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THE SEARCH FOR NEW ANTI-PLASMODIAL AGENTS: FATE *OF MITRAGYNA INERMIS, PSEUDOCEDRELA KOTSCHYI* AND *MORINGA OLEIFERA*

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DECLARATION

I hereby declare that this thesis, submitted towards the MPhil degree, is my own work. It contains to the best of my knowledge, no material previously accepted for the award of any other degree of a University nor published by any person, except where due acknowledgement has been made in the text.

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DEDICATION

I dedicate this work to my parents, Mr Isaac Orman and Mrs. Regina Orman, my siblings and to all my loved ones.

ABSTRACT

Malaria treatment keeps failing because of resistance. With recent speculations about genes likely to be coding for Artemisinin resistance, it has become pertinent to extend the search for new agents. This study was undertaken to evaluate the *In-vivo* antiplasmodial effects of traditionally used medicinal plants for malaria treatment. It also sought to evaluate them for possible future compound (s) isolation and drug development. Leaves and stem bark of *Moringa oleifera* (Moringaceae), twigs of *Mitragyna inermis* (Rubiaceae) and leaves of *Pseudocedrela kotschyi* (Meliaceae), were selected based on ethnopharmacological studies, collected and authenticated in the Department of Herbal Medicine, KNUST, Kumasi. The samples were dried at room temperature for a month and then pulverized. Aqueous and methanolic extracts were prepared and stored under refrigeration (-18 ^{o}C) until use. Samples of the powdered plant materials were also kept for investigation. Phytochemical screening was carried out on both powdered plant materials and the extracts. The extracts were also evaluated for their *In-vivo* antiplasmodial activity. Initial screening was carried out on the aqueous extracts of *Moringa oleifera* leaves (ML) and stem bark (MSB) at 500 and 750mg/kg respectively, and *Pseudocedrela kotschyi* leaves (PK) and *Mitragyna inermis* twigs (MT), both at 500 mg/kg for 7 days. Extracts were administered orally to *Plasmodium berghei* infected ICR mice (25-30 g). The most effective aqueous extract, ML, was then evaluated at 250, 500, 750 and 1000 mg/kg using Artemether-Lumefantrine (A/L) at 4 mg/kg as reference drug. Both Four-Day Suppressive Test and seven days of Curative Test were conducted. The weights of the animals, physical signs showing either clinical deterioration or wellness and survival of the animals were also monitored. The methanolic extracts of *Moringa oleifera* leaves (MOR), *Pseudocedrela kotschyi* leaves (PSD) and *Mitragyna inermis* twigs (MIT), each at 500 mg/kg, were also evaluated on *P. berghei* infected BALB/c mice (20-25 g) using A/L at 4 mg/kg as reference drug. The weights of the experimental animals were also monitored. Chromatographic fingerprints for the extracts were developed using reversed high

performance liquid chromatography. The aqueous extracts obtained were MT (16.736 g; 1.67%), PK (16.6736 g; 3.33%), MSB (23.3355 g; 1.17%) and ML (24.1123 g; 2.41%). The methanolic extracts were MOR (16.4624 g; 11.65%), MIT (33.9513 g; 2.26%) and PSD (60.019 g; 5.91%). Phytochemical screening showed presence of alkaloids, tannins, coumarins, phytosterols, flavonoids and glycosides, with some variations in the compositions in the aqueous and methanolic extracts. The suppressions from the initial screening were as follows; ML (84% - 99.15%; AUC = 521.3), MSB (65% - 96.88%; AUC = 491.6), MT (47.67% – 84.49%; AUC = 340.8) and PK (22.47% - 82.91%; AUC = 275.0). ML extracts (250-1000 mg/kg) exhibited significant reduction in parasitemia in the four-day suppressive test ($F_{6,49}$ = 4.309; p =0.0014). However, 250 mg/kg (69.31%; $p < 0.001$) and 500 mg/kg (77.26%; $p < 0.001$) extracts exhibited relatively higher activities compared to 750 mg/kg (25.28%; $p < 0.001$) and 1000 mg/kg (07.12%; $p > 0.05$). In the curative test, similar results were obtained with significant parasitemia reduction for 250 mg/kg (AUC = 52.52 ± 6.732; $p < 0.01$) and 500 mg/kg (AUC = 49.62 ± 3.804; $p < 0.01$) compared to the positive control group (AUC = 101.3 ± 14.32). In addition, physical signs such as piloerection, lethargy and decreased locomotor activity were observed to be progressive in all experimental groups except A/L. Finally, survival analysis showed that although 750 mg/kg and 1000 mg/kg groups recorded relatively higher mortalities, statistical analysis didn't show any significant difference. In evaluating the methanolic extracts, percentage suppressions by day-3 post infection were as follow; MOR (67.08%), MIT (44.37%) and PSD (25.79%). Parasite reduction ratio (PRR), an indication of extract potency, was observed to be declining with day for the extracts whereas that of A/L kept increasing exponentially. The chromatograms developed gave indication of the complexity of the extracts and also showed feasibility of optimizing the experimental conditions for preparative HPLC fractionation of the extracts.

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"I will bless the LORD at all times: His praise will continually be in my mouth" – Psalm 34:1

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CHAPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

Malaria is a potentially deadly parasitic disease of global public health relevance, caused by a protozoan of the genus *Plasmodium*. Five species of the parasite are known to cause human infection, and these are *Plasmodium ovale, Plasmodium malariae, Plasmodium vivax, Plasmodium falciparum* and *Plasmodium knowlesi*. So far, the most virulent of the parasites is *Plasmodium falciparum*, which also accounts for the majority of severe illnesses, complications and deaths from malaria (Shapiro & Goldberg 2006; Okwa 2012). In Africa, most malaria cases are caused by *Plasmodium falciparum* and accounts for 90-98% of cases, while the rest are contributed by the other species especially *Plasmodium malariae* and *Plasmodium ovale* (Sory *et al*. 2009; Okwa 2012). *Plasmodium falciparum* was reported to be the major causative species causing 100% malaria cases in Ghana as at 2012 (WHO 2013a).

Malaria is an important cause of death and illness in children and adults, especially in tropical countries. In Ghana, malaria is termed to be endemic and perennial in all parts, with seasonal variations more pronounced in the Northern part of Ghana (PMI 2013). According to the 2010 national census, 24.2 million Ghanaians are at risk of malaria infection. Children under five years and pregnant women however stand a higher risk of severe illness due to declined immunity. Malaria transmission tends to be less intense in large urban centres (PMI 2013).

According to the health facility data of the Ghana Health Service (GHS), malaria is the number one cause of morbidity and mortality in children under five years of age, accounting in recent years for 33% of hospital deaths among them and about 38% of all out - patient illnesses and also, 36% of all hospital admissions. Each year, between 3.1 and 3.5 million

cases of clinical malaria are reported in public health facilities, 900,000 cases of which are in children under five years and 3,000 - 4,000 result in in - patient deaths (PMI 2013).

The control of malaria requires an integrated approach, including prevention that deals primarily with vector control and prompt treatment with effective antimalarials (WHO 2010). Early diagnosis and prompt treatment are fundamental components of the World Health Organization (WHO) global strategy for malaria control. Correct use of an effective antimalarial drug will not only shorten the duration of malaria illness but also reduce the incidence of complications and the risk of death (Bosman *et al*. 2001), especially among the most vulnerable groups such as pregnant women, children under 5 years and foreigners in malaria endemic countries with low immunity to the disease (PMI 2013).

Management of malaria has seen a lot of changes, mainly as a result of resistance development of *P. falciparum* against antimalarials in use. For instance, Chloroquine, which used to be one of the most effective drugs has now been proven to be ineffective in malaria treatment (Bray *et al*. 1998). The same applies to monotherapies like Halofantrine and Mefloquine. Currently, WHO recommends a combination therapy involving any of the artemisinins in the treatment of uncomplicated malaria in most parts of the world. This recommendation has emanated from evidence of studies revealing the increasing rates of resistance development with the use of monotherapies (WHO 2010). Thus, combinations like Artesunate - Amodiaquine, Artemether - Lumefantrine, Atovaquone - Proguanil, Chloroquine - Proguanil, and Mefloquine – Sulphadoxine - Pyrimethamine have all been proposed by the WHO, some of which appear in most Antimalarial Drug Policies of member countries (Bosman *et al*. 2001).

Plasmodium resistance to antimalarial medicines is one of the major obstacles in the fight against malaria. Comprehensive, up-to-date understanding of the scope of antimalarial

resistance is essential for protecting the recent advances in malaria control (WHO 2010). Without regular monitoring and reporting of antimalarial drug resistance, the disease burden and the economic costs of malaria will rise dramatically. In addition, ineffective treatment resulting from drug resistance might lead more patients to rely on the unregulated private sector, increasing the risk of reliance on monotherapy, substandard and counterfeit medicines and subsequently leading to further spread of drug resistance (WHO 2010).

However, the wonder of nature has always created avenues for combating the menace. There is a school of thought that the solution to the mystery of the development of resistance of *Plasmodium* parasites rests in the use of traditional medicinal plants (Maranz 2012). There are pieces of evidence of medicinal plant preparations being employed in the treatment of malaria in Ghana and other African countries (Idowu *et al*. 2010; Abiodun *et al*. 2011; Maranz 2012; Bero *et al*. 2009; Asase *et al*. 2005). In popular African thought, there is a conviction that the continent's vast traditional pharmacopeia includes potent indigenous therapies that can outperform medicines from industrialized countries (Maranz 2012).

However, with respect to malaria, the current success of Artemisinin Combination Therapy (ACTs) in reducing transmission rates across the continent has not been matched yet by results from comparable indigenous counterparts (Maranz 2012). This may be due to a lot of reasons, for example, if an African plant therapy with artemisinin - like epidemiological effects exists, it is either not currently in widespread use, or its effects are flying under the radar due to lack of objective scientific evidence of efficacy and safety (Maranz 2012).

The counter argument often heard in Africa is that although highly effective cures do exist, they constitute hidden knowledge, with the secret formulas held by few old men, who will not even on their deathbeds transmit the information to their own sons (Maranz 2012). If this

is true, then the tradition of secrecy serves no public health purpose, nor does it have any future other than extinction.

Notwithstanding all these challenges, the story behind the discovery of the artemisinins seeks to provide headway in the discovery of bioactive constituents from medicinal plants for combating malaria. Through thorough research conducted by scientists who were enrolled on the 'Programme 523' in China, the artemisinins were discovered and this has been a major breakthrough in the fight against malaria (Li *et al*. 2006).

With the recent reportage on the resistance development of *Plasmodium falciparum* against the artemisinins in some parts of Cambodia and Thailand (Heseltine 2010), it calls on scientists especially medicinal chemists to 'go back to the drawing board', to come up with new solutions. Armed with information on the folkloric uses of traditional medicines in different parts of Africa, it paves a very good platform to validate the uses of such plants and to further explore them unto the discovery of novel compounds. When established to possess significant activity, they can be developed into drugs for the treatment of the disease, most especially those emanating from resistant strains of *Plasmodium falciparum* parasites.

