine + tetracycline, or doxycycline or clindamycin. In the case of severe *falciparum* malaria, quinine and artemisinin derivatives (artesunate, artemether, artemotil, dihydroartemisinin) are basically used (WHO, 2012).

2.1.6 The current status of antimalarial drugs

There are a number of approaches for the prevention and control of malaria. These include vector avoidance (i.e. insecticide treated bed nets), vector control (residual indoor and outdoor mosquito spraying), vaccine development and chemotherapy (Rotimi, 1988) However, with a successful vaccine still in the works, our current best defense against malaria is chemotherapy, and in populations where prophylactic treatment is simply too expensive, quick acting drugs that clear *Plasmodium* infections are most often used. Currently used antimalarial drugs fall into several categories: aminoquinolines, arylaminoalcohols, aminoquinolines, artemisinins, antifolates, inhibitors of the respiratory chain and antibiotics (Schlitzer, 2014). Quinine, which is derived directly from *Cinchona* spp., was isolated in 1820 and was the only drug in pure form used to treat malaria for more than one hundred years (Rotimi, 1988). In the 1940's, chloroquine was introduced as a less expensive and more effective alternative and quickly became the mainstay of antimalarial drug therapy and pioneer of the malaria eradication era in the 1950's. The natural endoperoxide, artemisinin, was discovered from the traditional Chinese medicinal plant *Artemisia annua* in 1972 and influenced the semi-synthesis of several analogues, which have become the current first choice in front line antimalarial treatment (Mann *et al.,* 2008). Unfortunately, the

successes of modern drug development were not long lived. When chloroquine resistant *P. falciparum* strains first began to appear in Africa during the late 1970s (Salawu *et al.,* 2008), medicinal advantage over malaria began to fade. Since then, antimalarial chemotherapy has been dominated by the cyclical development of new drugs (often as modifications of existing drugs) and the subsequent appearance of drug resistance. This fact is nowhere more disturbing then in reports of resistance to artemisinin in Southeast Asia (Oloyede*.,* 2005). The therapeutic 'arms race' between new drug entities and resistance is a constant concern as the synthetic drug pipeline dries up (Stray, 1998). Antimalarial drugs are also becoming progressively morecostly in a time when increasing world population, climate change, and political distress are exacerbating an overall inability to afford antimalarial treatment (Okafor *et al.,* 2018). These two problems resistance and cost must be addressed for malaria to be successfully controlled.

2.1.7 Antimalarial compounds and their mechanism of action

Antimalarials are used in three different ways: prophylaxis, treatment of *falciparum* malaria, and treatment of non-*falciparum* malaria. Prophylactic antimalarials are used almost exclusively by travellers from developed countries who are visiting malaria endemic countries. Treatment protocols for *falciparum* malaria vary, depending on the severity of the disease; fast-acting, parenteral drugs are best for severe, life threatening disease. In addition, treatment protocols for *falciparum* malaria vary geographically and depend on the resistance profiles for strains in particular regions. Non-*falciparum* malarias, in contrast, rarely are drug resistant. In addition, *P.vivax* and *P.ovale* have dormant liver stages that can cause relapses months to years after an infection is cleared, so they need to be treated with an additional agent that can clear this stage. The antimalarials in common use come from following classes of compounds: the quinolines (chloroquine, quinine, mefloquine, amodiaquine, primaquine), the antifolates

(pyrimethamine, proguanil and sulfadoxine),the artmisinin derivatives (artemisinin, artesunate, artemether, arteether) and hydroxyl-naphthaquinones (atovaquine).

2.1.8 Malaria vaccine and its challenges

2.1.8.1 Vaccines

Our relationship with parasites has been a long one on the evolutionary scale. The methods adopted by parasites to thrive and colonize living organisms are truly fascinating. Along with basic features such as fecundity and resistant cyst structures, the parasites exhibit a fine-tuning of modifications in response to the attack by the host immune system. While the host fights the parasites through its armoury of immune as well as certain behavioural responses, the parasites appear to use the host immune responses towards quorum sensing, limiting their own number, but surviving. The human malarial parasite, *Plasmodiumfalciparum*, which appears to have an ancient origin and has evolved in parallel with humans (Olajide, 2011), is known to possess a complex arsenal of defences against man, and therefore the efforts to generate an effective malaria vaccine have been fraught with obstacles.

A good estimation of the current burden of malaria has been difficult, but nevertheless it is apparent that over one million persons succumb to malaria every year in Africa (Krishna *et al.,* 2009). Devising an effective malaria vaccine would certainly help in limiting such morbidity. Over the years, numerous attempts have been made to develop a vaccine against malaria. The possibility of using inactivated sporozoites was first demonstrated in 1910 in avian malaria (Guchu *et al.,* 2007). It was followed by studies in 1941 that showed immunization with irradiated sporozoites could prevent infection. Besides irradiated sporozoites, the other observation that holds promise for a vaccine

comes from the documented ‘clinical immunity’ observed in adult residents of malaria endemic areas (Ekanem and Yusuf, 2005).

2.1.8.2 Challenges for an effective malaria vaccine

Lack of good animal models for testing of human malaria vaccines, the difficulties in evaluation of efficacy of the vaccine in endemic areas, and the lack of understanding of the immunosuppressive mechanisms of the parasite are the foremost reasons for the failure of an effective vaccine. The use of murine models to demonstrate robust protection has failed to stand true in most human trials. One of the possible reasons for the same is because we use unnatural rodent hosts, and not the natural host (tree shrews) of the murine malaria (Chinezun *et al.,* 2017).

Vaccine development and field trials are lengthy and expensive. In most of the trials, including those in naïve volunteers, it is important to note that sterile immunity was not observed in a large proportion of the subjects for a significant length of time. This has important implications. Are children from endemic areas in a position to take frequent vaccination doses? The RTSS as well as irradiated sporozoite vaccine might prove useful for transient visitors to endemic areas, such as tourists or military personnel. However, to manage the current burden of malaria in endemic areas, alternative methods such as insecticide spraying, insecticide-treated bed nets, long-lasting insecticidal nets and combination drug therapies should be used to their maximum.

2.2 The Role of Plants in Antimalarial Drug Discovery

The estimated 300,000 species of higher plants contain a pharmacopeia of complex and unique chemical compounds that are employed *in* anti-pathogenic activity and reproduction (Ayodele *et al.,* 2013). Humans have taken advantage of this fact by utilizing plants medicinally for millennia. In our current medicinal repertoire, the plant

derived compounds quinine, artemisinin, and their derivatives account for more than half of the WHO's accepted antimalarial remedies (WHO, 2013). Quinine, being derived from *Cinchona* spp. and artemisinin, derived from *Artemisia annua*, represent inspiring and triumphant stories of ethnobotanical knowledge influencing the discovery and development of potently bioactive compounds that have been used to save countless lives. However, more than 1,200 plant species are known to be traditionally used for malaria treatment (Alshawsh and Eyasu, 2017). This ethnobotanical knowledge may similarly serve as a guide in elucidating novel antimalarial compounds of comparable or superior activity to quinine and artemisinin. In fact, hundreds of antimalarial compounds have already been described from plants and other natural sources, some of which hold clinical appeal (Kaur *et al.,* 2009).

While a select number of these compounds have garnered attention as drug candidates, few have been viewed as integrated botanical therapies. On the one hand, this neglect originates in the highly complex nature of bioactive plant extracts, with many compounds of varied activity. For example, quinine, long thought to be far superior to crude cinchona bark, has been shown to be less effective than a mixture of cinchona alkaloids (Alshawsh and Eyasu, 2017). On the other hand, plant extracts containing important medicinal compounds are often scientifically dismissed because their activities cannot be traced to a single active chemical. The biological activity of extracts *in situ* may contrast sharply with that measured in bioassays of isolated components (Ahmed, 2014). For this reason, drug discovery programs have failed to capture the value of multi-functional, multi-faceted agents such as phyto-medicines and botanical therapeutics.

Luckily, with a little effort, previously complex plant extracts can now be subject to isolation and fractionation methods that preserve phytochemical integrity and

deliberately seek to quantify incidences of chemical interactions. In this manner, we can begin to understand and exploit the entire range of efficacies of phytochemical mixtures and their key components. This is truly important considering recent calls from the World Health Organization for the discontinuation of all artemisinin monotherapies, while favoring the use of Artemisinin Combination Therapies (ACTs; mixtures of slow acting artemisinins with longer acting quinolone antimalarials in an effort to delay resistance) (WHO, 2012). Chemical complexity found in synthetically, carefully constructed drug therapy programs and naturally in plants is believed to help delay or avoid the emergence of resistant *Plasmodium* strains.

