ANTIPLASMODIAL ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *ANACARDIUM OCCIDENTALE* AND *CYMBOPOGON CITRATUS*

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CERTIFICATION

This is to certify that this thesis has been examined and approved for the award of the degree of

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DECLARATION

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

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DEDICATION

This work is dedicated to my late father, Sha'a Kiri for his immeasurable contribution to my education.

TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

CHAPTER TWO
LITERATURE REVIEW

CHAPTER THREE
MATERIALS AND METHODS

CHAPTER FOUR
RESULTS

CHAPTER FIVE
DISCUSSION

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABSTRACT

The search for antimalarial compounds was necessitated by *Plasmodium falciparum* resistance to most antimalarial drugs. In endemic countries where malaria is prevalent, medicinal plants are often used to treat malaria. In this study, the *in vitro* antimalarial activities of crude aqueous and ethanolic extracts of *Anacardium occidentale and Cymbopogon citratus*, were evaluated against *P. falciparum*. Fourteen fresh blood samples obtained from infected children and adults aged 15 to 25 years were tested against the plant extracts. Standard microtest technique of schizont maturation and parasite growth assay was used to culture fresh isolates. Acute toxicity of the aqueous and ethanolic extracts *A. occidentale* and *C. citratus* was tested on albino rats. Anti-inflammatory activity was determined using xylene induced ear oedema and the analgesic properties of plants' extracts were evaluated using acetic acid-induced writhing in mice. The SSPS Statistical tool was used to calculate mean parasite growth and percentage parasite inhibition. The t-test was used to analyse the data. There was a significant ($p = 0.05$) reduction in the number of parasitized cells relative to the control. Ethanol extract of *A. occidentale* exhibited higher antimalarial activity of 75.6% than aqueous IC_{50} of 11.7µg/ml followed by aqueous extract of *A. occidentale* with 72.4% activity and IC_{50} of 16.0µg/ml. The aqueous extract of *C. citratus* had an activity of 69.0%, IC_{50} of 23µg/ml. Ethanol extract of *C. citratus* had the lowest antimalarial activity of 67.9%, IC_{50} of 30µg/ml. All the four extracts showed moderate antimalarial activity. None of the extracts exhibited any sign of toxicity in rats, and all showed a good measure of anti-inflammatory activity. The extracts at doses 50mg/kg and 100mg/kg body weight produced various analgesics responses in a dose dependent manner. There was a significant ($P < 0.05$) reduction in writhing at 100mg/kg body weight in both aqueous and ethanolic extracts of *A. occidentale and C. citratus*. This result shows that the aqueous and ethanolic extracts of *A. occidentale* and *C. citratus* possess promising antimalarial activities which can be exploited for malaria therapy, and also justifies the traditional use of the

plants in malaria treatment. Further work is suggested to isolate, identify and characterize the active constituents from the plants.

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Malaria is a life threatening and one of the most important of all parasitic diseases (Ouattara *et al*., 2006). It may result from the infection by any protozoan parasites of the genus *Plasmodium.* Four species of malaria parasites are naturally known to infect humans. These are *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax, and Plasmodium ovale. P. falciparum* causes the most serious form of the disease. *P. knowlesi*, a newly recognised fifth species is important in a small geographical range in Oceania (DFID, 2010). *Plasmodium knowlesi* is a zoonosis that causes malaria in macaques but can also infect humans (Fong, Cadigan and Coatnney, 1971). It is recognized as a frequent cause of potentially fatal malaria in adults in Malaysian Borneo (Cox-Singh *et al*., 2008).

The disease which can be fatal is transmitted to humans through the bite of female mosquitoes of the genus *Anopheles.* The parasite matures and reproduces sexually in the female anopheline mosquito vector which is the parasites' definitive host. Man is the intermediate host. The symptoms of the disease include fever, headache, chills, loss of appetite, vomiting, shivering, general body weakness, joint pains, dizziness and drowsiness (Bruce-Chwatt, 1985). All the symptoms may not present in a single patient and if they do, the severity varies from one person to another. Confirmation is usually done by laboratory examination of blood for the presence of parasites (Avwioro, 2010).

Consequences of malaria include childhood deaths, anaemia, low birth-weight, epilepsy, and neurological problems. These consequences of malaria, compromise the health and development of millions of children throughout the tropical world (RBM, 2006a).

Malaria is one the most prevalent and debilitating tropical diseases. It is estimated that nearly half of the world's population in 104 countries live at risk of the disease (PATH 1990; Dexter, 2002; WHO, 2012). Ninety percent of malaria cases in the world are estimated

to be in Africa where the disease is endemic (WHO, 2008a; Kachur, MacArthur and Slutsker, 2010), particularly West and Central Africa, are attributed to a combination of very high transmission and weaker health services (DFID, 2010). Malaria is Africa's leading cause of infant mortality (20 per cent) and constitutes 10 per cent of the continent's overall disease burden. It accounts for 40 per cent of public health expenditure, 30-50 per cent of inpatient admissions, and up to 50 per cent of outpatient visits in areas with high malaria transmission (RBM, 2006b). Of the annual conservative estimate of 1 - 2.7 million deaths due to malaria globally, about 90% of these occur in Africa alone (Day, 1997; Dexter, 2002) with children under five years of age and pregnant women most severely affected (WHO, 2012).

1.2 MALARIA HIGH RISK GROUPS

Malaria infection cuts across people of all ages, sexes and occupations, but is most common among four categories of people who are referred to as "malaria high risk group" (Maartens and Ellis, 1990); FMOH, 1991; Najera, Liese and Hammer, 1992). These are i) Children between the ages of 6 months to 5 years, who have lost passive immunity acquired from mothers, ii) Pregnant women, possibly because immunity is depressed during pregnancy, iii) Sickle cell disease (SCD) patients (sicklers) and iv) Non-immune immigrants and travelers from non-endemic areas.

Malaria is the major cause of crises in SCD patients Molta, Sha'a, Watila and Oguche, 2005) and the commonest and most important cause of morbidity and mortality and precipitates both haemolytic and infarctive crises among SCD patients in malaria endemic countries (Fleming, 1989; Oniyangi and Omari, 2006). So preventing malaria in people with SCD may help to reduce crises and all problems associated with it. Long-life drugs to prevent malaria infections are often recommended for people with SCD living in malaria endemic areas (Oniyangi and Omari, 2006).

Malaria infection among pregnant women is a major public health problem and is one of the commonest causes of complication in pregnancy (Okonofua and Abejide, 1996). The burben of malaria infection in pregnancy is cause chiefly by *P. falciparum* the most common malaria species in Africa. At least 30 million pregnancies occur among women in malarious areas of Africa, most of who reside in area of relatively stable malaria transmission (RBM, 2001). Pregnant women are particularly vulnerable to malaria as malaria reduces a woman's immunity to the disease, making her more susceptible to malaria infection and increasing the risk of illness, severe anaemia and death. For the unborn child, maternal malaria increases the risk of spontaneous abortion, stillbirth, premature delivery and low birth weight- leading cause of child mortality (WHO, 2003).

1.3 SOCIO-ECONOMIC IMPLICATIONS OF MALARIA

Malaria is often referred to as the disease of poverty and the cause of poverty. The disease has a measurable direct and indirect cost, and has been known to be a major obstacle to socioeconomic development (RBM, 2010). It has been observed that children from households classified as poor had a significantly higher chance to get malaria (Krefis *et al*., 2010). The direct cost of malaria in malaria endemic regions shows that individuals or households encounter direct cash expenses in malaria prevention and treatment. This include household expenses on insecticide treated nets (ITNs), insecticides for indoor spray, hospital or doctor's consultation fees, antimalarial drugs, transportation to health facility, support for the patient and sometimes a patient relation during hospital stay (RBM, 2010; Mia *et al*., 2012).

In Nigeria, it was estimated that households are willing to pay an average of about Naira 7,324 (USD 61 per month for control of malaria, which is an excess of about Naira 2,715 (USD 22.6) over the cost they bear (protection, treatment and indirect costs) (Jimoh, Sofola, Petu and Okorosobo, 2007). Again in Khartoum, Sudan, average monthly

expenditure on malaria treatment per household was estimated at USD 1.7; this reduces the average monthly income per household by 0.8% (Mustafa and Babiker, 2007).

Indirect costs of malaria include household loss of productivity due morbidity and mortality. This might be expressed as cost loss of workdays or absenteeism from employment and the value of unpaid work done in the home by both men and women (RBM, 2010). Malaria also hinders children's schooling and social development through both absenteeism and sometimes permanent neurological damage associated with severe malaria infection in children. Another indirect cost of malaria which cannot be measured in monetary terms is the human pain and suffering caused by the disease. However, the level of indirect cost measurement varies considerably with individuals and households (Mia *et al*., 2012).

At the level of nations, malaria imposes substantial costs to governments through the maintenance of health facilities, purchase of drugs, public health interventions against malaria, such as indoor insecticides spraying, insect treated nets, loss of days of work which results into loss of income and loss of opportunities for joint economic ventures and tourism (CDC, 2012). The socioeconomic impact of malaria is greater among the poorest countries of the world, especially those in Sub-Saharan Africa (Goodman, Hanson, Mills, Wiseman and Worrall, 2003). There is no doubt that malaria endemicity hinders national prosperity due to its influence on social and economic decisions. It has been observed that economic growth in countries with high malaria prevalence has generally been lower than in countries without malaria (Africa@home, 2012). The disease therefore, presents a serious economic burden on economic development. As a result of the awareness of economic consequences due to malaria, African countries now allocate more resources to antimalarial drugs development as a major element of poverty reducing strategies (Africa@home, 2012).

In summary, where malaria prospers most, human societies have prospered least. The global distribution of per-capita gross domestic product shows a striking correlation between malaria and poverty, and malaria endemic countries have lower rates of economic growth

(Sachs and Malaney, 2002). There is no doubt that malaria has a tremendous implication on socioeconomic development of endemic countries.

1.4 SCREENING OF TRADITIONAL MEDICINAL PLANTS FOR ANTIMALARIAL ACTIVITIES

In sub-Saharan Africa and other parts of the world where malaria is endemic, herbal remedies are commonly used to treat the disease. The understanding is that traditional medicinal plants that are employed for treatment of malaria represent a potential for discovery of lead molecules for development into potential antimalarial drugs (Muthaura, Keriko, Derese and Rukunga, 2007). In most African and Asian countries, about 80% of the population rely on medicinal plants for primary health care (WHO, 2008b), and since the discovery of artemisinin as an effective antimalarial isolated from the herb plant *Artemisia annua* (Klayman, 1985), a lot of interests have shifted to plant sources as antimalarial agents. The screening of plants known to cure malaria in ethnomedicine is therefore an important strategy in the treatment and control of malaria. *A. occidentale* (cashew) and *C. citratus* (lemongrass) are some of the medicinal plants traditionally used to treat malaria in Nigeria (Akueshi, 1999). However, little scientific information about their antimalarial activities against *Plasmodium* species and safety for use is available. It is important therefore, to investigate the antimalarial activities of these plants to determine their potentials as new antimalarial compounds.

1.5 STATEMENT OF RESEARCH PROBLEM

Despite more than a century of efforts to control or eradicate malaria, the disease remains a major growing threat to public health and economic development of countries in the tropical and sub-tropical world is largely due to *P. falciparum* resistance to most antimalarial drugs (Bickii, Tchouya, Tchouankeu and Tsamo, 2007)

In Nigeria, malaria transmission occurs throughout the year round, and the country accounts for a quarter of all malaria cases in the World Health Organization(WHO), African region (WHO, 2008). Most malaria cases are caused by *P. falciparum,* although they remain uncomfirmed. There is no evidence of a systematic decline in malaria burden; in addition, the increasing trend in the number of cases and deaths is due to improvements in reporting (WHO, 2008). The search for antimalarials among our local herbs is therefore necessary. Malaria is one of the most important infectious diseases in tropical and sub-tropical regions, and continues to be a major global health problem, with over 40% of the world's population exposed to various degree of malaria risk (Tangpukdee, Duangdee, Wilairatana and Krudsood, 2009).