1.1.1 PROBLEM STATEMENT AND JUSTIFICATION

The Cambodia - Thailand border has historically been known as the place of origin for the emergence of resistance to antimalarials. The region was the first to show signs of *Plasmodium falciparum* resistance to Chloroquine, Sulphadoxine - Pyrimethamine and Mefloquine (Heseltine 2010). Upon first detection of falciparum resistance against the artemisinins, in this region, therapeutic efficacy studies were conducted. It was realized that between 2002 and 2005, there were records of high failure rates and increased parasite clearance time recorded even with the introduction of ACTs. Thus, WHO published a global

report warning of the potential emergence of artemisinin resistance in the region. Subsequently, WHO called for international ban of monotherapy and in confirmation of the suspicions of resistance, two cases of Artesunate resistance were reported in Tasanh, Cambodia (Heseltine 2010). In the World Malaria Report 2012, resistance to artemisinins has now been detected in 4 countries of the Greater Mekong sub region: Cambodia, Myanmar, Thailand and Viet Nam (WHO 2013b).

Considering the stepwise increment in the reportage of resistance development of *Plasmodium falciparum* against the currently used drugs in the management of malaria, there is a great need to find alternative ways for treatment. Just like *Cinchona sp.* and *Artemisia annua* proved to be miraculous plants and had therefore created a platform for a paradigm shift in antimalarial research, hidden are African medicinal plants yet to be discovered which may possess very significant antimalarial activity. Due to the wide diversity of compounds present in plants, it is possible to discover novel bioactive compounds, likely with new mechanisms of action to fight resistant strains of *Plasmodium* parasites. It is for these reasons that the current project was undertaken.

1.1.2 HYPOTHESIS

From evidence of traditional use of the selected medicinal plants in malaria treatment, it is theorized that they contain bioactive constituents responsible for their antimalarial activities. Thus, extraction, isolation, identification and characterization of these bioactive constituents may offer lead to compounds which could be developed into new antimalarial agents.

1.1.3 OVERALL AIM

The main aim of this research is to evaluate the antiplasmodial activities of three selected medicinal plants, documented to be employed traditionally to treat malaria in Ghana (Asase *et al*. 2005).

1.1.4 SPECIFIC OBJECTIVES

The specific objectives set were as follows:

1. To carry out crude aqueous extraction of the medicinal plants and establish their *In-vivo* antiplasmodial activities using *Plasmodium berghei* animal models.
2. To carry out methanolic extraction of the plant samples that exhibit significant activity from the initial investigation and also establish their *In-vivo* antiplasmodial activities.
3. To carry out phytochemical investigation on the plant materials employed for the study.
4. To develop chromatographic fingerprints for the bioactive extracts.

1.2 LITERATURE REVIEW

1.2.1 BIOLOGY OF MALARIA INFECTION

Until recently, nearly all human malaria infections were caused by four species of obligate intracellular protozoa of the genus *Plasmodium* and these are *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium vivax* and *Plasmodium falciparum* (Shapiro & Goldberg 2006). The recent addition has been *Plasmodium knowlesi*, which was discovered by Vincke and Lips (Thurston 1950) and used to be misdiagnosed as *Plasmodium malariae* infection (Cox-Singh *et al*. 2008). It was known to cause infection in animal models and recently have been

established to cause human infection. The parasite was named after Dr. Robert Knowles (White 2008).

Although transmission can be through transfusion of infected blood, congenitally, and by sharing needles, usually malaria infection is transmitted by the bite of infected female *Anopheles* mosquitoes (Shapiro & Goldberg 2006). The *Anopheles* genus happen to host all *Plasmodium* species infecting humans. Other species from the mosquito genera *Aedes, Culex, Mansonia* and *Theobaldia* can also transmit malaria but not to humans (Okwa 2012). Sporozoites from the mosquito salivary glands rapidly enter the circulation after a bite and localize via specific recognition events in hepatocytes, where they transform, multiply, and develop into tissue schizonts (Okwa 2012; Shapiro & Goldberg 2006). Below is a figure illustrating the different stages of the parasite in both host and vector (See fig. 1.1 below). This primary asymptomatic tissue (that is, either pre-erythrocytic or exoerythrocytic) stage of infection lasts for 5 to 15 days, depending on the species (Shapiro & Goldberg 2006). Tissue schizonts then rupture, each releasing thousands of merozoites which enter the circulation, invade erythrocytes, initiating the erythrocytic cycle (Clark & Schofield 2000). Once the tissue schizonts burst in *P. falciparum* and *P. malariae* infections, no forms of the parasite

remain in the liver. On the contrary, for *P. vivax* and *P. ovale* infections, tissue

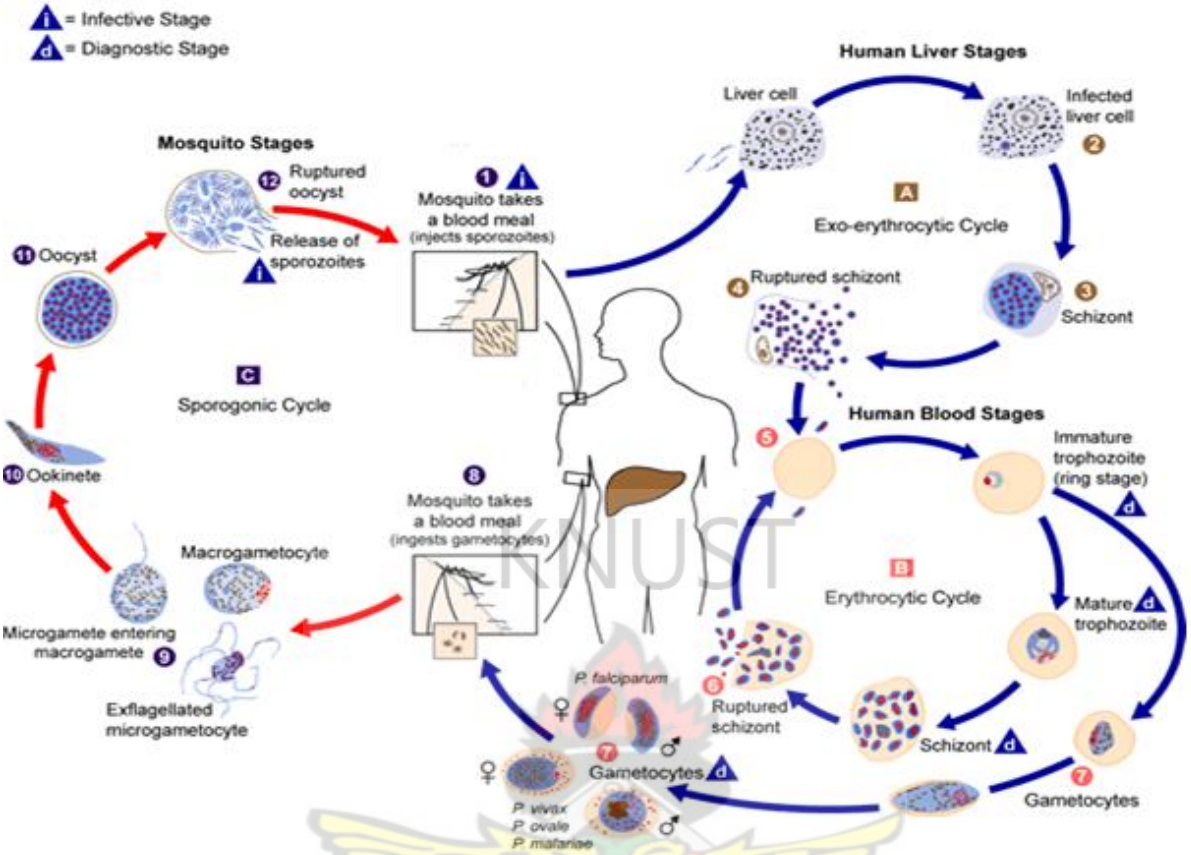


Figure 1.1 - Life cycle of the Plasmodium falciparum in human host

(1) – Infected female *Anopheles* mosquito takes a blood meal from uninfected host and injects sporozoites of parasites into the blood. **(2-3) –** Migration of sporozoites into hepatocytes where multiplication takes place to produce liver schizonts. **(4-6) –** Liver schizonts rupture to produce merozoites which then attack the red blood cells through surface proteins. Other merozoites in the case of *P. vivax* re-infect hepatocytes to produce hypnozoites. Blood merozoites then replicate through the ring, trophozoite and the schizonts stages after which the host RBC ruptures to release the merozoites to re-infect new RBCs. **(7) –** Some of the blood merozoites transform into male and female gametocytes. **(8-12) –** Gametocytes are then picked up by a mosquito during a new bite. The gametes undergo sexual reproduction going through different stages to finally produce sporozoites which migrate to the salivary glands of the mosquito; ready to be delivered on the next bite. **A –** Stages of the parasite in the liver**. B –** Asexual stages of parasite in the blood**. C –** Sexual stages in the female *Anopheles* mosquito (CDC 2014)**.**

parasites (hypnozoites) persist, later producing relapses of erythrocytic infection months to years after the primary attack (Okwa 2012; Wells *et al*. 2010). Once plasmodia parasites enter the erythrocytic cycle, they cannot reinvade the liver; hence, there is no tissue stage of infection for malaria contracted by transfusion (Shapiro & Goldberg 2006). In erythrocytes,

most parasites undergo asexual development from young ring forms to trophozoites and finally to mature schizonts (Bannister *et al*. 2000; Shapiro & Goldberg 2006; Okwa 2012) .

Schizonts-containing erythrocytes rupture, each releasing 6 to 32 merozoites depending on the *Plasmodium* species. It is this process that produces febrile clinical attacks (Clark & Schofield 2000; Clark & Cowden 2003). The merozoites invade more erythrocytes to continue the erythrocytic cycle, which proceeds until either death of the host or modulation by drugs or acquired partial immunity (Miller *et al*. 2002). The periodicity of parasitemia and febrile clinical manifestations depends on the timing of schizogony of a generation of erythrocytic parasites. For *P. falciparum*, *P. vivax*, and *P. ovale*, it takes about 48 hours to complete this process (White 1997); for *P. malariae*, about 72 hours is required (Shapiro & Goldberg 2006).

For erythrocyte invasion, merozoites bind to specific ligands on the red cell surface (Miller *et al*. 2002; Sibley 2004; Clark & Schofield 2000; Clark & Cowden 2003). *P. falciparum* has a family of binding proteins that can recognize a number of host cell molecules, including glycophorins A, B, and C, as well as *band* 3. It is able to invade all stages of erythrocytes and therefore achieving high parasitemia (Shapiro & Goldberg 2006). In the case of *P. vivax*, it is more selective in its binding; there is a need for the recognition of the Duffy chemokine receptor protein as well as reticulocyte-specific proteins. Thus, it will not establish infection in Duffy-negative individuals and will only invade reticulocytes. Because of this restricted subpopulation of 'suitable' erythrocytes, *P. vivax* rarely exceeds 1% parasitemia in the bloodstream. *P. ovale* is similar to *P. vivax* in its preference for young red blood cells, but the mechanism of its erythrocyte recognition is unknown. *P. malariae* recognizes only senescent red cells, maintains a very low parasitemia, and typically causes an indolent infection. (Shapiro & Goldberg 2006).