2.3 Natural Products in Drug Discovery

Medicinal plants use is widespread. Lifesaving and essential drugs from medicinal plants such as morphine, digoxin, aspirin, and emetine were introduced into modern therapeutics several centuries ago. However, plants have been used as drugs for over millennia by human beings (Sittie, 2005). Plants historically have served as models in drug development for some major reasons: the first being that plants are unique chemical factories capable of synthesizing large numbers of highly complex and unusual chemical substances. It has also been estimated by the World Health Organization (WHO) that about 80% of the population of the developing countries rely exclusively on plants to meet their health care needs (WHO, 2012). The second reason is that biologically active substances derived from plants have served as templates for synthesis of pharmaceuticals; while the third reason concerns the fact that highly active secondary plant constituents have been instrumental as pharmacological tools to evaluate physiological processes (Paddon and Keasling, 2014).

2.3.1 Safety issues in herbal medicine: implications for the health professions

Adverse effects of herbal medications may be intrinsic or extrinsic. The patient's age, genetic constitution, nutritional state, concomitant diseases and concurrent medication may affect the risk and severity of adverse events, following consumption of large amounts or a wide variety of herbal preparations, or long-term use (Tiwari *et al*., 2011).

Intrinsic effects are those of the herb itself and are characterised, as for pharmaceuticals, as type A (predictable, dose-dependent) and type B (unpredictable, idiosyncratic) reactions. Yohimbine, an alkaloid found in *Pausinystalia yohimbe* bark that has 2-adrenoceptor antagonist activity, is taken for male impotence, and can cause hypertension and anxiety in a predictable, dose-related manner (type A reaction); it has also been associated with the serious idiosyncratic reactions of bronchospasm and increased mucus production when taken in normal doses by a patient with severe allergic dermatitis (type B) (Wu *et al*., 2013). Type A reactions with herbal preparations also include effects with deliberate overdose or accidental poisoning and interactions with pharmaceuticals. Extrinsic effects are not related to the herb itself, but to a problem in commercial manufacture or extemporaneous compounding. Potential failures to adhere to a code of Good Manufacturing Practice, while not specific to herbal medicine, can occur, particularly in developing countries where such a code is not in place (Adewoye *et al*., 2010). This makes it more difficult for medical practitioners and other health professionals to assess the adverse effects of herbal preparations compared with pharmaceuticals.

2.3.1.1 Misidentification

It is difficult to track and identify adverse effects of herbal ingredients, as the plants can be named in four different ways -- the common English name, the transliterated name, the Latinized pharmaceutical name, and the scientific name. It is essential that plants are

referred to by their binomial Latin names for genus and species; misidentification can occur when other names are used. For example, the scientific name of the Chinese herb that is variously transliterated as "dong quai", "dong guai", "danggui" and "tang kuei" is *Angelica polymorpha* (formerly *sinensis*). The common English name "angelica" and the Latinized name "Radix Angelica" could refer either to this species, which is used in Australia or to the European species *Angelica archangelica*, depending on the country of origin (Drew and Myers, 1997). Misidentification can result in erroneous associations being made, with potential clinical implications. Plant material can be misidentified at the time of the manufacturer's bulk purchase or when wild plants are picked (Adewoye *et al*., 2010).

2.3.1.2 Lack of standardization

The therapeutic/toxic components of plants vary depending on the part of the plant used, stage of ripeness, geographic area where the plant is grown, and storage conditions. Therefore, batch-to-batch reproducibility of plant material should be assessed in the production of marketed products, but, in practice, product variation in herbal medicines can be significant. The content of ginsenoside, the glycosylated steroid to which most of the biological activity of ginseng (*Panax ginseng*) has been ascribed, was examined in 50 commercial brands of ginseng sold in 11 countries. In 44 of these products, the concentration of ginsenoside ranged from 1.9 % to 9 % w/w; six products contained no ginsenoside, and one of these six contained large amounts of ephedrine (Adewoye *et al*., 2010).

2.3.1.3 Contamination

During growth and storage, crude plant material can become contaminated by pesticide residues, microorganisms, aflatoxins, radioactive substances and heavy metals; lead, cadmium, mercury, arsenic and thallium have been reported as contaminants of some

overseas herbal preparations. In a case series of five patients in the United Kingdom with lead poisoning from Asian traditional remedies, the preparations implicated contained 6 %-60 % w/w lead by weight. The Australian Code of Good Manufacturing Practice specifies detection of microorganisms and leaves estimation of other contaminants (not specified in internationally recognized pharmacopoeial standards) to the discretion of manufacturers. (Kosalec *et al*., 2009)

2.3.1.4 Substitution

A report of nine cases of rapidly progressive interstitial nephritis in young women taking a Belgian slimming treatment led to the discovery that *Aristolochia fangchi*, containing the nephrotoxic component aristolochic acid, had been introduced in place of *Stephania tetrandra.* Eighty cases have now been identified and more than half of these patients developed terminal renal failure.

2.3.1.5 Adulteration

The intentional use of pharmaceutical adulterants has been reported. Cases of acute interstitial nephritis, reversible renal failure, loss of blood pressure control and peptic ulceration have been reported with a product called "Tung Shueh" pills, taken for arthritic complaints. The product contained mefenamic acid and diazepam, neither of which was included on the label. Adulterants can also be added by unethical herbalists compounding preparations for individual patients. In a recent court case, a Chinese herbalist was prosecuted for adding a steroid cream to a herbal preparation, which produced severe facial erythema in a patient.

2.3.1.6 Incorrect preparation/dosage

The processing of crude plant material carried out by a manufacturer, computer-based manufacturing (CAM) practitioner or the patient is a major determinant of the

pharmacological activity of the finished product. A Western Australian patient had a heart attack when he failed to follow a herbalist's instructions to boil aconite (a restricted plant in Australia) in three pints of water for one hour and take the decanted liquid; the patient increased the dose and shortened the boiling time. Boiling changes the alkaloid composition, rapidly reducing the plant's toxicity, and can substantially reduce microorganism contamination. Another point to consider is that the activity of crude plant material may differ from that of the purified constituents, as some constituents may modify the toxicity of others.

2.4 *Anogeissus leiocarpus* (DC.) Gill and Peer

2.4.1 Distribution and habitat

Anogeissus leiocarpus is typical substance of woodlands and savannas of the Sudanian regional center of endemism. The plant has large ecological distribution ranging from the boarders of Sahara up to the out layer humid tropical forests. It is a tree with a wide range, growing from Senegal to Cameroon and extends to Ethiopia and East Africa. It grows in dry forests and gallery forests (Ouedraogo *et al*., 2013; Hennenberg, 2005)

2.4.2 Botanical description

Anogeissus leiocarpus is a deciduous tree species that can grow up to 15–18 m of height and measure up to 1m diameter. The bark is grayish and scaly. The branches often drooping and slender, the leaves alternate, ovate–lanceolate in shape, 2-8 cm long and 1.3-5 cm across. The leaves are also acute at the apex, attenuate at the base and pubescent beneath. Inflorescence globose heads is 2cm across and yellow. The flowers are bisexual and petals are absent. Its fruits are globose cone like heads, each fruit is broadly winged, dark grey and 3cm across. It can reproduce by seeds as well as vegetative propagation (Ouedraogo *et al*., 2013).

Plate I: ***Anogeissus leiocarpus***

Source: El Ghazali *et al*. (2003)

2.4.3 Taxonomic description

Kingdom: Plantea

Subkingdom: Tracheobionta

Super Division: Spermatophyta,

Class: Magnoliopsida

Order: Myrtales

Family: Combretaceae

Genus: *Anogeissus*

Species*:* *leiocarpus*

2.4.4 Traditional uses

Traditionally, *Anogeissus leiocarpus* (DC.) have many reported uses. In Sudanese traditional medicine the decoction of the barks is used against cough (El Ghazali *et al*., 2003). Sticks of the plant are used for orodental hygiene in rural populations of Nigeria. The end of the sticks is chewed into fibrous brush which is rubbed against teeth and gum. Traditional practitioners in Ivory Coast use the plant for parasitic disease like malaria, trypansomiasis, helminthasis and dysenteric syndrome (Okpekon, 2004). Togolese traditional medicine has recorded it use against fungal infections such as dermatitis and mycosis, also the decoction of leaves is used against stomach infections (Batawila, 2005). Also, the plant is used for the treatment of diabetic, ulcers, general body pain, blood clots, asthma, coughing and tuberculosis (Victor, 2013).

2.4.5 Chemical constituents

Preliminary research works on phytochemical screening of the *Anogeissus leiocarpus* stem bark for the major secondary constituents showed that, the plant was rich in tannins and having appreciable quantities of flavonoids, terpenes and saponins, however it was devoid of alkaloids and anthraquinones (Elegami, 2002 and Salau *et al*., 2013). Polyphenolic compounds like 3,3,4-tri-Omethylflavellagic acid, 3,3,4-tri-O-methylflavellagic acid-4—Dglucoside, gentisic, protocatechuic, gallica acid, chebulagic acid, chebulinic acid and ellagic acid have been reported to be isolated from the plant. Hence, flavogallonic acid, bislactone, castalagin and ellagic acid were isolated from the bark (Shuaibu, 2008). There are eight flavonoids, namely, 4H - 1 - Benzopyran - 4 - one, 7- [(6-deoxy- α-Lmannopyranosyl) oxy] - 5- hydroxy- 2- (4 – hydroxyl - 3 - methoxyphenyl), cathecin, quercetin, isoquercetin, vitexin, kaempferol andprocyanidin B2 reported to have been isolated from the leaves of the plant. Five triterpens and triterpene glycosides were also isolated. They are; sericoside, its related aglyconesericic

acid, rachelosperoside; its related aglyconerachelosperogenin, and arjungenin (Chaabi, 2008).