Malaria endemic regions of the world are faced with an unprecedented situation in which affordable treatment options are rapidly losing therapeutic efficacy because of some degree of resistance (Soni and Gupta, 2009). As a consequence of drug resistance, drugs like quinine, chloroquine, primaquine and mefloquine are ineffective in treating malaria in many endemic regions of the world (Schlizer, 2007). Another problem relating to drug resistance in *P. falciparum* is the occurrence of cross-resistance among drugs belonging to the same chemical family (WHO, 2001a). Eventhough no clinically relevant artemisinin resistance has been reported yet,   it is likely to occur since artemisinin resistance has been obtained in laboratory animals (Meshnik, 2002). *Plasmodium falciparum* is reported to have reduced *in vivo* susceptibility to artesunate in Western Cambodia, historically part of a site of emerging antimalarial-drug resistance (Dondorf *et al*., 2009). Measures for containment are urgently needed to limit the spread of these parasites from Western Cambodia and to prevent a major threat to current plans for eliminating malaria.

1.6   JUSTIFICATION FOR THE STUDY

Some of the reasons for increase in mortality due to malaria include; *P. falciparum* resistance to most anti-malarial drugs, *Anopheles* mosquitoes resistance to insecticides, environmental changes, war and civil disturbances, travels and cross border movements (Omotayo, 2003). The main reason, however, is parasite resistance to antimalarials which complicates the problem of treatment. In addition, malaria puts a heavy economic burden on developing countries by exhausting health system resources and associated loss of economic activity (Ziegler *et al*., 2002). In the absence of a functional, safe, inexpensive and widely available malaria vaccine, the effort to develop new antimalarial drugs from local plants traditionally reputed to cure malaria becomes profoundly important.

One of the strategies in the search for new anti-malarial compounds   is the study of active constituents (metabolites) of medicinal plants (Mustof, Sholikhah and Wahyuono, 2007). Generally, scientific information about antimalarial activity of plants traditionally acclaimed to cure malaria is very limited or does not exist in some cases. Phytochemical screenings of medicinal plants are not only used to search for bioactive agents but also help to reveal the presence of agents in plants which serve as starting products for the partial synthesis of some useful drugs (Sofowora, 1993). It is therefore important to screen medicinal plants for antimalarial activity in order to ascertain their potentials as sources of new anti-malarial compounds. The reputed efficacies of *Anacardium occidentale* and *Cymbopogon citratus* have been documented (Odugbemi, Akinsulire, Aibinu and Fabiku, 2007). However, lack of scientific proof claimed by traditional healers in Nigeria necessitates a scientific study on these plants. It is in light of this, that *Anacadium occidentale* and *Cymbopogon citratus* have been screened for antimalarial activities.

1.7   AIM OF THE STUDY

To screen *Anacardium occidentale* (bark) and *Cymbopogon citratus* (leaves) for antimalarial activity.

1.8   THE OBJECTIVES OF THE STUDY

i.   To identify the active antimalarial components of *Anacardium occidentale* and *Cymbopogon citratus*.

ii. To assess/evaluate the antimalarial activities of crude extracts of *A. occidentale* and *C. citratus* using *in vitro sensitivity* test.

ii. To compare the antimalarial efficacies of crude extracts of *A. occidentale* and *C. citrates.*

iii. To determine the acute toxicity of the crude extracts on albino rats (*Ratus norvegicus*).

iv. To determine the anti-inflammatory and analgesic activities of both plant extracts in mice (*Apodemus sylvaticus*).

1.9 RESEARCH HYPOTHESES

i. There is no antiplasmodial activity in the aqueous and ethanolic extracts of *A. occidentale* and *C. citratus*.

ii. Aqueous and ethanolic extracts of *A. occidentale* and *C. citratus* are not toxic to albino rats.

iii. The aqueous and ethanolic extracts of *A. occidentale* and *C. citratus* do not possess anti-inflammatory activities.

iv. The aqueous and ethanolic extracts of *A. occidentale* and *C. citratus* do not possess analgesic properties.

CHAPTER TWO
LITERATURE REVIEW

2.1 MALARIA TREATMENT: A HISTORICAL BACKGROUND

Effective treatment of malaria originated in the 17th century with quinine, extracted from the back of the cinchona tree (*Cinchona officinalis*) (PATH, 1990). For more than three centuries quinine and other alkaloids of cinchona tree, such as quinidine, cinchonine and cinchonidine were the only effective drugs available for the treatment of malaria (Bruce-Chwatt, 1985).

In recent times, however, synthetic antimalarials were introduced as they were found to be superior to quinine and also less toxic (Bruce-Chwatt, 1985). These include 8-amino quinolines e.g. premaquine; 4- aminoquinolines e.g chloroquine and amodiaquine; biguanides e.g proguanil, diaminopyrimidines e.g. pyrimethemine, sulfones and sulfonamides and antibiotics e.g. tetracycline.

Of these synthetic antimalarials, chloroquine was found to be excellent both as curative and preventive, with a very wide range of other useful qualities. It is non-toxic (safe), reliable, inexpensive and highly effective and a rapid blood schizontocide against all forms of malaria parasites. It was therefore adopted as the first line drug of choice (PATH, 1990; Bruce-Chwatt, 1985).

In the 1960's, however, resistant strains of *P*. *falciparum* began to emerge in South – East Asia and Latin America (PATH, 1990). In Africa, resistant *falcipanum* malaria was first reported in Kenya (Fogh, Jepsen and Effersoe, 1979) and since then it has spread throughout the continent, with resistance first recorded in Nigeria 1989 (Lege – Oguntoye *et al*., 1989; Daniel and Molta, 1989).

The widespread resistance to chloroquine and other synthetic anti malarials necessitated   the   development   of   alternative   second   –line   drugs   such   as

Sulphadoxine/Pyrimethamine, Pyrimethamine/Sulphaline, Unfortunately cases of resistance to these second line drugs became widespread and persistent.

It is worthy to note that the emergence of widespread resistance of *P. falciparum* to chloroquine, the drug of choice, led to additional studies which produced new recent and effective antimalarial drugs the Artemisinin derivatives (Odugbemi, Akinsulire, Aibinu and Fabiku, 2007). These artemisinin based compounds which were developed first in China fortunately happened to arrive on the market just at a time when resistance to earlier drugs was becoming a very serious issue (Anderson, 2007). The World Health Organisation recommended that all countries experiencing resistance to monotherapies such as chloroquine, primaquine should use artemisinin-based combination therapies in order to ensure high cure rate of *P. falciparum* malaria and to reduce the spread of resistance (WHO, 2006). As a result, majority of falciparum malaria endemic countries have adopted ACTS as first line treatment and the deployment of ACTS in the public sector has increased exponentially in recent times (WHO, 2006). In order to delay eminent emergence of resistance, artemisinins are now combined with long-acting drugs, especially with amodiaquine, mefloquine, lumefantrine or sulfadoxine-pyrimethamine (Ehrhardt and Meyer, 2009). Currently four artemisinin combination therapies (ACTs) are recommended for treatment of malaria: Artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine and artesunate –sulfadoxine – pyrimethamine (WHO, 2008).

2.2 RESISTANCE TO ANTIMALARIAL DRUGS AND USE OF ARTEMISININ COMBINATION THERAPY (ACT)

Resistance is defined as 'the ability of a parasite strain to survive and/ or multiply despite the administration and absorption of a drug in doses equal to or higher than those usually recommended but within limits of tolerance of the subject' (WHO, 1973). The development and spread of *P. falciparum* resistance to most commonly used antimalarial drugs is a major challenge in the control of malaria, since it hampers our capacity to roll back

malaria (Olliaro, 2005). Mayer, Bruce, Kochurova and Stewart (2009) pointed out that parasite resistance has caused some of the least expensive traditional antimalarial drugs to be ineffective. They added that, because there is concern that resistance will emerge against the current first-line drugs such as the ACTs, there is currently great interest in discovering the next generation of antimalarial drugs.

A strategy that has received much attention recently to combat drug resistance is the use of combination of antimalarial drugs, such as mefloquine, sulfadoxine/pyrimethamine (SP), or amodiaquine, with an artemisinin derivative (Anonymous, 2006.) Currently four artemisinin combination therapies (ACTs) are recommended for treatment of malaria: Artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine and artesunate - sulfadoxine – pyrimethamine (WHO, 2009).

Bloland (2003) seems to have somehow a contrary view of malaria therapy using ACT in Africa. He said that while ACT undoubtedly holds tremendous promise as a malaria treatment, the reality of ACT today is somewhat problematic. One problem he noted was availability; that substantial quantities of quality artemisinin drugs are actually hard to come by. The second problem is cost. ACTs are far more expensive than currently used treatments and more than most African economies can sustain. Be that as it may, ACTs are being used as first- line drugs for malaria treatment in most African countries (WHO, 2009).

However, there is the fear among scientists that the most common and most debilitating malaria parasite in the tropics, *P. falciparum,* might soon develop resistance to artemissinin based combination drugs. Bacon *et al.* (2007) pointed out that although the artemisinin derivatives retain excellent efficacy, the selection of resistance to the artemisinins is a matter of time. He observed that since treatment courses are generally short, the artemisinin component is dependent upon the partner drug for adequate clinical efficacy. Furthermore, when resistance to the partner drug reaches critical level, the efficacy of ACTs would fall and would no longer be suitable for malaria treatment.

2.3 MEDICINAL PLANTS AS ALTERNATIVE SOURCES OF NEW ANTIMALARIAL COMPOUNDS

Alshawsh, Mothana, Al-shamahy, Alsllami and Lindequist (2007) pointed out that the problem created by drug-resistant strains of malaria especially *P. falciparum* in malaria therapy has accelerated antimalarial drug research over the last two decades. While synthetic therapeutic agents continue to dominate research, attention has increasingly been directed to natural products. Similarly, Ogbunugafor, Okochi, Okpuzor and Emeka (2008) observed that drug resistant strain of *P. falciparum* has compromised malaria therapy and has led to the search for new lead compounds in medicinal plants used in folk medicine for the treatment of the diseases. In search of new, safe, affordable and effective antimalarial drugs, phytochemical screening of extracts of plants used in traditional medicine becomes necessary as pointed out by Bickii, Tchouya, Tchouankeu and Tsamo (2007).

Medicinal plants have been part of African cultures for centuries, therefore the search for newer, more effective anti-malaria drugs is a major challenge (Anderson, 2007) and a very welcome development, and it is believed that traditional plants which have been used to cure malaria are bound to give us effective antimalarial compounds (Anderson, 2007). In addition, plants commonly used in traditional medicine are assumed to be safe due to their long usage in the treatment of diseases according to knowledge accumulated over centuries (Ajaiyeoba, Folade, Ogbole, Okpako and Akingoye, 2006).

2.4 *IN VITRO* AND *IN VIVO* ANTIMALARIAL ACTIVITIES OF MEDICINAL PLANTS REPUTED TO TREAT MALARIA

The literature on researches conducted in the tropics and sub-tropics on the *in vitro* and *in vivo* antimalarial activities of plants traditionally used in the treatment of malaria and related fevers is vast. Screening of medicinal plants for antimalarial activities is a strategy employed by scientists in the malarious regions of the world to validate or justify or support

the use of such plant remedies in malaria therapy. These researches are also efforts to produce the next effective, affordable and safe antimalarial drugs (Anderson, 2007).

In vitro antiplasmodial evaluation/studies involves the use of micro-test well plates for the assessment of the response of fresh isolates of *Plasmodium* species obtained from malaria patients (Flyg, Perlmann H, Perlmann P, Esposito and Berzins, 1997; WHO, 2001b; Basco and Ringwald, 2007) or from continues cultures of chloroquine sensitive or resistant strains of *P. falciparum* (O'Neil *et al*., 1986; Ngemenya *et al*., 2006; Wan Omar, 2007). On the other hand, the evaluation of *in vivo* plasmodial activity normally involves the innoculation of experimental animals (mice or rats) with *Plamodium berghei* followed by administration of drug or extract to observe therapeutic effects (Abosi and Raseroka, 2003; Okokon, Udokpoh and Essiet, 2006; Bickii, Tchouya, Tchouankeu, 2007; Ogbunufagor, Okochi, Okpuzor and Emeka, 2008).