1.2.2 CLASSIFICATION OF ANTIMALARIALS

Antimalarials can be categorized according to the stage of the parasite they affect and also by their proposed use for either prophylaxis or treatment. The various stages of the malaria life cycle in the host differ from each other not only in their morphology and metabolism but also in their drug sensitivity (Block 2011). For this reason, the classification of antimalarial drugs is best done in the context of the life cycle (Shapiro & Goldberg 2006; Miller *et al*. 2013). As the knowledge of the parasite and its life cycle grows, it has become possible to target parasite molecules that are unique to it and necessary for its survival (Miller *et al*. 2013). Currently, there are four possible stages for drug therapy:

a) Kill the sporozoites injected by the mosquito and/or prevent the sporozoites from entering the liver.
b) Kill the hypnozoites residing in hepatocytes and/or prevent them from becoming merozoites.
c) Kill the merozoites in the blood and/or prevent them from developing into gametocytes.
d) Kill the gametocytes before they can enter the mosquito and reproduce into zygotes (Block 2011).

1.2.2.1 SEXUAL STAGE IN THE MOSQUITO

Infection of the host begins with the bite of a mosquito and the injection of threadlike sporozoites into the blood stream to undergo both exo-erythrocytic and erythrocytic stages of infection. In addition to that, some of the merozoites after a number of re-infections, differentiate into male and female gametocytes which are then ingested by another mosquito upon a second blood meal (Okwa 2012; Shapiro & Goldberg 2006). The sexual stage of the life cycle therefore comprises these two stages which ensure the continuity of the infection in the host.

Gametocyte infection of the mosquito is dependent on the prevalence, duration and density of gametocyte carriage in the human host (Targett *et al*. 2001; Drakeley *et al*. 1999; Hogh *et al*. 1998)**.** In terms of density, as gametocyte density falls below 2µL, transmission becomes impossible. In addition to the mentioned factors, are also humoral (gametocyte antibodies) and leukocyte factors that affect gametocyte infection of vectors (Drakeley *et al*. 1999)**.**

Due to the obligatoriness of the *Anopheles* sp. mosquito acting as the vector for the transmission of the infection, making it an indispensable tool in the infection process, inhibition of it will therefore offer a great stride in the curtailment of the disease (Okwa 2012). Methods like the use of insecticides (such as Pyrethrins and Pyrethroids, Malathion, etc.) to kill the vectors and the use of insecticide treated nets (ITN) as well as a chemical repellent, DEET (that is, *N*,*N*-diethyl-meta-toluamide) to prevent direct contact with vector during the peak of their activity have been employed (USEPA 2014). Biological control methods used include bacteria such *Bacillus thuringiensis israelensi* and *Bacillus sphaericus* (USEPA 2014). However, the problem of resistance has caught up with these chemicals rendering the approaches quite obsolete nowadays. Recently, one of the feasible approaches adopted is the use of transgenic mosquitoes from genetic manipulations, which express antiparasitic genes in their midgut epithelium. This technique renders the resultant mosquitoes inefficient in transmission (Ito *et al*. 2002).

1.2.2.2 HEPATIC STAGE

Within minutes of release from the bite of an infected female *Anopheles* mosquito into the host, *Plasmodium* sporozoites reach the mammalian liver, where they invade hepatocytes and either lie dormant or develop over several days, eventually forming schizont which serve as prelude to a blood stage infection (Delves *et al*. 2012; Shapiro & Goldberg 2006; Cowman & Crabb 2006). Compounds which target the parasite stages in the liver offer protection from

infection by eliminating hypnozoites reservoirs formed by *P. vivax* and *P. ovale* (Wells *et al*. 2010).

1.2.2.2.1 8-Aminoquinolines

The only current clinically approved medication for treating hypnozoites is Primaquine [**1**], a member of the class of antimalarials known as the 8-Aminoquinolines. Other agents in this group that have faced out are Methylene Blue [**2**] and Pamaquine [**3**] due to issues of toxicity (Shapiro & Goldberg 2006; Wells *et al*. 2010). Little is known about the antimalarial action of the 8-aminoquinolines but it is believed that Primaquine is converted to electrophiles acting as oxidation-reduction mediators. Such an activity contribute to antimalarial effects by generating reactive oxygen species (ROS) or by interfering with electron transport in the parasite (Shapiro & Goldberg 2006; Bates *et al*. 1990). The free radicals can then react with nucleophiles at a variety of specific ring positions and with thiols and glutathione (Wells *et al*. 2010). In addition, infected erythrocytes and hepatocytes contain ferrous iron that facilitates the generation of oxygen radical species. Mitochondrial membrane modification is also suggested as part of its mechanism of action. There is also a consideration of a relation between the haemolytic effects of the agents as side effects and the mechanism of hypnozoites inhibition (Wells *et al*. 2010). The wakeup call for the discovery of new agents in this class for the continuous fight against the hypnozoites has been hampered by the following observations: (1) the reactive metabolites generated are short-lived and difficult to confirm in patients. (2) The safety margin of agents is dependent on the relative reactivity of the activated species towards the putative hypnozoites target compared with the erythrocyte targets. Increased selectivity could be the end product of altering the physicochemical properties of the compound to make it more likely to accumulate in the liver, and less likely to accumulate in the erythrocyte (Wells *et al*. 2010).

Primaquine can cause mild-to-moderate abdominal distress in some individuals but these symptoms often are lightened by taking the drug at mealtime. Mild anaemia, cyanosis (from methaemoglobinemia), and leucocytosis could show up but are less common (Wells *et al*. 2010). Chloroquine and dapsone have been shown to be synergistic with Primaquine in producing methaemoglobinemia. Pre-screening of patients for Glucose-6-Phosphatase Dehydrogenase (G6PD) deficiency is recommended before initiating therapy (Wells *et al*. 2010). Granulocytopenia and agranulocytosis are unusual complications of therapy and these are usually connected with overdosage (Gogtay & Kamtekar 2006).

In the quest to develop new analogues of primaquine with desirable properties, several compounds have been synthesized, but the most effective among them are RC-12 [**4**], Tafenoquine [**5**] and Bulaquine [**6**] (Wells *et al*. 2010). RC-12 [**4**], also known as N- (2-bromo-4,5-dimethoxyphenyl)-N-[2-(diethylamino)ethyl]-N',N'-diethyl-1,2-ethanediamine, has been shown to be effective in primate models of relapsing malaria. It is however not clear if the chemical processes that kill hypnozoites are the same as those causing haemolysis; a case that will call for separation of these activities, if possible to enhance clinical significance (Canfield & Rozman 1974). Tafenoquine [**5**], has a relatively longer plasma half-life and has actually gone through clinical trials with three days of treatment at dose of 200 mg (Shanks & Oloo 2001; Milhous 2001). Bulaquine [**6**], known as N-(3-acetyl-4,5-dihydro-2-furanyl)-N-(6-methoxy-8-quinilinyl)-1-4-pentadiamine), is a pro-drug of Primaquine. In clinical studies, it was shown to have similar efficacy as primaquine. No haemolysis was reported after treating three G6PD-deficient patients with 25 mg bulaquine, whereas 30 mg primaquine was observed to cause falls in haematocrit in four of such patients (Krudsood *et al*. 2006; Valecha *et al*. 2001). In another study, it was discovered that NPC-1161B [**7**] also possessed antimalarial activity. In addition to the antihypnozoite activity, it was established to inhibit exflagellation *In-vitro* and oocyst production in mosquito vector. This result suggested that,

either the drug did not need metabolic activation or it exhibited poly-pharmacology by acting through metabolite-independent mechanism for activity (Delves *et al*. 2012).

Recent modifications of primaquine have demonstrated that replacing the methoxy group with a bulky tertiary butyl group, retains the pharmacological activity, but reduces the haemolytic potential of primaquine in rodent models (Jain *et al*. 2004). Another promising approach has been an introduction of a second nitrogen into the ring system at the 5-position, producing naphthyridine analogues of primaquine, that retain blood-stage activity but with no haemolysis (Zhu *et al*. 2007).

1.2.2.3 ERYTHROCYTIC STAGE

From the hepatic stage, the liver merozoites migrate into the blood where they attack the erythrocytes to initiate second phase of asexual reproduction. In the erythrocyte, the parasite goes through different developmental stages; from the ring form to the trophozoites, then to the schizont stage, which finally breaks out of the erythrocyte. The released merozoites attack new erythrocytes to continue the cycle (Shapiro & Goldberg 2006).

The end product of haemoglobin digestion is the release of heme as Fe [II]–Protoporphyrin IX (Fe [II] PPIX) which quickly oxidizes to Fe [III] PPIX (hematin), a product that destroys the parasite by membrane damage and lysis through the release of ROS (Goldberg *et al*. 1991; Tilley *et al*. 2011; Sherman 1979; Shapiro & Goldberg 2006; Francis *et al*. 1997; Miller *et al*. 2013). The malaria parasites circumvent heme toxicity by dimerising the molecules and then converting them into inert crystals called hemozoin. They also degrade heme through peroxidative or glutathione-mediated pathways (Becker *et al*. 2004; Kawazu *et al*. 2008; Loria *et al*. 1999).

There are clinically accepted drugs that inhibit the formation of the hemozoin thereby exposing the parasites to the harmful effects of Fe (III) PPIX.

1.2.2.3.1 Natural Alkaloids

The first group of drugs to be employed for the treatment of malaria were alkaloids extracted from the *Cinchona* sp. tree which were later identified to possess the quinoline nucleus just as the 8-aminoquinolines (**Sec. 1.2.2.2.1**) and other classes that are discussed below. The cinchona tree is named after the Countess of Cinchon, who according to legend was cured of malaria in 1630 by a powder made from its bark (Foley & Tilley 1998; Foley & Tilley 1997). The plant had been widely used in the 17th century (Wallace 1996).

However, due to the variations in the quality of the bark preparations, there was a need to isolate the active ingredients (Foley & Tilley 1997). Thus crude mixture of crystalline alkaloids were extracted from the bark by Gomes. Some of the alkaloids that have been isolated and identified from the bark are Quinine [**8**] and Quinidine [**10**], an enantiomeric pair and their desmethoxy analogues, Cinchonidine [**9**] (for quinine) and Cinchonine [**11**] (for quinidine). The stereochemistry differs at positions 8 and 9 with quinine and cinchonidine being (S,R) and quinidine and cinchonine being (R,S) (Block 2011).

Quinine is lethal to all *Plasmodium* schizonts and the gametocytes from *P. vivax* and *P. malariae*, but not that of *P. falciparum*. Due to its narrow therapeutic window, it is not suitable for chemoprophylaxis and it's usually associated with cinchonism as adverse effects from administration of high doses (Block 2011). Some of the effects are tinnitus, headache, nausea, and disturbed vision.