2.4.6 Pharmacological studies

2.4.6.1 Antimicrobial activity

Results of *in vitro* antibacterial activity of *Anogeissus leiocarpus* carried out in Nigeria using agar diffusion assay against bacteria responsible for infections caused by multi-drug resistant *Pseudomonas aeruginosa* and methacillin resistant *Staphylococcus aureus* showed that most of the activities were associated with the methanolic and aqueous extract, with some activities being associated also with ether and chloroform fractions (Taiwo, *et al*., 1999). Hence, the aqueous and methanol extract of the bark, fruit, and leaves showed high activity against standard *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* and clinical isolates of *Staphylococcus aureus, Escherichia coli, Pseudomonas aerugenosa* and *Proteus vulgaris*. Chloroform extract exhibited practically no activity against all standard organisms (Elegami, 2002). The ethanol extract of stem bark of *Anogeissus leiocarpus* inhibited the growth of standard strains of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* (Kubmarawa, 2007). The compound, 3,3,4-Tri-o-methylflavellagic acid glucoside isolated from the stem bark possesses antimicrobial effect on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* (Adigun *et al*., 2000). A study carried out in Togo to investigate antifungal activity of *Anogeissus leiocarpus* against 20 pathogenic fungi, demonstrated that hydro-ethanolic extract possesses *in vitro* antifungal properties, their MIC were in a range of 0.25-4.00 mg/mL (Batawila, 2005).

2.4.6.2 Anti-helmintic activity

Research work conducted to investigate *in vitro* Anti-helmintic potential of crude ethanolic leaf extracts of *Anogeissus leiocarpus* relative to that of the commercial anthelmintic febendazole against eggs and infective larvae of *Haemonchus controtus* showed significant dose dependant inhibition of egg hatch and larval motility (Ademola and Eloff, 2011). However, the potency of the plant extract was comparable to that of febendazole, the finding suggests that this plant could yield natural alternative treatment for *Haemonchus controtus* (Ademola and Eloff, 2011). Study carried out in sheep naturally infected with gastrointestinal nematodiasis has indicated that aqueous leaf extract of the plant produced dose dependant reduction in the fecal egg count in the treated groups when compared to the untreated controls. Hence, the results revealed that there was reduction in the number of worms recovered from gastrointestinal tract of sheep treated with 400mg/kg of the extract for three days than the untreated control (Agaie and Onyeyili, 2007).

In a recent study, administration of ethanolic extract of the roots induced a moderate fecal egg reduction (81 %) and adult worm-burden reduction (87 %) against *Haemonchus contortus* and *Trichostrongylus colubriformis* (82 %) (Soro, 2013). The plant exhibited high efficacy against adult *Strongyloïdes papillosus* (100 %), *Gaigeria pachyscelis* (90 %), *Cooperia curticei* (100 %), and *Oesophagostomumco lumbianum* (95 %) but low efficacy against *Trichostrongylus axei* (67 %) and *Trichuris globulosa* (79 %) (Soro, 2013). .*Anogeisus leiocarpus* could find a potential application in the control of warm parasites.

2.4.6.3 Antiplasmodial activity

A study conducted to evaluate antiplasmodial activity of plant traditionally used for malaria in Ivory Coast showed that the strongest *in vitro* antiplasmodial activity was

found in the dichloroethane extract of *Anogeissus leiocarpus* leaves, compared to activity reported in literature for ethanolic extract of *Artemisia annua* (Vonthron-Senecheau, 2003). However, the study showed that biological efficacy of the plant extract is not due to *in vitro* cytotoxicity. Another study against chloroquine resistant strain of *Plasmodium falciparum* was concluded that methanol extract of leaves and roots of the plant were strongly active against malaria in this in vitro model (Okpekon, 2004). The butanol, ethyl acetate and methanol extracts of *Anogeissus leiocarpus* stem bark were screened for in *vitro* antiplasmodial activity; the better activity was found in the butanol fraction of the plant (Shuaibu, 2008).

2.4.6.4 Trypanocidal activity

Research work that evaluates *in vitro* trypanocidal effect of *Anogeissus leiocarpus* root methanol extract against *Trypansoma brucei* and *Trypansoma congolense* at concentrations of 4 mg/ml, 2 mg/ml and 0.4 mg/ml was reported to cause cessation or reduction in motility of the parasites in extract treated blood compared to that of parasite loaded control blood without extract taken as a measure of trypanocidal activity (Atawodi, 2003). It was also found that there is only slight reduction in motility in *T. congolense* and drastically reduced motility in *T. brucei* compared to control. Methanol extract of leaves, roots and stem barks of the plant showed interesting *in vitro* trypanocidal activity (Okpekon, 2004). The aqueous butanol fractions of the methanol extract of *Anogeissus leiocarpus* were associated with *in vitro* trypanocidal activity against four strains of *Trypanosoma* species. Castalagin isolated from these fractions showed trypanocidal activity on both, the human and domestic animal parasite causing trypanosomiasis (Shuaibu, 2008).

2.4.6.5 Antioxidant and hepatoprotective activities

Methanol and ethyl acetate extracts of the plant were investigated for their 1,1-diphenyl-2- picryl hydrazyl (DPPH) free radical scavenging activity and Ferric reducing antioxidant power (FRAP). Thus, the results revealed that plants exhibited scavenging ability and strong reducing ability (Alajide, 2011; Victor and Grace, 2013). Additionally the methanol extract of the stem bark of the plant was reported to have strong *in vivo* antioxidant, hepatoprotective andameliorative activities on hepatocellular injury following pre and post-treatment with carbon tetrachloride (CCl_4). Therefore, this may suggest a protective effect on human carcinogenesis, diabetes, asthma, atherosclerosis, and other degenerative diseases that are associated with free radicals. This activity may be attributed to flavonoids, phenolic acids, and tannins (Atawodi *et al*., 2011).

2.4.6.6 Toxicological studies

Investigation from research work of Agaie, (2007) showed that oral acute toxicity of the aqueous leaf extract of the plant in rats recorded no death at oral doses up to 3200 mg/kg body weight However the rats showed signs of depression and in appetence, while using intraperitoneal route rats showed dose dependant signs of toxicity ranging from in appetence, depression, unsteady gait, tremor and respiratory distress to death. Thus, there were no gross changes observed in rats that died following extract administration. In addition, histopathological changes were also not observed in all organs except the lung which showed congestion, oedema and bronchitis. These observations therefore suggested that the aqueous leaf extract of the plant could be used with some degree of safety especially by oral route (Agaie, 2007). Previous study found that the extracts of the plant were lethal to mice within five seconds after intravenous injection of 8 mg/kg body weight and within 60 seconds after intraperitoneal injection of 20 mg/kg body weight (Rotimi, 1988). Doses of 50 mg/kg given orally produced no

detectable toxicity, the post mortem result of the dead mice showed no pathological changes in the organs and viscera.

2.5 Phytochemicals

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are secondary metabolites and include glycosides, alkaloids, carotenoids, terpenoids, saponins, coumarins and antraquinones. These chemicals are produced by plants to protect themselves but have been shown to protect humans against diseases and pests. Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Tiwari *et al*., 2012).

2.5.1 Alkaloids

Alkaloids are the largest single class of plant phytochemicals, occurring in approximately 20 % of all plant species, alkaloid heterogeneous group compounds containing nitrogen with an amino acid as a precursor. Most of them have pharmacological properties in low concentrations. In sufficient amounts, these compounds are poisonous to livestock, herbivores and/or humans. Many alkaloids have been used as narcotics, stimulants, poisons and more important commercially as pharmaceuticals (Qiu, *et al*., 2014). Alkaloids such as morphine, cocaine and quinine were of the earliest compounds derived from plants. Many sub-groups of alkalolds have been reported to possess antiplasmodial properties, with high to moderate to low activity either *in vitro* or *in vivo*, or both (Lu, 2011). For example, echitamine (a major alkaloid) showed low antiplasmodial activity, and corialstonine and corialstonidine (two minor quinoline alkaloids) showed some antiplasmodial activity*in vitro*; whilst benzylisoquinoline alkaloids reticuline and laudanosine exhibit moderate antiplasmodial activity *in vitro* and laudanosine has some antiplasmodial activity *in vivo.* Indole

alkaloids (31 types), on the other hand, showed high antimalaria activity both *in vitro* and *in vivo.* The mode of action of alkaloids on the *Plasmodium* parasites appears to be due to the inhibition of the compound with the haemazoin formation.