Jenett-Siems, Mockenhaupt, Bienzle, Gupta and Eich (1999) studied the *in vitro* antiplasmodial activity of Central American medicinal plants. Their result revealed antiplasmodial activity *in vitro* of some of the remedies tested and concluded that selection of plants by ethnobotanical criteria may provide promising sources of potential antimalarial lead compounds. The antimalarial activity of *Swartzia madascariensis, Cumbretum glutunosum* and *Tinospora bakis*, Burkina Faso medicinal plants was evaluated by Ouattara *et al*. (2006). They reported that the extracts of *S. madagascariensis, C. glutunosum* and *T. Bakis* possess some measure of antimalarial activity.

The *in vitro* antiplasmodial activity of *Enicostemma littorale,* a plant traditionally used for malaria treatment in India, was evaluated by Soni and Gupta (2009). Their report demonstrated the antiplasmodial activity of *E. littorale* against *P. falciparum* and the potential antimalarial action of the plant and its active phytoconstituents. Ngemenya *et al.* (2006) similarly screened some products of *Turreanthus africanus*, a plant used in traditional medicine to treat malaria in Southwest Cameroon. Their result showed that *T. africanus* has

weak antiplasmodial activity, which probably when combined with other antiplasmodial plants results in enhanced antimalarial effect.

Kayembe, Taba, Ntumba, Tshiongo and Kazadi (2010) investigated the *in vitro* antimalarial activity of 20 quinones isolated from 4 plants used by traditional healers in the Democratic Republic of Congo. They reported that quinones isolated from *Cassia alata*, *C. occidentalis, Garcinia kola* and *Ocimum basilicum* have interesting antimalarial activities $IC_{50} < 1$ µg/ml for 12 of them. In the same vein, Bero *et al.* (2009) evaluated the *in vitro* antiplasmodial activity of crude extracts of 12 plant species traditionally used in Benin for the treatment of malaria in order to vadidate their use. Their study justified the traditional use of some of the investigated plants to treat malaria in Benin. They concluded that the dichloromethane extracts of *Acanthospermum hispidum* aerial parts, *Keetia leucantha* (leaves and twigs), *Carpolobia lutea* aerial parts and *Strychnos spinosa* leaves showed promising antiplasmodial activities. Ademowo, Nneji and Adedapo (2007) also investigated the *in vitro* antimalarial activity of methylene blue (MB) against field isolates of *P. falciparum* from children in Southwest Nigeria. Their preliminary study showed that MB has a potential to be used as potent schizonticidal antimalarial.

The *in vivo* antimalarial activity of root bark and leaves of *Vernonia amygdalina* (bitter leaf) was evaluated by Abosi and Raseroka (2003). The leaf extract produced 67% suppression of parasitaemia while the root-bark produced 53% suppression, justifying the use of the plant parts for malaria treatment in herbal medicine. Ogbunogafor, Okochi, Okpuzor and Emeka (2008) also studied the tolerance and antiplasmodial activity of *Ritchea longipedicellata* in *Plasmodium berghei*. Their result revealed a dose dependent therapeutic activity, which means a higher dose is required to clear the parasites from the blood stream once infection has been established. The result also justifies the use of *R. longipedicellata* as an antimalarial in herbal medicine in Nigeria. Okokon, Udokpoh and Essiet (2006) studied the *in vivo* antimalatial activity of stem bark of *Mammea Africana*, a plant used traditionally

by the Ibibios of the Niger Delta region of Nigeria for the treatment of malaria and related fever. They reported that *M. Africana* possesses antimalarial activity and therefore justifies its folkloric use as an antimalarial. Innocent *et al*. (2009) screened traditionally used plants in Tanzania for *in vivo* antimalarial activity in mice to establish validity of their claims. Their work revealed that the extracts of six plants used in traditional medicine exhibited *in vivo* antimalarial activity, but three had very weak activity. They concluded that *Caesalpinia bonducella* root and *Cassia abbreviate* leaf ethanol extracts were the most promising.

2.5 ACUTE TOXICITY TESTING

The first step in toxicological investigation of an unknown substance is the acute toxicity test (Lorke, 1963). The index of acute toxicity is the LD_{50} – the lethal dose that can kill 50% of experimental animals. Acute toxicity studies are usually necessary for any pharmaceutical intended for human use. The information obtained from these studies is useful in choosing doses for repeat-doses studies (CDER, 1996).

On acute toxicity of *A. occidentale*, Tedong *et al*. (2007) carried out the acute toxicity and subchronic toxicity of *A. occidentale* leaves. After oral administration of extract, they found out that doses of extract less than 6g/kg body weight are not toxic. In addition, The LD_{50} of the extract determined in mice of both sexes after oral administration was 6g/kg, and therefore concluded that toxic effects of *A. occidentale* hexane extract occurred at higher doses than those employed in the models of antidiabetic activities. Similarly, Ofusori *et al*. (2008) studied the effect of ethanolic extract of cashew stem bark *A.occidentale* on the brain and kidney of Swiss albino mice. They reported the non-toxic effect of ethanol extract of the plant on the brain and kidney parenchyma of mice.

The acute toxicity of *C. citratus* has also been investigated as indicated in the literature. Idowu, Soniran, Ajana and Aworinde (2009) undertook ethnobotanical survey of antimalatial plants in Ogun State, Southwest Nigeria, the survey revealed that *C. citratus* is

non-toxic. Similarly, Souza Formigoni, Lodder, Gianotti Filho, Ferreira and Carlini (1986) studied the pharmacology of lemon grass, in which they prepared an infusion from leaves of the plant and administered orally to rats for two months. The infusion did not induce any toxic effect, suggesting that lemon grass used in Brazilian folk medicine has no toxic properties.

2.6 ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES OF MEDICINAL PLANTS

Inflammation is a localized protective reaction of cells or tissues of the body to allergic or chemical irritation, injury and or infection, the symptoms of which are characterized by pain, heat, swellings and loss of function (Iwalewa, McGraw, Naidoo and Eloff, 2007). It is the protective attempt by the organism to remove injurious stimuli as well as initiate healing process for the tissue (Divya *et al*., 2009).

Inflammatory disorders and related diseases have been treated with plants materials or plant-derived formulations in various cultures all over the world from time immemorial (Rathore, Mahdi, Paul, Saxena and Das, 2007; Krishnaswamy, 2008; Mueller, 2010). Similarly, due to adverse side effects experienced in the use of conventional analgesics such as non-steroid anti-inflammatory drugs (NSAIDs) and opionates, pain relievers with little or no side effects are being searched for all over the world as alternatives to Non-Storoidal Anti-inflammatory Drugs (NSAIDs) (Zulfiker *et al*. (2010). The search into plants with reputed folkloric used as pain-relievers should be seen as fruitful and logical reseach strategy in the search for new analgesic drugs (Elisabetsky, Amador, Albuquerque, Nunes and Cavalho, 1995).

From the literature, various plant compounds have been shown to exhibit anti-inflammatory and analgesic properties. Mueller, Hobiger and Jungbauer (2010) carried out the antimalarial activity of extracts from fruits, herbs and spices. Their research revealed the

anti-inflammatory activity of various fruits, herbs and spices in a lipopolysaccharide-stimulated model, with chili pepper demonstrating the highest anti-inflammatory potential. Analgesic and anti-inflammatory properties of extracts from Bulbils of *Dioscorea bulbifera* in mice and rats was conducted by Mbiantcha *et al*. (2011). Their work revealed that the oral administration of aqueous and methanolic extracts caused significant anti-inflammatory activity on paw oedema induced by hisidine, serotomin and formalin. Kamal *et al*. (2009) demonstrated that *A. occidentale* aqueous extracts inhibits inflammatory mediators. Their finding indicated that *A. occidentale* extract is an effective protector against monocyte recruitment in inflammation vessel. In a study of analgesic and anti-inflammatory activities of *Asparagus africanus* root extract, Hassan H.S., Ahmadu and Hassan A.S. (2008) reported that the plant extract has potential dose-dependent analgesic and anti- inflammatory activities. They comfirmed the use of the plant in traditional medicine as a pain reliever and in the treatment of inflammatory diseases such as rheumatism and chronic gout.

Sutharson, Lila, Prasanna, Shila and Rajan (2007) investigated the anti-inflammatory and anti-nociceptive (analgesic) activities of methanolic extract of the leaves of *Fraxinus floribunda*, and reported significant anti-inflammatory effects in all the models used at the doses of 200mg/kg and400mg/kg. They also showed that anti-nociceptive evaluation at the dose of 400mg/kg had a significant activity against control, relieving effect being through the peripheral and central mechanism of action of the extract. The study of analgesic and anti-inflammatory effects of the ethylacetate extract of the leaves of *Pseudocedrella kotchyii* (Musa *et al*., 2008) revealed that the extract (50 and100mg/kg i.p.) exhibited significant activity ($p < 0.5$) against acetic acid-induced writhings in a dose dependent manner. They also reported that a slight effect against the raw egg albumin-induced oedema in the anti-inflammatory activity.

Similarly, the investigation of anti-inflammatory, analgesic and anti-pyretic properties of *Madhaca indica* (Shekhawat and Vijayvergia, 2010) revealed that the

methanolic extract at 50, 100 and 200mg/kg body weight showed a significant anti-inflammatory activity. They further revealed the extract reduced acetic acid-induced pain. Furthermore, Zulfiker *et al*. (2010) evaluated the *in vivo* analgesic activity of ethanolic extracts of two medicinal- *Scoparia dulcis* and *Ficus racemosa*, and reported significant analgesic activity of both plant extracts at oral doses of 100 and 200mg/body weight in the tested models, acting both centrally and peripherally.

CHAPTER THREE
MATERIALS AND METHOD

3.1   EXPERIMENTAL PLANTS

3.1.1 *Anacardium occidentale*

A. *occidentale*, popularly known as cashew is a plant belonging to the family Anacardiaceae (Plate 1). It is found throughout the tropics in West Africa. Leaf and bark infusion relieve tooth ache and sore gum (NNMDA, 2006). It is used as food and raw materials for confectionary and chocolatery. The bark and leaves are traditionally used for malaria cure (Odugbemi, Akinsulire, Aibinu and Fabiku, 2007; Akueshi, 1999). Aqueous extract of the plant showed significantly reduced oxidative-derived cell injury and cell signalling molecules expression which involved in the initial events of atherogenesis *in vitro* (Kamal *et al*., 2009). A hexane extract leaf of *A. occidentale* is non-toxic via the oral route in mice, at least up to a maximum dose of 14 g/kg (Tedong *et al.,* 2007).

3.1.2 *Cymbopogon citratus*

C. citratus, commonly called lemon grass, is a plant belonging to the family Gramineae (Plate 2). It is found in the Sudan savanna and rain forest zones of Nigeria. Lemon grass is used in traditional medicine for the treatment of malaria, jaundice, hypertension, as an analgesic and as an antiseptic of the urinary apparatus (Onabanjo, Agbaje and Odusote, 1993; Akueshi, 1999 ; Odugbemi, Akinsulire, aibinu and Fabiku, 2007; Idowu, Soniran, Ajana and Aworinde, 2009). The plant is widely used as herbal tea prepared from fresh or dried leaves and produces a pleasant aroma (Leite *et al.*, 1986). It is also reported to exhibit anti-oxidant activity by scavenging of peroxide anion and inhibition of the enzyme xanthine oxidase and lipid peroxidation in human erythrocytes (Cheel, Theoduloz, Rodriguez and Schmeda-Hirschmann, 2005). The essential oils of the plant are reported to have bactericidal effect against *Helicobacter pylori* without the development of acquired

resistance (Ohno *et al.*, 2003). Lemon grass oil is an essential oil used in deodorants, skin care products, fragrances, insect repellents, and for aromatherapy.

3.2 COLLECTION OF EXPERIMENTAL PLANTS PARTS

Fresh bark of *Anacardium occidentale* and leaves of *Cymbopogon citratus* were collected in Jos. Plants were identified by a taxonomist at the Federal College of Forestry, Jos and Herbarium sample specimens with voucher numbers FCFBM 020 and FCFBM 005 respectively, were deposited at the Botanical Museum of the College.