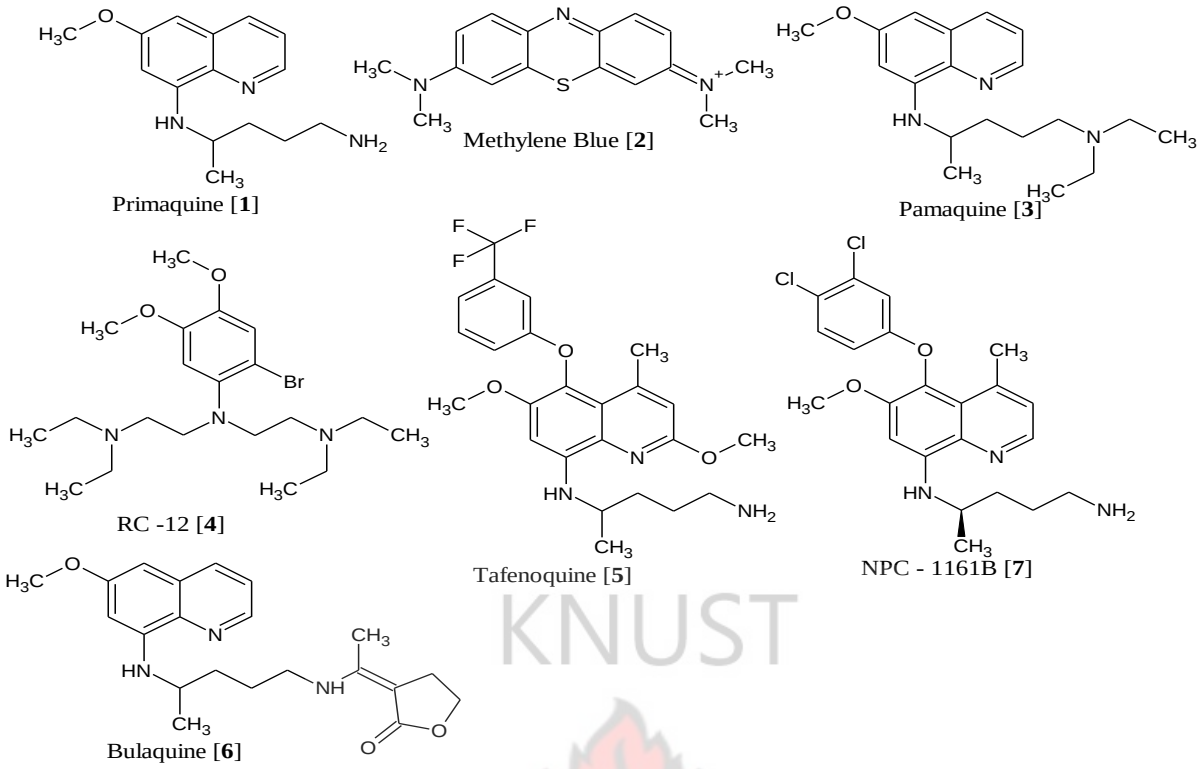


Figure 1.2 - 8-Aminoquinolines Class of Antimalarials with activity against liver stage of P. vivax

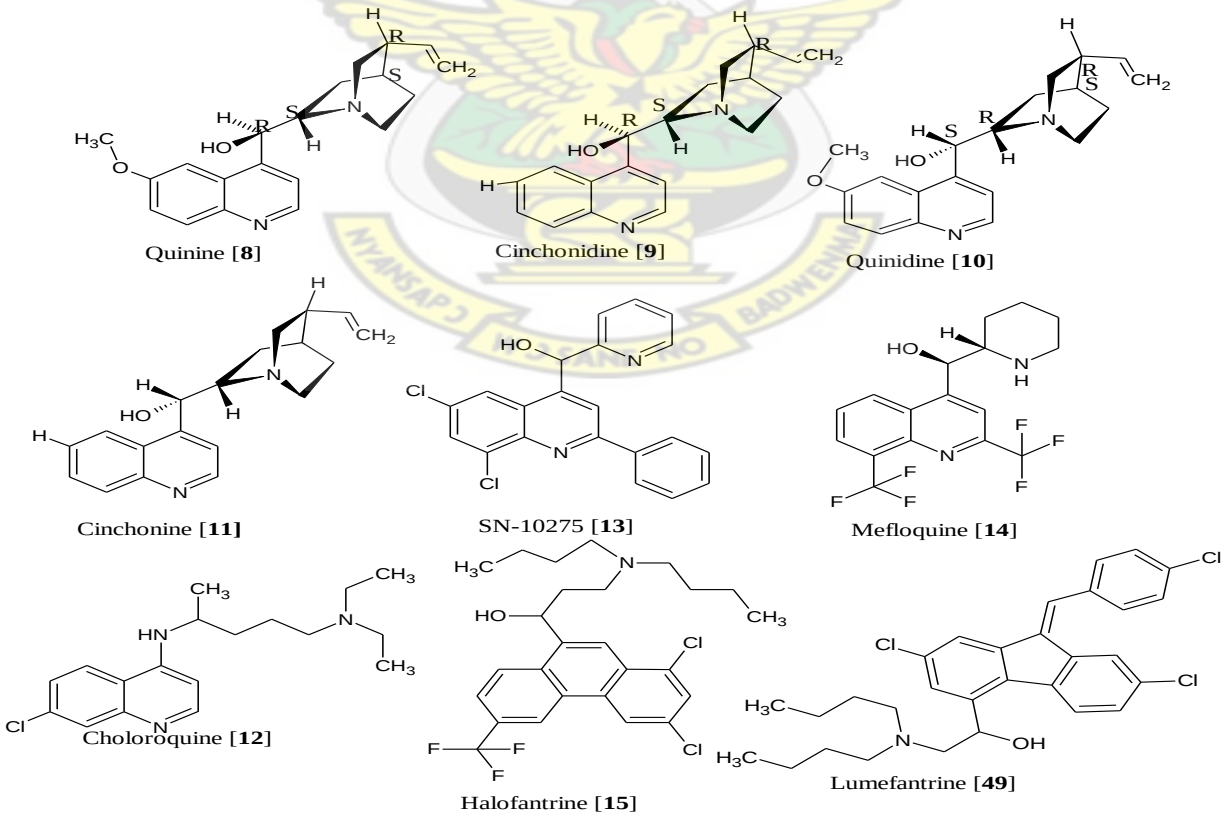


Figure 1.3 - Isolated quinoline alkaloids and synthetic quinolinemethanols with blood schizontocidal activity

The stereoisomer, quinidine, is also a schizontocide and for that matter indicated in the parenteral form as quinidine gluconate for severe malaria in the United States (CDC 2004). However, its primary indication is in cardiac arrhythmias (Block 2011; STG 2010).

The quinolinemethanols, with the alkaloids inclusive, had for a long time been the mainstay in the treatment of uncomplicated malaria. Most of these compounds resulted from the chemical modification of the quinine nucleus in the quest to reduce the toxicity effects and also optimize the activity (Foley & Tilley 1997). In 1940, the first of these products, Chloroquine [Fig 1.3 (**12)**] (technically falls under the 4-aminoquinolines to be considered in the next section) was synthesized but was plagued with photosensitive side effects making it undesirable though it was very effective (Foley & Tilley 1998). Later on, in contribution to the search for new antimalarial agents after the Second World War, a large number of piperidyl quinolinemethanols were explored for antimalarial activity in avian models due to the closeness in structure to quinine (Pullman *et al.* 1948). In the study, SN-10275 [**13**] or 6,8-dichloro-2-phenyl-α-2-piperidyl-4-quinoline methanol, gave highest indication of activity (Pullman *et al*. 1948). Further studies yielded Mefloquine [**14**] and Halofantrine [**15**] which possessed higher activities than chloroquine and have been subjects of quite a number of studies, especially Mefloquine (Jiang *et al*. 1982; Shamiss *et al*. 1996; Croft & Garner 1997; Kocisko & Caughey 2006; Schlagenhauf 1999). In addition to this repertoire is Lumefantrine [**49**] which is usually combined with Artemether [**27**] (see **Sec. 1.2.2.3.3** below). These agents together with the 4-Aminoquinolines (see **Sec. 1.2.2.3.2** below) have similar mechanism of action and this is explained in the section.

1.2.2.3.2 4-Aminoquinolines

This group has a substitution at the same position 4 as quinine and have an asymmetric carbon equivalent to quinine's C-9 position. A significant difference of this class from the cinchona alkaloids is replacement of the 6-methoxy on quinine with a 7-chloro substituent, as

can be seen in Chloroquine [**12**]. An ample study of the antimalarial properties of chloroquine revealed that chemical modification of the position and nature of the substituents on the quinoline nucleus tend to have a more noticeable influence on the biological activity than do modifications of the 4'-diethylamino-1-methylbutylamino side chain (Coatney *et al*. 1953). In addition to that, it was also observed that chloroquine derivatives containing a chlorine substituent in either the 4- or 7- position, were substantially less active as compared to the 5- and 6-chloro forms (Raynes *et al*. 1995). Furthermore, removal of the 6-chloro substituent led to a 14 fold reduction in activity compared with chloroquine. However, activity was retained when the carbon backbone of the alkylamine side chain contained between two-five carbons, irrespective of the number of branches and asymmetry of the chiral carbon atom (Slater 1993). Armed with these information, Amodiaquine [**16**], Hydroxychloroquine [**17**] and Sontoquine [**18**] were synthesized. Some of the recent agents that have been developed are Piperaquine [**19**] and Hydroxypiperaquine [**20**], which were the most potent of bisquinolines synthesized (Raynes *et al*. 1995; Raynes 1998), Tert-butyl Isoquine [**21**], Pyronaridine [**22**], Naphthoquine [**23**] and Mepacrine [**24**] but have recently faced out due to issues of resistance with monotherapy (Block 2011).

These compounds act against the blood stages of the malaria parasite and to be specific, against only those stages during which the parasite is actively degrading haemoglobin. They therefore interfere with the feeding process of the parasites (Foley & Tilley 1997; Foley & Tilley 1998). Most of the knowledge on the mechanism of action of 4-aminoquinolines have emanated from studies using chloroquine, hence a discussion on it will focus on chloroquine as a prototype. Chloroquine (CQ) is a diprotic weak base (drugs in this class are weakly basic) with pKa_1=8.1and pKa_2= 10.2. The drug is only taken up to a very limited extent by uninfected erythrocytes but concentrated in several thousand-fold inside the malaria parasite (Yayon *et al*. 1984; Hawley *et al*. 1996). In its unprotonated or unionised form, chloroquine

can cross the membranes of the parasitized erythrocyte and move down the pH gradient to accumulate in the acidic food vacuole (pH 5-5.2) where it becomes protonated. Once protonated, the drug becomes membrane impermeable and locked up in the acidic food vacuole (Fitch *et al*. 1974). In this proposed mechanism, the level of CQ accumulation depends on the difference in pH between the external medium (cytosol) and the food vacuole (Yayon *et al*. 1984; Fitch *et al*. 1974). Another school of thought holds it that chloroquine uptake in *Plasmodium falciparum* is determined by binding to Ferriprotoporphyrin IX (FP[III]IX) and so when specific proteinase inhibitors block the degradation of haemoglobin and stop the generation of FP[III]IX, CQ uptake is inhibited (Bray *et al*. 1998; Bray 1999). In the food vacuole, chloroquine is proposed to target FP[III]IX, which usually is in the form of hematin (H_2O/HO–Fe[III]PPIX), its l-oxo dimer ((Fe[III] $PPIX)_2O$) or crystalline β-hematin ($(Fe[III]PPIX)_2$) to form complexes (Fe[III] PPIX–quinoline complexes) (Egan 2006; Meshnick 1990; Kouznetsov & Gomez-Barrio 2009) and this has been confirmed in studies by Slater and other authors (Slater 1993; Martiney *et al*. 1996). The formation of complex prevent the incorporation of Fe[III]PPIX into hemozoin leading to a build-up of toxic heme molecules and heme-chloroquine complexes within the parasite eventually destroying the membranes by a lipid peroxidation mechanism and killing the parasite by lysis (Foley & Tilley 1997; Foley & Tilley 1998; Bray *et al*. 2005) (see fig. 1.5 below).