2.5.2 Flavonoids

Flavonoids, the most common of the phenolic compound found throughout the plant kingdom. Flavonoids are sub-groups into flavones, flavonols, flavanones, chalcones, anthocyanins and isoflavones (Gao and Ji, 2010). This class of compounds is made up of a C_6C_3-moity and has shikimic acid as a precursor. Pharmacological activities of flavonoids such as anti-inflammatory, antimicrobial, antioxidant and antitumor activities, decreasing capillary fragility, and anti-diarrhoeal properties have been documented (Lopez-Lazaro, 2009). Other studies also reported the antimalarial activity of flavonoids, whereby many sub-groups within flavonoids are reported to possess notable antiplasmodial activities. For example flavones displayed moderate activity, whereas the isoflavonoids (3-phenylbenzoprans) have higher antiplasmodial activity. The mechanism of tested flavonoids inhibits the influx of L-glutamine and myoinositol into infected red blood cells (RBCs) (Georges *et al*., 2011).

2.5.3 Saponins

Saponins are group of naturally occurring oily glycosides that foam freely when shaken with water. They occur in a wide variety of plants, including acacia, soapwort, soaproot, California pigweed, and many others. Saponins have been, and sometimes still are, used as cleaning agents and as foam producers, notably in fire-extinguishing fluids (Francis, 2002). They have a bitter taste and when ingested orally are practically nonpoisonous to warm-blooded animals. When injected directly into the bloodstream, however, they are dangerous and quickly dissolve red blood cells. Hydrolysis of saponin, brought about by acids or by enzymes, gives a sugar (often, but not necessarily, glucose) and a sapogenin,

the latter being either a triterpene or a steroid. Some of the sugars and saponins are useful as raw materials for synthesis of steroid hormones (Azobuogu, 2012).

2.5.4 Glycosides

Glycosides are a general term referring to the group of chemicals having at least one sugar moiety linked through its anomeric carbon to another molecule. Glycosides are grouped based on the structure of the aglycone including anthracene derivative, flavonoid, cardenolide and cyanogenic glycosides. The aglycones are released from the glucosides by hydrolysis and the pharmacological activity is found in the aglycone part. Glycosides are relatively polar due to the presence of one or more sugars in the molecule. Most glycosides can be extracted with polar solvents such as water, acetone, ethanol, or a mix of these (Kim *et al.*, 2004). However cardiac glycosides with their bulky steroidal aglycone have appreciable solubility in chloroform. When water is used for extraction, there is a possibility of enzymic degradation of the glycosides to aglycones by the action of glycosidase co-extracted from the plant material. However, this is prevented if boiling water is used or if a significant proportion of alcohol or ammonium sulphates are added to the extract. In plants glycosides serve several purposes including defence and prevention of decay of damaged tissues (Guchu *et al*., 2007).

2.5.5 Terpenoid

Terpenoids, also called Isoprenoids, are the major group of natural compounds, consisting of more than 40 000 different molecules. The terpenoid biosynthetic pathway produces both primary and secondary metabolites that are of great significance to plant growth and persistence. Isoprenoid are well-defined as secondary metabolites using molecular structures comprising carbon backbones that are made up of isoprene (2-methylbuta- 1, 3-diene) units. Isoprene comprises five carbon atoms and as a

consequence, the number of carbon atoms in any terpenoids is a multiple of five. The isoprenoid consist of two isoprene units, with ten carbon atoms. Among the primary metabolites produced by this pathway are: the abscisic acid (ABA); gibberellic acid (GAs) and cytokinins; the carotenoids; plastoquinones and chlorophylls involved in photosynthesis; the ubiquinones use for respiration; and the sterols that modify membrane structure (Doucette, *et al.,* 2013). Many of the isoprenoids are commercially valuables because of their use as flavours and fragrances enhancer in in foods and cosmetics e.g. menthol, nootkatone and sclareol or because they are important for the quality of agricultural products, such as the flavour of fruits and the fragrance of flowers like linalool (Yang, 2007).

2.5.6 Tannin

Tannins are polyphenol that occur naturally in plant. They have a characteristic of binding and precipitating proteins and influence the nutritive properties of many foods eaten by humans and feedstuff eaten by animals. Tannins occur in fruits such as grapes, blueberry, tea, chocolate, legume forages, legume trees like *Acacia* spp., *Sesbania* spp., in grasses that is; sorghum and corn. Tannins are oligomeric compounds with various structured units with free phenolic groups, having molecular weight of about 500 - 20,000, soluble in water, with exception of some high molecular weight structures that are able to bind proteins and form insoluble or soluble tannin-protein complexes. In the past, tannins have been viewed as one of the anti-nutrients of plant origin because of their capability to precipitate proteins, inhibit the digestive enzymes and decline the absorption of vitamins and minerals (Khattab *et al.,* 2010). However, over decades, there is increasing interest in tannins as bioactive component of foods as well as biological antioxidants (Souza *et al.,* 2007). Several health benefits have been

recognized for the intake of tannins and some epidemiological associations with the decreased frequency of chronic diseases have been established (Serrano *et al*., 2009).

2.5.7 Phenols

Phenolic compounds are one of the most widely spread compounds among phytochemicals. They act as natural antioxidants and antinitrosating agents which are of great significance in plant development (Karim *et al*., 2015). They are involved in various processes comprising rhizogenesis, vitrification, and resistance to biotic and abiotic stress and redox reactions in soils. They also provide protection against invading organisms, function as signal molecules, act as allelopathic compounds, and affect cells of plant (Ndakidemi and Dakora, 2003), and animals and may function as pesticides. They are also functional components of the rhizosphere and its soil organic matter. They have long been recognised as allelochemicals for weed control phytoestrogens in animals and plant defence molecules. If the frequent intake of phenolic compounds is reduced, the susceptibility to cancer development, hence the name chemo-preventive agents (D′Ischia *et al*., 2006). The antioxidant effect of phenolic compounds is determined by their radical scavenging activities and consequent inhibitory action on lipid peroxidation under oxidative stress situations (Rigobello *et al*., 2004).

2.5.8 Anthraquinones

Anthraquinones are orange or red polar compound with bitter taste; they are derived from anthracene (main nucleus for anthraquinone compounds). These compounds are found to possess certain therapeutic potentials including laxative, purgative and anti-inflammatory properties. Several anthraquinones isolated from herbs displayed antiplasmodial activity and may destroy the parasites through different modes. For example, these bioactive compounds have been found to be cytotoxic due to its

aldehyde group at C-2. Furthermore, anthraquinones may be potential DNA intercalators due to its cyclic planar structure (Sittie, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Collection, identification and processing of plant material

The fresh leaves of *A. leiocarpus* and C. longa were collected in Chanchaga area, Minna, Niger state, Nigeria. Based on ethnobotanical description and with the help of local traditional healers around Chanchaga area, Minna, Niger state. The plants were identified and authenticated with the help a Senior Botanist in the Department of Plant Biology, Federal University of Technology, Minna. The fresh leaves of the two plants were cleaned, from extraneous materials, air-dried under shade at room temperature then cut and reduced to appropriate size by grinding with an electric mill in the laboratory of Animal Biology.

3.1.2 Laboratory animal

Fifty-four male Swiss albino mice, weighing between 20 to 22 g was purchased from the Animal Holding unit of School of Life Sciences, Federal University of Technology, Minna. All animals were fed with formulated feeds and water was administered *ad libitum.* The caring and experimental use of the mice was in accordance with the National Institute of Health Guidelines for Care of Laboratory Animals. The animals were acclimatized for 14 days prior to their randomization into the various experimental groups.

3.2 Methods

3.2.1 Crude and alkaloid extraction of plant materials

Crude Extraction**:** A 200 g of the powdered plant sample was percolated in 1600 ml of absolute methanol and kept in shade for 48 hours after which it was filtered. The filtrate

was collected in a beaker and the solvent was removed under reduced pressure using rotary evaporator (Adebayo *et al.,* 2003).