3.3   PREPARATION OF EXTRACTS

3.3.1 Extraction of Plant Material

Fresh stem bark of *A. occidentale* and leaves of *C. citratus* were   separately cut into pieces and air dried in the laboratory at room temperature. The stem bark of *A. occidentale* took two weeks to dry while the leaves of *C. citratus* took five days to dry. The dried pieces were then made to powder using laboratory grinder. Eighty grammes (80g) of the dried powdered form of each plant material were exhaustively extracted with water in a soxhlet apparatus for 72 hours. Another 80g powder of each plant was extracted with ethanol in soxhlet extractor for 72 hours. All the extracts were concentrated to dryness on a water bath and weighed. The extracts were then stored in airtight containers and kept in a refrigerator at 4ºC to protect from light and moisture till used (Sutharson *et al.,* 2007). Extracts were stored in the refrigerator at the Obstetrics and Gynaecology (O and G) Research Laboratory, Jos University Teaching Hospital.

3.4   PHYTOCHEMICAL SCREENING OF CRUDE EXTRACTS

The preliminary phytochemical analyses of the plant extracts was carried out using the thin - layer chromatography (TLC).   The standard screening test using standard

procedure was utilized for detecting the active principles/constituents (Harborne, 1984). This was done at the Pharmacognosy Department, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.

3.4.1 Test for Alkaloids

About 0.5g of each extract was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath; 1ml each of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated similarly with Draggendorff's reagent. Turbidity or precipitation with either of these reagents was observed as preliminary evidence for the presence of alkaloids in the extracts (Harborne, 1984; Evans, 1989).

3.4.2 Test for Saponins

About 0.5g of each plant extract was shaken with water in a test tube.Frothing which persists on warming was accepted as a preliminary evidence for the presence of saponins (Evans, 1989).

3.4.3 Test for Tannins

About 0.5g of each plant extract was stirred with 1ml of distilled water, filtered, and ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate was evidence of the presence of tannins (Evans, 1989).

3.4.4 Test for Anthraquinones

About 0.5g of each of the extracts was taken in a dry test tube and 5ml of chloroform was added and shaken for 5 minutes. The extract was filtered, and the filtrate shaken with an equal volume of 100% ammonia solution. A pink violet or red colour in the ammoniacal layer (lower layer) indicated the presence of free anthraquinones (Evans, 1989).

3.4.5 Test for Glycosides

One hundred grammes of each extract was taken in a test tube and 2.5ml of dilute sulphuric acid was added and boiled in a bath of water for 15 minutes. This was cooled and

neutralized with 20% potassium hydroxide solution. Five ml of a mixture of Fehlings solution A and B added and boiled for 3 minutes. A brick red precipitate indicated the hydrolysis of a reducing sugar, an indication of glycoside (Harborne, 1984).

3.4.6 Test for Cardiac Glycocides (Keller Kiliani test)

About 0.5g of extract diluted to 5ml in water and 2 ml of glacial acid was added containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic layer a greenish ring may form just above the brown ring and gradually spread throughout this layer (Harborne, 1984; Evans, 1989).

3.4.7 Lieberman's Test Steroids and Terpenes

A little quantity of each extract was dissolved in chloroform and 1ml of acetic anhydride was added, then 2 drops of conc. sulphuric acid was added. A pink colour which changes to bluish green on standing is indicative of the presence of steroid and terpenes (Evans, 1989).

3.4.8 Test for Flavonoids

Two grammes of the extract was completely detanned with acetone. The residue was extracted in warm water after evaporating on water bath. The mixture was filtered while hot. The filtrate was cooled and used for the following tests (Evans, 1989).

3.4.8.1 Lead acetate test for flavonoids

Five ml of detanned water extracted was added to lead acetate solution. A yellow coloured precipitate indicates the presence of flavonoids (Evans, 1989).

3.4.8.2 Sodium hydroxide test for flavonoids

Five ml of 20% sodium hydroxide was added to equal volume of the detanned water extract. A yellow solution indicates the presence of flavonoids (Evans, 1989).

3.4.9 Test for Carbohydrates

One hundred mg of each extract was dissolved in 3ml of distilled water and mixed with few drops of 10% Molisch reagent solution. Then 1ml of concentrated sulphuric acid was carefully added down the side of the inclined tube so that the acid formed a layer beneath the aqueous solution without mixing it. A redish or violet ring at the junction of the liquids was observed indicating the presence of carbohydrate (Harborne, 1984; Evans, 1989).

3.5 BLOOD SAMPLES COLLECTION AND *IN VITRO* STUDY

3.5.1 Inclusion Criteria

The following inclusion criteria were set for this research:

  i.   Children and adults age 6 months and above respectively,

  ii. Axillary temperature $\geq 37.5^{o}C$ and or history of fever in the preceding 48 hours,

  iii. Patients who have not taken any anti − malarial in the last 2 weeks,

  iv. Informed or written consent of patient/parent/guardian sought and accepted.

3.5.2. Exclusion Cretaria

  i. Children below the age of six months,

  ii. Axillary temperature $<37.5^{o}C$

  iii. Patients who have taken antimalarial drugs in the last 2 weeks

  iv. Lack of informed consent

3.5.3 Screening for *Plasmodium falciparun*

Patients who satisfied the inclusion criteria were screened for *P. falciparun* infection. Thick and thin smears of blood samples obtained from finger prick using sterile disposable lancets were prepared on clean slides. Prepared slides were stained for 10

minutes in 10% Giemsa solution prepared in phosphate buffer of pH 7.3 solution and examined microscopically for parasites (Molta *et al.,* 1992; Molta, 1993).

Patients who had single infection of *P*. *falciparum* with parasitaemia of 1000/µl and not more than 100,000/µl of blood were included into the *in vitro* test.

3.5.4   Collection of *Plasmodium falciparum* Blood samples

Fourteen fresh blood samples of *P. falciparum* were obtained from symptomatic patients attending the out-patient clinic of the General Hospital, Damboa, Borno State. They were children and adults between the ages of 5 and 25years.

Three to five millilitres of blood was collected into ethylenedi- aminetetra acatate (EDTA) bottles from patients with a confirmed diagnosis of *P*. *falciparum* malaria. The fresh blood samples were centrifuged at 2000rpm for 10 minutes and the plasma was removed. Blood pellets were washed twice in Rapid Prototyping and Manufacturing Institute (RPMI) medium (GIBCO USA) before use for parasite cultivation (Flyg *et al.*, 1997). Patients were then treated with Artemether - Lumefantrine (COARTEM) or Amodiaquine - Artesunate combination drugs.

3.5.5   Preparation of Culture Medium

Culture medium was prepared by dissolving 10.43g RPMI 1640 powder (Invitrogen), 6g of N-2 hydroxyethyl piperazine-N-2- ethane sulphonic acid (HEPES), 2g of NaHCO3 and 0.5ml gentamacin (from 50mg/ml stock) in 1 litre of distilled-deionised water. The medium was filtered and stored at -20ºC in aliquots of 45ml. Before cultivation, every aliquot was supplemented with 5ml of 5% Albumax II (Cranmer, Magowan, Liang, Coppel and Cooke, 1997).

3.5.6 Preparation of Extracts Solution

Aqueous extracts of *A. occidentale* and *C. citratus* were first dissolved in distilled water while ethanol extracts were dissolved in methanol, sonicated for 10 minutes and diluted in distilled-deionised water, and 2mg/ml solution of each was prepared. The 2mg/ml solution was further diluted in 9ml of the malaria culture medium to give 200μg/ml stock solution (Clarkson *et al*., 2004). Both extracts were tested in 6 serial two-fold dilutions with a final concentration range of 1.56-100μg/ml in 96 wells microtitre plates according to manufacturer's instructions (Becton Dickinson Labwares, USA).

3.5.7 *In Vitro* Assay

The assay was performed in duplicate. Using a micropipette, 100 μl of distilled water was first distributed into well plates, after which 100μl of culture medium containin extracts of *A. occidentale* and *C. citratus* at various concentrations were added into well plates. One hundred microlitres of parasite culture (isolates) were finally added into each microtitre well plate. The plates were incubated in CO_2 condition at 37ºC in candle jar for 24-30 hours. After incubation, the red blood cells were harvested and transferred to a clean microscopic slide to form a series of thick blood films. The films were stained for 10 minutes in 10% Giemsa stain (pH 7.3). Parasite growth was counted in 10 microscopic fields and the mean calculated. The control was considered as 100% growth.

The percentage inhibition with concentration was calculated using the formula: (WHO, 2001b; Ngemenya *et al*., 2006).

[(% parasitaemia in control wells – %parasitaemia of test wells)/(%

parasitaemia of the control)] × 100

The IC_{50} values, concentration required to inhibit parasite growth by 50% were determined by linear interpolation from the parasite growth inhibition curves (concentration

versus percent inhibition) generated from each parasite-extract interaction (Mustofa, Sholikhah and Wahyuono, 2007).

3.5.8 Thresholds for *In Vitro* Antimalarial Activity

The thresholds for the *in vitro* antimalarial activity of the plant extracts were obtain according to Gessler, Nkunya, Nwasumbi, Heinrich and Tonner (1994). It is classified as follows: extract with IC_{50} less than 10µg/ml is considered very good, from 10 to 50µg/ml is moderate and over 50µg/ml is considered to have low activity.

3.6 COLLECTION OF EXPERIMENTAL ANIMALS

Animals used in this study were adult male and female mice (*Apodemus sylvaticus*) and albino rats (*Ratus norvegicus*) obtained from the animal house of the University of Jos. These animals were acclimatized for the period of 7 days to room temperature and humility before they are used. They were housed in standard cages and maintained on standard animal pellets and water *ad libitum*. All experiments involving animals were conducted in the animal house of the University of Jos.

3.7 ACUTE TOXICITY TEST

The acute toxicity of plant extracts were tested on albino rats (*R. norvegicus)* as described by Tanira, Shah, mohsin, Ageel and Qureshi (1996). Sixty five rats were divided into 13 groups of 5 rats each. The first group served as control and the remaining 12 groups were further subdivided into 4 major groups of 3 sets of rats each. Each set of rats in each major group were orally administered 500mg/kg or 1000mg/kg or 3000mg/kg of extracts. Control rats were kept under the same conditions without any treatments. The animals were routinely inspected for appearances or signs of toxicity

such as tremors, weakness and refusal of feeds, fallng off of hair, coma or even death after 48 hours.

3.8   ANTI-INFLAMMATORY TEST

3.8.1 Topical Oedema : Xylene Induced Oedema

The effect of aqueous and ethanolic extracts of *A. occidentale* and *C.   citratus* on topical acute oedema was assessed using xylene- induced ear oedema in mice as described by Okoli, Akah and Ezugworie (2005). Swiss albino mice (*A. sylvaticus*) (20-25g) received topical application (5g/ear) of aqueous extract of *A. occidentale*, ethanol extract of *A. occidentale*, aqueous extract of *C. citratus* or ethanol extract of *C. citratus* on the anterior surface of the right ear while xylene (0.08ml) was instantly applied on the posterior surface of the same ear. The control animals received 0.2ml of distilled water on the anterior surface and 0.08ml xylene on the posterior surface. The left ears were left untreated.

After 3 hours of xylene application, oedema produced was measured with micrometer screw guage (Moore and Wright, England) (Inoue, Mori, Shibata and Koshihara, 1989). The difference in thickness of ear from right treated and left untreated ears was calculated and used as a measure of oedema (Okoli, Akah and Ezugworie, 2005). The level of inhibition (%) of oedema was calculated using the relationship:

Inhibition (%) = 100[1 − (Et/Ec)],

Where Et =average oedema of treated ear,

Ec =average oedema of treated control.

3.9   TEST FOR ANALGESIC PROPERTIES OF PLANTS' EXTRACTS.

3.9.1 Acetic Acid-induced Writhing Test.

The analgesic properties of the plants extracts was evaluated using acetic acid induced writhings in mice (Winter, Risley and Nuss, 1963; Ahmed, Selim, Das and Choudhuri, 2004). In this method, acetic acid was injected intraperitoneally to the experimental animals and the response was the contraction of the abdominal muscles and the stretching of the hind limps. Fifty winstar mice were randomly divided into 10 groups of five each. The control, (group I) received distilled water (10ml/kg body weight, group II received the standard drug- Piroxicam (20mg/kg body weight) and the test groups (groups III, IV, V, VI, VII, VIII, IX and X) were treated with 50mg/kg or 100mg/kg body weight of water or ethanol extracts. After 30 minutes of drug administration, 0.7% acetic acid solution was given to each mouse at the dose of 0.1ml/kg body weight. The number of writhing was counted for 15 minutes, starting 5 minutes after acetic acid injection. A significant reduction in the number of writhing in the groups treated with extracts compare to control was considered to be a positive analgesic response (Sutharson *et al.*, 2007; Musa *et al.*, 2008). The percentage of inhibition of writhing offered by the extracts to the animals was calculated and compared with the control. It was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\overline{Nc} - \overline{Nt}}{\overline{Nc}} \times 100$$

Where $\overline{Nc}$= mean number of writhing in control group; $\overline{Nt}$= mean

number of writhing in treated group.