1.2.2.3.3 Artemisinin and its derivatives

In the 1960s when drug-resistant malarial parasites developed and spread rapidly in Southeast Asia and Africa, and existing antimalarial drugs, such as quinine, chloroquine, and pyrimethamine - sulphadoxine became less efficient, it became eminent for the introduction of new generation of antimalarial drugs (Wang *et al*. 2010). The end result from such study in China was the discovery of Quinghaosu which was named Artemisinin [**25**]. This compound, a sesquiterpene lactone, was isolated from *Artemisia annua* (Brown 2010).

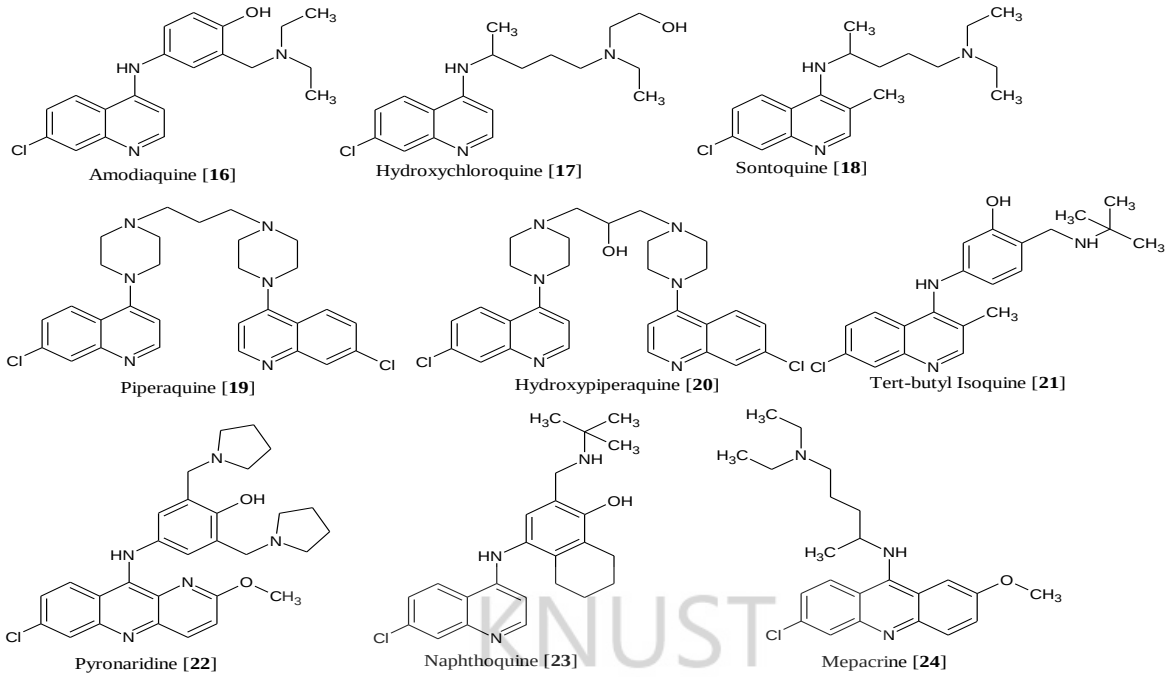


Figure 1.4 - 4-Aminoquinolines

Due to poor solubility and bioavailability as well as reduced activity of this compound, semisynthetic approaches by other researchers led to the synthesis of its derivatives, namely Artesunate [**26**], Artemether [**27**], Arteether [**28**], Dihydroartemisinin [**29**] and 10-Deoxoartemisinin [**30**] (Lemke 2007; Block 2011; Bray *et al*. 2005). They have a short duration of action but very effective in parasite clearance. This make them unsuitable for monotherapy and also increase the risk of resistance development against them. They however form the backbone of combination therapies (that is, ACTs) used to fight resistant *Plasmodium* parasites in several countries (WHO 2013b).

In the treatment of advanced cases of *P. falciparum* malaria, the water-soluble derivative of artemisinin (that is, artesunate) is desirable, which can be injected intravenously. Recently it has replaced quinine and other drugs as the drug of choice in severe or complicated malaria (Sinclair *et al*. 2011; Dondorp *et al*. 2005; Dondorp *et al*. 2010; Lubell *et al*. 2011). Early studies conducted to elucidate the mechanism of action of this class of compounds focused on the ability of the compounds to inhibit heme polymerisation.

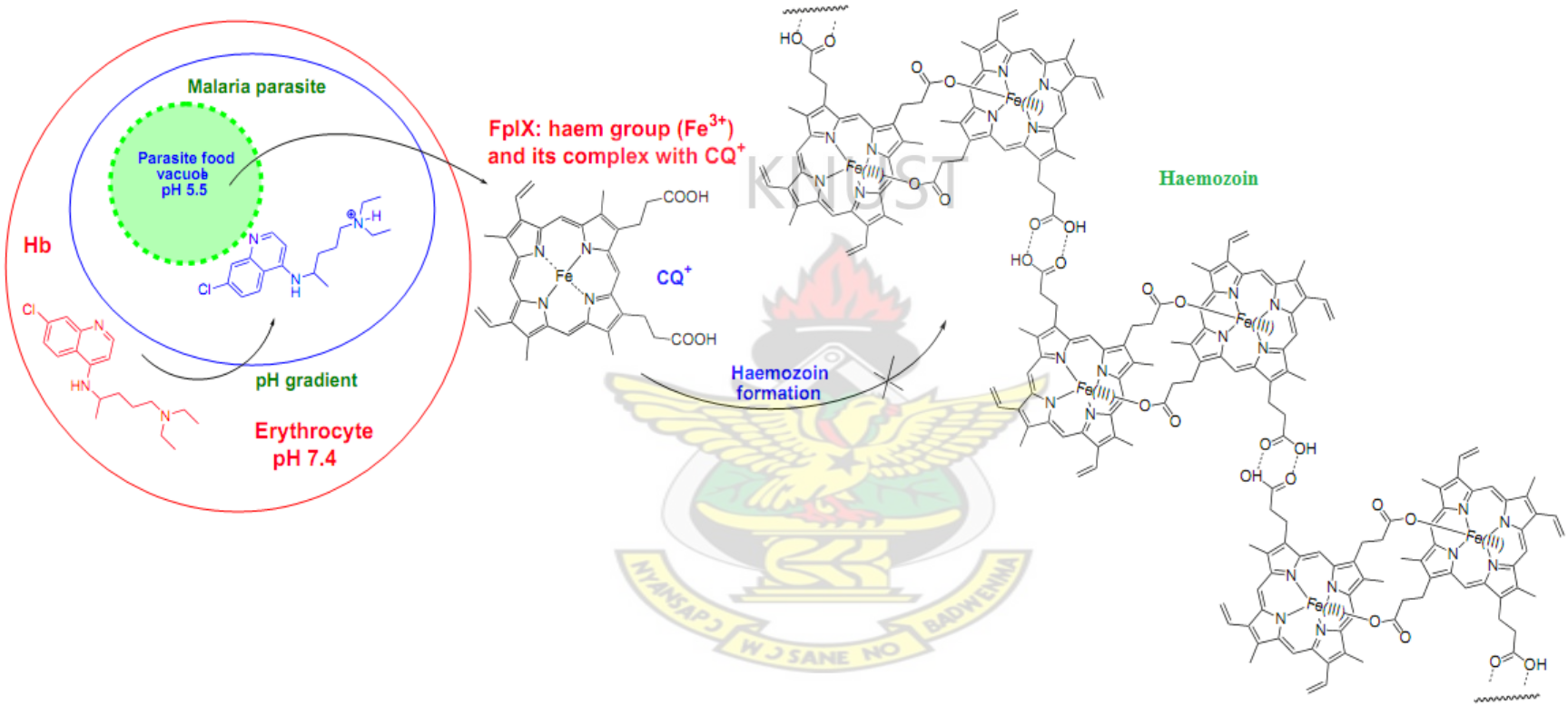
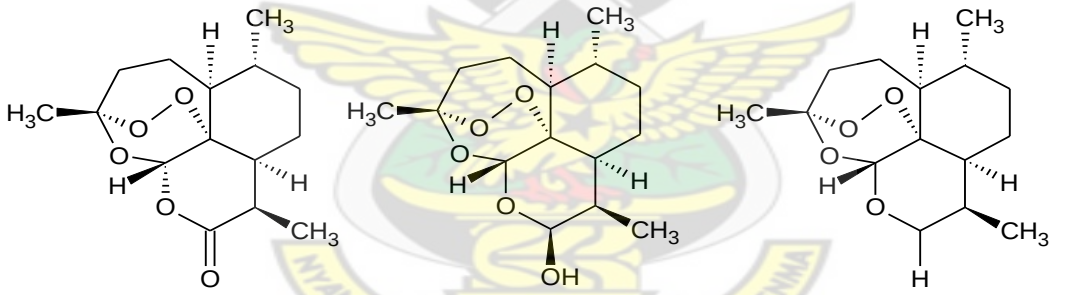
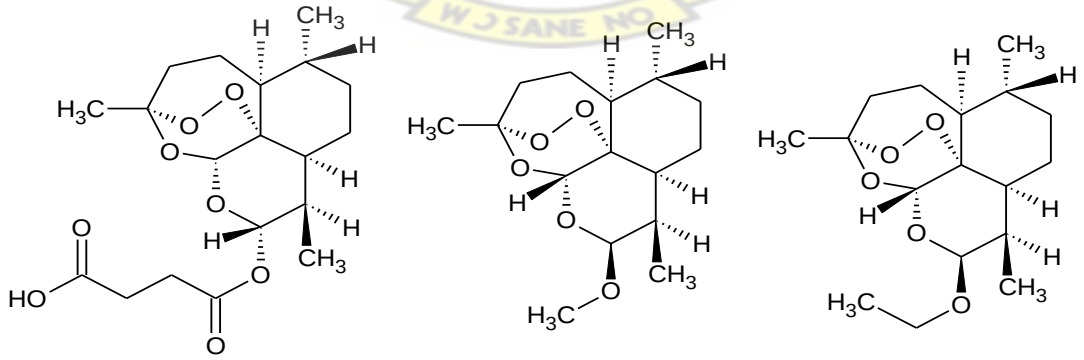


Figure 1.5 - Possible mode of action of Chloroquine and other Aminoquinolines

Thus, *In-vitro* studies carried out confirmed inhibitory effects of these compounds against β-haematin (Pandey *et al*. 1999; Basilico 1998; Asawamabasakda *et al*. 1994) in an attempt to link their mechanism of action to that of previously discussed groups. However, a later study by Haynes *et al*, proved otherwise. Their study focused on Artemisinin [Figure 1.6 (**25)**], Dihydroartemisinin [**29**] and 10-Deoxoartemisinin [**30**] where the latter compound, though known to possess antimalarial activity, failed to inhibit β-haematin *In-vitro* (Haynes *et al*. 2003). They argued that Artemisinin and Dihydroartemisinin may be unstable under aqueous conditions employed for the hemozoin studies. Thus, a ring-opening product (Haynes *et al*. 1999) which may form, binds to the heme and inhibit hemozoin formation. On the contrary, 10-Deoxoartemisinin, which has no oxygen at C-10, is less able to undergo ring opening under aqueous conditions and this was confirmed (Haynes *et al*. 2003).



