ISOLATION AND CHARACTERISATION OF SECONDARY METABOLITES WITH ANTIPLASMODIAL ACTIVITY IN SELECTED MEDICINAL PLANTS OBTAINED FROM NIGER STATE, NIGERIA

BY

SULAIMAN, Rukayyah

PhD / SLS/ 2015/783

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

FEBRUARY, 2021

ABSTRACT

Malaria continues to be a global burden as the efficacy of most anti-malarial drugs have been compromised by the evolution of resistant parasites. Plants present unlimited sources of novel substances with many therapeutic potentials. This study was designed to obtain bioactive compounds with antiplasmodial potential and high safety margin from selected medicinal plants that could be useful against both sensitive and resistant parasites. Eight medicinal plants; namely *Agelanthus dodoneifolius, Securidaca longepedunculata, Neocarya macrophylla, Merremia hederacea, Zanthoxylum zanthoxyloides, Leptadenia hastata, Polycarpea linearfolia* and *Lophira alata* collected in Niger State, Nigeria, extracted in methanol were subjected to phytochemical screening and acute toxicity study using standard methods. The plant extracts were screened for antiplasmodial activity *in vitro* against chloroquine-sensitive (CQS) strain, NF54 and chloroquine-resistant strain (CQR) K1 of *Plasmodium falciparum* while the *in vivo* activity was tested against CQS(NPK) strain of *Plasmodium berghei* at 100, 200 and 400 mg/kg bw. Subchronic toxicological screening of the most active extracts (*P. linearfolia* and *L. hastata*) was carried out at 200 mg/kg bw orally for 28 days in rats. Biochemical and haematological parameters were monitored while histopathological examination of the liver, kidney, heart and spleen of test animal and control groups were also undertaken. The most active extracts *in vivo* were subjected to bioassay guided fractionation. ^{1}H NMR, ^{13}C NMR and HPLC-ESI/MS were used to characterize active compounds from the potent fractions. The plant extracts contained a variety of phytochemicals, except phytosteroids that was absent in *A. dodoneifolius*. Acute toxicity study revealed *P. linearfolia* was safest with LD_{50} value of 4500 mg/kg bw. *Z. zanthoxyloides* recorded highest *in vitro* inhibition against CQS and CQR with IC_{50} of 1.07 and 1.31 µg/ml respectively. In the *in vivo* activity of the tested extracts, the parasite density of *L. hastata* (576 parasites/µl) and *P. linearfolia* (473 parasites/µl) at 400 mg/kg bw were comparable to the standard control (431 parasites/µl). Mean survival time of *L. hastata* and *P. linearfolia* treated groups were also comparable to the standard in the range of 31-35 days. Biochemical parameters of the rats for the 2 extracts administered subchronically revealed a significant ($p<0.05$) increase in creatinine, bilirubin and HDL concentrations when compared to the control. However, the LDL concentrations of the treated groups decreased significantly ($p<0.05$). Serum glucose concentration decreased while AST activity increased significantly ($p<0.05$) for *P. linearfolia* treated group when compared to the control. RBC count, haemoglobin concentration and haematocrit of *P. linearfolia* treated group decreased significantly($p<0.05$) compared to the control. White blood cell indices, of *L. hastata* group increased significantly($p<0.05$) compared to the control. Histopathological result showed normal cell architecture of all the organs analysed except the spleen that showed hyperplastic lymphoid follicles in *L. hastata* treated group. The six fractions of *L. hastata*: Lh1, Lh2 and Lh3 had IC_{50} value higher than10 µg/ml, while Lh4A, Lh4B and Lh5 had IC_{50} values of 4.24, 8.50 and 7.24 µg/ml. All the six fractions of *P. linearfolia,* had IC_{50} higher 10 µg/ml except Tk3 with a value of 2.40 µg/ml. Administration of fractions Lh3 of *L. hastata* and Tk3 of *P. linearfolia* resulted in complete parasite clearance on day 14 post infection. Spectral analysis identified two antiplasmodial compounds: 2,2' –methylene bis(6-tert-butyl-4-methylphenol) and Sclerinone A in *P. linearfolia* extract, and 2,2' –methylene bis(6-tert-butyl-4-methylphenol) in *L. hastata*. These antiplasmodial agents could serve as templates for the synthesis of new antimalarial drugs operating synergistically against both CQ sensitive and resistant strains of *Plasmodium falciparum.*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Plate

Page

LIST OF ABBREVIATIONS

PvRBP2 = *P. vivax* reticulocyte binding protein 2

RDTs = Rapid diagnostic tests

LDH =lactate dehydrogenase

HRP2=Histidine-rich protein-2

PCR = polymerase chain reaction

LAMP = loop-mediated isothermal amplification

EXP1= exported protein 1

ACT = artemisinin combination therapy

LLIN =long-lasting insecticidal nets

SNP = single-nucleotide polymorphism

SCD = Sickle cell disease

SCT = sickle cell trait

G6PD = Glucose-6-phosphate dehydrogenase

NADPH = nicotinamide adenine dinucleotide phosphate

RBC= red blood cells

PK =Pyruvate kinase

ACTs = artemisinin-based combination therapies

HCl =hydrochloric acid

PUFA=polyunsaturated fatty acid (PUFA)

NMR= Nuclear magnetic resonance

HPLC= High performance liquid chromatograph

GC = gas chromatography

LC = liquid chromatography

SFC = supercritical fluid chromatography

TLC = thin layer chromatography

PC = paper chromatography

CC=column chromatography

MS= mass spectrometer

LC-MS=Liquid chromatography-mass spectrometry

GC-MS=Gas chromatography-mass spectrometry

APCI =atmospheric pressure chemical ionization

API =atmospheric pressure ionization

ESI = electrospray ionization

UV = Ultra-violet

Uv-vis = Ultra-violet-visible

TLC = thin layer chromatography

ESI MS = electrospray ionization mass spectroscopy

HPLC ESI MS = High performance liquid chromatograph electrospray ionization mass spectroscopy

IT-MS = ion trap mass spectrometer

TOF-MS = time-of-flight mass spectrometer

Q-MS = quadrupole mass spectrometer

QQQ-MS =Triple-quadrupole mass spectrometry

CRP=C-reactive protein

ALT= alanine aminotransferase

AST=aspartate aminotransferase.

LC-Q-TOFMS = Liquid chromatography quadrupole time of flight mass spectrometry

LC QQQ-MS = liquid chromatography triple-quadrupole mass spectrometry

RP = reversed-phase

TFA = tri-fluoroaceticacid

DAD = diode array detectors

PDA = photodiode array detectors

TCD = thermal conductivity detector

MS = mass spectrometer

ECD = electron capture detector

FID = flame ionization detector

SFC = Supercritical fluid chromatography

NMR = Nuclear Magnetic Resonance

MRI =magnetic resonance imaging

δ = chemical shifts

TMS= tetramethylsilane

TSP = trimethylsilylpropanoic acid

CLA = conjugated linoleic acid

CQS =Chloroquine sensitive strain

CQR =Chloroquine resistant strain

DMSO = dimethyl sulphoxide

TAG =Triglycerides

HDL = High density lipoprotein

PEG = polyethylene glycol

ALP = alkaline phosphatase

pNPP = p-nitrophenyl phosphate

DEA = Diethanolamine (DEA)

ALT = alanine aminotransferase

AST = aspartate aminotransferase

EDTA = Ethylene diamine tetraacetic acid

PCV = packed cell volume

RBC count = red blood cell count

Hb = Hemoglobin concentration

TWBC = total white blood cells

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Malaria is an infection caused by Plasmodium protozoan parasite, a major human health threat with almost half of the world's population at risk (Asrar *et al.,* 2014, WHO, 2015). Malaria is a typical example of a disease that influences the efficiency of individuals, families and the society as a whole (Naghibi *et al.*,2013). The global incidence of malaria cases in 2015 is 214 million, with African continent being most susceptible (WHO, 2015). The global malaria mortality recorded in 2015 is 438, 000. Africa region has the highest (90%) mortality rate, followed by south East region (7%) and Eastern Mediterranean region (2%) with having the least mortality rate. Nigeria and Democratic Republic of Congo carries 35 % of these deaths burden (WHO, 2016).

The human malaria is caused by six *Plasmodium* genus species, *P. malariae, P. falciparum, P. vivax, P. ovale, P. knowlesi* and *P. simium.* The later is solely found in the Brazilian Atlantic Forest (Grigg and Snounou, 2017). *P. falciparum* is the most virulent specie and important cause of morbidity and mortality (Akkawi *et al*., 2014). Female *Anopheles* mosquitoes are the vectors for transmission of malaria parasites. (David-Bosne *et al.*,2013). These *Plasmodium* parasites are transmitted by over 70 species of *Anopheles* mosquitoes (Thota and Yerra, 2016).

Clinical manifestation of malaria include fever, protraction and anaemia. Acute forms of the disease can result in delirium, metabolic acidosis, multi organ system failure, coma and death eventully if untreated (Fidock *et al.*,2004). Proinflammatory cytokines are released as part of the pathogenesis of acute malaria (Aguiar *et al.*,2012[a]) and these cytokines contribute to the suppression of erythropoiesis. There are many anti-inflammatory and analgesics drugs in the market use in modern treatment but they are

not devoid of adverse drug effects (Osadebe and Okoye, 2003). Thus, new drugs with less detrimental effects are needed and warranting their search.

The problem of mosquito vector resistance to insecticides and parasite resistance, especially *Plasmodium falciparum* to the commonly available antimalaria drugs has resulted in recrudescence of malaria (Naghibi *et al.*,2013). Therefore, new drugs need to be studied and produced from plants that have not yet been exposed to drug pressure in order to triumph over the issue of emerging resistance and putting into cognisance the safety and affordability of the new drug. Floral diversity offers the hope for such novel agents

Medicinal plants have been used in the treatment of parasitic diseases since time immemorial. Plants like *Cinchona succiruba* (Rubiaceae) have history for the treatment of malaria infection. Numerous compounds isolated from rich natural resources form various structures for optimization to obtain improved therapeutics (Vangapandu *et al.*,2007). Some of such components include alkaloids, flavonoids and terpenoids.

Alkaloids are physiologically active nitrogenous base secondary metabolites found in plants, fungi, bacteria and marine organism (Kaur *et al.*,2009). Literature search indicated that a host of antiplasmodial alkaloids have been derived from African flora, ranging from indole alkaloids, amides, cryptolepines and many yet to be identified (Onguine-Amoe *et al.*,2013). Several flavonoids from medicinal plants, as well as from dietary sources have been found to possess *in vitro* and *in vivo* antiplasmodial actvity against both sensitive- and resistant- strains of *P. falciparum*. Examples of flavonoids having antimalarial activity are acacetin, genistein, baicaclein, kaempferol, chrysin, hesperetin, quercetin, isoquercetin, luteolin, naringenin and myricetin, just to name a few with such potency (Rudrapal and Chetia, 2017). Terpene is the second non-nitrogenous secondary metabolite apart from flavononids, having numerous reports of

its efficacy as antiplasmodial agent (Hussein and El-Anssary, 2018). The common ones are artemisinin and its derivatives (examples include artemether, artesunate, arteether and B-dihydroartemisinin) that has come as an alternative to Chloroquine in malaria treatment (Brown, 2010). Similarly, nerolidol, balsaminoside B, farnesol, karavilagenin C, limonene, and the karavoates B and D are also terpenes that have exhibited greater *in vivo* and *in vitro* antimalarial activity against strains of *P. berghei* and *P. falciparum* respectively (Grabriel *et al.*,2016 and Batisa *et al.*,2009). Many of these bioactive compounds are yet to be discovered. The use of such compounds as antiplasmodial agents can only be enchanced when empirical evidences exist for their efficacy and toxicity profile. They could also be used as template for the synthesis of more active, less toxic drug derivatives because drug efficacy, pharmacology and toxicity are important parameters in the selection of compounds for development.

1.2 Statement of the Research Problem

Despite intensive efforts to control malaria, its devastating heatlh effects remain unabated. Ninety five percent of global malarial burden is in sub-Saharan Africa. Most vulnerable group are pregnant women and children under the age of five. The numbers of easily available, affordable and effective antimalarial drugs are few. Viable multistage vaccines for the disease are unavailable. The situation is further compounded by the spread and intensification of drug resistant malaria parasites. The morbidity and mortality from the diseases are on upward trend. WHO recent estimates put annual global malaria infection at 350-500 million, with about 1-2 million fatalities. The disease accounts for over 100,000 deaths, 60% outpatient visits and 30% hospitalizations in Nigeria (WHO, 2016).

The economic impacts of malaria infection in endemic countries cause significant loses in gross domestic product annually. In Africa 12 billion dollars is lost due to malaria infection every year and the disease also consumes forty percent of all public health expenditure. (Zelefack *et al.*,2012). Malaria is a major obstacle to economic development and a cause of poverty in areas of it's prevalence. In Africa, 89 % of malaria control schemes and commodities are financed by global programs and 11 % by local governments (Nkumama *et al.*,2017).

1.3 Justification for the Study

Antimalaria drugs such as sulphadoxine-pyrimethamine, artemisinin and chloroquine have been compromised by drug resistant *P. falciparum*. Fixed dose ACT drug regimens are also being threatened by treatment failure. Sourcing for effective new drugs especially from plant sources is thus justifiable**.** Quinine, the original antimalarial alkaloid was isolated from *Cinchona ledgeriana* and Artermisinin from *Artemisia annua*, hence plants may provide yet another active antimalaria drug. The threat from malaria thus necessitates screening of medicinal plants with reputation in the treatment of the disease for leads that can be developed into new generation of cures**.** Hence, there is need to source and advance the course for new drugs development from medicinal plants having folklore background in the malaria treatment with the aim of surpassing the problem of emerging resistance, such drugs should be safe and affordable. The selected plants are claimed by traditional healers to be of importance in the management of malaria.

Herbal medicaments have gotten global application, acceptability and efficacy which have predispose millions of people to the fundamental but rarely reported problems of herbal toxicity and mis-adventuring. Most herbal treatments are in the forms of crude or

partially purified extracts which are often dispensed over a period of time with the chances of accruing organ toxicity and even death among users (Yuan *et al.*,2016). The need for *in vitro* and *in vivo* toxicological profiling of such new drugs as an integral component in their development is imperative (Jigam *et al.*,2012). Such routine evaluations include acute toxicity studies which are critical as they provide a rough idea about the nature of the medicinal extract in addition to determining safe doses for clinical use. Sub-chronic and chronic studies are necessary in the elucidation of target organs of toxicity and demonstration of dose-response relationships.

1.4 Aim and Objectives of the Study

1.4.1 Aim

The aim of this study is to isolate and characterize secondary metabolite with antiplasmodial potential from selected medicinal plants in Niger State, Nigeria.

1.4.2 Objectives

The objectives of this study are to:

i. determine the percentage yield of the crude extracts from selected medicinal plants
ii. determine the qualitative and quantitative phytochemical constituents of the crude plant extracts
iii. determine acute toxicity of the crude extracts.
iv. screen crude plant extracts for antiplasmodial activity
v. determine subchronic toxicological effects of the most active extracts
vi. fractionate the most active extract and test for their antiplasmodial activity.
vii. determine the spectral characteristics of the most active fraction

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Malaria Infection

2.1.1 Life cycle of malaria parasite

Malaria transmission occurs from one person to another through the bite of infected female *Anopheles* mosquitoes. The parasite goes through a multifaceted life cycle in both the mosquito and in human. *Plasmodium* parasites undergo two cycles of asexual division (schizogony, or merogony) in man and a single sexual reproductive cycle (sporogony) in mosquito (Figure 2.1). The cycle begins when an infective sporozoites from the salivary glands of infected female *Anopheles* mosquito is injected into human host blood stream during a blood meal. The sporozoites together with the saliva that the mosquito uses as an anticoagulant, that stops the blood from coagulating in the mosquito's proboscis or mouth parts (WHO, 2010).

Once inside the human being, the sporozoites go straight to the liver and attack the hepatocytes. In the hepatocytes, there is division of a single parasite, leading to the generation of many thousands of new parasite (the first massive replication). This is a process called exoerythrocytic schizogony (asexual reproductive phase) and occurs over 7-21 day period. The enlarged hepatocyte (liver schizont), finally bursts, freeing thousands of merozoites into the blood stream, in vesicles (merosomes) generated in hepatocytes to prevent their capture by Kupffer cell (Burda *et al.*,2017). This phase is endured by the liver differently across different species. This description of the liver phase infection applies to *P.falciparum* and *P. malariae* lasting for an average of 6 days. A slightly different cycle occurs for *P. vivax* and *P. ovale*, as a number of the parasites that originally enter the liver cells do not become schizonts immediately, but enter a kind of sleeping phase called hypnozoites. These parasites which are dormant, are

responsible for the relapses that occur at intervals after the first attack of malaria. The liver stage last an average of 10 and 15 days in *P. vivax* and *P. ovale* respectively (Miller *et al.*,2013).

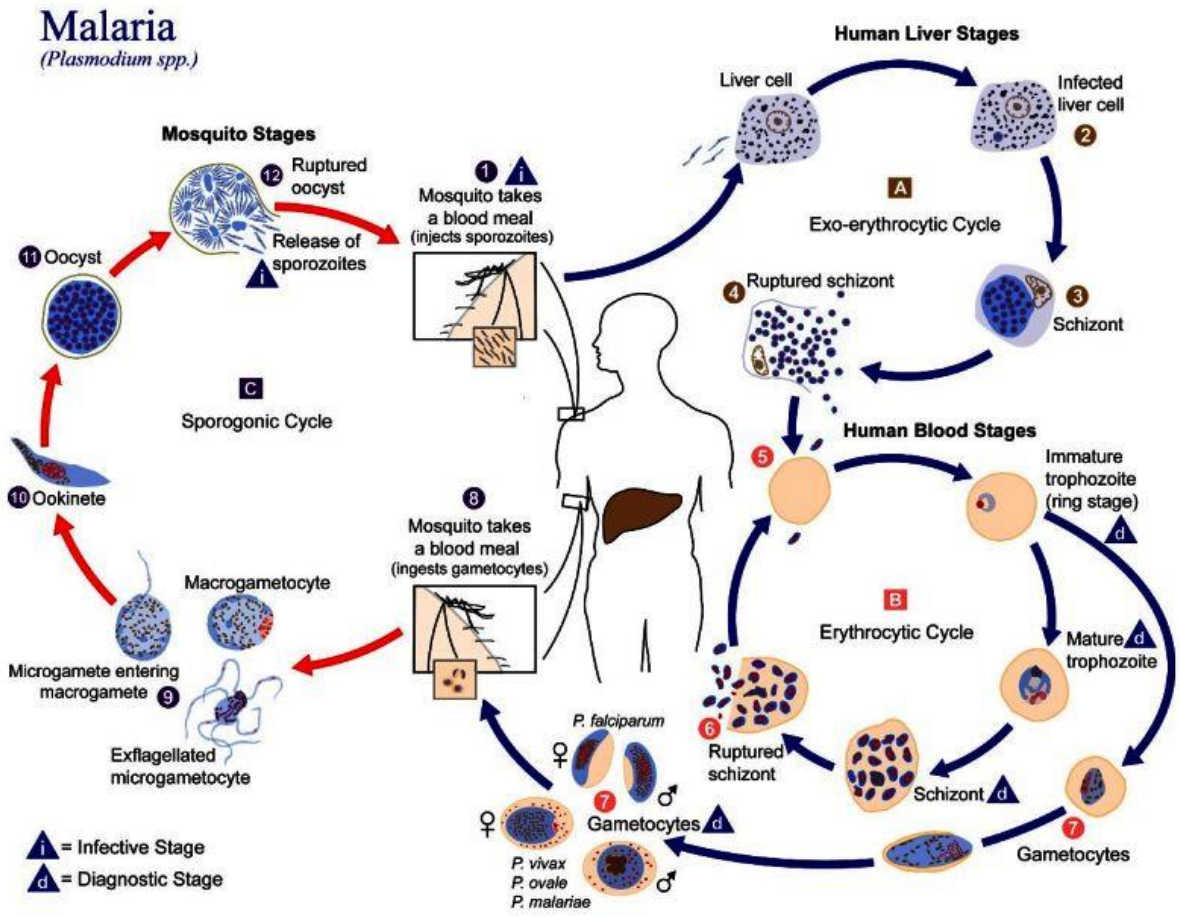


Figure 2.1: Life Cycle of Malaria Parasite in Man and Mosquito

Source: Klein, (2013).

The merozoites released, quickly stick to red blood cells, one merozoite infects a single erythrocyte. The invasion process is complex, depending on various cell mechanisms that allow the parasite to attach, reorient and strike, forming the parasite vacuole known as parasitophorous vacuole (Cowman *et al.*,2012).

The parasite starts its second cycle of asexual division once in the red blood cells, passing through various stages. The early trophozoite, known as the "ring stage," is beginning to develop, expanding to a mature trophozoite with a high metabolic index. Without cytokinesis, multiple nuclear divisions are formed in the late stage, creating schizonts. The schizont contains an average of 32 merozoites produced on the lysis of the erythrocyte. The whole process can take approximately 36–48 h in *P. falciparum*, 48 h in *P. vivax*, and can go up to 72 h in *P. malariae*, but in *P. knowlesi* the duration is 24 h, which is one attribute that leads to high human virulence (Martinelli and Cullenton, 2018). Cell lysis correlates with fever symptoms, immune system reaction to the bloodstream release of hemozoin and other parasite products (Miller *et al.*,2002).

The parasite can follow another development path within the erythrocytes, differentiating into gametocytes. Such gametocytes are consumed by the female Anopheles mosquito during a blood meal from an infected individual and this marks the beginning of the parasite's sexual reproductive cycle. Blood meal gametocytes create 4-8 flagella. Each flagellum separates from the parent body, swimming in the abdomen of the mosquito through the coagulating blood and undergoing a meiotic division of the parasite.

The gametocytes develop inside the mosquito gut to form male and female gametes that are fertilized and form the zygote. This zygote turns into an ookinete that penetrates the midgut and becomes an oocyst. Under several cellular divisions, thousands of sporozoites are released from oocysts, which migrate to the salivary glands of the mosquito to be expelled during the next blood meal with the anticoagulant factor found in saliva (Meibalan and Marti., 2017).

2.1.2 Pathogenesis

Normal red blood cells have life span over the course of 120 days Most species of Plasmodium show a preference for reticulocyte invasion (1–2 days), which typically accounts for less than 1% of total red blood cells. *P. vivax* has the sternest reticulocyte choice, resulting in a much lower density of parasites in human infections compared to the lethal *P. falciparum*, which is much less limited and can lead to high parasitemia. The *P. vivax* parasite ligand PvRBP2 has been identified as being preferentially binding to reticulocytes, likely associated with limitation in *P. vivax* infection (Cowman *et al.*,2017). Often, *P. falciparum* parasite isolates show reticulocyte preference. An association was observed between loss of erythrocyte selectivity and severity of disease that could be associated with reduced reticulocyte preference (Chotivanich *et al.*,2000). Surprisingly, such a correlation in Africa did not hold up, indicating regional differences in the use of the invasion route (Deans *et al.*,2007). *P. knowlesi* has demonstrated a related ability to adapt and colonize an extended host niche in human red blood cell (Lim *et al.*,2013). This is becoming increasingly important, with increased transmission of *P. knowlesi* to humans in Southeast Asia (Moon *et al.*,2013).

Severe malaria pathogenesis is associated with pain and the release of proinflammatory cytokines (Aguiar *et al.*,2012[a]). Inflammation and pain are common nonspecific manifestations of many diseases. The most normal biological response to a variety of stimuli and local injury is inflammation. Inflammatory reactions can be caused by chemical and physical trauma, foreign species and antigen-antibody reactions and are often compounded by tissue discomfort (because of increased tissue pressure during oedema formation or inflammatory mediators) or even cell damage caused by swelling or edema (Yam *et al.*,2009). As such, the use of anti-inflammatory agents can be

effective in the therapeutic treatment of inflammatory reaction-related pathological conditions.

2.1.3 Malaria diagnosis

Early parasitological diagnosis of malaria is the foundation for the treatment and control of malaria. Two options are used in malaria diagnosis, Laboratory diagnosis or Presumptive diagnosis. In presumptive (clinical) diagnosis, treatments of malaria are centered on clinical signs, such as fever but malaria is not the only cause of fever. This practice is common where diagnostic tools are not available. Therefore, many people are mis-diagnosed causing abuse of drug and thus wasting the drug. In Laboratory diagnosis a test is run to ascertain the presence of malaria, there are different method to confirm malaria in the laboratory (Nkumama *et al.*,2017). This includes:

2.1.3.1 Diagnosis by rapid diagnostic test (RDT)

Rapid diagnostic tests for malaria (RDTs) are immunochromatographic tests that detect the presence of antigens of malaria released from parasite infected erythrocytes. These tests are very specifici and sensitive compared to microscopy, with some loss of sensitivity when parasite densities are very low (Maltha *et al.*,2013). Malaria detection by RDTs is based primarily on detecting one or more of the three antigens; lactate dehydrogenase (LDH), histidine-rich protein-2 (HRP2) and aldolase

From the set, only HRP2 is used solely for *P. falciparum* detection due of its exclusive expression in the species of human, while aldolase and LDH are non-specific as they are produced by all human malaria parasites

LDH can also be used to detect *P. falciparum* infection directly, its sensitivity is lower than HRP2, so HRP2 has been used for *P. falciparum* detection in almost all RDTs (Pati *et al.*,2018). The experiments do not require specialized equipment or electricity and

within 30 minutes produce results. Systematic reviews have shown the reliability and cost-effectiveness of RDT. The usability of these assessments eliminates the need for a technical expert and allows a wide range of workers to use them, which can drastically decrease the presumptive use of anti-malarial medicines (Boyce *et al.*,2018). HRP2-based tests showed a decreased specificity because HRP2 can still be found in the blood stream a week after treatment (Nkumama *et al.*,2017).

2.1.3.2 Diagnosis by light microscopy

In endemic countries, microscopy is still known as the "gold standard" of malaria diagnosis. Microscopy can detect parasites in the range of 50–500 parasites/μL, it is cheap and allows species and parasite density to be defined (Berzosa *et al.*,2018). This approach has been used to identify parasites in the peripheral blood since the late 1800s from stained blood smears. All plasmodium parasites have distinctive features that can be distinguished using microscopy and in addition this method is most preferred due to its high level of accuracy in diagnosing malaria. Basically, two types of blood films are being used in malaria microscopy. Thin and thick blood films. The thin films are similar to normal blood films and enable identification of species because the presence of the parasite in this preparation is best preserved while the thick films allow the microscopist to monitor a greater volume of blood and are about 11 times more sensitive than the thin film, but the presence of the parasite is much more blurred and distinguishing between the different species can be much more difficult (Adebayo, 2016). Microscopy is cheap and can be very specific and sensitive when handled by experts. The reliability of microscopic diagnosis in Nigeria is often jeopardized by poor equipment, unqualified personnel, high workload and inadequate power supply (WHO, 2010).

2.1.3.3 Molecular detection of malaria

WHO advises that molecular malaria detection such as nucleic acid amplification tests should only be considered for epidemiological study. The use of this technique as a malaria diagnostic tool in sub-Saharan Africa is difficult due to availability of equipment, maintenance of chemicals and and trained personnel needed for the work. Although polymerase chain reaction (PCR) is the most sensitive method available with lowest parasite detection limit in the range of 2–5 parasites/μL, it is not suitable for field use, as it is a costly and compslex process (Berzosa *et al.*,2018).

Recently loop-mediated isothermal amplification (LAMP), a nucleic acid based diagnostic tool has been developed. It has equal sensitivity compared to PCR but cheaper, easier and faster. It can be used to target the cause of malaria infections by mass screening and treatment (MSAT) (Nkumama *et al.*,2017).

2.1.4 Drugs used in the treatment of malaria

2.1.4.1 Quinine

The first antimalarial medication to be isolated from the bark of the cinchona tree in 1820 is Quinine and it has been used as one of the most effective treatments for malaria (Achan *et al.*,2011). Cinchona plants are used by Peruvian Indians as folk medicinal items in South America and in the 1700s, they were introduced in Europe (Dolabela *et al.*,2012). Quinine is extracted commercially through solvents extraction from wild species Cinchona grown in South America or Indonesia (Pan *et al.*,2018). Resistance was first reported in the 1980s (Bunnag *et al.*,1996) and as of 2006, quinine was no longer used as a front-line treatment for malaria but is still on the WHO's Model List of Essential Medicines (MLEM) (Model List of Essential Medicines (MLEM), 2018) for the treatment of severe malaria in cases where artemisinins are not available.

2.1.4.2 Mepacrine

Mepacrine (a.k.a quinacrine) was predominantly used throughout the Second World War as a prophylatic, sold under the trade name Atabrine (Green, 1932). This compound is a derivative of methylene blue, another anti-malarial that was discovered in 1891 and found to be an effective treatment for malaria (Guttman *et al.*,1891; Schimer *et al.*,2003). Its use has declined over the years but methylene blue and its derivatives are, however, the subject of increasing current interest (Lu *et al.*,2018) and it is currently in clinical trials as a combination with primaquine (vide infra). Mepacrine itself is no longer used today due to the high chance of undesirable side effects such as toxic psychosis (Edwin *et al.*,2019).

2.1.4.3 Chloroquine

Developed in the 1940s, chloroquine and its 4-aminoquinoline derivative are still commonly used as antimalarial medications. Nevertheless, the efficacy of the drugs has decreased significantly since in the 1960s, due to the development of drug resistance by *P. falciparum* strains, which resulted in a substantial mortality rate associated with malaria (Guerin *et al.*,2002). During the 1940s, chloroquine (CQ) was used to treat all forms of malaria with few side effects (Loeb, 1946). Resistance to CQ was first reported in the 1950s and over the years many strains of malaria have developed resistance. Indeed, resistant strains (K1, 7GB, W2, Dd2, etc.) of the malaria parasite are now used in potency evaluation assays as a way of showing efficacy (Mushtaque, 2015). Chloroquine is on the MLEM for the treatment of *P. vivax* in regions where resistance has not developed (MLEM, 2018).

2.1.4.4 Mefloquine

Mefloquine is a derivative of 4-quinolinemethanol obtained by complete synthesis. This anti-malarial drug was launched in 1985 and can be used to treat moderate or mild malaria (Fidock *et al.*,2004). Mefloquine was developed in the 1970s by the United States Army (Trenholme *et al.*,1975) and is still used today, also being one of the medicines on the MLEM. Originally introduced for the treatment of chloroquine-resistant malaria, it has been used as both a curative and a prophylactic drug. Resistance was first reported in 1986 (Brasseur *et al.*,1986). It is thought that the structurally related quinoline drugs (such as quinine, mepacrine, chloroquine and mefloquine) act through the disruption of haemoglobin digestion in the blood stage of the parasite (Foley, 1997). These drugs are commonly used in combination with a complementary drug (e.g. mefloquine and artesunate, sold as Artequin™) to reduce the chance of resistance development to the quinoline family of compounds. Mefloquine is commonly sold in its racemic form under the brand name Lariam®, however it is no longer widely used due to the perception of central nervous system toxicity that has been suggested to affect a large number of its users (Nevin and Croft, 2016).

2.1.4.5 Qinghao

In the 1970s, the leaves of Qinghao (*Artemisia annua* L.) was the source of yet another antimalaria drug. This compound was clinically active against strains of malaria resistant to chloroquine (Tu, 2011). However, opioid resistance was observed recently in some countries in South Asia: Cambodia, Viet Nam, Thailand, Myanmar and the Democratic People's Republic of Laos (WHO, 2017).

2.1.4.6 Artemisinin

Artemisinin was first isolated in 1971 by Tu Youyou from the plant *Artemisia annua*, a herb that has commonly been used in Chinese traditional medicine (Qinghaosu Antimalaria Coordinating Research Group, 1979). Due to the great positive impact of artemisinin in combating malaria, Youyou was awarded the joint Nobel Prize in Physiology or Medicine in 2015 for "her discoveries concerning a novel therapy against malaria" (The Nobel Prize in Physiology or Medicine 2015). Artemisinin has been shown to be efficacious against all multi-drug resistant forms of *P. falciparum*. A great number of artemisinin derivatives have been produced. Artesunate, arteether (artemotil), artemether and β-dihydroartemisinin (DHA) are the best-known analogs of artemisinin (Sriram *et al.*,2004). Artemisinin and its semi-synthetic forms showed better efficacy than quinine in both adults and children (Zhang *et al.*,2012). These semi-synthetic derivatives are prodrugs which are transformed to the active metabolite, dihydroartemisinin.

The use of artemisinins has been integral in the fight against malaria with ACT making up the majority of modern day treatments (Eastman and Fidock, 2009). Although slow to develop, the first report of resistance to artemisinin was in western Cambodia in 2008 (Noedl *et al.*,2008). Ten years later, in February of 2018, a report was published identifying more than 30 independent cases of artemisinin resistance in Southeast Asia, specifically with resistance to the dihydroartemisinin–piperaquine combination therapy (Amato *et al.*,2018). The mechanism of action (MoA) through which artemisinin acts has been widely debated (O'Neill *et al.*,2010). The most accepted theory is that the molecule is activated by haem to generate free radicals, which in turn damage proteins required for parasite survival (Wang *et al.*,2015; Tilley *et al.*,2016). Still, evidence for a number of other possible mechanisms have been found. In 2013, a computational

approach was taken to determine the MoA based around previous studies which identified haem and PfATP6 (Ca^{2+} transporter) as potential MoAs (Shandilya *et al.*,2013). More recently in 2015, artemisinin was shown to be associated with the up-regulation of the unfolded protein response (UPR) pathways which may be linked to decreased parasite development (Mok *et al.*,2015). Another study showed that artemisinin is a potent inhibitor of *P. falciparum* phosphatidylinositol-3-kinase (PfPI3K) (Mbengue *et al.*,2015).

Currently, the most effective treatment for *P. falciparum* malaria is artemisinins in conjunction with other antimalarial medications, known as based on artemisinin combination therapy (ACT). Presently, five ACTs are available. Four are artesunate-based variants in combination with mefloquine, sulfadoxine-pyrimethamine and amodiaquine (two formulations). The last one is arthemether in combination with lumefantrine (WHO, 2017). Notwithstanding, both artemisininin and artesunate components of the various ACTs and the non-artemisininin-based mixture of atovaquone and proguanil have already developed resistance (Newman and Cragg, 2016).

2.1.4.7 Dihydroartemisinin

Dihydroartemisinin, the most common derivative of artemisinin today, is a prodrug, which is metabolized in the body to pharmacologically active artemisinin (Zani *et al.*,2014). A vital S-transferase membrane glutathione, exported protein 1 (EXP1) of *P. falciparum* can be inhibited by artesunate (Lisewski *et al.*,2014).

2.1.4.8 Amodiaquine

Amodiaquine was first synthesized in 1948 (Berliner *et al.*,1948). It is mainly used for the treatment of uncomplicated *P. falciparum* malaria when used in combination with artesunate and is commonly sold under the trade name Camoquine® or Coarsucam™ (Bompart *et al.*,2011). Similar to chloroquine, amodiaquine's MoA is thought to involve complexation with haem and inhibition of haemozoin formation (Combrinck *et al.*,2013).

2.1.4.9 Piperaquine

Piperaquine was developed in the 1960s as a part of the Chinese National Malaria Elimination Programme (Chen *et al.*,1982). Initially used throughout China as a replacement for chloroquine, resistance led to its diminished use as a monotherapy. While the MoA of piperaquine is not completely understood, studies have suggested that it acts by accumulating in the digestive vacuole and inhibiting haem detoxification through the binding of haem-containing species (Eastman and Fidock, 2009). These days, piperaquine is used as a partner drug with dihydroartemisinin (commonly sold under the trade name Eurartesim®).

2.1.4.10 Lumefantrine

Lumefantrine (a.k.a. benflumetol) was first synthesized in 1976 as a part of the Chinese anti-malarial research effort "Project 523" which also resulted in the discovery of artemisinin (Cui and Zhuan Su, 2009). It is currently sold under the trade name Coartem®. The exact MoA of lumefantrine is unknown, however studies suggest that it inhibits nucleic acid and protein synthesis through the inhibition of β -haematin

formation by complexation with haemin (Combrinck *et al.*,2013). Lumefantrine is currently used only in combination with artemether.

2.1.4.11 Proguanil

Proguanil and atovaquone Proguanil was first reported in 1945 as one of the first antifolate anti-malarial drugs (Curd *et al.*,1945), while atovaquone was first reported in 1991 for the treatment of protozoan infections (Hudson and Randall, 1991). The combination of these, commonly sold as Malarone™, has been marketed by GlaxoSmithKline (GSK) since the early 2000s, and has proven to be a very effective anti-malarial due to the synergistic effect of the two components. This is, in large part, due to the different MoAs for each compound. Atovaquone acts as a cytochrome bc1 complex inhibitor which blocks mitochondrial electron transport (Fry and Pudney, 1992). Proguanil (when used alone) acts as a dihydrofolate reductase (DHFR) inhibitor through its metabolite, cycloguanil (CG) which disrupts deoxythymidylate synthesis. When used in combination with atovaquone however, proguanil does not act as a DHFR inhibitor but has instead been shown to reduce the concentration of atovaquone required for treatment (Srivastava and Vaidya, 1999). Generic atovaquone/proguanil is still available today for the treatment of chloroquine-resistant malaria.

2.1.4.12 Pyrimethamine and sulfadoxine pyrimethamine (PYR)

Pyrimethamine and sulfadoxine pyrimethamine (PYR) was developed in the early 1950s by Gertrude Elion and George Hitchings and is now sold under the trade name Daraprim™ (Russell PB, Hitchings GH 1951). The development of pyrimethamine was a part of the efforts that won Elion, Hitchings and Black the joint Nobel Prize in Physiology or Medicine in 1988 for “their discoveries of important principles for drug treatment”. Sulfadoxine was developed in the early 1960s (Laing, 1965). It is no longer

used as a preventative drug due to high levels of resistance. The combination of pyrimethamine and sulfadoxine was approved for use for the treatment of malaria in 1981 and is now commonly sold under the trade name Fansidar®. Both drugs are known to target the parasite folate biosynthesis pathway (Lumb *et al.*,2011). Pyrimethamine inhibits dihydrofolate reductase, while sulfadoxine inhibits dihydropteroate synthetase.

2.1.4.13 Pyronaridine

Pyronaridine was first synthesized in the 1970s at the Institute of Chinese Parasitic Disease (Chang *et al.*,1992). It has been found to be efficacious against chloroquine resistant strains and has been in use for over 40 years, sold under the trade name Pyramax® (in combination with artesunate). Like lumefantrine, pyronaridine has been found to act through the inhibition of β-haematin formation (Croft *et al.*,2012).

2.1.4.14 Tafenoquine

Tafenoquine (TQ) was first discovered in 1978 at the Walter Reed Army Institute of Research. It was approved by the United States Food and Drug Administration for use as the first new single-dose for the radical treatment of *P. vivax* malaria in over 60 years (US FDA, 2018). TQ is thought to be a prodrug which is metabolized to the active quinone TQ, however the MoA is not well known (Ebstie *et al.*,2016). It is currently sold under the brand name Krintafel.

2.1.4.15 Halofantrine

Halofantrine, developed between the 1960s and 1970s by the Walter Reed Army Institute of Research (Cosgriff, 1982), halofantrine was initially used for treatment against all forms of the Plasmodium parasite. Its use has diminished over time due to a

number of undesirable side effects, such as the potential for high levels of cardiotoxicity. It is only used as a curative drug and not for prophylaxis due to the high toxicity risks and its unreliable pharmacological properties. Halofantrine is still used, under the brand name Halfan™, but only in cases where patients are known to be free of heart disease and where infection is due to severe and resistant forms of malaria (Croft, 2007).

2.1.5 Malaria prevention and control strategies

Taking specific actions have been proved to lessen illnesses and deaths due to malaria. Such actions include:

2.1.5.1 Use of long-lasting insecticidal nets (LLIN) and indoor residential spraying

Malaria-causing mosquitoes bite at night when people should be under the LLINs. LLINs have insecticides bound in their fibres and can be effective for 3-5 years. LLINs have reduced complications like anemia, low birth weight and miscarriage in pregnant women.

Epidemological survey has shown that insecticides and long-lasting insecticidal nets users, along side with environmental management control have reduced the incidence of malaria by 1/3 and mortality due to malaria by half (Bhatt *et al.*,2015). Such strategies rely on a small number of insecticides, with only the resistance of pyrethroids available for LLINs. The development and spread of resistance to insecticides in mosquitoes is therefore a major threat to malaria control. Resistance to pyrethoid is now widespread in Africa with, Zambia, Tanzania, Zimbabwe, Benin, Uganda, Cameroon, Angola, Kenya, Democratic Republic of Congo and Madagascar all inclusive (Ranson and Lissenden, 2016).

2.1.5.2 Environmental management

Environmental management reduces the population of mosquito in a community. Best practices including: development of good drainage systems, screening of windows, filling pot holes with sand, drainage of stagnant waters, controling and covering water reservoirs (WHO,2017).

2.1.5.3 Intermittent preventive treatment of malaria in pregnancy

The World Health Organization has advised that expectant mothers be given a single dose of three sulfadoxine-pyrimethamine tablets at 16 and 36 weeks of gestation to prevent malaria in pregnancy. Sulfadoxine-pyrimethamine tablet is a mixture of 25 mg of pyrimethamine and 500 mg of sulfadoxine (Burrows *et al.*,2013).

2.1.5.4 Use of personal protection and knock-down insecticides and repellants

In addition to the use of LLINs, people should be encouraged to use personal protection measures against mosquito bites (Nkumama *et al.*,2017).

2.1.5.5 Prophylactic drugs

Most of the medicines used for malaria treatment can also be used for prevention. Current medicines used include, mefloquine (Lariam), doxycycline and proguanil hydrochloride combination with atovaquone (Jacqurioz and Croft, 2009). The selection of medication used depends on which drugs the parasites in the region are immune to, side effects of such drug and other factors. The drugs are taken 2weeks before visiting malaria endemic area and must continue for another 4weeks. With the exception of atovaquone and proguanol, which must be started just two days before visit and finished seven days later. Normally, prophylactic drugs are limited to short-term travelers to

malaria regions; due to it negative effect from long-term use and high cost (Roestenberg *et al.*,2009).

2.1.5.6 Genetic control of malaria mosquitoes

Genetically modifying the wild mosquito population to suppress its ability to transmit human pathogens i.e. malaria parasites have been known for over a decade. Nevertheless, it was a major challenge to translate this achievement into the field (ie transgenes are difficult to spread to wild mosquito populations). About 50% of the progeny will bear the transgene when a mosquito carrying an anti-plasmodium transgene mate a wild mosquito. When these offspring encounter wild mosquitoes again, the transgene will decrease in the next generation to 25% frequency. In other words, the transgene is slowly faded out and the only way to overcome the fading is by introducing transgenic males at an extremely high rate (i.e. at numbers approaching the wild population) and constantly repeating the process (Mclean and Jacobs-Lorena, 2016). Recessive genes that make female mosquitoes infertile have been detected as homozygous knockouts. If any of these genes undergo mutagenic chain reaction (MCR), the gene[cas 9-g RNA] copies itself into the gene in the homologous chromosome essentially knocking the gene out of the germline. As a result, almost all female MCR carriers are infertile while males remain unchanged (Hammond *et al.*,2016). Two other genes in their MCR cassette targeting surface proteins of malaria parasite and coding for monoclonal antibodies have been shown to inhibit the growth of parasites in Indian malaria vector, *Anopheles stephensi* (Gantz *et al.*,2015).

2.1.5.7 Vaccines

There is presently no commercially available malaria vaccine, but ongoing efforts are being made to develop vaccines. Futhermore, the most promising candidate for the

vaccine against *P. falciparum*-caused malaria is RTS, S / AS01, which is still under clinical trials (Pan *et al.*,2018).

2.1.6 Genetic Resistance to Malaria infection

Some genetic variations are known to confer protection to malaria infection. These include: Alpha globin chain disorder, sickle cell disease, beta-thalassemia, pyruvate kinase deficiency, glucose-6- phosphate dehydrogenase deficiency, duffy antigens and dantu hybrid gene

2.1.6.1 Alpha globin chain disorder

Alpha-globin chain disorders are prevalent in most malaria endemic regions (WHO, 2015) with gene mapping studies suggesting natural selection through malaria infection (Hedrick, 2012). The clinical manifestations reflect the number of deletions and /or insertions. Single or dual deletions (α-thalassemia trait) have asymptomatic phenotype or manifest mild anaemia, whereas patients with 3 deletions (HbH) or HbH/HbCS have more severe anaemia requiring transfusions. The frequency of α-globin chain disorders is high in Southeast Asia (up to 40%), a region in which multidrug parasite resistance (including to artemisinin derivative) is well known (Woodrow and White, 2017).

2.1.6.2 Sickle cell disease

Sickle haemoglobin (HbAS) is caused by a single-nucleotide polymorphism (SNP) in the globin chain leading to a Glu6Val substitution. It affords protection against mortality from falciparum malaria in heterozygous individuals (Parikh and Rosenthal, 2008). Sickle cell disease (SCD) refers to a group of disorders caused by β-globin gene mutations that produce sickle – shaped erythrocytes. They include the homozygous pattern (HbSS; sickle cell anaemia) and the combination of a single mutation with

another abnormal β globin gene allele (SC, S-β^0 thalassemia). Sickled cells are more easily eliminated by the reticuloendothelial system and more readily undergo hemolysis, leading to anaemia (Sugiarto *et al.*,2018). Patients with sickle cell disease can either spontaneously or in response to stress such as infections (including malaria) undergo rapid decompensation and develop an acute clinical event or 'crisis'. Sickle cell disease is the most common monogenic disease globally with its highest prevalence in malaria-endemic regions of sub-Saharan Africa, reflecting the selection pressure associated with malaria infection (Piel *et al.*,2014). For patients with SCD, infection still occurs but the disease does not progress (Piel and Weatherall, 2014), as the dysfunctional haemoglobin S ultimately prevents further erythrocyte invasion and intra-erythrocyte growth of Plasmodium. Individuals heterozygous for β-globin gene mutations have sickle cell trait (SCT or HbAS) with a lower number of circulating sickled erythrocytes. Although sickle cell trait erythrocytes have increased fragility, patients are not typically anemic and develop symptoms only if they are severely hypoxic or dehydrated. There is strong evidence for protection against malaria in sickle cell trait, but the evidence in sickle cell disease is less consistent (Sugiarto *et al.*,2018).

2.1.6.3 Beta–thalassemia

Beta thalassemia (β-thalassemia) is a group of heritable blood disorders characterized by a genetic deficiency in the synthesis of beta-globin chains. In the homozygous state, beta thalassemia (ie, thalassemia major) causes severe, transfusion-dependent anemia, while in the heterozygous state, the beta thalassemia trait (ie, thalassemia minor) causes mild to moderate microcytic anemia. The total incidence of symptomatic individuals annually is estimated at 1 in 100,000 throughout the world and 1 in 10,000 people in the

European Union (Galanello and Origa, 2010). β-thalassemia trait afford some protection against falciparum malaria (Sugiarto *et al.*,2018).

2.1.6.4 Glucose 6-phosphate dehydrogenase deficiency

Glucose-6-phosphate dehydrogenase (G6PD) is a metabolic enzyme that catalyzes the first reaction in the pentose phosphate pathway (PPP), providing energy for the red blood cell in the form of nicotinamide adenine dinucleotide phosphate (NADPH). NADPH enables RBC to counterbalance oxidative stress through oxidant agents (Baldwin *et al.*,2015). The G6PD gene is located on the X chromosome, as such, it is more prevalent in men (Mayer *et al.*,2009). The G6PD deficiency is the most common cause of hereditary hemolytic anemia and is more prone to oxidative stress from the reduced production of NADPH. The clinical picture of G6PD deficiency involves different degrees of disease severity, which might include hemolytic anemia, neonatal hyperbilirubinemia and asymptomatic cases (Li *et al.*,2012). Glucose-6-phosphate dehydrogenase deficiency results in red blood cells that are more prone to suffering damage. The invasion of malarial parasites aggravates this condition, making the cells highly prone to phagocytosis. Infected G6PD-deficient erythrocytes are morphologically different compared to non-infected ones and are thus hyper prone to phagocytosis (Manganelli *et al.*,2013). Roughly 400 million people living in tropical and subtropical regions exhibit a Glucose-6-phosphate dehydrogenase deficiency (Lelliott *et al.*,2015).

2.1.6.5 Pyruvate kinase deficiency

Pyruvate kinase (PK) is another enzyme associated with energy production, which is also an important factor in the susceptibility to malaria. Its deficiency has been

associated with the reduced survival and increased phagocytosis of parasite-infected erythrocytes (deMendonca *et al.*,2012). Pyruvate kinase deficiency leads to altered membrane rigidity of erythrocytes, thus preventing Plasmodium invasion. Furthermore, pyruvate kinase deficiency significantly reduces the intracellular concentration of glucose, a vital source of energy for the intra-cellular life cycle of Plasmodium. As such, both G6PD and pyruvate kinase have been recognized as important drug targets against *P. falciparum* (Withrock *et al.*,2015). Pyruvate kinase deficiency is the second most common cause of hereditary nonspherocytic hemolytic anemia in humans. PK catalyzes the rate-limiting step of glycolysis, and the energy for erythrocytes is derived from glycolysis, as RBC lack mitochondria. The PK gene is highly pleomorphic and includes 59 SNPs and several loss-of-function variants that might be associated with decreased resistance to malaria (Berghout *et al.*,2012).

2.1.6.6 Duffy antigen

Unlike thalassemais and enzymopathies, the Duffy antigen represents an extracellular-based resistance mechanism. The Duffy antigen, which is a receptor for chemokines, is present on the surface of erythrocytes. This antigen is also an obligatory binding site for the malarial toxin secreted by *P. vivax*. A single nucleotide polymorphism (SNP) in the promoter region controlling expression of the Duffy antigen receptor for chemokines gene (DARC or Duffy; rs2814778), prevents erythrocytic expression and results in the Duffy-negative blood phenotype (Lelliott *et al.*,2015). Duffy is a ligand for a *P. vivax* merozoite protein called the Duffy-binding protein (PvDBP) and the Duffy-PvDBP interaction is crucial for merozoite invasion of the erythrocyte (Gething *et al.*,2012). When there is a point mutation in the Duffy gene, it leads to lack of expression of the encoded receptor and thus negates *P. vivax* toxin attachment. Therefore, Duffy-negative

individuals are protected against *Plasmodium vivax* infection and innocuous polymorphism is the most common in Papua New Guinea and Western Africa, which may explain why infection of *P. vivax* is uncommon in these areas of the world (Mueller *et al.*,2013). In contrast *P. vivax* is common in other malaria endemic areas, such as Asia and South America, where populations do not carry this polymorphism (Howes *et al.*,2011).

2.1.6.7 Dantu hybrid gene

It has been identified that a particular variant that encodes hybrid glycophorin proteins is associated with defense against severe malaria. The reference haplotype carries three glycophorin genes, two of which (GYPA and GYPB) are expressed as proteins on erythrocyte surface. The malaria protective haplotype carries five glycophorin genes, including two hybrid genes that codes the Dantu blood group antigen. The GYPB is replaced by two Dantu hybrid gene to confer protective effect against malaria. This variant decreases the risk of severe malaria by 40 % and has increased frequency in parts of Kenya, yet it appears to be absent from West Africa (Leffler *et al.*,2017).

2.2 Description of Plants used for the Study

The control of malaria has remained a challenge, world health organization (WHO) recommends the use of artemisinin-based combination therapies (ACTs) for treatment of malaria. Despite this guideline, many people in Africa and Asia where malaria is endemic still use herbal medicine to treat malaria, due mainly to its availability and for the purpose of saving cost (Bankian, *et al.*,2018). Medicinal plants have organs that contain chemical substances that could be used for therapeutic purposes or are precursors for drug synthesis (Haruna *et al.*,2017)

Eight medicinal plants (Table 2.1) with folklore base were investigated for antiplasmodial activity. These are:

2.3.5 *Maranthes polyandra*

Maranthes polyandra or *Parinari polyandra* Benth plant is found in West Africa, extending from Mali to Sudan. The plant is known as 'Abo idofin' and 'Gwandan kwosa' in Yoruba and Hausa languages in Nigeria (Odetoye *et al.*,2016). *Maranthes polyandra* belongings to the family of Rosaceae. The tree is about 8m in high with glossy leaves that are elliptical and usually rounded at both ends. The fruits are smooth and usually rounded at both ends. The fruits are smooth and about 2.5cm long having a deep red or blackish purple colour depending on the variety. The fresh seed kernel contains between 31-60% oil depending on the variety and season of harvest, but is not considered edible (Odetoye *et al.*,2014). *M. polyandra* Benth flowers between January and August and produces fruit between March and October. Although the fruits are scarely edible, the leaves are said to be chewed like kola. Oil from the seed is used in the production of alkyd polymer resin, a main raw material in the paint industries and for biodiesel production (Motojesi *et al.*,2011). *Maranthes polyandra* is a plant widely used in used in traditional medicines for a number of aliments. The bark is used for fever, crushed leaves in treatment of fractures and powered roots for curing syphilis (Tor-Anyiin *et al.*,2015). Traditionally the plant is used in Africa as a remedy for malaria disease (Feitosa *et al.*,2012). The oil has been used for the production biodesel (Amos *et al.*,2016).

2.3.6 *Leptadenia hastata*

Leptadenia hastata (pers) decne belong to the family of Asclepiadececae. It is widely distributed in tropical African (Mailafiya *et al.*,2017). *Leptadenia hastata* is a voluble herb with creeping latex stem, glabrescent leaves, glomerulus and recemus flowers as well as follicle fruit. *Leptadenia hastata* serve wild food during seasonal changes and have medicinal uses in many areas. (Umaru *et al.*,2018). Locally, it has been used to manage onchocrciasis, scabies, hypertension, catarrh skin disease and wound healing (Haruna *et al.*,2017). Traditional healers also use it to treat sexual impotence, trypanosomiasis and acute rhinopharyngitis (Bayala *et al.*,2018). Pharmacologically, *Leptadenia hastata* has been reported to have antidiabetic (Ukwuanli and Igbokwu, 2015), antiandrogenic (Bayala *et al.*,2011), anti-implantation (Garba *et al.*,2013), anti-trypanasonal (Haruna *et al* 2017) anti-oxidant (Isaac and Fasihuddin, 2018), anti-fungal and anti-bacterial (Umaru *et al.*,2018) activities. In addition to these, Aluh (2018), has also reported its membrane stabilization potential, while Bello *et al.*,(2011) reported it hypoglycemic and hypolipidemic effects.

2.3.7 *Merremicae hederacea*

Merremicae hederacea (Burm.f) Hallier is a herbaceous, glabrous twiner and climber, belonging to the family of Convolvulaceae, sometimes rooting at the nodes (Akobundu *et al.*,2016). It is a medicinal plant common to riverine forest and grassland, often grow on sandy soil and it is distributed in Africa, Tropical Asia and Australia (Simoes *et al.*,2015). The leaves are simple, ovate, 1.5-5cm long and 1.5-4cm wide (Charles *et al.*,2012). Flowers are solitary or in lax branched inflorescences, stalk 1-7cm long. Flowers are white or pale to bright yellow, bell-long shaped. Its flowering period is December to February (Simoes *et al.*,2015). The fruits are globoid or broadly conical

capsules, indistinctive 4-angled, valves wrinled (Akobundu *et al.,*2016). The seeds are pubescent or glabrous and 2.5mm long (Carles *et al.,*2012). *Merremicae hederacea* is use for treatment of fever. It is also used as a detergent by dwellers of riverine area in Africa before the advent of soap.

2.3.8 *Securidaca longepedunculata*

Securidaca longepedunculata is commonly known as the violet plant. It is a medicinal herb, grown in Savanna belonging to the family Polygalaceae (Sanusi *et al.,*2015). The roots are taken orally either powdered or as infusions for treating chest complaints, headache, inflammation, malaria, tuberculosis, infertility problems, veneral diseases, constipation, urethral discharges, dysentery, rheumatism, fibroditis, toothache, cough, snakebite and wound dressing (Sanusi *et al.,*2015). *Securidaca longepedunculata* have been reported to have numerous pharmacological activities. Abonyi *et al.* (2014) have reported the methanolic leave extract of *S, longepedunculata* to have antioxidant activity. Anti-inflammatory activities have also been found in petroleum ether and methanolic fractions of *S. longepedunculata* at 66.63s and 53.13% respectively (Okoli *et al.,*2006). Furthermore, Karou *et al.* (2012) and Ngonda *et al.* (2012) validated its antifungal activities against *Fusarium oxysporum* and *Candida albicans.* In addition, antiparasitic activities against *Trypanosome brucei* rhodesiense and *Heligmosomides polgyrus* have also been reported (Freiburghous *et al.,*1996 and Adiele *et al.,*2013).

2.3.9 *Polycarpaea linearifolia*

Polycarpaea linearifolia (DC) is commonly known as Mouth or Speech of bird, the Hausas called it ‘Baakin Suudaa’ while the Yorubas refer to it ‘Asamórítaná’ (put flowers in a head) (Burkill, 1985). *Polycarpaea linearifolia* (DC.) DC is an annual herb

of cultivated or fallowed land, belonging to the family of caryophyllaceae. It grows on grassland Savanna to about 60cm high, occurring throughout region from Senegal to Nigeria. It resembles *Polycarpeae carymbosa* (old man's white head) but can be distinguished by the globose totally white flower heads. *P. linearifolia* have narrow, parallel-sided and linear leaves (Hutchinson *et al*, 1954). Infusion of *P. linearifolia* is used to combat fatigue whole plant is use as fodder, although horses and cattles sometimes shun the plant (Burkill, 1985). Locally, *P. linearifolia* is used to treat fever. Scientific research base on this plant is scare.

Of the three plants, *P. linearfolia*, *P. corymbose* and *P. eriantha* belonging to Caryophyllaceae family, only *Polycarpaea corymbose* lam have been of interest to phytochemist, literature search has not shown any work or report on *P. linearfolia* and *P. eriantha*. *P. corymbose* is an herb of annual or perennial, small shrubs with taproots slender to stout, stems erect, branched, terete, leaves opposite, sometime appearing whorled. It is a medicinal plant used to traditionally to treat various ailments (Sindhu and Manorama, 2012). Extracts of *P. carymbosa* have been reported to have antitumor (Balamurugan *et al.,* 2013), antimicrobial (Sindhu and Manorama, 2012) and antidiabetic (Balamuruga *et al.,* 2013) activities. It has also been reported to be a fertility enhancer (Mohen *et al.,* 2013). Compounds that have been isolated from *P. carymbosa* include camelliagenin A, Lupeol, A1-barrigenol, stimastanol, apoanagallosaponin IV, n-hexadecanoic acid, 5-hydroxymethyl furfural along with some phenols and flavonoids (Modi and Shah, 2017).

2.3.10 *Agelanthus dodoneifolius*

Agelanthus dodoneifolius (synonymous to *Tapinanthus dodoneifolius*) DC Danser (Lorenthanareae) is a unbiquist which largely grow in west Africa. The plant is found

on many host trees such as *Mangnifera indica*, *Parkia biglobas*a *Ziziphus spina-christs* and *Azadirachta indica* (Builder *et al*, 2012). Despite their destructive nature to their host, the plant is generally used medicinally for treatment of various ailments (Inusa *et al.,* 2018). *Agelanthus dodoneifolius* also known as African misteloe is used as a remedy to treat wounds, stomach ache, diarrhea, cholera, nervous confusion, cardio vascular and respiratory diseases (Ouedraogo *et al.* 2007). Some of these claims have been validated by researchers. Ouedraogo *et al.,* (2011) reported the hypotensive property of the dodoneine present in *Agelanthus dodoneifolius.* Antioxidant and anti-inflammatory activities have also been reported by Boly *et al.,* in 2015 and 2016 respectively. Modulatory, antiplasmodial and antibacterial activity of *Agelanthus dodoneifolius* have been validated by the works of Boly *et al.* (2011), Builder *et al.* (2012) and Inusa *et al.* (2018) respectively.

2.3.11 *Zanthoxylum zanthoxyloide*

Zanthoxylum zanthoxyloide Lam (Rufaceae) also known as *Fagara zanthoxyloide* is an indigenious plant used widely as chewing stick for teeth cleaning in west Africa (Ynalvez *et al.,*2012). It is a common component of rain forest vegetation found most abundantly in the drier parts of south-western Nigerian. The plant is also used in folklore medicine to treat different diseases including stomach diseases, rheumatism, and leprous ulceration, and also as antislucking, antiparasitic antiseptic and analgesic agent (Oralidiya *et al.,*2010). Pharmacologically, *Zanthoxylum zanthoxyloide* have been proved to have antioxidant (Chaaib *et al.,*2003), antibacterial (Misra *et al.,*2013) antimicrobial (Anne *et al.,*2013), anti-inflammatory (Folasade *et al.,*2006), antiparasitic (Azando *et al.,*2018) anti-sickling (Nurain *et al.,*2010 and Imaga, 2013) and anticancer (Patel *et al.,*2010) activities. It has been reported that the extract of this plant contains

aporphines, lembetarine, berberine, magnoflorine, furoquinoltines, 8-methoxydictamine, skimmanine, 3-dimethylallyl-4-methony-2-quinoline, fagaronine, dihydroavicine, chelerythrine and canthine-6-one (Orafidiya *et al.*,2010).

2.3.12 *Lophira alata*

Lophira alata Banks ex C. F. Gaertn is a plant that belongs to Ochnaceae family. It is usually straight, without buttress roots but sometimes with swollen base and is usually clear of branches up to about 30m with globrous twigs and found in subtropical and tropical moist lowland forest of Cameroon, Congo, Ivory Coast, Equatorial Guinea, Gabon, Ghana and Nigeria (Ibukunoluwa *et al.*,2015). It is a highly valued timber that is well-renowned for use in local wood industry for furniture production. These seeds are flat, circular (diameter about 1.5-2cm). Its seeds are bulging and elongated weighing about 1.0g each. Seedings grow rapidly and produce abundant dark red roots which are coarsely branched and devoid of root hairs (Onguene *et al.*,2011). Traditionally, the bark is used in treatment of inflammation, toothache and as analgesic. Some of its parts are also used in the treatment of malaria (Ibukunoluwa *et al.*,2015). The use of the leaves as an antimalaria agent has been validated (Falade *et al.*,2014). *Lophira alata* also have antiviral potential against hepatitis B virus, (Negbenebor *et al.*,2017).

Table 2.1: Botanical, Common and Local Names of Plants used in this Study

Scientific name	Common name	Local names		
		Hausa name	Yoruba name	Igbo name
Merremia hederacea	Ivy woodrose		Ata koko/adere eko	
Leptadenia hastata	Akamongot /Anvara	Yadiya /Dan zindri	Iran-aji- Igbo	Isanaje-Igbo
Neocarya macropylla	Neou oil tree/Ginger bread plum	Gawasa/Bakar rura	Rura	
Lophira alata	Ironwood	Namijin kade	Iponhon	Okopia
Polycarpaea linearifolia		Baakin/Baakin sudaa	Asamoritana	Eyin-ire
Securidaca longepedunculata	Violet tree	Uwar magunguna/ Sanya	Ofodo/ ipeta	Ezeogwu
Zanthoxylum zanthoxyloidess	African satinwood	Fasakuwari	Ata/ Orin-ata	Aya
Agelanthus dodonefolium	African misletoes	Kauchi/itche/ kawshyi	Agenerigbo/ Afomo onisana	Elozie

Neocarya macrophylla

Merremia hederacea

Agelanthus dodoneifolius

Securidaca longepedunculata

Securidaca longepedunculata

Leavwes

Securidaca longepedunculata root

Polycarpea linearfolia

Polycarpea linearfolia

Lophira alata

Leptadenia hastata

Leptadenia hastata

Zanthoxylum zanthoxloide

Plate I: Pictures of Plant Parts Used

2.4 Natural Plant Secondary Metabolites

Medicinal plants constitute a significant part of greenery and are generally dispersed in Nigeria. The pharmacological assessment of plant constituents is a built-up strategy for identification that could lead to the development of a novel and safe medicinal agent (Alluri *et al.*,2005). The screening of traditional medicinal plants is not only for natural lead compounds against malaria disease but also safe active compound (Ch'ng *et al.*,2013). Notwithstanding a few classes of common items of natural origin exhibit antiplasmodial potential displayed by many herbs utilized in customary medication, the most significant and different bioactivity has been detected in sesquiterpene, alkaloids, and lactone quassinoids. Non alkaloidal natural compounds with antiplasmodial and antimalarial properties belonging to the classes of terpenenes, limonoids, flavonoids, chromone, xanthones and related compounds are also been reviewed (Naghibi *et al.*,2013).