3.10 STATISTICAL ANALYSIS

The SSPS was used to calculate mean parasite growth and percentage parasite inhibition. The student t-test was used to statistically analyze the data and values of P≤ 0.05 were considered significant.

Plate 1: *Anacardium occidentale* (Cashew)

Plate 2: *Cymbopogon citratus* (Lemon Grass)

CHAPTER FOUR
RESULTS

4.1 PHYTOCHEMICAL PROPERTIES OF PLANTS EXTRACTS

The phytochemical properties of plant extracts revealed the presence of saponins, tannins, flavonoids, carbohydrates, cardiac glycosides, alkaloids, steroids and anthaquinones (Table 1). *Anacardium occidentale* showed the presence of five constituents and *C. citratus* the presence of seven with tannins, flavonoids, carbohydrates and cardiac glycosides being common in both experimental plants.

4.1.1 Yield of Extracts

Table 2 shows crude extract and solvent extraction of experimental plants. Water solvent yielded more of extracts than ethanol in both plants. Similarly, *A. occidentale* yield was more than *C. citratus* yield in both solvents.

4.2 *IN VITRO* ASSAY

The crude extracts were tested mainly on the trophozoites of *Plasmodium falciparum*. Table 3 shows mean parasite growth at various concentrations. There was seven concentrations of the extracts, there was a significant ($p = 0.05$) reduction in the number of parasitised cells compared to control. The basic measurement of antimalarial activity used in this study was the reduction in number of parasitised cells in the test cultures after 24 hours incubation.

The mean percentage inhibition of erythrocytes invasion *by P. falciparum* isolates for the four extracts ranged between 9.40-15.19% at the lowest concentration of 1.56µg/ml and 67.91-75.64% at the highest concentration of 100.00µg/ml (Table 4).

Among the four extracts tested in this study, the ethanol extract of *A. occidentale* gave the highest antimalarial activity of 75.64%, with IC_{50} of 11.70µg/ml (Table 5). This is followed by aqueous extract of *A. occidentale* with parasite growth inhibition of

72.38% and IC_{50} of 16.00µg/ml. Aqueous extract of *C. citratus* had parasite growth inhibition of 69.42%, IC_{50} of 23µg/ml. The ethanol extract of *C. citratus* has the lowest parasite growth inhibition of 67.91% with IC_{50} of 30.00µg/ml (Table 5). However, the difference in antimalarial activities of extracts of *A. occidentale* and *C. citratus* is insignificant ($p > 0.05$).

Table 1: Phytochemical Properties of *Anacardium occidentale* and *Cymbopogon citratus*

Constituents	Extracts			
	Anacardium occidentale		*Cymbopogon citratus*	
	Aqueous	ethanol	Aqueous	ethanol
Alkaloids	_	_	+	+++
Saponins	++	+	_	_
Tannins	++	++	++	++
Flavonoids	+	++	++	++
Carbohydrates	+	+	+	+
Steroids	_	_	_	+++
Anthraquinones	_	_	_	+
Cardiac glycosides	_	+++	++	++

Legend;

+++ = very much

++ = much

+ = little

− = nil

Table 2: Yield of Extracts of *Anacardium occidentale* and *Cymbopogon citratus*

Plant	Solvent	Weight of plant (g)	wt of extract (g)	Yield (%)
Anacardium occidentale	Aqueous	80	25.85	32.32
	Ethanol	80	11.79	14.74
Cymbopogon citratus	Aqueous	80	18.85	23.56
	Ethanol	80	7.68	9.60

Table 3: Mean number of Parasitised Red Blood Cells at Various Concentrations.

Extracts	Concentration of extracts (µg/ml)						
	1.56	3.13	6.25	12.5	25	50	100
	% Mean parasite growth ± SE						
AO Aqueous	88.4±0.3	76.0±0.4	64.7±0.5	52.5±0.5	42.3±0.6	35.0±0.7	27.6±0.7
AO Ethanol	84.8±0.5	71.7±0.6	60.1±0.8	48.5±0.8	38.7±0.8	31.0±0.8	24.4±0.7
CC Aqueous	90.6±0.1	79.1±0.6	69.0±0.7	58.5±0.6	48.9±0.7	39.9±0.7	30.6±0.6
CC Ethanol	88.0±0.5	80.7±0.5	71.6±0.6	61.6±0.7	52.4±0.8	43.9±0.9	32.1±0.7

Legend: AO = *Anacardium occidentale*; CC = *Cymbopogon citrates;* $P = 0.05$ compared to control.

SE = Standard Error

Table 4*:* Inhibition (%) of *Plasmodium falciparum* Isolates (trophozoite stages) by Plant Extracts.

	Concentration of extracts (µg/ml)						
Extract	1.56	3.13	6.25	12.5	25	50	100
AO Aqueous	11.6	24.03	35.26	47.5	57.72	65	72.38
AO Ethanol	15.19	28.29	39.94	51.49	61.35	69.03	75.64
CC Aqueous	9.4	24.03	31.01	41.47	51.1	60.15	69.42
CC Ethanol	11.98	19.28	28.44	38.41	47.58	56.07	67.91

Legend: AO = *Anacardium occidentale,*

CC = *Cymbopogon citratus*

Table 5: Antimalarial Activity and Inhibition Concentration (IC_{50}) of Aqueous and Ethanolic Extracts of *Anacardium occidentale* and *Cymbopogon citratus*

Extract	Antimalarial Activity (%)	IC_{50} (µg/ml)
AO Aqueous	72.38	16.0
AO Ethanol	75.64	11.7
CC Aqueous	69.42	23.0
CC Ethanol	67.91	30.0

Legend: AO = *Anacardium occidentale*; CC = *Cymbopogon citratus*

Figure 1 is the growth inhibition curve of *P. falciparum* by aqueous extract of *A. occidentale* generated from parasite-extract interaction, with IC_{50} value of 16.0μg. Figure 2 also shows the inhibition of *P. falciparum* by ethanolic extract of *A. occidentale* which produced the IC_{50} of 11.70μg/ml.

Figure 3 is the inhibition curve of *P. falciparum* by aqueous extract of *C. citratus* also generated from parasite-extract interaction, which had the IC_{50} of 23.0μg/ml. Figure 4 is similarly the inhibition of *P. falciparum* by the ethanolic extract of *C. citratus*, giving the IC50 of 30.0μg/ml. Inhibition concentration (IC_{50}) values were determined by linear interpolation from the growth inhibition curves.

Figure 5 shows comparative parasite inhibition by aqueous extracts of *A. occidentale* and *C. citratus* and Figure 6 is the comparative parasite inhibition by ethanolic extracts of *A. occidentale* and *C. citratus*. In both cases, ethanolic and aqueous extracts of *A. occidentale* showed higher inhibition than extracts of *C. citratus*. With regard to concentrations administered, dose-dependent antimalaria activity was clearly shown for all the crude extracts. The percentage inhibitions are higher with increasing concentrations. Figure 7 is percentage inhibition of aqueous and ethanolic extracts of *A. occidentale* and *C. citratus* showing dose-dependent antimalarial activity in all the extracts.

Figure 8 shows the percentage inhibition of parasite growth by aqueous and ethanolic extracts of *A. occidentale* and *C. citratus* at various concentrations (1.56μg/ml-100μg/ml). At 1.56μg/ml, the ethanolic extract of *A. occidentale* showed the highest inhibition of parasite growth. This was followed by the ethanolic extract of *C. citratus*, the

lowest being the aqueous extract of *C. citratus*. At 3.13µug/ml, ethanolic extract of *A. occidentale* exhibited the highest inhibition (28.29%) of parasites growth. The aqueous and ethanolic extracts showed equal strength (24.03%) in parasite growth inhibition.

At 6.25µg/ml and 12.5µg/ml, ethanolic extract of *A. occidentale* again showed the highest inhibition followed by aqueous extracts of *A. occidentale*. In the same vain, highest inhibition of growth at 25.0µg/ml, 50.0µg/ml and 100.0µg/ml was displayed by ethanolic extract of *A. occidentale,* and the least growth inhibition was displayed by ethanolic extract of *C. citratus.*