Alkaloid Extraction: The extraction of the alkaloid was done using the continuous extraction method using the Soxhlet apparatus. Four hundred grams (400 g) of powdered plant was weighed and packed in a cheese cloth bag which served as an extraction thimble. The thimble was then placed into a suitable jar with cover. The sample was moistened with sufficient amount of 95 % ethanol. This was made alkaline with sufficient quantity of ammonia T.S. and mixed thoroughly. The sample in the thimble was macerated overnight, and then placed in the Soxhlet extractor on the next day. Sufficient amount of 95 % ethanol was placed in the solvent flask (4.8 liters). The sample was extracted for about 3 – 4 hours. The ethanol extract was filtered and was concentrated in a Soxhlet distilling apparatus at 60 ^{o}C. The crude alkaloid extract was further treated with 1.0 N hydrochloric acid. This was filtered and the filtrate was collected. The filtrate was alkalinified with ammonia T.S. and placed in a separatory funnel. Measured quantities of chloroform was added into the separatory funnel, mixed and shaken for about five times and allowed to separate into two layers. The lower layer of chloroform contained the alkaloids and the upper layer the aqueous portion. The upper layer was extracted until the last chloroform extract was found negative to Dragendorff's reagent. The combined chloroform extract was concentrated in Soxhlet distilling apparatus at 60 ^{o}C and evaporated in water bath maintained at that temperature until semi-dry. The residue was weighed and percentage yield was calculated using the formula:

$$\% \text{ yield} = \frac{\text{Weight of the alkaloidal residue (g)}}{\text{Weight of powdered } \textit{Anogeissus leiocarpus} \text{ (g)}} \times 100$$

3.2.2 Determination of bioactive metabolites in the crude plant extracts

The methanol extracts of the two plant crude extracts was screen for the presence of bioactive metabolites such as alkaloids, flavonoids and saponnins following the protocol described by (Evans, 2002; Sofowora, 2006).

3.2.3 Determination of acute oral toxicity of the crude extract of the two plants

The acute oral toxicity for the determination of LD50 of extracts' crude methanol extract was assessed using method of Lorke (1983).

3.2.4 Parasite and standard inoculation

P. berghei NK65 chloroquine-sensitive strain was obtained from National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. Parasite was maintained through serial blood passage in mice wherein the mice previously infected with *P. berghei* and with high parasitemia level will serve as the donor. Blood samples was taken from the donor and diluted with phosphate-buffered saline such that 0.2ml injected intraperitoneally into the experimental animals contained 1 x 10^7 infected erythrocytes.

3.2.5 Antiplasmodial bio-assay

The method described by Ryley and Peters (20) was use in this study. The animals was infected with *P. berghei* NK 65 and then divided into nine groups of three mice per group. Parasitemia was established 72 hours after infection and was taken as day 0. Group I normal control, Group II will receive quinine (Q) 100 mg/kg only. Group III (150 mg/kg b wt) and IV (300 mg/kgbwt) will receive methanolic leaf extract of *A. leiocarpus,* respectively.

Group V (150 mg/kg b wt) and VI (300 mg/kg b wt) will receive methanolic leaf extract of *Curcuma longa*. Group VII (150 mg/kg b wt) and VIII (300 mg/kg b wt) combination Alkaloid extract each of *A. leiocarpus and C. longa*. Group IX was infected and not-

treated. Administration was done orally once daily for four days for the standard drugs or the extract/vehicle respectively.

Table 3.1: Experimental Set-up

Extract	Dose (mg/kg b. wt)	Plat A (no. of mice)	Plan B (no. of mice)
Crude	150	3	3
	300	3	3
Alkaloid	150	3	3
	300	3	3
Combination of Alkaloid	150	3	3
	300	3	3
Normal Control	0.2 NS	3	3
Positive Crude	4mg/kg Standard dry	3	3
Negative	Infected untreated	3	3

3.2.6 Parasitemia determination

Blood samples was collected by bleeding via the tail vein of *P. berghei* infected mice and thin blood smears was made on microscope slides, fixed in methanol and stained with 10 % Giemsa solution (Merck, Tokyo, Japan) and observed under the binocular microscope (Olympus, Japan). The percentage parasitemia will be determined by counting the percentage parasitized RBC for at least four different fields (Khin *et al.,* 2017).

3.2.7 Determination of packed cell volume (PCV)

The packed cell volume (PCV) of each mouse was measured before infection, on day 4 after infection and day 8 after treatment. For this purpose, blood was collected from tail end of each mouse in hapatorinized micro-haematocrit capillary tubes up to 3/4th of their length.

The tube was sealed and placed in a microhaematocrit centrifuge with the seal end out ward. The blood was centrifuge at 12,000rpm for 5 minutes. Then the tube will the result was read using microhaematocrit and the volume of erythrocytes was measured (Bantie *et al*., 2014).

3.2.8 Determination of mean survival time

Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse. The mean survival time (MST) was calculated as follows:

$$\text{MST} = \frac{\text{sum of survival time of all mice in a group}}{\text{Total number of mice in that group}}$$

3.3 Data Analysis

The results were expressed in terms of mean ± standard error. Parameters in the groups was compared by one-way (ANOVA) and Duncan multiple range test using the computer software Statistical Package for Social sciences (SPSS) version 2.2.0. All data was analyzed at a 95% confidence interval and values were considered statistically significant at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Phytochemical composition

4.1.1.1 Qualitative phytochemicals

Table 4.1 presents the qualitative phytochemical screening of crude methanol leaf extract of *Curcuma longa* and *Anogeissus leiocarpus*. The results revealed the presence of flavonoids, tannins, saponins, alkaloids, steroids, cardiac glycoside, anthraquinone and terpenes but absent of phlobatannins in *Curcuma longa. Anogeissus leiocarpus* on the other hand contains only flavonoids, tannins, saponins, alkaloids, cardiac glycoside, anthraquinone and terpens.

4.1.1.2 Quantitative phytochemicals

Table 4.2 presents the quantitative phytochemical compositions of crude methanol leaf extract of *Curcuma longa* and *Anogeissus leiocarpus*. The results revealed that phenol (273.04±6.35 mg/100g) is the most abundant phytochemical components of *Curcuma longa* followed by tannins **(**205.30±7.43 mg/100g) while alkaloids (9.39±0.43 mg/100g) is the least. *Anogeissus leiocarpus* on the other hand also recorded367.15±7.92 of phenol as it most abundant phytochemical followed by alkaloids **(**194.75±8.01 mg/g) while saponins (39.00±0.20 mg/100g) was the least

Table 4.1 Qualitative Phytochemical Composition of Methanol Leaves and Stem Extracts of *Curcuma longa* and *Anogeissus leiocarpus*

Phytochemical	*Curcuma longa*	*Anogeissus leiocarpus*
Flavonoid	+	+
Tannin	+	+
Saponin	+	+
Alkaloid	+	+
Steroids	+	-
Phlobatannins	-	-
Cardiac glycoside	+	+
Anthraquinone	+	+
Terpenes	+	+

Keys:(+) = present, (-) = absent

Table 4.2 Quantitative Phytochemical Composition of Crude Methanol Extracts of *Curcuma longa* and *Anogeissus leiocarpus*

Phytochemical	Curcuma longa (mg/100g)	*Anogeissus leiocarpus* (mg/100g)
Tannin	205.30±7.43^{d}	97.54±11.79^{b}
Saponin	31.66±1.38^{b}	39.00±0.20^{a}
Phenol	273.04±6.35^{e}	367.15±7.92^{e}
Flavonoid	65.88±2.67^{c}	165.00±0.56^{c}
Alkaloid	9.39±0.43^{a}	194.75±8.01^{d}

Data are Mean±SEM of triplicate determination. Values followed by different superscript alphabet are significantly different

4.1.2 Acute toxicity test

In the acute toxicity studies, no death was recorded in the animals that received methanol extract of *Anogeissus leiocarpus* and *C. longa* up to a dose of 5000 mg/kg body weight. Furthermore, the animals did not show any changes in general behaviour and other physiological activities like giddiness, sniffing, aggressiveness, tachypnoea, or convulsion. From the above toxicity studies, the lethal dose (LD_{50}) of the two plants extract were greater than 5000 mg /kg body weight in rats (Table 4.3).

Table 4.3 Acute Oral Toxicity Profile of Methanol Extract of *C. longa* and *A. leiocarpus*

Group	Dosage (mg/kg)	Number of mice	Mortality/Toxicity *C. longa*	Mortality/Toxicity *A. leiocarpus*
Phase 1				
1	10	3	0/3	0/3
2	100	3	0/3	0/3
3	1000	3	0/3	0/3
Phase 2				
4	1600	3	0/3	0/3
5	2900	3	0/3	0/3
6	5000	3	0/3	0/3

4.1.3 Anti-plasmodial bioassay

4.1.3.1 *In-vivo* Anti-plasmodial activity of crude and alkaloidal extract of *A. leiocarpus*

4.1.3.2 Effect of crude and alkaloidal extract of *A. leiocarpus* on parasitaemia count in *P. berghei* – infected mice

Table 4.4 showed the result of parasite count in *P. berghei* – infected mice treated with chloroquine (registered standard drugs), methanol and alkaloids extract of *Anogeissus leiocarpus* compared with the control (infected untreated) mice. The parasitaemia count of infected untreated group increased throughout the study period while infected treated with chloroquine (registered standard drugs), methanol extract of *Anogeissus leiocarpus* at 100 and 200 mg/kg body weight and alkaloid fraction of *A. leiocarpus* at 100 and 200 mg/kg body weight decreases in a dose dependent manner compared with the negative control (infected untreated) mice.