Artemisinin [**25**] Dihydroartemisinin [**29**] 10-Deoxoartemisinin [**30**]



Artesunate [**26**] Artemether [**27**] Arteether [**28**]

Figure 1.6 - Artemisinin and derivatives (Bray *et al*. 2005)

The key pharmacophore in artemisinin is the 1,2,4-trioxane unit, an endoperoxide linkage which is crucial for expression of antiparasitic activity (Bray *et al*. 2005). The endoperoxide linkage has been proposed to generate free radicals which interact with the parasites. Thus, artemisinins which lack a peroxidic oxygen are devoid of antimalarial activity (Krishna *et al*. 2004; Bray *et al*. 2005). Some authors consider artemisinins as pro-drugs which produce bioactive free radicals for eliciting activity in the body (Krishna *et al*. 2004).

In the infected erythrocyte, the endoperoxide linkage undergo either homolytic or heterolytic reductive cleavage in the presence of Fe^{2+} ions (Bray *et al*. 2005), which is usually available in the food vacuole after the breakdown of haemoglobin in the form of free Fe^{2+} or heme-bound iron (Butler *et al*. 1998). Through a Fenton-like reaction (Prousek 2007), the cleavage leads to the generation of alkoxy radicals that subsequently rearrange into carbon-centred radical species (Posner *et al*. 1995; Posner *et al*. 1996; Bray *et al*. 2005; Krishna *et al*. 2004). It is proposed that final alkylation by these reactive carbon radical intermediates of biomacromolecules such as haem, specific proteins and other targets, result in the death of malaria parasites (Bray *et al*. 2005). Another school of thought contrary to the iron-induced homolytic endoperoxide cleavage hypothesis is that artemisinins act as a masked source of hydroperoxide (Haynes *et al*. 1999). In this proposal, following specific non-covalent binding of artemisinin with a given target protein, heterolytic cleavage of the endoperoxide bridge results and formation of an unsaturated hydroperoxide is followed by capture from water (or other nucleophile). The process provides a reactive hydroperoxide capable of irreversibly modifying protein residues by direct oxidation (Bray *et al*. 2005). The hydroperoxides released can also undergo Fenton-like reactions leading to the generation of hydroxyl radicals, adding up to the total radicals which target amino acids in the malaria parasites (Haynes *et al*. 1999; Bray *et al*. 2005).

The difficulty with the semi-synthetic artemisinin compounds has to do with all derivatizations, requiring Artemisinin [**25**] as starting material, which usually has very low yield (0.01–0.8% yield) upon extraction from *Artemisia annua* (Klayman 1985; Liu *et al*. 2003; Abdin *et al*. 2003). Thus, several groups have carried out total synthesis of endoperoxides which have been established to be active against the parasites (Taylor *et al*. 2004; Sato *et al*. 2011; Borstnik *et al*. 2002; Tang *et al*. 2004). Some of these compounds include Fenozan B0–7 [Fig. 1.7 (**31)**], the dispiro tetraoxanes [**32, 33**], synthetic analogue of Yingzhaozu A [**34**] and more recently 1,2,4-trioxolanes [**35, 36**], with some even recently going through different stages of clinical trials, for example, OZ-277 (Schlitzer 2008; Dong *et al*. 2010; Dong 2002; Vennerstrom *et al*. 2004) and OZ-439 (Miller *et al*. 2013).

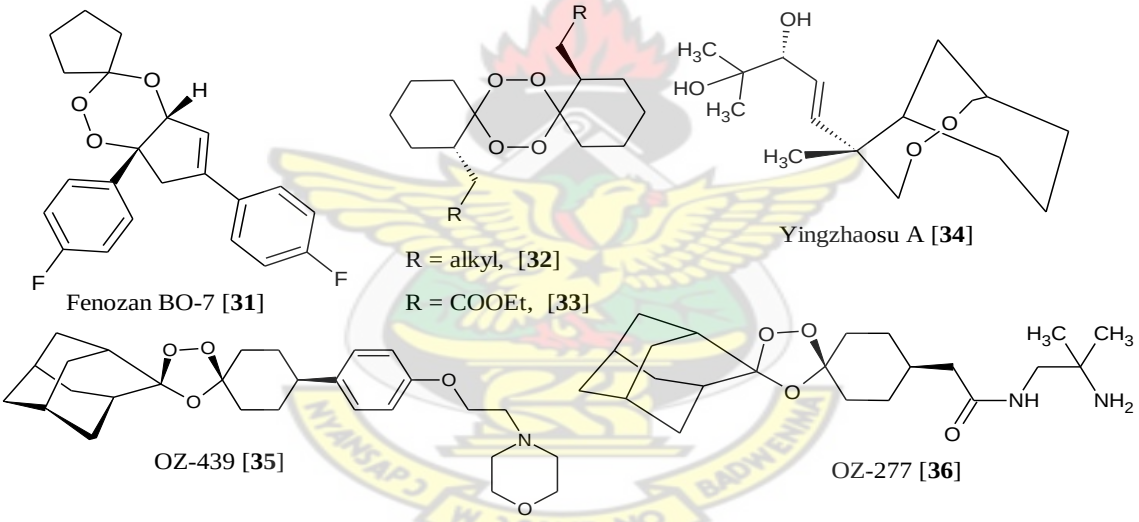


Figure 1.7 - Synthetic Endoperoxides with antimalarial activity

1.2.2.3.4 Antibiotics and other miscellaneous agents

Over the years, the treatment protocol for malaria has evolved to include antibiotics established to possess antimalarial activity. World Health Organization recognizes some antibiotics for the treatment of uncomplicated malaria (Bosman *et al*. 2001). For example, Clindamycin [**37**], a semi-synthetic lincomycin, which is employed in the treatment of uncomplicated malaria among pregnant women in combination with quinine in Ghana (MOH 2009; STG 2010) due to synergistic effects (Losert *et al*. 2000). Clinical trials of its use as

monotherapy is also documented (Lell & Kremsner 2002). Clindamycin is established to inhibit *Plasmodium falciparum In-vitro* in a dose-dependent manner, with a possible site of action being the apicoplast (Seaberg *et al*. 1984). Doxycycline [**38**], an example of the tetracyclines, is also used in malaria treatment, preferably in chemoprophylaxis (Schuhwerk & Behrens 1998; Ohrt 1997; Pang *et al*. 1987). It inhibits protein synthesis in the *Plasmodium* by reversibly inhibiting the 30S ribosomal subunit (Block 2011). It is usually the preferred of the tetracyclines because of its longer half-life, reliable absorption and better safety profile in patients with renal insufficiency, where it may be used with caution. It is relatively water insoluble but very lipid soluble. It may be given orally or intravenously (WHO 2010). Doxycycline can also be combined with quinine in places of high quinine resistance (Bosman *et al*. 2001). Azithromycin [**39**], belonging to a new class of azalid macrolide antibiotics, is also employed in malaria chemoprophylaxis. It is structurally similar to erythromycin but is better tolerated, has a broader antimicrobial spectrum of action, and provides prolonged tissue levels. It is an efficient blood schizonticide but has a relatively slow action. There is report on its use for monotherapy but several studies have documented its benefit in combination therapies (Andersen *et al*. 1995; Na-Bangchang & Kanda 1996; Taylor & Richie 1999; Andersen & Ager 1994; Andersen *et al*. 1998). Thiostrepton [**40**] is a natural cyclic oligopeptide antibiotic, derived from strains of *Streptomyces azureus* and *Streptomyces laurentii*. This compound is established to exert antimalarial activity (McConkey *et al*. 1997) by binding to ribosomal RNA (rRNA) (Clough *et al*. 1997; Rogers *et al*. 1997).

Sulphonamides like Sulphadoxine [**41**] and diaminopyridines, for example Proguanil [**43**] and Pyrimethamine [**42**] have played very instrumental role in the treatment of malaria for several years. Sulphadoxine [**41**] is usually combined with Pyrimethamine [**42**] whiles Proguanil is comes combined with Atovaquone [**44**]. The combinations are considered blood

schizontocides (Block 2011). Sulphadoxine interferes with the parasite's ability to synthesize folic acid by blocking the incorporation of p-aminobenzoic acid (PABA) to form dihydropteroic acid whiles pyrimethamine inhibits the reduction of folic acid to its active tetrahydrofolate coenzyme form, which is normally needed for many important reactions involving pyrimidine biosynthesis (Olliaro 2001). The focus in the parasite is regeneration of N^5, N^{10}-methylene tetrahydrofolate from dihydrofolate, formation of which is blocked by sulphonamides. The synthesis of thymidine 5'-monophosphate (a pyrimidine analogue) from deoxyuridine 5'-monophosphate is a universal reaction in all cells forming DNA. Thus combining the two offers synergism (Shapiro & Goldberg 2006; Block 2011). Proguanil is a pro-drug metabolized into Cycloguanil [**45**]. Unlike the former combination, the latter combination have mechanisms of inhibition unrelated. While Atovaquone [**44**] is a selective inhibitor of the *Plasmodium's* mitochondrial electron transport system, Cycloguanil is a dihydrofolate reductase inhibitor just like pyrimethamine. However, their use in malaria treatment have been inundated by resistance development recently. Notwithstanding that, they still find use in some places where they are employed in combinations with other antimalarial agents in the quest to minimise risk of resistance development (Bosman *et al*. 2001). Other antibiotics that have been established to exert antimalarial effects are Ciprofloxacin [**46**], which act against DNA Gyrase and Rifampicin [**47**], acting against RNA polymerase all in the Apicoplast of the parasite (Dahl & Rosenthal 2007) and Fosmidomycin [**48**] (Lell *et al*. 2003; Missinou *et al*. 2002) and Mirincamycin [**49**] (Held *et al*. 2010).