In all concentrations, the ethanolic extracts of *A. occidentale* took the lead in inhibition of parasite grow.
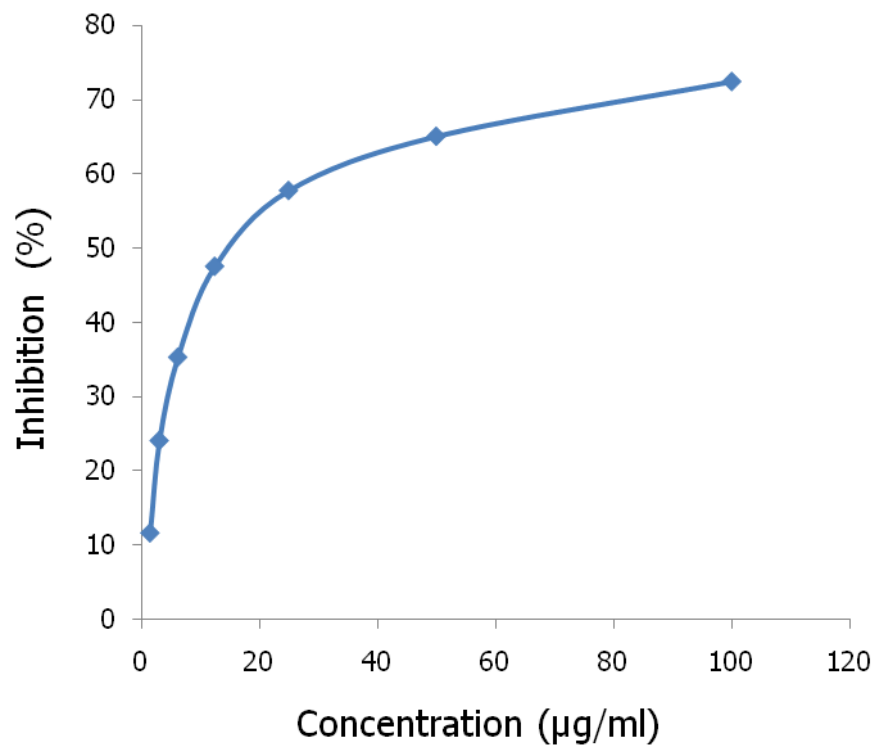
**Figure 1: Inhibition of *Plasmodium falciparum* Isolates by Aqueous
Extract of *Anacardium occidentale***
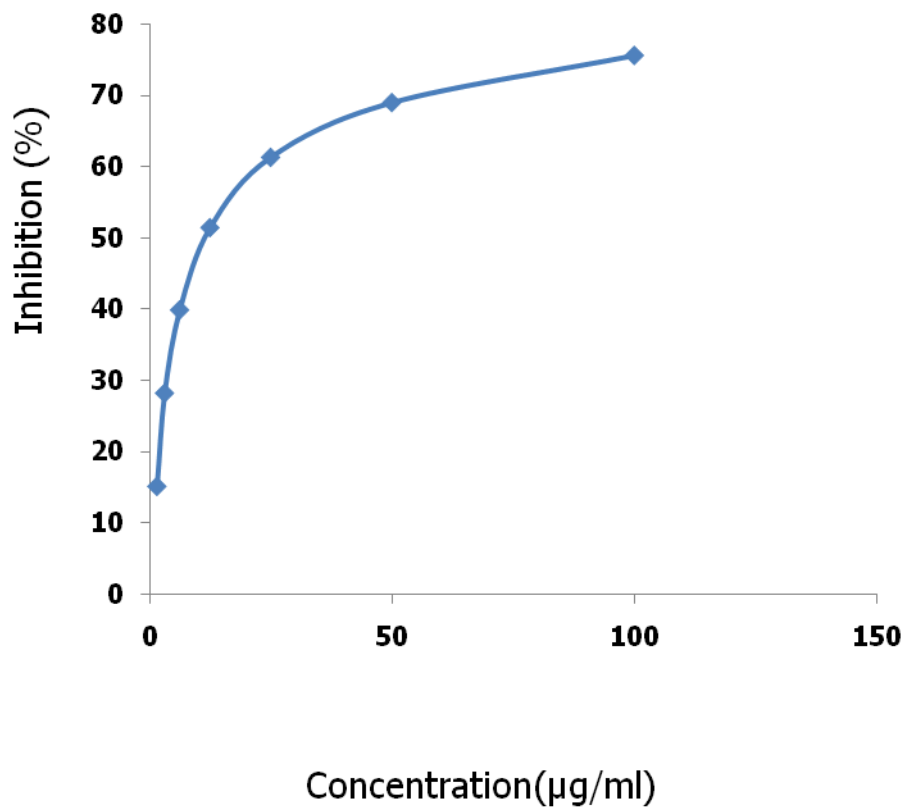
Figure 2: Inhibition of *Plasmodium falciparum* Isolates by Ethanol Extract of *Anacardium occidentale*
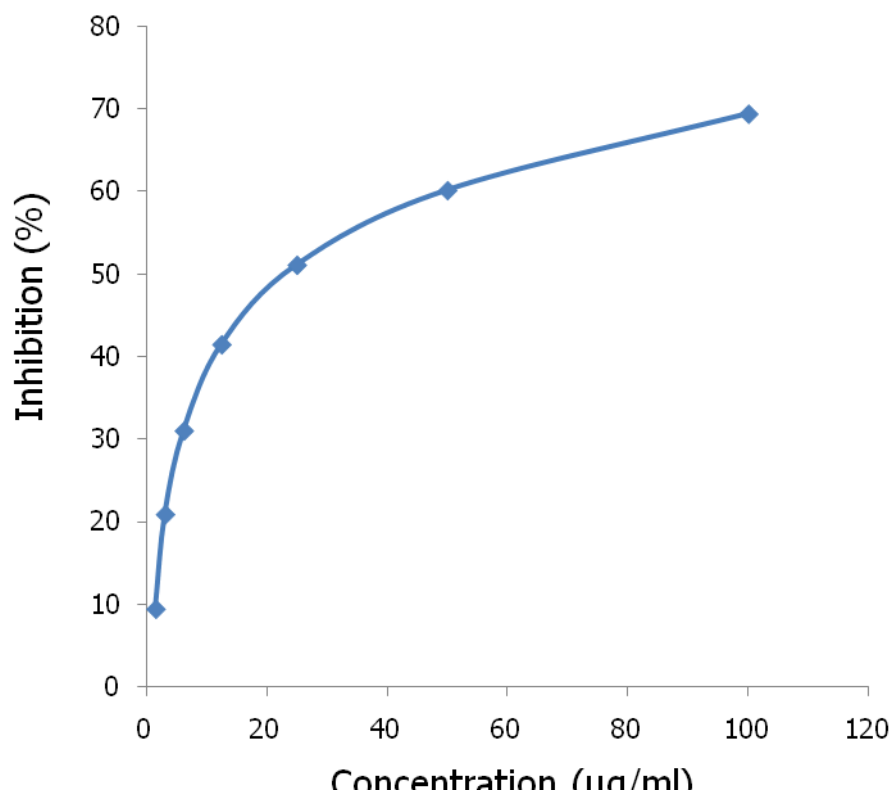
Figure 3: Inhibition of *Plasmodium falciparum* Isolates by Aqueous Extract of *Cymbopogon citratus*
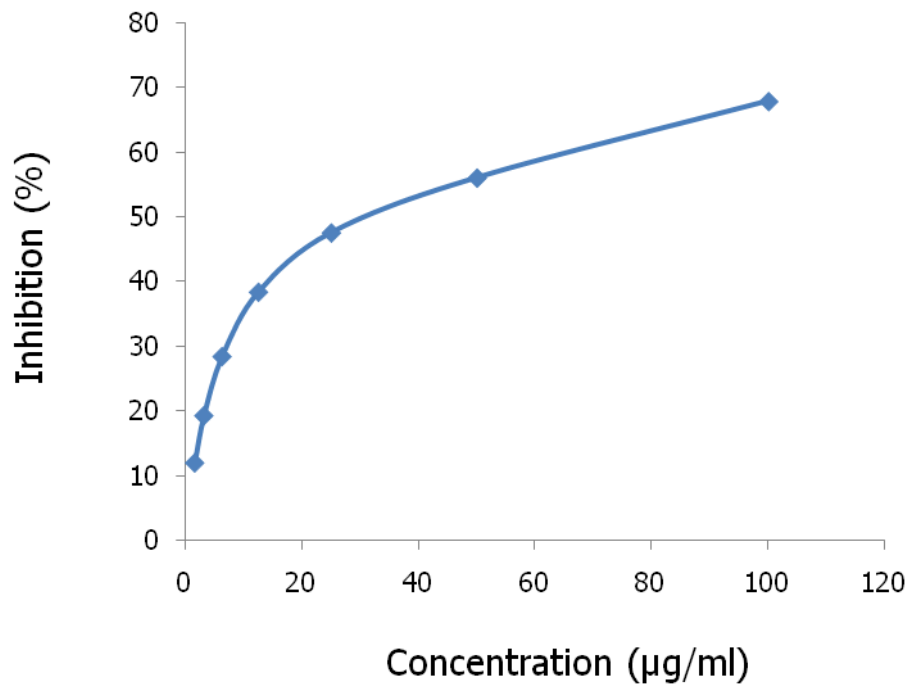
Figure 4: Inhibition of *Plasmodium falciparum* Isolates by Ethanol Extract of *Cymbopogon citratus*
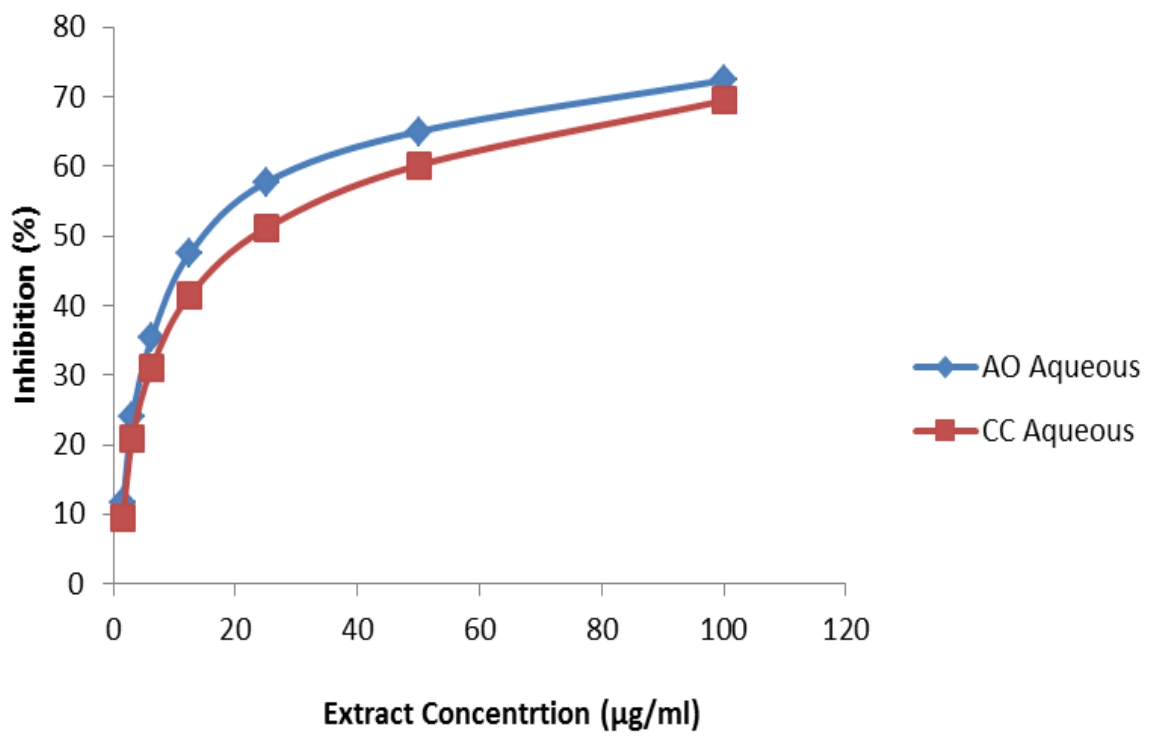
**Figure 5: Comparative Parasite Inhibition by Aqueous Extracts
of *Anacardium occidentale* and *Cymbopogon citratus***
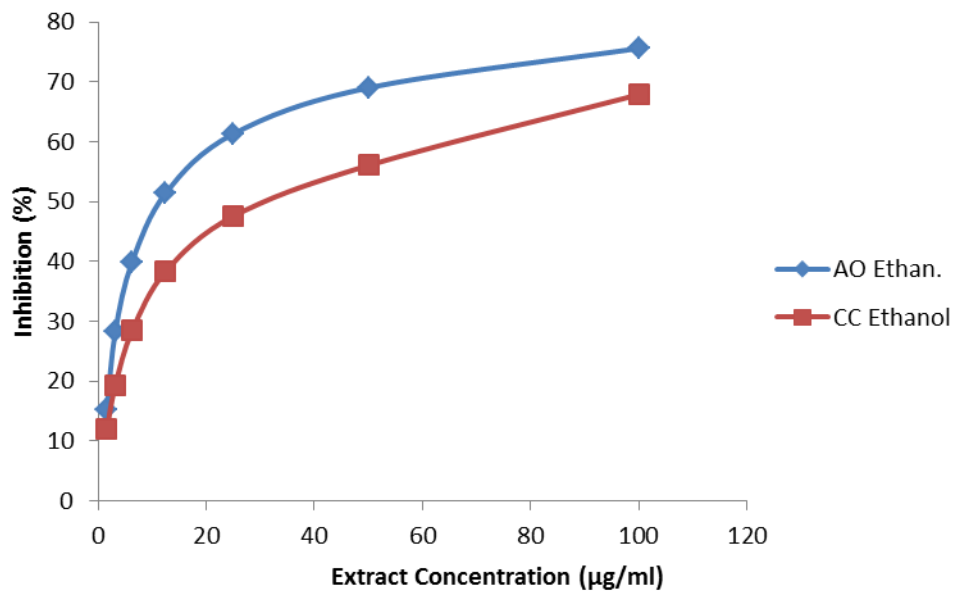
Figure 6: Comparative Parasite Inhibition by Ethanol Extracts
of *Anacardium occidentale* and *Cymbopogon citratus*
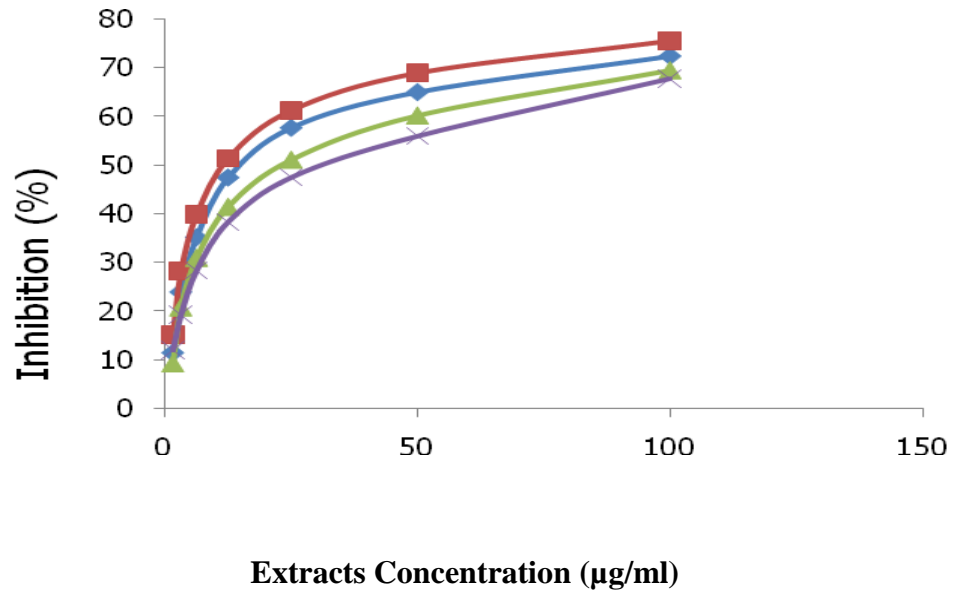
Legend:

■ Ethanol extract of *A. occidentale*

▰ Aqueous Extract of *A. occidentale*

▲ Aqueous extract of *C. citratus*

X Ethanol Extract of *C.citratus*

Figure 7: The Percentage Inhibition of the Four Extracts Showing Dose-dependent Antimalarial Activity
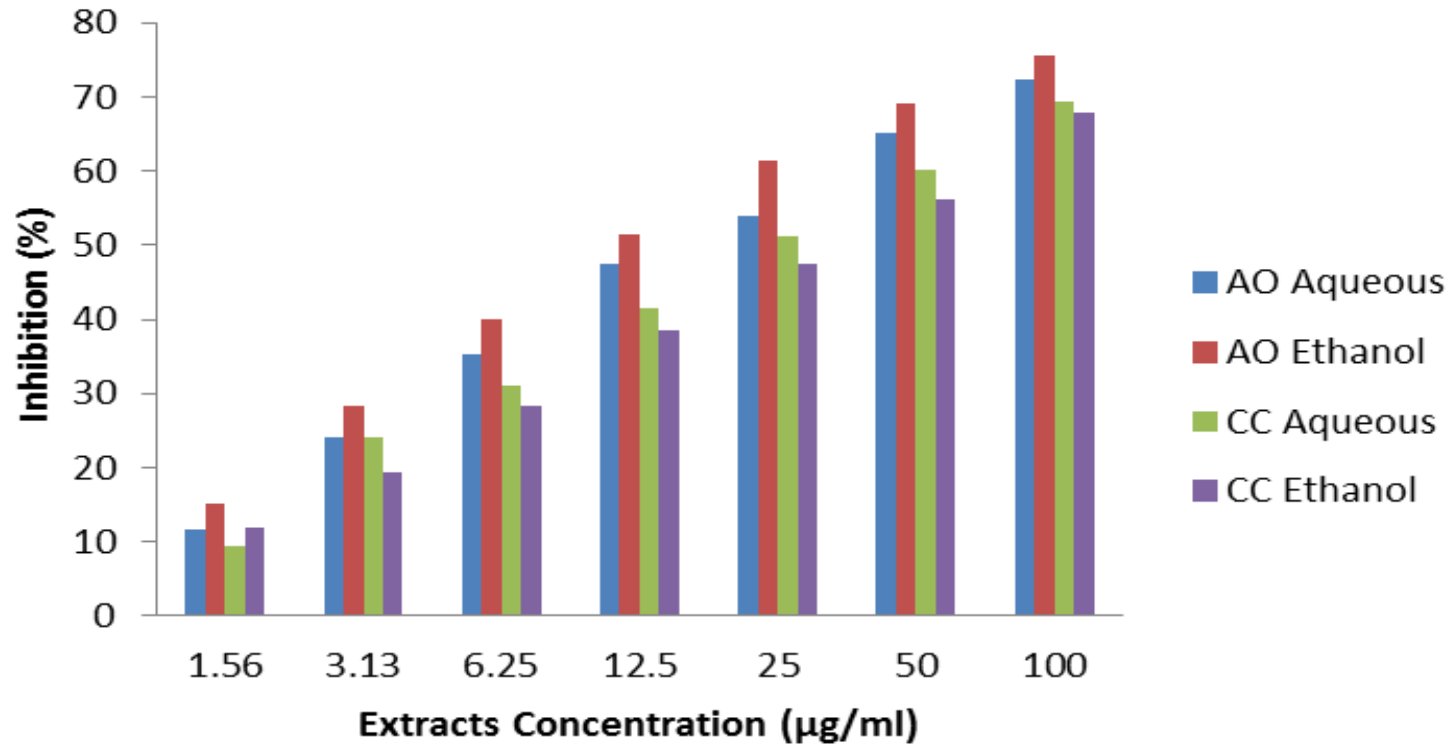
Figure 8: Percentage Inhibition of Parasite Growth by Aqueous and Ethanolic Extracts of *Anacardium occidentale* and *Cymbopogon citratus* at Various Concentrations (1.56µg/ml- 100µg/ml)

Legend: AO = *Anacardium occidentale*   CC = *Cymbopogon citratus*

4.3   ACUTE TOXICITY

The oral administration of aqueous and ethanolic extracts of *A. occidentale* and *C. citratus* in the doses of 500mg/kg, 1000mg/kg and 3000mg/kg body weight did not cause any major sign of acute toxicity. No deaths of Albino mice were recorded even up to 72 hours after oral administration. However, ethanol extract of *A. occidentale* administered at 3000mg/kg body weight showed signs of weakness, all other parameters such as tremors, refusal to feed, falling of hair and coma were not observed (Table 6).

4.4   ANTI-INFLAMMATORY TEST: TOPICAL OEDEMA

All the ears of treated mice exhibited topical oedema induced by xylene. There was a significant difference in sizes of oedema ($P \leq 0.05$) between the treated right and the untreated left ears in all extracts tested. On the effect of extracts on acute oedema, aqueous extract of *C. citratus* exhibited highest (55.6%) inhibition than other extracts (Table 7). Ethanol extract of *A.occidentale* followed with 54.0 %. Water extract of *A. occidentale* exhibited the lowest inhibition (43.0 %).

Table 6: Acute Toxicity of Aqueous and Ethanolic Extracts of *Anacardium occidentale* and *Cymbopogon citratus* in Mice

Extract	Dose (mg/kg)	Number of mice	Toxic Symptoms	Dead Mice	D/T	% Mortality
AO Aqueous	500	5	None	0	0/5	0
	1000	5	None	0	0/5	0
	3000	5	None	0	0/5	0
AO Ethanol	500	5	None	0	0/5	0
	1000	5	None	0	0/5	0
	3000	5	Weakness of body	0	0/5	0
CC Aqueous	500	5	None	0	0/5	0
	1000	5	None	0	0/5	0
	3000	5	None	0	0/5	0
CC Ethanol	500	5	None	0	0/5	0
	1000	5	None	0	0/5	0
	3000	5	None	0	0/5	0
Control	-	5	None	0	0/5	0
Total		65	1	0	0	0

Legend: AO   =   *Anacardium   occidentale*,   CC   =   *Cymbopogon   citratus*

Table 7: Effects of Extracts on the Topical Oedema of the Mouse Ear

Extract	Dose (mg/ear)	Oedema (mm) ± SE	Inhibition (%).
AO aqueous	5.0	0.0072 ± 0.0002	43.0
AO ethanol	5.0	0.0058 ± 0.0002	54.0
CC aqueous	5.0	0.0056 ± 0.0006	55.6
CC ethanol	5.0	0.0064 ± 0.0004	49.2
Control	—	0.0126 ± 0.0004	—

n = 5   P ≤ 0.05 compared to untreated ears. SE = Standard Error

AO = *Anacardium occidentale*.

CC = *Cymbopogon citratus.*

4.5 ANALGESIC PROPERTIES OF PLANTS' EXTRACTS

4.5.1 Acetic Acid-induced Writhing Test

The effects of aqueous and ethanolic extracts of *A. occidentale* and *C. citratus* on acetic acid induced writhing in mice are presented in Tables 8 and 9 respectively. Both doses (50mg/kg and 100mg/kg body weight) showed reduction in writhings in a dose dependent manner. Reduction in writhings at the dose of 50mg/kg body weight in both aqueous and ethanolic extracts of the two plants was not significant ($P > 0.05$), pain inhibitions range is 24.3 to 27 %. However, at the dose of 100mg/kg body weight, there was a significant ($P < 0.05$) reduction in writhings induced by acetic acid, with pain inhibitions ranging from 52.7% to 55.4 % (Tables 8 & 9). The intraperitoneal administration of Piroxicam, the standard drug showed a significant ($P < 0.05$) reduction in writhings and gave pain inhibition of 70.3%. Comparatively, the control drug, Piroxicam had a higher pain inhibition than the aqueous and ethanolic extracts of *A. occidentale* and *C. citratus*.

Table 8: The effects of Aqueous and Ethanolic Extracts of *Anacardium occidentale* on Acetic Acid Induced Writhing in Mice

Groups	Treatment	Dose (mg/kg)	Writhings (X± SE)	% Inhibition
I	Distilled water	10ml/kg	14.8±0.3	_
II	Piroxicam	20	4.4±0.4	70.3
III	AO Aqueous	50	11.0±0.5	25.7
IV	AO Ethanol	50	10.8±0.4	27.0
V	AO Aqueous	100	6.8±0.4	54.1
VI	AO Ethanol	100	6.4±0.5	55.4

Legend: AO = *Anacardium occidentale*

SE = Standard Error

Table 9: The Effects of Aqueous and Ethanolic Extracts of *Cymbopogon citratus* on Acetic Acid Induced Writhing in Mice

Groups	Treatment	Dose (mg/kg	Writhings (X± SE)	%Inhibition
I	Distilled water	10ml/kg	14.8±0.3	_
II	Piroxicam	20	4.4±0.4	70.3
III	CC Aqueous	50	11.2±0.4	24.3
IV	CC Ethanol	50	11.0±0.4	25.7
V	CC Aqueous	100	6.8±0.4	54.1
VI	CC Ethanol	100	7.0±0.7	52.7

Legend**:** CC = *Cymbopogon citratus*

SE = Standard Error

CHAPTER FIVE
DISCUSSION

5.1 ANTIMALARIAL ACTIVITIES OF *ANACARDIUM OCCIDENTALE* AND *CYMBOPOGON CITRATUS*

The emergence of widespread resistance of *Plasmodium* species to most antimalarial drugs has led to a more vigorous and concerted research in traditional medicinal plants for the treatment of malaria. *Anacardium occidentale* and *Cymbopogon citratus* are two such plants which are traditionally used for the treatment of malaria in Nigeria, but for which there are no scientific proof of their efficacies. The results of the present study revealed that *A. occidentale* and *C. citratus* exhibited potent antimalarial activity against *P. falciparum* isolates *in vitro*. Similarly, (Odugbemi, Akinsulire, Aibinu and Fabiku, 2007) reported that the two plants have been traditionally claimed to relieve fever and cure malaria. This study provides a scientific evidence for the claims.

Highest antimalarial activity was observed with the ethanol extract of *A. occidentale* which had parasite growth inhibition of 75.64%, followed by the water extract of *A. occidentale* which had the parasite growth inhibition of 72.4 (Table 6). The ethanol extract of *A. occidentale* showed highest antimalarial activity in all concentrations. *A. occidentale* (leaves and bark) are used by traditional healers for treatment of asthma, diabetes, urinary disorders, diarrhea. They contain the saponins, tannins and flavonoids. These compounds are responsible for the observed antimalarial activities of the extracts though the active constituents are yet to be identified.

Alkaloids are one of the major classes of compounds possessing antimalarial activity, and one of the oldest and important antimalarial drugs, quinine belongs to these compounds (Dharani *et al*., 2008). The presence of alkaloids in *C. citratus*

extracts might have contributed to antimalarial activities exhibited by the plant extracts. Also, flavonoids are compounds with a widespread occurrence in the plant kingdom which have also been detected in *Artemisia* species. They are reported to have exhibited significant *in vitro* antimalarial activity against *P. falciparum* (Chanphen, Thebtaranonth, Wanauppathamkul and Yuthavong, 1998). Their presence in *A. occidentale* and *C. citrarus* extracts justify the antimalarial activities exhibited by the plants' extracts.

Both aqueous and ethanolic extracts of *A. occidentale* had higher antimalarial activity than extracts of *C. citratus* which had parasite inhibition of 69.4% (aqueous extract) and 67.9% (ethanol extract). Traditional healers in Nigeria usually use decoctions of *A. occidentale* and *C. citratus* in combination with other plant parts such as leaves of *Vernonia amygdalina* (bitter leaf) and leaves of *Azadirachta indica* (neem tree) to treat malaria and fever, which results into enhanced antimalarial effect (Odugbemi, Akinsulire, Aibinu and Fabiku, 2007; Avwioro, 2010). Occasionally, some people administer it alone as an antimalarial therapy (Omotayo, 2003).