4.1.3.3 Effect of crude and alkaloidal extract of *A. leiocarpus* on weight changes in *P. berghei* – infected mice

The effect of crude and alkaloidal extract of *Anogeissus leiocarpus* on weight changes in *P. berghei* – infected mice are shown in table 4.5 The weight gain of all experimental mice decreases after infection (48 hrs post infection). The weight of infected untreated group further decreases after treatment. However, the weight of infected mice treated with chloroquine (registered standard drugs), methanol extract of *Anogeissus leiocarpus* at 100 and 200 mg/kg body weight and alkaloid fraction of *A. leiocarpus* at 100 and 200 mg/kg body weight increases in a dose dependent manner after treatment compared with the negative control (infected untreated) mice.

Table 4.4 Effect of Crude and Alkaloidal Extract of *A. leiocarpus* on paracitaemia Count in *P. bergei* – Infected Mice

EXRACT TYPE	DOSE (mg/kg)	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Crude	100	35.13 ± 0.04^{a}	36.75 ± 0.16^{b}	35.11 ± 0.01^{e}	17.16 ± 1.03^{e}	10.03 ± 0.04^{d}
	200	38.11 ± 3.12^{a}	35.17 ± 1.31^{b}	28.32 ± 1.32^{d}	15.02 ± 0.03^{d}	9.37 ± 0.16^{d}
	400	33.89 ± 0.17^{a}	32.63 ± 1.29^{a}	25.07 ± 1.01^{c}	10.16 ± 0.81^{b}	5.31 ± 0.07^{c}
Alkaloid	100	36.74 ± 2.16^{a}	35.39 ± 1.39^{b}	27.33 ± 1.16	24.31 ± 1.11^{f}	18.31 ± 1.31^{e}
	200	35.31 ± 2.87^{a}	35.14 ± 1.09^{b}	24.16 ± 0.91^{c}	13.01 ± 0.14^{c}	6.03 ± 0.66^{c}
Alkaloid (A+B)	100	38.91 ± 0.48^{a}	34.21 ± 1.02^{b}	21.14 ± 0.01^{b}	11.02 ± 1.21^{b}	2.11 ± 0.04^{b}
Negative Control		35.24 ± 1.86^{a}	36.07 ± 0.96^{b}	39.07 ± 0.76^{f}	39.13 ± 1.07^{g}	40.11 ± 0.08^{f}
Positive Control		33.41 ± 3.49^{a}	32.32 ± 2.31^{a}	19.07 ± 1.01^{a}	8.03 ± 0.03^{a}	1.01 ± 0.04^{a}

Values followed by the same superscript alphabets on the same column are not significantly different at p>0.05. Values are presented in mean ±standard error of three replicates

Table 4.5 Effect of Crude and Alkaloidal Extract of *A. leiocarpus* on Body Weight in *P. bergei* – Infected Mice

EXRACT TYPE	DOSE	Before Infection	72 Hours After Infection	After Treatment
Crude	100	28.14 ± 0.03^{b}	24.75 ± 1.31^{a}	25.67 ± 0.76^{c}
	200	28.41 ± 0.01^{b}	26.34 ± 1.43^{a}	$28.31 \pm 1.41e$
	400	27.31 ± 1.31^{a}	23.21 ± 2.16^{a}	26.31 ± 0.01^{d}
Alkaloid	100	28.61 ± 0.05^{b}	25.63 ± 1.89^{a}	25.10 ± 0.03^{c}
	200	26.02 ± 2.16^{a}	24.81 ± 2.14^{a}	23.45 ± 0.01^{b}
Alkaloid (A+B)	100	26.41 ± 1.07^{a}	24.10 ± 0.04^{a}	25.32 ± 0.04^{c}
Negative Control		25.41 ± 0.83^{a}	23.23 ± 0.31^{a}	21.04 ± 0.06^{a}
Positive Control		26.17 ± 1.15^{a}	24.14 ± 0.01^{a}	25.01 ± 0.12^{c}

Values are presented in mean ±standard error of three replicates. Values followed by the different superscript alphabets on the same column are significantly different at $p<0.05$.

4.1.3.4 Effect of crude and alkaloidal extract of *A. leiocarpus* on PCV in *P. bergei* – infected mice

The effect of crude and alkaloidal extract of *Anogeissus leiocarpus* on PCV in *P. bergei* – infected mice are shown in Table 4.6. The PCV of all experimental mice decreases after infection (48 hrs post infection). The PCV of infected untreated group further decrease after treatment, however the PCV of infected mice treated with chloroquine (registered standard drugs), methanol extract of *Anogeissus leiocarpus* at 100 and 200 mg/kg body weight and alkaloid fraction of *A. leiocarpus* at 100 and 200 mg/kg body weight increases in a dose dependent manner after treatment compared with the negative control (infected untreated) mice.

4.1.3.5 *In-vivo* Anti-plasmodial activity of crude and alkaloidal extract of *C. longa*

4.1.3.6 Effect of crude and alkaloidal extract of *C. longa* on paracitaemia count in *P. bergei* – infected mice

Table 4.7 showed the result of parasite count in *P. bergei* – infected mice treated with with chloroquine (registered standard drugs), methanol and alkaloids extract of *C. longa* compared with the control (infected untreated) mice. The parasitaemia count of infected untreated group increased throughout the study period while infected treated with chloroquine (registered standard drugs), methanol extract of *C. longa* at 100 and 200 mg/kg body weight and alkaloid fraction of *C. longa* at 100 and 200 mg/kg body weight decreases in a dose dependent manner compared with the negative control (infected untreated) mice. The alkaloidal fraction exhibited higher antiplasmodial activity comparable the standard control group

Table 4.6 Effect of Crude and Alkaloidal Extract of *A. leiocarpus* on Body Weight in *P. bergei* – Infected Mice

EXRACT TYPE	DOSE	Before Infection	72 Hours After Infection	After Treatment
Crude	100	42.31 ± 0.01^{a}	37.68 ± 0.07^{b}	45.23 ± 0.03^{d}
	200	42.56 ± 0.21^{a}	33.40 ± 0.03^{a}	39.32 ± 0.14^{b}
	400	43.12 ± 0.03^{a}	38.53 ± 1.21^{b}	41.31 ± 0.32^{c}
Alkaloid	100	42.16 ± 1.21^{a}	41.32 ± 1.16^{c}	41.34 ± 0.78^{c}
	200	44.12 ± 0.07^{a}	44.25 ± 0.03^{d}	44.56 ± 0.89^{d}
Alkaloid (A+B)	100	43.60 ± 1.31^{a}	38.63 ± 0.01^{b}	38.76 ± 1.31^{b}
Negative Control		42.06 ± 0.81^{a}	38.14 ± 1.11^{b}	33.06 ± 0.12^{a}
Positive Control		41.16 ± 0.92^{a}	39.35 ± 1.31^{b}	39.56 ± 0.05^{b}
Normal		42.38 ± 0.01^{a}	43.06 ± 1.12^{c}	42.31 ± 0.32^{c}

Values are presented in mean ±standard error of three replicates. Values followed by the different superscript alphabets on the same column are significantly different at $p<0.05$.

Table 4.7 Effect of Crude and Alkaloidal Extract of *C. longa*on Paracitaemia Count in *P. bergei* – Infected Mice

EXRACT TYPE	DOSE (mg/kg)	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Crude	100	33.81 ± 0.11^{a}	34.01 ± 0.01^{e}	32.15 ± 1.16^{e}	28.11 ± 0.01^{d}	12.03 ± 0.02^{e}
	200	34.12 ± 0.11^{a}	31.25 ± 0.03^{d}	24.11 ± 0.01^{d}	11.12 ± 0.10^{c}	7.11 ± 1.89^{d}
	400	34.21 ± 0.12^{a}	28.41 ± 0.01^{c}	20.41 ± 0.05^{c}	10.13 ± 0.03^{c}	3.10 ± 0.04^{c}
Alkaloid	100	33.06 ± 0.17^{a}	26.11 ± 0.07^{b}	12.11 ± 0.01^{a}	7.11 ± 0.04^{a}	2.12 ± 0.05^{b}
	200	35.11 ± 0.81^{a}	24.10 ± 0.05^{a}	11.23 ± 0.04^{a}	6.10 ± 0.03^{a}	0.07 ± 0.01^{a}
Negative Control		35.24 ± 1.86^{a}	36.07 ± 0.96^{f}	39.07 ± 0.16^{f}	39.13 ± 1.07^{e}	40.11 ± 0.08^{f}
Positive Control		33.41 ± 3.49^{a}	24.32 ± 0.31^{a}	11.07 ± 1.01^{b}	8.03 ± 0.03^{b}	0.05 ± 0.01^{a}

Values are presented in mean ±standard error of three replicates. Values followed by the different superscript alphabets on the same column are significantly different at $p<0.05$.