2.4.1 Alkaloids

Alkaloids are remarkably different collections of blends in the plant realm, containing a nitrogen atom located in the heterocyclic ring and a ring structure having a striking biological action on animals and human when administered in little amount (Aguiar *et al.*,2012[b]). There are several classification base on their biosynthestic precursor, pharmacological action and chemical properties, but the common and most accepted classification is the one based on chemical entity (Kaur *et al.*,2017).

Classification based on biosynthetic precursor include indole (alkaloids of tryptophan origin), piperidine (alkaloids of lysine origin), pyrrolidine containing alkaloid synthesized from ornithine, imidazole (alkaloids derived from histidine) and phenylethylamine (alkaloids derived from tyrosine) alkaloids (Kumar, 2014).

Classification of alkaloids based on pharmacological action include strychnine with reflex excitability action, morphine with analgesic property, boldine with laxative property, lobeline capable of respiratory stimulation, pilocarpine with miotic potential, aconitine having neuralgia property, quinine as antimalaria, ergonivine as oxytoic and ephedrine as bronchodilator (Kumar, 2014). Classification of alkaloids could also be based on basic heterocyclic nucleus (chemical). These include pyrrolidine, piperidine alkaloids, pyrrolizidine, tropane, quiniline, isoquinoline aporphine, indole, imidazole, diazocine, purine, steroidal, amino and diterpene alkaloids (Kaur *et al.*,2017).

Alkaloids have also been classified based on the presence or absence of heterocyclic ring. They can therefore be heterocyclic (typical) alkaloids and non- heterocyclic (atypical) alkaloids (Evans, 1996). Among the active components in plants that are of utmost significance are alkaloids. Some alkaloids have been successfully developed into chemotherapeutic drugs, such as tetrandine and piperine with anticancer properties, quinine and its derivatives as antiplasmodial agents (Aguiar *et al.*,2012[b]).

Literature search indicates a host of antiplasmodial alkaloids have been derived from African flora, ranging from indole alkaloids, amides, cryptolepines and many yet to be identified (Onguine-Amoe *et al.*,2013). The use of such compounds as antiplasmodial agents can only be enchanced when empirical evidence exists for their efficacy and toxicity profile. They could also be used as template for the synthesis of more active, less toxic drug derivatives because drug efficacy, pharmacology and toxicity are important parameters in the selection of compounds for development.

Garavito *et al.* (2006) reported *Abuta grandifolia* alkaloidal crude extract exhibited *in vivo* model activity, inhibiting 66% of the parasite growth at 250 mg/kg/day. Alkaloids based antimalarials are divided into naphthylisoquinolines, bisbenzylisoquinolines,

protoberberines and aporphines, indoles, manzamines, and miscellaneous alkaloids (Kaur *et al.*,2009).

Dioncopeltine A, dioncophylline B and dioncophylline C are Naphthylisoquinoline alkaloids isolated from *Triphyophyllum peltatum* (Dioncophyllaceae) extract, exhibited high antiplasmodial activity in *P. berghei* infected mice (Francois *et al.*,1997). Sener *et al.* (2003) also isolated four naphthyliisoquinolines of alkaloids, lycorine, crinine, tazettine, and galanthamine exhibiting antimalarial activity at different potencies, from amaryllidaceae plants namely *Pancratium maritimum, Leucojum aestivum*, and *Narcissus tazetta*, found in Turkey.

Mambu *et al.* (2000) reported strong antiplasmodial activity of bisbenzylisoquinoline alkaloids (curine and isochondodendrine) isolated from the stem bark of *Isolonaghe squiereina* (IC_{50} = 353 and 892 nM, respectively). *Pseudoxandra cuspidata* bark has good antimalarial activity and furthermore, its bio-assay-guided fractionation by Roumy *et al.* (2006) led to the isolation of an unusual azaanthracene alkaloid (IC_{50} = 42.92 lM), a bis-benzylisoquinoline alkaloid (1S, 1'R)-rodiasine (IC_{50} = 1.14 lM) along with alkaloids O-methyl-punjabine and O-methyl-moschatoline.The antimalarial activity of this bark was mostly due to rodiasine, which was most active and least toxic of these alkaloids.

Wright *et al.* (2000) evaluated antimalarial activities of several protoberberine group containing alkaloids, which exhibited promising antiplasmodial activities. Dehydrodiscretine (IC_{50} =0.64 lM) and berberine (IC_{50} = 0.96 lM) were the most active. Hadranthine A and B are 4,5-dioxo-1-azaaporphinoids obtained from *Duguetia hadrantha*. Hadranthine A exhibited *in vitro* antimalarial activity against *P. falciparum* with IC_{50} = 120 ng/mL, while B was inactive (Muhammed *et al.*,2001).

10-Hydroxyusambarensine, a tertiary phenolic bisindole alkaloid isolated from the roots of *Strychnos usambarensis* displayed modest antimalarial activity (IC_{50} = 0.16 lg/mL, W2 strain) (Frédérich *et al.*,1999). Paulo *et al.* (2000) also isolated seven alkaloids from *Cryptolepiss anguinolenta* and tested against K1 and T996 strains of *P. falciparum.* The indolobenzazepine alkaloid cryptoheptine was the second most active in this series after cryptolepine (IC_{50} = 0.8 and 0.23 lM, respectively).

Enantiomers of 8-hydroxymanzamine A, manzamine F and a manzamine dimer, possessing antiplasmodial activities were isolated from Indo-Pacific sponge (Petrosiidae) (El Sayed *et al.*,2001).

Wright *et al.* (2002) reported an extensive study on two novel alkaloids, lepadins E and F isolated from a tropical marine tunicate Didemnum specie., which showed significant antiplasmodial activity (IC_{50} = 0.4 and 0.2 lg/mL against K1 strain, and 0.9 and 0.3 lg/mL against NF54 strain, respectively). Chea *et al.* (2007) also reported the antimalarial activities of four major alkaloids from *Stephania rotunda*: dehydroroemerine (IC_{50} = 0.36 lM), tetrahydropalmatine (IC_{50} = 32.6 lM), xylopinine (IC_{50} = 52.3 lM) and cepharanthine (IC_{50} = 0.61 lM).

2.3.2 Flavonoids

Flavonoids are a group of essential low molecular weight substances based on 2-phenyl-chromone nucleus of plant origin (Figure. 2.2). They are polyphenol containing structure which are secondary metabolites produced by plants. Flavonoids contains C6—C3—C6 rings and are commonly found either in an esterified or glycosylated form, consisting of, namely rings A and B linked by three carbon-ring C (Isola *et al.*,2014). They are biosynthesized from derivatives of acetic acids/phenylalanine by means of shikimic acid pathway (Rudrapal and Chetia, 2017 and Wang *et al.*,2018[a]).

Flavonoids perform different biological activities in plants where they are synthesized, and also in the life of bacteria and animals that feed on plants. Flavonoids confer both the aroma and colour observed in flowers and fruits, so as to entice pollinators (Griesbach, 2005).

2.3.2.1 Classification of flavonoids

Flavonoids can be divided into various groups relying upon the carbon of the C ring to which the B ring is linked and the level of oxidation and unsaturation of the C ring as shown in Figure 2.2. Whenever the B ring of flavonoids is connected to position 3 of the C ring, such flavonoids are referred to as isoflavones. However, when the B ring is connected to the C ring at position 4, such flavonoids are known as neoflavonoids. The flavonoids having the B ring connected to the C ring at position 2 are in addition grouped into a few subclasses based on the specific features of C ring structure. The subclasses include: flavones, flavanones, flavanonols, flavonols, anthocyanins, flavanols or catechins, and chalcones (Sore *et al.*,2018).

a) **Flavones:** One of the most significant subclass of flavonoids are flavones. Usually, flavones contain a ketone group in position 4 of the C ring and double bond in between position 2 and 3 of the C ring. Greater number of flavones of fruits and vegetable source commonly possess hydroxyl functional group in the 5^{th} position of the A ring, whereas presence of hydroxyl group in different positions of A ring, mostly in position 7 or 3′ and 4′ of the B ring, do change based on the ordered characterization of the specific fruit or vegetable. Apigenin, tangeritin and luteolin, shown in figure 2.2 are members of flavones subclass of flavonoids. Tageretin, nobiletin and sinensetin are polymethoxylated flavones majorly contained in the peels of citrus fruits (Manach *et al.*,2004).

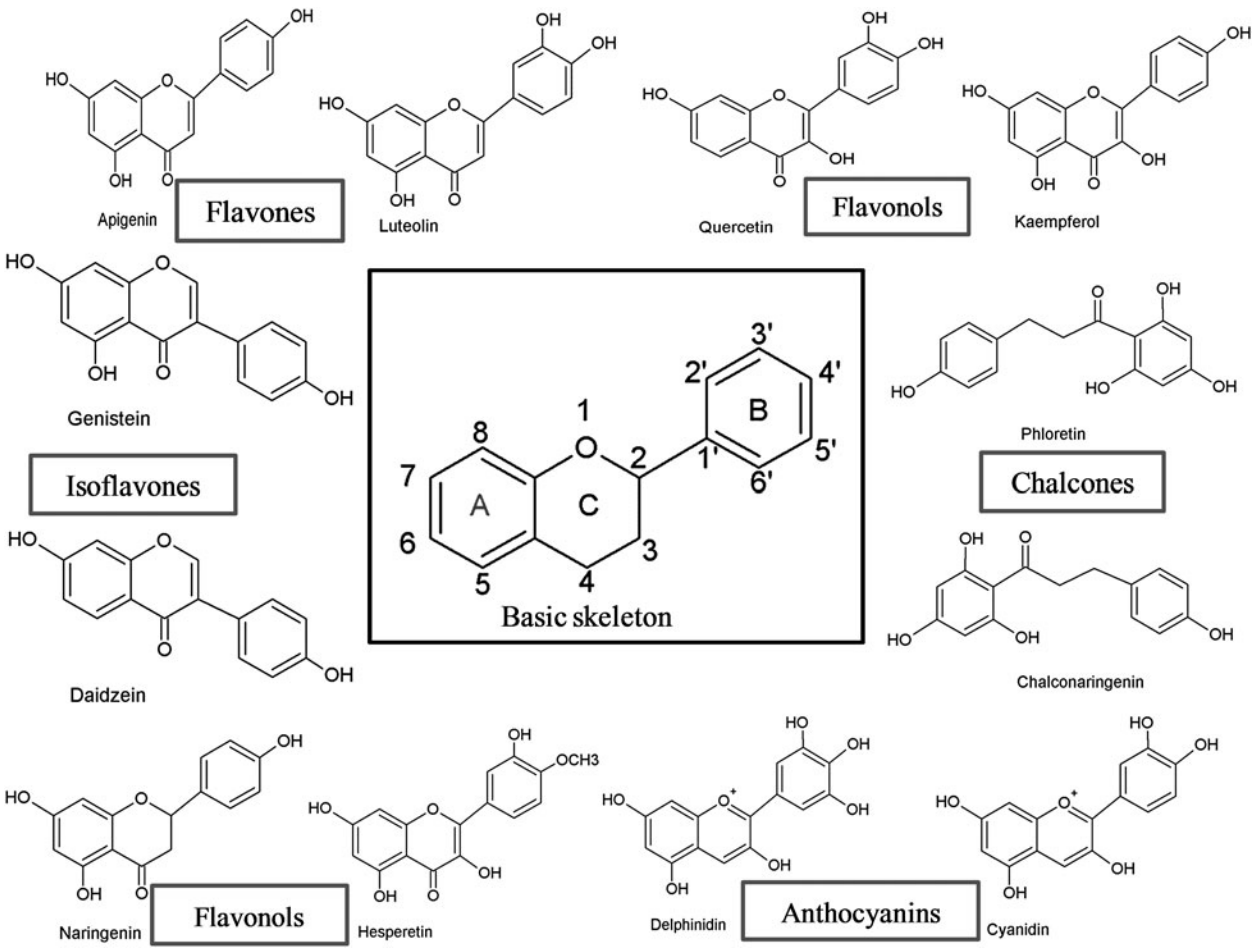


Figure 2.2: Classes of flavonoids and their basic skeleton structure
Source: Panche *et al.*,2016.

b) **Flavonols:** Flavonoids that contain ketone group are called flavonols. Flavonols are the basic units of proanthocyanins. Unlike flavones, the hydroxyl group on the C ring in flavonols is usually at position 3. The hydroxyl group could also be glycosylated as observed in flavones. Just as in flavones, the pattern of hydroxylation and methylation in flavonols are widely different. When the pattern of glycosylation is put into consideration, flavonols seem to be the most common and biggest subclass of flavonoids present in vegetables and fruits (Iwashina, 2013). Quercetin, fisetin, kaempferol and myricetin are the most studied flavonols (Panche *et al.*,2016).

c) **Flavanones:** Dihydroflavones, majorly known as flavanones possess a saturated C ring. The sole difference between the structures of flavanones and flavonoids is that, unlike flavones, the double bond between positions 2 and 3 in flavanones is saturated (Wang *et al.*,2018[a]). Generally, Flavanones have been found to be present in all citrus fruits; lemons, grapes and oranges. Eriodictyol, hesperitin and naringenin are members of this group of flavonoids. The bitter taste observed in the peels and juice of citrus fruits are due to the presence of flavanones (Panche *et al.*,2016).

d) **Isoflavonoids:** One of the most distinctive and largest subgroup of flavonoids are isoflavonoids. Leguminous plants and soyabeans are the major plants from which isoflavonoids are predominantly found. Experimental reports have also shown that isoflavonoids are present in microorganisms. Genistein and daidzein are examples of isoflavonoids (Sore *et al.*,2018).

e) **Flavanols:** These are Catechins or flavan-3-ols. The 3-hydroxy derivatives of flavanones are dihydroflavonols also known as flavanonols, or catechins. Flavanols possess hydroxyl group that is usually attached to C ring at position 3. The absence of double bond between positions 2 and 3 in flavanols has been reported (Sandhar *et al.*,2011). This is unusual in many of the known flavonoids.

f) **Neoflavonoids:** The class of polyphenolic containing flavonoids are called neoflavonoids. The presence of a 4-phenylchromen support without hydroxyl group substitute at position 2 of neoflavonoids differentiate them from flavonoids that possess a 2-phenylchromen-4-one backbone (Sore *et al.*,2018).

g) **Chalcones**: Chalcones are a subclass of flavonoids. They are characterized based on the lack of C ring that is usually present in the structure of flavonoid skeleton as shown in Figure 2.2. Therefore, they are mostly referred to as open-

chain flavonoids. phloridzin, arbutin, phloretin and chalconaringenin are the major members of class of flavonoids called chalcones (Panche *et al.*,2016).

h) **Anthocyanins**: The colours in flowers, fruits and plants are due to the presence of a group of pigments called anthocyanins. The most studied anthocyanins include malvidin, cyanidin, delphinidin, peonidin and pelargonidin. Anthocyanins predominantly occur in outer layers of several fruits. Acylation or methylation of the hydroxyl groups on both B and A rings as well as pH determine the colour of anthocyanin (Iwashina, 2013).

2.3.2.2 Flavonoids with antiplasmodial activity

Quercetin and its derivatives belonging to the class of flavonols have been reported to exhibit significant antiplasmodial activity against *P. yoeliinigeriensis*, chloroquine sensitive and resistant strains of *P. falciparum* (Ganesh *et al.*,2012, Hassan *et al.*,2013 Ezenyi *et al.*,2014). Similarly, kempferol and its derivatives are also known flavonoids that have been reported to have antiplasmodial activity (Cai *et al.*,2016). Erythrinasinate B and lupeol are also flavonoids with antiplasmodial activity against Chloroquine sensitive strain of *P. falciparum* (Chukwujekwu *et al.*,2016). Nymphaeol A, B, C and solophenol D are flavonoids isolated from *Marcaranga tanarius* with antiplasmodial activity against *P. falciparum* strain 3D7 (Marliana *et al.*,2018). Recently, Artonin A, E, F and biflavanonone 1 and 2 have potency against Chloroquine resistant strain K1 (Sore *et al.*,2018).

There are two main targets of the mechanism of flavonoids that is capable for inhibiting the growth of Plasmodium:

1) malaria parasite food vacuoles by inhibiting the process of hemoglobin degradation.

2) membranes formed intraerytrhocytic stage malaria parasite i.e. New Permeation

Pathway (NPP) by inhibiting the transport of nutrients needed by parasites (Meler *et al.*,2018). It has also been reported that flavonoids could also act probably by preventing the inflow of myoinositol and L-glutamine into infected erythrocytes during intraerytrhocytic phase of *Plasmodium* life cycle (Rasoanaivo *et al.*,2011).

2.3.3 Terpenes

Terpenes are non nitrogenous plant secondary metabolites, that are synthesized from isoprene unit (five carbon unit). They are the most abundant secondary metabolite in nature (Ivanescu *et al.*,2015). Terpenes are classified based on the number of five carbon units they contain as shown in Figure 2.3. Ten- carbon terpenes, which contain two isoprene units are called monoterpenes, examples include geraniol and limonene. 15-Carbon terpenes, that contain three isoprene units are sesquiterpenes, example is farnesol. 20-carbon terpenes with four isoprene units are diterpenes while 30-carbon terpenes with six isoprene units are triterpene and 40-carbon terpenes having eight isoprene units are called tetraterpene, lycopene and β-carotene belong to this class of terpenes. Terpenes with 50 and above carbon atoms are polyterpenes (Katerova *et al.*,2012).

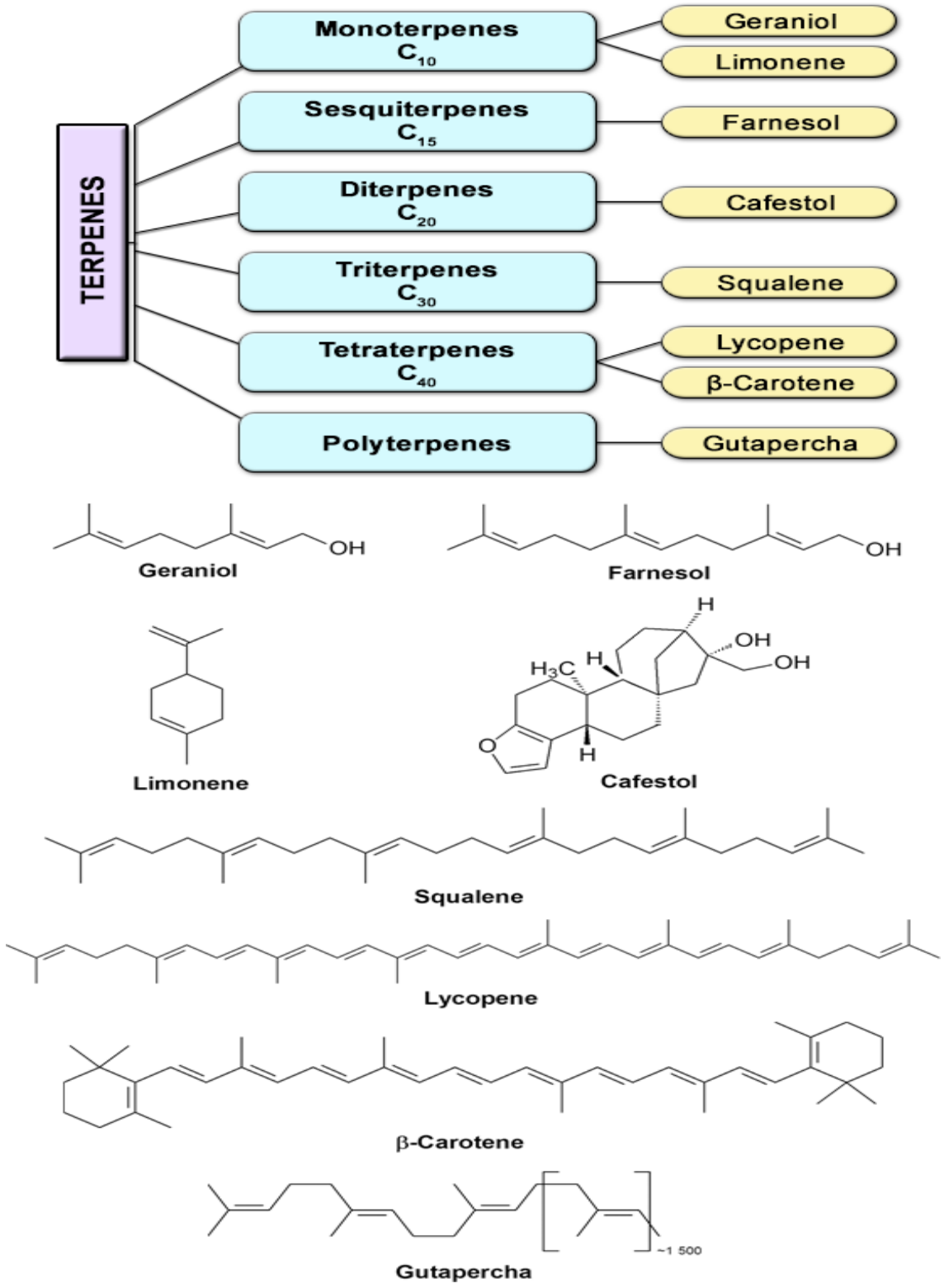


Figure 2.3: Classes of Terpenes and some examples
Source: Katerova *et al.*,(2012).

Studies have shown that numerous terpenes and their derivatives gotten from different sources ranging from marine fungi to terrestrial plants are capable of killing parasites that cause malaria, especially *P. falciparum* (Mayer *et al.*,2009).

Several terpenes have been reported to have antiplasmodial activity. Examples include artemisinin derived from the plant *Artemisia annua*, artemether, artesunate and dihydroartemisinin that are commonly known and use to treat malaria. They are the class of compound reccommeded by WHO to be used in combined thrapy (Li *et al.*,2007). Isomukaadial acetate, naghibione is a sesquiterpene with *in vitro* and *in vivo* antiplasmodial activity against Chloroquine sensitive *Plasmodium falciparum* and *P. berghei* (Nyaba *et al*, 2018 and Naghibi *et al.*,2015) Linalool, nerglidol, farnesol and limonene are all terpenes, having antiplasmodial activity *in vitro* and *in vivo* (Goulart *et al.*,2004).

There are two major mechanisms by which terpenes elicit antiplasmodial activity. The first involves blocking the degradation of hemoglobin and inhibition of toxic heme biocrystalization (Afshar *et al.*,2016). Artemisinin and its derivatives act using this mechanism. Due to the presence of endoperoxide bridge, they interact directly with the intraparasitic iron to form free radical which alkylates malaria-specific protein, thus damaging the microogranelles and membranes of the parasite (Haynes *et al.*,2013). The second mechanism of action involves inhibition of isoprene biosynthesis (Goulart *et al.*,2004). Other mechanisms include induction of oxidative stress *via* generation of H_2O_2 and NO. Bakuchiol is monoterpene with antiplasmodial activity and acts as oxidative stress inducer *via* generation of H_2O_2 and NO, causing mitochondrial membrane depolarization and DNA fragmentation leading to parasite cell death (Cheema *et al.*,2017).

2.5 Review of Analytiacal Methods

The hyphenated techniques enable the natural product researchers to use extremely powerful new tools to achieve excellent separation efficiency as well as to obtain online complementary spectroscopic data of the peak of interest within a complex sample mixture. Two major factors play a vital role in natural product research, the separation and purification of bioactive constituents in crude plant samples and their accurate structural identification (Patel *et al.*,2010). Rapid and accurate detection of bioactive phytocompounds is crucial in the investigation of crude plant samples for lead against pathological conditions (Boligon and Athyade, 2014). The knowledge of different types of analytical procedures is essential for any researcher who has to choose the most suitable technique for the choice of sample, considering the equipment available for use. The best methods employed in determination of bioactive compounds are chromatographic techniques, particularly high-performance liquid chromatography (HPLC) with different detection methods, followed by gas chromatography (GC). Due to the difficulty associated with these techniques coupled with their being expensive and time consuming nature, some analytical techniques that are inexpensive and readily available to the laboratories, such as thin layer chromatography (TLC) and spectrophotometric methods (UV-Vis) (Ivannescu *et al.*,2015).

2.5.1 UV-Vis Spectrophotometry

Spectrophotometric technique measures the amount of a chemical constituent based on the intensity of light absorbed when a beam of light is allowed to pass through the sample solution. The principle upon which the technique is based is that individual constituent transmits or absorbs light within a wavelength range. Also, spectrophotometric technique can be employed in measuring the quantity of a known chemical constituent (Bunaciu *et al.*,2013). Although, spectrophotometry is a simple,

cheap and handy technique in the laboratories, except for some analytes without particular chromophore groups within the molecule, a problem may ensue, because they may not have substantial absorption within UV-Vis wavelength. Also, absence of specific functional groups may prevent some analytes from being able to undergo chemical reaction with specific compounds in order to produce colored products. For these reasons, analysis using UV-Vis spectrophotometry is not an easy task (Lawal *et al.*,2012).

2.5.2 Chromatography

Chromatographic techniques contribute significantly in the area of natural product research, especially regarding identification, separation, and characterization of bioactive compounds from plant sources (Costa *et al.*,2015). Chromatography is an analytical technique used for the separation, purification and identification of constituents from the mixture. It works on the principle of differential interaction (partitioning) of solutes between two different phases, viz., the stationary phase and the mobile phase (Ravaili *et al.*,2011). Chromatography could be for preparative or analytical purposes. Analytical purpose of chromatography is to determine the chemical composition of a sample while Preparative purpose is to purify and collect one or more components of a sample (Patel, 2018).

Differences in the distribution coefficient between the stationary and mobile phases is the major principle upon which chromatographic separation technique is based. Depending on the nature of the mobile phase i.e liquid, gas or solid, chromatographic technique is classified as liquid chromatography (LC), gas chromatography (GC), and supercritical fluid chromatography (SFC). However, based on regular forms of the stationary phase, chromatography is classified as thin layer chromatography (TLC),

paper chromatography (PC) and column chromatography (CC). Generally, among the LC methods, column chromatography using liquid as mobile phase is commonly. The modification of liquid column chromatography gave birth to High performance liquid chromatography (HPLC) (Pang *et al.*,2016). Based on its capability to separate complex samples, liquid column chromatography has greater advantage. When mixtures are to be separated, LC is the best option but cannot be used in getting the required information about the structure of the constituent compounds, when used alone except if coupled to a detection system (Liu and Liu, 2016).

The interface of the chromatographic method with a mass spectrometer (MS) detection system is efficient to provide rapid qualitative determination and identification of unknown compounds from natural products extracts (Yang *et al.*,2009). Liquid chromatography-mass spectrometry (LC-MS) combines the great separation ability of complex samples by LC and the high qualitative ability of mass spectrometry which has been used widely in medicinal research because of its high sensitivity and selectivity (Wu *et al.*,2013). MS is commonly used in drug research because of its high sensitivity, high selectivity and its capability to provide information on both structural characteristics and the molecular mass of a compound. Mass spectrometry finalizes the qualitation by the use of structural information and molecular mass and then completes quantitation using the relationship of the peak and compound content that the peak represented. Both atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) are the major components of atmospheric pressure ionization (API) of MS (Korfmacher *et al.*,2005). For many types of compounds, ESI has high sensitivity. For the analysis of volatile compounds and the less polar ones, APCI is suitable while ESI is suitable for polar and nonvolatile compounds (Allwood and Goodacre, 2010).

In ESI-MS, purification of sample and procedures for derivative development are somewhat simple when compared with traditional GC/MS techniques. The connection between ionization procedure and applicable analytes is as depicted in Figure 2.4. Since HPLC alone, can measure a large number of compounds, the use of APCI or ESI as the ionization protocol now allows for measuring of broader range of natural compounds as well.

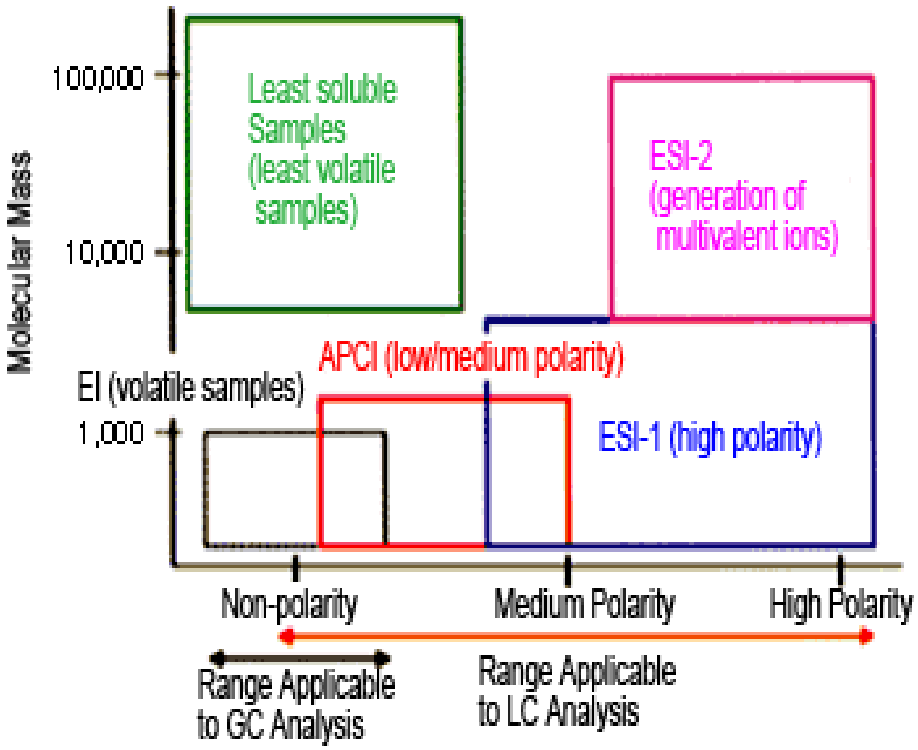


Figure. 2.4: Ionization Methods and Applicable Compounds
Source: Johnson, (2000)

ESI-MS is a tool for studying heat labile and non-volatile biomolecules that are not responsive to analysis by other general techniques especially at at femto-mole quantities in micro-litre sample volumes. In addition to HPLC capability for molecular fractionation before mass spectrometric analysis, HPLC/ESI-MS is now a powerful technique with the ability to analyse either small or large molecules of different polarities in a multifarious biological sample (Ho *et al.*,2003). ESI mode is ideal for

LC–MS analysis of secondary metabolites from plants. It is a soft ionization technique capable of producing small fragmentation patterns through electrical energy, which allows the ions to transfer from liquid to gaseous phase (Steinmann and Ganzera, 2011) before being analyzed in mass spectrometer (Ho *et al.*,2003). Ionic compounds having high polarity are best analysed using ESI (Figure 2.5). Both positive and negative ionization modes are used in natural product analysis (Cuyckens and Claeys, 2004).

The transfer of analyte from solution to gaseous phase requires three major steps:

i. Charged formation droplets from capillary tip with high voltage from where the solution of the analyte is injected.
ii. The evaporation of solvent from the charged droplet.
iii. Formation of gaseous phase ion which is maintained at a high voltage (2.5–6.0 kV) to the wall of the surrounding chamber ((Banerjee and Mazumdar, 2012).

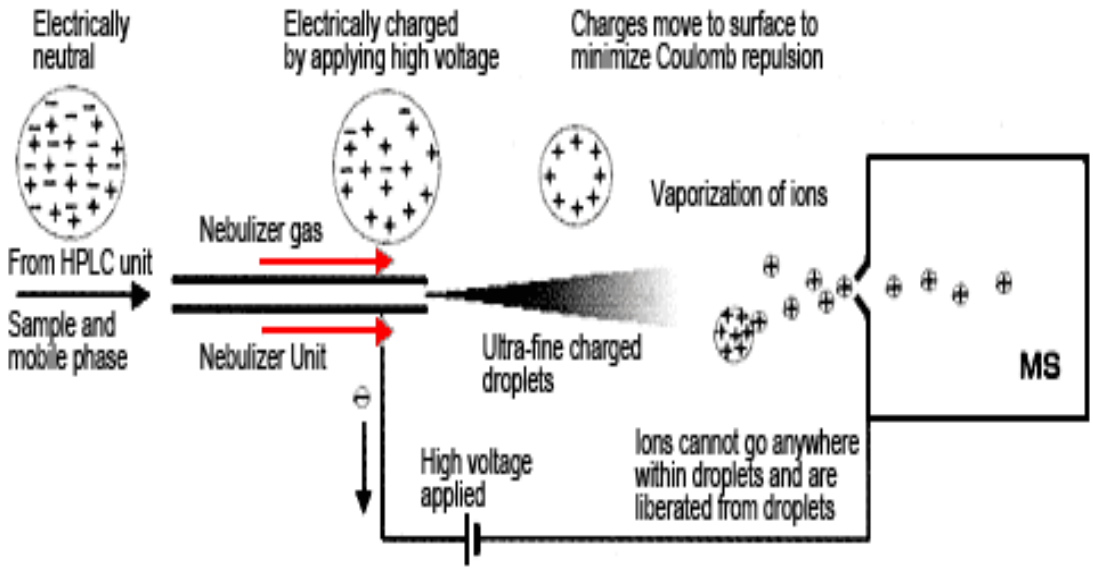


Figure 2.5 Evaporation of Ions in Electrospray Ionization (ESI)
Source: Ashcroft, (1997)

Chemical ionization, a principle employed in CI for GC-MS, is equally employed in the atmospheric pressure chemical ionization (APCI) as the alternative technique (Figure

2.6). Although there is similarity in the interface design in ESI, there still exist, differences in the ionization principle, making it most suitable majorly for compounds having medium and low polarity. Atmospheric pressure chemical ionization vaporizes both the sample molecules and solvent when the sample solution is sprayed into a heater (already heated to 400 °C) using gaseous nitrogen, N_2. To generate ions required for stable reaction, corona discharge ionizes the solvent molecules. Between the sample molecules and reaction ions (molecule- ion reaction), protons are usually transferred to ionize the sample molecules by either removing or adding a proton. Generally, the ion-molecule reactions involve different patterns, such as electrophilic addition reactions and proton-transfer reactions. Just like ESI, mainly deprotonated molecules (or protonated molecules) are identified. Thus, its use in analyzing compounds that do not undergo ionization in water and highly fat-soluble compounds (Ashcroft, 1997 and Bruins, 1998).

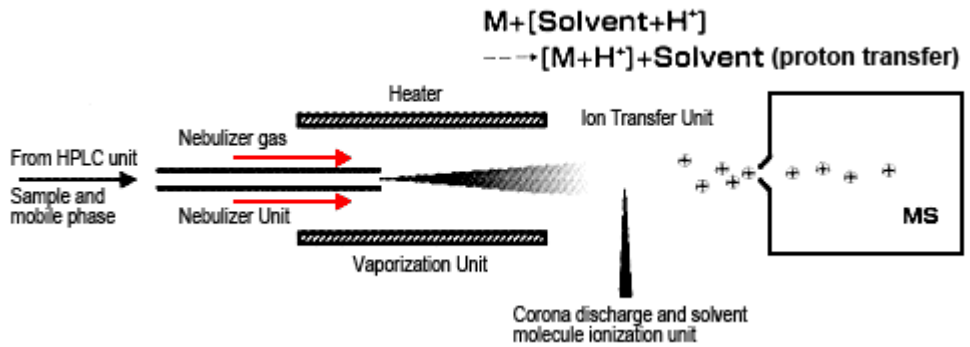


Figure 2.6: Ion-Molecule Reactions in Atmospheric Pressure Chemical Ionization (APCI) Source: Bruins, (1998).

Based on the differences between mass analyzers used, conventional MS analyzers include ion trap mass spectrometry (IT-MS), time-of-flight mass spectrometer (TOF-MS) and quadrupole mass spectrometer (Q-MS), and (Marshall and Hendrickson, 2008). Two or more mass spectrometry that are working together are usually refers to as

Tandem mass spectrometry. Triple-quadrupole mass spectrometry (QQQ-MS) is the general type of tandem mass spectrometry in use. Fragments of molecular ions that are generated by first-stage mass spectrometry can be induced by tandem mass, from the fragments and parent relationship, accurate structural information of the molecule can be obtained for unknown compounds (Steinmann and Ganzera, 2011). Liquid chromatography quadrupole time of flight mass spectrometry (LC-Q-TOFMS) is a better choice in qualitative analysis while liquid chromatography triple-quadrupole mass spectrometry (LC QQQ-MS) gives better quantitative analysis. Using specific standard with known concentration, quantitative analysis can be better performed. Structures of unknown compounds can be measured using qualitative analysis and predict their designations based on information available from database and previous research literatures (Pang *et al.*,2016).

2.4.2.1 High-performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) is an important liquid chromatography (LC) technique used for the segregation of different components in mixtures. It is also used for the identification and quantification of compounds in the process of drug development and has been used over the world since decades (Chawla and Ranjan, 2016). The main principle of separation in this technique is adsorption. This robust analytical technique is mainly used for the qualitative analysis of non-volatile classes of compounds such as phenolics, terpenoids, and alkaloids (Harborne, 1998). It is highly efficient and provides rapid and better analytical separation with higher sample loading capacity (Long *et al.*,2014). Sample derivatization is not required before analysis in this method (Huang *et al.*,2007).

The column is a major component of HPLC. The column contains the particles used as stationary phase. The stationary phases usually have a particle size ranging between 3

and 50 μm packing contained in a column with 2–5 mm pore size (Oniszczuck and Hawryl, 2011). Silica is the most commonly used column packing material for its reliable strength and rigidity, relative inertness and ability to be modified chemically (Priyadarishini *et al.*,2016). Most of the separations are carried out on reversed-phase (RP) columns (Oniszczuck and Hawryl, 2011). RP columns used in HPLC are more desirable and widely used for the analysis of multiple phytoconstituents (Patel *et al.*,2012). Routine HPLC methods use RP octadecyl silica columns because they offer many important benefits in HPLC columns such as good efficiency, high sample loading, durability and wide commercial availability (Priyadarishini *et al.*,2016).

Mobile phase selection depends on the type and nature of compounds to be separated by HPLC. Generally, water and organic solvents such as methanol and acetonitrile along with small concentrations of acetic acid, formic acid and tri-fluoroaceticacid (TFA) are used for the separation of phenolic compounds from plant samples in RP-HPLC (Bonta, 2016).

Detectors play a significant role in maintaining accuracy and stability and are useful for reducing the loss during the separation of a specific compound from complex plant samples (Oniszczuck and Hawryl, 2011). Detectors are the important component of mass spectrometer that generates a signal from incident ions through secondary electrons or by the induced current. The detectors are used according to the nature of compounds to be characterized (Steinmann and Ganzera, 2011). Commonly detectors used are UV detectors, diode array detectors (DAD), photodiode array detectors (PDA) (Chandra *et al.*,2014), UV-Visible detectors and fluorescence detectors (Stalikas, 2010).

Once the ionized sample is introduced into the mass spectrometer, the molecular mass of the compound is calculated based on mass to charge ratio (m/z)(Robards, 2003).

There are many types of mass analyzers routinely used such as magnetic (B)/electric (E) sector mass analyzer, time of flight (TOF) and quadrupole analyzers (Steinmann and Ganzera, 2011). Time of flight is best for qualitative analysis while quadrupole analyzers are best for quantitative analysis (Pang *et al.*,2016).

2.4.2.2Gas chromatography (GC)

Gas Chromatography is a highly sophisticated analytical procedure in which the mobile phase is gaseous. It works on the principle of partition and adsorption. Supply of carrier gas in a high pressure cylinder with attendant pressure regulators and flow meters. Carrier gas: He (common), N_2, H_2 and argon- methane (He is preferred due to high thermal conductivity. N_2 is preferable when large consumption of carrier gas is employed.) A gas flow rate of 25 to 150 mL/min is preferred for the packed column whereas a flow rate of 1 to 25 mL/min for the open tubular column while applied pressure is 1-4atm (Coskun, 2016**).** Sample in liquid form is injected using microsyringe. The injector must be kept at some high temperature to change into vapour phase for easy analysis. The sample is directly injected into heated port which has a temperature higher than that of the oven. The volume to be injected for a packed column and capillary column are 1-20 µL and 10-3 µL, respectively. For a reasonable GC analysis the detector needs to be sensitive (10-8-10-15 g solute/s), operate at high temperature (0-400°C), stable and reproducible, linear response, wide dynamic range, fast response, simple (reliable), non-destructive, uniform response to all analytes. The detectors used are thermal conductivity detector (TCD), atomic emission detector, thermionic detector, mass spectrometer (MS), electron capture detector (ECD), flame photometric detector and flame ionization detector (FID) (Ivanescu *et al.*,2015 and

Priyadarshini *et al.*,2016). The number of peaks obtained determines the number of components in a sample. The amount of a given component in a sample is determined by the area under the peaks. The retention time aids in the identity of components

The advantages of gas chromatography are very high-resolution power of complex mixture components, very high sensitivity with TCD, even a small sample size is sufficient and fast analysis with relatively good precision and accuracy. It can be used for both qualitative and quantitative analysis (Priyadarshini *et al.*,2016). Parts of the main disadvantages of gas chromatography is that it is only good for non-polar and thermostable compounds (non-volatile or less volatile compounds and thermolabile compounds detection is impaired). The compounds need to undergo derivatization or transformation into stable degradation products is needed before running gas chromatograph (Ivanescu *et al.*,2015).

2.4.2.3 Thin-layer chromatography

Thin-layer chromatography (TLC) plays a crucial role in the early stage of drug development when information about the impurities and degradation products in drug substance and drug product is inadequate. TLC works on the principle of adsorption. However, adsorption and partition or a combination of both is usually present. The solutes with more affinity towards stationary phase travel slower and vice versa (Lee and Webb, 2003). TLC is a "solid-liquid adsorption" chromatography. In this method stationary phase is a solid adsorbent substance coated on glass plates. All solid substances used in column chromatography as adsorbent material (alumina, silica gel, and cellulose) can be utilized. In this method, the mobile phase travels upward through the stationary phase. The solvent travels up the thin plate by means of capillary action. During this process, it also drives the mixture spotted on the lower parts of the plate upwards with different flow rates. Thus the separation of analytes is achieved. This

upward travelling rate depends on the polarity of the material, solid phase, and of the solvent (Coskun, 2016).

In cases where molecules of the sample are colorless, florescence, radioactivity or a specific chemical substance can be used to produce a visible coloured product so as to identify their positions on the chromatogram. Formation of a visible colour can be observed under UV light. The position of each solute in the mixture can be measured by calculating the ratio between the distances travelled by the solute and the solvent. This measured value is called retention factor (RF) (Donald *et al.*,2006)

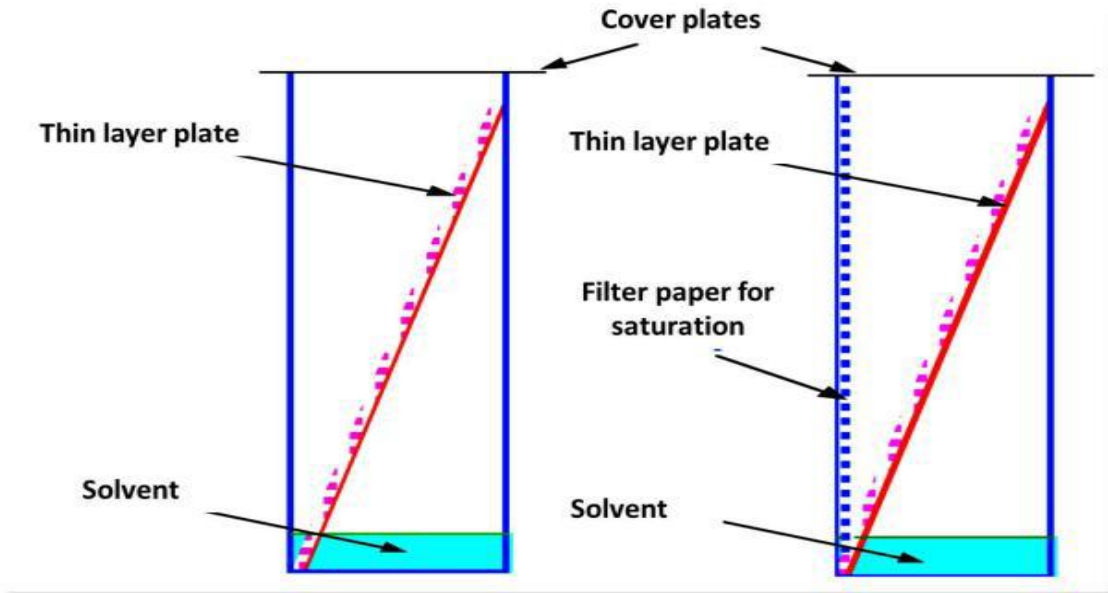


Figure 2.7: Thin Layer plate development (Donald *et al.*,2006)

2.5.3 Nuclear Magnetic Resonance (NMR) spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical technique used in quality control and research for determining the content and purity of a sample as well as its molecular structure. NMR is also the most powerful technique used to obtain structural information, and therefore it can help to understand the structure of components in complex mixture **(**Caligian *et al.*,2007**)**. It can be applied to liquid and/or

solid materials and has become increasingly popular in the field of drug discovery. It has been used for structural determinations of biopolymers, for example proteins or nucleic acids. NMR is also used in medicine for magnetic resonance imaging (MRI) (Gunther, 2015). NMR spectroscopy is one of the very few techniques, along with X-ray technology which works only for compounds that can be crystallized, that can provide information about the regio/stereo chemistry of a molecule (Spyros & Dais, 2013). The nondestructive character, and the high accuracy, and reproducibility of NMR analysis, which is often performed without using any separation and purification steps, gives it tremendous potential in analyzing plant components (Ohtsuki *et al.*,2016 and Otte *et al.*,2014)

Nuclear magnetic resonance imaging is based on the emission and absorption of energy in the radio frequency range of the electromagnetic spectrum basis **(**Sobeli *et al.*,2014**)**. NMR spectroscopy refers to the behaviour of atoms subjected to a magnetic field. This analytical technique is based on the magnetic properties of certain nuclei that have an odd mass number or an even mass number but odd atomic number (Levitt, 2005). Atoms with an odd mass number such as ^{1}H, ^{31}P and ^{13}C possess the quantum property of ‘‘spin’’ and behave as dipoles aligning along the axis of an applied magnetic field. During relaxation following excitation, radiofrequency signals are generated which can be expressed as a frequency spectrum (Tognarelli *et al.*,2015). Relaxation refers to the phenomenon of nuclei returning to their thermodynamically stable states after being excited to higher energy levels. The energy absorbed when a transition from a lower energy level to a high energy level occurs and energy is released when the opposite happens (Guzeler and Parlak, 2016).

Relaxation time is called to the time taken for relaxation. There are two types of relaxation.

1. Spin – lattice relexation T1: Release of energy by excited nuclei to their general environment

2. Spin – spin relaxation T2: Release energy is transferred to a neighboring nucleus by nucleus.

NMR spectrometer basically consists of four main sections.

1. A magnet that generates the static magnetic field (i.e Magnet containing highly homogeneous magnetic field in of pole ends)

2. Very stable a radio frequency transmitter: the NMR probe that hosts the sample and is used to deliver the radio frequency pulses

3. Radio frequency receiver: a console that contains all the electronics required for RF pulse generation, signal detection, and sample temperature control

4. Recorder (Monitor) (Figure 1).: a computer that is used to control the console, the probe, and the magnet, as well as for processing of the NMR data (Hatzakis, 2019).

^{1}H NMR and ^{13}C NMR spectroscopies are used for analyses of all organic compounds frequently (Guzeler and Parlak, 2016). Nuclear magnetic resonance is a non-destructive, multinuclear, multiparametric and often non-invasive technique, successfully employed in plant and mammalian biochemistry **(**Curtis *et al.,*2000**)**.

The most common NMR observables are chemical shifts (δ), scalar coupling (*J* coupling), and signal area.

In one-dimensional (1D) NMR spectrum is a plot of signal intensity (y-axis) against frequency (x-axis) called chemical shift (δ). Chemical shift determines the location of a signal in the NMR spectrum and provides information about the chemical environment of nuclei. It is expressed in parts per million (ppm) in reference to an internationally recognized internal standard, such as tetramethylsilane Si (CH3)4, (TMS) or trimethylsilylpropanoic acid (TSP), which is usually set to 0 ppm. Expressing chemical

shift in ppm instead of Hz renders it independent of the basic operational frequency of the spectrometer and makes data obtained in different spectrometers easily comparable to each other (Hatzakis, 2019). Therefore, chemical shift is defined in terms of the difference in resonance frequencies between the nucleus of interest (i.e. 1H) and a reference nucleus (i.e. 1H of TMS) by means of a dimensionless parameter δ. The δ values are positive if the sample absorbs to high frequency of the reference absorption (Gerothanassis *et al.*,2002). Typical chemical shift range values for ^{1}H NMR are from 0 to 12 ppm, for ^{13}C from 0 to 220 ppm and for ^{31}P from−30 to 200 pm (Martin *et al.*,2006). Peaks in the MR spectra are also called resonances. Some metabolites may be split into two (doublet) or more sub-peaks. The area beneath the peak represents the concentration of the metabolite. Absolute quantification of metabolites is theoretically possible, but can be difficult to achieve accurately due to spin lattice and spin-spin relaxaztion (Tognarelli *et al.*,2015).

Signal area is the area of the NMR signal that is proportional to the number of nuclei giving rise to the signal and thus can be used to provide information about the number of nuclei, which is of specific importance for identification purposes. In addition, the signal area can be used under certain conditions for quantitative NMR (qNMR) analysis for the determination of the relative or absolute concentration of one or more compounds (Hatzakis, 2019).

For solution state NMR analysis, the sample is usually resuspended in an appropriate NMR solvent, which also contains a reference compound such as TMS (for nonpolar solvents) or TSP (for polar solvents). After the NMR acquisition, a set of FIDs is obtained. The spectral processing for the conversion of FIDs to spectra includes the standard procedures that are used in any other type of NMR experiments, such as

Fourier transform, phase correction, baseline correction (Xi and Rocke, 2008), and signal referencing (Rusilowicz *et al.*,2014).

Most quantitative NMR studies involve ^{1}H NMR analysis because of the increased sensitivity of proton nuclei. However, because ^{1}H NMR spectra often suffer from extensive overlap due to the short spectral width and the presence of scalar coupling, ^{13}C NMR is becoming a very attractive approach for food analysis due to its high spectral resolution. This is in contrast to other fields, such as pharmaceutical analysis, where limited amounts of samples are generally an issue. ^{13}C NMR analysis can be extremely effective when using Broadband Observe (BBO) NMR probes, which are optimized for the observation of heteronuclei such as 13C (Williamson and Hatzakis, 2017). Conversely, ^{13}C NMR is not the preferable method for the determination of minor compounds that appear in very low concentrations. The determination of minor compounds can also be an issue with ^{1}H NMR due to the overlap of their signals with those of major compounds.

The element carbon consists of the stable isotopes ^{12}C and ^{13}C with 98.9 % and 1.1 % natural abundance respectively. Only the ^{13}C nucleus has a magnetic moment with I = 1/2, while the 12C nucleus of the major isotope is non-magnetic. Therefore, nuclear magnetic resonance spectroscopy of carbon, which is of great interest for organic chemistry, is limited to the investigation of ^{13}C. The magnetic moment of the 13C is smaller than that of the proton by a factor of 4. Consequently, 13C is less sensitive for the NMR experiment than the proton. Moreover, the low natural abundance renders its detection more difficult and, therefore, ^{13}C NMR spectroscopy is by far less sensitive than 1H-NMR (Gerothanassis *et al.*,2002).

Proton nuclear magnetic resonance (NMR) spectroscopy is both a novel and inviting alternative to traditional methods such as gas chromatography (GC), which can require time-consuming sample derivatization. Proton NMR analysis approach has potential application in the dairy industry as a screening technique for total conjugated linoleic acid (CLA) concentrations in large numbers of cheese samples and in other dairy products (Prema *et al.*,2013).

NMR displays lower sensitivity (nano- to milli-molar) but samples remain intact and NMR spectral pro-files have been extensively categorized (Wishart *et al.*,2007). Additional benefits include robustness and reproducibility (Heather *et al.*,2013). Furthermore, the method is rapid, non-destructive, uses minimal sample volumes, and requires limited sample preparation (Ryan *et al.*,2011). These qualities make in vitro MRS a reliable technique for identification of structures (Dumas *et al.*,2014).

The major disadvantages of NMR include a failure to detect mdetabolites with low concentrations, particularly those <5 mM (Ryan *et al.*,2011). Moreover, some metabolites can be effectively ‘‘hidden’’ in spectra if they are co-resonant with higher concentration metabolites (Heather *et al.*,2013). Furthermore, data analysis can be very complex and requires expert interpretation when identifying potential metabolites of importance.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and reagents

All chemicals and reagents were of analytical grade. N-hexane, chloroform, methanol, diethyl ether, petroleum ether, ethyl acetate and sodium hydrogen carbonate were obtained from BDH chemical, Poole England.Chloroquine phosphate and Artesunate from Sigma Alrich, Germany were used.

3.1.2 Collection of plant materials

Eight (8) plants namely: *Agelanthus dodoneifolius* (leaves), *Seciridaca longepedunculata (*root bark), *Neocarya macrophylla* (stem bark), *Merrimia hederacea* (whole plant), *Zanthoxylum zanthoxyloides* (stem bark), *Leptadenia hastata* (whole plant), *Polycarpea linearfolia* (whole plant) and *Lophira alata* (stem bark) used locally in the management of malaria were collected in Niger State, Nigeria. Taxonomic identification and authentication were performed at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu- Abuja, where voucher specimens were deposited.

3.1.3 Parasites

Chloroquine sensitive *Plasmodium berghei* strains were obtained from Nigeria Institute of Medical Research (NIMR), Lagos and maintained in the laboratory by serial passage in mice. Chloroquine sensitive and resistant *Plasmodium falciparum* strains were obtained from University of Western Cape, South Africa.

3.2 Methods

3.2.1 Preparation of crude extracts

The plant samples were air dried at 35-38 ^{o}C temperature and pulverized. 500 g of pulverised plant samples were exhaustively extracted by using methanol under reflux at 60 ^{o}C. The filtrates were concentrated in a rotary evaporator and the concentrate was placed in a fume cupboard for complete evaporation of solvent. Extracts were stored in air tight container in a refrigerator at -8 ^{o}C.

3.2.2 Qualitative and quantitative phytochemical screening of extracts: Plant samples were tested for the presence of some phytochemicals using standard procedures as described by Tiwari *et al.*,(2011) and quantified using Edeoga *et al.*,(2005) methods.

3.2.2.1 Qualitative Test

a. **Tests for alkaloids detection:** Extracts were dissolved singly in dilute Hydrochloric acid and filtered. Filterates were subjected to the following tests:
    i. **Alkaloids detection by Mayer's test:** Potassium Mercuric Iodide (Mayer's reagent) was added to the filtrates. There upon the formation of a yellow coloured precipitate proved the presence of alkaloids.
    ii. **Alkaloids detection by Wagner's test:** Wagner's reagent (Iodine in Potassium Iodide) was added to the filtrates and the presence of alkaloids was affirmed with formation of brown/reddish precipitate.
    iii. **Alkaloids detection by Dragendroff's test:** Dragendroff's reagent (solution of Potassium Bismuth Iodide) was added to the filtrate and consequently the presence of alkaloids was revealed with the formation red precipitate.

iv. **Alkaloids detection by Hager's test:** Hager's reagent (saturated picric acid solution) was added to the filterate. A yellow coloured precipitate confirmed the presence of alkaloids.

b. **Detection of glycosides:** Extracts were hydrolysed with dilute hydrochloric acid (HCl) and the presence of glycosides were tested.

i. **Modified Borntrager's test:** Iron (III) Chloride solution was added to the digested extract and immersed in boiling water for about 5 minutes. The mixture was allowed to cool and treated with equal volumes of benzene. Ammonia solution was added to the separated benzene layer. Presence of anthranol glycosides was confirmed by rose-pink colour formation in the ammonical layer.

ii. **Legal's test:** Digested extracts were subjected to sodium nitropruside in pyridine and sodium hydroxide treatment. The presence of cardiac glycosides was ascertained with the formation of pink to blood red colour.

iii. **Cardenolides's test (Keller-killani's test):** Two millilitre of glacial acetic acid containing one drop of ferric chloride solution was added to 5 ml of each extract. This was followed by treatment with 1ml of concentrated sulphuric acid. A brown ring interface cofirmed the presence of deoxysugar characteristic of cardenolides or a violet ring in the acetic acid layer that appeared below the brown ring indicated cardenolides. (Akinyemi *et al.*,2005).

c. **Detection of saponins**

i. **Froth test:** Aliquot part (2 g) of powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was

mixed with 5 ml of distilled water and shaken vigorously for stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion (Edeoga *et al.*,2005).

ii. **Foam test:** Aliquot part (0.5 g) of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

d. **Detection of phytosterols**

i. **Salkowski's test:** Aliquot part (2 g) of extracts were treated with 10 ml of chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenes.

ii. **Libermann Burchard's test:** Aliquot portion (2 g) of extracts were treated with 10ml of chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

e. **Detection of phenols**

Ferric chloride test: Aliquot portion (0.5 g) of extracts was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

f. **Detection of tannins**

Gelatin test: To aliquot portion (0.5 g) of the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

g. **Detection of flavonoids**

i. **Alkaline Reagent test:** Aliquot portion (0.5 g) of extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

ii. **Lead acetate test:** Aliquot portion (0.5 g) of extracts were treated with few drops of lead acetate solution. Formation of yellow coloured precipitate indicates the presence of flavonoids.

h. Detection of diterpenes

Copper acetate test: Aliqant portion (0.5 g) of extracts were dissolved in 2 ml of distilled water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

3.2.2.2 Quantitative phytochemicals analysis

Estimation of phytochemicals such as flavonoids, alkaloids, saponins and total phenols were carried out using the methods by Edeoga *et al.*(2005).

The method of Bohm and Koupai-Abyazan (1994) described by Ogbonna *et al.* (2016) for flavonoids determination was used. Ten grams of each of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125mm). The filtrate was transferred into evaporating dish and placed on a water bath until a constant weight was maintained (Edeoga *et al.*,2005).

Harborne's (1973) Method of alkaloid determination as described by Ezeonu and Ejikeme (2016) was used. Aliqout (5 g) of the samples were weighed into 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allow to stand

for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Edeoga *et al.*,2005)

Obadani and Ochuko (2001) method as described by Khan *et al.*(2011) was used in saponin determination. Twenty grams of each plant sample was weighed into a conical flask and 100 cm^3 of 20 % aqueous ethanol will be added. The samples were heated over a hot water bath for 4 hours with continuous stirring at 55 ^oC. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ^oC. The concentrate was transferred into a 250 ml seperatory funnel and 20 ml of diethyl ether was added and shaked vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 50 ml of n-butanol was added to the extract and was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation, the samples were dried in the oven to a constant weight, the saponin content was calculated as percentage.

Total phenol was determined using Edeoga *et al.*(2005) method. Two grams of each plant sample was defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 hours. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 minutes. Aliquot (5 ml) of the extract was pipetted into a 50 ml flask, and then 10 ml of distilled water was added. Two millilitre of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples

were made up to mark and left to react for 30 minutes for colour development. The absorbance was measured at 505 nm.

2.2.3 Determination of safe and lethal Dose (LD_{50})

Acute toxicity was determined using Lorke's method as modified by Chinedu *et al.* (2013). This method is divided into stages, with the outcome from each stage determining the next step to take (i.e, whether to terminate or proceed to the next stage).

Stage 1

This is the initial stage and it requires four animals. These animals were divided into four groups of one animal each. Then different doses of the test substance were administered to the different animals. The animals were observed for 1 hour post-administration and then 10 minutes every 2 hours interval for 24 hours. The behavioral signs of toxicity and also mortality were recorded. Where no mortality is recorded at this stage, the testing should proceed to stage 2.

Stage 2

This stage involves three animals, which were divided into three groups of one animal each. Different doses of the test substance (higher than those used in stage 1) were administered to the different animals and then observed for 1 hour after administration and periodically for 24 hours. Behavioral signs of toxicity were noted and mortality as well. If no mortality occurred, testing proceeded to stage 3.

Stage 3

This stage also required three animals which were distributed into three groups of one animal each. Various high doses of test substance (with 5000 mg/kg as the highest) were administered to the different animals. Observation is done for 1 hour after administration and then 10 minutes every 2 hours for 24 hours. Behavioral toxicity signs

and also mortality was recorded. This is the final stage of testing and where no mortality is recorded at this stage, the LD_{50} of the test substance is said to be greater than 5000 mg/kg and hence has a high degree of safety.

Table 3.1: Recommended Doses for Acute ToxicityAnalysis

Stage	Recommended doses (mg/kg bdw)			
1	10	100	300	600
	50	200	400	800
2	1000	1500	2000	
3	3000	4000	5000	

LD_{50} was calculated using the formula below:

$$LD_{50} = \frac{[M_0 + M_1]}{2}$$

Where M_0 = Highest dose of test substance that gave no mortality,
M_1 = Lowest dose of test substance that gave mortality (Chinedu *et al.*,2013).

3.2.4 Antiplasmodial activities

3.2.4.1. *In vitro* antiplasmodial assay

Chloroquine sensitive strain NF54 and Chloroquine resistant strain K1 of *Plasmodium falciparum* were used for *in vitro* blood stage culture to test the antiplasmodial efficacy of different plant extracts. The culture was maintained according to the method described by Trager and Jensen (1976) as modified by Ferrer *et al.*,2008. Fresh O positive human erythrocytes was suspended at 4% haematocrit in Rosewell Park Memorial Institute (RPMI) 1640 (Sigma) containing 0.2 % sodium bicarbonate, 0.5 albumax I, 45 µg/L hypoxanthine, and 50 µg/L gentamicin and incubated at 37 ^{o}C under a gas mixture of 5 % oxygen, 5 % carbon dioxide and 90 % nitrogen. Infected

erythrocytes were transferred into fresh complete medium for propagation of culture (Kaushir *et al.*,2015).

The extracts were evaluated for their antiplasmodial activity against NF54 and K1 strains of *Plasmodium falciparum* and was cultured in sealed flasks at 37 ^{o}C, in a 3 % O_2, 5 % CO_2 and 91 % N_2 atmosphere in RPMI 1640, 25mM HEPES, pH 7.4, supplemented with heat inactivated 10 % human serum and human erythrocytes to achieve a 2 % haematocrit. Parasites were synchronized in the ring stage by serial treatment with 5 % sorbitol and studied at 1% parasitemia. Plant extracts was prepared as 1mg/ml stock solutions in dimethyl sulphoxide (DMSO), diluted as needed for individual experiments, in triplicate. The stock solutions were diluted in supplemented RPMI 1640 medium so as to have at most 0.2% DMSO in the final reaction medium. An equal volume of 1% parasitemia, 4% hematocrit culture were added and gently mixed thoroughly. Negative controls contained equal concentrations of DMSO. Positive controls contained 1µM Artesunate. Cultures were incubated at 37 ^{o}C for 48 h (one parasite erythrocytic life cycle). Parasites at the ring stage were fixed by replacing the serum medium by an equal volume of 1% formaldehyde in phosphate buffer solution. Aliquot (50 µl) of each culture were added to 5 ml round-bottom polystyrene tubes containing 0.5 ml 0.1% Triton X- 100 and 1 nM YOYO nuclear dye (Molecular Probes) in phosphate buffer solution (PBS), and parasitemia of treated and control cultures were compared (Singh and Rosenthal, 2001).