The *in vitro* antimalarial activity of *C. citratus* has similarly been reported by Bidla *et al.* (2004), who recorded antimalarial activity of 75.2% at 40μg/ml which is comparatively higher than what was recorded in this report: 69.4% (water extract) and 67.9% (ethanol extract) at 100μg/ml, considering the concentration of 40μg/ml used. The finding of this work also agrees with Tchoumbougnang, Zollo, Dagne and Mekonnen (2005) in which the essential oils of *C. citratus* leaves was tested against *P. berghei* in a 4 day suppressive *in vivo* test. The difference in result might be due to the different methods employed. Similarly, the antiplasmodial activity of extracts of *C. citratus* partly tallies with Melariri, Campbell, Etusim and Smith (2011) in which the combination of *C. citratus* and *V. amygdalina extracts* gave a good suppression of malaria parasites in mice.

Comparatively, both water and ethanol extracts of *A. occidentale* exhibited higher antimalarial activities (water extract 72.40%, IC_{50} 16.00μg/ml; ethanol extract 75.64%, IC_{50} 11.70μg/ml) than water and ethanol extracts of *C. citratus* (water extract 69.42%, IC_{50} 23.00%, ethanol extract 67.91%, IC_{50} 30.00%), but the differences are insignificant. This is reflected in comparative parasite inhibition by water and ethanol extracts, with *A. occidentale* being slightly higher than *C. citratus* in almost all concentrations (Figures 5 and 6). It is interesting to note that the *in vitro* antimalarial activity of *A. occidentale* is being reported for the first time in this study

Based on Gessler *et al.* (1994) thresholds for *in vitro* antimalarial activity, the water and ethanol extracts of *A. occidentale* with IC_{50} of 16.0μg/ml and 11.7μg/ml respectively, are considered as moderate antimalarial activity. Similarly, the water and ethanol extracts of *C. citratus* with IC_{50} of 23μg/ml and 30μg/ml respectively have moderate antimalarial activity, although IC_{50} values are much higher compared to IC_{50} values of *A. occidentale* (16.0μg/ml and 11.7μg/ml) reported in this study.

All the four extracts showed dose-dependent antimalarial activity against *P. falciparum* isolates (Figure 7). Dose-dependent pattern of activity have been reported against *P. falciparum* in Borrelidin, a potent antimalarial (Ishiyama *et al.*, 2011); 4-Aminoquinolines (Aguiar *et al.*, 2012) and small-molecules histone methyltransferase (Malmquist, Moss, Mecheri, Sherf and Fuchter, 2012). Also, dose-dependent antimalarial activities against *P. berghei* have been observed in fractions of leaves of *Ageratum conyzoides* in mice (Ukwue *et al.*, 2010) and ethanolic crude extracts of leaf and root of *Carpolia lulea* (Okokon, Effiong and Ettebong, 2011). These patterns of antimalarial activity suggests that extracts or drugs would exhibit higher antimalarial activities at higher concentrations.

5.2 ACUTE TOXICITY IN MICE

Acute toxicity describes the adverse effects or safety of a substance that result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). Acute toxicity testing usually attempts to determine dose-dependent adverse effect that may occur (Walum, 1998).

The results obtained in the acute toxicity test imply that aqueous and ethanolic extracts of *A. occidentale* and *C. citratus* at the dosages tested (500mg/kg, 1000mg/kg and 3000mg/kg body weight) in this work was not toxic to the mice. The extracts can therefore be considered safe since with 6-fold increase in the therapeutic doses of 500mg/kg to 3000mg/kg body weight, no deaths or major signs of toxicity were observed. This finding agrees with Tanira *et al.* (1996) that the plants extracts at the dose of 500mg/kg, 1000mg/kg and 3000mg/kg did not cause any deaths and only the dose of 3000mg/kg showed signs of reduced locomotor activity.

Furthermore, the finding agrees with Idowu, Soniran, Ajana and Aworinde (2009) whose report showed that *C. citratus* is non- toxic. It is also in agreement with Tedong *et al.* (2007) who reported that *A. occidentale* is non-toxic at the dosages tested. Similarly, the result of this work also agrees with Ofusori *et al*. (2008), who reported the non-toxic effect of ethanolic extract of cashew stem bark on the brain and kidney parenchyma of mice.

5.3 ANTI-INFLAMMATORY ACTIVITY

Anti-inflammation properties have been observed in flavonoids, tannins and alkaloids (Yogamoorthi and Priya, 2006; Iwalewa, McGraw, Naidoo and Eloff, 2007). It is therefore possible that the anti-inflammatory effect observed in water and ethanol extracts of *A. occidentale* and *C. citratus* may be attributed to their flavonoids, tannins and alkaloids contents as also observed by Musa *et al.* (2008). Furthermore, the anti-

inflammatory properties exhibited by *A. occidentale* extracts agree with that of Kamal *et al.* (2009) who reported that *A. occidentale* extract is an effective protector against monocyte recruitment in inflammatory vessel. In addition, the anti inflammatory effects exhibited by these extracts to topical model of acute inflammation justify the traditional use of the plants leaves and bark in the management of painful inflammatory conditions.

5.4 ANALGESIC PROPERTIES OF PLANTS' EXTRACTS

Pain sensation is said to be one of the reasons why people seek medical attention, and therefore analgesics are commonly prescribed to relieve pain (Cuartero *et al.*, 2006). The use of analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs), aspirin and opionates as pain killers/relievers have not been successful in all cases due to adverse side effects such as gastric lesions and liver damage (Zulfiker *et al.*, 2010). The strategy should therefore be the search for clinically new and useful analgesics which have negligible or no adverse effects. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Shekhawat and Vijayvergia, 2010). The findings of the present study reveal that aqueous and ethanolic crude extracts of *A. occidentale* and *C. citratus* at doses of 50 and 100mg/kg body weight exhibited analgesic effects against chemical pains (writhings) induced by acetic acid (Sha'a, Oguche and Ajayi, 2011). The effects were significant (P< 0.05) at the dose of 100mg/kg body weight. The acetic acid induced abdominal writhing is widely used for evaluating analgesic properties of extracts and drugs (Winter, Risley and Nuss, 1963; Ahmed, Selim, Das and Choudhuri, 2004; Du, Yu, Ke, Wang and Qian, 2007).

The model which evaluates peripheral antinociceptive (analgesic) activity is very sensitive and able to detect analgesic effects of compounds at dose levels that

may appear inactive in other methods (Bentley, Newton and Srarr, 1981). Another report revealed that acetic acid induced writhing response in mice is a simple, rapid and reliable model to evaluate peripheral type of analgesic action of herbal and other drugs (Shinde *et al*., 1999). However reports from Le Bar, Gozariu and Cadden (2001); Malec, Mandryk and Fidecka (2008) showed that the acetic acid induced abdominal writhing in mice is a nonspecific test and Sresponse to both central and peripheral analgesia is therefore used for evaluating analgesic properties.

In the present work, the aqueous and ethanolic extracts of *A. occidentale* and *C. citratus* as well as piroxicam similarly showed a significant inhibitory effect on acetic acid induced writhing. The results suggest that the extracts possess peripheral analgesic properties and their mechanisms of action may be mediated through inhibition of local peritoneal receptors as similarly reported by Mbiantcha *et al*. (2010).

However, irrespective of whether the model evaluates peripheral analgesic Action only or non-specific, the result of this work validates the use of these plants as analgesics in ethnomedicine in Nigeria. In addition, their analgesic properties confer on them added advantages as antimalarials and antibacterials in traditional medical practice. On mode of action, local peritoneal receptors are suspected to be partly involved in abdominal constriction response (Bentley, Newton and Srarr, 1983). The injection of acetic acid is reported to induce the release of mediators of pain such as prostaglandins and other cyclokinase (Divya *et al*., 2009; Nkeh-Chungag *et al*., 2010). This suggests that the extracts of *A. occidentale* and *C. citratus* acted by inhibiting the actions of cycloxygenase which is said to be responsible for producing prostaglandins from arachidomic acids (Nkeh-Chungag *et al*., 2010).

The phytochemical screening of aqueous and ethanolic extractsof *A. occidentale* and *C. citratus* showed the presence of alkaloids, tannins and flavonoids. It is therefore possible that the analgesic effects observed may be attributed to the flavonoids and

tannins, since analgesic and anti-inflammatory activities have earlier been observed in these compounds (Karumi, Onyeyili ang Ogugbuaja, 2003)). The analgesic effects produced by crude extracts of both experimental plants validate their use in traditional medical practice as analgesics. It also gives them curative advantage as antimalarials and antibacterials.

5.5   CONCLUSION

The results of this study show that both bark of *A. occidentale* and the leaves of *C. citratus* possess moderate antimalarial activity. Comparatively, the extracts of *A. occidentale* exhibited higher antimalarial activity than extracts of *C. citratus.* This result has established the rationale for the traditional use of the plants in the treatment of malaria, and showed that medicnal plants which have reputations for antimalarial properties can be screened in order to ascertain their efficacy and determine their potentials as sources of new antimalarial drugs.

Extracts of *A. occidentale* and *C. citratus* have been found to be non-toxic and therefore safe for malaria therapy and perhaps for treatment of other ailments. The extracts of experimental plants showed good measure of anti-inflammatory activities and therefore justify the traditional use of the plants leaves and bark in the management of painful inflammatory conditions. The findings of the present study reveal that aqueous and ethanolic crude extracts of *A. occidentale* and C. *citratus* at doses of 50 and 100mg/kg body weight exhibited analgesic effects against chemical pains (writhings) induced by acetic acid.

5.6 CONTRIBUTION TO KNOWLEDGE

This research work as contribution to knowledge reports, (**1**)   the *in vitro* antimalarial activity of *A. occidentale* and *C. citratus*, thereby supporting the use of the

plants' parts for malaria treatment in traditional medicine; in addition, the *in vitro* antimalarial activity of *A. occidentale* being reported for the first time. (**2**) Acute toxicity or non-toxic effects of plants used in traditional medicine is very important in determining its safety and efficacy. This research reports the non-toxic and safe use of the extracts of *A. occidentale* and *C. citratus* not only in malaria, perhaps in the traditional use of the plants to cure or treat other diseases. (**3**) Also as a contribution to knowledge, the anti-inflammatory and analgesic activities of *A. occidentale* and *C. citratus* reported in this research gives them therapeutic advantage as antimalarial agents.

5.7 RECOMMENDATIONS FOR FURTHER WORK

One of the key challenges to the fight against malaria is not just to develop effective and safe antimalarial drugs, but also to make sure they are available to local communities and people at an affordable price to allow wide spread use (Omotayo, 2003). *A. occidentale and C. citrates* are popular, especially in rural and sub-urban centres in Nigeria. As potential sources of antimalarial agents, they should be subjected to further research to study their active constituents, so that their products would be available and widely used by the people. In addition, since the current strategy in malaria therapy is combination therapy to prevent or delay resistance of parasites, it would be proper to test the effect of combining the two plants' products for malaria treatment such as reported here.

Considering the socio-economic burden of malaria on poor households especially in Africa, the screening of medicinal plants commonly used for curing malaria should be encouraged with a view to making the research findings and such antimalarial compounds available to the people. This would reduce the economic burden of the disease on the poor. A similar research work should be conducted on acute toxicity

using extracts of the same experimental plants, involving more animal models over a longer period of time. This would give a broad picture of safety of using the plant products for malaria therapy. This research used the topical oedema of the mouse ear to determine the anti-inflammatory properties of plant extracts. Further work should involve the use of other experimental models such as Carraeenan-induced rat paw oedema, cotton pellet induced granuloma for evaluating the anti-inflammatoty activities of the plant extracts, and the results be compared with this work.

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APPENDIX

APPENDIX A

Table 9: List of Medicinal Plants used in the investigation.

Botanical name	Family	Part used	Site of collection	Traditional uses
A. occidentale	Anacardinaceae	Bark	Jos	
				Antimalarial
				Antibacterial
				Toothache
				Soregum
C. citratus	Poaceae	Leaves	Jos	Antimalarial
				Antiseptic
				Hypertension
				Diuretic
				Antibacterial