4.1.3.7 Effect of crude and alkaloidal extract of *C. longa* on weight changes in *P. bergei* – infected mice

The effect of crude and alkaloidal extract of *C. longa*on weight changes in *P. bergei* – infected mice are shown in Table 4.8. The weight gain of all experimental mice decreases after infection (48 hrs post infection). The weight of infected untreated group further decreases after treatment. However, the weight of infected mice treated with chloroquine (registered standard drugs), methanol extract of *C. longa*at 100 and 200 mg/kg body weight and alkaloid fraction of *C. longa*at 100 and 200 mg/kg body weight increases in a dose dependent manner after treatment compared with the negative control (infected untreated) mice.

4.1.3.8 Effect of crude and alkaloidal extract of *C. longa* on PCV in *P. bergei* – infected mice

The effect of crude and alkaloidal extract of *C. longa*on PCV in *P. bergei* – infected mice are shown in Table 4.9. The PCV of all experimental mice decreases after infection (48 hrs post infection). The PCV of infected untreated group further decrease after treatment, however the PCV of infected mice treated with chloroquine (registered standard drugs), methanol extract of *C. longa* at 100 and 200 mg/kg body weight and alkaloid fraction of *C. longa* at 100 and 200 mg/kg body weight increases in a dose dependent manner after treatment compared with the negative control (infected untreated) mice.

Table 4.8 Effect of Crude and Alkaloidal Extract of *C. longa*on Body Weight in *P. bergei* – Infected Mice

EXRACT TYPE	DOSE	Before Infection	72 Hours After Infection	After Treatment
Crude	100	25.13 ± 0.13^{a}	23.18 ± 0.04^{b}	22.31 ± 0.03^{b}
	200	23.12 ± 1.27^{a}	21.13 ± 0.01^{a}	22.89 ± 0.02^{b}
	400	23.89 ± 1.29^{a}	21.01 ± 0.02^{a}	23.41 ± 0.10^{b}
Alkaloid	100	24.10 ± 1.14^{a}	21.71 ± 0.07^{a}	22.47 ± 0.07^{b}
	200	25.32 ± 1.07^{a}	23.82 ± 0.02^{b}	24.25 ± 0.13^{b}
Negative Control		25.41 ± 0.19^{a}	23.87 ± 0.07^{b}	20.19 ± 0.31^{a}
Positive Control		26.17 ± 0.18^{a}	24.60 ± 0.01^{c}	25.38 ± 0.19^{c}
Normal		25.32 ± 0.38^{a}	25.07 ± 1.17^{d}	24.98 ± 0.79^{c}

Values are presented in mean ±standard error of three replicates. Values followed by the different superscript alphabets on the same column are significantly different at $p<0.05$.

Table 4.9: Effect of Crude and Alkaloidal Extract of *C. longa*on Body Weight in *P. bergei* – Infected Mice

EXRACT TYPE	DOSE	Before Infection	72 Hours After Infection	After Treatment
Crude	100	42.17 ± 1.89^{a}	41.07 ± 0.27^{b}	41.82 ± 0.07^{c}
	200	42.81 ± 1.07^{a}	40.87 ± 0.89^{b}	41.01 ± 0.01^{c}
	400	41.79 ± 0.26^{a}	41.01 ± 0.40^{b}	41.14 ± 0.03^{c}
Alkaloid	100	41.89 ± 0.79^{a}	41.19 ± 0.04^{b}	42.01 ± 0.06^{d}
	200	42.01 ± 1.01^{a}	41.01 ± 0.17^{b}	39.81 ± 0.04^{b}
Negative Control		42.06 ± 0.81^{a}	38.14 ± 1.11^{a}	33.06 ± 0.12^{a}
Positive Control		41.16 ± 0.93^{a}	39.35 ± 1.31^{a}	39.56 ± 0.05^{b}
Normal		42.38 ± 0.01^{a}	43.06 ± 1.12^{b}	42.31 ± 0.32^{d}

Values are presented in mean ±standard error of three replicates. Values followed by the different superscript alphabets on the same column are significantly different at $p<0.05$.

4.1.4 Mean survival time

Effect of the crude and alkaloidal extract of *Anogeissus leiocarpus* and *Curcuma longa* on mean survival time of *P. berghei* infected mice are shown in Table 4.10. Group of mice treated alkaloid fraction of *Curcuma longa* at 100 mg/kg bw had the highest mean survival time of 31.12±0.79 days comparable to the mice treated with the chloroquine (32.00±0.00 day) while the infected untreated mice survive only for 16.23±0.03 days. The crude extract also exhibited dose dependent increase in survival days of the treated mice. Similarly, mice treated with 200 mg/kg bw *A. leiocarpus* exhibited higher survival time of 30.56±1.03 days when compared with the crude extract treated mice.

4.1.5 Haematological parameters

Effect of the crude and alkaloid extract of *Anogeissus leiocarpus* on haematological parameters in *P. berghei* infected mice are shown in Table 4.11. There was significant decrease ($P<0.05$) in the values of haemoglobin concentration (Hb) and red blood cell (RBC) count of the infected untreated groups in comparison with the uninfected not treated, and infected treated groups. Also, significant increase ($P<0.05$) was observed in white blood cell (WBC) count of the infected untreated groups when compared with the normal control, infected treated groups. The group of mice treated with alkaloidal extract exhibited higher value of haematological indices than the crude extract and chloroquine treated mice

Table 4.10 Effect of the Crude and Alkaloidal Extract of *Anogeissus leiocarpus* and *Curcuma longa* on Mean Survival Time of *P. berghei* Infected Mice

Extract	Dose (mg/kg b. wt)	*Curcuma longa*	*Anogeissus leiocarpus*
Crude	100	26.02±0.03^{b}	27.92±0.23^{b}
	200	27.45±0.23^{b}	26.26±0.54^{b}
	400	29.32±0.87^{c}	30.05±1.04^{c}
Alkaloid	100	31.12±0.79^{d}	27.42±0.65^{b}
	200	29.32±0.02^{c}	30.56±1.03^{c}
Alkaloid (A+B)	100	31.89±0.49^{d}	31.91±0.23^{c}
Positive Control		32.00±0.00^{d}	32.00±0.00^{d}
Negative Control		16.23±0.03^{a}	16.23±0.03^{a}

Values are presented in mean ±standard error of three replicates. Values followed by different superscript alphabets are not significantly different at $p<0.05$.

Table 4.11 Effect of Crude Methanol Extract and Alkaloidal Fraction of *Anogeissus leiocarpus* on Haematological Parameters in *P. bergei* Infected Mice

	GROUP	HB (g/dl)	MCV (Fi)	MCH (pg)	MCHC (g/dl)	RBC (%)	PLC (%)	TWBC (%)
Crude	100	16.95±0.05C	41.00±13.00a	14.75±0.70a	31.80±0.85a	9.63±0.35c	552.00±1.00	7.35±0.50a
	200	6.25±0.25a	49.50±2.50b	23.50±0.50d	46.35±0.35e	3.21±1.00a	140.30±0.30b	24.15±0.50c
	400	16.95±0.05C	46.50±0.50b	17.50±0.50b	35.25±0.2b	7.25±0.25c	253.30±0.30d	5.39±0.20a
Alkaloid	100	13.30±0.20b	61.00±1.00c	19.50±0.50c	29.50±0.20a	5.45±0.05b	200.50±0.50c	4.21±0.50a
	300	14.41±1.02c	59.12±2.01b	18.87±0.03 b	31.22±0.46 a	6.41±0.37c	211.03±0.89 c	5.31±0.46 a
Alkaloid (A+B)	100	13.23±1.29c	57.39±0.35 d	18.19±0.21 b	32.01±0.86 a	6.81±1.07 c	201.98±0.38 c	8.14±2.09b
	Untreated	7.80±0.15a	44.50±0.50b	16.50±0.50b	41.00±1.00d	4.20±2.80b	121.00±1.00a	25.33±0.50d
	Standard Normal	13.25±0.25c	45.50±0.50b	19.00±1.00b	37.50±0.50c	6.25±0.25b	181.00±49.00c	7.29±0.20b

Values followed by the same superscript alphabets on the same column are not significantly different at p>0.05. Values are presented in mean ±standard error of three replicates. Keys: Hb=Haemoglobin, MCV= Mean cell volume, MCH= Mean cell haemoglobin, MCHC=

Mean cell haemoglobin concentration, RBC= Red blood cell, PLC= Platelet count, TWBC= Total white blood cell, PCV=Packed cell volume

4.2 Discussion

In the current study, the two plants showed presence of therapeutic metabolites. The high levels of phytochemicals including alkaloids, flavonoids, phenols and saponins in *C. longa* rhizome established in this study, is an indication of its potential for medicine and therapy. This is because the pharmacological impacts of plants are based on these compounds (Jigam *et al.,* 2017). The results revealed that phenol (273.04±6.35 mg/100g) is the most abundant phytochemical components of *Curcuma longa* followed by tannins **(**205.30±7.43 mg/100g) while alkaloids (9.39±0.43 mg/100g) is the least. Tannins and flavonoids have recorded antimicrobial, antioxidant and anti-inflammatory characteristics. Flavonoids are a class of plant metabolites that, through pathways and signal cells, are believed to provide health benefits. In addition to antioxidant function, flavonoids have anti-inflammatory, anti-viral, anti-cancer, and antimicrobial activities (Umar et al., 2012). Phenols are plant components that can be used as hormone and immune stimulating anti-inflammatory modulators (Okwu and Morah, 2007). The haemostatic and astringent physiological characteristics of tannins were stated to promote wound healing and inflamed mucus membrane. Typical of the position is their strong anti-oxidant ability and stabilization (Trease and Evans, 1989). In addition, medicinal plants produce these metabolites from secondary metabolic activities and primarily to ward off infections from plant pathogens and insectivorous plants.