The IC_{50} values, which is the concentration required to inhibit schizont growth by 50%, was determined by linear interpolation from the schizont growth inhibition curves (Log of concentration versus percent inhibition) generated from parasite-extract interaction (Mustofa *et al.*,2007).

3.2.4.2 *In vivo* antiplasmodial screening

Four days curative test as described by Fidock *et al.*,2004 was used. Mice were pre-screened by microscopy for thin and thick tail tip blood smears. This is necessary to exclude the possibility of test animals harbouring rodent *Plasmodium* species. This is a procedure whereby mice are infected and left for 72 hours before treatment with test and standard as in Rane test or established infection (Jigam *et al.*,2012). The screening of the crude extracts was divided into two phases. For each phase, forty-two mice were used for four different extracts, control and the standard. Swiss albino mice weighing between 15-19 g were selected. Each extract had three dose levels consisting of three mice. One group served as control, one as standard and the other as the test groups for 100, 200 and 400 mg/kg bw of crude extracts. The groups were infected with *Plasmodium berghei* (approximately 1 x 10^7 infected red cells) by the intraperitoneal routes. The animals were left for 72hours for the infection to be established. On D3 i.e, after 72 hours of infection, the extracts were administered orally once daily for 4 days to the test groups from D3 to D7 while control group was given 0.9w/v of normal saline during the period. 5 mg/kg bw of Chloroquine phosphate was administered to serve as a standard control. The thick and thin blood smears from the tail blood were prepared for each animal on a single slide on D4, D7, D14, D28 and D42 (i.e Day 4, 7,14,28 and 42 post infection). Thick and thin films stained with Giemsa were prepared for the microscopic examination of malaria parasites, the thin films was fixed with methanol and all films were stained with 3% Giemsa stain of pH 7.0 for 30 minutes as recommended by WHO (2000) blood films were examined microscopically using 100X (oil immersion) objectives as described by WHO (2010). The thick films were used to determine the parasite densities while thin films were used to identify the parasite species and infective stages (WHO, 2001). Parasite density per microliter of blood

(parasitemia) was estimated from the thick film, taking the number of leucocytes per microliter of blood as 8,000 and expressed as follows

Parasite density/μ1 = $\frac{\text{parasite count x 8,000}}{\text{No of WBC counted}}$ (WHO, 2010).

3.2.5 Toxicological analysis

Thirty-six Wister rats were grouped into three of twelve rats each. Group I and II were administered 200mg/kg bw of *P. linearfolia* and *L. hastata* extracts orally on alternate days. Group III received 0.1ml of physiological saline. Three rats were selected randomly on weekly basis and sacrificed. Blood samples were collected in plain and heparinazed bottles for biochemical and hematological analysis respectively.

3.2.5.1 Biochemical Analysis

Biochemical parameters such as glucose, total protein, triacylglyceride, cholesterol, bilirubin, albumin, aminotransferases and alkaline phosphatase were monitored over a period of five weeks. The blood samples for biochemical parameters were collected in plain bottles

i. **Estimation of Serum Glucose in rats administered crude methanol extracts of *L. hastata* and *P. linearfolia***

Serum glucose was determined using Randox Glucose Kit.Glucose is oxidized in the presence of glucose oxidase to give glucoronic acid and hydrogen peroxide. The later, under catalysis of peroxidase with phenol and 4 – aminophenalzone form a red – violet quinoneimine dye as indicator (Raba and Mottola, 1995)

$$Glucose + O_2 + H_2O \rightarrow \text{Glucuronic acid} + H_2O_2$$

$$2H_2O_2 + 4 - \text{amino phenazone} + \text{phenol} \rightarrow \text{quinoneimine} + 4H_2O$$

Three test tubes were used. To the first tube (standard), 10 µl of the standard solution was pipette, and 1000 µl of reagent was added. To the second test tube (reagent blank),

1000 µl of reagent was pipette then 10 µl of distilled water. To the third tube, 10 µl of sample was pipette, and 1000 µl of reagent added.

The contents were mixed and incubated at 37 ^{o}C for 10 minutes. The absorbance of test was measured at 546nm against blank and glucose concentration was determined using.

$$\text{Glucose concentration (mmol/L)}) = \frac{\text{A sample}}{\text{A standard}} \times \text{conc of standard (mmol/L)}$$

Normal range = 4.2-6.4mmol/L

ii. **Estimation of total serum proteins in rats administered crude methanol extracts of *L. hastata* and *P. linearfolia***

The determination serum total protein was carryout with Total Protein Randox kit. This test is base on the fact that cupric ions, in an alkaline medium, interact with protein peptides bonds resulting in the formation of a colored complex compound which is proportional to the concentration of protein present in the sample.

Three test tubes were used. To the first tube (blank) 0.02 ml of distilled water was pipette and 1.0ml of reagent added. To the second test tube (standard) 0.02 ml of standard solution was pipette and 1.0 ml of reagent added. To the third test tube (test) 0.02 ml of sample was pipette and 1.0ml of reagent 1 added. The contents were mixed and incubated at 37 ^{o}C for 10 minutes. Absorbance of sample (A sample) and of the standard (A standard) were read against the reagent blank at 546nm. The total protein concentration was determined using:

$$\text{Total protein conc.}\,(\text{g/dL}) = \frac{\text{A sample}}{\text{A standard}} \times \text{conc. of standard (g/dL)}$$ (Burtis *et al.*, 2012)

.

iii. **Estimation of serum albumin in rats administered crude methanol extracts of *L. hastata* and *P. linearfolia***

Albumin binds to 3, 3', 5, 5'-tetrabromo-m-cresol sulphenephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578nm, the absorbance is directly proportional to the concentration of albumin in the sample (Tietz, 1987).

1ml of R (BCG concentrate) was measured into test tubes labeled as reagent blank, standard and sample. Aliqout portion (3µl) of respective sample was added to test tubes and the content was mixed and incubated for 10minutes at 37 ^{o}C. Absorbance was read at 600nm (Tietz, 1987).

iv. **Estimation of serum urea in rats administered crude methanol extracts of *L. hastata* and *P. linearfolia* extracts**

Urease hydrolyzes urea to ammonia and carbon iv oxide. The ammonia formed further react with a phenolic chromagen and hydrochlorite to form a blue colored complex. Intensity of colour formed is directly proportional to the concentration of urea in the sample.

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease } 2NH_3 + CO_2} \text{Ammonia} + \text{Phenol} + \text{Indophenol(blue compound)}$$

Procedure

Three test tubes were labelled sample, standard and blank. Aliquot (5 µl) of serum, standard and distilled water was measured in to respective test tubes. Aliquot (50 µl) of R1 (Sodium nitroprusside and urease) was added to each tube and incubated at 37 ^{o}C for 5 minutes. 1.25 ml of R2 (Phenol) and R3 (hydrochlorite) were added to all tubes and incubated for 15minutes at 37 ^{o}C. Absorbance of sample was read against sample blank at 530 nm (Kumar *et al.*,2000).

v. **Estimation of serum creatinine in rats administered crude methanol extracts of *L. hastata* and *P. linearfolia* extracts**

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The intensity of the colored complex formed is directly proportional to the creatinine concentration.

Aliquot (50 µl) of respective sample was measured in to test tubes labeled reagent blank, sample and standard, and 500 µl working reagent (picric acid and sodium hydroxide) was added. The content was mixed properly and absorbance was read within 30 seconds at 505 nm. Normal range 0.5-1.1 mg/dl (Toora and Rajagopal, 2002).

vi. **Estimation of serum total cholesterol in rats administered crude methanol extracts of *L. hastata* and *P. linearfolia* extracts**

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction products, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces colored complex. The color intensity is proportional to cholesterol concentration and absorbance was measured at 500 nm. The reaction sequence is as follows:

$$\text{Cholesteryl ester} + H_2O \xrightarrow{\text{cholesteryl ester hydrolase}} \text{Cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest} - 4 - \text{en} - 3 - \text{one} + H_2O_2$$

$$2H_2O_2 + 4 - \text{aminophenazone} + \text{phenol} \xrightarrow{\text{peroxidase}} 4 - (\text{benzoquinone} - \text{monoimino}) - \text{phenazone} + 4\,H_2O$$

Desirable cholesterol levels are considered to be those below 5.17mmol/l (200 mg/dL) (Hamid and Omar, 2003).

Procedure

Aliqout portion (500 µl) of reagent was measured into three different test tubes labeled as blank, standard and tests. Aliqout portion (5µl) of distilled water, standard cholesterol and serum were added to blank, standard and test testtubes. The content was mixed and incubated for 5 minutes at 37 ^{o}C. Each of these was aspirate after the regent blank in monza spectrophotometer at 500nm and respective absorbance was displayed.

R is a solution compose of 80mmol/l Pipes buffer, 0.25mmol/l 4-Aminoantipyrine, 6 mmol/l phenol, 0.5 u/ml Peroxidaes,0.15 u/ml Cholestrol esterase and 0.10 u/ml Cholestrol oxidase.

vii. **Determination of serum triglycerides in rats administered crude methanol extracts of *L. hastata* and *P. linearfolia* extracts**

Estimation of Triglycerides (TAG) was carried out using Randox Triglycerides kit. The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

$$\text{Triglyceride} + \text{water} \rightarrow \text{Glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \rightarrow \text{Glycerol} - 3 - \text{phosphate} + \text{ADP}$$

$$\text{Glycerol} - 3 - \text{phosphate} + O_2 \rightarrow \text{Dihydroxuacetoxic} + \text{phosphate} + H_2O_2$$

$$2H_2O_2 + 4 - \text{amino phenazone} + 4 - \text{chlorophenol} \rightarrow \text{quinoneimine} + \text{HCL}4H_2O$$

Three test tubes were used and to the first (reagent blank), 0.01ml of the distilled water was pipetted and 1.0ml of reagent added. To the second tube (standard) 0.01ml of the standard solution was pipetted and 1.0ml of the reagent was added. To the third tube, 0.01ml serum was pipette, and then 1.0ml of the reagent added.

The content of each test-tube was mixed properly and incubated at 37 ^{o}C for 5minutes. The absorbance of sample (A sample) and standard (A standard) against blank was measured at 546nm (Penumarthy *et al.*,2013).

Concentration of triglycerides was calculated using:

$$\text{Triglycerides conc.}(\text{mg/dL})) = \frac{\text{A sample}}{\text{A standard}} \times \text{conc of standard}\ (\text{mg/dL}).$$

viii. **High density lipoprotein (HDL) cholesterol of rats administered crude methanol extracts of *L. hastata* and *P. linearfolia* extracts**

Direct HDL method was used for the estimation of serum HDL concentration. The basic principle of the method is as follows. The apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL-chol is detected under the assay conditions.

The method uses sulfated alpha-cyclodextrin in the presence of Mg^{2+}, which forms complexes with apoB containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL-cholesterol measurement. The reactions are as follows:

$$(1)\text{Apo B containing lipoproteins} + \alpha - \text{cyclodextrin} + Mg^{2+} + \text{dextran}SO_4 \rightarrow \text{soluble non} - \text{reactive complexes with apo B} - \text{containing lipoproteins}$$

$$(2)\text{HDL} - \text{cholesteryl esters} \xrightarrow{\text{PEG}-\text{cholesteryl esterase}} \text{HDL} - \text{unesterified cholesterol} + \text{fatty acid}$$

$$(3)\ \text{Unesterified chol} + O_2 \xrightarrow{\text{PEG}-\text{cholesteryl esterase}} \text{cholestenone} + H_2O_2$$

$$(4)\ H_2O_2 + 5 - \text{aminophenazone} + N - \text{ethyl} - N - (3 - \text{methylphenyl}) - N'\text{succinyl ethylene diamine} + H_2O + H^+ \xrightarrow{\text{peroxidase}} \text{quinoneimine dye} + H_2O$$

Absorbance is measured at 500 nm (Jacobs *et al.*,1990).

Test tubes were labeled standard, test and control. Aliquot portion (100µl) of respective samples were added to the tubes and 250 µl of diluted precipitant was added to all tubes. The content of each test tube was mixed and incubated for 10minutes 37 ^{o}C. They were centrifuged at 4000 rpm for 10 minutes. Spectrophotometer was zero with blank supernatant at 500 nm and absorbance values were read using supernatant of tests.

ix. **Estimation of low-density lipoprotein (LDL)-cholesterol of rats administered *L. hastata* and *P. linearfolia* extracts**

Most of the circulating cholesterol is found in three major lipoprotein fractions: very low density lipoproteins (VLDL), LDL and HDL.

$$[\text{Total chol}] = [\text{VLDL} - \text{chol}] + [\text{LDL} - \text{chol}] + [\text{HDL} - \text{chol}]$$

LDL-cholesterol was calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship:

$$[\text{LDL} - \text{chol}] = [\text{Total chol}] - [\text{HDL} - \text{chol}] - [\text{TG}]/2$$

Where [TG]/2 is an estimate of VLDL-cholesterol and all values are expressed in mmol/l.

LDL carries most of the circulating cholesterol in man and when elevated contributes to the development of coronary atherosclerosis. LDL-cholesterol is measured to assess risk for coronary heart disease and to follow the progress of patients being treated to lower LDL-cholesterol concentrations. Desirable levels of LDL-cholestrol are those below 130 mg/dL in adults and 110 mg/dL in children (Jacobs *et al.*,1990).

x. **Estimation of serum sodium of rats administered crude methanol extracts of *L. hastata* and *P. linearfolia***

Sodium reacts with a selective chromagen to produce a chromophore whose absorbance varies directly as the concentration of sodium in the test specimen. Normal serum sodium range is 135-150 mEq/l. (Adrogue, 2005)

0.5 ml 0f sodium reagent was measured into testtubes labeled blank, standard and test. 5 µl of sodium reagent respective sample was added. The content of each test tube was mixed and incubated at room temperature for 5miuntes. Absorbance of test and standard was read against reagent blank at 630 nm (Tietz, 1987).

xi. **Estimation of Serum Potassium in Rats Administered Crude Methanol Extracts of *L. hastata* and *P. linearfolia***

The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produre a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range of 2-7 mEq/l (Viera and Wouk, 2015).

Test tubes were labeled standard, test and control. 1ml of potassium reagent was added to all tubes and 10µl of respective samples were added to the tubes. The content of each test tube was mixed properly and incubated for 3minutes at room temperature. Spectrophotometer was zero with blank at 500nm and absorbance values were read (Tietz, 1987).

xii. **Estimation of serum alkaline phosphatase (ALP) activity of rats administered crude methanol extracts of *L. hastata* and *P. linearfolia***

The estimation of ALP activity was done using Randox ALP kit. ALP hydrolyses p-nitrophenyl phosphate (pNPP) producing phosphate and p-nitropenol at alkaline pH. The speed at which the p-nitrophenolate anion (yellow) appears is read at 405nm, which is directly proportional to the enzymatic activity of the sample (Kim and Wyckoff, 1991).

$$\text{p} - \text{Nitrophenyl phosphate} + \text{H}_2\text{O} \xrightarrow{\text{ALT}} \text{Phosphate} + \text{p} - \text{Nitrophenol}$$

Diethanolamine (DEA) regulates the reaction's pH and acts as acceptor of the phosphate release by phosphatase (Transphosphorylation), resulting in activation of the reaction. DEA has the best condition terms of activation and buffering when p-NPP is used as substrate.

Aliquot portion (0.01 ml) of the sample was measured into a test-tube and 0.5ml of ALP reagent was added. The content of the test-tube was mixed and the absorbance was read at 405nm. The activity of ALP was calculated by multiplying the absorbance of sample by 2760, to give the activities in u/L.

xiii. **Determination of Alanine Aminotransferase (ALT) Activity of Rats Administered Crude Methanol Extracts of *L. hastata* and *P. linearfolia***

Estimation of Alanine Aminotransferase was done using Randox ALT kit. The activity of ALT is measured by measuring the concentrations of the product of the reaction catalyze by ALT. ALT catalyze the amino conversion reaction between alanine and α-ketoglutaric acid to produce pyruvic acid and glutamic acid at pH 7.4 and 37℃. Then 2, 4-dinitro–phenylhydrazine is added to react with pyruvate to form 2, 4-dinitro–phenylhydrazone. 2, 4-dinitro–phenylhydrazone is reddish brown under alkaline

conditions. Absorbance was read spectrophotomerically at 546 nm (Reitman and Frankel, 1957). ALT activity can be obtained from standard calibration curve.

$$\text{Oxoglutarate} + \text{L} - \text{Alanine} \xrightarrow{\text{ALT}} \text{L} - \text{Glutamate} + \text{Pyruvate}$$

Two test tubes were used for reagent blank and sample. To the reagent blank, 100 µl of solution R, was pipetted, then 20 µl of distilled water added. To the sample tube, 100 µl of solution R, was pipetted and 20 µl of serum added. The content of each test tube was mixed and incubated at 37 ^{o}C for 30 minutes. Aliquot portion (100 µl) of R2 was added to each test tube. The content was mixed and incubated at 37 ^{o}C for 10 minutes. Aliquot portion (1000 µl) of sodium hydroxide was added. The contents of each tube was mixed and absorbance of sample (A sample) against blank was read at 546 nm after 5 minutes. The activities of alanine aminotransferase were read from standard calibration curve.

xiv. **Estimation of Serum Aspartate Aminotransferase (AST) Activity of Rats Administered Crude Methanol Extracts of *L. hastata* and *P. linearfolia***

Estimation of AST was done using AST Randox kit. The activity of AST is measured by measuring the concentrations of the product of the reaction catalyze by AST. AST catalyze the amino conversion reaction between aspartate and α-ketoglutaric acid to produce oxoloacetate and glutamic acid at pH 7.4 and 37℃.

$$\text{Oxoglutarate} + \text{L} - \text{Aspartate} \xrightarrow{\text{AST}} \text{L} - \text{Glutamate} + \text{Oxaloacetate}$$

2, 4-dinitro–phenylhydrazine is added to react with oxaloacetae to form 2, 4-dinitro–phenylhydrazone to form a colored complex oxaloacetate hydrazone. AST is measured by monitoring the concentration of oxoloacetate hydrazone formed with 2, 4 – dinitrophenylhydrazone (Reitman and Frankel, 1957).

Two test tubes were used. To the first tube (reagent blank), 100 µl of solution R1, was pipetted, and then 20 µl of distilled water added. To the second tube (sample), 0.1ml of solution R, was pipetted and 20 µl of serum added. The content of each test tube was

mixed properly and incubated at 37 ^{o}C for 30 minutes. 1000 µl of R2 was added to each test tube. The content of each test tube was mixed properly and incubated at 37 ^{o}C for 10minutes. 1000 µl of sodium hydroxide was pipetted into each test tube. The content of each testtube was mixed and the absorbance of sample (A sample) was read against blank at 546 nm after 5 minutes. The activity of AST was obtained from a standard calibration of AST activity (in u/l) against absorbance.

xv. **Estimation of Serum Bilrubin of Rats Administered Crude Methanol Extracts of *L. hastata* and *P. linearfolia***

Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Serum total bilirubin concentration was determined based on the following procedure. Aliquot part (200 µl) of R1 (Sulphanilic acid/ hydrochloric acid) was measured in to test tubes labeled sample blank and sample. Aliquot portion (50 µl) of R2(sodium nitrate) was added to the sample test tube. 1000 µl of R3 (caffeine) was added to all tubes and 200µl of sample was added. The content of the tubes was mixed and incubated for 5 minutes at 37 ^{o}C. 1ml of R4 (Tartrate) was added and incubated at 37 ^{o}C for 15 minutes. Absorbance of sample was read at 560 nm against sample blank. Absorbance value was multipled by 10.8 to obtain bilirubin concentration (Puppalwar *et al.*,2012).

3.2.5.2 Haematological assessment

Blood samples for hematological parameters were collected in Ethylene Diamine Tetraacetic Acid (EDTA) bottles. The blood samples were mixed gently to achieve good anticoagulation. Haematological parameters measured include packed cell volume

(PCV), red blood cell count (RBC count), Hemoglobin concentration (Hb), total white blood cells (TWBC), lymphocytes, monocytes, granulocytes and platelets count over a period of five weeks using automated hematological analyser.

3.2.5.3 Histopathological analysis

Histopathological screening of kidney, spleen, heart, and liver were carried out. At the end of the treatment period, the rats were sacrificed and liver, heart, spleen and kidney tissues collected in sterile saline. Freshly dissected organs from each animal were cut rapidly and fixed in buffered neutral formalin (10%). The tissues were dehydrated in ascending grades of ethanol (70 %, 80 %, 90 %, 95 % and 100 %), cleared in 2 changes of Xylene (to make it translucent for optical differenciation), impregnated with 2 changes of molten paraffin and finally embedded in wax. Tissue sections of 4-5 µm in thickness were cut with a rotary microtome and stained with hematoxylin and eosin (Bancroft and Sevens, 1990).

3.2.6 Bioassay guided fractionation of active extracts

The use of plants as therapeutic agents may have several goals such as the isolation or the production of bioactive compound with new structures (Boly*et al.*,2015). Further purification of the active crude extract was done using the following chromatographic techniques.

Column chromatography: The active extracts were subjected to column chromatography. Silica gel was use as the stationary phase while the eluting solvent was varied from non polar to polar solvents. Open-column silica gel chromatography of the extract of *P. linearfolia* was carried out with a gradient of increasing polarity using n-hexane, chloroform, ethylacetate and methanol. This afforded several fractions that

were pooled on the basic of their TLC profile. The combined fractions were then dried under a fume-hood.

The fractions were tested for antiplasmodial activity as described earlier and most active fraction was subjected to thin layer chromatography (TLC). Silica gel prepared in TLC plate was used as a stationary phase with toluene: acetone: ethanol: ammonia (40:40:6:2) as the mobile phase. The thin-layer chromatography (TLC) chamber (9" x 4 ½") was lined with filter paper. The solvent system used was prepared in a separate flask and sufficient amount poured into the TLC chamber. The most active extract was dissolved in chloroform and spotted to about 1 ½ cm apart on a silica gel H coated glass plate with the use of capillary tubing until the spot point is visibly clear. A 10-centimeter mark above the spot was placed. The plate was now introduced into the TLC developing chamber. The solvent was allowed to reach the lower edge of the adsorbent, but the spot points were not allowed to be immersed. The cover was placed and the system was maintained until the solvent ascended to the point 10 cm above the initial spots. The TLC plate was then viewed under the long wavelength of the UV light (366 nm). The distance of each spot from the point of origin was measured and then recorded. The spots were sprayed with Dragendorff's reagent and then observed. Orange spots indicated the presence of alkaloids. The Rf values of the sample were computed as follows:

Rf = distance traveled by the solute/ distance traveled by the solvent

Thin-layer chromatography was repeated and the spots identified as alkaloid were isolated from the TLC plate. Alkaloid containing spots are scraped and dissolved in chloroform for isolation of the semi-pure alkaloid. Then, this was filtered to remove

silica gel and the filtrate was placed in water bath for evaporation of the solvent (Gonzales *et al.*,2014).

3.2.7 Spectral analysis

The most active fractions were analyzed using ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectroscopy (NMR), High Performance Liquid Chromatography Electrospray Ionization linked mass spectrometry (ESI/MS).

3.2.8 Data analysis

Results were analyzed using ANOVA, with Statistical package MINITAB version 14. The results are expressed as mean ± Standard error of mean ($\bar{x}$±SEM) of 3 replicates Values with the same superscript are significantly ($p<0.05$) same while those with different superscript are significantly ($p>0.05$) different along the column.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Extracts Yield

The percentage yields of crude extracts of the selected medicinal plants is presented in Table 4.1. The yields varied from 8.54 in *S. longepedunculata* to 1.41 in *L. alata*. This represents the quantities in grams of extracts recovered after extraction relative to the initial weight of the plant materials.

Table 4.1: Percentage Yield of Selected Medicinal Plants

Crude plant extract	Yield (%)
Merremia hederacea	2.57
Polycarpaea linearifolia	2.34
Securidaca longepedunculata	8.54
Leptadenia hastata	7.15
Neocarya macropylla	2.12
Zanthoxylum zanthoxyloides	2.50
Lophira alata	1.41
Agelanthus dodoneifolium	7.77

4.1.2 Phytochemical Contents of the selected medicinal plants

The presence of various phytochemicals in the selected plant samples is presented in Table 4.2 below. Alkaloids, flavonoids, tannins, diterpenes and saponins were detected

in all the plant samples. Phenols were only detected in *P. linearifolia, A. dodonefolium* and *L. hastata* while phytosterols were absent in *A. dodoneifolium.*

Table 4.2: Qualitative Phytochemical Contents of Selected Plant Extracts

Extract	Alkaloids	Phenols	Flavonoids	saponins	Phytosterols	Tannins	Diterpenes
M.h	+	-	+	+	+	+	+
P.l	+	+	+	+	+	+	+
S.l	+	-	+	+	+	+	+
L. h	+	+	+	+	+	+	+
N. m	+	-	+	+	+	+	+
Z. z	+	-	+	+	+	+	+
L. a	+	-	+	+	+	+	+
A. d	+	+	+	+	-	+	+

Key: Mh=*Merremia hederacea*, Pl=*Polycarpaea linearifolia*, Sl= *Securidaca longepedunculata*, Lh=*Leptadenia hastata*, Nm=*Neocarya macropylla*, Zz=*Zanthoxylum zanthoxyloides*, La=*Lophira alata*, Ad=*Agelanthus dodoneifolium*, + = present, and - =absent

The results for the quantitative phytochemical screening of the selected medicinal plants are presented in Table 4.3., with *Merremia hederacea* having the highest alkaloidal content and *Leptadenia hastata* having the lowest. Flavonoids were most abundant in *A. dodoneifolium* and lest in *Z. zanthoxyloide.* Similarly, saponins were most abundant in *S. longepedunculata* and *L. alata*. Tannins and total phenols were most abundant in *A. dodoneifolium.*

Table 4. 3: Quantitative Phytochemical Contents of Selected Plant Extracts

Extracts	Alkaloids	Flavonoids	Saponins	Tannins	Total phenol
Mh	16.60±0.11^{e}	19.90±0.05^{d}	45.40±0.13^{d}	10.72±0.08^{a}	30.40±0.50^{c}
Pl	1.63±0.08^{a}	12.73±0.17^{b}	33.23±0.12^{c}	12.20±0.20^{b}	24.90±0.05^{a}
Sl	1.87± 0.06^{a}	24.47±0.26^{e}	49.10±0.05^{e}	17.28±0.13^{d}	41.53±0.41^{c}
Lh	1.27±0.03 ^{a}	20.57±0.26^{d}	46.37±0.48^{d}	17.64±0.14^{d}	39.43±0.12^{e}
Nm	1.42±0.03^{a}	19.80±0.00^{d}	26.00±0.12^{a}	12.47±0.06^{b}	28.04±0.03^{b}
Zz	4.07±0.18^{b}	8.00 ± 0.00^{a}	29.10±0.10^{b}	13.83±0.08^{c}	23.40±0.23^{a}
La	5.23±0.03^{c}	14.73±0.03^{c}	49.90±0.05^{e}	13.13±0.13^{c}	29.00±0.28^{b}
Ad	7.83±0.03^{d}	31.50±0.25^{f}	36.00±0.60^{c}	19.40±0.11^{e}	50.60±0.23^{f}

Key: Mh=*M. hederacea*, Pl=*P. linearifolia*, Sl= *S. longepedunculata,* Lh=*L. hastata*, Nm=*N. macropylla*, Zz=*Z. zanthoxyloides*, La=*L. alata* and Ad=*A.dodoneifolium*

N.B: Results are expressed as $\bar{x}$ ±SEM, values with the same superscript are significantly ($p<0.05$) the same while those with different superscript are significantly ($p>0.05$) different along the column.

4.1.3 Acute toxicity of crude extracts (Pre LD_{50}) and safe dose determination

The median lethal dose (LD_{50}) of the crude extract is presented in Table 4.4. *Polycarpaea linearifolia* has the highest LD_{50} (4500 mg/kg bw) while *Merremia hederacea, Zanthoxylum zanthoxyloide* and *Agelanthus dodoneifolium* had the least. The safe doses were selected below the LD_{50} of the crude plant extracts.

Table 4.4: Median Lethal Dose (LD_{50}) of Crude Extracts

Extract	LD_{50}(mg/kgbw)
Merremia hederacea	1750
Polycarpaea linearifolia	4500
Securidaca longepedunculata	2500
Leptadenia hastata	2500
Neocarya macropylla	2500
Zanthoxylum zanthoxyloides	1750
Lophira alata	3500
Agelanthus dodonefolium	1750

4.1.4 Antiplasmodial activity

4.1.4.1 *In vitro* antiplasmodial activity of the selected crude plant extracts

The results of the *in vitro* antiplasmodial activities of crude extracts is presented in Table 4.5. *Zanthoxylum zanthoxyloides* produced the best *in vitro* antiplasmodial activity with IC_{50} value of 1076.4 ng/ml against Chloroquine sensitive strain and 1315.1 ng/ml against Chloroquine resistant strain while *Polycarpaea linearifolia* (with IC_{50} value of 7536.7 ng/ml against Chloroquine sensitive strain and 6917.3 ng/ml against Chloroquine resistant strain) and *Leptadenia hastata* (with IC_{50} value of 6349.2 ng/ml against Chloroquine sensitive strain) recorded the least activity against the *P. falciparum. Agelanthus dodonefolium* had the least *in vitro* activity (IC_{50} of 7084.1

ng/ml) against Chloroquine resistant strain of *P. falciparum.* All other extracts exhibited moderate (IC_{50} value <10000 ng/ml ie 10 µg/ml) *in vitro* antiplasmodial activity.

Table 4.5: *In vitro* Antiplasmodial Activity of Crude Extracts against NF54 and K1

Extracts/standards	IC_{50}(ng/ml)CQS(NF54)	IC_{50}(ng/ml)CQR(K1)
Lophira alata	4544.8 ± 244.6^{b}	5243.7 ± 404.9^{b}
Leptadenia hastate	6349.2 ± 372.8^{d}	6845.0 ± 356.8^{c}
Merremia hederacea	5129.0 ± 365.4^{b}	5562.0 ± 101.1^{b}
Zanthoxylum zanthoxyloides	1076.4 ± 56.4^{a}	1315.1 ± 121.6^{a}
Securidaca longepedunculata	4655.6 ± 89.6^{b}	5109.4 ± 207.9^{b}
Neocarya macropylla	5216.6 ± 77.7^{b}	5519.3 ± 221.8^{b}
Polycarpaea linearifolia	7536.7 ± 127.7^{d}	6917.3 ± 248.3^{c}
Agelanthus dodonefolium	6278.8 ± 46.9^{c}	7084.1 ± 309.6^{c}
CQ (nM)	10.94/3478.92	310.68/98796.24
ART (nM)	9.24/3215.52	10.94/3650.52

Legend: CQS=chloroquine sensitive strain, CQR=Chloroquine resistant strain, CQ=chloroquine, ART= Artesunate

N.B: Results are expressed as $\bar{x} \pm SEM$, values with the same superscript are significantly ($p<0.05$) same while those with different superscript are significantly ($p>0.05$) different along the column.

4.1.4.2 *In vivo* antiplasmodial activity of crude plant extracts

The antiplasmodial activity of *Leptadenia hastata* gainst *Plasmodium berghei* (NK65) in mice is presented in Figure 4.1 below. Mice treated with 100 and 200 mg/kg bw of

Leptadenia hastata did not clear parasites and these groups did not survive to day 28. However, the activity of *Leptadenia hastata* at 400 mg/kg bw was comparable to the standard group treated with 5 mg/kgbw of Chloroquine (Figure 4.1). The activity of *Leptadenia hastata* was in a dose dependent manner. Neither the test groups nor the standard survive to day 42 while the control died after day 14.

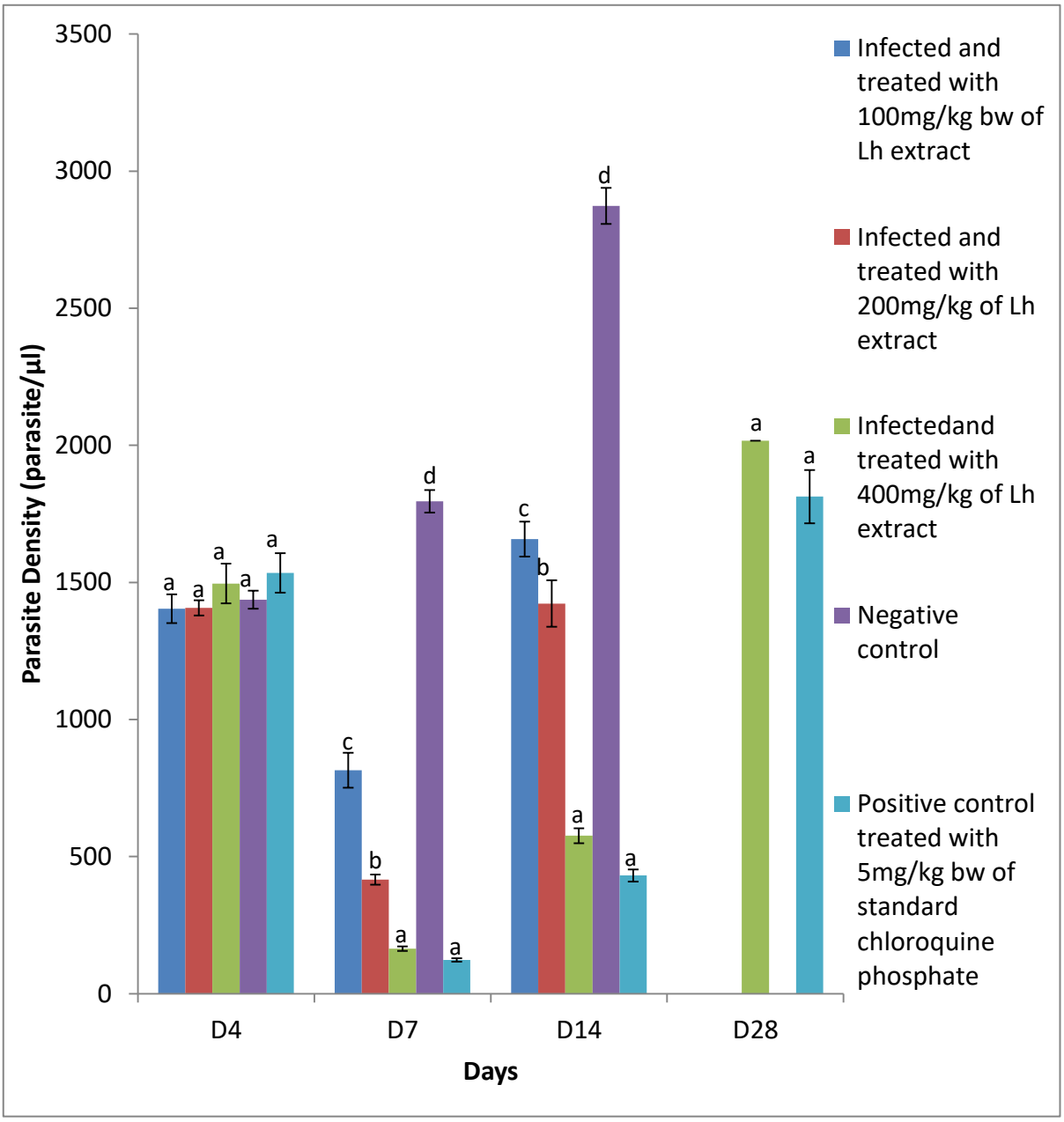


Figure 4.1: Parasite density of *P. berghei* infected mice treated with *Leptadenia hastata*

Key:Lh = *Leptadenia hastate,* Negative control= group infected with *P. berghei* without treatment

Positive control= infected group treated with the standard drug (5 mg/kg bw chloroquine phosphate)

The *in vivo* antiplasmodial activity of *Lophira alata* extract is presented in Figure 4.2. None of the *P. berghei* infected mice treated with 100, 200 and 400 mg/kgbw of *Lophira alata* cleared the parasites completely from circulation and the activities were lower compared to the standard group. The activity of *Lophira alata* was also in a dose dependent manner.

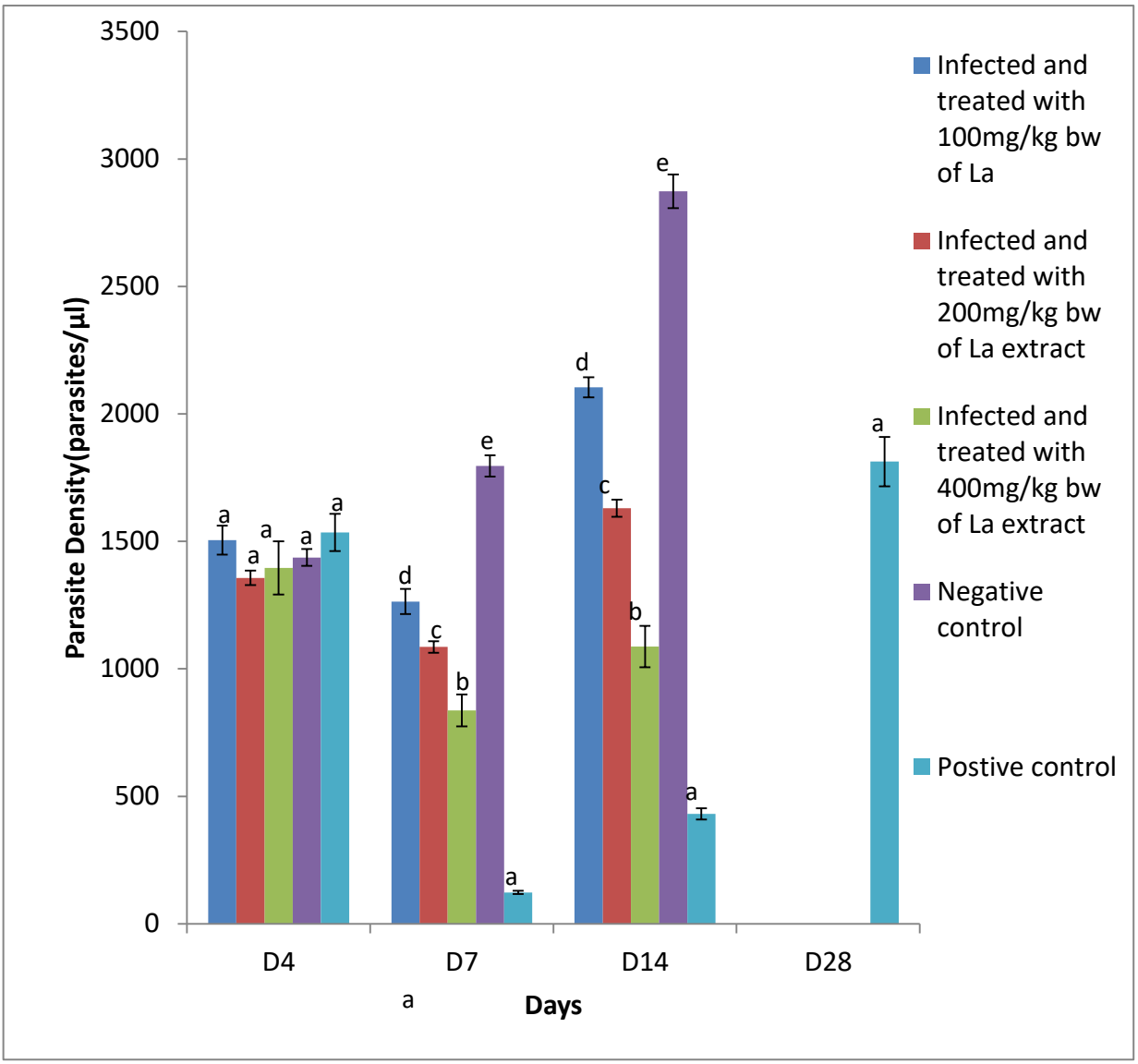


Figure 4.2: Parasite density of *P. berghei* infected mice treated with *Lophira alata*

Key: La = *Lophira alata*, Negative control= group infected with *P. berghei* without treatment and Positive control= infected group treated with 5 mg/kg bw of chloroquine phosphate (the standard drug)

The activity of *Merremia hederacea* during the antiplasmodial screening in *P. berghei* infected mice is presented in Figure 4.3. The activity increased as the dose increased. However, 100, 200 and 400 mg/kg bw of *Merremia hederacea* extract did not clear parasites completely from circulation and could not survive to day 28. The activities of these groups were lower compared to the standard group.

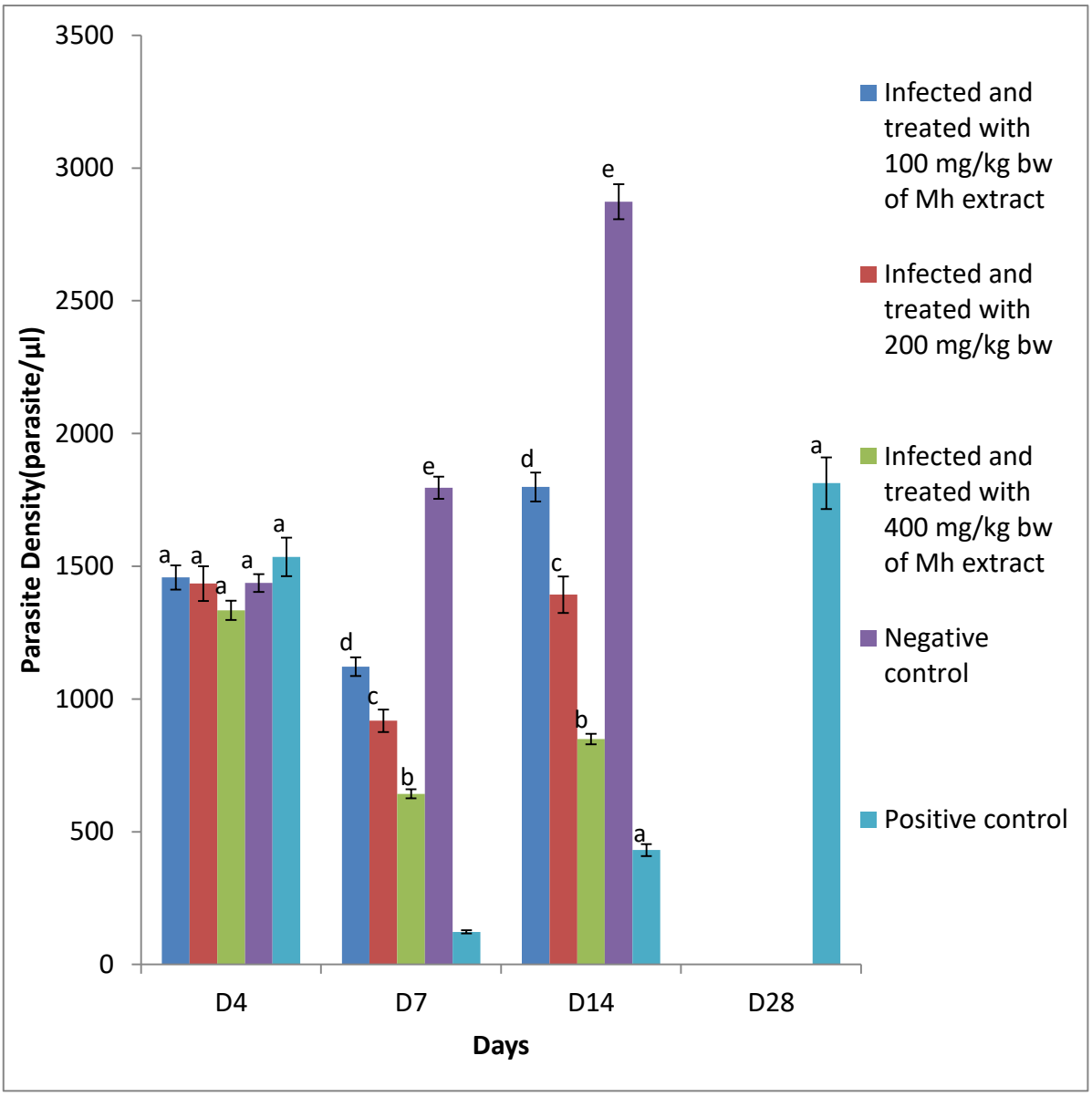


Figure 4.3: Parasite Density of *P. berghei* infected mice treated with *Merremia hederacea*

Key: Mh= *Merremia hederacea*, Negative control= group infected with *P. berghei* without treatment and Positive control= infected group treated with 5 mg/kg bw chloroquine phosphate (standard drug)

In vivo antiplasmodial activity of *Zanthoxylum zanthoxyloide* in NK65 infected mice is presented in Figure 4.4. The activity of this extract also followed a dose-dependent manner. However, none of the groups treated with 100, 200 and 400 mg/kg bw cleared parasites completely and none survived to day 28. The activity of this extract at these dose levels were lower compared to the standard group.

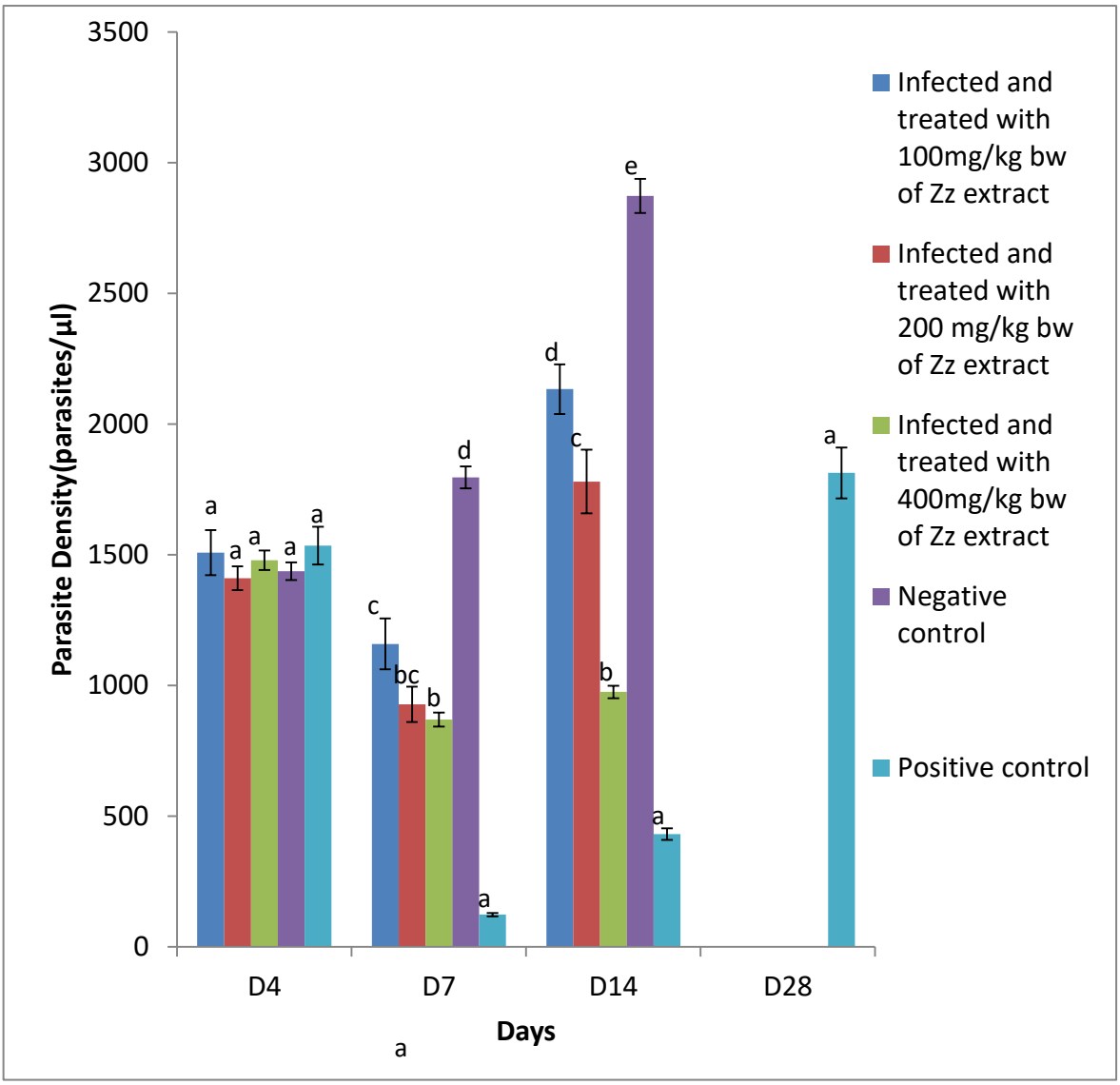


Figure 4.4: Parasite density of *P. berghei* infected mice treated with *Zanthoxylum zanthoxyloide*.

Key: Zz= *Zanthoxylum zanthoxyloide*, Negative control= group infected with *P. berghei* without treatment while Positive control= infected group treated with 5 mg/kg bw chloroquine phosphate (standard drug)

Neocarya macrophylla has shown some *in vivo* antiplasmodial activity as presented in Figure 4.5. The activity of this extract at 100, 200 and 400 mg/kg bw could not clear parasites from circulation and all mice in these group died before day 28 except one (1) mouse treated with 100 mg/kg bw. This extract exhibited an inverse relation between concentration and antiplasmodial activity. The activity of this extract at these dose levels was low compared to the standard group treated with 5 mg/kg bw of Chloroquine.

This extract had an inverse relationship between concentration and antiplasmodial activity.

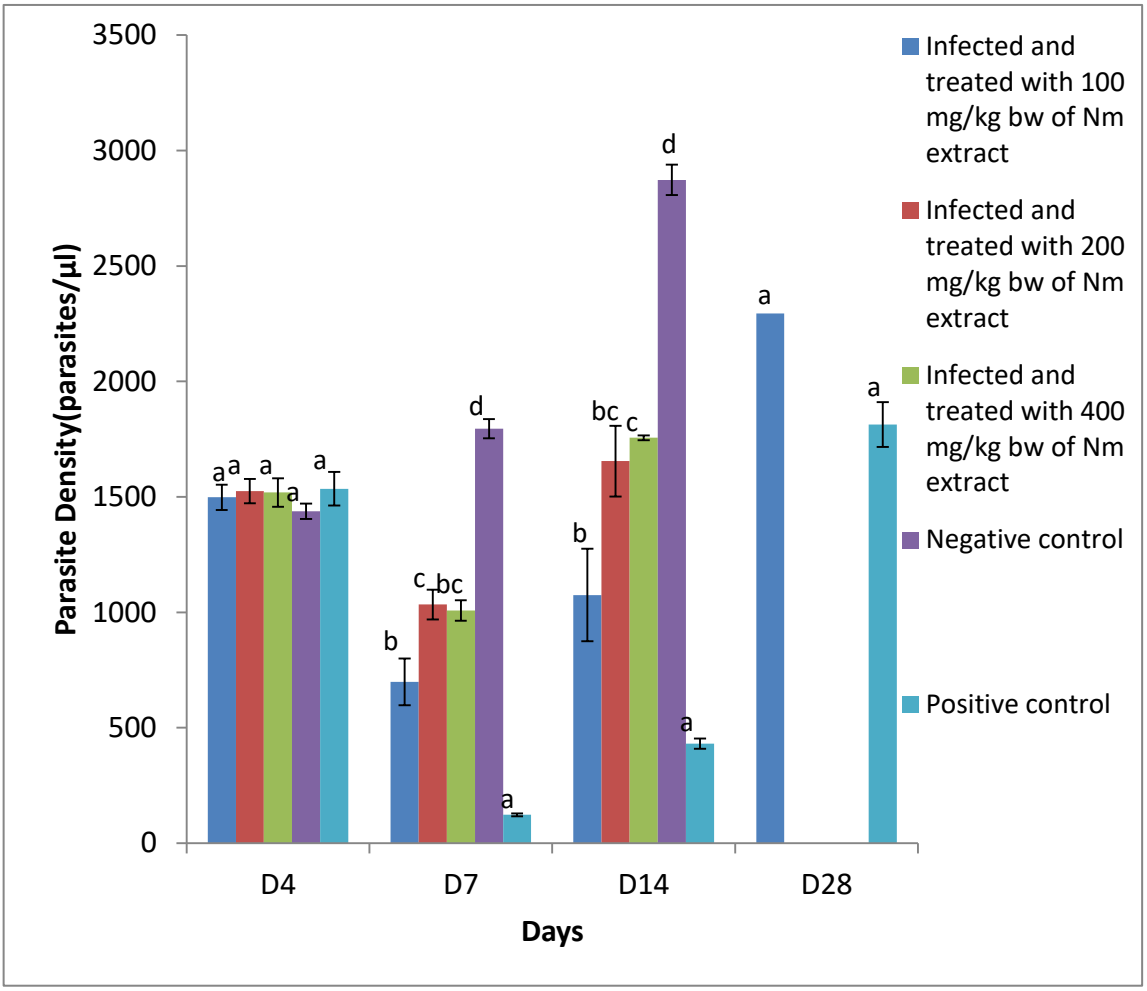


Figure 4.5: Parasite density of *P. berghei* infected mice treated with *Neocarya macrophylla*.

Key: Nm= *Neocarya macrophylla*, Negative control= group infected with *P. berghei* without treatment and Positive control= infected group treated with 5 mg/kg bw of chloroquine phosphate (the standard drug)

The *in vivo* antiplasmodial activity of *Securidaca longepedunculata* in NK65 infected mice is presented in Figure 4.6. Although the activity is dose dependent, higher doses were toxic to the mice. The group teated with 400 mg/kg bw of *Securidaca longepedunculata* died before the control group (before day 14), but had lower parasite density compared to groups treated with 100 and 200 mg/kg bw of *S. longepedunculata.*

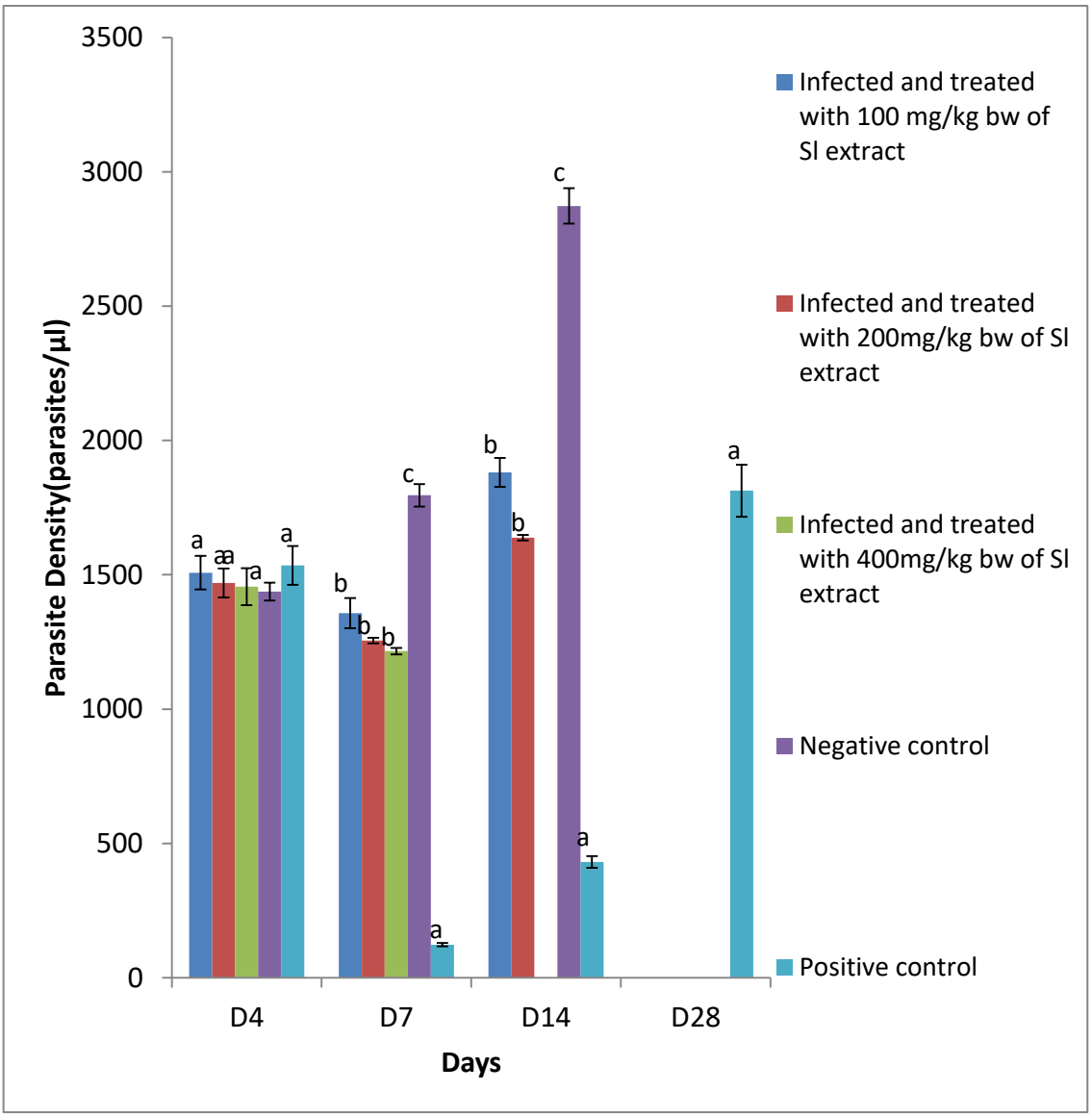


Figure 4.6: Parasite density of *P. berghei* infected mice treated with *Securidaca longepedunculata*

Key: Sl= *Securidaca longepedunculata*, Negative control= group infected with *P. berghei* without treatmen and Positive control= infected group treated with 5 mg/kg bw of chloroquine phosphate

In vivo antiplasmodial activity *Agelanthus dodoneifolium* against *Plasmodium berghei* infected mice is presented in Figure 4.7. 100, 200 and 400 mg/kg bw of *Agelanthus dodoneifolium* did not clear parasites from circulation. Increasing dose of *Agelanthus dodoneifolium* have resulted in little but no significant ($p<0.05$) change in the activity of this extract. The activity of the standard group was better than the *A. dodoneifolium* test groups.

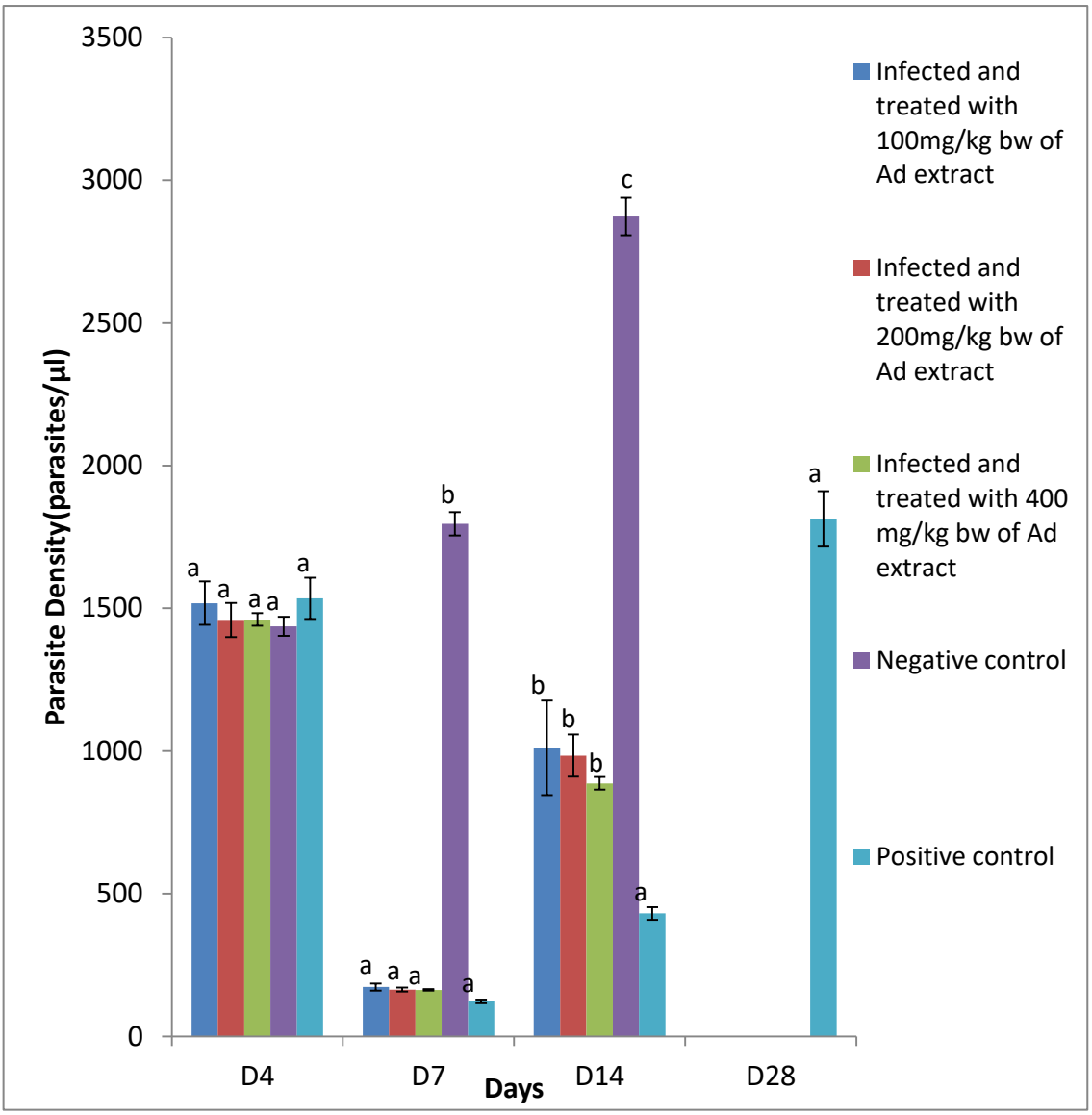


Figure 4.7: Parasite density of *P. berghei* infected mice treated with *Agelanthus dodoneifolium*

Key: Ad= *Agelanthus dodoneifolium*, Negative control= group infected with *P. berghei* without treatment and Positive control= infected group treated with 5 mg/kg bw of chloroquine phosphate (standard drug)

Antiplasmodial activity against *Plasmodium berghei* infected mice for *Polycarpaea linearifolia* extract is presented in Figure 4.8. At 100 and 200 mg/kg bw of *Polycarpaea linearifolia* extract, parasites were not cleared from circulation and mice in these groups did not survive to day 28. This extract also exhibited a dose dependent inhibition. The activity of *P. linearifolia* extract at 400 mg/kg bw is comparable to the standard group, and the mice in this group also survived beyond day 28.