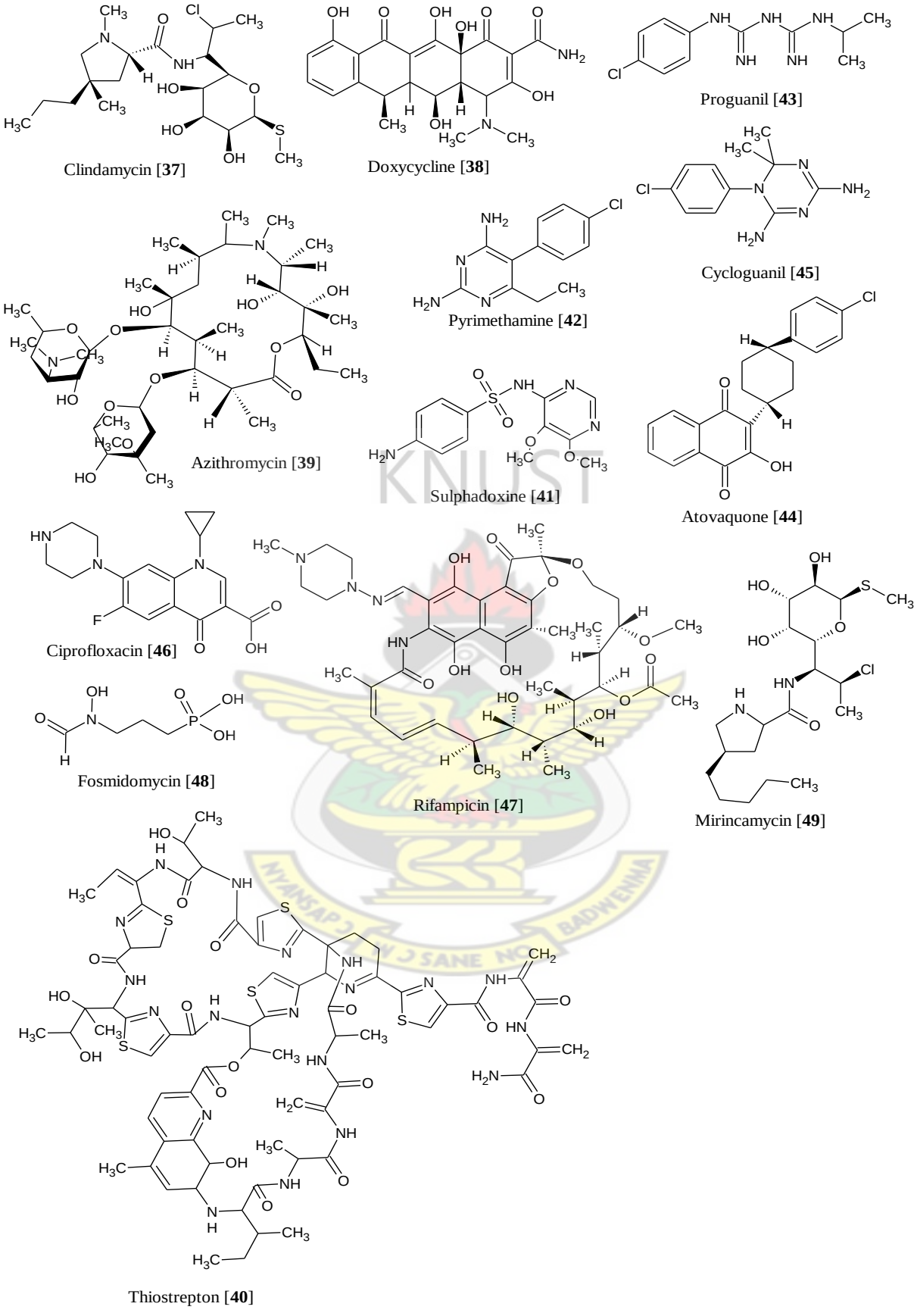


Figure 1.8 - Antibiotics with antimalarial activity

1.2.2.4 GAMETOCYTE STAGE

Ultimately after several cycles of asexual multiplication, some of the merozoites after invading the red cells differentiate into either male or female gametocytes. This stage of the parasite links the vector to the host, especially when the vector can only ingest the gametocytes to continue with the life cycle of the parasite and also the next stage to be ingested into the vector cannot be in the asexual form (Talman *et al*. 2004). The stimulating factor for gametocytogenesis in the host has not yet been identified but different authors have reported of inducing gametocytes differentiation *In-vitro* for their studies. Some of these include addition of red cell lysate (Smalley *et al*. 1981), human serum and lymphocytes (Ponnudurai *et al*. 1989), mammalian hormones (J W Barnwell *et al*. 1989; Ockenhouse *et al*. 1989), high levels of reticulocytes (Roberts *et al*. 1985), some inhibitors of nucleic acid synthesis (Berendt *et al*. 1989) including antifolates (Ockenhouse 1992) and also chloroquine (Sherman *et al*. 2003; Baruch *et al*. 1995). It has however been established that the ratio of production of asexual forms to gametocytes is 1:10 (Kitchen & Putnam 1942) while others even report 1:156 (Eichner *et al*. 2001).

Antifolates, for example, sulphadoxine-pyrimethamine, are associated with post-treatment prevalence (Targett *et al*. 2001) and increased density of gametocytes (Robert *et al*. 2000). This could be attributed to drug induced release or redistribution of gametocytes (Targett *et al*. 2001). Patients carrying gametocytes are most probably carriers of resistant parasites, fuelling the spread of antimalarial resistance and this is possible as the slower clearance and prolonged presence of asexual parasites associated with resistance increases gametocytogenesis (Barnes & White 2005). Hence, the need to concentrate on the elimination of this stage of parasite development to reduce transmission on the whole and also to reduce the rate of resistance development contributed by them. However, some authors hold a contrary view on the effect of eliminating gametocyte on resistance development (Hastings

2006). The argument from the angle of population genetics is that, gametocytocidal agents reduce transmission of drug-sensitive forms to a greater extent than the resistant ones due to susceptibility, thereby increasing the spread of the latter (Hastings 2006).

Gametocytes are usually divided into five stages of development. The first three stages of the sexual parasites are sequestered, and they are potentially susceptible to the drugs meant for the asexual stage of infection. However in stage 4, they move into circulation and by stage 5, the gametocytes circulate and are resistant to all drugs except the 8-aminoquinolines (Barnes & White 2005; Araújo *et al*. 2005; Gogtay & Kamtekar 2006). Antimalarial drugs established to be active against the asexual stages of *P. vivax*, *P. malariae* and *P. ovale* also act against the gametocytes, but only affect the first three stages of *P. falciparum* gametocyte development (Bunnag *et al*. 1980). The artemisinin derivatives may affect the fourth stage gametocytes, but only the 8-aminoquinolines are active against mature forms (ter Kuile *et al*. 1993; Bunnag *et al*. 1980). Notwithstanding this observation, the clinical use of artemisinins have resulted in the decline in infectivity (Chen *et al*. 1994) and transmissibility of parasites from host to vector (Price *et al*. 1996).

1.2.3 TRADITIONAL MEDICINAL PLANTS AND MALARIA

The number of available and effective antimalarial drugs is fast declining most especially because of drug resistance-associated mutations in the parasite genes such as *P. falciparum* chloroquine resistance transporter (*pfcrt*), *P. falciparum* multi-drug resistant 1 (pf*mdr1*) and genes coding for antifolates resistance (*dhfr*/*dhps*). This has led to widespread resistance to all known classes of antimalarial compounds with recent addition being spouts of artemisinin resistance in regions in Cambodia and Thailand (Valderramos *et al*. 2010; Wellems *et al*. 1990; Heseltine 2010). While there is much need for more antimalarial agents, the drug development pipeline remains woefully thin, with little chemical diversity. Currently no clinically accepted alternative to the valuable artemisinins have been developed (Grimberg &

Mehlotra 2011). Some of the approaches that are currently being adopted to discover new agents to fight the resistance menace include (a) enhancing the efficacy of currently used agents by combination (b) developing new drugs from agents used to treat other infectious diseases with known safety profile; (c) chemical modification of already existing antimalarial agents to enhance their activity; (d) discovering new agents from natural sources (which will be the focus of discussion in this section); (e) high throughput screening of diverse chemical libraries to discover lead compounds and (f) discovery of new agents through parasite genome-based ("targeted") studies (Grimberg & Mehlotra 2011).

Traditional medicinal plants have for a very long time played an integral role in the treatment of malaria. Before the advent of orthodox antimalarial medicines, traditional medicinal plants were used for treatment. *Cinchona sp*. bark was identified as early as 1627 to be used by the Native Americans in Peru for treating malaria (Lederberg 2000). The *Cinchona* bark having gained popularity, was then taken to Europe in the seventeenth century and empirically used to treat fever and pain until 1820, when the French chemists Pelletier and Caventou isolated the active ingredient, quinine (Faurant 2011). For centuries to come, quinine and its semisynthetic derivatives produced after the Second World War enjoyed monopoly till concerns about resistance started to surface. Amidst the plight inflicted by resistance development to quinine and its analogues, the research programme 523 (named after its official starting date, 23rd of May 1967) was launched in China and this not only led to the discovery of artemisinin but also new quinoleine derivatives that have helped curb the menace (Li *et al*. 2006). Getting back to the crossroad, especially now that the reality of resistance to the 'mighty' artemisinins hits us, there is a need to salvage the situation. Maranz argues that if there exist a potential African plant with artemisinin-like effects, then it is either currently not in widespread patronage and thus, its effects are flying under the radar (Maranz 2012). This has sparked up a new interest in antimalarial plant research. From the knowledge

of ethnopharmacological studies on traditional medicinal plants used in different parts of the globe, it has become feasible to design further studies to isolate compounds from these plants and optimize them into new lead compounds in the quest to discover new antimalarial agents. A number of studies have been conducted to identify medicinal plants with significant antiplasmodial or antimalarial activity (Omoregie & Sisodia 2012; Melariri *et al*. 2011; Bero *et al*. 2005; Awe *et al*. 1998; Oseni & Akwetey 2012; Abiodun *et al*. 2011; Köhler *et al*. 2002; Soh & Benoit-Vical 2007), and isolate and characterize the bioactive compounds (Bero *et al*. 2009; Khalid *et al*. 2007; Isaka *et al*. 1999; Kraft *et al*. 2000; Carraz *et al*. 2008; Frederich *et al*. 2008) for further consideration in drug discovery and development.

At this point, a discussion on the selected medicinal plants for the study would be beneficial. These are *Mitragyna inermis* (Willd) O. Kuntze (Rubiaceae), *Pseudocedrela kotschyi* (Schweinf.) Harms (Meliaceae), and *Moringa oleifera* Lam. (Moringaceae).