This study also revealed the presence of various important medicinal phytochemicals in crude extract of *Anogeissus leiocarpus*. *Anogeissus leiocarpus* on the other hand also recorded367.15±7.92 of phenol as it most abundant phytochemical followed by alkaloids **(**194.75±8.01 mg/g) while saponins (39.00±0.20 mg/100g) was the least. Although alkaloids have been reported by Theresa *et al*. (2016) and Victor and Grace (2013) to be absent in the methanolic leaf extracts of *Anogeissus leiocarpus* while the

results obtained by (Mann *et al*., 2008; Sore *et al*., 2012) is in agreement with this study. This disparity could be as a result of different stress undergo by the plant and reactions to environmental, climatic and soil factors. All these contribute to the composition of the plant.

Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and their synthetic derivatives are use as basic medicinal agent for their analgesic, anti-plasmodial and antibacterial effect (Stray, 1998). Saponin has been reported to have anti-inflammatory and analgesic activity (Trease and Evans, 1989). Flavonoids are the most diversified groups of phenolic compound found in plant. Its biological activities include; antibacterial, anti-inflammatory, protection against ulcers, vineses and antitumor effect (Okwu and Okwu, 2004). Flavonoids are also free radical scavengers, super antioxidant and potential water soluble substances which prevent oxidative cell damage and have strong anti-ulcer activity (Karim *et al.*, 2015).

In this current investigation, crude methanol extract of *A. leiocarpus* and *C. longa* rhizome had a high safety bracket, as no death occurred within 24 hours of extract administration up to 2000 mg/kg body weight extract dose. This is an indication that the tested dose is safe for oral administration. According to the Guidelines recommended by OECD on the acute oral toxicity testing based on LD_{50}, *A. leiocarpus* and *C. longa* extract could be assigned to the class 5 (LD_{50}>2000 mg/kg bodyweight), as designated to be the lowest toxicity class (OECD, 2001). The use of herbal medicine traditionally, is an old practice in most regions of the world and has played a pivotal role in drug bioprospecting (Heinrich *et al*., 1998). Administration of herbal preparations without any standard dosage coupled with inadequate scientific studies of their safety has raised concerns on their toxicity. However, toxicity studies in animals help to assess the potential health risk in humans caused by intrinsic adverse effects of chemical

compounds present in plant extracts (Ashafa *et al*., 2010). Acute toxicity tests the toxicological response of an experimental organism to single or instant sample exposure (OECD, 2001). The first guide to pharmacological and toxicological testing (Lorke, 1983) is to assess the acute toxicity of an unknown substance.

In the present study, the parasitaemia count of *P. berghei* infected mice treated with methanol extract of *C. Long*a,, 100, 200 and 400 mg/kg body weight, decreases in a dose dependent manner compared with the negative control (infected untreated) mice. This antimalarial potency of the plant could be attributed to the aforementioned secondary metabolites in the plants. Kosalec *et al*. (2009) documented that phytochemicals are responsible for antiplasmodial properties of medicinal plants. The mechanism of inhibition of the phytochemical components on the *Plasmodium* may be due to the impairment of various enzyme systems such as those involved in energy production as well as the interference with the integrity of the cell membrane and structural component synthesis (Okwu and Morah, 2007). They might be individually and/or collectively responsible for the exhibited antiplasmodial activity. Indeed, these groups of compounds were previously reported to have significant inhibitory effects on *P. falciparum* (Kaur *et al.,* 2009). This finding agrees with the results of previous *In vitro* and *in vivo* studies of antimalaria effects of *Curcuma longa* (Nandakumar *et al*., 2006; Martinelli *et al.,* 2008).

Similarly, this study shows that the alkaloid fraction of *A. leiocarpus* and *C. longa* demonstrated higher antiplasmodial activity that is comparable with the standard control. The proposed mechanism of antiplasmodial effect of alkaloids is by elevation of erythrocytes oxidation and inhibition of the plasmodium protein synthesis (Sofowora, 1993). Alkaloids have been reported in literature of different plant species as having different extents of antimalarial activity. Several classes of phytoconstituents are

responsible for the antimalarial activity of plants including alkaloids, terpenes, steroids, and flavonoids. Alkaloids are considered as an important group exhibiting diverse biological activities, particularly antimalarial activity. They constitute an important class of structurally diversified compounds that are having the nitrogen atom in the heterocyclic ring and are derived from the amino acids (Kaur and Arora, 2015).

Haematological parameters generally provide information on deleterious effects of foreign components and parasite on the blood and also explain blood-related functions of chemical compounds (Betancourt-Alonso *et al*., 2011). Such analysis is relevant to risk evaluation because changes in the haematological system are highly predictive for human toxicity, when data are translated from animal studies (Umar *et al*., 2012). In the present study, the continuous loss of PCV in *P. berghei*-infected mice could be attributed to RBC destruction, due to either parasite multiplication or spleen reticuloendothelial cell action as the presence of many abnormal RBC stimulates the spleen to produce many phagocytes (Chinchila *et al*., 1998). This massive destruction leads to a decrease in erythroid precursors and erythropoiesis inhibition, usually resulting in the death of the patient (Okafor, *et al.,* 2018). Anaemia is a preventable cause of death in malaria-infected children under five years and pregnant women (WHO, 2006). Treatment of the infected mice with the crude extract and alkaloid fraction of *A. leiocarpus* caused a significant ($P<0.05$) increase in the PCV when compared with the untreated control. The alkaloid extracts from both plant was found to exhibited higher effect on PCV. This could be due to the higher antiplasmodial effect of the alkaloids fractions and as a result of sustaining the availability of new RBCs produced in the bone marrow. It could also be an indication of haematopoietic properties of the alkaloid (Nandakumar *et al.,* 2006).

White blood cells defend the body against infections or any foreign body. The significant increase in WBC recorded in infected untreated mice may indicate immunological response by the mice to the infection which augmented the production of more WBC (Bashir et al., 2015), to cope with the stress induced by the parasite

The loss of body weight is associated with progression of infection followed by appetite decreases, and the animal loses condition as a result, there is wasting. The decreased supply of oxygen because of the anemia is also an important factor (Yusuf *et al*., 2012). However, animals, which received crude extract and the alkaloid extract gained weight compared to the negative control groups. This shows that because of reduction in parasitemia and prevention of drop in PCV as a result of the plasmodium suppressive effect of the alkaloid fraction against plasmodium infection, physical status of the treated mice was improved. Similar observations have been made by other researchers (Abubakar *et al*., 2005; Alli *et al*., 2011).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The crude extracts of *Anogeissus leiocarpus* and *Curcuma longa* leaf have appreciable amounts of phytochemicals with medicinal reputations. They are relatively safe upon oral acute doses in mice. This work is a confirmation of the inherent medicinal property of *Anogeissus leiocarpus* and *Curcuma longa* since they showed activity in vivo against *Plasmodium berghei* infected mice. The crude extract showed decrease parasite replication and improvement in PCV and body weight of the infected treated mice. However, alkaloidal fractions from the two plants exhibit interesting activities by significantly reduced the level of parasitemia count, and improve PCV and weight gain of infected mice comparable to the standard control. Furthermore, this study justifies the use of *Anogeissus leiocarpus* and *Curcuma longa* in traditional medicine for treatment of malaria. As such, the plants alkaloidal extracts can be formulated into supplements to enable more people benefit from it and as well commercialize the drug since it is already being used for treatment albeit in the crude form.

5.2 Recommendations

Based on the results obtained from this study the following recommended were suggestions.

1. The *Anogeissus leiocarpus* and *Curcuma longa* leaf extract has shown promising anti-malaria, activity, the next step would be to further identify and characterize the active alkaloid for structural elucidation.

2. The mechanism by which these alkaloids exerts its effect can also be determined, this could be of help even in other areas of research and as well if there be need to formulate synthetic analogues of the compound in the future.

3. Other phytochemical component of the plants can also be further analysed to ascertain the extent of their therapeutic activity. This might bring insight into the role of these components in the effect that the plant exerts in its various pharmacological roles.

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