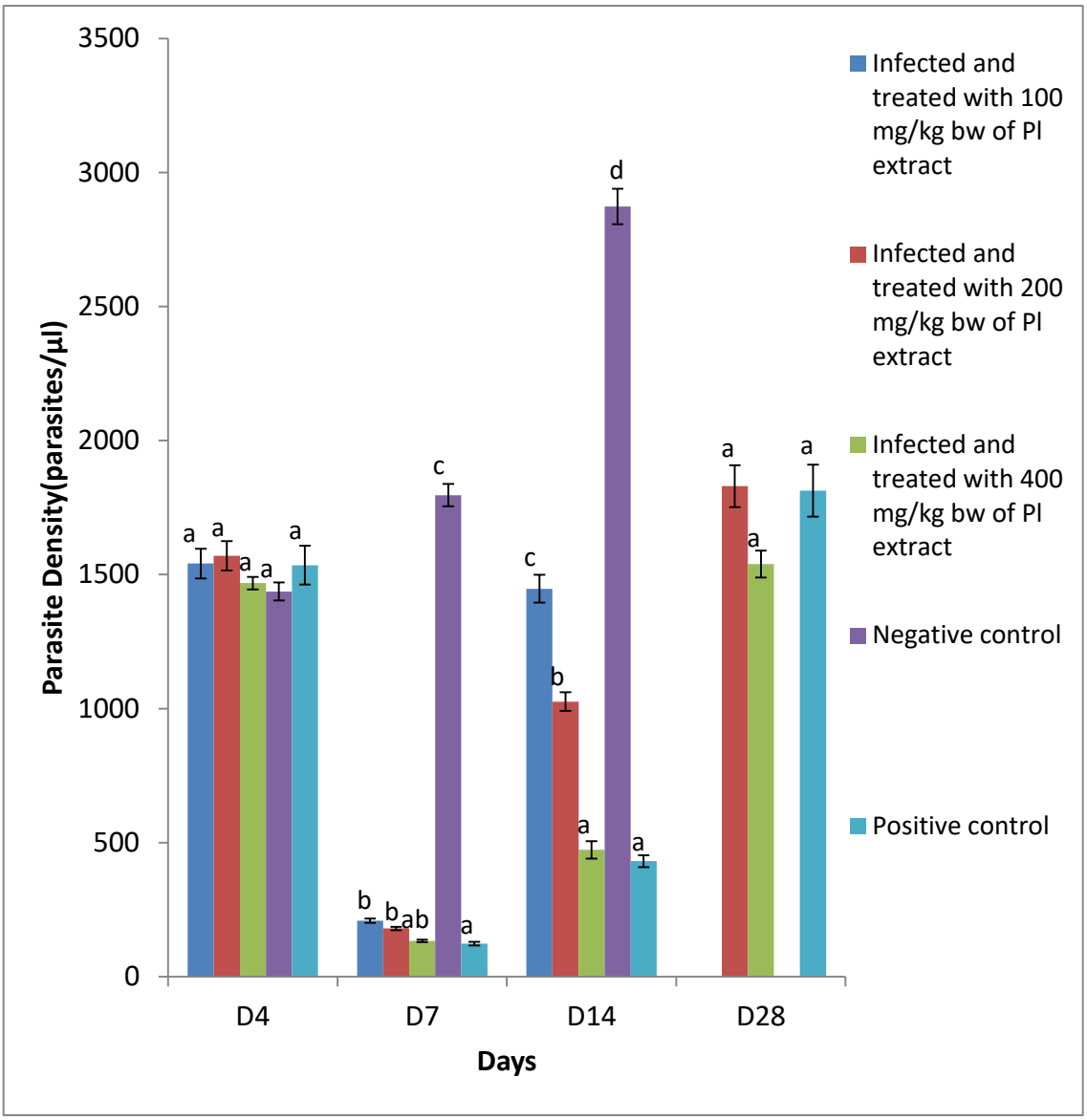


Figure 4.8: Parasite density of *P. berghei* infected mice treated with *Polycarpaea linearifolia*

Key: Pl= *Polycarpaea linearifolia*, Negative control= group infected with *P. berghei* without treatment and Positive control= infected group treated with the standard drug (chloroquine phosphate).

4.1.4.3 Mean Survival Time for mice treated with *L. hastata, L. alata, M. hederacea* and *Z. zanthoxyloide* extracts

The mean survival time of mice treated with 100, 200 and 400 mg/kg bw of different crude extracts (*Leptadenia hastata*, *Lophira alata*, *Merremia hederacea* and *Zanthoxylum zanthoxyloides*) is presented in Table 4.6. *Leptadenia hastata* at 400mg/kgbw have the highest mean survival time (32 days) that is comparable to the standard group (35days) while *Merremia hederacea* and *Lophira alata* had the least

mean survival time (24 days). There was an increase in mean survival time as the treatment dose increased.

Table 4.6: Mean Survival Time of Mice Treated with *L. hastata, L. alata, M. hederacea* and *Z. zanthoxyloides*

Group/Extracts	Dose (mg/kg bw)	Mean survival time(days)
Leptadenia hastata	100	19.33 ± 0.33^{b}
	200	23.33 ± 0.88^{bc}
	400	31.67 ± 1.00^{d}
Lophira alata	100	20.00 ± 0.57^{bc}
	200	21.67 ± 0.33^{bc}
	400	23.67 ± 1.20^{bc}
Merremia hederacea	100	19.67 ± 0.67^{b}
	200	23.33 ± 1.20^{bc}
	400	24.67 ± 0.88^{bc}
Zanthoxylum	100	21.33 ± 2.03^{bc}
zanthoxyloides	200	$23.33\ \pm0.67^{c}$
	400	26.60 ± 0.57^{c}
Standard	5	35.33 ± 1.33^{d}
Control		16.00 ± 0.57^{a}

Values with the same superscript are significantly ($p<0.05$) the same while those with different superscript are significantly ($p>0.05$) different along the column.

4.1.4.4 Mean Survival Time of mice treated with *N. macropylla, S. longepedunculata, A. dodonefolius* and *P. linearifolia* extracts.

The mean survival time of mice treated with 100, 200 and 400 mg/kg bw of *Neocarya macropylla, Securidaca longepedunculata, Agelanthus dodonefolium* and *Polycarpaea linearifolia* extracts is represented in Table 4.7. The mean survival of groups treated with *N. macropylla* and *S. longepedunculata* decreased as the dose increased. *P.*

linearifolia at 400mg/kgbw had mean survival time (31days) that is comparable the standard group (33 days). The control group had the least mean survival time of 16 days.

Table 4.7: Mean Survival Time of Mice Treated with *N. macropylla, S. longepedunculata, A. dodonefolium* and *P. linearifolia*

Group/Extracts	Dose (mg/kg bw)	Mean survival time(days)
N. macropylla	100	31.00 ± 6.00^{d}
	200	26.33 ± 0.88^{cd}
	400	17.67 ± 2.60^{b}
S. longepedunculata	100	21.33 ± 0.67^{bc}
	200	19.33 ± 0.33^{b}
	400	8.33 ± 0.330^{a}
A. dodonefolium	100	19.00 ± 1.53^{b}
	200	22.67 ± 0.33^{bc}
	400	25.00 ± 0.00^{c}
P. linearifolia	100	21.33 ± 0.67^{bc}
	200	24.67 ± 0.33^{c}
	400	30.67 ± 0.88^{d}
Standard	5	33.00 ± 1.00^{d}
Control		16.33 ± 0.33^{a}

Values with the same superscript are significantly ($p<0.05$) the same while those with different superscript are significantly ($p>0.05$) different along the column.

4.1.4.5 *In vivo* antiplasmodial activity of *L. hastata* and *P. linearifolia* when 800 mg/kg bw was given on the first day of treatment

The *in vivo* antiplasmodial activity of *L. hastata* and *P. linearifolia* when 800 mg/kg bw was administered on the first day of treatment (D4) and 400 mg/kg bw on the remaining 3days (D5, D6 and D7) is presented in Figure 4.9 At day 14 there was no parasite in test groups and their activities were comparable to the standard which received double dose on the first of treatment.The test and the standard groups survived beyond day 42 while the control group died before day 28.

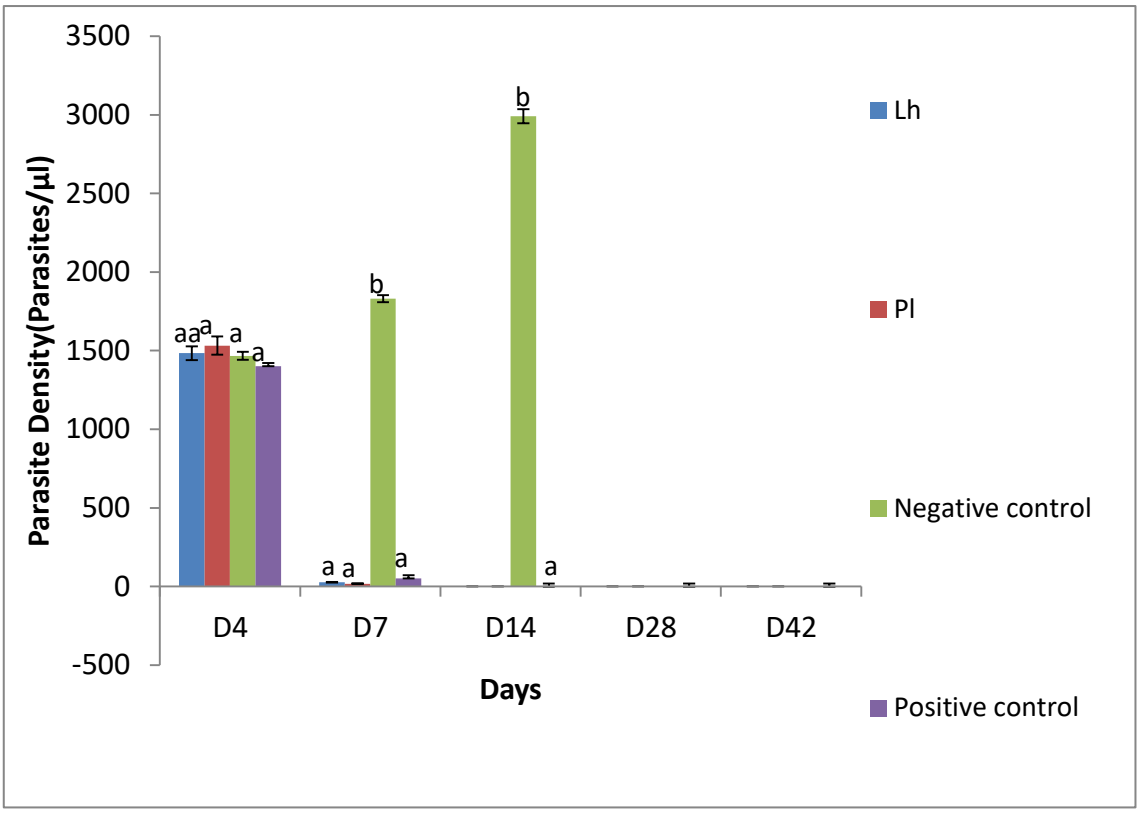


Figure 4.9: *In vivo* Antiplasmodial Activity of *Leptadenia hastata* and *Polycarpaea linearifolia* when treated with 800 mg/kg bw first day of treatment.

Key: Lh=parasite density of infected mice treated with 800 mg/kg bw *L. hastata* extract on the day 4 and 400 mg/kgbw on the days 5, 6 and 7.

Pl = parasite density of infected mice treated with 800 mg/kg bw *P. linearifolia* extract on the day 4 and 400 mg/kg bw on the days 5, 6 and 7.

Control= infected untreated group, Standard= mice treated with 10mg/kgbw of Chloroquine phosphate on the day 4 and 5 mg/kg bw on the days 5, 6 and 7.

4.1.5 Subchronic toxicological screening of crude extracts in rats

4.1.5.1 Some biochemical parameters assayed in rats

The serum glucose concentration of rats administered different crude plant extract is presented in Table 4.8. Serum glucose concentration of test group administered *P. linearifolia* decreased steadily from week1 to week4 and at the end of week4 glucose concentration decreased significantly compared to the control group. There was no significant ($p<0.05$) difference in glucose concentration of *L. hastata* group and the control group.

Table 4.8: Serum Glucose Concentration (mmol/l) levels in Wister Rats

Extract	Week1	Week2	Week3	Week4
P. linearifolia	6.77 ±0.136[b]	5.03 ± 0.449[a]	4.70 ± 0.445[a]	3.76 ±0.199 [a]
L. hastata	3.67 ±0.064[a]	7.39 ± 0.287[b]	4.85 ± 0.051[a]	5.61 ± 0.176[b]
Control	5.47± 0.156[b]	6.27 ± 0 .463[ab]	4.73 ±0.074[a]	6.29 ±0 .299[b]

Key: *P. linearifolia* =*P. linearifolia* test group, *L. hastata* =*L. hastata* test group and C = Control group

N.B: For all tables with superscript, results are expressed as mean± standard error of mean (X±SEM), values with the same superscript are significantly ($p<0.05$) same while those with different superscript are significantly ($p>0.05$) different along the column.

The results for the serum total proteins concentration is represented in table 4.9. None of the extracts significantly ($p<0.05$) altered the total protein content at the end of the four weeks period.

Table 4.9: Serum Total proteins Concentration (g/dl) levels in Wister Rats

Extract	Week1	Week2	Week3	Week4
P. linearfolia	8.75 ±0.186[a]	4.86 ±0.188[a]	6.72 ±0.337 [a]	5.66 ± 0.261[a]
L.hastata	8.97 ±0.138[a]	7.18±0.309[bc]	7.44 ±0.528 [a]	6.02 ±0.271 [a]
C	9.15 ± 0.290[a]	6.72 ±0.384[b]	7.72 ±0.173 [a]	6.83 ± 0.647[a]

Key: *P. linearifolia* =*P. linearifolia* test group, *L. hastata* =*L. hastata* test group and C = Control group

The results of the serum albumin concentration in Wister rats dosed with *P. linearfolia* and *L. hastata* extracts is presented in Table 4.10. At the end of the four weeks, there was no significant ($p<0.05$) difference in albumin concentration of test groups compared to the control group.

Table 4.10: Albumin (g/dl) levels of Rats dosed *P. linearfolia* and *L. hastata* extract.

Extract	Week1	Week2	Week3	Week4
P. linearfolia	3.08 ±0.236[a]	1.84 ±0.140[a]	1.89±0.123 [a]	2.02 ±0.233[a]
L.hastata	2.74 ±0.392[a]	2.17± 0.092[a]	2.81 ±0.065[b]	2.28±0.138[a]

Control	2.53± 0.200[a]	2.01± 0.331[a]	3.00 ±0.209[b]	2.92±0.131[a]

Key: *P. linearifolia =P. linearifolia* test group, *L. hastata =L. hastata* test group and C = Control group

Serum urea concentration in wister rat dosed with *P. linearfolia* and *L. hastata* extracts over the four weeks study period is presented in Table 4.11. Serum urea concentration did not change signicantly compared to the control in week1,3 and 4 but there was a significant (p>0.05) increase in serum urea of group dosed with *L. hastata* at week 2. At the end of week4 serum urea concentrations of rats dosed with *P. linearfolia* and *L. hastata* extracts were all comparable to the control group.

Table 4.11: Serum Urea Concentration (mmol/l) in Wister Rats

Extract	Week1	Week2	Week3	Week4
P. linearfolia	3.53±0.118[a]	7.70±0.201[a]	6.59±0.084[ab]	6.14 ±0.305[a]
L.hastata	4.54±0.176[a]	11.06±0.166[b]	5.03 ±0.438[a]	4.32 ±0.303[a]
Control	5.37±0.436[a]	7.30± 0.185[a]	5.54±0.547[ab]	6.74±0.638[a]

Key: *P. linearifolia =P. linearifolia* test group, *L. hastata =L. hastata* test group and C = Control group

Serum creatinine concentration of rats administered, *P. linearifolia* and *L. hastata* over four weeks is presented in Table 4.12. At week 1, 2 and 3 there no significant (p<0.05) difference in serum creatinine concentration of *P. linearifolia* and *L. hastata* groups compared to the control. Test groups at week4 had significant (p>0.05) increase in serum creatinine concentration compared to the control but the increase is more pronounced in *L. hastata* group.

Table 4.12: Serum Creatinine Concentration (mmol/l) levels in Wister Rats

Extract	Week1	Week2	Week3	Week4
P.linearfolia	0.83 ±0.061[a]	0.72 ±0.037[a]	0.94 ±.082[a]	1.20 ± .026[ab]

L.hastata	0.80 ±0.078[a]	0.87 ± 0.084[a]	0.97 ± .062[a]	1.25 ±.020[b]
Control	0.79 ±0.014[a]	0.93 ± 0.153[a]	0.83 ± .163[a]	0.79 ± .116[a]

Key: *P. linearifolia =P. linearifolia* test group, *L. hastata =L. hastata* test group and C = Control group

The cholesterol concentrations of rats administered different crude extract is represented in Figure 4.10. Only rats that receive *L. hastata* had a regular pattern (ie continuous decrease) in cholesterol content compared to the control over the study period. *P. linearifolia* had increased cholesterol concentration for first three weeks and a decrease in the fourth week.

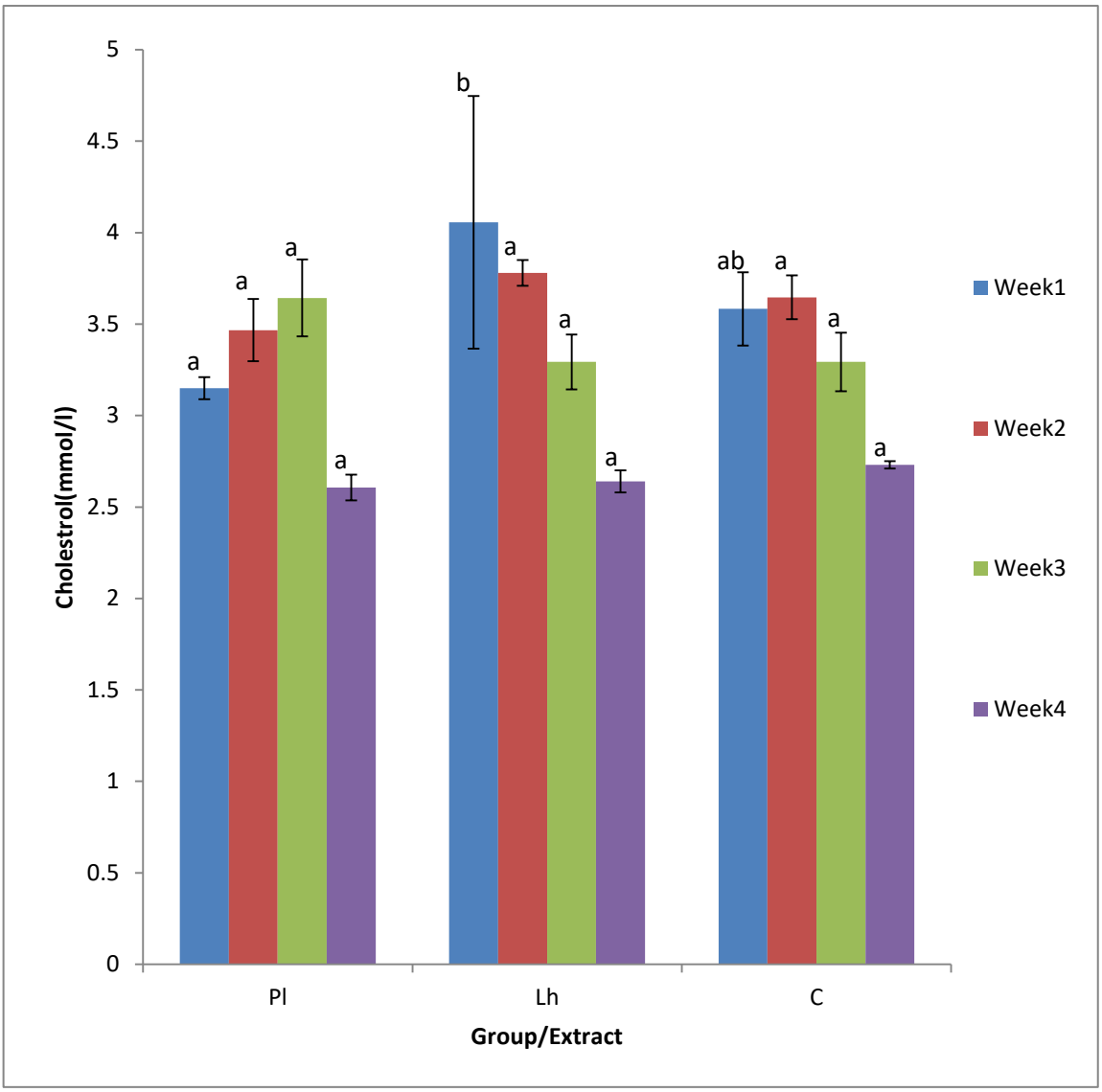


Figure 4.10: Cholesterol levels of Rats admininstered *P. linearfolia* and *L. hastata* extracts.

Key: Pl= group administered *P. linearifolia*, Lh= group administered *L. hastata* and C= control group

Serum triacylglycerol levels of rats administered *P. linearfolia* and *L. hastata* extract is presented Figure 4.11. None of the extracts showed regular pattern for triacylglycerol concentration in rats.

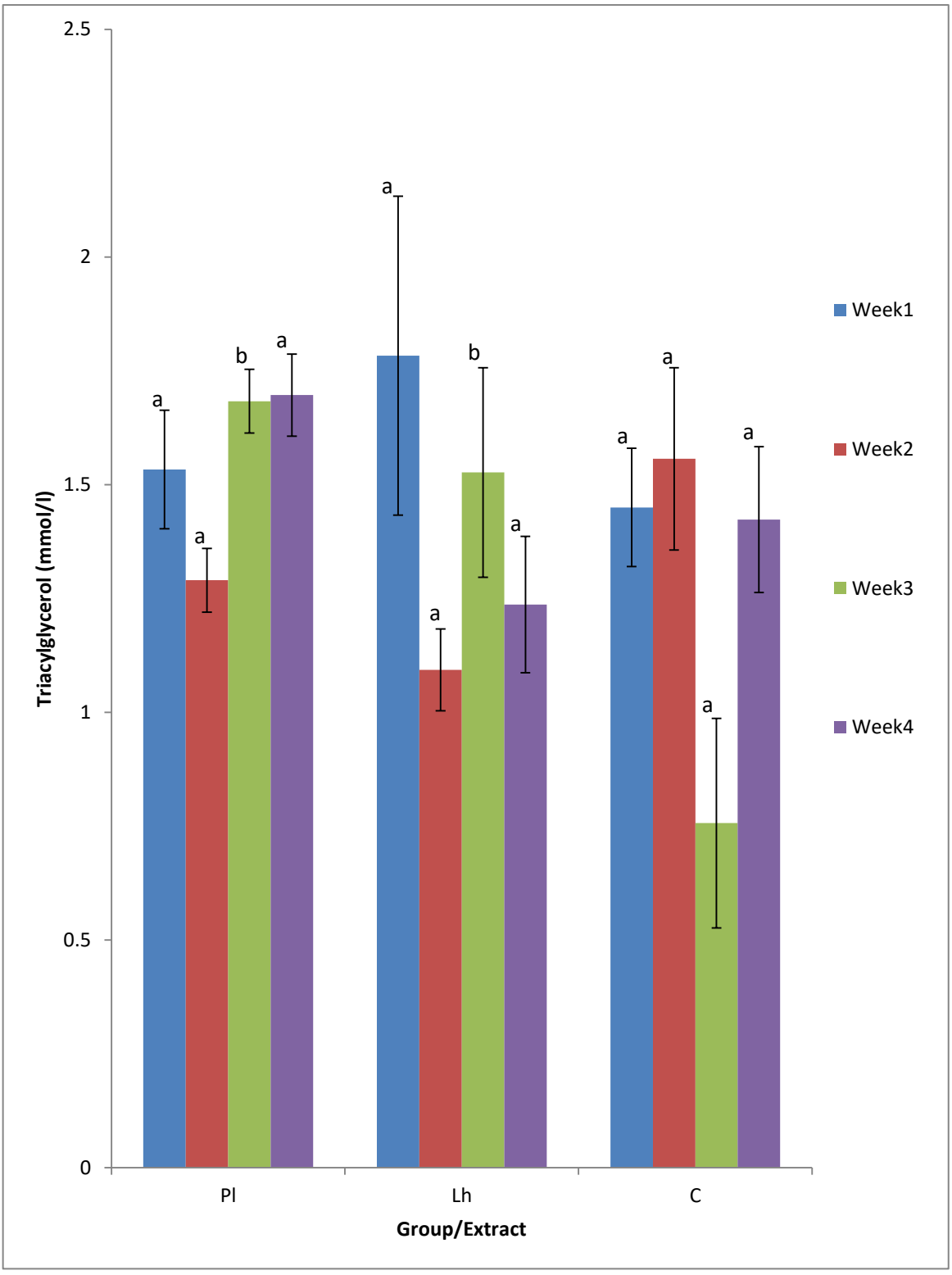


Figure 4.11: Triacylglycerol levels in Rats administered *P. linearfolia* and *L. hastata* extracts.

Key: Pl= group administered *P. linearifolia*, Lh= group administered *L. hastata* and C= control group

The high density lipoprotein (HDL) content of the blood of rats administered *P. linearfolia* and *L. hastata* extracts.is represented in Figure 4.12. HDL levels of group administered *P. linearifolia* and *L. hastata* were significantly higher compared to the control group throughout the four weeks period.

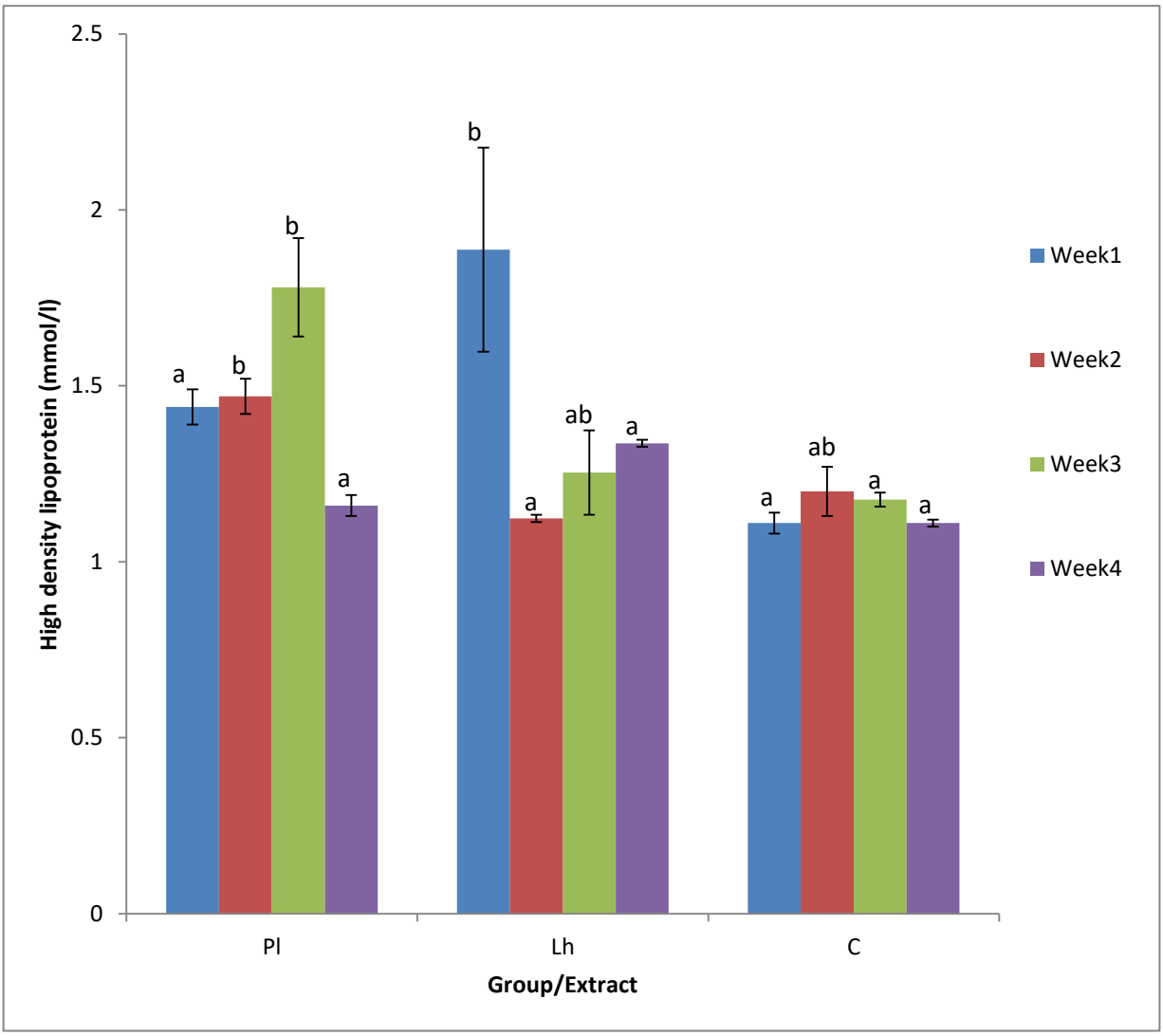


Figure 4.12: High Density Lipoprotein levels in tests and control rats.

Key: Pl= group administered *P. linearifolia*, Lh= group administered *L. hastata* and C= control group

Serum low density lipoprotein (LDL) levels in rats given *P. linearfolia* and *L. hastata* extracts is presented in Figure 4.13. LDL concentration of test groups decreased

significantly compared to the control, except in week2 where LDL concentration of *L. hastata* group was significantly higher than the LDL level of the control.

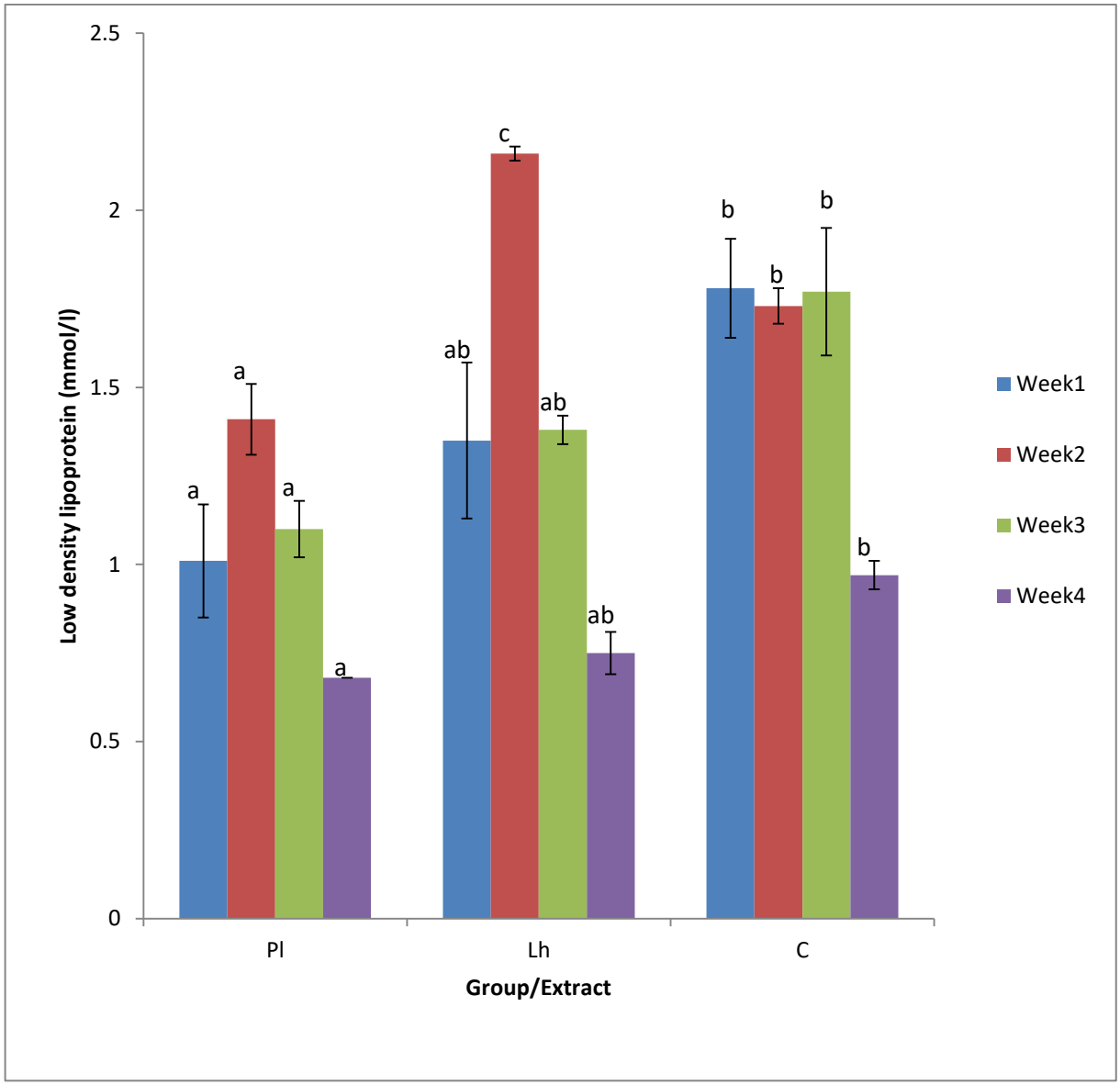


Figure 4.13: Low Density Lipoprotein levels in Rats administered *P. linearfolia* and *L. hastata* extracts.

Key: Pl= group administered *P. linearifolia*, Lh= group administered *L. hastata* and C= control group

The results for serum sodium concentration in rats administered *P. linearfolia* and *L. hastata* extract is represented in table 4.13. There was no significant ($p<0.05$) difference

in sodium concentration of groups administered *P. linearfolia* and *L. hastata* extract compared to the control group.

Table 4.13: Serum Sodium(mEq/L) levels in Wister Rats

Extract	Week1	Week2	Week3	Week4
P.linearfolia	134.63±2.96 [a]	138.40± 6.93[a]	129.17± 2.53[a]	137.50±0.89[a]
L.hastata	134.43±13.17[a]	133.37± 5.41[a]	133.10± 2.10[a]	138.07±1.03[a]
Control	138.50± 4.90[a]	130.23± 4.61[a]	141.97± 2.81 [a]	131.87±2.44[a]

Key: *P. linearifolia =P. linearifolia* test group, *L. hastata =L. hastata* test group and C = Control group

The result for serum potassium concentration during the study period is represented in Table 4.14. None of extract studied gave a specific pattern in potassium concentration but at end of week 4, the concentrations of *P. linearifolia* and *L. hastata* groups were comparable to the control (ie no significant ($p<0.05$) difference between tests and the control).

Table 4.14: Serum Potassium (mmol/l) levels in Wister Rats

Extract	**Week1**	**Week2**	**Week3**	**Week4**
P.linearifolia	4.96 ± 0.180[ab]	9.01 ± 0.229[b]	3.90 ± 0.479[a]	7.78 ± 0.328[a]
L.hastata	4.02 ±0.495[a]	6.45 ± 0.386[a]	3.64 ±0.521[a]	7.81 ± 0.061[a]
Control	6.75 ± 0.549[b]	5.93 ±0 .428[a]	4.13 ± 0.066[a]	7.78 ± 0.393[a]

Key: *P. linearifolia =P. linearifolia* test group, *L. hastata =L. hastata* test group and C = Control group

Table 4.15 shows the serum *Alkaline phosphatase* (ALP) activities in rats over four weeks. There was no statistically significance difference in ALP activities of tests compared to the control group at week.

Table 4.15: Serum Alkaline Phosphatase Activity (U/L) levels in Rats dosed with different Crude Plant Extracts

Extracts	Week1	Week2	Week3	Week4
P.linearifolia	234.98± 3.93[a]	221.67± 9.70[a]	170.08 ± 10.27[a]	160.12±29.51[a]
L.hastata	155.52 ± 8.77[a]	162.03± 2.60[a]	160.20 ± 9.99[a]	147.83± 9.92[a]
Control	162.26 ± 2.82[a]	162.35± 3.77[a]	148.27 ± 11.66[a]	133.31± 8.62[a]

Key: *P. linearifolia* =*P. linearifolia* test group, *L. hastata* =*L. hastata* test group and C = Control group

Serum *Alanine aminotransferase* activity over four weeks period is presented in Table 4.16. At week1 the test group had a decreased ALT activity compared to the control group. Administration of *P. linearifolia* and *L. hastata* had no significant ($p<0.05$) difference in ALT activity compared to the control at the end of week 2, 3 and 4.

Table 4.16: Serum Alanine Aminotransferase (U/L) levels of Rats dosed with different Plant Crude Extracts

Extracts	Week1	Week2	Week3	Week4
P.linearifolia	6.65 ±0.749[a]	9.22 ±0.199[a]	7.74 ± 0.123 [a]	8.46±0.057[a]
L.hastata	7.97 ±0.499[ab]	9.63 ±1.052[a]	8.42 ± 0.159 [a]	11.06 ±0.745[a]
Control	9.68 ±0.868[b]	7.66 ±0.800[a]	8.28 ±0.877[a]	10.74 ±0.843[a]

Key: *P. linearifolia* =*P. linearifolia* test group, *L. hastata* =*L. hastata* test group and C = Control group

Serum *Aspartate Aminotransferase* (AST) activities of rats administered different crude extracts is presented in Table 4.17. At week 1,2 and 3 there was no significant ($p<0.05$) difference in AST activity of *P. linearifolia* and *L. hastata* test groups. *P. linearifolia* group recorded the highest AST activity at the end of week4 treatment (ie there was a significant ($p>0.05$) increase compared to the control). *L.hastata* test group had values comparable to the control group.

Table 4.17: Serum Aspartate Aminotransferase (U/L) of Rats dosed with different Plant Crude Extracts

Extracts	Week1	Week2	Week3	Week4
P.linearifolia	8.91 ±1.100 [a]	7.74 ± 0.123 [a]	8.22 ± 0.477 [a]	14.983 ± 0.658[b]
L.hastata	10.45 ±0.694 [a]	8.42 ± 0.159 [a]	9.46 ± 1.545 [a]	9.806 ±1.063 [a]
Control	10.73 ±0.749[a]	8.28 ±0.877[a]	10.21 ± 0.456 [a]	9.160±0.760[a]

Key: *P. linearifolia* =*P. linearifolia* test group, *L. hastata* =*L. hastata* test group and C = Control group

Bilirubin concentration of rats dosed with different plant extracts is presentred in Table 4.24 below. In week 1, 2 and 3 there was no significant ($p<0.05$) difference in bilirubin concentration of the *P. linearifolia* and *L. hastata* compared to the control. There was a significant ($p>0.05$) increase in bilirubin concentration at the end of week4 of *L. hastata* test group compared to the control but *P. linearifolia* group had no significant ($p<0.05$) difference compared to the control group.

Table 4.18: Serum Bilirubin (mg/dl) of Rats treated with different Plant Crude Extracts

Extracts	Week1	Week2	Week3	Week4
P.linearifolia	0.36 ±0.115[a]	0.72 ± 0.023[a]	0.37 ± 0.115[a]	1.25 ± 0.128[ab]
L.hastata	0.93 ±0.095[a]	0.76 ± 0.036[a]	0.88 ± 0.105[a]	1.55 ±0.240[b]
Control	0.88 ±0.102[a]	0.73 ± 0.080[a]	0.813±0.113[a]	0.96 ±0.085[a]

Key: *P. linearifolia =P. linearifolia* test group, *L. hastata =L. hastata* test group and C = Control group

4.1.5.2 Haematological parameters assayed

The white blood cell counts in week1of rats dosed with *P. linearifolia* and *L. hastata* extracts is presented in Figure 4.24. Group administered *L. hastata* had a total leucocyte count greater than the control group while *P. linearifolia* was comparable to the control. *L. hastata* had lymphocytes value higher than the control while *P. linearifolia* group had value lower than the control but not significantly different. The *L. hastata* group had the lowest monocytes count. Rats that received *P. linearifolia* had the highest granulocyte count in week1

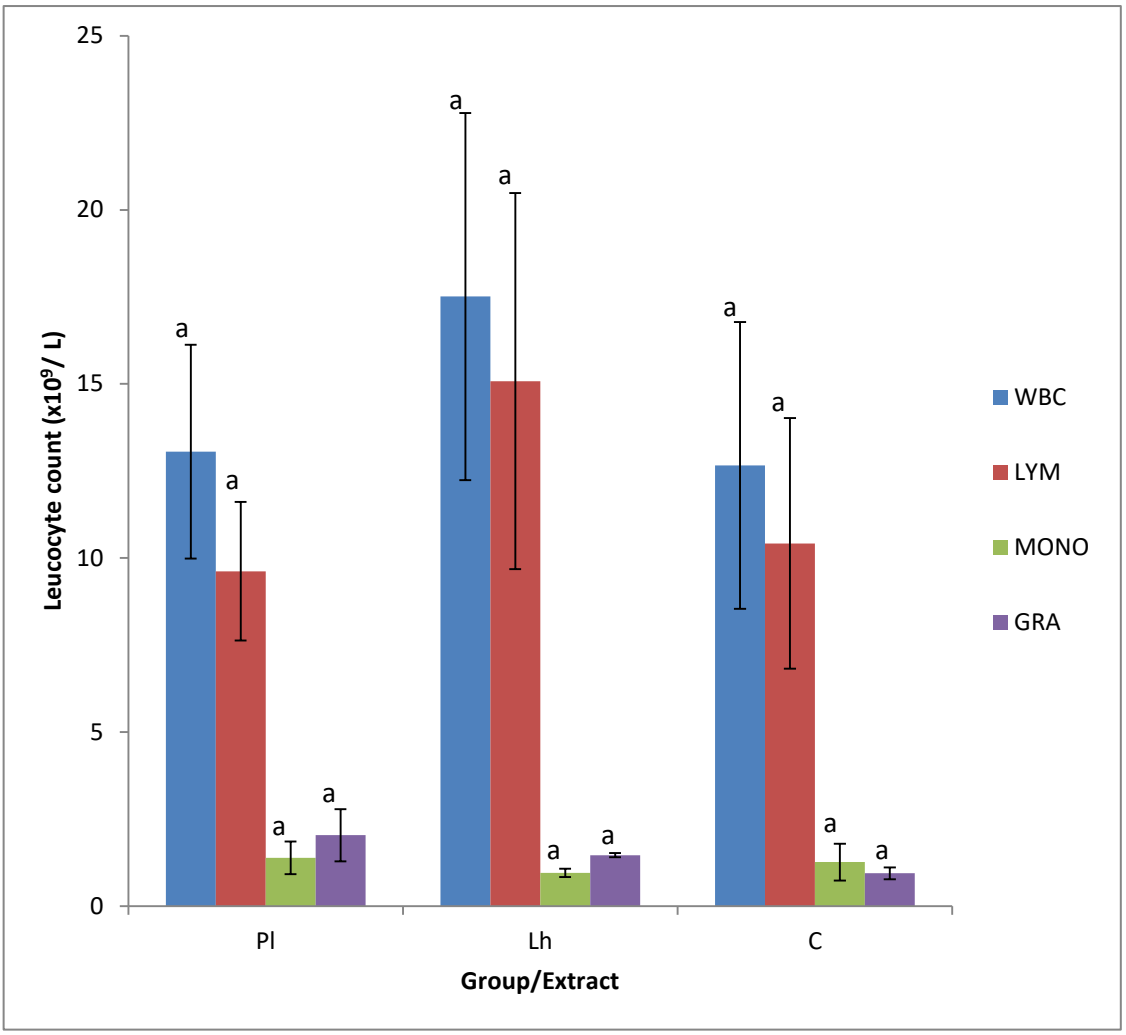


Figure 4.14: White Blood Cell Indices of Rats Dosed with *P. linearifolia* and *L. hastata* extracts at Week1.

Key: WBC=white blood cells, LYM=lymphocytes, MONO=monocytes, GRA=granulocytes, Pl= group administered *P. linearifolia*, Lh= group administered *L. hastata* and C= control group.

The result for white blood cell indices in week 2 is represented in figure 4.15. Total leucocytes were higher in rats given *P. linearifolia* and *L. hastata* compared to the control. Lymphocytes we in groups dosed with *P. linearifolia* and *L. hastata* groups were comparable to the control group. Similarly, *P. linearifolia* and *L. hastata* groups were most abundant in term of circulating monocytes in week 2. All test groups have circulating monocytes significantly higher than the control group. *L. hastata* had exceptional higher granulocyte count in comparism to the control.

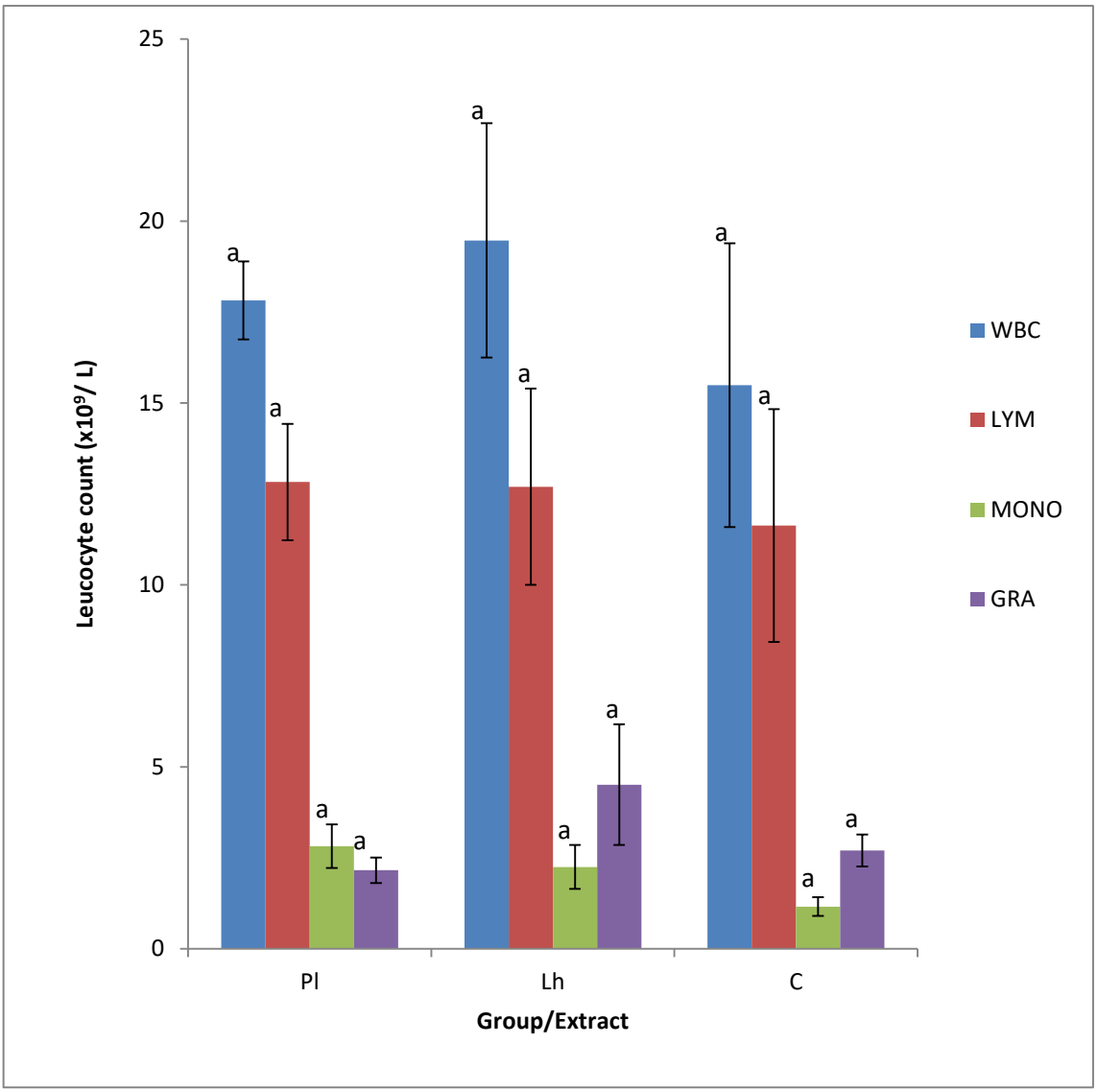


Figure 4.15: White Blood Cell Indices of Rats Dosed with *P. linearifolia* and *L. hastata* at week2

Key: WBC=white blood cells, LYM=lymphocytes, MONO=monocytes, GRA=granulocytes, Pl= group administered *P. linearifolia*, Lh= group administered *L. hastata* and C= control group.

Figure 4.16 below represents the white blood indices of rats administered *P. linearifolia* and *L. hastata* extracts. *P. linearifolia* group had the highest total leucocyte count while *L. hastata* group had the least compared to the control. *L. hastata* group had lymphocyte count lower than the control in week 3. Still within week3 *P. linearifolia* group had the

highest granulocyte count while *L. hastata* group recorded the least granulocyte count in week 3.

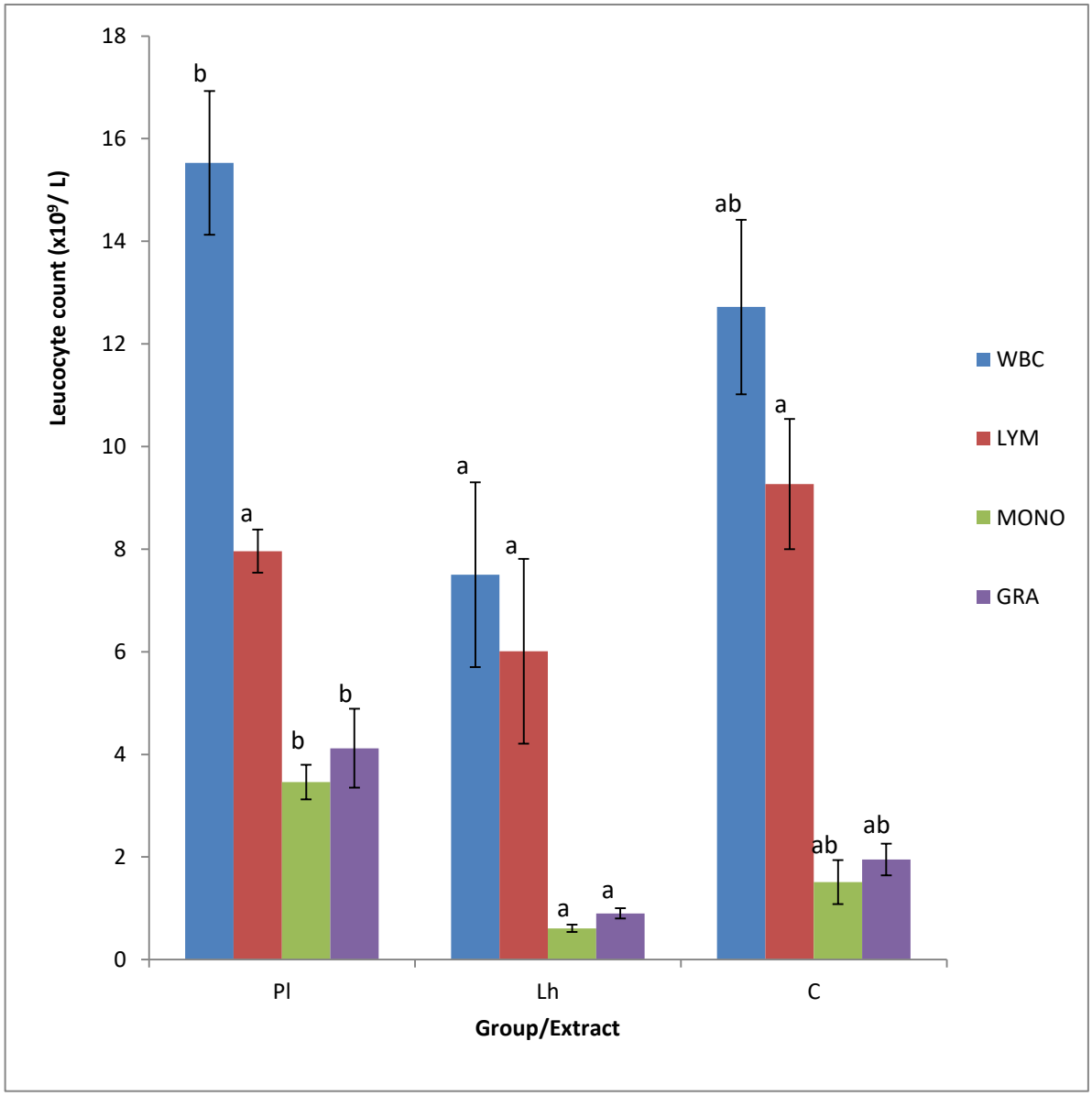


Figure 4.16: White Blood Cell Indices of Rats Dosed with Different Extracts at Week3.

Key: WBC=white blood cells, LYM=lymphocytes, MONO=monocytes, GRA=granulocytes, Pl= group administered *P. linearifolia,* Lh= group administered *L. hastata* and C= control group.

The white blood cell indices in week 4 of rats dosed with *P. linearifolia* and *L. hastata* extracts is presented in Figure 4.17. Total white blood cell counts of the groups were comparable to the control. Similarly, lymphocyte count of *L. hastata* group was higher

compared to control group. *L. hastata* had the highest count for monocytes while *P. linearifolia* had monocytes comparable to the control group. Granulocyte count for *L. hastata* group was higher than the control while *P. linearifolia* had a lower count compared to the control.

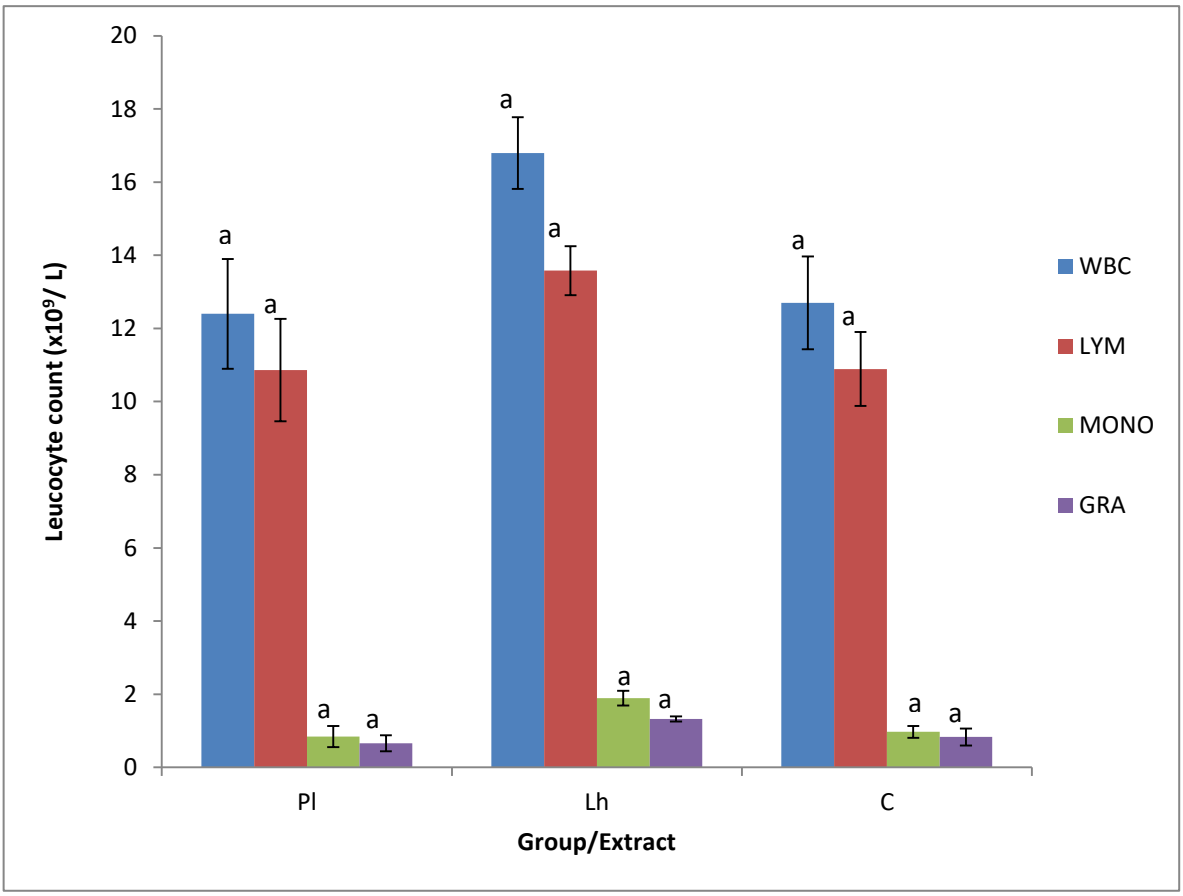


Figure 4.17: White Blood Cell Indices of Rats Dosed with Different Crude Extracts at Week4.

Key: WBC=white blood cells, LYM=lymphocytes, MONO=monocytes, GRA=granulocytes, Pl= group administered *P. linearifolia*, Lh= group administered *L. hastata* and C= control group.

The result for platelet count of rat groups dosed with *P. linearfolia* and *L. hastata* is presented in Table 4.19. There was no significant ($p<0.05$) difference in platelet count of the test groups compared to the control at week 1 and 2 but the group treated with *P. linearfolia* had a significant ($p>0.05$) increase in platelet count compared to the control while *L. hastata* group was significantly lower compared to the control. There was no

significant ($p<0.05$) difference in platelet count of the test groups compared to the control at week4.

Table 4.19: Platelet count (x 10^9/l) in rats dosed with *P. linearfolia* and *L.hastata* extract

Extract	**Week1**	**Week2**	**Week3**	**Week4**
Pl	272.50 ±82.523[a]	723.33±52.045[a]	620.33 ± 67.760[b]	634.00± 35.837 [a]
Lh	266.67± 98.727[a]	683.33± 27.290[a]	234.33 ± 55.103[a]	422.67± 23.918 [a]
C	364.6 7±81.068[a]	569.33± 71.265 [a]	358.33 ± 46.160[ab]	522.33 ±16.300 [a]

Key: Pl= group administered *P. linearifolia*, Lh= group administered *L. hastata* and C= control group.

The result of red blood cell count of rats dosed with *P. linearifolia* and *L. hastata* extracts is represented in Figure 4.18. RBC count of *P. linearifolia* group was on a continuous decrease from week 1 to 4 compared to the control while the red blood cell count of *L. hastata* group did not follow any regular pattern..

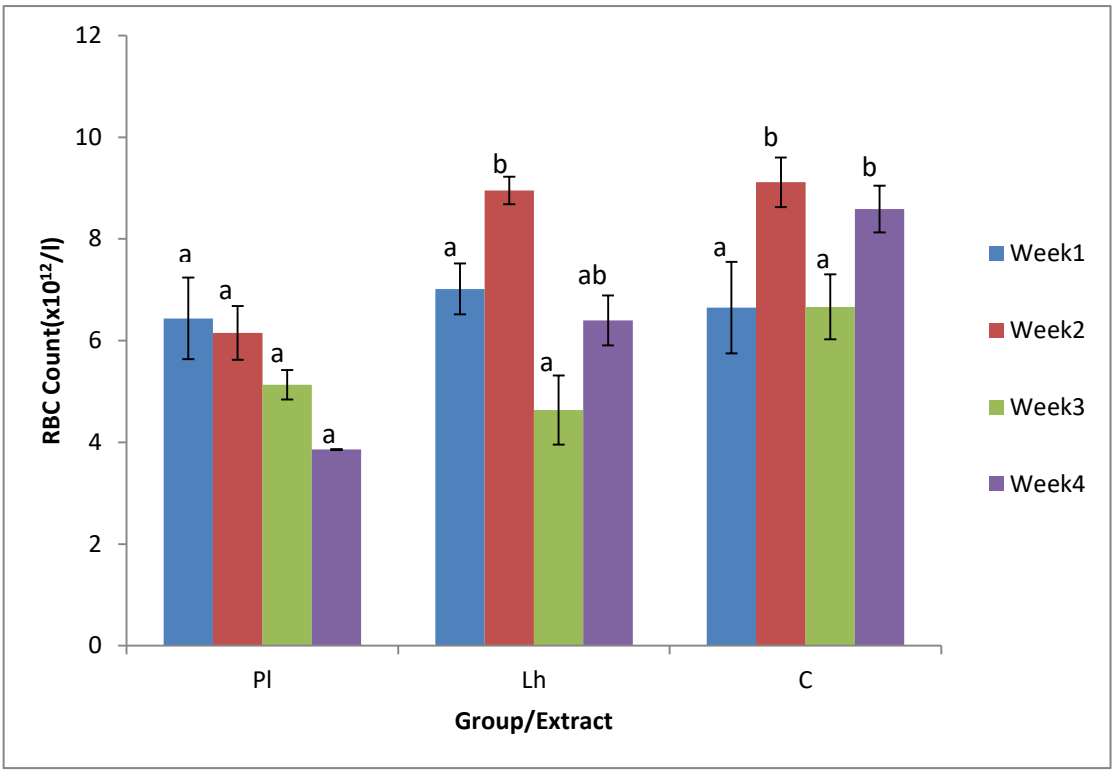


Figure 4.18: Red Blood Cell Counts of Rats Dosed with Different Plant Extracts

Key: Pl= group administered *P. linearifolia*, Lh= group administered *L. hastata* and C= control group.

The results of haemoglobin concentration in rats dosed with crude extract of *P. linearifolia* and *L. hastata* is represented in Figure 4.19. Haemoglobin concentration of *P. linearifolia* group was continuously decreasing on weekly basis compared to the control group while *L. hastata* group did not follow any regular pattern.

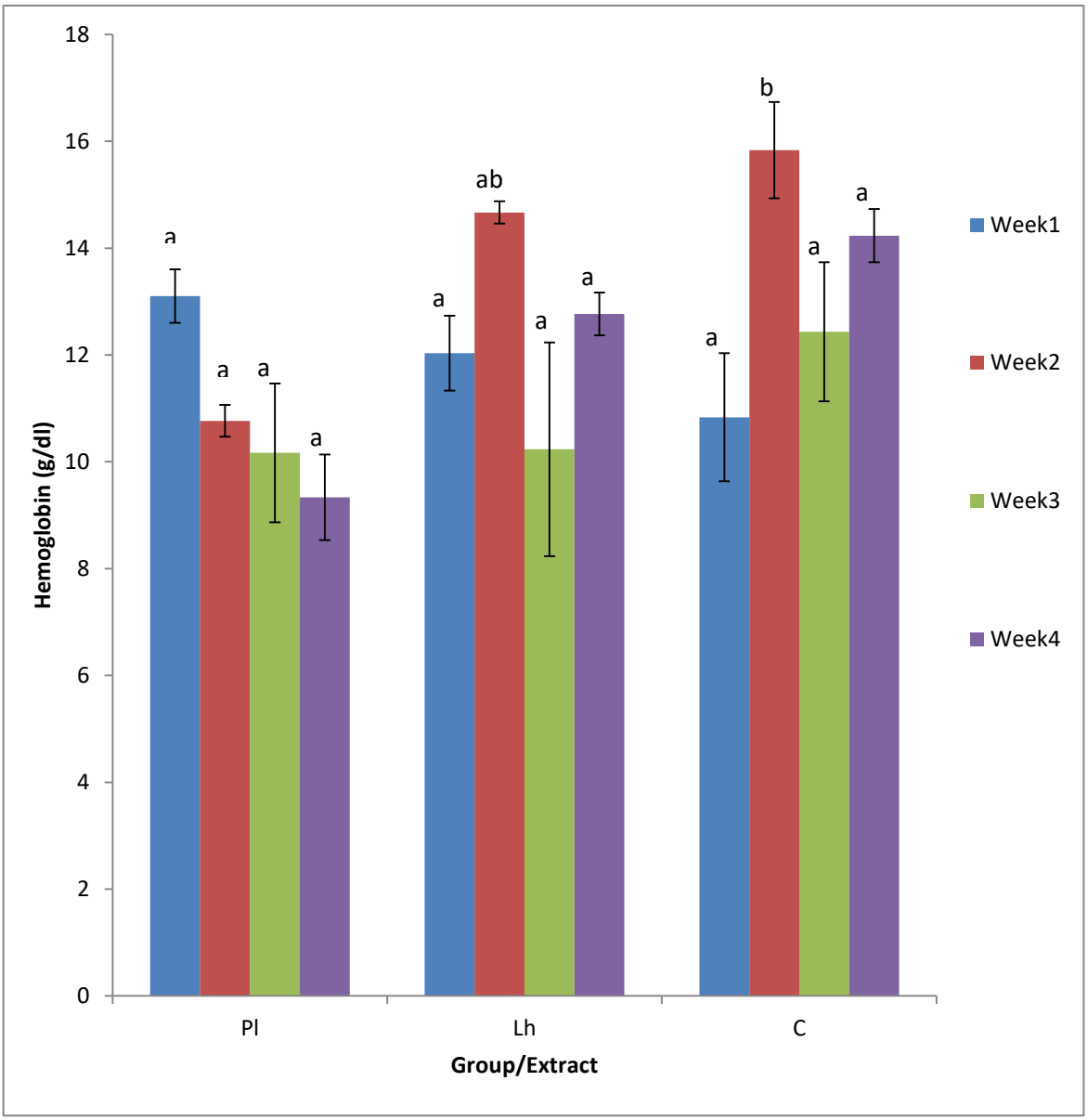


Figure 4.19: Haemoglobin Concentration of Rats Dosed Different Crude Extracts

Key: Pl= group administered *P. linearifolia*, Lh= group administered *L. hastata* and C= control group.

The result for hematocrit in rats administered crude extracts of *P. linearifolia* and *L. hastata* is presented in Figure 4.20. *P. linearifolia* group had decreased hematocrit value compared to the control over the study period. *L. hastata* group had an increased hematocrit value compared to the control except in week3 that low hematocrit was recorded.

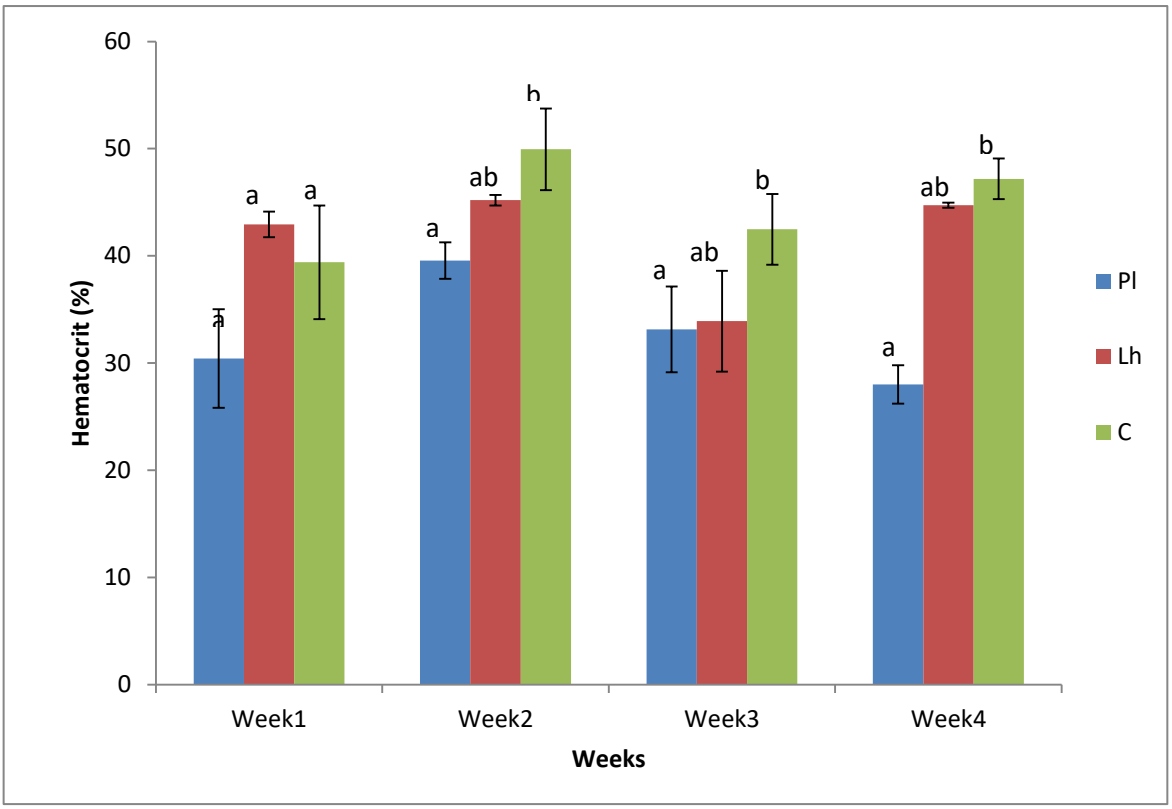


Figure 4.20: Hematocrit of rats dosed with *P. linearfolia* and *L. hastata* extracts

Key: Pl= group administered *P. linearifolia*, Lh= group administered *L. hastata* and C= control group.

4.1.5.3 Histopathological effect of most active extract in rats

The histology results are presented in Plates I-XII. Plates I, II and III represent the micrograph sections of rat liver admininstered *P. linearfolia*, *L. hastata* extracts and the control group. The histoarchitecture of the liver sections revealed intact hepatocytes with normal hexagonal arches and no significant pathology in the liver section of *P. linearfolia* and *L. hastata* treated groups.

The administration of *L. hastata* and *P. linearifolia* extracts in the subchronic toxicity study did not reveal any damage to the liver, kidney and the heart, but hyperplastic lymphoid follicles were seen in the spleen of rat administered *L. hastata* extract.

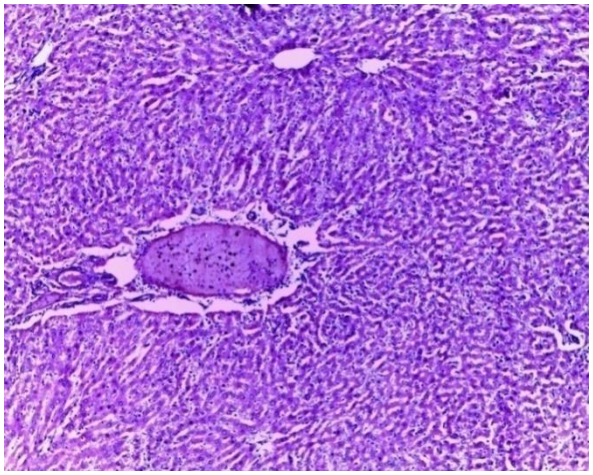


Plate II: Micrograph section of rat liver administered *P. linearifolia* (H & E, X100).

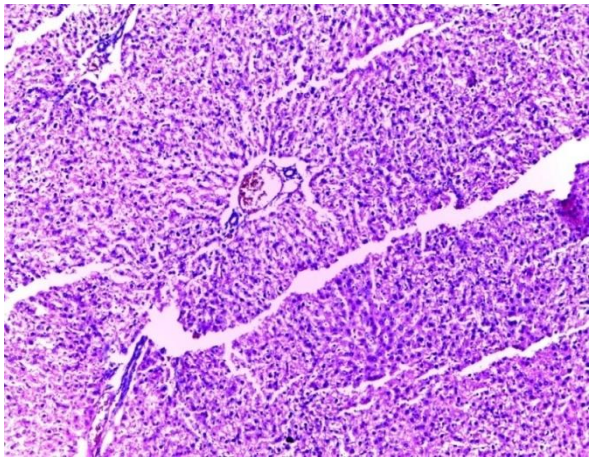


Plate III: Micrograph section of rat liver administered *L. hastata* (H & E, X100).

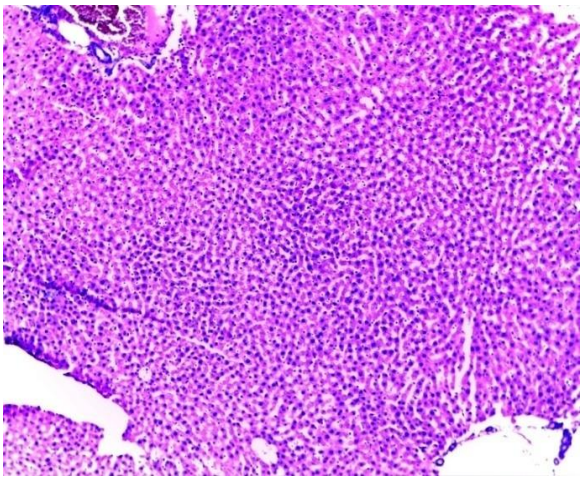


Plate IV: Micrograph of liver section of control group (H & E, X100)

The histology result of groups dosed with *P. linearfolia* and *L. hastata*, and the control group are represented in Plates IV, V and VI. The histoarchitecture of the kidney sections had normal and intact glomerulus and bowman's capsule. There was no pathology in the kidney section of test groups compared to the control.

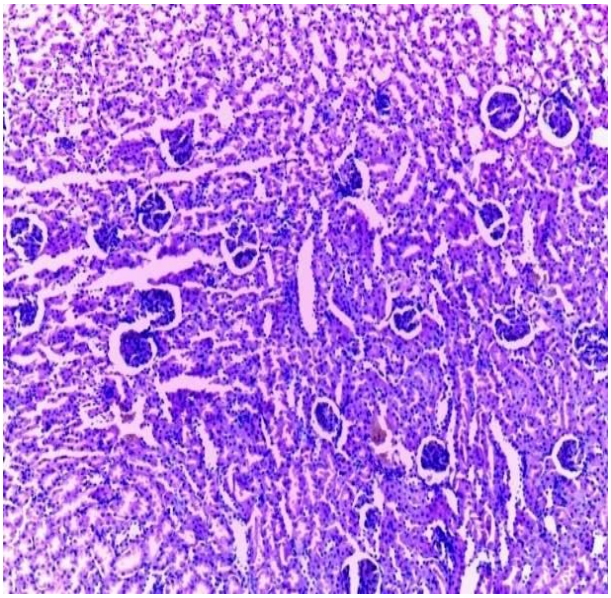


Plate V: Micrograph section of rat kidney administered *P. linearifolia* section (H & E, X100).

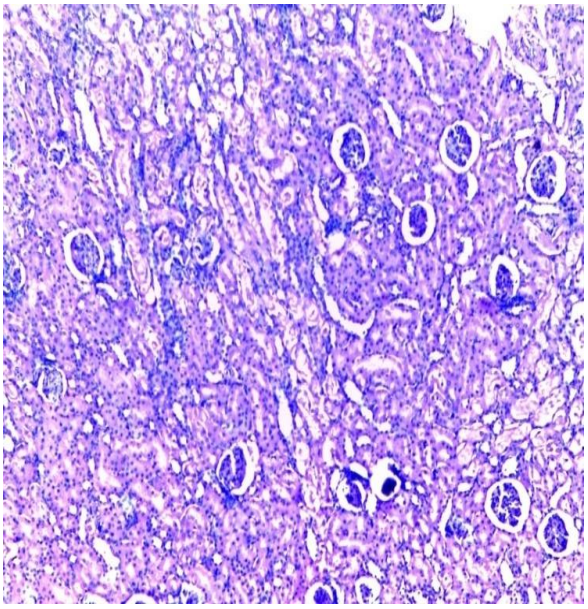


Plate VI: Micrograph section of rat kidney administered *L. hastata* (H & E, X100).

.

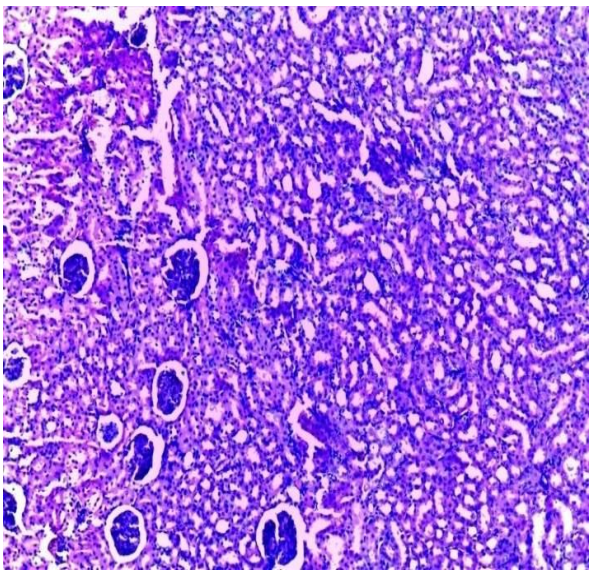


Plate VII: Micrograph of kidney section of control rat (H & E, X100).

The spleen monography sections of rats administered *P. linearfolia* and *L. hastata* with the control are presented in Plates VII, VIII and VIII respectively. Histoarchitecture of spleen section of *P. linearfolia* group is comparable to the control groupwith normal white and red pulps while group treated with *L. hastata* had hyperplastic lymphoid follicles.

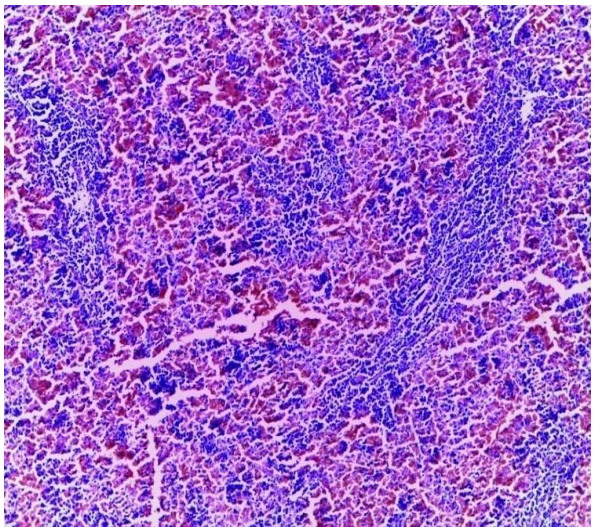


Plate VIII: Micrograph section of rat spleen administered *P. linearifolia* (H & E, X100).

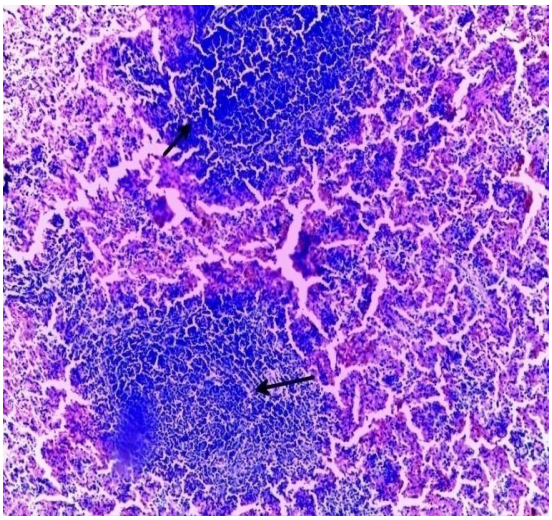


Plate IX: Micrograph section of rat spleen administered *L. hastata* (H & E, X100).

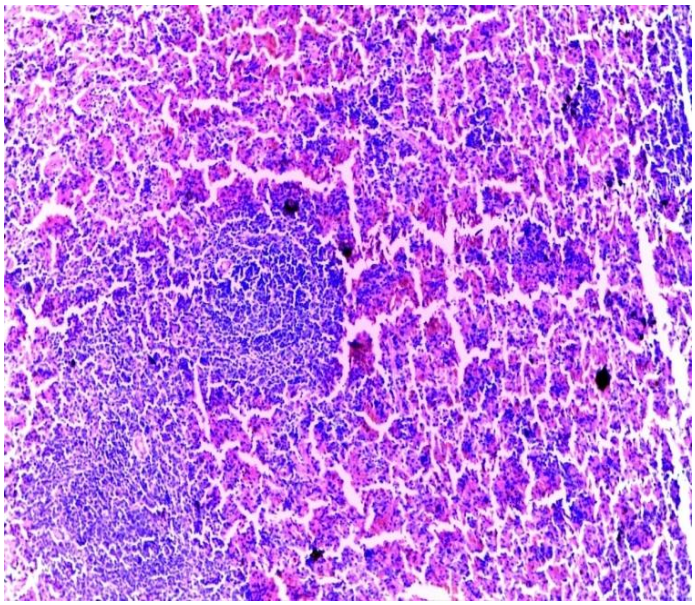


Plate X: Micrograph of spleen section of control (H & E, X100).

The heart monography sections of rats administered *P. linearfolia* and *L. hastata* with the control are presented in Plates X, XI and XII respectively. Monograph sections of heart of test groups are comparable to the control group with normal histoarchitecture of the myocardium.

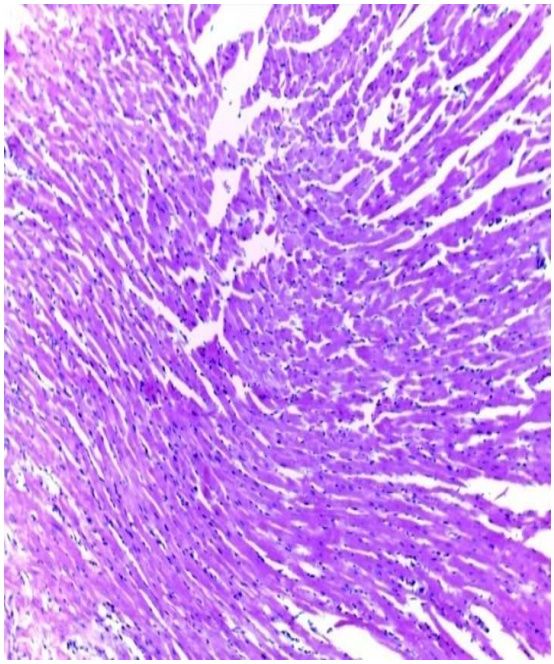


Plate XI: Micrograph section of rat heart administered *P. linearifolia* (H & E, X100).

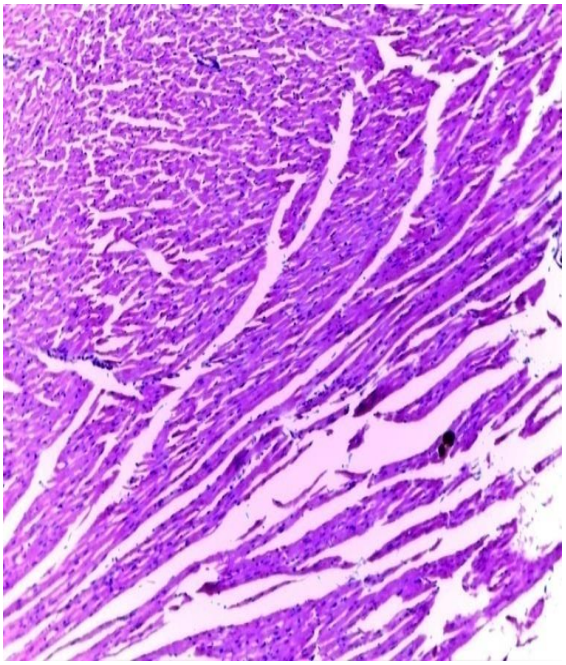


Plate XII: Micrograph section of rat heart administered *L. hastata* (H & E, X100)

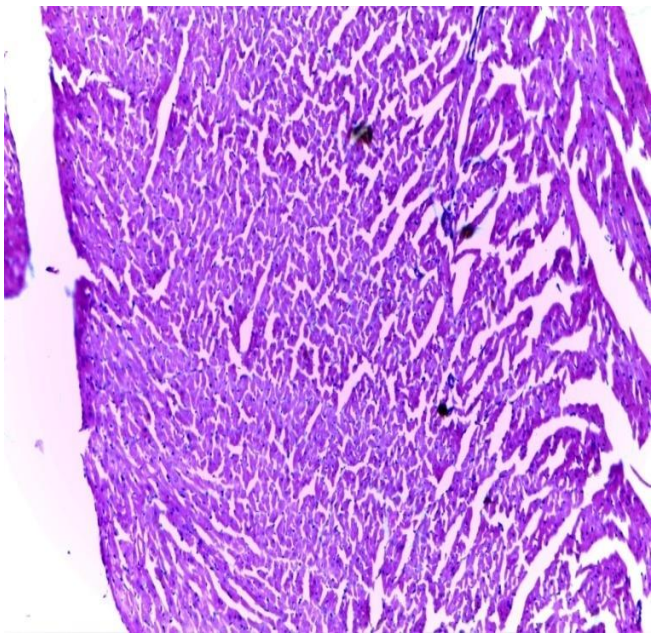


Plate XIII: Micrograph of heart section of control rat (H & E, X100)

4.1.6 Bioassay guided fractionation

Fractionation of *L. hastata* and *P. linearfolia* extracts gave six fractions each. Lh1, Lh2, Lh3, Lh4A, Lh4B and Lh5 were fractions from *L. hastata*. Fractions from *P. linearfolia* were Tk1, Tk2A, Tk2B, Tk3, Tk4 and Tk5.

4.1.6.1 *In vitro* antiplasmodial activity of column fractions of *L. hastata* and *P. linearfolia* extracts

The *in vitro* antiplasmodial activities (IC_{50}) of *L. hastata* and *P. linearfolia* fractions is presented in Table 4.20. Fractions Lh1, Lh2 and Lh3 had IC_{50} value above 10 µg/ml *in vitro* antiplasmodial against NF54 *P. falciparum* strain while fractions Lh4A, LhB and Lh5 had IC_{50} values of 4.24, 8.50 and 7.24 µg/ml in *in vitro* antiplasmodial test against *P. falciparum.* All *P. linearfolia* fractions had IC_{50} greater than 10 µg/ml, except Tk3 that had the best activity with IC_{50} value of 2.4 µg/ml.

Table 4.20: *In vitro* Antiplasmodial Activities of *L. hastata* and *P. linearfolia* Fractions

Fraction	Activity (µg/ml)
Lh1	>10
Lh2	>10
Lh3	>10
Lh4A	4.24
Lh4B	8.50
LH5	7.24
Tk1	>10
Tk2A	>10
Tk2B	>10
TK3	2.40
Tk4	>10
Tk5	>10

Key: Lh1, Lh2, Lh3, Lh4A, LhB and Lh5 are *L. hastata* fractions while Tk1, Tk2A, Tk2B, TK3, Tk4 and Tk5 are *P. linearfolia* fractions.

4.1.6.2 *In vivo* antiplasmodial activities of *L. hastata* and *P. linearfolia* fractions

The parasite density of *P. berghei* infected mice treated with *L. hastata* fractions is presented in Figure 4.21. In contrast to the *in vitro* antiplasmodial activity Lh3 had the best *in vivo* antiplasmodial activity of the *L. hastata* fractions, with no parasite seen in the blood film at day 7 and parasite density was zero. Other *L. hastata* fractions had antiplasmodial activities but did not clear parasites completely from circulation, resulting in their death before day 42.

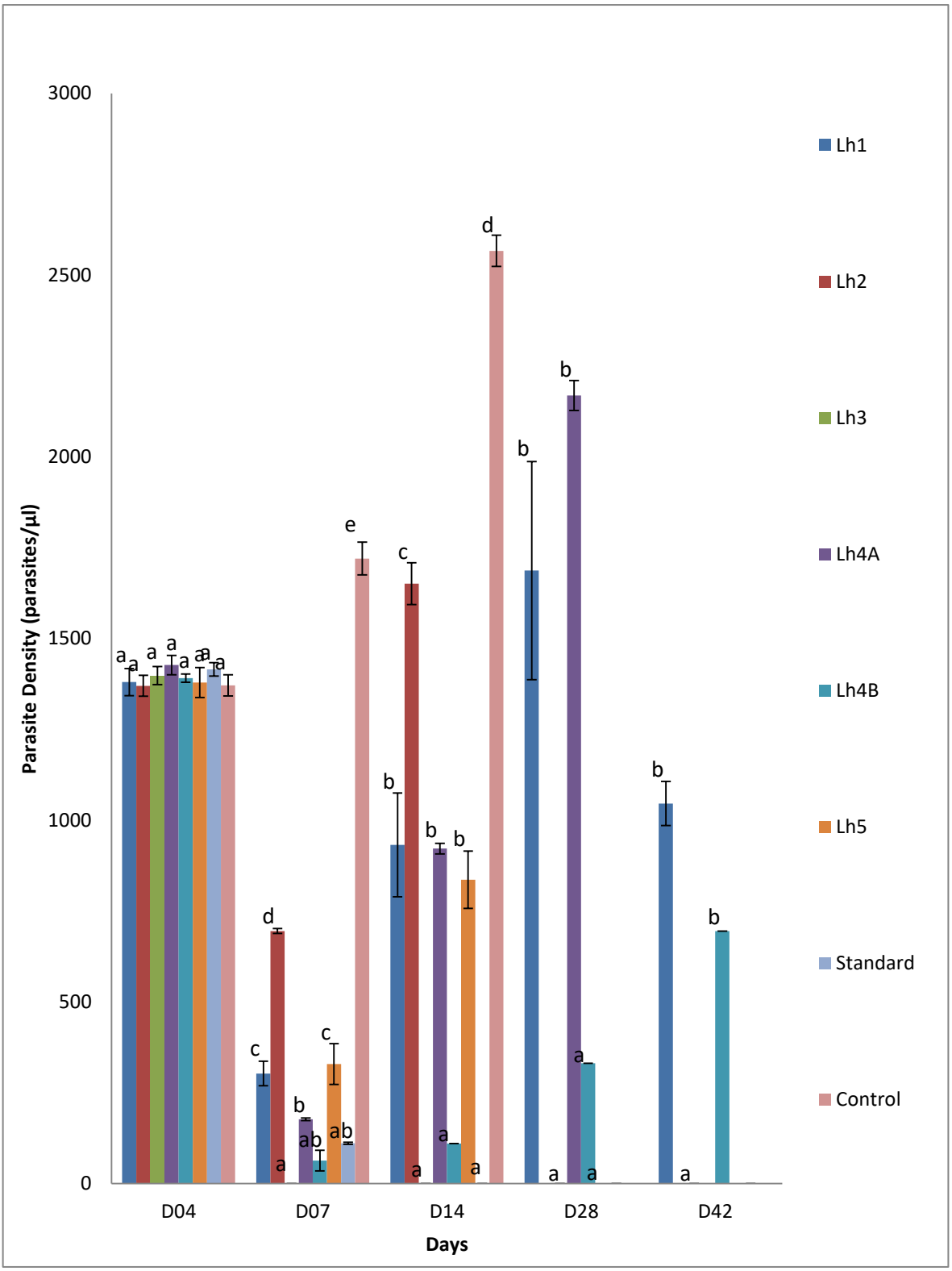


Figure 4.21: *In vivo* antiplasmodial activity of *Leptadenia hastata* fractions

Key: Lh1, Lh2, Lh3, Lh4A, LhB and Lh5 are groups treated with various *L. hastata* fractions.
Standard= infected group treated with standard drug (chloroquine phosphate), Control= infected untreated group

The parasite inhibition of *L. hastata* fractions is presented in Table 4.21. The parasite inhibition of Lh3 fraction is comparable to the standard while other fractions have inhibition below 100.

Table 4.21: Parasite Inhibition of *L. hastata* Fractions on Day 14

L. hastata Fractions	Parasite inhibition (%)
Lh1	63.69
Lh2	35.68
Lh3	100.00
Lh4A	64.08
Lh4B	98.28
Lh5	67.43
Standard	100.00
Control	0.00

Key: Lh1, Lh2, Lh3, Lh4A, LhB and Lh5 are groups treated with *L. hastata* fractions

The *in vivo* antiplasmodial activities of *P. linearfolia* fractions is presented in Figure 4.22. Tk3 fraction was the only fraction of *P. linearfolia* that completely cleared parasites from circulation by day 14 with a parasite density of zero. Others did not clear parasites from circulation and parasite density kept rising until the mice died.

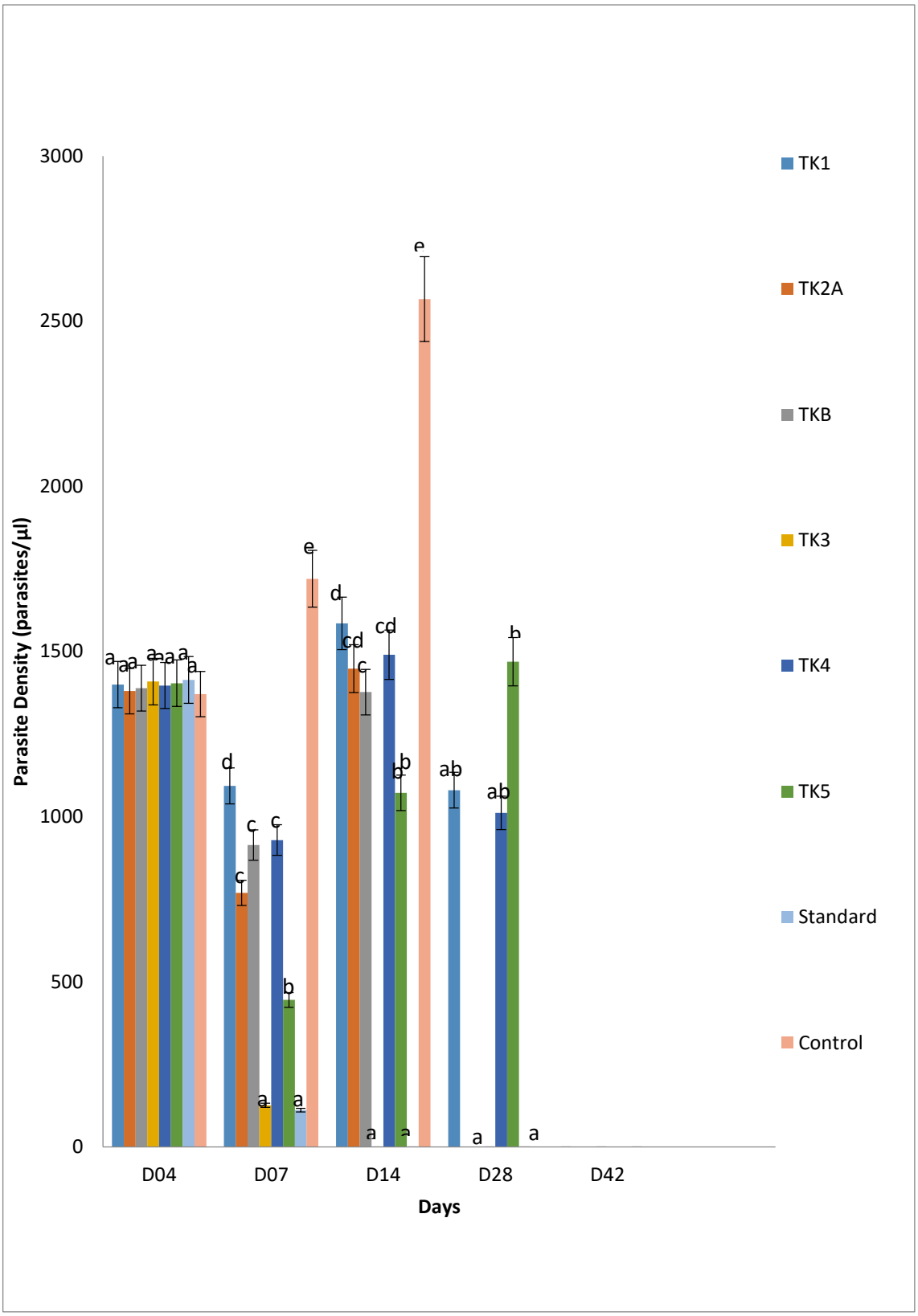


Figure 4.22: *In vivo* antiplasmodial activities of *P. linearifolia* fractions.

Key: Tk1, Tk2A, Tk2B, TK3, Tk4 and Tk5 groups treated with various fractions of *P. linearfolia*. Standard= infected group treated with standard drug (chloroquine phosphate), Control= infected untreated group

The parasite inhibition of *P. linearfolia* fractions is presented in Table 4.22 The parasite inhibition of Tk3 fraction (100) is comparable the standard (100) while other fractions have inhibition below 100.

Table 4.22: Parasite Inhibition of *P. linearfolia* Fractions on Day 14

P. linearfolia Fractions	Parasite inhibition (%)
Tk1	38.12
Tk2	43.59
Tk3	46.36
Tk4A	100.00
Tk4B	30.26
Tk5	58.28
Standard	100.00
Control	0.00

Key: Tk1, Tk2A, Tk2B, TK3, Tk4 and Tk5 groups treated with various fractions of *P. linearfolia*.

4.1.6.4 Weight Variations in Mice Treated with *Polycarpea linearfolia* Fractions Over a Period of 42 days.

The variations in weight of mice treated with *P. linearfolia* fractions is presented in Table 4.23. Only group treated with Tk3 fraction survived to day 42 and had weight (27g) that was significantly the same with the weight (31g) of standard group that also survived to day 42.

Table 4.23: Weight (g) Variations of Mice Treated with *Polycarpea linearfolia* Fractions Over a Period of 42 days.

Fractions	D04	D07	D14	D28	D42
TK1	16.40±0.71[a]	17.21±0.73[a]	17.43±1.96[ab]	10.63±0.27[b]	0.00±0.00[a]
TK2a	17.15±0.69[a]	17.66±0.66[a]	18.66±2.25[ab]	0.00±0.00[a]	0.00±0.00[a]
TK2b	17.33±1.17[a]	17.84±1.35[a]	18.81±0.60[ab]	0.00±0.00[a]	0.00±0.00[a]
TK3	16.81±0.86[a]	17.74±0.77[a]	20.20±1.30[ab]	26.18±3.01[c]	26.78±1.19[b]
TK4	16.31±0.58[a]	16.99±0.69[a]	18.50±0.78[ab]	17.76±0.93[bc]	0.00±0.00[a]
TK5	16.17±0.69[a]	18.54±0.90[a]	22.33±0.98[b]	22.26±1.36[bc]	0.00±0.00[a]
Standard	16.87±044[a]	17.55±0.34[a]	21.11±0.34[b]	27.00±0.43[c]	31.30±1.08[b]
Control	16.70±0.41[a]	17.20±0.58[a]	14.89±0.62[a]	0.00±0.00[a]	0.00±0.00[a]

Key: Tk1, Tk2A, Tk2B, TK3, Tk4 and Tk5 groups treated with various fractions of *P. linearfolia*.

4.1.6.5 Mean survival time of mice treated with *Polycarpea linearfolia* fractions

The mean survival time of mice treated with *P. linearfolia* fractions is represented in Table 4.24. The mean survival times of Tk3 (42 days) and Tk5 (36 days) were comparable to the standard group (42 days). The survival time of mice treated with other fractions were similar and significantly lower compared to the standard group.

Table 4.24: Mean Survival Time of Mice Treated with *Polycarpea linearfolia* Fractions

Fractions	Mean Survival Time (days)
TK1	24.25 ± 1.30^{ab}
TK2a	26.75 ± 1.17^{ab}
TK2b	28.00 ± 2.67^{ab}
TK3	42.00 ± 0.00^{b}
TK4	27.25 ± 1.44^{ab}
TK5	36.50 ± 3.20^{b}
STANDARD	42.00 ± 0.00^{b}
CONTROL	16.50 ± 0.29^{a}

Key: Tk1, Tk2A, Tk2B, TK3, Tk4 and Tk5 groups treated with various fractions of *P. linearfolia*. Standard= infected group treated with 5mg/kg bw of standard drug (chloroquine phosphate), Control= infected untreated group

4.1.6.6 Weight (g) variations over a Period of 42 days of mice treated with *Leptadenia hastata* fractions

The variations in weight of mice treated with *L. hastata* fractions is presented in Table 4.25. At D04 and D07 there was no significant ($p<0.05$) difference in weight of mice treated with fractions and standard compared to the control. Lh1, Lh2 and Lh4a had a significant ($p>0.05$) decrease in weight compared to the standard group at D14 and D28. Mice treated Lh3 fraction had weight that was significantly the same with the weight of standard group that also survived to day 42.

Table 4.25: Weight Variations Over a Period of 42 days for Mice Treated with *Leptadenia hastata* fractions

Fractions	D04	D07	D14	D28	D42
Lh1	17.91±0.85[a]	17.87±0.87[a]	17.54±0.79[ab]	17.16±1.41[a]	-
Lh2	18.62±1.40[a]	18.04±1.29[a]	15.78±1.53[a]	-	-
Lh3	18.55±0.36[a]	18.20±0.32[a]	19.11±1.03[b]	22.16±0.57[b]	25.35±0.66[b]
Lh4a	17.37±1.11[a]	17.04±1.04[a]	15.20±0.30[a]	13.88±1.51[a]	-
Lh4b	18.25±1.81[a]	17.93±1.62[a]	17.21±1.06[ab]	21.20±1.34[b]	21.15±3.22[ab]
Lh5	18.02±0.96[a]	17.39±0.75[a]	16.07±0.78[ab]	18.36	19.98[a]
Standard	16.87±0.44[a]	17.56±0.34[a]	21.11±0.34[b]	27.00±0.43[c]	31.30±1.08[b]
Control	16.70±0.41[a]	17.15±0.59[a]	14.89±0.62[a]	-	-

Key: Lh1, Lh2, Lh3, Lh4A, LhB and Lh5 are groups treated with *L. hastata* fractions. Standard= infected group treated with standard drug (chloroquine phosphate), Control= infected untreated group.

4.1.6.7 Mean survival time (days) of mice treated with *Leptadenia hastata* fractions

The mean survival time of mice treated with *L. hastata* fractions is presented in Table 4.26. The mean survival time of Lh3 (42days) was comparable to the standard (42days) group. The survival time of mice treated with other fractions were significantly lower compared to the standard group.

Table 4.26: Mean Survival Time of Mice Treated with *Leptadenia hastata* Fractions

Fractions	Mean Survival Time (days)
Lh1	38.00 ± 2.35^{bc}
Lh2	24.00 ± 0.58^{ab}
Lh3	42.00 ± 0.00^{c}
Lh4a	33.51 ± 0.50^{bc}
Lh4b	38.00 ± 4.00^{bc}
Lh5	27.00 ± 5.02^{b}
STANDARD	42.00 ± 0.00^{c}
CONTROL	16.50 ± 0.29^{a}

Key: Lh1, Lh2, Lh3, Lh4a, Lhb and Lh5 are groups treated with *L. hastata* fractions

4.1.7 Spectral Analysis

4.1.7.1 UV-visible Spectral Results

a) UV-visible Spectral of Tk3 fraction of *P. linearfolia*

The UV-visible Spectral of Tk3 fraction of *P. linearfolia* is presented in Appendix A1. This spectrum revealed ten components in this fraction with maxium absorption wavelength (λmax) in the range of 240 to 362 nm.

b) UV-Visible Spectral of Lh3 fraction

The UV-visible spectral of Lh3 fraction of *L. hastata* is presented in Appedix A2. This spectral revealed the presence of nine components in the fraction with maxium absorption wavelength (λmax) in the range of 251-671 nm.

4.1.7.2Nuclear Magnetic Resonance Results

NMR spectra of active fractions were recorded on a Bruker Advance 400MHz spectrometer. The ^{1}H and^{13}C NMR of Tk3 and Lh3 fraction are presented in Appendices B1and B2, Appendix C1 and C2 respectively.

Chemical shift for Tk3 H NMR were at 0.5, 1.3, 2.1, 2.6, 2.8, 3.3, 3.4, 4.8, 4.9 and 5.5ppm.^{1}H NMR spectra analysis indicated two methyl singlets at δ_H0.5 and 1.3ppm

with resonance at 1.3ppm very strong. Another triplet methyl proton is indicated at 3.4, this shows that the carbon atom to which this proton is attached has an electronegative element attached and from the δ_H value (3.4ppm) it shows the element is oxygen (RO-C-**H**, δ range is 3.2-3.8ppm). A strong duplet of two methylene group (-CH_2) appeared at 4.9, this indicate that the proton is bonded to a carbon atom that is doubled (R-C=C-**H**), which is a characteristics of unsaturated ring strucuture while the single resonance shift at 5.5ppm is also a methylene group (=C-**H**). The chemical shift between 1.8-3.0ppm is associated with proton attached to carbon atom of C=O, C=C, and aromatic groups.

The 13C NMR in deuterated methanol (MeOD) data showed 18 signals, revealed δ_c 13, 23, 29, 30,32,33,35,38,40,47,49,61,63,68,70,82,89, 162 and 200 including a carbonyl resonance at 200. Chemical shift indicating the presence of a moiety include δ_c at 33 ppm for C4 of flavonoids (with resonance range between 28-35 ppm), δ_c at 47.58 and 48 ppm for non-oxygenated C2 and C3 (reference range of 40-85 ppm), another non-oxygenated aromatic carbon atom at δ_c 89 ppm (with reference range of 90-125 ppm).

Distortionless Enhancement Polarisation Transfer (DEPT) revealed odd number of hydrogens is attached to the carbon atoms at δ_c 15,68,78,82 and 89ppm while carbon atoms at δ_c of 22, 23, 29,30,62,62 have even number hydrogen atom attached to the carbon atom. Single hydrogen atom was found bonded to carbon atoms at δ_c 30, 48, 49, 68, 78, 82, and 89.

The ^{1}H and^{13}C NMR of TK3 fraction in comparism to sclerinone A is presented in Table 4.27.

Table 4.27: The NMR Spectrum of Tk3 Fraction and Sclerinone A in Deuterated Methanol (MeOD)

Carbon position	Tk3 δ_H (ppm)	Literature of sclerinone A δ_H Sclerinone A	Multiplicity	Tk3 δ_c (ppm)	Literature of sclerinone A δ_c (ppm)	Assignment
1	5.5	5.55(1H, d)	d		116.8	(CH_2)
	5.4	5.34(1H, d)	d			
2	-	6.02(1H, dd)	dd		139.6	(CH)
3			q	81.43	80.2	(C)
4					212.2	(C=O)
5		2.85(2H, m)	m	33.54	32.8	(CH_2)
6	2.8	2.85(1H, m)	m	48.31	49.0	(CH)
7				68.31	71.4	(C)
8		6.80 (1H,d)	d		155.4	(CH)
9		5.90(1H, d)	d		125.6	(CH)
10				200	202.7	(C=O)
11			q	46.9	45.5	(C)
12	0.9	1.06(3H, s)	s	-	25.1	(CH_3)
13		1.06(3H, s)	s	22.3	21.4	(CH_3)
14	1.3	1.34(3H, s)	s	22.85	23.1	(CH_3)
15		1.54(3H, s)	s		25.2	(CH_3)

Key: s =singlet, d =doublet, dd =doublet of doublet, m=multiple, and q =quadruplet

The ^{1}H and^{13}C NMR of TK3 fraction in comparism to 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] is presented in Table 4.28.

Table 4.28: The NMR Spectrum of Tk3 Fraction and 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] in Deuterated Methanol (MeOD)

Carbon position	Tk3 δ_H (ppm)	Literature of δ_H 2, 2'-methylenebis [6-tert-butyl-4-methylphenol]	Tk3 δ_c (ppm)	Literature of δ_c (ppm) 2, 2'-methylenebis [6-tert-butyl-4-methylphenol]
1	5.4	5.35		146.6(C)
2				126.2(C)
3	6.7	6.73		128.4(C)
4				134.3(C)
5	6.9	6.97		127.3(C)
6				138.4(C)
1'	5.4	5.35		146.6(C)
2'				126.2(C)
3'	6.7	6.73		128.4(C)
4'				134.3(C)
5'	6.9	6.97		127.3(C)
6'				138.4(C)
13	3.8	3.96	29.37	30.1(-CH2)
14	2.3	2.34	22.35	21.9(-CH_3)
15	2.3	2.34	22.35	21.9(-CH_3)
16	1.3			
17		1.35	31.69	31.6(-CH_3)
18		1.35	31.69	31.6(-CH_3)
19		1.35	31.69	31.6(-CH_3)
20				
21		1.35	31.69	31.6(-CH_3)
22		1.35	31.69	31.6(-CH_3)
23		1.35	31.69	31.6(-CH_3)

The ^{1}H and^{13}C NMR of Lh3 fraction in comparism to2, 2'-methylenebis [6-tert-butyl-4-methylphenol] is presented in Table 4.29.

Table 4.29: The NMR Spectrum of Tk3 Fraction and 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] in Deuterated Methanol (MeOD)

Carbon position	Lh3 δ_H (ppm)	Literature of δ_H 2, 2'-methylenebis [6-tert-butyl-4-methylphenol]	Lh3 δ_c (ppm)	Literature of δ_c (ppm) 2, 2'-methylenebis [6-tert-butyl-4-methylphenol]
1	5.4	5.35	Q	146.6(C)
2			127.11	126.2(C)
3	6.7	6.73	128.28	128.4(C)
4			131.96	134.3(C)
5	6.9	6.97	127.11	127.3(C)
6			Q140	138.4(C)
1'	5.4	5.35	Q	146.6(C)
2'			127.11	126.2(C)
3'	6.7	6.73	128.28	128.4(C)
4'			131.96	134.3(C)
5'	6.9	6.97	127.11	127.3(C)
6'			Q140	138.4(C)
13	3.8ss	3.96	29.71	30.1(-CH2)
14	2.3	2.34	21.02	21.9(-CH_3)
15	2.3	2.34	21.02	21.9(-CH_3)
16			34.7	
17	1.35	1.35	31.96	31.6(-CH_3)
18	1.35	1.35	31.96	31.6(-CH_3)
19	1.35	1.35	31.96	31.6(-CH_3)
20			34.7	
21	1.35	1.35	31.96	31.6(-CH_3)
22	1.35	1.35	31.96	31.6(-CH_3)
23	1.35	1.35	31.96	31.6(-CH_3)

4.1.7.3 HPLC-ESI-MS Spectral Result

a) **HPLC-ESI-MS Spectral Analysis of Tk3 fraction**

The HPLC-ESI-MS Spectral result of Tk3 faction is represented in Appedix E1. This spectrum revealed the presence of nine major compounds with retention time of 2.12, 9.51, 14.51, 16.02, 19.39, 21.90, 24.75, 26.62 and 32.06, having molecular weight of 163,153,159,173,187, 151, 265, 339 and 163 respectively.

b) **HPLC-ESI-MS Spectral Analysis of Lh3 fraction**

The HPLC-ESI-MS spectral result of Lh3 is represented in Appedix E2. This spectrum yields a composite peak with unresolved compounds that could not be interpreted by the mass spectrometer.

The result of the compounds detected during high performance liquid chromatography electro-spray ionization mass spectrometer is presented in Table 4.27. Nine compounds were detected within 30 minutes of running the fractions. Compounds 1,2, 3, and 9 were sugars with molecular weight of 163,153,159 and 163 and the corresponding double bond were 3,6,3 and 3 respectively. Compounds 4,5 and 6 were more of nitrogen containing compounds with molecular weight of 173,187 and 151 with corresponding double bond of 2, 3 and 6 respectively. Compound 7 was a sesquiterpene ($C_{15}H_{21}O_4$) with molecular weight of 265 and 6 double bonds. Compound 8 was a flavonoid ($C_{23}H_{31}O_4$) with 9 double bonds and had a molecular weight of 339.

Table 4.30: HPLC ESI MS Data of Compounds in the most active Fraction TK3

S/N	Retention time(min)	Molecular weight(m/z)	Molecular formula	Double bond Equivalence
1	2	163	$C_5H_7O_6$	2.5
2	10	153	$C_7H_5O_4$	5.5
3	14.5	159	$C_7H_{11}O_4$	2.5
4	16	173	$C_3H_{13}N_2O_6$	1.5
			$C_8H_{13}O_4$	2.5
5	20	187	$C_9H_{15}O_4$	2.5
			$C_4H_{15}NO_6$	1.5
6	22	151	$C_8H_7O_3$	5.5
			$C_3H_7N_2O_5$	1.5
7	25	265	$C_{15}H_{21}O_4$	5.5
8	27	339	$C_{23}H_{31}O_4$	8.5
9	32	163	$C_5H_7O_6$	2.5

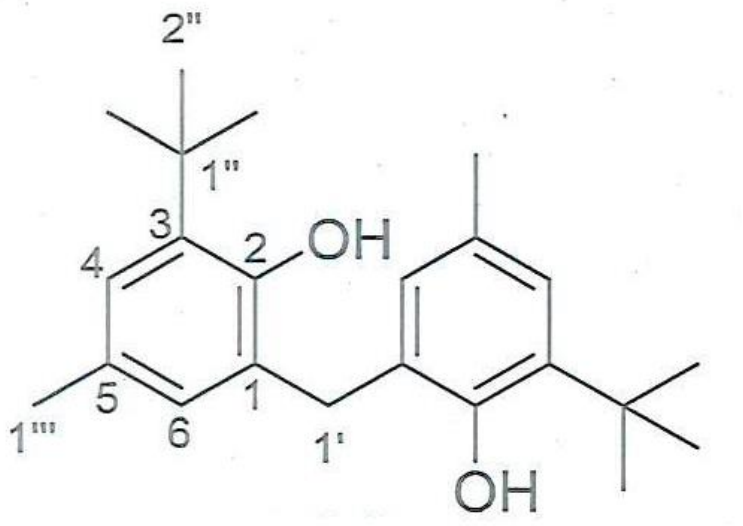


Figure 4.23: Structure of compound 8 (2, 2'-methylenebis [6-tert-butyl-4-methylphenol])

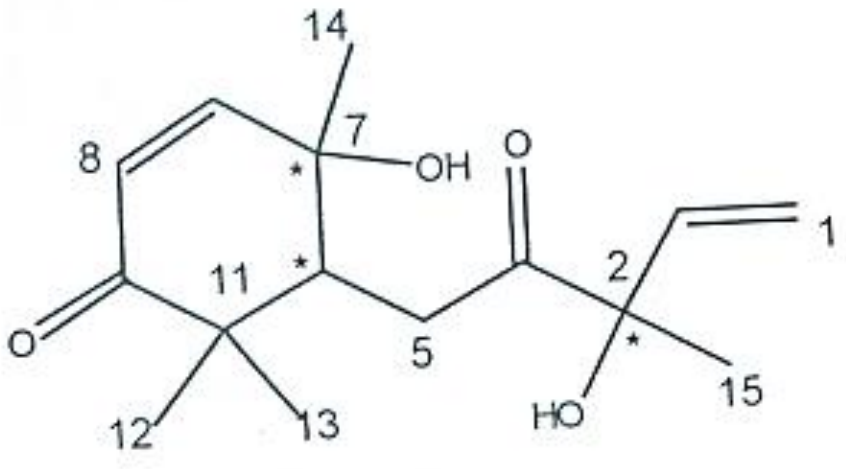


Figure 4.24: Structure of compound 7 (Sclerinone A).

4.2 Discussions

L. hastata, A. dodoneifolius and *S. longepedunculata* with higher yield in contrast to *Merremia hederacea, Z. zanthoxyloide, P. linearifolia, N. macropylla* and *Lophira alata* (Table 4.1). The variations in the percentage yield recorded in this study could be attributed to difference in plant part surface area, season of collction, geographical location among others. Plant part used also determines the yield and type of phytoconstituent present in an extract. For example, when leave samples are extracted, the extract is expected to be abundant in chlorophyll (Azwanida, 2015).

Lowering particle size have been reported to increase surface contact between samples and extraction solvents. However, coarse smaller samples give better yield than fine powdered samples (Azwanida, 2015).

Similarly, geographical location influence yield and bioactivity. According to Issa *et al.* (2008), plants from colder regions have been reported to have higher yield and better activities than those from warmer regions. Finally, the season of plant collection also affects extract yield. For example, monoterpenes and sesquiterpene are both essential oil that have higher yield in dry season (Castelo *et al.*, 2008). Hot extractions, such decoction, soxhlet extraction, and extraction under reflux are suitable for extracting heat-stable compounds, hard plants materials (e.g. roots and barks) usually resulted in more oil-soluble compounds compared to cold extractions such as maceration and infusion (Azwanida, 2015).

The extract yield could also be influenced by temperature, solvent types and extraction duration/time (Tambunan *et al.*, 2017). Extraction at temperature between 0-60 ^{o}C tends to be faster and increases extract yield, although heat-labile compounds are destroyed at higher temperatures. Furthermore, non-polar solvent such as n-hexane, diethyl ether, petroleum ether,

and chloroform extract non-polar compounds while polar solvent such as ethylacetate, acetone, ethanol and water extract polar substances/compounds. Increase in extraction time also increase yield. Ethanol and hydroalcohol extracts have been reported to give highest extraction yield with maximum presence of phytoconstituents (alkaloids, saponins, tannins and flavonoids) compared to the other solvents such as petroleum ether, chloroform and water (Azwanida, 2015). However, temperature, solvent types and extraction time were kept constant in this study.

Phytochemical profile revealed the presence of alkaloids, flavonoids, tannins, diterpene, and saponins in all the plant extracts, while phenols were detected in *P. linearifolia*, *L. hastata* and *A. dodoneifolius* (Table 4.2 and Table 4.3). The successful use of these plants traditionally in treating malria infections could be as a result of individual or synagestic action(s) of the detected phytochemicals. These phytochemicals have been reported to have antiplasmodial activities (Kaur *et al.,* 2009).

All drugs have side effects, but the extent of their impact and severity varies from mild to severe (such as damage to vital organs) and even death in some cases (Alshammari, 2014). The Safe dose observed for all the plant extracts used (Table 4.4) allows for further investigation on their antiplasmodial activity as their LD_{50} were all below the bench mark of 5000 mg/kg bw (Lorke 1983). Efficacy and safety are two decisive factors that affect the viability of chemical entity while furthering the drug discovery pipeline (Siramshetty *et al.,* 2016).

Increase in dosage of all the extracts resulted in increased parasite clearance in the blood of all groups tested. Except the group treated with crude methanol extract *Agelanthus dodoneifolius*, in which increased dosage did not significantly increase parsite clearance and *N. macrophyll* extract showed an inverse correlation between parasite density and conacentration (Figure 4.1 - 4.8).

Although antiplasmodial activity of crude aqueous of *A. dodoneifolius* have been reported by Builder *et al.* (2012) to be dose dependent. The difference in observations could be due high polarity of the active component which would have been more aboundant in the aqeous extract than in the methanol extract used in this study. The difference in observation could also be attributed to variations in phytoconstituents due to difference in season of collection, host plant and location.

Futhermore all the tested extracts exhibited significant ($p>0.05$) antipasmodial activity below 200 mg/kg bw except *Agelanthus dodoneifolius* which showed no significant ($p<0.05$) reduction in parasitamia below 200 mg/kg bw. A similar observation was made by Builder *et al.* (2012) for *Agelanthus dodoneifolius* below 200 mg/kg bw.

The inverse relationship between concentration and parasite density of mice treated with *N. macrophyll* extract, could be due to, the presence of an antiplasmodial agent and its antagonist. So, at lower concentration (100 mg/kg bw) the effect of the antagonist is minimal but as the concentration increases the effect of the antagonist becomes more evident and pronounced. A similar report has been observed by Nascimento *et al.* (2000). Alternatively, nitric oxide could have been involved. Nitric oxide has been reported to have an inverse correlation with malaria infection (ie the lower the concentration of nitric oxide, the higher the severity of malaria disease (Weinberg *et al.,* 2009). It is possible that *N. macrophyll* extract contain a phytoconstituent capable of decreasing nitric oxide concentration. Inflammation and infection are known to reduce nitric oxide concentration (Levine *et al.,* 2013).

In vitro systems used in screening for antimalarial activity have short falls, particularly as they do not take into account pharmacodynamics of the test substance and the role of the immune system in controlling the infection. For this reason, *in vivo* test of the extracts against *P. berghei*

were also carried out in this study the. Generally, all the extracts exhibited *in vitro* and *in vivo* antiplasmodial activities, However, *L. hastata* and *P. linearfolia* methanol extracts which had the lowest *in vitro* antiplasmodial effect had the highest *in vivo* antiplasmodial effect. This could atributed to the biotransformation of the active compounds responsible for the antiplasmodial activity which could have increased their antiplasmodial activity.

Furthermore, *P. linearifolia* and *L. hastata* were most active against the asexual erythrocytic stage of *P. berghei* in a dose dependent chemosuppression of parasitemia (Figure 4.1 and 4.8). Mice treated with these extracts had prolonged survival time (Table 4.6 and Table 4.7). Unlike in the *in vitro* tests against *P. falciparum* in which the extracts (*L. hastata* and *P. linearifolia*) had the least activity compared to other extracts. However, *Z. zanthoxyloides* with the best activity *in vitro* (Table 4.5) had moderate activity *in vivo* (Figure 4.4), this could be due to: (1) host metabolism resulting in biodegradation of the active compound (2) biological difference between *P. falciparum* and *P. berghei* (Falade *et al.*, 2014).

Death of infected mice treated with high dose of *S. longepedunculata* extract before the infected untreated control group (Figure 4.6) could be attributed significant ($p<0.05$) increased saponin content of this extract. Saponins are known to have strong hemolytic effect (Bihonegn *et al.*, 2019). This effect could be responsible for the ability of the extract to reduce red blood cells count and hemoglobin concentration (Sulaiman *et al.*, 2019) coupled with the antiparasite effect of destroying infected red blood cells. Additiomnally, Biotransformation of *S. longepedunculata* might have produced a toxic metabolite that can attack cells and damage tissues which could have resulted in the deaths recorded. Some medications (e.g Acetaminophen Halothance/Isoniazid) have been reported to produce metabolites that are more toxic than the parent compound. (Murray *et al*, 2008).

Base on the observed antiplasmodial activity of the selected plant extracts, *L. hastata* and *P. linearfolia* extracts were selected for further studies. Drug safety is one of the cardinal reasons for sourcing a new medication or withdrawing an existing drug from the market (Alshammari, 2014).

At the end of week 4 there was no significant ($p<0.05$) difference in serum glucose concentration of group administered *L. hastata* compared to the control while group administered *P. linearfolia* extract had a significant ($p>0.05$) decreased serum glucose concentration at the end of week 4 compared to the control. The ability of this extract to significant ($p>0.05$) decrease the serum glucose is of advantage to the host (man), because malaria parasite which depends exclusively on exogenous glucose supply as energy source. They cannot store energy in form of glycogen as a result the metabolism of glucose in infected red blood cell is about 100times greater than non-infected red blood cell (Olayemi *et al.*,2012). During malaria infection pro-inflammatory cytokines (TNF-α, IL1B, IL-6) are released, the released of the cytokines causes increase in blood glucose concentration (Kiely *et al.*,2007). High level of serum glucose enhances the growth and proliferation of malaria parasite and thus increases the severity of malaria infection. The growth and proliferation of malaria parasite is impaired below 5.5 mM. (Humeida *et al.*,2011). All artemisia species seem to have a hypoglycemic effect. For example, treatment of rats with *Artemisia annua* aqueous extract reduced serum glucose after 4 weeks from 110 to 70mg (Mojarad, 2005). So, any extract such as *P. linearfolia* with hypoglycermic effect will short chain the malaria parasite of its essential energy source.

The average blood glucose of children under 6 years is 8.3mM (which favours growth and proliferation of malaria parasite) for adult normaglycemic 3.0–7mM reason for susceptibility of children under the age of five to malaria infection

There was no significant change in serum total proteins concentration of test groups compared to the control group. Proteins involved in inflammatory response (example CD80, ITGAV), cellular adhesion and constituent of erythrocyte membrane are increased in malaria. Elevated serum protein levels with malaria indicate a rising step trend with incremental levels as the diseases progress to severe stage (Renterward *et al.*,2018). Protein markers of oxidative stress were found elevated in anemic malaria patient while markers of endothelial activation (angiopoitein I & II), platelet adhesion and muscle (*cratine kinase, carbonic anhydrase* III) and tissue damage were linked to cerebral malaria (Bachman *et al.*,2017). The ability of the extract to maintain a stable protein concentration is not likely to aggrevate the effect of increase in protein concentration in malaria.

Furthermore, there was no significant ($p > 0.05$) difference in serum albumin concentration of test groups compared to the control group in this study. Plasma albumin levels have been shown to be lower in both severe and mild malaria as compared to non-infected individuals. This is because plasma albumin is a negative acute phase protein, the level of which falls as a result of malaria infection, probably because of an increase in its trans-capillary escape rate (Kwena *et al.*,2012). Red blood cells infected with malaria are prone to oxidative stress (Fevang *et al.*,2018) and albumin is a strong extracellular antioxidant, accounting for forty percent antioxidant capacity in healthy human serum (Roche *et al.*,2008). Malaria parasite growth has been reported to increase with increase in albumin concentration (up to 10 g/L) in *in vitro* study.

Albumin prevent peroxidation of polyunsaturated fatty acid (PUFA), opposing the haemolytic effect of polyunsaturated fatty acid (Fevang *et al.*,2018). The binding of Albumin to polyunsaturated fatty acids prevents peroxidation and the formation of reactive oxygen species. Also, albumin has a free thiol at the Cysteine 34 place, which in free form has a significant

capacity to scavenge hydroxyl radicals due to the large albumin pool in the body (Soeter *et al.*,2019). Since was no significant change in albumin concentration of the test group compared to the group in this study, means that the antioxidant capacity of albumin will be maintained.

Similarly, there was no significant ($p<0.05$) difference in serum urea concentrations of the tests compared to the control, although, the creatinine concentration of the tests was increased compared to the control. This increase did not exceed the normal creatinine level (0.7 ton1.3 mg/dL (61.9-114.9 mol/L) in men and 0.6 to 1.1 mg/dL (53.0-97.2 mol/L) in women (Abcar *et al*., 2004)) and there was no concomitant increase in serum urea to suspect a kidney damage. During starvation and fever, creatinine concentration has been observed to be increased due to significant muscle wasting (Kwena *et al.*,2012). Uremia weakens crucial defenses required for protection against infection and also cause generalized inflammation that is linked to recessive cardiovascular disease (Betjes 2013). Urea and creatinine are markers of kidney derangement. Urea, creatinine, total and conjugated bilirubin have been reported be significantly higher in malaria infection. Parasitemia had a positive correlation with increase in urea and creatinine (Hermansyah *et al.*,2017). A decreased serum levels of urea, creatinine, sodium and potassium have also been associated with renal impairment (Elbadawi *et al.*,2013). Extract or compound with antiplasmodial activity should not have of these effects (as observed in this study) but be able to allieviate these problems.

The group of rats that received *L. hastata* had continuous decrease in serum cholesterol content compared to the control over the study period while group of rats administered *P. linearifolia* had increased cholesterol concentration for the first three weeks and a decrease in the fourth week. The general decrease in cholesterol concentration could decrease membrane fluidity and

permeability of the red blood cells, thereby inhindering the invasion of parasite in to the cell. Lipid profile is altered in malaria infected persons to facilitate increase in membrane fluidity and permeability for cell lysis and consumption of lipids by the parasites (Chukwuocha and Eke, 2011). Plasmodium species requires lipid for anabolic reaction such as membrane formation during parasite replication in the hepatocytes and red blood cells (Visser *et al.*,2013). Cholestrol, HDL and LDL are lower in malaria patients (Viera and Rivera, 2017). Cholesterol concentration is positively associated with coronary heart disease (Naito, 2003), the cause of primary hypercholesterolaemia are associated with over production of very low density lipoprotein (VLDL), increased conversion of VLDL to low density lipoprotein (LDL), LDL enriched with cholesteryl esters, defective and decreased LDL receptor number while, secondary hypercholesterolaemia are associated with hypothyroidism, obesity, physical inactivity, fat rich diet and obstructive liver disease (Al-Omar *et al.*,2010).

The serum triacylglycerol concentrations of rats administered *P. linearifolia* and *L. hastata* over the four weeks period did not follow any regular pattern. Triacylglycerol promotes hemozoin formation, by implication it is involved in haem detoxification (Visser *et al.*,2017). Triacylglycerol are major portion of lipids found in lipid droplet in late trophozoite and schizont stage of *P. falciparum* (Nawabi *et al.*,2003). Evaluation of lipid profile for abnormalities in the course of malaria treatment is important, since long term changes can directly influence risk of obesity and coronary diseases.

The main function lipoproteins (HDL and LDL) in the body is for lipid transportation. HDL for transport of lipid from peripheral tissue to the liver for detoxification while LDL reverse HDL function. (Orimadegun and Orimadegun, 2015). HDL levels of group administered *P. linearifolia* and *L. hastata* were significantly higher compared to the control group throughout

the four weeks period. HDL has been reported to be toxic to malaria parasite at higher concentration (2.4 mg/ml). HDL causes abnormal maturation and death of trophozoites in the infected erythrocytes (Visser *et al.*,2013).

Woestijne *et al.* (2013) reported that 0.1mmol/L increase in HDL concentration was associated with a 5% reduced risk of coronay heart disease. Another study has shown that increased HDL cholesterol by 0.1mmol/L reduces the risk of coronary disease by 2% in men and 3% (Smellie *et al.*,2007). Therefore, increase by 0.2 as a difference between the test and the control of rat administerd *P. linearfolia* and *L. hastata* extract will reduced/ decrease risk of coronary heart disease by 6% in men and 9% in women.

The serum sodium and potassium concentrations of the *P. linearfolia* and *L. hastata* administered group were significantly the same compared to the control. Electrolyte derangement, especially decrease in potassium and sodium concentration are not to be over looked during treatment regimes that alleviate malaria disease, because these derangements are part of clinical signs of malaria (Ikekpeazu *et al.*,2010). Corrections of fluid and electrolyte imbalance form a major component of the treatment of critical ill patients in modern intensive care settings. Hyponatraemia and hypokelamia could be a good marker for severity of the disease (Singh *et al.*,2015). Electroyte imbalance is a common clinical manifestation in many infectious diseases including malaria. Hyponatraemia and hyperkalaemia develop due to Plasmodium infection (Rani *et al.*,2015). Some antimalaria drugs such as spiroindolene exhibit their antiplasmodial effect by inhibiting *Plasmodium falciparum* ATPase (PfATPase 4) activity countering the influx Na^+ and disrupting parasite Na^+ regulation and hinders it proliferation (Spillman *et al.*,2013). In this studies, *P. linearfolia* and *L. hastata* did not cause any significant

alteration in sodium and potassium concentration indicating their ability to maintain electrolyte balance resulting from Plasmodium infection.

There was no significant change in the activities of A LT and ALP between the test groups and the control. Although there was an increase in AST activity of group administered *P. linearfolia*. Since ALT a major liver damage marker was not increased other sources of AST may have been affected. These include red blood cells.

As the use of herbal medicine is rapidly increasing globally, the potential toxicity of herbal drugs in particular drug induced injury has become a serious medical problem or isuse.

Discontinuation of toxic herbs can reverse the toxic effect of the herb (Liu *et al.*,2016). Kupffer cells, stellate cells and neutrophils contribute to pathogenesis of hepatic toxicity. Kupffer cells, and neutrophils are source of proinflammatory cytokines, and chemokines, reactive oxygen and nitrogen species which promotes oxidative stress in injury induced by toxicant and ischemia. Activated stellate cells synthesize collagen whose overproduction lead to hepatic fibrosis and cirrhosis (Jaesche *et al.*,2002).

There was no significant ($p<0.05$) difference in total white blood cell indices of group administered *P. linearfolia* extract compared to the control. But there was a significant ($p>0.05$) increase in total white blood cell indices of *L. hastata* group compared to the control. Total white blood cell (WBC) count is often elevated during infections, and it is one of the nonspecific markers of inflammation (Hussani and Harris, 2007). Increased synthesis of lymphocytes can be a warning sign or cause bone marrow failure, resulting in shut down synthesis of other normal cells. Other conditions presenting with a lymphocytosis, such as follicular lymphoma, marginal zone lymphoma, mantle-cell lymphoma or hairy-cell leukaemia often have clinical features, such as anaemia, splenomegaly or lymphadenopathy (Smellie *et al.*,2007). Increased esinophile and

basophile (granulocytes) is associated with allergies and some inflammatory state (Wang *et al.*,2018[b]) while increased monocytes may indicate presence of medication, autoimmune or blood disorder. This condition is a normal immune response to an event such as some medications, infection, injury or inflammation (Feng *et al.*,2018). Depletion of these white blood cells is responsible for the immunosuppression in malaria (Silva Junior *et al.*,2017). Granulocyte count of group administred L. *hastata* extract was higher than the control while those administerd *P. linearifolia* extract were lower than the control. Increased granulocyte-macrophages alleviate or counter adverse effects associated with malaria and boost immune response. So it is proposed that *L. hastata* plant extract could stimulate immune response during treatment regime with the extract and decrease the chances of futher infection.

Rats administered *L. hastata* had the highest count for monocytes while *P. linearifolia* had monocytes count that was comparable to the control group. Haemozoin, the end product of heamoglobin degradation in malaria inhibits the host monocytes functions. This is one of the immune modulatory effect of malaria parasite during infection (Jackson *et al.*,2004). Extract capable of increasing circulating monocytes could boost immune system against plasmodium parasites infection.

There was no significant ($p<0.05$) difference in platelets count of the test groups compared to the control in this study. There is a reduction in circulating platelet in malaria. Alacra (2009), reported that platelet survival, revealed a shortened life span to 2–3 days in malaria, in comparison to 7–10 days in normal controls. Platelets initiate and support inflammatory processes by secretion of numerous biologically active substances such as platelet activation factor, platelet derived growth factor, platelet factor 4, IL-1 and beta thromoglobulin in

inflammatory conditions (Konca *et al.*,2014). In acute malaria, plateleterit (PCT) percentage has been reported to be low (Leal-Santos *et al.*,2013).

There was a significant ($p>0.05$) decrease in red blood cell count, haemoglobin concentration and hematocrit of group dosed with *P. linearfolia* extract compared to the control. Similarly, *Colacasia esculenta* leaf extract have been reported to decrease haemoglobin concentration, hematocrit and red blood cell count (Ufelle *et al.*,2018).

Decrease in red blood cell count, haemoglobin concentration and hematocrit value have been reported to confer anemia (Osei-Bimpong *et al.*,2012). Anemia affects about 800 million children and woman worldwide, with 97% occurring in low and middle-income countries (Matos *et al.*,2016). Malaria causes anemia and therefore call for concern.

None of the two active extracts elicited pathology in any of the organs investigated, except the spleen of rats that received *L. hastata* extract. The monography of this spleen showed hyperplastic lymphoid follicles. Hyperplastic lymphoid follicle is an inflammatory disorder that causes enlargement of the lymphoid tissue due to antigen stimulus and that is a reversible process once the stimulus is lifted (Socolovsky *et al.*,2008).

Severe malaria causes splenomegahy, hepatomegaly and discolouration of major organs like the spleen, liver, kidney, lungs and the brain (Bashir *et al.*,2012). A chronic inflammatory process either progress to initiate fibrosis or result in repair. Alternatively, exposure to xenobiotic (plant extract) might lead to adaptation process, which resists inflammation and leukocytosis. Hauser *et al.* (1995) suggested that adaptation process to a certain extent may be responsible for non-significant rise in total leukocyte count with regard to the period of exposure.

Drug induced organ injury is one of the most challenging toxicity disorders faced by pathologist (Andrade *et al.*,2019). Efficacy and safety are two decisive factors that affect the viability of chemical entity while furthering the drug discovery pipeline (Siramshetty *et al.*,2016).

Fractionation led to obtaining purified fraction with increase antiplasmodial activity. Lh3 and Tk3 fractions of *L. hastata* and *P. linearfolia* extract respectively, had better antiplasmodial activity compared to their crude extracts. 800 mg/kg bw of *L. hastata* and *P. linearfolia* crude extracts on the first day and 400 mg/kg bw on the remaining 3days was required to cleared parasites from circulation where as, only 200 mg/kg bw of Lh3 and Tk3 fractions was required to cleared parasites from circulation within 4 days of treatment. Purification of crude extract has been reported to enchance antiplasmodial activity (Mbouna *et al.*,2018).

Tk3 fraction was most active both for *in vitro* and *in vivo* antiplasmodial screening of *P. linearfolia* fractions. Similar activity has been observed in dichloromethane fraction of *Trema orientalis* extract. (Olanlokum *et al.*,2017). This implies that the bioactive compound(s) responsible for the activity is/are active both *in vitro* and *in vivo*.

Although Lh3 fraction had lower *in vitro* antiplasmodial activity than some fractions (Table 4.20)., it was the most active fraction in *in vivo* antiplasmodial test (Figure 4.21). Reasons could be that the active principle(s) or bioactive compound(s) responsible for the activity could have been biotransformed to a more potent metabolite to elicit better activity in the *in vivo* study. Additionally, absorption, distribution, metabolism and excretion properties of a compound could translate to its improved pharmacokinetic properties. Simply, stated Lh3 might have been more rapidly absorbed, well distributed, minimally metabolically degraded and not rapidly eliminated, and therefore more likely to rapidly achieve peak levels in the blood, maintain the desired levels

(n-fold above the IC_{50}) for longer duration, before falling to low trough levels and ultimately being cleared by the body (Chung *et al.,*2015) which made it have better *in vivo* activity than fractions that performed better than it in the *in vitro* study.

The UV-Visible spectral results of fraction Tk3 revealed ten (10) absorbance peak while its HPLC-ESI MS showed nine (9) peaks (Appendix A1 and Appendix E1). The maxium absorptions at wavelengths 350nm and 240nm of UV-Visible spectral of fraction Tk3 are were suspected to be those of rings A and B respectively of chalcone (Awouafack *et al.,*2013). This was confirmed by the HPLC- ESI MS result. The fact that chalcone has two maxium absorption in the UV-Visible spectrum, accounts for the increase by one peak of the detected components in UV-Visible spectrum compared to the HPLC- ESI MS spectral.

Chemical shift for Tk3 H NMR were at 0.5, 1.3, 2.1, 2.6, 2.8, 3.3, 3.4, 4.8, 4.9 and 5.5ppm (Appendix B1).^{1}H NMR spectra analysis indicated two methyl singlets at δ_H 0.5 and 1.3ppm with very strong resonance at 1.3ppm. Another triplet methyl proton was indicated at 3.4 ppm, this shows that the carbon atom to which this proton is attached has an electronegative element attached and from the δ_H value (3.4ppm) it shows the element is oxygen (RO-C-**H**, δ range is 3.2-3.8ppm). A strong duplet of two methylene group (-CH_2) appeared at 4.9 ppm, this indicate that the proton is bonded to a carbon atom that has a double bond (R-C=C-**H**), which is a characteristics of unsaturated ring strucuture while the single resonance shift at 5.5ppm is also an indication of a methylene group (=C-**H**). The chemical shift between 1.8-3.0 ppm is associated with proton attached to carbon atom of C=O, C=C, and aromatic groups. From this, it could be infer that the chemical shift at 2.1, 2.6 and 5.5 ppm are due to the unsaturation of the flavonoid ring structure or aromatic ring associated with flavonoids.The resonance at 2.1 and 2.6 ppm are due to proton of C2 and C3 in flavonoids with resonance range of 2-3 ppm while 4.9 and 5.5

ppm represent proton of the methylene group of flavonoids. Additionally, H NMR analysis in deuterated methanol (MeOD) showed the presence of flavonoids moiety which is revealed by three proton of doublet- doublet at δ_H 4.9, 3.4 and a singlet at 1.3 ppm.

The 13C NMR in deuterated methanol (MeOD) data showed 18 signals, revealed δ_c 13, 23, 29, 30,32,33,35,38,40,47,49,61,63,68,70,82,89, 162 and 200 including a carbonyl resonance at 200 (Appendix C1). Chemical shift indicating the presence of a moiety include δ_c at 33 ppm for C4 of flavonoids (with resonance range between 28-35 ppm), δ_c at 47.58 and 48 ppm for non-oxygenated C2 and C3 (reference range of 40-85 ppm), another non-oxygenated aromatic carbon atom at δ_c 89 ppm (with reference range of 90-125 ppm).

The carbonyl carbon at δ_c 200 ppm (δ_c of C=O has a range of185-220 ppm) is due to the presence of the sesquiterpene. The carbonyl carbon has the lowest intensity. This is due the fact that no hydrogen is attached, so it has longer relaxation time. Quarternary carbons tend to have long relaxation time.

Distortionless Enhancement Polarisation Transfer (DEPT) revealed odd numbers of hydrogen atoms are attached to the carbon atoms at δ_c 15,68,78,82 and 89 ppm while carbon atoms at δ_c of 22, 23, 29,30,62,62 have even number hydrogen atoms attached to them. A single hydrogen atom was found bonded to carbon atoms at δ_c 30, 48, 49, 68, 78, 82, and 89 (Appendix D1).

HPLC ESI MS of TK3 fraction revealed nine compounds in the fraction (Appendix E1). The compounds 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] and sclerinone A which were observed at peak 7 and 8 have been reported with antiplasmodial activity (Sore *et al.*,2018 and Gao *et al.*, 2015). High-performance liquid chromatography–electrospray ionization time-of-flight mass spectrometry (HPLC–ESI-TOFMS) was used because it is a feasible method for

establishing metabolic profiling of traditional medicine due to its inherent characteristics of accurate mass measurements and high resolution. Moreover, its high sensitivity ensures that components of minor amounts will not be excluded, thereby ensuring a detailed and complete metabolic profiling (Li *et al.*, 2013). Positive electrospray ionization (ESI^+) was selected as the ionization mode for the TOFMS experiments because the metabolites can be detected with greater ion intensities using this ionization mode, and hence this mode provided richer information on the metabolites.

Mass spectra were obtained with Electrospray Ionisation Mass spectrometer- (ESI- MS). Profiles of the target compounds were elucidated on the basis of the single-ion chromatograms of protonated molecules registered in MS mode. This was coupled to aAccuTOF to determine the exact molecular weight and elemental composition of the unknown compound and with the aid of the in-built library, a search that matches the compound was displayed and the compound was identified as 2, 2'-methylenebis [6-tert-butyl-4-methylphenol]. 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] is suspected to be responsible for the antiplasmodial activity of fraction TK3 fraction. The molecular weight of the identified 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] estimated from Electrospray Ionisation Mass Spectrometer experiment was 339 (Table 4.27) and was found to correspond to the known molecular weight of the compound. The molecular formula $C_{23}H_{31}O_2$, with eight degree of unsaturation was deduced from the single-ion chromatograms of protonated molecules registered in MS mode.

Earlier researchers have reported the presence of 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] from *Rhodospaera rhodanthema* extract with a noteworthy antiplasmodial property with IC_{50} of 2.7μM (Eaton, 2015).This compound belongs to the flavonoids subclass called chalcone or open chain flavonoids that do not have the C ring of the basic flavonoids

structure. Flavonoids are known to exert antiplasmodial activity by chelating with nucleic acid base pairing of the parasite (Okokon *et al.*,2017), thereby producing plasmodicidal effect. Other modes of action include modulation of host immunity to tackle disease and inhibition of plasmodial enoyl-ACP reductase (FAB I enzyme) – a key regulator of type II fatty synthases (FAS-II) in *P. falciparum* (Mamoun *et al.*,2010). Flavonoids may also bind parasite's serine threonine kinase with high affinity and affect its development (Ferreira *et al.*,2010)

Specially, Chalcones are present in fruits, vegetables and medicinal plants (Panche *et al.*,2016). It is of special interest that chalcones are found to be potential inhibitors of α-glucosidase, which is an effective target on glucose homeostasis (Hummel *et al.*,2012). 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] is a more potent antiplasmodial agent compared to other cholcones (cholcones A and B having IC_{50} of 3.1 and 14 μM) isolated from *Polygonum senegalense* with antiplasmodial activity against *P. falciparum* D6 and W2 strains (Sore *et al.*,2018).

On the subject of structure activity relationship, absence of a prenyl group on 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] could be one of the reasons for its higher antiplasmodial activity and reduced cytotoxicity compared to other flavonoids with a prenyl group. On the order hand, presence of phenyl group on some flavonoids has been reported to decrease antiplasmodial activity and increase cytotoxicity (Zelefack *et al.*,2012). Methylation of some flavonoids also increases antiplasmodial activity. For example, methyl gallate has better antiplasmodial activity with IC_{50} of 0.0025μg/ml compared to gallic acid with IC_{50} of 0.0130μg/ml (Khasanah *et al.*,2017). In this study, methyl substituent at C-3 and or C-4' of 2, 2'-methylenebis [6-tert-butyl-4-methylphenol], is suspected to be responsible for effective inhibition the growth of *P. falciparum*.

Additionally, Pharmacokinetic effects of compound can be reviewed from three aspects of physical properties of the compound which are electronic effects, hydrophobic property and molecular size (Marliana *et al.,*2018). The presence of methyl substituent also increases lipophilic properties of compound.

The lipophilic aspect contributes to the activity of the compound in terms of its ability to bypass the semi-permeable lipid membrane of parasite. Furthermore, this can enable the compound enter the food vacuole and inhibits the formation of heme polymers (hemozoin) thereby resulting in parasite death. The lipophilic of nature of compounds is also associated with the mechanism of inhibition of the New Permeation Pathway (NPP) by inhibiting the transport of nutrients needed by parasites (Meler *et al.,*2018).

According to established norms, the inhibition effect of an isolated compound is considered to be very active if IC_{50} vaule is<5µg/ml (Pachiappan *et al.,*2017) hence the isolate 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] is a potent antiplasmodial agent with IC_{50} of 2µg/ml. This is the first report on the isolation of 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] from *P. linearifolia* having antimalarial property. Previous study on synthetic 2,2'-methylenebis (4-methyl-6-tert-butylphenol) has revealed that the LD50 of this compound is greater than 5g/kgbw by oral administration (Takagi *et al.,*1994). Flavonoid-based antimalarial drugs are expected to have high therapeutic efficacy against resistant malaria with minimal toxicities (Rudrapal and Chetia, 2017). It has also been reported to suppress weight gain by 0.6%, a good quality of an antiplasmodial agent (Aguiar *et al.,*2012[a]).

Further, in order to delineate anti-age-dependent neuropathology more in-depth, structure-dependent stimulation manner of flavonoids was investigated and the role of ring B

hydroxylation has been implicated (Lei *et al.*,2012). 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] has B ring hydroxylation. Therefore, it could also be an anti-aging agent.

Increased production of reactive oxygen species during malaria results in consumption and depletion of endogenous scavenging compounds such as glutathione, ascorbic acid and α-tocopherol. 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] is a well-known antioxidant, Ortega *et al.,* (2017) reported 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] to have a strong antioxidant property. Its radical scavenging activity is more than that of Trolox standard antioxidant. Ring A hydroxylation may contribute less to anti-oxidant activity than ring B, since *ortho*-dihydroxyl group in ring B is more easily oxidizable than ring A *meta*-dihydroxylation (Chen *et al.*,2012). As an antioxidant, 2, 2'-methylenebis [6-tert-butyl-4-methylphenol], has an additive effect to the endogenous scavenging compounds (Kumar and Pandey, 2013).

Generally, the mechanisms underlying the antioxidant property of flavonoids are free radical scanvenging and transition metal ion chelating activity. Due to reducing activities of phenolic hydroxyl groups, flavonoids are able to donate hydrogen. Along with delocalization of phenoxy radical products, flavonoids can protect against various disease damage from reactive oxygen species (Verma *et al.*,2012).

The sesquiterpenoid found in faction TK3 (Table 4.27) has a molecular formula of C_{15}-$H_{21}O_4$ with six units of unsaturation on the bases of HRESIMS (m/z 265.1442). The ^{13}C NMR spectrum of resolved 15 carbon signals that were assigned with the aid of DEPT techniques in to four methyls (including acetyl resonating at δ 31.6) seven quaternary carbon including one keto and one carboxylic carbonyl resonating at δC 160.0 and 200.0, two all carbon quaternary center at δ C 47.5 and 49.0.

The ^{1}H-NMR spectrum, the resonances of four methyl and an exchangeable hydroxy 0.9, 1.3 3.3 and doublet 4.8 and 5.0. The above found abilities account for three out of the six degree of unsaturated and the remaining three degree suggest that it is a tricyclic sesquiterpenoid. This findings is similar to the research of Zhu *et al.*, (2017), that reported procehemiketal a with molecular formula $C_{15}H_{12}O_4$ with six double on the basis of HRESIMS (m/z 265.1443$(M+H)^+$ 287.1263 (M + Na) and 303.1001 (M + K). The ^{13}C NMR spectrum of 1 resolved 15 carbon signed that were assigned with the aid of DEPT and HSQC techniques in to four methyl (including one acetyl resonating at δC 31.6) seven quaternary carbons (including one keto and one carboxylic carbonyl resonating at δC 200.0 and 168.0 and two all carbon quaternary centers δC 50.0 and 49.5. Three out of the six degree of unsaturation were accounted for and the remaining three degree suggests that the compound is a tricyclic sesquiterpenoid.

Gao *et al* (2015) also isolated a yellowish-brown oil liquid with UV max of 241 nm and HR-ESIMS m/z 265.1143 with a molecular formular of C_{15} $H_{21}O$ and calculated the molecular weight to be 265.1445. this agrees with the results of this study, in which the compound has UV max of 240nm and a molecular weight of 265.1474 (HR-ESIMS m/z) and calculated for 265.1440 with elemental composition of $C_{15}H_{21}O_4$ with six degree of unsaturation. The variation in UV max could due to the presence of other compounds in the fraction. This compound has been identified as Sclerienone A (Nyongbela *et al.*,2009).

The *in vitro* antimalaria test of Sclerienone A, (oil substance with HR -MS (negative ion mode): m/z 265.1511 C_{15} H_{21} O_4 (calculated for C_{15} H_{21} O_4, 165.1440) against chloroquine sensitive (NF54 strain of the *P. falciparum* parasite gave IC_{50} values > 1000ng/ml (Nyongbela *et al.*,2009). However, this metabolite is not primarily responsible for the antiplasmodial activity of fraction

TK3 but biotransformation of Sclerinone A to more an active antiplasmodial agent could have enchance the activity of this fraction. Presence of hydroxyl group in the identified sesquiterpene increases its antiplasmodial activity, because replacement of hydroxyl groups with methoxy groups reduced activity of phloroglucinol mallotajaponin C. Increased alkenyl chain length have also been reported to increase antiplasmodial activity (Harinantenaina *et al.*,2013).

Sclerienone C is an only substance with Rf of 0.5 (hexane: Et0Ac, 1:2), UV/Vis xmax (MeOH): 255nm ESI-APCI-MS (positive ion mode): m/2 (%) = 266 ($M + NH_4^+ - H_2O$), 249 ($M + H^+ - H_2O$) (100), 231 ($M + H^+ - 2H_2O$) (negative ion mode): m/3 (%) = 325 (M + AcO) (100), 265 ($M-H^+$). HRMS –ESI m/2 ($M+N_a^+$) calculated for $C_{15}\ H_{22}\ O_4\ Na^+$ 286.141 found 289.141. This sesquiterpene was screened *in vitro* against NF54, a strain of *Plasmodium falciparum* parasite sensitive to known antiplasmodial drug, sclerianone C gave IC_{50} values of 5.1 and 4.4 µg/ml respectively using chloroquine and artemisinin as reference drugs (Nyongbela *et al.*,2016).

Inflammation has been implicated in the pathogenesis of malaria (A) and Sclerienone C has been reported to have anti-inflammatory effect. A recent research by Zhang *et al.,* (2014) in which sesquiterpenoid from *Inula britennica* was isolated and identified with HRESIMS (positive), m/z 265.1455 calculated for $C_{15}\ H_{21}\ O_4$, 265.1434. The compound which is called 1β, 6∝-Dihydroxy-5xH-eudesma-4(15), 11 (13)-dien-12,8β-olide, had inhibitory effect against lipopolysaccharide (LPS) induced nitric oxide production in RAW 264.7 macrophages (Zhang *et al.*,2014), revealing its mechanism of anti-inflammatory activity. Costa *et al* (2018) have also tried to identify the mechanism of how this sesquiterpene elicit its antioxidant activity via anti-glycation potential but discovered the mechanism is not through anti-glycation potential but could be through free radical scavenging. This same sesquiterpene, isolated from *Chloranthu sanhuiensis* has been investigated for neuroprotective effect (Xu *et al.*,2018).

Individually, compounds 2, 2'-methylenebis [6-tert-butyl-4-methylphenol] had an IC_{50} of 5.1µg/ml while compound Sclerienene C had IC_{50} of 2.71µg/ml against NF54 strain of *Plasmodium falciparum* (Eaton, 2015 and Nyongbela *et al.*,2016)The synergistic effect of these two antiplasmodial compounds in fraction Tk3 could be responsible for the enhanced and increased antiplasmodial activity (IC_{50} of 2µg/ml.) Generally, the criteria for antiplasmodial activity against *P. falciparum* used by World Health Organization are: pronounced activity for IC_{50}< 5 µg/ml, good activity for 5 IC_{50}< 10µg/ml, moderate activity for 10 < IC_{50}< 20, low activity for 20 < IC_{50} < 40 µg/ml and inactive for IC_{50}< 40 µg/ml (Nyongbela *et al.*,2016). Hence fraction TK3 has a pronounced antiplasmodial activity.

The antiplasmodial activity of this fraction is suspected to be through multiple mechanisms of action. The flavonoids play a role in blocking the formation of hemozoin by the formation of free heme complexes with active compound in the food vacuoles of the parasite. The heme free (Fe3+) is highly toxic because it can cause highly reactive oxygen species which can trigger oxidative reactions so that the parasites die (Marliana *et al.*,2018). Therefore, the parasite converts it into a non-toxic substance by forming a polymer from the heme residues by a coordination bond between Fe3+ heme with another heme hydroxyl group to form the *β*-hematin molecule, further forming a larger aggregate called hemozoin. This formation process of hemozoin could be hindered by 2,2'-methylenebis [4-methyl-6-tert-butylphenol]. However, inhibition of isoprenoid biosynthesis was proposed for flavonoids. Unlike quinoline-based antimalarials which are basic in nature, flavonoids possess acidic character (due to the phenolic –OH groups) which impedes their entry into the acidic food vacuole (FV) of parasites, and hence they do not directly interfere with the action of enzymes involved in the haemoglobin degradation process, the site of action of all major group of antimalarial drugs (Ntie-Kang *et*

al.,2014). Rudrapal *et al.* (2013), also supported the opinion that flavonoids might act at the same site within the parasite but with a different mechanism of action. It is attributed that flavonoids exert their antimalarial action by targeting certain functional biomolecules (protein, enzymes, DNA etc.) that are essential for parasite survival. The phenolic –OH groups of such polyphenolic flavonoids (hydroquinones) is readily converted to a stable phenoxy radical anion (semiquinones) under cellular oxidative stress (*in vivo*) which in turn exerts either oxidative damage on cellular components of parasites or direct tissue damage by irreversible covalent interaction with parasitic structural proteins or DNA. In such circumstances, the antioxidant property of flavonoids could be the basis of their antimalarial action.

The potentially active antiplasmodial agents in this fraction also include a sesquiterpene. Some sesquiterpene such as nerolidol and its derivatives elicit antiplasmodial activity by inhibition of isoprenoid biosynthesis and hemozoin formation (Saito *et al.*,2016). This identified sesquiterpene could be acting using these same mechanisms. This could be the reason why this fraction is more potent compared to the other fraction and also why it is the only fraction whose IC_{50} against the resistant strain decreased compared to Chloroquine sensitive strain (Table 4.20). In addition, the mode of action of quinolines and artemisinin derivatives has been established to be by inhibition of haemin polymerisation through their binding with haemin (Kayemba *et al.*, 2012). The apicoplast and digestive vacuole are distinct cellular compartments (organelles). Their physiological processes are targeted by different drugs (Howe *et al.*,2013). As the flavonoid is inhibiting hemozoin formation in the food vacuole or biosynthesis of isoprenoids, similarly the sesquiterpeneis exhibiting dual mechanistic actions against *P. falciparum* by inhibiting the biosynthesis of isoprenoids in *Plasmodium* parasite and interferes in the

prenylation of proteins of the digestive vacuole, thus affecting the overall process of hemozoin formation in the parasite. Because Ubiquinones and menaquinones are synthesized through the same methylerythritol phosphate (MEP) pathway for isoprenoid synthesis in plasmodium parasite. Inhibition of this pathway deprives the parasite of these molecules which are essential for its survival. Ubiquinone, also known as coenzyme Q, is an important electron carrier which is actively synthesized in the mitochondria of *Plasmodium* parasites and exerts an important protective antioxidant effect in the parasite (Nowick and Kruk, 2010) while menaquinone (MQ), also known as vitamin K2, is a fat-soluble vitamins and an important electron receiver of the respiratory chain in *Plasmodium falciparum*. Inhibition of menaquinone results in decreased parasite growth (Tonhosolo *et al.*,2010).

The presence of these bioactive compounds in *P. linearfolia* have not been reported in *P. carymbosa* lam that belong to the same family with *P. linearfolia*

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Z. zanthoxyloide crude methanol extract had a pronounced *in vitro* antiplasmodial activity with IC_{50} of 1.0 µg/ml. *L. hastata* while *P. linearifolia* crude methanol extract had an outstanding *in vivo* antiplasmodial activity with mean survival time and parasite density comparable to the standard drug treated group. *P. linearifolia* extract crude methanol extract had the highest safety margin of 4500 mg/kg bw when compared to other the plants screened. Lh3 fraction from *L. hastata* was most active in *in vivo* antiplasmodial test. Tk3 fraction from *P. linearifolia* was most active in both *in vitro* and *in vivo* antiplasmodial tests. Spectral characterization revealed the presence 2,2'-methylene bis (6-tert butyl- 4-methyl phenol) in both Lh3 and Tk3 fractions. Sclerinone A was also identified in Tk3 fraction of *P. linearifolia.* These identified antiplasmodial agents could serve as templates for the synthesis of new drugs against both CQ sensitive and resistant strains of *P. falciparum.*

5.2 Recommendations

i. The neeed for further study on efficacy of combination therapy using the identified compound.

ii. Structural modifications may lead to the development of more pharmacologically acceptable antimalaria drugs from the plants studied.

REFERENCES

Abcar, A. C, Chan, L. & Yeoh, H. (2004). What to do for the patient with minimally elevated creatinine level? *The Permanente Journal*, 8 (1):51-53.

Abonyi, O., Uzoegwu, P. N., Ezugwu, A. I., Uroko, R.I., Ani, O.O., Onyemuche,T. N. & Anigbogu, J. U., (2014). *In vitro* antioxidant profile of methanolic leaf extract of *Securidaca longepedunculata*. *Journal of Dental and Medical Sciences*, 13, 75-81.doi:10.9790/0853-131157581.

Abugri, D. A. & McElhenney, W. H. (2013). Extraction of Total Phenolic and Flavonoids from Edible Wild and Cultivated Medicinal Mushrooms as Affected by Different Solvents. *Journal of Natutral Products and. Plant Resource*, 3, 37-42.

Achan, J., Talisuna, A. O., Erhart, A., Yeka, A., Tibenderana, J. K., Baliraine, F. N., & D'Alessandro, U. (2011). Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. *Malaria Journal*, 10, 144, 1-12.

Adebayo, F. A. (2016). Improving laboratory –based diagnosis of malaria. 1st edition, Edoson publisher, Lagos, pp27-30.

Adiele, R. C., Fakae, B. B. & Isuzu, I. U. (2013). Antihelmintic activity of *Securideca longepedunculata* (Family polygalaceae) root extract in mice *in vitro* and *in vivo*. *Asian Pacific Journal of Tropical Medicine*, 6, 841-846.

Adiukwu, P. C., Amon, A. & Nambatye, G. (2011). Pharmacognotic, antiplasmodial and antipyretic evaluation of aqueous extract of *Vernonia amygdaline* leaf. *International Journal of Biology and Chemical Science*, 5,709-716.

Afsar, F. H., Deleizer, A., Asnashad, S., Vaez, H., Zolali, E. & Asghasian, P. (2017). Screening of antimalarial activity of different extract obtained from three species of Scrophularia growing in Iran. *Pharmaticautical Research*, 17, 660-676.

Aguiar, A. C., Santos, R. de M., Figueiredo, F. J., Cartopassi, W.A., Pimental, A.S., Franca, T.C., Meneghetti, M.R. & Kretti, A.U. (2012) [a.] Antimalarial activity and mechanism of action of two novel 4-aminoquinolines against Chloroquine resistant parasite. *PLOS ONE*, 7, e37259.

Aguiar, A. C., Rocha, E. M., Souza, N. B., Franca, T. C. & Kretti, A. U. (2012[b]). New approaches antimalarial drug discovery and development-A review. *Mem Inst Oswaldo,RIO de Janeiro*, 107, 831-845.

Akinyemi, K. O., Oladapo, O., Okwara, C. E., Ibe, C. C. & Fasure, K. A. (2005). Screening of crude extracts of six medicinal plants used in South-west Nigeria unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity, *BMC complementary and Alternative Medicine*, 5,6. Doi:10.1186/1472-6882-5-6.

Akkawi, M., Jaber, S., Abu-Lafi, S., Qutab, M., Abu-Rmeleh, Q. & Lutgen, P.L. (2014). HPLC separation and *in vitro* antimalarial studies of Artemisia annua plants from two different origins: Cameroon verse Lexembourg. *Malaria World Journal*, 5,11.

Akobundu, O. I., Ekeieme, F., Agyakwa, C. W. & Ogazie, C. A. (2016). A handbook of West African Weeds, International Institute of Tropical Agricuture, Ibadan, pp133.

Aktar, K. & Foyzun, T. (2017). Phytochemistry and pharmacological studies of *Citrus macroptera*: a medicinal plant review. *Evidence-Based Complementary and Alternative Medicine*, 2017:(9789802):1-7. Doi: 10.1155/2017/9789802.

Alacra, (2009). Infections – Malaria. Life Science Analytics Inc. (LSA) Biomedical Data Co. Copyright 2009 Alacra Inc., 100 Broadway, Suite 1100, New York, NY 10005. Life Science Analytics, Report, p. 257.

Albuquerque, A. (2014). Nodular lymphoid hyperplasia in gastrointestinal tract in adult patient: a review. *World Journal of Gastrointestinal endoscopy*, 6(11), 534-540. doi:10.4253/wjge.v6i11.534.

Ali, N., Ahmed, G., Shah, S., Ghias, M. & Khan, I. (2011). Acute toxicity, brine shrimp cytotoxicity and relaxant activity of fruits of *Callistemon citrinus* Curtis. *BMC Complementary and Alternative Medicine*, 11,99. Doi:10.1186/1472-6882-11-99.

Allwood, J. W. & R. Goodacre, R. (2010). An introduction to liquid chromatography-mass spectrometry instrumentation applied in plant metabolomic analyses. *Phytochemical Analysis*, 21, 33-47. doi:10.1002/pca.1187.

Al-Omar, I. A., Eligail, A. M., Al-Ashban, R. M. & Shah, A.H. (2010). Effect of falciparum malaria infection on blood cholesterol and platelets. *Journal of Saudi Chemical Society*, 14, 83-89. Doi:10.1016/j.jscs.

Alshammari, T. M. (2014). Drug safety: the concept, inception and its importance in patient's health, *Saudi Pharmaceutical Journal*, 24, 405-412.

Aluh D. O. (2018). Evaluation of membrane stabilization activity of leaf extracts *Leptadenia hastata* (pers) decne. *Acta Scientific Pharmaceutical Sciences*. 2, 22-27.

Amato, R., Pearson, R. D., Almagro-Garcia, J., Amaratunga, C., Lim, P., Suon, S.,… Kwiatkowski, D. P. (2018). Origins of the current outbreak of multidrug-resistant malaria in southeast Asia: a retrospective genetic study. *Lancet Infectious Diseases*, 18 (3), 337-345.

Amos, O., Oguniyi, D. S. & Odetoye T. E. (2016). Production of Biodiesel from *Parinari polyandra* B. seed oil using Biobase catalyst. *Nigeria Journal of Technology Development*, 13, 26-30. doi:http//dx.dol.org/10.4314/njtd.v13i.

Andrade, R. J., Aithal, G. P., Bjornsson, E. S., Neill, K., Kullak-ublick, A. G. & Larrey, D. (2019). Clinical practice guidelines: drug –induced liver injury, *Journal of Hepatology*, 70,1222-1261.

Anne, I. O., Andrew, O. O. & Idu, M. (2013). Phytochemical and antimicrobial activity of *Zanthoxylum zanthoxyloides* root used as chewing stick in Nigeria, *The Journal of Phytopharmacology*, 2 (6), 1-7.

Annie- Bligh, A., Ogegbo, O. & Wang, Z.T. (2017). Flavonoids by HPLC, K.G. Ramawat, J.M. Merillion (Eds.), Natural Products, Springer-Verlag, Berlin Heidelberg, 2013, 2107-2144.

Ashcroft, A.E. (1997). "Ionization Methods in Organic Mass Spectrometry", Analytical Monograph Series, Royal Society of Chemistry, Cambridge, UK. pp99.

Asrar, A., Neyaz, K. M. & Hassan, S. I. (2014). Exploiting unique structurall and functional properties of malarial glycolytic enzymes for antimalarial drug development. *Malaria Research and Treatment*, 65,13. http://dx.doi.org/10.1155/2014/451065.

Awouafack, M.D., Tane, P. and Morita, H. (2017). Isolation and structural characterization of flavonoids. *Intechnopen*, 3, 45-59.

Awouafack, M. D., Tane, P. and Eloff, J. N. (2013). Two new antioxidant flavones from the wigs of *Eriosema robustum* (Fabaceae). *Phytochemistry Letters*, 6(1),62-66.

Azando, E. Olounlade, A., Hounzangbe-Adote, M., Tamtta, T. Fabre, N. &Valentin, A. (2017). Controledesparasitoses gastro-intestinaales ovine par 1'hule essentielle de *Zanthoxylum zanthoxyloide* (*Fagara zanthoxyloide*). *Revenue de medicine veterinaire*, 168, 205-212.

Azwanida, N.N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitations. *Medicinal and Aromatic Plants*, 4,1-6. Doi:10.4172/2167-0412.1000196.

Babuselvam, M, Ravikumar, S., Mohamed Farook, K. A., Abideen1, S., Peer Mohamed, M. & Uthiraselvam, M. (2012). Evaluation of anti-inflammatory and analgesic effects on the extracts of different parts of *Excoecaria agallocha* L. *Journal of Applied Pharmaceutical Science*, 2 (9), 108-112.

Bachman, J., Burle, F, Pramna, S. Conte, L., Brown, B, J, Orimodegen A. E, Ajetunmobi K. F. A., Afolabi, N. K, Akikunmi, F., Samuel, O. Akinbami, F. O, Shounbi, W. A, Kamjof, C. & Nilson, P. (2014). Affinity Proteomics reveals elevated muscle Protein in Plasma of children with cerebral malaria, *Pathogen*, doi.org/10.1371/journal.ppat.1004038.

Balamurugan, K., Nishanthini, A. & Mohan, V. R. (2013)[a]. Antidiabetic and antihyperlipidaemic activity of ethanol extract of *Polycarpaea corymbose* (L.) Lam while plants in alloxan induced diabetic rats. *International Journal of Pharmaceutical and Chemical Sciences*, 2, 757-763.

Balamurugan, K., Nishanthini, A. & Mohan, V. R. (2013)[b]. Anticancer activity of ethanol extract of *Polycarpaea carymbosa* (L.) Lam while plant against altonascities trymphoma. *International Journal of Pharmacology and Biosciences*, 4, 296-303.

Baldwin, M. R., Li, X., Hanada, T., Liu, S. C. & Chishti, A. H. (2015). Merozoite surface protein 1 recognition of host glycophorin A mediates malaria parasite invasion of red blood cells. *Blood*, 125, 2704-2711. doi:10.1182/blood-2014-11-611707.

Banerjee, S. & Mazumdar, S. (2012). Electrospray ionization mass spectrometry: a technique to access the information beyond the molecular weight of the analyte. *International Journal of Analytical Chemistry*, 2012 (2012) 282574. doi:10.1155/2012/282574.

Banroff, J. D. & Stevens, A. (1990). A theory and practice of histological techniques, 3rd edition, Churchilllivingstone Edinburgh. London, Melborne and New York pp

Bansal, A., Chhabra, V., Rawal, R. K. & Sharma, S. (2014). Chemometrics: A new scenario in herbal drug standardization, *Journal of Pharmaceutical Analysis*, 4, 223–233.

Bashir R., FazalulRahiman S. S., Hasballah, K., Chong, W. C., Tahib, H. Yam, S. F., Jabbarzare, M., Tie, T. H., Omman, F., Moklas, M.A.M., Abdullah, W. O., & Ameh, Z., (2012). *Plasmodium berghei* ANKA infection ICR mice as a model of cerebral malaria. *Iranian Journal of Parastology*, 7(4), 62-74

Batisa, R., SilverJunior, A. & deOlivera, A. B. (2009). Plant-derived antimaterialagents: New leads and efficient photomedicine. Part II. Non-Alkaloid Natural products, *Molecules*, 14, 3037-3072. doi:10:3399:/molecules14083037.

Bayala, B., Halabalaki, M., Ouedraogo, A., Keiler, M. A., Tamboura, H. & Vollmer, G. (2018). *Leptadenia hastata* Pers.(Decne) a promising source for natural compounds in biomedical applications, *American Journal of Drug Discovery and Development*, 8, 1-10.

Bayala, B., Telefo, P. B., Bassole, I. H. N., Tamboura, H. H., Belemtougri, R. G., Sawadogo, L., Malpaux, B. & Dacheux, J. L. (2011). Anti-spermtogenic activity of *Leptadenia hastata* (Pers) decne leaf stems aqueous extracts in Male wister rats. *Journal of Pharmacology and Toxicology*, 6(4), 391-399. doi:10:3923/jpt.2011.391.399.

Bello, A., Aliero A. A., Saidu, T. & Muhammed, S. (2011). Hypoglycaemic and hypolipidaemic effects of *Leptadenia hastata* (pers) decne I alloxan induced diabetic rats, *Nigeria-Journal of Basic and Applied Science*, 19, 187-192.

Berghout, J., Higgins, S., Loucoubar, C., Sakuntabhi, A., Kain, K.C. & Gros, P. (2012). Genetic diversity in human erythrocyte pyruvate kinase. *Genes and Immunity*, 13(1), 98-102. Doi:10.1038/gene.2011.54.

Berliner, R. W., Earle, D. P., Taggart, J. V., Zubrod, C. G., Welch, W. J., Conan, N. J., Bauman, E., Scudder, S. T. Shannon, J. A. (1948). Studies on the chemotheraphy of the human malarias. VI. The physiological disposition, antimalarial activity, and toxicity of several derivatives of 4-aminoquinoline. *Journal of Clinical Investigation*, 27 (3), 98-107. doi: 10.1172/JCI101980.

Berzosa, P., de Luciol, A., Romay‐Barja1, M., Herrador, Z., González, V., García, L., Fernandez, A., Morales, M., Ncogo, P., Basilio Valladares, B., Riloha, M. & Benito, A. (2018). Comparison of three diagnostic methods (microscopy, RDT, and PCR) for the

detection of malaria parasites in representative samples from Equatorial Guinea, *Malaria Journal*, 17, 1-12. doi.org/10.1186/s12936-018-2481-4.

Betjes M.G. (2013). Immune cell dysfunction and inflammation in end stage renal disease, *Nature Review Nephrology*, 9,255-265. Doi:10.1038/nrneph-2013-44.

Bhatt, S., Weiss, D. J., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., Battle, K., Moyes, C. L., Henry, A., Eckhoff, P. A., Wenger, E. A., Briret, O., Penny, M. A., Smith, T. A., Bennett, A, Yukich, J., Eisele, T. P., Griffin, J. T., Fergus, C. A., Lynch, M., Lindgren, F., Cohen, J.M., Murray, C.L.J., Smith, D.L., Hay, S.I., Cibulskis, R.E. & Gething, P.W. (2015). The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015, *Nature*, 526, 207-211. doi:10.1038/nature15535.

Bhatt, S., Weiss, D. J., Cameron, E., Bisanzio, D., Mappin, B., Dalrmphe, H., Battle, K. E., Moye, C. L., Henry, A., Eckchoff, P. A., Wanger, E. A., Briety, O., Penny, M. A., Smith, T. A., Bennett, A., Yukch, J., Eisde, T. P., Griffin, I. I., Fergus, C. A., Lynch, M., Lindgren, F., Cohen, J. M., Murray J. C., Smith, D. L. & Hay, S. I. (2015). The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature*, 526, 207-211. doi:10.1038/nature15535.

Biamonte, M. A., Wanner, J. & Roach, K. G. L. (2013). Recent advances in malaria drug discovery. *Bioorganic Medical Chemistry Letters*, 23, 2829-2843. doi:10.1016/j.bmcl.2013.03.067.

Bihonegn, T., Giday, M., Yimar, G., Animut, A. & Sisay, T. (2019). Antimalarial activity of hydromethanolic extract and its solvent fractions of *Vernonia amygdaline* leaves in mice infected with *Plasmodium berghei*. *SAGE Open Medicine*, 7, 1-10

Blasco, B., Leroy, D. & Fidock, D. A. (2017). Antimalarial drug resistance: Linking *Plasmodium falciparum* parasite biology to the clinic. *Nature Medicine*. 23, 917-928. DOI: 10.1038/nm.4381.

Bohm B. A. & Koupai-Abyazan R. (1994). Flavonoid and Condensed tannins from leaves of *Vaccinum raticulation* and *Vaccinum calcyimium*. *Pacific Science*, 48, 458-463.

Boligon, A. A. & Athayde, M. L. (2014). Importance of HPLC in analysis of plant extracts. *Autin Chromatography*, 1(3),2.

Boly, R., Dessy, S. I Kohnen, S., Kini, F., Lompo, M., Monithys, A., Guisson, I., Dubois, J., Deby Duport, G., Serteyn, D. & Franck, T. (2011). Modulatory activities of *Agelanthus dodoneifolius* (Laranthaceace) extracts on stimulated equine neutrophils and myeloperoxidase activity, *International Journal of Molecular Medicine*, 28, 261-270.d0:10.3892/ijmm.2011.695

Boly, R., Franck, T., Kohnen, S., Lomgo, M., Guasson, I. Dubois, J., Sertejn Didier, S. & Mouths-Mickalad, A. (2015). Evaluation of Antradical and anti-inflammatory activities of ethylacetate and butanolic subtractions of *Agelanthus dodoneifolius* (DC.) Polhill and Wiens (Loranthanceae) using equine myeloperonidase and both PMA-activited

neutrophils and HL-60 cells. *Evidence Based complementary and Alternative medicines*, 1-9. doi.org/10.1155/2015/7075 24.

Boly, R., Lamkami, T., Lompo, M., Dobois, J & Guisson I. P. (2016). DPPH free radical scavenging activity of two extracts from *Agelanthus dodoneifolius* (Laranthaceae) leaves, *International Journal of Toxicological and Pharmacological Research*, 8, 29-34.

Bompart, F., Kiechel, J. R., Sebbag, R., & Pecoul, B. (2011). Innovative public-private partnerships to maximize the delivery of anti-malarial medicines: lessons learned from the ASAQ Winthrop experience. *Malaria Journal*, 10 (143), 1-9.

Bonkian, L. N., Yerbanga, R. S., Koama, B., Soma, A., Cisse, M., Yalea, I., Tinto, H, Onedraoge, J. B, Guigemale, T. R. & Couldbaly M. T. (2018). *In vivo* antiplasmodial activity of two Sahelian plant extracts on *Plasmodium berghei* ANKA infected NMRI mice. *Evidence-Based Complementary and Alternative Medicine*, 1-4 doi:org/10.1155/2018/6859632.

Bonta, R. K. (2017). Application of HPLC and ESI-MS techniques in the analysis of phenolic acids and flavonoids from green leafy vegetables (GLVs). *Journal of Pharmaceutical Analysis*. 7 (2017), 349–364. Doi:10.1016/j.jpha.2017.06.005.

Borneo, R., León, E. A., Aguirre, A., Ribotta, P. & Cantero, J. J. (2008). Antioxidant capacity of medicinal plants from the Province of Cordoba (Argentina) and their *in vitro* testing in model food system. *Food Chemistry*, 112, 664-670.

Botma, J., Mongongoa, L. F., Jaftha, A. D. & Ransburg, W, J (2012). Reference Ranges for plalelet indices using system XE2100 Blood analyser, *Medical Technology South African*, 26 (2), 17-21.

Boyce, M. R., Menya, D., Turner, E. L., Laktabal, J. & Prudhomme-Omeara, W. (2018). Evaluation of malaria rapid diagnostic test (RDT) use by community health workers: a longitudinal study in western Kenya. *Malaria Journal*, 17, 1-11. Doi:10.1186/s12936-018-2358-6.

Boyom, F. F., Kemgne, E. M., Tepongning, R., Ngouana, V., Mbacham, W. F., Tsamo, E., Zollo, P. H., Gut, J. & Rosenthal, P. J.(2009). Antiplasmodial activity of extracts from seven medicinal plants used in malariatreatment in Cameroon. *Journal of Ethnopharmacology*, 123, 483–488.

Brasseur, P., Druilhe, P., Kouamouo, J., Brandicourt, O., Danis, M., & Moyou, S. R. (1986). High level of sensitivity to chloroquine of 72 *Plasmodium falciparum* isolates from southern Cameroon in January 1985. *American Journal of Tropical Medicine and Hygiene*, 35, 711-716.

Brown, G. D (2010). The Biosynthesis of Artemisinin (Qinghaosu) and the phytochemistry of *Artemisia annua* L. (Qinghao). *Molecules*, 15, 7603-7698. doi:10.3390/molecules15117603.

Bruins, A.P. (1998). Mechanistic aspects of electrospray ionization. *Journal of Chromatogram Analysis*, 794, 345-357.

Builder, M. I., Uguru, M. O., & Aguiyi, C. (2012). Antiplasmodial potential of African Mistletoe:*Agelanthus dodoneifolius* Polh and Wiens. *Indian Journal of Pharmaceutical Sciences,* 74, 223-229.

Bunaciu, A. A., Hoang, V. D. & Aboul-Enein, H. A. (2013). Applications of differential spectrophotometry in analytical chemistry. *Critical Reviews in Analytical Chemistry*, 43, 125–130. doi: 10.1080/10408347.2013.803357.

Bunnag, D., Karbwang, J., Na-Bangchang, K., Thanavibul, A., Chittamas, S., & Harinasuta, T. (1996). Quinine-tetracycline for multidrug resistant falciparum malaria. *Southeast Asian Journal of Tropical Medicine and Public Health*, 27, 15-18.

Burda, P. C., Caldelari, R. & Heussler, V. T. (2017). Manipulation of the host cell membrane during plasmodium liver stage egress. MBio. **8**:e00139-e00117. doi:10.1128/mBio.00139-17

Burkill, H. M. (1985). The useful plants of West Tropical Africa, Vol.1.

Burrows, J. N., Hooft, V. R., Möhrle, J. J., Oeuvray, C. & Wells, T. N. (2013). Designing the next generation of medicines for malaria control and eradication. *Malaria Journal*, 12, 187. doi: 10.1186/1475-2875-12-187.

Burtis, C. A., Ashwood, E. R. & Bruns, D. E. (2012). eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, Fifth Edition*. St. Louis, MO:Elsevier Saunders, pp 509-567.

Cai, S., Risinger, A. L., Nair, S., Peng, J., Anderson, T. J. C., Du, L., Powell, D. R., Mooberry, S. L. & Cichewicz, R. H. (2016). Identification of Compounds with efficacy against malaria parasites from common North American plants. *Journal of Natural Products*. 79, 490-498. doi: 10.1021/acs.jnatprod.5b00874.

Caligiani, A., Acquotti, D., Palla, G. & Bocchi, V. (2007). Identification and quantification of the main organic components of vinegars by high resolution **1**H NMR spectroscopy. *Analytica Chimica Acta*, 585**,** 110–119. doi: 10.1016/j.aca.2006.12.016.

Castelo, A. M., Ambrose, O. A., Mohammed, M., Haruna, M. K. & Jacobus, N. E. (2008). Effect of geographical location on the yield and bioactivity of *Anoigeissus leiocarpus*, *Journal of Pharmacy and Bioresources*, 5(2), 68-72. Doi:10.4314/jpb.v512.52995.

Cedillo-Rivera, R., Ramfrez, A., & Munoz, O., (1992). A rapid colorimetric assay with the Tetrazolium salt MTT and Phenazine Methosulfate (PMS) for viability of *Entamoeba histolytica*. *Archives of Medical Research*, 23, 59–61.

Ch'ng, J., Mok, S., Bozdech, Z., Lear, M. J., Boudhar, A. Russell, R., Nosten, F. & Tan, K. S. (2013). A whole cell pathway screen reveals seven novel chemosensitizers to combat Chloroquine resistant malaria. *Scientific Reports*, 3,1734. doi:10.1038/srep01734.

Chaaib, F., Queiroz, E. F., Ndjoko, K., Diallo, D. & Hosttehman (2003). Antifungal and antioxidant compounds from the root bank of *Fagara zanthoxyloides*, *Planata Medicine*, 69:310-315. Doi: 10.1055/s 2003.38877

Challand, S. & Wilcox, M. (2009). A clinical trial of the traditional medicine *Vernonia amygdaline* in the treatment of uncomplicated malaria, *Journal of Alternate and Complementary Medicine*. 15(11),1231-1237.

Chandra, S., Khan, S., Avula, B., Lata, H., Elsohly, M. A. & Khan, I. A. (2014). Assessment of total phenolic and flavonoids content, antioxidant properties and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: a comparative study. *Evidence Based Complementary and Alternative Medicine*, 2014, 253875. doi: 10.1155/2014/253875.

Chang, C., Lin-Hua, T., & Jantanavivat, C. (1992). Studies on a new antimalarial compound: pyronaridine. *Transaction of the Royal Society of Tropical Medicine and Hygiene*, 86, 7-10.

Charles, A. Joseph, M. & Alex, R. V. (2012). Phytochemical investigation of *Merremia hederacea*. *Journal of Natural Product and Plant Resource*, 2, 486-489.

Chawla, G. & Rajan, C. (2016). Principle, Instrumentation, and Applications of UPLC: A Novel Technique of Liquid Chromatography. *Open Chemistry Journal*, 2016, *3*, 1-16. doi:10.2174/18742201603010001.

Chea, A., Hout, S., Buna, S. S., Tabatadze, N., Gasquet, M., Azas, N., Elias, R., & Balansard, G. (*2007*). Antimalarial activity of alkaloids isolated from *Stephania rotunda*. *Journal of Ethnoparmacology*, 112, 132-137.doi: 10.1016/j.jep.2007.02.005.

Cheema, H. S., Singh, A., Pal, A., Bhakuni, R. S. & Darokar, M.P. (2017). Antimalarial activity, mechanism of action and drug interaction study of active constituent isolated from the dried seeds of *Psoralea corylifolia*. *International Journal of Pharmacognosy and Phytochemical Research*, 9 (3); 364-375. Doi: 10. 2525 8/phy to. v9: 2.8088.

Cheerbrough, M. (2000). District laboratory practice manual in Tropical countries part 2. Cambridge University Press.

Chen, L., Qu, F. Y., Zhou, Y. C. (1982). Field observations on the antimalarial piperaquine. *Chinese Medical Journal (English Edition)*, 95, 281-286.

Chen, Y. H., Yang, Z. S., Wen, C. C., Chang, Y. S., Wang, B. C., Hsiao, C. A. & Shih, T. L. (2012). Evaluation of the structure-activity relationship of flavonoids as antioxidants and toxicants of zebrafish larvae. *Food Chemistry*, 134(2), 717–724. doi: 10.1016/j.foodchem.2012.02.166.

Cheng, J., Ma, T., Liu, W., Wang, H., Jiang, J., Wei, Y., Tian, H., Zou, N., Zhu, Y., Shi, H., Cheng, X. & Wang, C. (2016). In *in vivo* evaluation of the anti-inflammatory and analgesic activities of compound Muniziqi granule in experimental animal models. *BMC Complementary and Alternative Medicine*, 16:20. dio 10.1186/s12906-016-0999-y.

Chinedu, E. Arome, D. & Ameh, F. S. (2013). A new method for determining acute toxicity in animal models. *Toxicology International*, 20, 224-246.doi: 10.4103/0971-6580.121674

Chotivanich, K., Udomsangpetch, R., Simpson, J. A., Newton, P., Pukrittayakamee, S., Looareesuwan, S., and White, N. J. (2000). Parasite multiplication potential and the severity of Falciparum malaria. *The Journal of Infectious Diseases*, 181, 1206–1209. doi: 10.1086/315353.

Chukwujewu, J. C. de Kock, C. A., Smith, P. J., Van Heerden, F. R. & Van Staden, J. (2016). Antiplasmodial activity of compounds isolated from *Erythrina caffra*, *South Africa Journal of Botany*, 106,101-103. Doi: 10.1016/. sab. 2016.05.019.

Chukwuocha, U. M. & Eke, K. N. (2011). Malaria parasite status and cholesterol level of malaria parasite in parts of Imo river basin of Nigeria. *Asian Pacific Journal of Tropical Medicine,* 993-996.

Chung, T. D. Y., Terry, D. B. & Smith, L. H. (2015). *In vitro* and *in vivo* assessment of ADME and PK properties during lead selection and lead optimization – guidelines benchmark and rules of thumb, Assay Guidance Manual, Eli Lilly and company and National Center for Advanced and Translational Sciences, pp 2004-2018.

Combrinck, J. M., Mabotha, T. E., Ncokazi, K. K., Ambele, M. A., Taylor, D., Smith, P. J., Hoppe, H. C. & Egan, T. J. (2013). Insights into the role of heme in the mechanism of action of antimalarials. *ACS Chemical Biology*, 8 (1), 133-137.

Cosgriff, T. M., Desjardins, R. E., Pamplin, C. L., Canfield, C. J., Doberstyn, E. B., & Boudreau, E. F. (1982). Evaluation of the antimalarial activity of the phenanthrenemethanol halofantrine (WR 171,669)*. *American Journal of Tropical Medicine and Hygiene*, 31, 1075-1079.

Coskun, O. (2016). Separation techniques: chromatography, North Clinic Istanbul, 3(2),156-60. Doi:10.14744/nci.2016.32757.

Costa, D. C., Costa, H. S., Albuquerque, T. G., Ramos, F., Castilho, M. C. & Sanches-Silva, A. (2015). Advances in phenolic compound analysis of aromatic plants and their potential applications. *Trends Food and Science Technology*, 45, 336–354. Doi:10.1016/j.tifs.2015.06.009.

Costa, E. V., Soares, L. N., Pinhiero, M. L. B., Maia B. H. L, Marques, F. A., Barison, A., Almeida, J. R. G., Souse I. L., Heetdt, G., Morgan, N. H., Acho, L. D. Lima, E. S., de Silva, F. M. & Koolen, H. F. (2018). Guainolidesequiterpene Lactones and aporphine alkaloids from the stem bark of *Guatteriafriesiana*, *Phytochemistry*, 145, 18-25. doi:10 1016/6.Phytochem. 2017.10.003.

Cowman, A. F., Christopher, J. T., Tham, W. & Duraisingh, M. T. (2017). The molecular basis of erythrocyte invasion by malaria parasites. Cell Host and Microbes, 22, 232-245. Doi:10.1016/j.chom.2017.07.003.

Cowman, A.F., Berry, D. & Baum, J. (2012). The cellular and molecular basis for malaria parasite invasion of the human red blood cell. *The Journal of Cell Biology*, 198:961-971.

Croft, A. M. (2007). A lesson learnt: the rise and fall of Lariam and Halfan. *Journal of the Royal Society of Medicine*, 100, 170-174.

Croft, S. L., Duparc, S., Arbe-Barnes, S. J., Craft, J., Shin, C. S., Fleckenstein, L.,… Rim, H. J. (2012). Review of pyronaridine anti-malarial properties and product characteristics. *Malaria Journal*, 11 (270), 1-28.

Cui, L. & Zhuan Su, X. (2009). Discovery, mechanisms of action and combination therapy of artemisinin. *Expert Review of Anti-Infective Therapy*, 7, 999-1013.

Curd, F. H. S., Davey, D. G., Rose, F. L. (1945). Studies on synthetic antimalarial drugs. *Annals of Tropical Medicine and Parasitology*, 39, 208-216.

Curtis S. D. A, Curini, R., Delfini, M., Brosio, E., D'ascenzo, F. & Bocca B. (2000). Amino Acid Profile in the Ripening of Grana Padano Cheese: A NMR Study. *Food Chemistry; 71*(4): 495–502. doi:10.1016/S0308-8146(00)00192-198.

Cuyckens, F. & Claeys, M. (2004). Mass spectrometry in the structural analysis of flavonoids. *Journal of Mass Spectrometry*, 39 (2004) 1–15. Doi; 10.1002/jms.585.

Daharo, E. R., Oporto P., A., Gimenez, A. M., Sauvin, V. J. & Ginsburg, H. (2002). A non – radiolabelled ferriprotoporphyrin IX biomineralisation inhibition test for the high throughput screening of antimalaria compounds. *Experimental Parasitology*, 100, 252-256.

David-Bosne, S., Florent, I., Lund-Winter, A., Hansen, J.B., Buch-Pdersen, M., Machillot, P., Maire, M., & Jaxel, C. (2013). Antimalarial screening via large scale purification of *Plasmodium falciparum* Ca^{2+} -ATPase 6 and in vitro studies. *FEBS Journal*, 280,5417-5419.

Deans, A. M., Nery, S., Conway, D. J., Kai, O., Marsh, K., & Rowe, J. A. (2007). Invasion pathways and malaria severity in Kenyan *Plasmodium falciparum* clinical isolates. *Infection and Immunity*. 75, 3014–3020. doi:10.1128/IAI.00249-07.

deMendonca, V. R., Marilda Souza Goncalves, M.S. & Barral-Netto, M. (2012). The host genetic diversity in malaria infection. *Journal of Tropical Medicine,* 940616, 1-17. doi:10.1155/2012/940616.

Dolabela, M. F., Oliveira, S. G., Peres, J. M., Nascimento, J. M., Póvoa, M. M. & Oliveira, A. B. (2012). *In vitro* antimalarial activity of six Aspidosperma species from the state of Minas Gerais (Brazil). *Annals of the Brazilian Academy of Sciences*, 84(4), 899–910.

Donald, P. L., Lampman, G. M., Kritz, G. S. & Engel, R. G. (2006). Introduction to Organic Laboratory Techniques. 4th edition, Thomson Brooks/Cole pp 797–817.

Dumas, M. E., Kinross, J. & Nicholson, J. K. (2014). Metabolic phenotyping and systems biology approaches to understanding metabolic syndrome and fatty liver disease. *Gastroenterology*, 146(1), 46–62. Doi: 10.1053/j.gastro.2013.11.001.

Eaton, A. L. (2015). Isolation and Synthesis of Bioactive Compounds from Plants. Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University. Blacksburg, VA pp151-155

Eastman, R. T., & Fidock, D. A. (2009). Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria. *Nature Reviews Microbiology*, 7 (12), 864-874.

Ebstie, Y. A., Abay, S. M., Tadesse, W. T. & Ejigu, D. A. (2016). Tafenoquine and its potential in the treatment and relapse prevention of *Plasmodium vivax* malaria: the evidence to date. *Drug Discovery and Development Therapy*, 10, 2387-2399.

Edeoga, H. O., Okwu, D. E. & Mbaebie, B. O. (2005). Phytochemical Constituents of some Nigeria medicinal plants. *African JournalofBiotechnology*, 49, 685-687.

Egan, T.J. (2008). Haemozoin formation. Molecular and Biochemical Parasitology, 157:127–136. doi.org/10.1016/j.molbiopara.2007.11.005.

El Sayed, K. A., Kelly, M., Kara, U. A., Ang, K. K., Katsuyama, I., Dunbar, D. C., Khan, A. A. & Hamann, M. T. (2001). New manzamine alkaloids with potent activity against infectious diseases. *Journal of American Chemical Society,* 123, 1804-1808.

Elbadawi, N., Ibrahim, E. K. & Dawoo, D. (2013). The effect of malaria on biochemical renewal function parameters in sudaness pregnant women. *Journal of call Biology and Biochemical Research*, 1(1), 4-7.

Evans, W.C. (1996). Pharmacoeial and related drugs of biological origin. InTrease and Evan's , WB saunders, London, pp357-415.

Ezenyi, I. C., Salawu, O. A., Kulkarni, R. & Emeje, M. (2014). Antiplasmodial activity aided isolation and identification of quercetin-40-methyl ether in *Chromolaena odorata* leaf fraction with high activity against chloroquine resistant *Plasmodium falciparum*. *Parasitology Research*, 113(12):4415–4422. doi: 10.1007/s00436-014-4119-y.

Ezeonu, C. S. & Ejikeme, C. M. (2016). Qualitative and quantitative determination of phytochemical contents of indigenous Nigeria softwood, *New Journal of Science*, 2016,5601327. Doi:10.1155/2016/5601327.

Falade, M. O., Akinboye, D. O., Gbotosho, G. O., Ajaiyeba, E. O., Happi, T. C., Abiodun, O. O. & Oduola, A.M.J. (2014). *In vitro* and *in vivo* antimalaria activity of *Ficus thonningii* Blume (Moraceae) and *Lophira alata* Banks (Ochnaceae) identified from ethnomedicine of the Nigerian Middle Belt, *Journal of Parasitology Research*, 1-6. Doi:10.1155/2014/972853.

Feitosa, E. A., Xavier, H. S. & Randav, K. P. (2012). Chrysobalaciceae: traditional uses, phytochemistry and pharmacology, *Brazilian Journal of Pharmacognosy*, 22, 1181-1186.doi:htp//dx.doi.org/10.1590/50102-695x20.

Ferreira, J.F., Luthria, D.L., Sasaki, T. & Heyerick, A. (2010) Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. *Molecules*, 15(5),3135–3170.

Feng, F., Zheng, G., Wang, Q., Liu, S., Liu, Z., Xu, G., Wang, F., Guo, M., Lian, X. & Zhang, H. (2018.) Low lymphocytes count and high monocytes count predicts poor prognosis of gastric cancer, *BMC Gastroenterology*, 18:148. Doi: 10.1186/s1287-018-0877-9.

Ferrer, J., Rosal, R. D., Vidal, D. M., Prats, C., Valls, J., Herreros, E. A., Lopez, D. & Gargolto, D. (2008). Effect of haematocrit layer-geometry on *Plasmodium falciparum* static thin layer in microculture. *Malaria Journal*, 7,203. Doi:10.1186/1475-2875-203.

Fevang, P., Havemann, K., Fevang, B. & Hostmark, A.T. (2018). Malaria and Malnutrition: Kwashiorkor Associated with Low Levels of Parasitaemia. *Malaria research and Treatment*,7153173:1-5. Doi:10.1155/2018/7153173.

Fidock, D. A., Rosenthal, P. J., Croff, S. L., Brun, R. & Nwaka, S. (2004). Antimalarial drug discovery: efficacy models for compound screening. *Nature Review*, 3,509-520. Doi:10.1038/nrd1416.

Folasade, S. I., Olukemi O. A. & Jones, M. O. (2006). Management of Sickle cell anemia in Nigeria with medicinal plants: cationic evolution of extracts and possible effects on the efficacy, *Journal of Biological Sciences*, 6, 100-102.

Foley, M., & Tilley, L. (1997). Quinoline antimalarials: mechanisms of action and resistance. *International Journal of Parasitology*, 27, 231-240.

Francois, G., Timperman, G., Eling, W., Assi, L. A., Holenz, J. & Bringmann, G. (1997) Naphthylisoquinoline alkaloids against malaria: evaluation of the curative potentials of dioncophylline C and dioncopeltine A against *Plasmodium berghei in vivo*. *Antimicrobial. Agents Chemotherapy*. 41, 2533.

Franklin, B. S., Ishizaka, S. T., Lamphier, M., Gusovsy, F., Hasan, H., Rose, J., Zheng, W., Ataide, M. A., de Oliveira, R. B., Goldenback, D. T. & Gazzindli, R. T. (2011). Therapeutical targeting of nucleic acid sensing toll like receptors prevent 0065perimental cerebral malaria. *Proceedings of National Academy of Science*, 108, 3689-3694.

Frédérich, M., Tits, M., Hayette, M. P., Brandt, V., Penelle, J., DeMol, P., Llabrès, G. & Angenot, L. (1999). 10'-Hydroxyusambarensine, a new antimalarial bisindole alkaloid from the roots of *Strychnos usambarensis*. *Journal of Natural. Products*, 62, 619. Doi:10.1021/np980375.

Freiburghous, F., Kaminsky, R., Nkunya, M. H. H. & Brun, R. (1996). Evaluation of African medicinal plants *in vitro* trypanocidal activity. *Journal of Ethnopharmacology*, 55,1-11.

Fry, M., & Pudney, M. (1992). Site of action of the antimalarial hydroxynaphthoquinone, 2-[trans-4-(4'-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone (566C80). *Biochemical Pharmacology*, 43, 1545-1553.

Gaertner, M., Muller, L., Roos, J. F., Cani, G., Santos, A. R. S., Niero, R., Calixto, J. F., Yunes, R. A., Delle Monache, F. & CechinelFehho, V. (1999). Analgesic triterpenes from *Sebastiania schottianan* roots. *Phytomedicine*; 6: 41- 44.

Galanello, R & Origa, R. (2010). Beta-thalassemia. *Orphanet Journal of Rare Diseases*, 5,1-15. doi.10.1186/1750-1172-5-11.

Gamaniel, K. S. (2000). Toxicity from medicinal plants and their products. *Nigeria Journal of Natural Products and Medicine*, 4, 4-8.

Ganesh, D., Fuehrer, H., Starzengruber, P., Swoboda, P., Khan, W. A., Reismann, J. A., Mueller, M. S., Chiba, P. & Noedl, H. (2012). Antiplasmodial activity of flavonol quercetin and its analogues in *Plasmodium falciparum*: evidence from clinical isolates in Bangladesh and standardized parasite clones. *Parasitology Research*, 110, 2289–2295. doi: 10.1007/s00436-011-2763-z.

Ganesh, M., Vasudevan, M., Kamalakannan, K., Kumar, A. S., Vinoba, M., Ganguly, S. & Sivakumar, T. (2008). Anti-inflammatory and analgesic effects of *Pongamia glabra* leaf gall extract, *Pharmacologyonline*, 1, 497-512.

Gantz, V. M., Jasinskiene, N., Tatarenkova, O., Fazekas, A, Macias, V. M., Bier, E. & James, A. A (2015). Highly efficient cas 9-mediated gene drive for population modification of

the malaria vector mosquito *Anopheles Stephensi*, *Proceedings of the National Academy of Sciences of the USA*, 112, E6736 – E6743. doi:10.1073/pnas.1521077172.

Gao, Y, Li, Y., Yu, X., Guo, S., Ji, X., San, T., Lan, C., Lavergne, V. Gnannoum, M. & Li, L. (2014). The impact of various platelet indices as prognostic markers of septic shock. PLOS ONE 9 (8) e103761 doi:10.1371/Journal/pone.0103761.

Gao, Y. Q., Guo, C., Zhang, Q., Zhou, W., Wang, C. C. & Goa, J. (2015). Asperaculanes A and B, two soquiterpenoids from the fungus Aspergillus aculeatus. *Molecules*, 20, 325-334. Doi:10.3990/molecules 20010325.

Garavito, G., Rincón, J., Arteaga, L., Hata, Y., Bourdy, G., Gimenez, A., Pinzón, R., & Deharo, E. (2006). Antimalarial activity of some Colombian medicinal plants. *Journal Ethnopharmacology*. 107, 460-462. Doi:10.1016/j.jep.2006.03.033.

Garba, A., Maurice, N. A., Maina, V. A., Baraya, Y. S., Owada, A. H., Sa'adatu, I., Gugong, V. T., Sada, A., Agang, I. & Hambolu, S. E. (2013). Effect of *Leptadenia hastate* leaf extract on embyofoetal development in white albino rats, *Greener Journal of Medical Sciences,* 3(4),121-128.

Garba, S. & Okeniyi, S.O. (2012). Antimicrobial activities of total alkaloids extracted from some Nigerian medicinal plants. *Journal of Microbiology and Antimicrobials*, 4, 60-63.

Gasparyan, A. Y., Ayvazyan, L., Mikhailidis D. P. & Kitas, G. D. (2011). Mean platelet volume: a link between thrombosis and inflammation. *Current Pharmaceutical Design*, 17: 47-58.

Gemayel, R. (2017). Malaria, metabolism and mathematical models. *The Federation of European Biochemical Societies Journal*, 284: 2553-2555.

Gemperline, P. (2006). Practical Guide to Chemometrics, CRC, Boca Raton, Florida, pp1.

Gerothanassis, I. P., 1 Troganis, A., Exarchou, V. & Barbarossou, K. I. (2002). Nuclear magnetic resonance (NMR) spectroscopy: Basic principles and phenomena, and their applications to chemistry, biology and medicine. *Chemistry Education:Research and Practice in Europe*, 3(2), 229-252.

Gething, P. W., Elyazar, I. R. F., Moyes, C. L., Smith, D. L., Battle, K. E., Guerra, C. A. Patil, A. P., Tatem, A. J., Patil, Howe, R. E., Myers, R. E., George, D. B., Horby, P., Wertheim, H. F., Price, R. N., Mueller, I., Baird, J. K. & Hay, S. I. (2012) A long neglected world malaria map: *Plasmodium vivax* endemicity in 2010. *PLoS Neglated Tropical Diseases*, 6(9):e1814. doi: 10.1371/journal.pntd.0001814.

Golwala, M. Z. Shah, H., Gupta, N., Seenivas, V. & Puliyel, J. M. (2016). Mean platelet volume (MPV), platelet distribution width (PDW) platelet count and plateleterit (PCT) as predicors of in-hospital paediatric mortality: a case control study. *African Health Sciences*, 16, (2) 356-362.

Gonzales, M. V. & Tolentino, A. G. (2014). Extraction and isolation of the Alkaloids from the *Samanea saman* (Acacia) Bark: Its Antiseptic Potential, *International Journal of Scientific and Technology Research,* 3(1), 119-124.

Goodman, C. D., Mollard, V., Louie, T., Holloway, G. A., Watson, K. G. & Mcfadden, G. I. (2014). Apicoplast acetyl CoA carboxylase of the human parasite is not targeted by cyclohexanedione herbicides. *International Journal for Parasitology*, 44,285-289. http://dx.doi.org/10.1016/j.ijpara.2014.01.007.

Goulart, H. R., Kimura, E. A., Peras, V. J., Couto, A. S., Duarte, F. A. & Katzin, A. M. (2004). Terpenes arrest parasite development and inhibit biosynthesis of lsoprenoids in *Plasmodium falciparum*. *Antimicrobial Agent and Chemotherapy*, 48(70), 2502-2509. Dol;10:1128/AAC.48.7.2502-209.2004.

Green, R. (1932). A report on fifty cases of malaria treated with Atebrin. A new synthetic drug. *Lancet*, 219, 826-829.

Griesbach, R. (2005). Biochemistry and genetics of flower color. Plant Breeding Reveiws 25, 89–114.

Grigg, M. J. & Snounou, G. (2017). *Plasmodium simium*: A Brazilian focus of anthropozoonotic vivax malaria? *The Lancet Global Health*, 5, e961-e962. DOI: 10.1016/S2214109X(17)30343-1.

Guerin, P. J., Olliaro, P., Nosten, F., Druilhe, P., Laxminarayan, R.., Binka, F., Kilama, W. L., Ford, N. & White, N. J. (2002). Malaria: Current status of control, diagnosis, treatment, and a proposed agenda for research and development. *The Lancet Infectious Diseases*, 2, 564– 573.

Gunther, H. (2015). NMR Spectroscopy: Basic Principles, Concepts and Applications in Chemistry, 3rd Edition, UK pp1.

Guttman, P., & Ehrlich, P. (1891). Ueber die wirkung des methylenblau bei malaria. *Berl Klin Wochenschr*, 28, 953–956.

Guzeler, N. & Parlak, Y. (2016). Nuclear magnetic resonance spectroscopy applications in foods. *Current Research in Nutrition and Food Science*, 4, 161-168. Doi: 10.12944/CRNFSJ.

Hamid, S. B. & Omar, I. C. (2003). Cholesterol determination of total cholesterol in serum by cholesterol oxidase and cholesterol esterase co-immobilized on to cellulose acetate membrane. *Pakistan Journal of Biological Sciences*, 6(15), 1340-1344. Doi:10.3923/pjb.2003.1340-1344.

Hammond, A., Galizi, R., Kyrou, K., Simoni, A., Sinisacalchi, C., Katsanos, D., Gribble, M., Baker, D., Marlos, E., Russell, S., Burt, A., Windbichler, N., Crisanti, A. & Nolan, A. (2016). A CRISPR – cas 9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nature Biotechnology*, 34, 78–83. Doi:10.1038/nbt.3439.

Harborne, B. (1998) Phytochemical methods: a g guide to modern techniques of plant analysis, 5th Edition, Chapman and Hall Ltd, London, 21-72.

Harborne, J. B. (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, UK,1973

Harinantenaina, L., Bowman, J. D., Brodie, P. J., Slebodnick, C. Callmander, M. W., Randrianaivo, R., Rasamison, V. E., Gorka, A., Roepe, P. D., Cassera, M. B. & Kingston, D. G., (2013). Antiproliferative and antiplasmodial dimeric phloroglucinol from *Mallotus oppostifolius* from Madagascar dry forest. *Journal of Natural Product*, 76, 388-393.

Haruna, A., Mann, A. & Ogboidoyi E. O (2017). Phytpchemical composition and antitrypanosonal activity of the leaf extract of *Leptatenia hastate* (pers) Decne. *Bayero Journal of Pure Applied Science,* 10, 292-299. Doi:org/10.4314/bajopas. VI0i2.47.

Hassan, S. W., verma, S., Srivastava, S. K. & Dwarm, S. (2013). Activity guided isolation and characterization of antiplasmodial agent of some local medicinal plants. *Nigeria Journal of Basic and Applied Science,* 21(3), 177. Doi:10.4314/njbas.v2li3.2.

Hatzakis, E. (2019). Nuclear magnetic resonance (NMR) spectroscopy in food science: a comprehensive review. *Comprehensive Reviews in Food Science and Food safety,* 18, 189-220. doi: 10.1111/1541-4337.12408.

Hauser, R., Elreedy, S., Hoppin, J. A., & Christiani, D. C. (1995). Upper airway response in workers exposed to fuel oil ash: Nasal lavage analysis. *Occupational and Environmental Medicine*, 52, 353-358.

Haynes, R. K., Cheu, K. W., N'Da, D., Coghi, P. & Monti, D. (2013). Considerations on the mechanism of action of artemisinin antimalarials: part 1—the 'carbon radical' and 'heme' hypotheses. *Infectious Disorders—Drug Targets*, 13, 217– 277.

Heather, L. C., Wang, X., West, J. A. & Griffin, J. L. (2013). A practical guide to metabolomic profiling as a discovery tool for human heart disease. *Journal of Molecular and Cellular Cardiology*. 55, 2–11. 40. Doi:10.1016/j.ymcc.2012.12.001.

Hedrick, P. W. (2012). Resistance to malaria in humans: the impact of strong, recent selection. *Malaria Journal*, 11:349.

Hermansyah, B., Titro, L. E., Sardgono, I. W., Endhati, A., T., Aritin, S., Budiath, N., Candi Kusuma, D., Substyonigish, E. & Berewtiha, N., (2017), Clinical features and servere malaria: protective effect of mixed Plasmodial malaria. *Asia Pacific Journal of Tropical Biomedicine*, 7(1), 4-9. doi.org/10.1016/j.apjtb.2016.11.001.

Ho, C. S., Lam, C. W. K., Chan, M. H. M., Cheung, R. C. K., Law, L. K., Lit, L. C. W., Ng, K. F., Suen, M. W. M. & Tai, H. L. (2003). Electrospray Ionisation Mass Spectrometry: Principles and Clinical Applications. *Clinical Biochemistry Review*, 24, 3-12.

Howe, R., Kelly, M., Jimah, J., Hodge, D. & Odom, A. R. (2013). Isoprenoid biosynthesis inhibition disrupts Rab5 localization and food vacuolar integrity in *Plasmodium falciparum*. *Eukaryot Cell*, 12, 215–223. Doi:10.1128/EC.00073-12.

Howes, R. E., Patil, A.P., Piel, F.B., Nyangiri, O.A., Kabaria, C.W., Gething, P.W., Zimmerman, P. A., Barnadas, C., Beall, C. M., Gebremedhin, A., Menard, D., Williams, T. N., Weatherall, D. J. & Hay, S. I. (2011). The global distribution of the Duffy blood group. *Nature Communication*, 2:266. doi: 10.1038/ncomms1265.

Huang, Z., Wang, B.& Eaves, D. H. (2007). Phenolic compound profile of selected vegetables frequently consumed by Africans Americans in the Southeast United States. *Food Chemistry*, 103, 1395–1402.

Hudson, A. T., Randall, A. W. (1991). Naphthoquinone derivatives. US5053432A (United States Patent).

Humeida, H., Pradel, G., Stich, A. & Krawinkel, M. B. (2011). The effect of glucose and insulin on in vitro proliferation of *Plasmodium falciparum*. *Journal of Diabetology*, 3,6.

Hummel, C.S., Lu, C., Liu, J., Ghezzi, C., Hirayama, B. A., Loo, D. D., Kepe, V., Barrio, J. R. & Wright, E. M. (2012). Structural selectivity of human SGLT inhibitors. *American Journal Physiology*, 302, 373–382. doi: 10.1152/ajpcell.00328.2011.

Hussani, S. P. & Harris, C. C. (2007). Inflammation and cancer an ancient link with novel potentials. *International Journal of Cancer*, 12(11), 237-238.

Hussein, R. A. & El-Anssary, A. A. (2018). Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. *Intechopen*, pp11-30. Doi:10.5772/intechopen.76139.

Hutchinson, J. Dalziel, J. M. &Keay, R. W. J. (1954). Flora of West Tropical Africa (FWTA), 2nd edition, vol(1) pp132.

Ibukunoluwa, M. R., Olusi, T. A. & Dada, E. O. (2015). Assessment of chemical compositions of three antimalaria plant from Akure, Southwestern Nigeria: A preliminary study. *African Journal of Plant Science*, 9(8), 313-319: doi:10.5897/AJPS2015.1297.

Ikekpeazu, E. J., Neboh, E. E., Aguchime, N. C., Maduka, I. C. & Anyanwu, E. G. (2010) Malaria Parasitemia Malaria: Effect on serum sodium and potassium levels, *Biology and Medicine*, 2(2), 20-25.

Imaga, N. A (2013). Phytomedicine and Nutraceuticals: Alternative therapeutic for Sickle cell anemia, *The Scientific-World Journal*, 2013, 1-12 doi:org/10.1155/2013 269659.

Inusa, A., Sanusi, S. B, Linatoc, A.C., Mainassara, M. M. & Ibrahim, M. A. (2018). Phytochemical and antibacterial screening of mistletoe (*Agelanthus dodoniefolius*) (DC) collected from shea butter three (*Vitelleria paradoxa*), *Science World Journal*, 13, 33-36.

Isaac J. U. & Fasihuddin A. B. (2018). Antioxidant and biological activities of *Leptadenia hastata* ethylacetate root extracts, *Medicinal and Analytical Chemistry International Journal*, 2, 0013.

Isoda, H., Motojima, H., Onaga, S., Samet, I., Villareal, M. O. & Han, J. (2014). Analysis of the erythroid differentiation effect of flavonoid apigenin on K562 human chronic leukemia cells. *Chemico-Biological Interactions*, 220, 269–277. doi:10.1016/j.cbi.2014.07.006.

Ivanescu, B. Micon, A. & Corciova, A. (2015). Sesquiterpene lactones from Artemisia genus: biological activities and methods of analysis. *Journal of Analytical Method in Chemistry*, 2015, 1-21. doi:10:1155/2015/247685.

Iwashina, T. (2013). Flavonoid properties of five families newly incorporated into the order Caryophyllales (Review). *Bulletin of National Museum of Nature and Science*, 39, 25–51.

Jackson, K. E., Klonis N., Ferguson, D. J. P., Adisa, A., Dogovski, C., & Tilley, L. (2004). Food vacuole-associated lipid bodies and heterogeneous lipid environments in the malaria parasite. *Plasmodium falciparum*. *Molecular Microbiology*, 54,109–122.

Jacobs, D. S., Kasten, B. L., DeMott, W. R. & Wolfson, W. L. (1990). Laboratory Test Handbook. 2nd Edn., Lexi-Comp Inc., Hudson, Cleveland, pp 219.

Jacquerioz, F. A. & Croft, A. M. (2009). Drugs for preventing malaria in travelers, *Cochrane Datebase System Revised*, 4, 6491.

Jaesche, H., Gores, G. J., cederbaum, A. I., Hinson, J. A., Passayre, D. & Lemasters, J. J. (2002). Mechanism of hepatoxicity. *Toxicology Sciences*, 65, 166-176.

Jigam, A. A., Abdulrazaq, U. T., Mahmud, H. A. & Tijani, F. O. (2012). Efficacy of Thoninga sanguine Vahl (Balanophoreceae) root extract against *Plasmodium berghei*, *Plasmodium chabaudi*, inflammation and Noioception in mice. *Journal of Applied Pharmaceutical Science*, 1, 47-51.

Johnson, D.W. (2000). A rapid screening procedure for the diagnosis of peroxisomal disorders: quantification of very long-chain fatty acids, as dimethylaminoethyl esters, in plasma and blood spots, by electrospray tandem mass spectrometry. *Journal of Inherited Metabolic Diseases*, 23, 475-486.

Juan, De A. & Tauler, R. (2003). Chemometrics applied to unravel multicomponent processes and mixtures: revisiting latest trends in multivariate resolution, *Analytica Chimica.Acta* 500, 195–210.

Kalra, B. S., Chawla, S., Gupta, P. & Valecha, N. (2006). Screening of antimalarial drug: An overview. *Indian Journal of Pharmacology*, 38, 5-12.

Karou, S. D., Tchacondo, T., Tchibozo, M. A. D., Anani, K., Ouattara, L., Simpore, J. & de Sousa, C. (2012). Screening of Togolese medicinal plants for new pharmacogical properties. *Pharmacognosy Research*, 4, 116-122.

Katalinic, V., Milos, M., Kulisic, T. & Jukic, M. (2004). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry*. 94, 550-557

Katerova, Z., Tdorova, D., Tasheva, K. & Sergiev, I. (2012). Influence of ultra violet radiation on secondary metabolites production. *Genetic and Plant Physiology*, 2,113-114.

Kaur, K. Jain, M., Kaur, T. & Jain, R. (2009). Antimalarial from nature. *Bioogganic and Medicinal Chemistry*, 17, 322-3356.

Kaur, R., Matta, T. & Kaur, H. (2017). Plant derived alkaloids. *Saudi Journal of Life Sciences*, 2, 158-189. doi:10.2127/haya.

Kaushik, N. K., Bagavan, A., Rahuman, A. A., Zahir, A. A., KAmaraj, C., Elango, G., Jayaseelan, C., Kirth, A.V., Sanyhoshkumar, L., Marimuthus S., Rajakumar, G., Tiwari, S. K. & Sahal, D. (2015). Evaluation of antiplasmodial activity of medicinal plants from Indian Buchpora and south Indian eastern Ghats. *Malaria Journal*, 10:1186/s12936-015-0564-z.

Kayemba, J. S., Taba, K. M., Ntumba, K. & Kazadi, T. K. (2016). *In vitro* antiplasmodial activity of 11 terpenes isolated from *Ocibumgratissium and cassicalata leaves*, screening of their binding affinity with haemin, *Journal of Plant Science*, 1,168-172. Doi:10.5539/jps.vIn2p.

Khan, A. M., Qureshi, R. A., Ullah, F., Gilani, A., Nosheen, A., Sahreen, S., Laghari, M. K., Ur-Rehman, S., Hussain, I. & Murad, W. (2011). Phytochemical analysis of selected medicinal plants of Margalla hills and surroundings. *Journal of Medicinal Plant Research*, 5(25), 6017-6023.

Khasanah, U., Waruyanti, A. W., Hafid, A. F. &Tanjung, M. (2017). Antiplasmodial activities of isolated polyphenol from *Alectryon serratus* leaves against 3D:7 *Plasmodium falciparum*. *PharmacognosyResearch*, 7, s57-s60.

Kiely, A. McClenaghan, N. H., Flutt, P. R. & Newsholme, P. (2007). Proinflammatory cytokines increase glucose, alanine and triacylglycerol utilization but inhibit insulin secretion in a clonal pancreatic β-cell line, *Journal of Endocrinology*, 195,113-123, doi:10.1677/JOE-07-0306.

Kim. E. E. & Wyckof, H. W. (1991). Reaction mechanism of alkaline phosphates based on crystal structures: Two metals in catalysis, *Journal of Molecular Biology*, 218, 449-464.

Klein, E. Y. (2013). Antimalarial drug resistance: a review of biology and strategies to delay emergence and spread. *International Journal of Antimicrobial Agent*, 41(4),311-317. Doi:10.1016/j.ijantimicag.2012.12.007

Kokate, C. K., Puhohit, A. P. & Gokhat, S. B. (2002). Pharmacognosy, 20th edition, Nirali publication, India, pp13-14.

Konca, C., Tekin, M., Colak, P., Uckardes, F. & Turgut, M. (2014). An overview of platelet indices for evaluating platelet function in children with scorpion envenomation. *EXCLI Journal*, 13: 801-808.

Konkle B. A. (2011). Acquired disorders of platelet function. *Hematology America Society Hematology Education Program*, 2011, 391-396.

Korfmacher, W. (2005). Principles and applications of LC-MS in new drug discovery. *Drug Discovery Today*, 10(20)1357-1367. Doi:10.1016/s1359-6446(05)03620.2.

Kronenberger, T., Linndner, J., Meissner, K. A., Sauer, M. F., Schettert, I. & Wrenger, C. (2014). Vitamin B6-dependent enzymes in human malaria parasite *Plasmodium falciparum*: A druggable target. *Biomedical Research International*, 11,108516. Doi:10.1155/2014/108516.

Kumar, H., Kumar, A., Kumari, P. & Tulsani, N. B. (2000). A test strip for estimation of urea in serum. *Indian Journal of Clinical Biochemistry*, 2, 124-127.

Kumar, S. & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *Scientific World Journal*, 2013, 1-16. doi.org/10.1155/2013/162750.

Kumar, S. (2014). Alkaloidal drugs: A review. *Asian Journal of Pharmaceutical Sciences and Technology*, 4, 107-119.

Kumar, S., Guha, M., Choubey, V., Maity, P. & Bandyopadhyay, U. (2007). Antimalarial drug inhibiting hemozoin (B-hematin) formation: A mechanistic update. *Life Sciences*, 80, 813-828.

Kumari, S. C., Yasmi, N., Hussain, M. R. & Babuselvam, M. (2015). *In vitro* anti-inflammatory and anti-arthritic property of *Rhizopora mucronata* leaves. *International Journal of Pharma Sciences and Research*, 6, 482-486.

Kwena, A., Wakhisi, J. & Mambo, F. (2012). Possible biochemical marker in *Plasmodium falciparum* malaria infected children wuth or without malnutrition at Webuye and Eldoret, Western Kenya. *Advances in Bioresearch*, 3 (2),49-54.

Laing, A. B. (1965). Treatment of acute *falciparum* malaria with sulphorthodimethoxine (fanasil). *British Medical Journal*, 1, 905-907.

Lambros, C., & Vanderberg, J. P., (1979). Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *Journal of Parasitology* 65, 418–420.

Lavine, B. & Workman, J. (2010). Chemometrics, *Analytical Chemistry*.82, 4699–4711.

Lawal, A., Umar, R. A., Abubakar, M. G., Faruk, U. Z. &Wali, U. (2012). FTIR and UV-Visible spectrophotometeric analyses of artemisinin and its derivatives. *Journal of Pharmaceutical and Biomedical Sciences*, 24, 6–14.

Leal-Santos, F. A., Silva, S. B., Crepaldi, N. P., Nery, A. F., Martin, T. O. & Alves-Junior, E. E. (2013). Altered platelet indices as potential markers of severe and complicated malaria caused by *Plasmodium vivax*: a cross sectional descriptive study, *Malaria Journal*, 12, 462.

Lee, D. C. & Webb, M. L. (2003). Pharmaceutical analysis. Canada: Blackwell Publishing Ltd.

Leffler, M. E., Band, G., Busby, G. B., Kirinen, K, Le, Q. S., Clarke, G. M., Bojang, K. A., Conway, D. J. (2017). Resistance to malaria through structural variation of red blood cells invasion receptors, *Science*, 356, 1122 – 1135. Doi:10.1126/Science. Amm6393.

Lei, Y., Chen, J., Zhang, W., Fu, W., Wei, H., Wang, Q. & Ruan, J. (2012). *In vivo* investigation on the potential of galangin, kaempferol and myricetin for protection of D-galactose-induced cognitive impairment, *Food Chemistry*, 135, 2702–2707. doi: 10.1016/j.foodchem.2012.07.043.

Lelliott, P. M., Brendan J McMorran, B. J., Foote, S. J. & Burgio, G. (2015). The influence of host genetics on erythrocytes and malaria infection: is there therapeutic potential? *Malaria Journal*, *14:289.* DOI 10.1186/s12936-015-0809-x.

Levine, A. B., Punihaole, D. & Levine, T. B. (2012). Characterization of the role of nitric oxide and its clinical applications. *Cardiology*, 122, 55-68. Doi: 10.1159/000338150.

Levitt, M. (2005). Spin dynamics: Basic nuclear magnetic resonance, 2nd edition, Chichester: John Wiley & Sons, pp230.

Li, Q., Weina, P. J. & Milhous, W. K. (2007). Pharmacokinetic and pharmacodynamic profiles of rapid-acting artemisinins in the antimalarial therapy. *Current Drug Therapy*, 2, 210–223.

Li, X., Marinkovic, M., Russo, C., McKnight, C. J., Coetzer, T. L. & Chishti, A. H. (2012). Identification of a specific region of *Plasmodium falciparum* EBL-1 that binds to host receptor glycophorin B and inhibits merozoite invasion in human red blood cells. *Molecular and Biochemical Parasitology*, 183, 23-31. doi: 10.1016/j.molbiopara.2012.01.002.

Li, Y., Wang, X., Zhao, H., Lv, L., Zhou, G., Chai, Y. & Zhang, G. (2013). High-Performance Liquid Chromatography–Electrospray Ionization Time-of-Flight Mass Spectrometry Analysis of Radix Saposhnikoviae for Metabolomic Research. *Journal of Chromatographic Science*, 51:99–106. doi:10.1093/chromsci/bms111.

Lim, C., Hansen, E., DeSimone, T. M., Moreno, Y., Junker, K., Bei, A., Brugnara, C., Buckee, C. O. &Duraisingh, M. T. (2013). Expansion of host cellular niche can drive adaptation of a zoonotic malaria parasite to humans. *Nature Communications*, 4, 1638. doi: 10.1038/ncomms2612.

Lisewski, A. M., Quiros, J. P. Adikesavan, A. K.; Miura, K.; Putluri, N., Eastman, R.T., Scanfeld, D., Regenbogen, S.J., Altenhofen, L., Llinas, M., Sreekumar, A., Long, C., Fidock, D. A. & Lichtarge, O. (2014). Supergenomic network compression and the discovery of EXP1 as a glutathione transferase inhibited by artesunate. *Cell*, 158, 916-928. doi: 10.1016/j.cell.2014.07.011.

Liu, C., Fan, H., Yazhno, L. & Xiao, X. (2016). Research advances on hepatotoxicity of herbal medicine in China. *Biomedical Research International*, 2016 (7150391), 1–14, dio:org/10.1155/2016/7150391

Liu, L. J. & Liu, J. N. (2016). A strategy for quality control of *Menispermum dauricum* DC based on cytotoxic activity and HPLC fingerprint analysis. *Indian Journal of Pharmaceutical Sciences*, 78 (1), 143–150.

Loeb, F. (1946). Activity of a new antimalarial agent, chloroquine (SN 7618). *Journal of the American Medical Association* (JAMA), 130, 1069-1070.

Long, Y., Zhang, W., Wang, F. & Chen, Z. (2014). Simultaneous determination of three curcuminoids in *Curcuma longa* L. by high performance liquid chromatography coupled with electrochemical detection. *Journal of Pharmaceutical Analysis*, 4, 325–330. doi: 10.1016/j.jpha.2013.10.002

Lorke D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 5, 275-287.

Loup, C., Lelievre, J., Benoit-vical, F. & Meunier, B. (2007). Trioxaquines and heme-artemisinin adducts inhibit the *in vitro* formation of hemozoin better than chlorquine. *Antimicrobial Agents and Chemotherapy*, 51, 3768-3770. Doi:10.1128/AAC.00239-07.

Lu, G., Nagbanshi, M., Goldau, N., Jorge, M. M., Meissner, P., Jahn, A., Mockkenhaupt, F. P. & Muller, O. (2018). Efficacy and safety of methylene blue in the treatment of malaria: a systematic review. *BioMed Central (BMC) Medicine*, 16 (59), 1-16.

Lu, J., Lin, J., Chen, X., Huang, M. & Wang, Y. (2012). Alkaloids isolated from natural herbs as anticancer agents. *Evidence Based Complementary and Alternative Medicine*, 2,1. doi: 10.1155/2012/485042.

Lumb, V., Das, M. K., Singh, N., Dev, V., Khan, W. & Sharma, Y. D. (2011). Multiple origins of *Plasmodium falciparum* dihydropteroate synthetase mutant alleles associated with sulfadoxine resistance in India. *Antimicrobial Agents Chemotherapy*, 55 (6), 2813-2817.

Mabry, T. J., Markham, K.R. & Thomas, M. B. (1970). The Systematic Identification of Flavonoids. Springer Verlag., New York. 2204pp.

Mailafiya, M. M, Pateh, U. U., Yusuf, A., Mohammed, G. T.& Sanusi, A. (2017). Isolation of B-sitesterol from the methanol leaf extract of leptedenia hastate (press) Decne (Asclepideceae). *Nigeria Journal of Pharmecuetical and Biomedical Research*, 2, 132-138.

Maltha, J., Gillet, P. & Jacobs, J. (2013). Malaria rapid diagnostic tests in endemic settings. *Clinical Microbiology and Infection*, 19, 399-407. doi: 10.1111/1469-0691.12151.

Mambu, L., Martin, M. T., Razafimahefa, D., Ramanitrahasimbola, D., Rasoanaivo, P. & Frappier, F. (2000). Spectral characterisation and antiplasmodial activity of

bisbenzylisoquinolines from *Isolonaghes quiereina*, *Planta Medicine*, 66, 537-540. Doi:10.1055/s-2000-8610.

Mamoun, C.B., Brigge, S. T. & Vial, H. (2010). Targeting the lipid metabolic pathway for the treatment of malaria. *Drug Development Research*, 7(1), 44-45. doi:10,1002/ddr.20347.

Manach C, Scalbert A, Morand C, Remesy, C. & Jimenez, L. (2004) Polyphenols: food sources and bioavailability. *Amerian Journal of Clinical Nutrition*, 79, 727–747.

Manganelli, G., Masullo, U., Passarelli, S. & Filosa, S. (2013). Glucose-6-phosphate dehydrogenase deficiency: disadvantages and possible benefits, *Cardiovascular and Hematology Disorder Drug Targets*, 13(1), 73-82. doi: 10.2174/1871529x11313010008.

Mărghitas, L. A., Dezmirean, D., Laslo, A. M., Olimpia, P. & Maghear, O. (2007). Validated method for estimation of total flavonoids in romanian propolis, *Bulletin USAMV-CN*, 63, 1-6.

Marliana, E., Hairani, R., Tjahjanaric, T. S. & Tanjung, M. (2018). Antiplasmodial activity of flavonoids from *Macaranga tranarius* leaves. *Earth and Environmental science*, 144, 1-7 do:10. 1088/1755-1315/144/1/01 2011.

Marshall, A. G. & Hendrickson, C. L. (2008). High-resolution mass spectrometers. *Annual Review of Analytical Chemistry*, 1, 579–599. doi:10.1146/annurev.anchem.1.031207.112945.

Martin, G., Akoka, J. S., & Martin, M. L. (2006). SNIF-NMR—Part 1: Principles. In G. A. Webb (Ed.), *Modern magnetic resonance, part 3,* Dordrecht, the Netherlands: Springer. pp. 1629.

Martin, G., Akoka, S., & Maryvonne, L. M. (2006). SNIF-NMR—Part 1: Principles. pp.1651–1658.

Martinelli, A. & Cullenton, R. (2018). Non-human primate malaria parasites: Out of the forest and into the laboratory. *Parasitology*, 145(1):41-54. Doi:10.1017/S0031182016001335.

Martiney, M. A., Cerami, A. & Slater, F. G. (1996). Inhibition of hemozoin formation in *Plasmodium falciparum* Trophozoite extracts by heme analogs: possible implication in the resistance to malaria conferred B- thalassemia trait. *Molecular Medicine*, 2, 236-246.

Matos, J. F., Dusse, L. M. D., Borges, K. B. G., de Castro R. L. Coura-vital, W. & Carvallo, M. G. (2016). A new index to discriminate between iron deficiency anemia and thalassemia trait. *Brazilian Journal of Hematology and Hematherapy*, 38 (3), 214-219. doi.org/10.1016/j.bjhh.2016.05.011.

Mayer, D. C., Cofie, J., Jiang, L., Hartl, D. L., Tracy, E., Kabat, J., Mendoza, L. H. & Miller, L. H. (2009). Glycophorin B is the erythrocyte receptor of *Plasmodium falciparum* erythrocyte-binding ligand, EBL-1. *Proceedings of National Academy of Science of United States America*, 106, 5348-5352. doi: 10.1073/pnas.0900878106.

Mayer, G. D. G. Bruce, M., Kochurova, O., Stewart J. K. & Zhou, Q. (2009). Antimalaria activity of cis-terpenone. *Malaria Journal*, 8, 1-14. doi: 10.1186/1475-2875-8139.

Mbengue, A., Bhattacharjee, S., Pandharkar, T., Liu, H., Estiu, G., Stahelin, R. V. Rizk, S. S., Njimoh, D. L., Ryan., Chotivanich, K., Nguon, C., Ghorbal, M., Lopez-Rubio, J., Pfrender, M., Emrich, S. Mohandas, N., Dondorp, A. M. and Wiest. O. & Haldar, K. (2015). A molecular mechanism of artemisinin resistance in *Plasmodium falciparum* malaria. *Nature*, 520 (7549), 683-687.

Mbouna, C. D. J., Koulpou, R. M. T., Keumoe, R., Tchokouaha, L. R.Y., Fokou, P. V., Tali, B. M., Sahal, D. & Boyom, F. F. (2018). *Malaria Journal*, 17:142. Doi:10.1186/s12936-018-2298-1.

McKerrow, J. H., Sun, E., Rosenthal, P. J. & Bouvier, J. (1993). The proteases and pathogenicity of parasitic protozoa. *Annual Review of Microbiology*, 47, 821–853.

Mclean, K. J., & Jacobs-Lorena, M. (2016). Genetic control of malaria mosquitoes. *Trends in Parasitology*, 32, 174 – 176. Doi: 10.1016/j.pt.2016.01.002.

Meibalan, E. & Marti, M. (2017). Biology of malaria transmission. Cold Spring Harbor Perspectives in Medicine. **7**:a025452. DOI: 10.1101/cshperspect.a025452.

Meler, A., Erler, H., & Beltz, E. (2018). Targeting channel and transport in protozoan parasite infections, *Frontier in chemistry*, 6, 1-23 doi: 10. 3389/cjem. 2018. 00088.

Messori, L., Piccioli, F., Eitler, B., Bergonzi, M.C., Bilia, A. R. & Vincieri, F. F. (2003). Spectrophotometric and ESI-MS/HPLC studies reveal a commonmechanism for the reaction of various artemisinin analogues with hemin. *Bioorganic and Medicinal Chemistry Letters*, 13, 4055–4057. doi.org/10.1016/j.bmcl.2003.08.032.

Miller, L. H., Baruch, D. I., Marsh, K. & Doumbo, O. K. (2002). The pathogenic basis of malaria. *Nature*, 415, 673-679. Doi: 10.1038/415673a.

Miller, L. H., Ackerman, H. C., Su, X. & Wellems, T. E. (2013). Malaria biology and disease pathogenesis: Insights for new treatments. *Nature Medicine*, 19, 156-167. DOI: 10.1038/nm.3073.

Misra, L. N, Wouatsa, N. A, Vyry, N. A, Kumar, S., Kumar, R. V. & Francois, T. (2013). Antibacterial, cytotoxic activities and chemical composition of fruits of two Cameroonian Zanthoxylum species. *Journal of Ethnophamacologyy*, 148, 47-80.do.org/10/106/j.jep.2013.03.069.

Model List of Essential Medicines. http://www.who.int/medic ines/publicatio ns/essen tialm edici nes/en/. Accessed 24th March, 2018.

Modi, K. & Shah, M. (2017). Phytochemical investigation and pharmacoynostic standardization of *Polycarpaea corymbose* lam. *Pharmaognose Journal*, 9, 895-899. Doi:10.5530/pj.2017.6.140.

Mohan, V. R., Balamuragan, K. & Sakthidevi, G. (2013). Fertility enhancement of *Polycarpaea corymbose* (L.) Lam (carophyllacene) whole plant on male albino rats, *Asian Journal of Pharmaceutical and Clinical Research*, 6(9), 151-155.

Mojarad, T. B. (2005). Effect of *Artemisia annua* extract on serum glucose, *Iranian Biomedicinal Journal*, 9(2)57-62.

Mok, S., Ashley, E. A., Ferreira, P. E., Zhu, L., Lin, Z., Yeo, T., Chotivanich, K., Imwong, M., Dhorda, M., Nguon, C., Li m, P., Amaratunga, C., Suon, S., Hien, T. T., Htut, Y., Onyamboko, M. A., Newton, P. N., Trpura, R., Woodrow, C. J., Miotto, O., Kwiatkowski, D. P., Nosten, F., Day, N. P., preiser, P. R., White, N. J., Dondorp, A. M., Fairhurt, R. M. & Bozdech, Z. (2015). Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. *Science*, 347 (6220), 431-435. Doi: 10.1126/science.1260403.

Moon, R.W., Hall, J., Rangkuti, F., Ho, Y. S., Almond, N., Mitchell, G. H., Pain, A., Holder, A. A. & Blackman, M. J. (2013). Adaptation of the genetically tractable malaria pathogen *Plasmodium knowlesi* to continuous culture in human erythrocytes. *Proceedings of National. Academy of Science of USA*, 110, 531–536. doi: 10.1073/pnas.1216457110.

Motojesi, O., Ogunlaja, A. S. & Amos, O. (2011). Variation in Lipid composition of the seed oil *Parinari polyandra* Benth. *Asian Journal of Applied Sciences*, 4 (2), 195-201.

Mueller, I., Galinski, M., Tsuboi, T., Arevalo-Herrera, M., Collins W. E. & King, C. L. (2013). Natural acquisition of immunity to *Plasmodium vivax*: epidemiological observations and potential targets. *Advanced Parasitology*. 81, 77-131. doi:10.1016/B978-0-12-407826-0.00003-5.

Muhammad, I., Dunbar, D. C., Takamatsu, S., Walker, L. A. & Clark, A. M. (2001). Antimalarial, cytotoxic, and antifungal alkaloids from *Duguetia hadrantha. Journal of Natural. Products*, 64, 559.

Mulabagal, V., & Tsay, H. (2004). Plant Cell Cultures - an alternative and efficient source for the production of biologically important secondary metabolites. *International. Journal of Applied Science and Engineering Technology*, 2, 29-48.

Murray, R. F., Hadiza, N., Wirth, S., Bassett, M. & Kelly, D. (2008). Drug-related hypertoxicity and acute liver failure. *Journal of Pediatric Gastroenterology and Nutrition*, 47, 395-405.

Mushtaque, M. (2015). Reemergence of chloroquine (CQ) analogs as multi-targeting antimalarial agents: a review. *European Journal of Medical Chemistry*, 90, 280-295.

Mustofa, J., Sholikhah, E. N. & Wahyuono, S. (2007). *In vitro* and in *vivo* antiplasmodial activity and cytotoxicity of extracts of *Phyllanthus niruri* L. herbs traditionally used to treat

malaria in Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health*, 38(4), 609- 615.

Naghibi, F., Esmaeili, S., Abdullah, N., Nateghpour, M., Taghvai, M., Kamkar, S. & Mosaddegh, M. (2013). *In vitro* and *in vivo* malarial evaluation of Myrtle extract, a plant traditionally used for treatment of parasitic disorders, Techniques, Plants Metabolomics, Beijing and Springer Science, Beijing, pp25-26

Naghibione, F., Ghafari, S., Esmaeli, S. & Jenettl- Stems K. (2015). Naghibione; A novel sesquiterpenoid with antiplasmodial effect from *Direma hyracanum* koso pol, root of a plant used in Traditional medicine, *Iranian Journal of Pharmaceutical Research*, 14, 961-968.

Naito, H., (2003). Coronary artery disease and disorders of lipid metabolism. In: Kaplan, L., Pesce, A., Kazmierczak, S. (Eds.), Clinical Chemistry, Theory, Analysis, Correlation, forth ed. Elsevier Science, pp. 603–611 (Chapter 33).

Nascimento, G. G. F., Locatelli, J., Freitas, P. C. & Silva, G. L. (2000). Antibacterial activity of plant extract and phytochemical on antibiotic resistance bacteria. *Brazilian Journal of Microbilogy*, 31, 247-256.

Nawabi, P., Lykidis A., Ji, D., & Haldar, K. (2003). Neutral-lipid analysis reveals elevation of acylglycerols and lack of cholesterol esters in *Plasmodium falciparum* infected erythrocytes. *Eukaryotic Cell*, 2,1128–1131. doi: 10.1128/ec.2.5.1128-1131.2003.

Ncokazi, K. K. & Egan, T. J. (2005). A colorimetric high throughput B-hematin inhibition screening assay for use in the search for compounds. *Analytical Biochemistry*, 404, 306-310.

Negbenebor, C., Johnson, J. T., Gbado, E. A. & Ekpo, G. (2017). Anti-Hepatitis B. viral potentials of ethanolic stem bark extract of *Vitex doniana* and *Lophira alata* in Wister rats, 4, 147-153.

Nevin, R. L., & Croft, A. M. (2016). Psychiatric effects of malaria and anti-malarial drugs: historical and modern perspectives. *Malaria Journal*, 15 (332), 1-13.

Newman, D. J. & Cragg, G. M. (2016). Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Products*, 79, 629–661. doi: 10.1021/acs.jnatprod.5b01055.

Ngemenya, M. N., Akam, T. M., Youg, J. N., Tane, P., Fanso-Free, S. N., Berzins, K. & Titanji, V. P. (2006). Antiplasmodial activity of some product from *Turrenthis Africana* (*Meliacae*). *African Journal of Heatlth Sciences*, 13, 33-39.

Ngo, T. V., Scarlett, C. J., Bowyer, M. C., Ngo, D. T. & Vuoug, Q. V. (2017). Impact of different extraction solvent on bioactive compounds and antioxidant capacity from the root of *Salacia chinesis* L. *Journal Food Quality*, 2017,1-8. Doi: 10.1155/2017/9305047.

Ngonda, F., Magombo, Z., Mpeketula, P. & Mwatseteza, J., (2012). Evaluation of Malawian *Vernonia glabra* (Steetz) Vatke leaf and *Seciridaca longepedunculata* (Fresen) root

extracts for antimicrobial activities. *Journal of Applied Pharmaceutical Science*, 2, 026-033.doi: 10.7324/JAPS.2012.21106.

Nkumama, I. N., Meara, W. P. O. & Osier, F. H. O. (2017). Changes in malaria epidemiology in Africa and new challenges for elimination. *Trends in Parasitology*, 33, 128 – 140.

Noedl, H., Se, Y., Schaecher, K., Smith, B. L., Socheat, D., & Fukuda, M. M. (2008). Evidence of artemisinin-resistant malaria in western Cambodia. *New England Journal of Medicine*, 359 (24), 2619-2620.

Nowicka, B. & Kruk, J. (2010). Occurrence, biosynthesis and function of isoprenoid quinones. *Biochmica Biophysica Acta*, 1797, 1587–1605. doi.org/10.1016/j.bbabio.2010.06.007

Ntie-Kang, F., Onguene, P. A., Lifongo, L. L. & Luc, M. M. (2014). The potential of anti-malarial compounds derived from African medicinal plants, part II: a pharmacological evaluation of non- alkaloids and non-terpenoids. *Malaria Journal*, 13, 81. doi.org/10.1186/1475-2875-13-81.

Nurain, I. O., Bewaji C. O., Johnson, J. S., Davenport, R. D. & Zhang, Y. (2017). Potential of three ethanomedicinal plants and antisickling agents. *Molecular Pharmaceutics* 14, 172-182.doi:10.1021/acs.molpharmaccut. 600767.

Nyaba, Z. N., Murambiwa, P., Opoku, A. R., Mukaratirwa, S., Shode, F. O. & Simdane, M. C. (2018). Isolation, characterization and biological evaluation of potent antimalarial drimane sesquiterene from. *Warburgia salutaris* stem bark. *Malaria Journal*, 17, 1-8.

Nyongbela, R. D., Karina, N., Bran, R., Wittlin, S., Mbah, J., Makolo, F., Akam, M., Wirmum, C., Efange, S., & Hostettman, K. (2009). Occurrence of sesquiterpene derivative in *Scleria striatonux* De wild (Cyperaceae), 4, 58. Doi: 10.177/1934578x0900400103.

Nyongbela, R. D., Makolo, F. L., Hoye, T. R. & Efange, S. M. N. (2016). Isolation and characterization of sclerinone C from *Scleria striatinux*. *Natural Product Communications*, 11, 5-6.

O'Neill, P. M., Barton, V. E., & Ward, S. A. (2010). The molecular mechanism of action of artemisinin—the debate continues. *Molecules*, 15 (3), 1705-1721.

Obadoni, B. O., & Ochuko, P. O. (2001). Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria. *Global Journal of Pure Applied Sciences*, 8, 203-208.

Odetoye, T. E., Afolabi, T. J. & Onifade, K. R. (2016). Effects of extraction process parameters on the quality characteristics of *Parinari polyandra* B. seed oil. *Nigeria Journal of Technology Development*, 13, 40-49. Doi:http/dx.dol.org/10.434/ijtd.v13i2.1.

Odetoye, T. E., Onifade, K. R., Abubakar, M. S. & Titiloye, J. O. (2014). Pyrolysis of *Parinari polyandra* Benth fruit shell for bio-oil production. *Biofuel Research Journal*, 3, 85-90.

Ogbonna, O. A., Ogbonna, P. C. & Dike, M. C. (2016). Phytochemical screening, quantitative estimate of bioactive compounds in *Spondias mombia* and *Azadirachta indica*. *Research Journal of Chemical Sciences*, 6(1), 38-40.

Okokon, J. E., Antia, B. S., MohanaKrishnan, D. & Sahal, D. (2017). Antimalarial and antiplasmodial activity of husk extract and ractions of *Zea mays*. *Pharmaceutical Biology*, 55(1), 1394-1400. Doi:1080/13880209.2017.1302966.

Okoli, C., Akah, P. & Ezugworie, U. (2006). Antiinflammatory activity of extracts root bark of *Seciridaca longepedunculata* Fres (Polygalaceae). *African Journal of Traditional complementary and Alternative Medicine*, 2, 54-63 doi:10.4314/ajtcamv3i131139.

Olanlokum, J. O., David, M. O. & Afolayan. A. J. (2017). *In vitro* antiplasmodial activity and prophylactic potential of extract and fractions of *Trema oriental* (Linn) stem bark, *BMC Complementary and Alternative Medicine*, 17:407. Doi: 10.1186/s12906-017-1914-x.

Olayemi, S. O., Arikawe, A. P., Akinyede, A., Oreagba A. I & Awodele, O. (2012). Effect of Malarial treatments on biochemical parameters and plasma pH of mice infected with *Plasmodium berghei*. *International Journal of Pharmacology*, 1-6, doi: 10.3923/ijp.2012.

Onguene, N. A. Ngonken, L. E. M., & Kuyper, T. W. (2011). Growth response of *Pterocerpus soyauxii* and *Lophira alata* seedlings to host soil mycorrhizal in oncula in relation to land use types, *African Journal of Microbiology Research*, 5, 2391-2399.

Onguine-Amoe, P., Ntiekong, F., Lifongo, L., Ndon, J., Sippl, W. & Mbaze, L. (2013). The potential of antimalaria compounds derived from Africa medicinal plants, part: a pharmacological evaluation of alkaloids and terpenoids. *Malaria Journal*. 12,449-470.

Oniszczuck, A. & Hawryl, A. (2011). Sample preparation of plant material, M. W. Hajnos, J. Sherma (Eds.), High Performance Liquid Chromatography in Phytochemical Analysis, CRC Press, Taylor & Francis Group, Boca Raton, pp107-142.

Orafidiya, L.O Akinkunmi, E.6., Oginni F. O. & Oluwamakin, A. (2010). Effectiveness of extracts waterman (Rutaceae) formulated as toothpastes, *Nigeria Journal of National Product and Medicine*, 14, 21-26 doi.org/10.4314/njnpm.v149!1i5.

Orimadegun, A. E. & Orimadegun, B. E. (2014). Serum apolipoprotein-A1 and cholesterol levels in Nigeria Childern with *Plasmodium falciparum* infection. *Medical Principles and Practice*, 24, 318-324. Doi:10.1159/000430812.

Ortega, M. J, Pantoja, J. J., Reyes, C. & Zubia, E. (2017). 5-Alkyl resorcinol derivatives from the Bryozoan *Schizomavella mamillata*: Isolation, synthesis and antioxidant activity, *Marine Drugs*, 15,344-361, doi:10.3390/md15110344.

Osadebe, P. O. & Okoye, F.B.C. (2003). Anti-inflammatory effects of crude methanolic extract and fractions of *Alchornea cordifolia* leaves. *Journal of Ethnopharmacology*, 8, 19-24.

Osei-Bimpong, A., Mclean, R., Bhonda, E. & Lewis, S. M. (2012). The use of white blood cell count and haemoglobin in combination as an effective screen to predict the normality of the full blood count. *International Journal of Laboratory Hematology*, 34:91-97.

Otte, D. A., Borchmann, D. E., Lin, C., Weck, M., & Woerpel, K. A. (2014). 13C NMR spectroscopy for the quantitative determination of compound ratios and polymer end groups. *Organic Letters*, 16, 1566–1569. Doi:10.1021/o1403776k.

Ouedraogo, M., Carreyre, H., Vandebrouck, C., Bescond, J. Raymond, G., Guisson, I., Cognard, C., Becq, F., Potreau, D. Cousson, A., Marrot, J. & Caustard, J. (2007). Structure elucidation of a dilhydropyrannone from *Tapinanthus dodoneifolius*. *Journal of Natural Products*, 70, 2009.doi: 10.1021/np070455*x*ccc

Ouedraogo, M., Ruiz, M., Vardedle, E., Carreyre, H., Cousterd J. M., Potrean, D., Sawaedogo, L. L., Ccgnard, C. Becg, F., Vandebrouck, C. & Bescond, J (2011). From the vasodilator and hypotensive effects of an extract *Agelanthus dodoneifolius* (DC) Danser (Loranthaceae) to the active compound dodoneine 10.1016/j.jep.2010.10.002.

Pachiappan, P., Rajamani, S., Sundaram, P. K., Sundaram, R., Paramasivam, D. & Govindasamy, B. (2017). Isolation, Structural elucidation and antiplasmodial activity of fucosterol compound from brown seaweed, *Sagassum linearfolium* against malarial parasite *Plasmodium falciparum*. *Natural Product Research*, 32, 1316-1319. doi: 10.1080/14786419.2017.1342081.

Pan, W., Xu, X., Shi, N., Tsung, S. W. & Zhong, H. (2018). Antimalarial activity of plants metabolites. *International Journal of Molecular Science*, 19(5), 1382. Doi:10.4490/ijms19051382.

Panche, A. N., Diwan, A. D. & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, 5, 1-15.Doi:10.1017/jns2016.41.

Pang, B., Zhu, Y., Lu, L., Gu, F. & Chen, H. (2016). The applications and features of liquid chromatography-mass spectrometry in the analysis of traditional Chinese medicine. *Evidence-Based Complementary and Alternative Medicine*, 2016(3837270):17. http://dx.doi.org/10.1155/2016/3837270.

Parapini, S., Basilico, N., Pasini, E., Egan, T. J., Olliaro, P., Taramelli, D. & Monti, D. (2000). Standardization of the physiocochemical parameters to assess *in vitro* the B-hematin inhibitory activity of antimalarial drugs. *Experimental Parasitology*, 96, 249-256.

Parikh, S. & Rosenthal, P. J. (2008). Human Genetics and Malaria: Relevance for the Design of Clinical Trials. *The Journal of Infectious Diseases*, 198:1255–1257. DOI: 10.1086/592223.

Patel, K. N., Patel, J. K., Patel, M. P., Raiput, G. C. & Patel, H. A. (2010). Introduction to hyphenated techniques and their applications in pharmacy. *Pharmaceutical Methods*, 1, 2–13. doi: 10.4103/2229-4708.72222

.

Patel, M. (2018). Chromatography principle and applications. *International Journal of Pharmacy and pharmaceutical Research*, 13, 288-293.

Patel, R. K., Patel, V. R & Patel, M. G. (2012). Development and validation of a RP-HPLC method for the simultaneous determination of Embelin, Rottlerin and Ellagic acid in Vidangadi Churna. *Journal of Pharmaceutical Analysis*, 2, 366-371. doi: 10.1016/j.jpha.2012.03.001.

Pati, P., Dhangadamajhi, G., Bal, M. & Ranjit, M. (2018). High proportions of pfhrp2 gene deletion and performance of HRP2-based rapid diagnostic test in *Plasmodium falciparum* field isolates of Odisa. *Malaria Journal*, 17, 1-11. Doi:10.1186/s12936-018-2502-3

Paulo, A., Gomes, E. T., Steele, J.,Warhurst, D. C. & Houghton, P.(2000) Antiplasmodial activity of *Cryptolepis sanguinolenta* alkaloids from leaves and roots, *Journal of Planta Medica*, 66(1), 30-34. DOI:10.1055/s-2000-11106.

Penn-Coutinho, J. Cortopassi, W. A., Oliveira, A. A., Costa, T. C. & Krettli, A. U. (2011). Antimalaria activity of potential inhibitors of *Plasmodium falciparum* Lactate dehydrogenase enzyme selected by docking studies. *PLosOne*, 6, e212337.

Penumarthy, S., Penmetsa, G. S. & Mannem, S. (2013). Assessment of serum level of triglycerides, total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol in periodontitis patients. *Journal of Indian Society of Periodontology*, 17(1), 30-35. Doi: 10.4103/0972-124x.10747.

Piel, F. & Weatherall, D. (2014). The a-thalassemias. *The New England Journal of Medicine*, 371, 1908-1916. doi: 10.1056/NEJMra1404415.

Piel, F. B., Patil, A. P., Howers, R. E., Nyangiri, O. A., Gething, P. W., Dew, M., Temperley, W. H, Williams, T. N., Weatherall, D. J. & Hay, S. I. (2014). Global epidemiology of sickle hemoglobin in neonates: a contemporary geostatistical model based map and population estimates. *Lancet*, 381, 142-152. Dol: 10.1016/SO 140-6736 (12) 61229-X.

Prema D, Pilfold J. L, KrauchiJ, Church J. S, Donkor K. K. & Cinel, B. (2013). Rapid determination of total conjugated linoleic acid content in select Canadian cheeses by **1**H NMR Spectroscopy. *Journal of Agriculture and Food Chemistry*, 61, 9915-9921. Doi: 10.1021/jf402627q.

Priyadarshini, R., Raj, G. M. & Shewade, D. G. (2016). Chromatography – the essence of bioanalysis. *European Journal of Biomedical and Pharmaceutical sciences*, 3, 366-377.

Puppalwar, P.V., Goswami, K. & Dhok, A. (2012). Review on evolution of methods of bilirubin estimation. *Journal of Dental and Medical Sciences*, 1(3), 17-28.

Qinghaosu Antimalaria Coordinating Research Group. (1979). Antimalarial studies on Qinghaosu. *Chinese Medical Journal (English Edition)*, 92 (12), 811-816.

Raba, J. & Mottola, H. A. (1995). Glucose oxidase as an analytical reagent. *Critical Review in Analytical Chemistry*, 25(1), 1-42.

Ramadhani, S., Nondo, O., Mainen, J. M., Erasto, P., Zofou, D., Njovendou, J. A., Ngemenya, M. N., Kidukuli, A. W., Masimba, P. J. & Titanji, P. K. (2015). Evaluation of the cytotoxic activity of the extracts from medicinal plants used for the treatment of malaria in Kagera and Lindi regions, Tanzania. *Journal of Applied Pharmaceutical Science*, 5, 7-12.

Rani, A., Akhtar, S., Nawaz, S. K., Irfan, S., Azam, S. & Arshad, M. (2015). Electrolyte disturbance and the type of malaria infection. *Iran Journal of Public Health*, 44(11), 1492- 1497.

Ranson, H. & Lissenden, N. (2016). Insecticide resistance in Africa Anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. *Trends in Parasitology*, 32, 187-196. doi: 10.1016/j.pt.2015.11.010.

Rasoanaivo, P., Wright, C. W., Willcox, M. L. & Gilbert, B. (2011). Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. *Malaria Journal*, 10, S4. http://dx.doi.org/10.1186/1475-2875-10-S1-S4.

Ravali, R., Phaneendra, M., Bhanu, J. K., Ramya, S. L. & Sushma, K. (2011). Recent trends in analytical techniques for the development of pharmaceutical drugs. *Journal of Bioanalysis Biomedicine,* S11, 1-7. doi: 10.4172/1948-593X.S11-002.

Reitman, S. & Frankel, S. (1957). A colorimeteric method for the determination of serum glutamic oxaloactic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28(1), 56-63. Doi:10.93/ajcp/28.1.56.

Renterward, P., Bergstrom, S., Orikliriza, J., Lindquist, E., Bergstrom, S., Svatin, H. A., Ayoglu, B., Uhlen, M., Wahlgren, M., Normark, J., Riback, U. & Nilsson, P. (2018). Level of human proteins in plasma associated with acute paediatric malaria. *Malaria Journal*, 17, 246. doi.org/10.1186/512936-018-256y.

Robards, K. (2003). Strategies for the determination of bioactive phenols in plants, fruit and vegetables. *Journal of Chromatography A*, 1000, 657–691.

Roche, M., Rondeau, P., Singh, N. R., Tarnus, E. & Bourdon, E. (2008). The antioxidant properties of serum albumin. *Federation of European Biochemical Societies Letters*. 582(13), 1783–1787.

Roestenberg, M., McCall, M. & Hopman, J. (2009). Protection against a malaria challenge by sporozoite inoculation, *New England Journal of Medicine*, 361,468-477.

Rosenthal, P. J., Olson, J. E., Lee, G. K., Palmer, J. T., Klaus, J. L., & Rasnick, D. (1996). Antimalarial effects of vinyl sulfone cysteine proteinase inhibitors. *Antimicrobial Agents and Chemotherapy* 40, 1600–1603.

Roumy, V., Fabre, N., Souard, F., Massou, S., Bourdy, G., Maurel, S., Valentin, A. & Moulis, C. (2006). Isolation and antimalarial activity of alkaloids from *Pseudoxandra cuspidata Planta Medicne*, 72(10), 894-898. DOI:10.1055/s-2006-947184.

Rudrapal, M. & Chetia, D. (2017). Plant flavonoids as potential source of future antimalarial lead. *Systematic Reviews in Pharmacy*, 8 (1), 13-18. Doi:10.5530/srp.2017.1.4

Rudrapal, M., Chetia, D. & Prakash, A. (2013). Synthesis, antimalarial and antibacterial activity evaluation of some new 4-aminoquonoline derivatives. *Medicinal Chemistry Research*, 22(8), 3703-3711. http://dx.doi.org/10.1007/s00044-012-0371-9.

Rusilowicz, M., O'Keefe, S., Wilson, J., & Charlton, A. (2014). Chemometrics applied to NMR analysis. In R. A. Meyers (Ed.). *Encyclopedia of analytical chemistry*. John Wiley & Sons, Ltd, NJ, USA.

Russell, P. B., & Hitchings, G. H. (1951). 2,4-Diaminopyrimidines as antimalarials. III. 5-Aryl derivatives. *Journal of American Chemical Society*, 73, 3763-3770.

Ryan, D., Robards, K., Prenzler, P. D. & Kendall, M. (2011). Recent and potential developments in the analysis of urine: a review. *Analytica Chimica Acta*, 684, 8-20. Doi:10.1016/j.aca.2010.10.035.

Saito, A. Y. Rodigue, A.A., Vega, D. Sussmann, R. A. C., Kimura, E. A. & Katzin, A. M. (2016). Antimalarial activity of the terpene nerolidol. *International Journal of Antimicrobial Agents*, 48(2016), 641-645. Doi:10.1016/j.ijantimicag.2016.08.017.

Sajid, M. & McKerrow, J. H. (2002). Review Cysteine proteases of parasitic organisms. *Molecular & Biochemical Parasitology* 120, 1–21.

Salehein, M. Sultana, N., Hossain, N. & Islam, R. (2016). Pharmacological effects of the phytochemicals of *Anethum sowa* L. root extract. *BMC Complementary and Alternative Medicine*, 16,464. Doi: 10.1186/s12906-016-1438-9.

Sandhar, H. K., Kumar, B., Prasher, S., Tiwari, P., Salhan, M. & Sharma, P. (2011). A review of phytochemistry and pharmacology of flavonoids. *Internationale Pharmaceutica Sciencia*, 1, 25-41.

Sandlin, R. D., Fong, K. Y., Whcht, J. K., Carrell, H. M. & Eyan T. J. (2014). Identification of B-hematin inhibitor in a high throughput screening effort reveal scaffolds with *in vitro* antimalarial activity. *International Journal for Parasitology*, 4, 316-325.

Sanusi J., Jibia A. B., Runka, J. Y., Ladi, S., Abubukar, A. A. & Rabiu, S. Z. (2015). Antimicrobial activity of aqueous and ethanol extract of violet plant (*Securidaca longepedunculata Fres*) on tested pathogenic bacteria. *International Journal of Pharmaceutical Sciences and Research*, 6, 3276-3284. Doi:10.13040/IJPSR.0975-8232.6(8)3276-84.

Schirmer, R. H., Coulibaly, B., Stich, A., Scheiwein, M., Merkle, H., Eubel, J., Becker, K., Becher, H., Muller, O., Zich, T., Schiek, W. & Kouyaté, B. (2003). Methylene blue as an antimalarial agent. *Redox Report*, 8 (5), 272–275.

Semenov, A., Olson, E. J. & Rosenthal, P. J. (1998). Antimalarial synergy of cysteine and aspartic protease inhibitors. *Antimicrobial Agents and Chemotherapy*, 42, 2254–2258.

Sener, B.; Orhan, I. & Satayavivad, A. (2003). Antimalarial activity screening of some alkaloids and the plant extracts from Amaryllidaceae. *Journal of Phytotherapy Research*, 17, 1220. DOI:10.1002/ptr.1346

Sha'a, A., Oguche, S., Watila, I. & Ikpa, T. F. (2011). *In vitro* antimlarial activity of the extracts of *Vernonnia amygdalinna* commonly used in traditional medicine in Nigeria. *Science World Journal*, 6,2.

Shandilya, A., Chacko, S., Jayaram, B. & Ghosh, I. (2013). A plausible mechanism for the antimalarial activity of artemisinin: a computational approach. *Scientific Report*, 3 (2513), 1-7.

Shenai, B. R., Sijwali, P. S., Singh, A., & Rosenthal, P. J. (2000). Characterization of native and recombinant falcipain-2, a principal trophozoite cysteine protease and essential hemoglobinase of *Plasmodium falciparum*. *Journal of Biological Chemistry,* 275, 29000–29010 http:dx.doi.org/10.1016/j.ijpddr.2014.08.002

Shoib, A. B. & Shahid A. M. (2015). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University for Science*, 9,449–454.

Shojaii, *A.*, Motaghinejad*a*, M., Norouzi*a*, *S.* & Motevalian, M. (2015). Evaluation of anti-inflammatory and analgesic activity of the extract and fractions of *Astragalus hamosus* in animal models, *Iranian Journal of Pharmaceutical Research*, 14, 263-269.

Siklos, M., BenAissa, M. & Thatcher, R. J. (2015). Cysteine proteases as therapeutic targets: does selectivity matter/A systematic review of calpain and cathepsin inhibitors. *Acta Pharmaceutica SinicaB*, 5(6), 506–519.

Silva Junior, G. B., Pinto, J. R., Guardao, E. J., Farias, G. M. N. & Daher, E. (2017). Kidney involvement in malaria an update. *Journal of the Sao Paulo Institute of Tropical Medicine*, 59, e53.

Simoes, A. R., Culham, A. & Carine, M. (2015). Resolving the unsolved tribes: a molecular phylogenetic framework for the Merremicae (convolulaceae). *Botanical Journal of Linnean Society*, 179, 374-387.

Sindhu, S. & Manorama, S. (2012). Antimicrobial activity of *Polycarpaea corymbose* lam (Caryophyllaceae) against human pathogens. *Journal of Chemical and Pharmaceutical Research*, 4, 4014-4019.

Singh, A. & Rosenthal, P. J. (2001). Comparison of efficacies of cysteine protease inhibitors against five strains of *Plasmodium falciparum*. *Antimicrobial Agents and Chemotherapy* 45, 949–951.

Singh, G., Ureahekar, A. D., Mahesweod, U. & Samant P., (2015). Malaria infection: Effects on electrolytes parameters. *Asian Academic Research Journal of Multidisciplenary*, 1(32), 211-220.

Siramshetty, V. B., Nickel, J., Omieczynski, C., Gohlke, B., Drwal, M. N. & Preissner, R. (2016). Withdrawn—a resource for withdrawn and discontinued drugs. *Nucleic Acids Research*, 44, D1080–D1086 doi: 10.1093/nar/gkv1192.

Smellie, W. S. A., Forth, J., Smart, S. R. S., Galloway, M. J., Irving, W., Bareford, D., Collinson, P. O., Kerr, K. G., Summerfield, G., Carey, P. J. & Minhas, R. (2007). Best practice in primary care pathology: review 7, *Journal of Clinical Pathology*, 60, 458-465. Doi:10.1136/jcp2006.04994.

Smilkstein, M, Sriwilaijaroen, N, Kelly, J. X, Wilairat, P. & Riscoe, M. (2004). Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. *Antimicrobial Agents and Chemotherapy*, 48, 1803–1806. doi: 10.1128/aac.48.5.1803-1806.2004.

Sobeli, C. & Kayaardi, S. (2014). Et Kalitesini BelirlemedeYeniTeknikler. *Gýda*; 39(4),251-258: (2014).

Soeters, P. B., Wolfe, R. R. & Shenkin, A. (2019). Hypoalbuminemia: Pathogenesis and Clinical Significance. *Journal of Parenteral and Enteral Nutrition*, 43 (2): 181–193. DOI: 10.1002/jpen.1451.

Socololovsky, M., Spaho, N., Ceto, D.G., Doglietto, F. and Fernandez, E. (2008). Reactive lymphoid follicular hyperplasia mimicking a peripheral nerve tumor. *Surgical Neurology*, 70,514-517. Doi:10.1016/j.surneu.2007.05.009.

Sore, H., Sanon, S. & Hilon, A. (2018). Antiplasmodial properties of plants isolated flavonoids and their derivatives. *International Journal of Herbal Medicine*, 6, 43-56.

Spillman, N. J., Allen, R. J., McNamara, C.W., Yeung, B. K., Winzeler, E. A., Diagana, T. T. & Kirki, K. (2013). Sodium ion regulation in the malaria parasite *Plasmodium falciparum* involves the cation ATPase PfATP4 and is a target of the spiroindolene antimalaria. *Cell Host and Microbe*, 13:227-237. Doi:10.1016/j.chom.2012.12.006.

Sriram, D., Rao, V. S., Chandrasekhara, K. V. & Yogeeswari, P. (2004). Progress in the research of artemisinin and its analogues as antimalarials: An update. *Natural Product Research*, 18, 503–527.

Srivastava, I. K., & Vaidya, A. B. (1999). A mechanism for the synergistic antimalarial action of atovaquone and proguanil. *Antimicrobial Agents Chemotherapy*, 43, 1334–1339.

Stalikas, C. D. (2010). Phenolic acids and flavonoids: occurrence and analytical methods, R. M. Uppu (Ed.), Free Radicals and Antioxidant Protocols, Springer Science Business Media LLC, pp65-90.

Stankovic, M. S. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L extracts. *Kragujevac Journal Science*. 33, 63-72.

Stead, P. (1998). Isolation by preparative HPLC, R.J.P. Cannell (Ed.) Methods in Biotechnology, Natural Products Isolation, Humana Press Inc, Totowa, New Jersey, pp165-208.

Steinmann, D. & Ganzera, M. (2011). Recent advances on HPLC/MS in medicinal plant analysis. *Journal of Pharmaceutical and Biomedical Analysis*, 55, 744–757. doi: 10.1016/j.jpba.2010.11.015.

Subhan, N., Aslam, A., Ahmed, F. &Shahid, I. Z. (2008). Antinociceptive and gastroprotective effect of the crude ethanolic extracts of *Excoecariaagallocha*linn, *Turkish Journal of Pharmaceutical Science.* 5, 143-154.

Sudaratana, R. K. & Jerapan, K. (2011). Malaria parasite carbonic anhydrase: inhibition of aromatic heterocycylic sulfonamides and its therapeutic potential. *Asian Pacific Journal of Tropical Biomedicine*, 1, 233-242. Doi:10.1016/S2221-1691(11)60034-8.

Sugiarto, S. R., Moore, B. R., Makani, J. & Davis, T. M. E. (2018). Artemisinin therapy for malaria in hemoglobinopathies: a systematic review. *Clinical Infectious Diseases*, 16(5):799-804. Doi:10.1039/cid/cix785.

Sulaiman, S. R., Jigam, A. A., Abubakar, A., Salau, B. R. and Taylor, D. (2019). Antiplasmodial activity and toxicological profile of alkaloids obtained from *Securidaca longepedunculata*, *Al-Hikmah Journal of Pure & Applied Sciences*, 8, 23-31.

Syafii, W., Sari, R. K., Cahyaningsih, U. & Anisah, L. N. (2016). Antimalarial activity of the fraction from Ethanol extract of *Strchonoslignstrina* Blume. *Research Journal of Medicinal Plant*, 10,403-408. Doi: 10.3923/rjmp. 2016.403.408.

Syros, A. & Dais, P. (2013). Nuclear magnetic resonance spectroscopy in food analysis. Royal Society of Chemistry,Cambridge, UK, pp67-86.

Takagi, A. Takada, K., Sai, K., Ochiai, T., Matsumoto, K., Sekita, K., Momma, J., Aida, Y., Saitoh, M. & Naitoh, K. (1994). Acute, subchronic and chronic toxicity studies of a synthetic antioxidant, 2,2'-methylenebis(4-methyl-6-tert-butylphenol) in rats. *Journal of Toxicological Science*,19, 77-88.

Tambunan, A. P., Bahtiar, A. & Tjandrawinata, R. R. (2017). Influence of extraction parameters on the yield, phytochemical TLC-Densitometeric quantification of quercetin and LC-MS profile and how to standardize different batches for long term from *Ageratum conyoides* L. leaves. *Pharmcognosy Journal*, 9,767-774. Doi:10.5530/pj.2017.6.121.

The Nobel Prize in Physiology or Medicine 2015. https ://www.nobel prize .org/nobel prize s/medic ine/laure ates/2015/. Accessed 24th March, 2018.

Thome, R., Lopes, S. C., Costa, F. T. & Verinaud, L. (2013). Chloroquine: modes of action of an undervalued drug. *Immunology Letters,* 153, 50–57. doi.org/10.1016/j.imlet.2013.07.004.

Thota, S. & Yerra, R. (2016). Drug discovery and development of antimalaria agents: Recent advances. *Current Protein and Peptides Science*, 17, 1.

Tietz, N. W. (1987). Fundamental of clinical chemistry, 3rd edition, W.B Saunders, Philadephia, pp328-329.(protein)

Tietz, N. W., Shuey, D. F. & Astles, J. R. (1987). Turbidimetric measurement of lipase activity-problem and some solutions. *Clinical Chemistry*, 33(9):1624-1629.(sodium)

Tietz. N.W. (1987). Fundamentals of Clinical Chemistry, W.B. Saunders Co. Philadelphia, pp. 874-940.

Tilley, L., Straimer, J., Gnädig, N. F., Ralph, S. A., & Fidock, D. A. (2016). Artemisinin action and resistance in *Plasmodium falciparum*. *Trends in Parasitology*, 32, 682–96.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. & Kaur, H. (2011). Phytochemical screening and extraction: a review. *Internationale Pharmaceutica Sciencia*, 1, 98-106.

Tognarelli, J. M., Dawood, M., Shariff, M. I. F., Grover, V. P., Crossey, M. M. E., Cox, I. J., Taylor-Robinson, S. D. & McPhail, M. J. W. (2015). Magnetic Resonance Spectroscopy: Principles and Techniques: Lessons for Clinicians. *Journal of Clinical and Experimental Hepatology*, 5, 320-328. doi: 10.1016/*j*.jceh.2015.10.006.

Tonhosolo, R., Gabrielm, H. B., Matsumura, M. Y., Cabral, F. J., Yamamoto, M. M., D'Alexandri, F. L., Sussmann, R. A., Belmonte, R., Peres, V. J., Crick, D. C., Wunderlich, G., Kimura, E. A., & Katzin, A. M. (2010). Intraerythrocytic stages of *Plasmodium falciparum* biosynthesize menaquinone. *Federation of European Biochimical Soceties Letters*, 584, 4761–4768. doi.org/10.1016/j.febslet.2010.10.055.

Toora, B. D. & Rajagopal, G. (2002). Measurement of creatinine by Jaffe's reaction determination of concentration of sodium hydroxide required for maximum colour development in standard, urine and free filtrate of serum. *Indian Journal of Experimental Biology*, 40(3), 352-354.

Tor-Anyiin, T. A., Anyan, J. V., Anger, G. & Anyam, J. N. (2015). Preliminary phytochemical screening and antimicrobial activity of dried seed extracts of *Meranthes polyandra*. *Journal Chemical Society of Nigeria*, 40, 22-27.

Trager. W. and Jensen, J. B. (1976). Human malaria parasites in continuous culture. *Science*, 193(4254), 673-675.

Trenholme, C., Williams, R., Desjardins, R., Frischer, H., Carson, P, Rieckmann, K., & Canfield, C. J. (1975). Mefloquine (WR 142,490) in the treatment of human malaria. *Science*, 190 (4216), 792-794.

Tse, E. G., Korsik, M., & Todd, M. H. (2019). The past, present and future of anti-malarial medicines. *Malaria Journal*, 18, 93, 1-21.

Tu, Y. (2011). The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. *Nature Medicine*, 17, 1217-20. doi: 10.1038/nm.2471.

Ufelle, S. A., Onyekwelu, K. O., Ghasi, S., Ezeh, C. O., Ezeh, R. C. & Eson, E. A. (2018). Effects of *Colocasia esculenta* leaf exctracts in anemia and normal wister rats. *Journal of Medical Sciences*, 38(3), 102-106.

Ukwuani, A. N. & Igbobikwu, M. O. (2015). *In vitro* antidiabetic effect of *Leptadenia hastata* leaves fractions. *Biosciences Research in Today's World*, 1, 40-46.

Umaru, I. J., Fasihuddin, A. B. & Umaru, H. A. (2018). Phytochemical, antifungal and anti-bacterial potential of *Leptadenia hastata* stem bark extract, *Medcave Online Journal of Toxicology*, 4, 263- 268. Doi:10.15 406/Moj.2018.04.00110.

US FDA approves *Krintafel* (tafenoquine) for the radical cure of *P. vivax* malaria 2018. https ://www.mmv.org/newsr oom/press -relea ses/us-fdaappro ves-krint afel-tafen oquin e-radic al-cure-p-vivax -malaria. Accessed 25th September, 2018.

Vagdatli, E. Gounari E., Lazaridous E., Katsibourlia, E. T., Kopoulou, F. & Labrianous, I. (2010). Platelet distribution width: a simple, practical and specific marker of coagulation. *Hippokratia*, 14, 28-32.

Vangapandu, S., Meenakshi, J., Kaur, K., Patil, P., Sanjay, R. P. & Rahul, J. (2007). Recent advances in antimalaria drug development. *Medicinal Research Reviews*, 27, 65-107.doi:org/10.1002/med.20062.

Verma, A. K., Singh, H., Satyanarayana, M., Srivastava, S. P., Tiwari, P., Singh, A. B., Dwivedi, A. K., Singh, S. K., Srivastava, M., Nath, C., Raghubir, R., Srivastava, A. K. & Pratap, R. (2012). Flavone-based novel antidiabetic and antidyslipidemic agents. *Journal of Medicinal Chemistry*, 2012;55(10),4551–4567. DOI: 10.1021/jm201107g.

Viera, J. F. & Rivera, G. B. (2017). Lipid levels in patients with uncomplicated malaria due to *Plasmodium falciparum*. *International Journal of Tropical Dieases and Health*, 24(3), 1-7.

Villas-Boas S. G., Roessner, U., Hansen, M. A., Smedsgaard, J. & Nielsen, J. (2007). Metabolomics analysis: an introduction, New Jersey, Wiley, pp77.

Visser, B. J., de Vries, S. G., Vingerling, R., Gritter, M., Kroon, D., Aguilar, L.C., Kraan, R.B., Wieten, R. W., Danion, F., Siouke, B., Adegnika, A. A., Agandii, S.T.,Kremsner, P. G., hanscheid, T., Mens, P. F., van Vugt, M. & Grobusch, M. P. (2017). Serum lipids and lipoproteins during uncomplicated malaria: a cohort study in Lambarene, Gabon.

American Journal of Tropical Medicine and Hygiene, 96 (5), 1205-1214. Doi:10.42269/ajtmh.16-0721.

Visser, B. J., Wieten, R.W., Nagel, I. M. & Grobusch, M. P. (2013). Serum lipids and lipoproteins in malaria a systematic review and meta-analysis. *Malaria Journal*, 12,442.

Wang, J., Zhang, C. J., Chia, W. N., Loh, C. C. Y., Li, Z., Lee, Y. M., He, Y., Yuan, L., Lim, T., Liu, M., Liew, C., Lee, Zhang, J., Lu, N., Lim, C., Hua, Z., Liu, B., Shen, H., Tan, K. S. & Lin, Q. (2015). Haem-activated promiscuous targeting of artemisinin in *Plasmodium falciparum*. *Nature Communications*, 6 (10111), 1-11.

Wang, T., Li, Q. & Bi, K. (2018)[a]. Bioactive flavonoids in medicinal plant: structure, activity, and biological fate. *Asian Journal of Pharmaceutical Science*, 13, 12-23. doi:10.1016/j.ajps.2017.08.004.

Wang, Y., Ge, X., Wang, Y., Xu, M., Gong, F., Tao, M., Wang, W., Shou, L., Chen, K., Wu, M. & Li, W. (2018)[b]. The values of applying classification and counts of white blood cell to prognostic-evaluation of resectable gastric cancers. *BMC Gastroenterology*, 18:99. Doi:10.1186/s1287-018-08120.

Weinberg, J. B., Lopansri, B. K., Mwaikambo, E. & Granger, D. L. (2009). Arginine, nitric oxide, carbon monoxide and endothelial function in severe malaria. *Current Opinion in Infectious Diseases*, 21(5), 468-475. Doi:10.1097/Qeo.0b013e32830ef5ef.

WHO (2001). *In vitro* micro test (Mark III) for assessment of *P. falciparum* to Chloroquine, mefloquine, quinine, amodiaquine, sulfadoxine/ pyrimethamine and artemisinin, CTD/MAL/9720 Rev 2. Geneva, Switzerland.

WHO, Basic Malaria microscopy.Part I. Learner's guide 2nd edition,WHO Graphics, Switzerland, pp9-10.

Williamson, K., & Hatzakis, E. (2017). NMR spectroscopy as a robust tool for the rapid evaluation of the lipid profile of fish oil supplements. *Journal ofVisualized Experiments* 123, e55547. Doi: 10.3791/55547.

Wilson, R. J. (2008). Sertyping *Plasmodium falciparum* malaria with S-antigen. *Nature*, 3(5755), 451-452.

Winter, C. A., Risley, E. A. & Nuss, G. V. (1962). Carrageenin induced oedema in hind paw of rats as an assay for anti –inflammatory drugs. *Processings of Society of Experimental Biology and Medicine*, 3, 544-561.

Wishart, D. S., Tzur, D., Knox, C., Eisner, R. Guo, A. C., Young, N., Cheng, D., Jewell, K., Arndt, D., Sawhney, S., Fung, C., Nikolai, L., Lewis, M., Coutouly, M. A. & Forsythe, I. (2007). HMDB: The Human Metabolome Database. *Nucleic Acids Research*, 35, D521– D526. Doi:10.1093/nar/gkl923.

Withrock, I. C., Anderson, S. J., Jefferson, M. A., McCormack, G. R., Mlynarczyk, G. A., Nakama, A., Lange, J. K., Berg, C. A., Acarya, S., Stock, L. M., Lind, M. S., Luna, K., Kondru, N. C., Manne, S., Patel, B. B., de la Rosa, B. M., Huang, K., Sharma, S., Hu, Y., Kanuri, S. & Carlson, S. A. (2015). Genetic diseases conferring resistance to infectious diseases. *Genes and Diseases*, 2, 247e254. Doi:10.1016/j.gendis.2015.02.008.

Woestijne, A. P., van der Graaf, Y., Liem, A., Cramer, M. J., Westerink, J. and Visseren, F. L. J. (2013). Low high density lipoprotein is not a risk factor for recurrent vascular events in patients with vascular disease on intensive lipid-lowering medication. *Journal of American college of Cardiology*, 62(20),1834-1841. Doi:10.1016/j.jacc.2013.04.101.

Wolfender, J. L., Rodriguez, S. & Hostettmann, K. (1998). Liquid chromatography coupled to mass spectrometry and nuclear magnetic resonance spectroscopy for the screening of plant constituents, *Journal of Chromatography A*, 794, 299–316.

Woodrow, C. J. & White, N. J. (2017). The clinical impact of artemisinin resistance in Southeast Asia and the potential for future spread. *FEMS Microbiology Review*, 41, 34–48.

World Health Organisation (2010). Basic malaria microscopy, part I: Learner's guide, 2nd edition, WHO graphics, Switzerland, pp9-10.

World Health Organisation (2016). Fact sheet on the world malaria report 2015.

World Health Organisation, World Malaria Report. 2015, WHO: Geneva.

World Health Organization (2010). Basic Malaria Microscopy Part1, 2nd edition, Switzerland, pp75.

World Health Organization (2015). World malaria report 2015, pp12.

World Health Organization (2015). World Malaria Report. Geneva: World Health Organization.

World Health Organization (2016). Key Facts: World Malaria Day.

World Health Organization (2017). World Malaria Report 2017; WHO Press: Geneva, Switzerland, 2017.

Wright, A. D., Goclik, E., König, G. M. & Kaminsky, R. (2002). Lepdins D-F: antiplasmodial and antitrypanosomal decahydroquinoline derivatives from the tropical marine tunicate Didemnum sp. *Journal of Medicine Chemistry*, 45,306-372.

Wright, C. W., Marshall, S. J., Russell, P. F., Anderson, M. M., Phillipson, J. D., Kirby, G. C., Warhurst, D. C. & Schiff, P. L. (2000). *In vitro* antiplasmodial, antiamoebic, and cytotoxic activities of some monomeric isoquinoline alkaloids. *Journal of Natural Product*, 63, 1638.

Wu, H., Guo, J., Chen, S., Liu, X., Zhou, Y., Zhang, X. & Xu, X. (2013). Recent developments in qualitative and quantitative analysis of phytochemical constituents and their

metabolites using liquid chromatography-mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 72, 267–291. doi: 10.1016/j.jpba.2012.09.004.

Xi, Y. & Rocke, D. M. (2008). Baseline correction for NMR spectroscopic metabolomics data analysis. *BMC Bioinformatics*, 9,324. Doi:10.1186/1471-2105-9-324.

Xu, J., Zhu, H., Zhang, J., Du, T., Guo, E., Liu, W., Lou, J., Ye, F., Feng, F. & Qu, W. (2018) sesquitesperiods from *Chloranthus anhuniences* with neuroprotective effects in PC12 cells. *Journals of Natural Products*, 81, 1391-1398 doi:10.1012/asc.jnatprod.

Xue, Z., duan, L., & Qi, X. (2015). Gas chromatography Mass spectrometry coupling World Health Organisation (2016). Fact sheet on the world malaria report 2015.

Yam, M. F., Ang, L.F., Ameer, O. Z., Salman, I. M., Abdul Aziz, H. & Asmawi1, M. Z. (2009). Anti-inflammatory and analgesic effects of *Elephantopus tomentosus* ethanolic extract. *Journal Acupuncture Meridian Studies,* 2, 280−287.

Yang, M., Sun, J., Lu, Z., Chen, G., Guan, S., Liu, X. Jiang, B., Ye, M. & Guo, D. A. (2009). Phytochemical analysis of traditional Chinese medicine using liquid chromatography coupled with mass spectrometry. *Journal of Chromatography A*, 1216, 2045-62. doi: 10.1016/j.chroma.2008.08.097.

Ynalvex, B. A., Cardenas, C., Addo, J. L, Adukpo, G. E., Dadson, B. A. & Addo-Mensah, A. (2012). Evaluation of antimicrobial activity of *Zanthoxylum zanthoxyloides* roof bark extracts. *Research Journal of Medicinal Plant*, 6, 149-159, doi:10. 39 23/rjmp. 2012.149.159.

Yuan, H., Ma, Q., Ye, L & Piao, G. (2016). The Traditional Medicine and Modern Medicine from Natural Products. *Molecules*, 21, 559; doi:10.3390/molecules21050559.

Yubin, J. I., Miao, Y., Bing, W., & Yao, Z. (2014). The extraction, separation and purification of alkaloids in natural medicine. *Journal of Chemical and Pharmaceutical Research*, 6, 338-345.

Zani, B., Gathu, M., Donegan, S., Olliaro, P. L. & Sinclair, D. (2014). Dihydroartemisinin-piperaquine for treating uncomplicated *Plasmodium falciparum* malaria. *The Cochrane Database of Systemic Review*, 1, 1–160.doi: 10.1002/14651858.CD010927.

Zelefack, F., Guilet, D., Valentin, A., Soh, R.C., Kom, B., Chevally, S., Ngouala, S.A., Tsamo, E., Fabre, N. & Dijojox-Franca, M. (2012). Antiplasmodial and cytotoxic activities of flavonoids and arybenzofuran derivative from *Morus mesozgia*. *Greener Journal of Biological Science*, 2, 20-24.

Zhang, X., Du, J., Ren, J., Ye, F., Xie, Y., Cheng, X., Yan, S., & Jin, H. (2014). Three new sequiterpene lactones from *Inula britannica*. *Archives of Pharmaceutical Research*, doi:10.1007/s12272-014-0388-3.

Zhang, Y. K., Ge, M. & Plattner, J. J. (2012). Recent Progress in the synthesis of antimalarial agents. *Organic Preparation and Procedures International*, 44, 340–374. doi:10.1080/00304948.2012.697708.

Zhu, H., Zhou, Q, Peng, C., Chen, M, Li, X., Lin, D & Xiong, Y (2017). Pocahemiketals A and B, two, new hemiketal with unprecedented sesquiterpenoid skeleton from *Pogosterman cablin*, *Fitioterapis*, 120, 67-71 `doi:org/10.1016/fitote 2017.05.013.

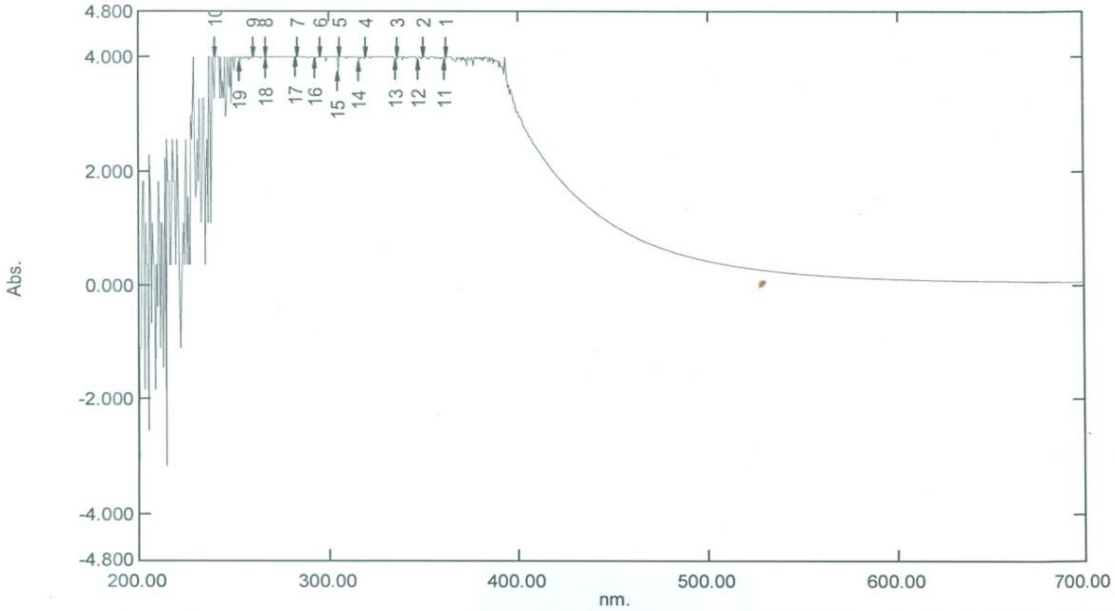
APPENDICES

Appendix A1: UV- visible spectrum of Tk3 fraction of *Polycarpea linearfolia*

Spectrum Peak Pick Report

09/20/2018 01:21:30 PM

Data Set: TK3 - RawData



Measurement Properties	
Wavelength Range (nm.):	200.00 to 700.00
Scan Speed:	Fast
Sampling Interval:	0.5
Auto Sampling Interval:	Enabled
Scan Mode:	Single
Instrument Properties	
Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.0 nm
S/R Exchange:	Normal
Attachment Properties	
Attachment:	None
Sample Preparation Properties	
Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	1% Phenol solution

No.	P/V	Wavelength	Abs.	Description
1	↑	362.00	4.000	
2	↑	350.00	4.000	
3	↑	336.50	4.000	
4	↑	319.50	4.000	
5	↑	305.50	4.000	
6	↑	295.50	4.000	
7	↑	283.00	4.000	
8	↑	267.00	4.000	
9	↑	260.00	4.000	
10	↑	240.00	4.000	
11	↓	361.50	3.947	
12	↓	347.00	3.946	
13	↓	335.00	3.954	
14	↓	316.00	3.930	
15	↓	305.00	3.759	
16	↓	293.00	3.970	
17	↓	282.50	3.998	
18	↓	266.50	3.984	
19	↓	253.00	3.924	

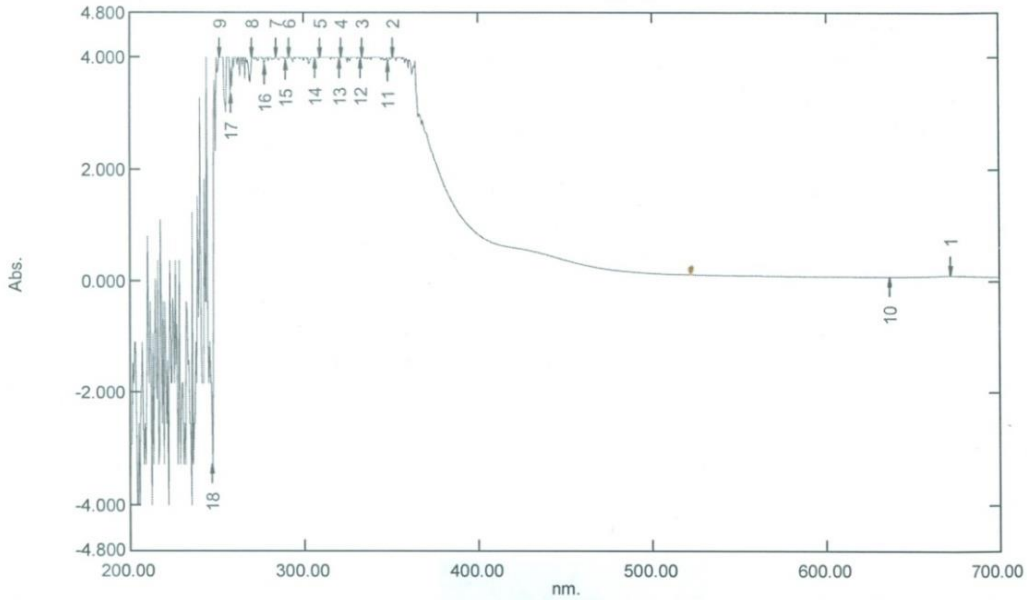
Page 1 / 1

Appendix A2: UV- visible spectrum of Lh3 fraction of *Leptadenia hastata*

Spectrum Peak Pick Report

09/20/2018 01:12:09 PM

Data Set: LH 3 CONC. - RawData

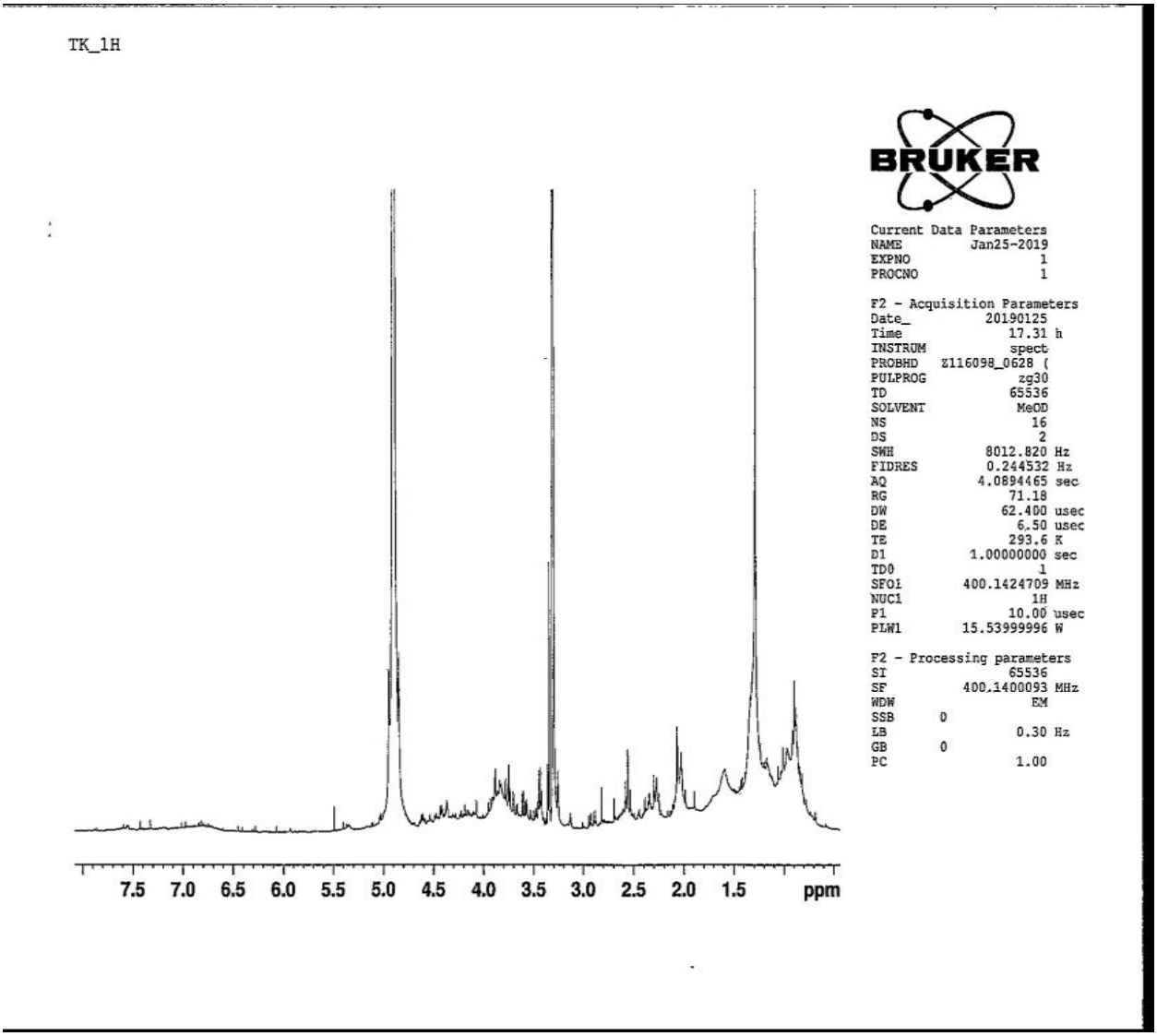


Measurement Properties	
Wavelength Range (nm.):	200.00 to 700.00
Scan Speed:	Fast
Sampling Interval:	0.5
Auto Sampling Interval:	Enabled
Scan Mode:	Single
Instrument Properties	
Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.0 nm
S/R Exchange:	Normal
Attachment Properties	
Attachment:	None
Sample Preparation Properties	
Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

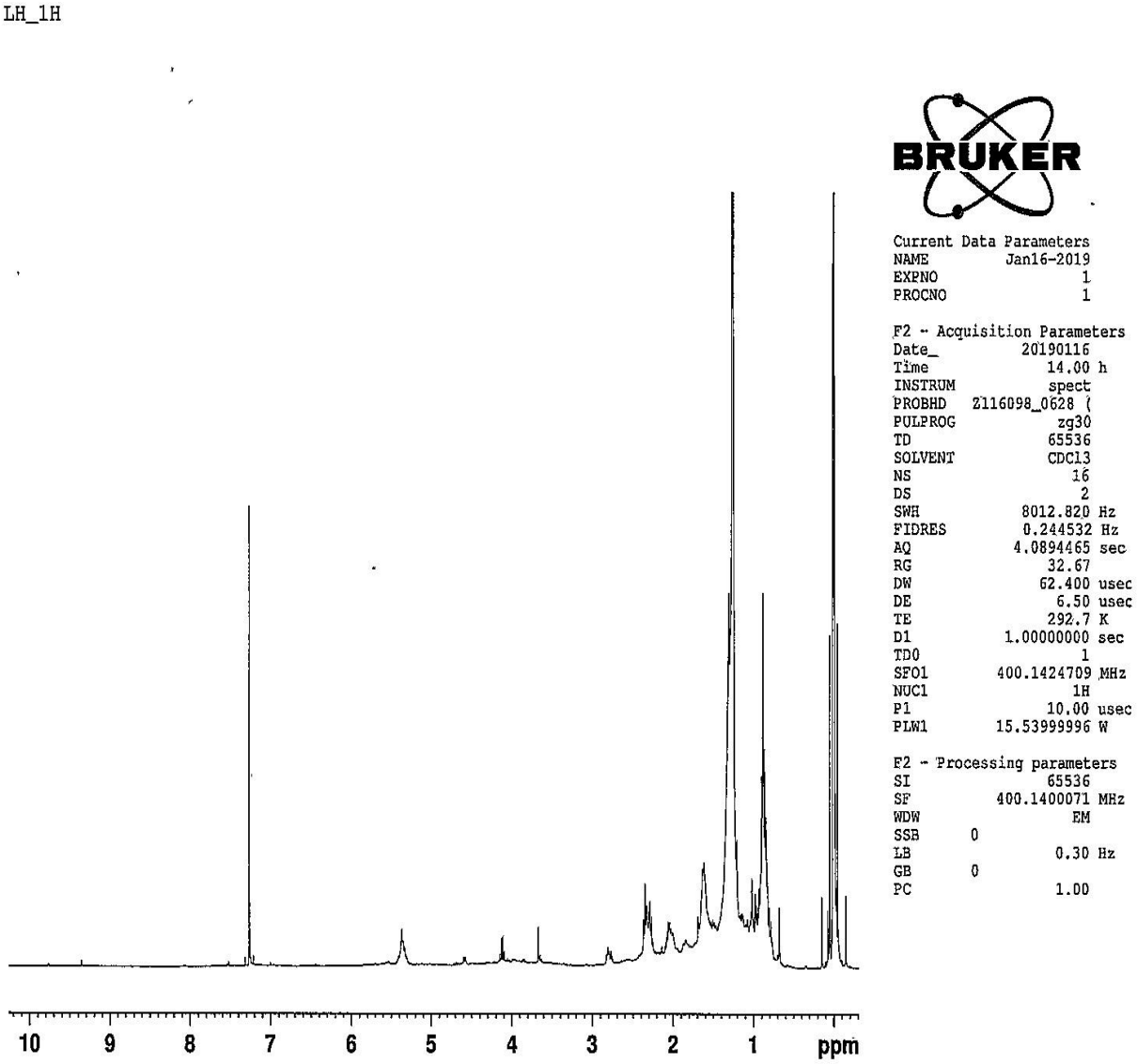
No.	P/V	Wavelength	Abs.	Description
1	↑	671.50	0.101	
2	↑	350.50	4.000	
3	↑	333.00	4.000	
4	↑	321.00	4.000	
5	↑	309.00	4.000	
6	↑	291.50	4.000	
7	↑	284.00	4.000	
8	↑	270.50	4.000	
9	↑	251.50	4.000	
10	↓	636.00	0.080	
11	↓	348.00	3.929	
12	↓	332.50	3.963	
13	↓	320.00	3.975	
14	↓	306.50	3.982	
15	↓	289.50	3.956	
16	↓	277.50	3.873	
17	↓	258.00	3.362	
18	↓	247.50	-3.273	

Page 1 / 1

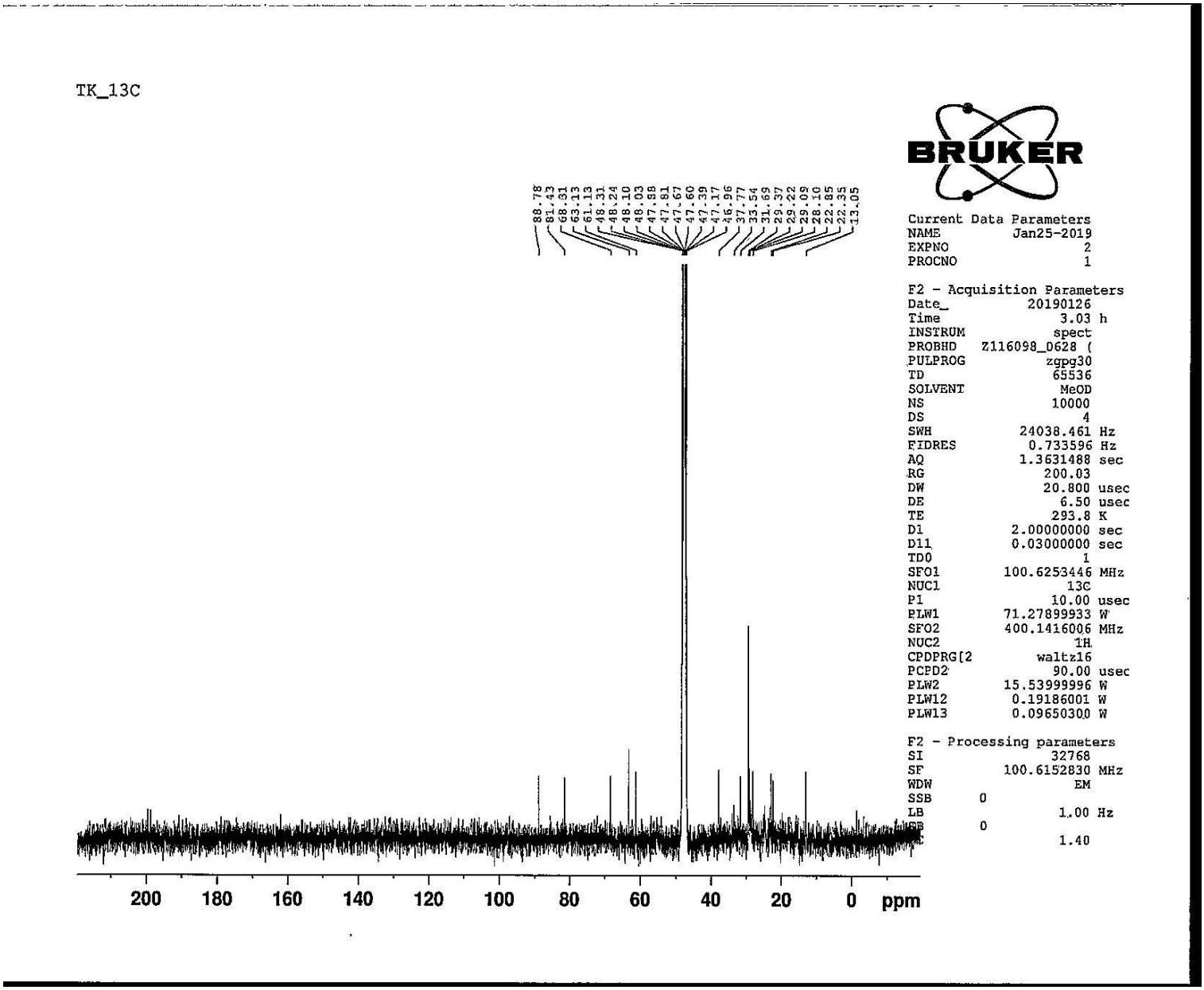
Appendix B1: ^{1}H Nuclear Magnetic Resonance spectrum of TK3 fraction of *Polycarpea linearfolia*



Appendix B2: ^{1}H Nuclear Magnetic Resonance spectrum of Lh3 fraction of *Leptadenia hastata*



Appendix C1: ^{13}C Nuclear Magnetic Resonance spectrum of Tk3 fraction of *Polycarpea linearfolia*



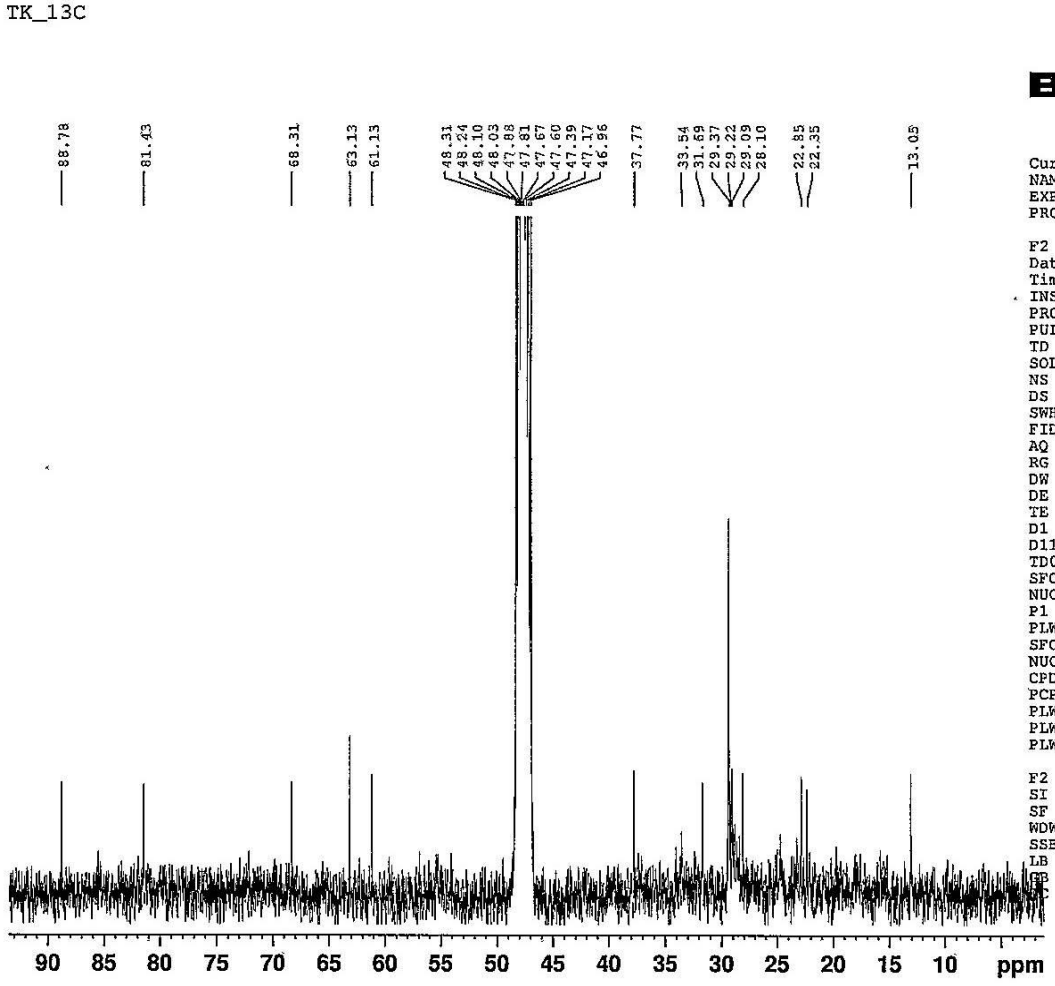
BRUKER

Current Data Parameters
NAME Jan25-2019
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20190126
Time 3.03 h
INSTRUM spect
PROBHD Z116098_0628 (
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 10000
DS 4
SWH 24038.461 Hz
FIDRES 0.733596 Hz
AQ 1.3631488 sec
RG 200.03
DW 20.800 usec
DE 6.50 usec
TE 293.8 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 100.6253446 MHz
NUC1 13C
P1 10.00 usec
PLW1 71.27899933 W
SFO2 400.1416006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 15.53999996 W
PLW12 0.19186001 W
PLW13 0.09650300 W

F2 - Processing parameters
SI 32768
SF 100.6152830 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
1.40

Appendix C2: ^{13}C Nuclear Magnetic Resonance spectrum of Tk3 fraction of *Polycarpea linearfolia* expanded



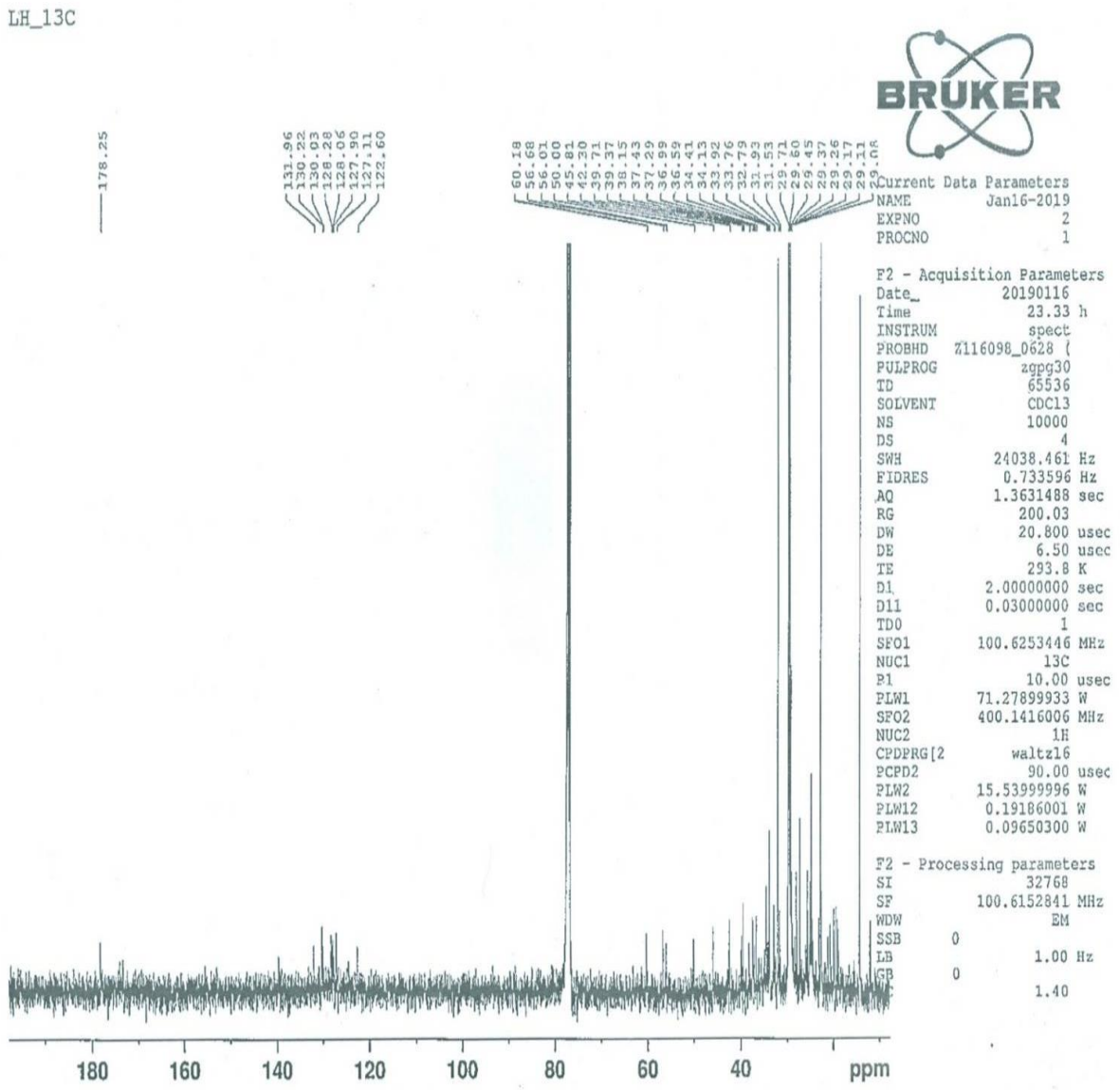
BRUKER

Current Data Parameters
NAME Jan25-2019
EXPNO 2
PROCNO 1

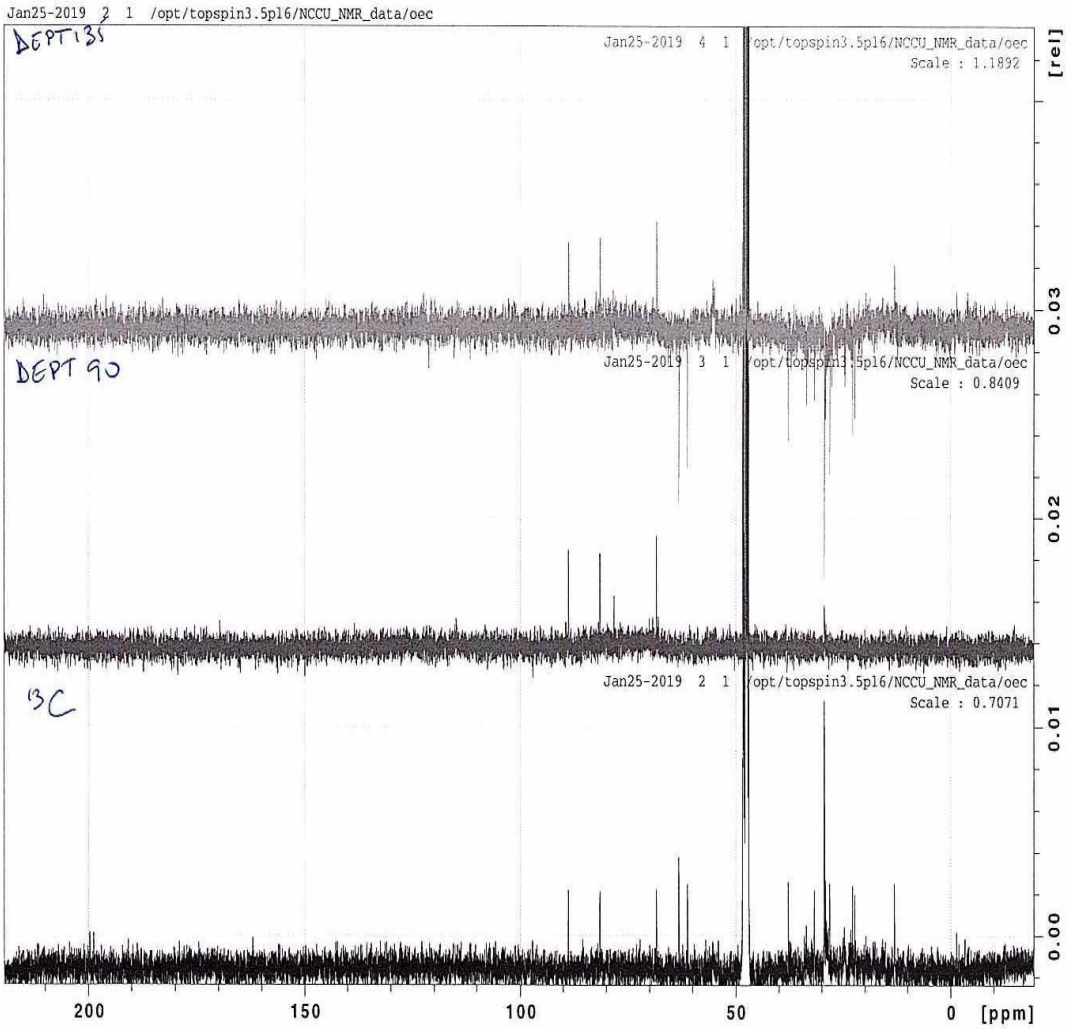
F2 - Acquisition Parameters
Date_ 20190126
Time 3.03 h
INSTRUM spect
PROBHD Z116098_0628 (
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 10000
DS 4
SWH 24038.461 Hz
FIDRES 0.733596 Hz
AQ 1.3631488 sec
RG 200.03
DW 20.800 usec
DE 6.50 usec
TE 293.8 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 100.6253446 MHz
NUC1 13C
P1 10.00 usec
PLW1 71.27899933 W
SFO2 400.1416006 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 15.53999996 W
PLW12 0.19186001 W
PLW13 0.09650300 W

F2 - Processing parameters
SI 32768
SF 100.6152830 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
1.40

Appendix C3: ^{13}C Nuclear Magnetic Resonance spectrum of Lh3 fraction of *Leptadenia hastata*

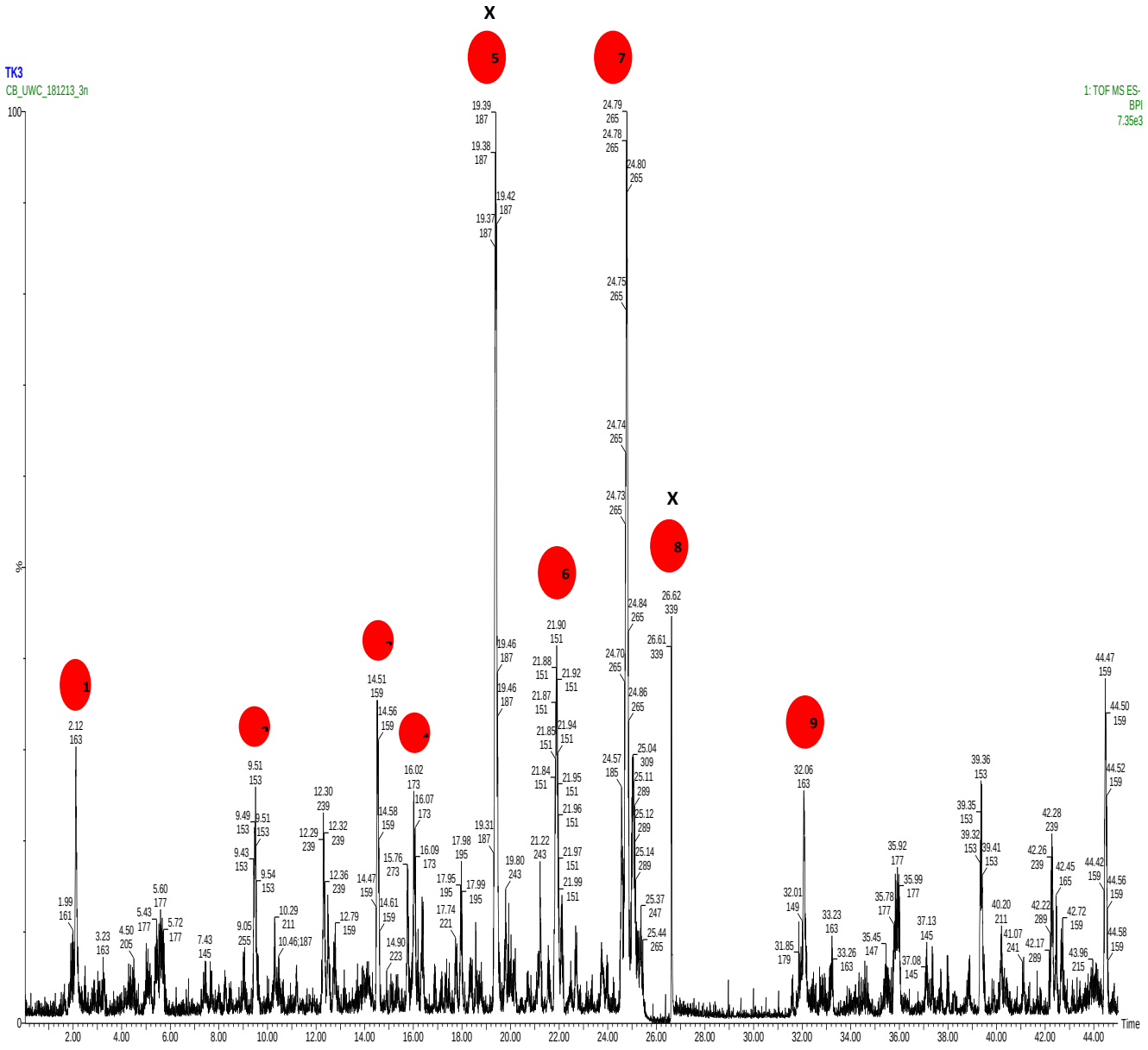


Appendix D1: ^{13}C Nuclear Magnetic Resonance spectrum DEPT of Tk3 fraction of *Polycarpea*



linearfolia

Appendix E1: HPLC ESI MS chromatogram of Tk3 fraction of *Polycarpea linearfolia*



Appendix E2: HPLC ESI MS chromatogram of Lh3 fraction of *Leptadenia hastata*