1.2.3.1 *MITRAGYNA INERMIS*

1.2.3.1.1 Botanical description of the plant

Figure 1.9 - Leaves and twigs of *Mitragyna inermis*

Mitragyna inermis (willd.) kuntze (family: Rubiaceae), with traditional names like; *giyeya* (Hausa)*; okobo* (Yoruba)*; okpetenyi* (Igbo); *dondoleyee* (Waale - Sissali), is a shrub growing in some parts of West Africa including Ivory Coast, Ghana, Mali and Senegal (Toure *et al*. 1996). It occurs generally in swampy savannah regions, flooded in the raining season. It can be recognized by the opposite pairs of thin leaves, small heads of white or yellowish flowers and the small blackish heads of fruits which remain on the tree for a long time (Tor-Anyiin & Orokpo 2012). It is 6-9 m in height with a diameter of 60-90 cm.

The tree has low branches and bushy crown and its bark is smooth, grey brown, with a few scales. The leaves are normally 6-12 cm long, 3.5-7.5cm broad and broadly elliptic rounding, at the base. It is also acuminate at apex, with short hairs, thin and light green in colour. The plant bears fruits between December and June and its fruits are usually dense dark brown or blackish balls, 12-18 mm diameter (Tor-Anyiin & Orokpo 2012). The individual fruits are club-shaped and are about 5 mm long. They also contain winged seed (Tor-Anyiin & Orokpo 2012).

1.2.3.1.2 Ethnopharmacological Studies

The fruits, leaves, stem-bark and roots of this plant have been documented to possess quite a number of medicinal properties. Infusions, decoctions or concoctions of different parts of it find use as an abortifacient and also in the management of many infections and diseases like abdominal irritation, abscesses, anaemia, arthritis, baby growth delay, chicken pox, conjunctivitis, constipation, cough, diabetes, diarrhoea, dysentery, fever, general weakness, gonorrhoea, headache, hepatitis B, infant umbilical pains, internal inflammation, respiratory infection, ringworm, scabies, syphilis, urinary tract infection, vomiting and wounds (Karou *et al*. 2011; Tor-Anyiin & Orokpo 2012). The traditional use in periodic fever and malaria treatment is also captured (Asase *et al*. 2005; Ollivier *et al*. 2005; Sam *et al*. 2011). Stem bark extracts of *M. inermis* have been shown to exhibit moderate to significant antibacterial activity against *Staphylococcus aureus, Escherichia coli, Streptococci pyogenes, Salmonella typhi* and *Proteus mirabolis* (Tor-Anyiin & Orokpo 2012). Zongo *et al*. also document the *In-vitro* antibacterial activity of total alkaloids from the leaves as well as their anticonvulsant effects (Zongo *et al*. 2009). Other ethnopharmacological effects that have been established for the plant include *In-vitro* antiplasmodial activity (Mustofa *et al*. 2000) and toxicity of its alkaloids (Traore *et al*. 2000); the cardiovascular properties of the aqueous extracts of the stem bark (Ouédraogo *et al*. 2004); the hypoglycaemic effects of ethanol extract from stem

bark (Adoum *et al*. 2012) and a pharmacological investigation of the alkaloids (Toure *et al*. 1996).

1.2.3.1.3 Non-Medicinal Uses

In the horticulture industry, the plant is cultivated for its ornamental benefits. The bark finds use in the production of materials for building, carpentry, fishing among others. There are reports of use of leaves serving as food for humans as well as foliage for animals (Burkhill 2004).

1.2.3.1.4 Chemical constituents isolated with their bioactivity

The leaves of *Mitragyna inermis* contain tetracyclic and pentacyclic oxindole and indole alkaloids which include uncarine D [**53**], rhyncophylline [**51**], isorhyncophylline [**50**], rotundifoline [**57**], isorotundifoilne, ciliaphylline, speciogynine, pteropodine (uncarine C), isopteropodine (uncarine E), uncarine F, mitraphylline [**52**], isomitraphylline and mitraciliantine (Toure *et al*. 1996; Shellard *et al*. 1971; Shellard & Sarpong 1969; Shellard & Sarpong 1970) (See figure 1.9 below). Some of these compounds have also been identified in different species of the *Uncaria* genus and have undergone partial synthesis (Hart *et al*. 1967). Corynoxine A [**55**] and B [**54**] have also been isolated from the plant (Shellard *et al*. 1971). The proportions of these compounds in the leaves have been shown to be variable and dependent on seasonal variations and geographical locations (Karou *et al*. 2011). For example, it was reported that isorhyncophylline and rotundifoline are the main alkaloids present in leaves collected in Ghana, while another study in Mali showed that these two compounds were present, but in low proportion, whereas uncarine D was the main alkaloid (Shellard & Sarpong 1970; Ollivier *et al*. 2005). Some of these alkaloids are also present in other genus of the same Rubiaceae family, for example, rhyncophylline and isorhyncophylline, which are present in the *Uncaria* genus (Shellard *et al*. 1971).

In one study, uncarine D exhibited cytotoxic activity against different cancerous cell lines (Muhammad et al. 2001). In another, a mild *In-vitro* antiplasmodial activity for uncarine D was demonstrated (Ollivier *et al*. 2005; Bero *et al*. 2009). 5-cholesten-3-phenyl-22, 24-β-diketone, a compound with a steroidal nucleus was isolated and established to possess hypoglycaemic effect (Adoum *et al*. 2012). From the bark of the plant, inermiside I and II, which are two 27-nor-triterpenoid glycosides have also been isolated (Cheng *et al*. 2002).

1.2.3.2 *PSEUDOCEDRELA KOTSCHYI*

1.2.3.2.1 Botanical description

Pseudocedrela kotschyi (Schweinf.) Harms, locally known in Ghana as *kpela* (Waale - Sissali), belongs to the Meliaceae family. It is common in the savannah woodland (Hutchison & Dalziel 1958; Shahina 1989). The tree is 20 metres in height with a wide crown, fissured bark and fragrant white flowers. The bark is bitter and exudes a dark-coloured gum (Lemmens 2008).

Figure 1.10 - Leaves of *Pseudocedrela kotschyi*

1.2.3.2.2 Ethnopharmacological Studies

The root bark is reported to be used in Togo as a febrifuge and in the treatment of gastro-intestinal diseases and rheumatism (Hutchison & Dalziel 1958). In Ghana, the twigs and leaves are employed in the treatment of malaria and stomach aches (Asase *et al*. 2005). The decoction is used as a wash for ulcers (Hutchison & Dalziel 1958; Oliver-Bever 1986). The roots and leaves are used to treat rheumatism and dysentery in some parts of Nigeria. In Northern Nigeria, the plant has also served purposes of occasional ingredient for use in arrow poison (Oliver-Bever 1986). In West Africa, it has been established that the root of *P*.

kotschyi is widely used as chewing sticks for dental cleaning (Akande & Hayashi 1997; Tapsoba & Deschamps 2006; Okunade & Adejumobi 2007; Kassim *et al*. 2009). It has found value in the treatment of toothache and internal wound in the Northern parts of Cote d'Ivoire. The root of the plant, which is also used to treat intestinal helminthiasis, has been found to be a potential source of antibacterial agents (Koné *et al*. 2004). The stem and root barks are documented to contain essential oils which exhibit low antiradical and antioxidant effects (Boyom *et al*. 2004). One study investigated *In-vitro* inhibitory effects on growth and development of the schizont stage of *Plasmodium falciparum* from the root extracts (Kassim *et al*. 2009) whiles another carried out isolation of compounds from the dichloromethane of the same roots and evaluated the antiprotozoal activities of the isolated compounds against *Leishmania donovani, Trypanosoma brucei rhodesiense, Trypanosoma cruzi*, and *Plasmodium falciparum* (Hay *et al*. 2007) (see **Sec 1.2.3.2.4** for the names of the isolated compounds). The ethanol extract of the fresh leaves have been evaluated for its antipyretic effects in rats (Akuodor *et al*. 2013). In addition to this, the n-butanol fraction from the ethanol extract of the leaves has further been shown to exhibit anti-nociceptive and anti-inflammatory activities in mice and rats respectively (Musa *et al*. 2005). The aqueous leaf extract of the plant is also proven to reduce the onset and the duration of the sleeping time induced by pentobarbitone in rats. It was also shown in the same study to increase the depression or sedation time followed by sleep (Anuka *et al*. 2005). The antimicrobial activity of different organic and aqueous extracts of the leaves, stem bark and roots have also been documented by some authors (Asase, Kokubun & Grayer 2008; Ayo *et al*. 2010; Adeniyi *et al*. 2010). Extracts of the plant in addition with other plants traditionally used in Nigeria, have been studied for their molluscicidal activity against laboratory-reared *Lymnaea natalensis* Krauss (Kela *et al*. 1989).

1.2.3.2.3 Non-Medicinal Uses

The wood is valued for high-class woodwork, furniture and cabinet making, and for construction. It resembles mahogany, but is heavier and harder. It is also used for doors, windows, frames, drums, barrels, canoes, mortars, bowls and gun-stocks. The wood is also suitable for flooring, interior trim, ship building, vehicle bodies, toys, novelties, carvings, turnery, veneer and plywood. The wood is as well used as firewood and for charcoal production (Lemmens 2008).

1.2.3.2.4 Chemical constituents isolated with their bioactivity

A bitter non-nitrogenous compound, pseudocedrelin, has been isolated from the bark and this has been shown to elicit piscidal activity (Oliver-Bever 1986). The leaves are shown to contain 3-O-rhamnosides of myricetin and quercetin, and 3-O-glucosides of the same aglycones which are responsible for antimicrobial activity (Asase, Kokubun & Grayer 2008). The roots have also been shown to be a good source of antiprotozoal agents against *Leishmania donovani*, *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi* and *Plasmodium falciparum* and these are phragmalin-type limonoid orthoacetates, namely, kotschyins A [**62**], B [**63**] and C [**64**], 7-deacetylgedunin [**58**] and 7-deacetyl-7-oxogedunin [**59**] (Hay *et al*. 2007). The latter, 7-deacetoxy-7-oxogedunin and pseudrelones A, B [**60**] and C, which are also limonoids, have also been isolated from the wood oil (Ekong & Olagbemi 1967; Taylor 1979; Niven & Taylor 1988). Investigation into the essential oils from the stem and root barks of the plants yielded mostly sesquiterpenoids. δ - Cadinene [**61**], was found to be the most abundant (that is, 31.3%) in the stem bark while the root bark oil contained a majority of oxygenated sesquiterpenoids, with cubebols, representing almost one-third of the extract. These compounds were shown to play a role in the antiradical and antioxidant effects of the extracts (Boyom *et al*. 2004).

1.2.3.3 *MORINGA OLEIFERA*

1.2.3.3.1 Botanical description

Figure 1.11 - Leaves of Moringa oleifera

Moringa oleifera (formerly known as *Moringa pterygosperma*) is one of the best known and most widely distributed and naturalized species of the monogeneric family, Moringaceae. It is commonly known as *horseradish tree* or *drumstick tree* (English); *atiuwuse, yevu-ti, kpokpoti* (Ewe) and *Obnukuo* (Dagari). The tree ranges in height from 5 to 10 m. It is found wild and cultivated throughout the plains, especially in hedges and in house yards. It thrives best under the tropical insular climate and is plentiful near the sandy beds of rivers and streams (Anwar *et al*. 2007). It tolerates a wide range of rainfall with minimum annual rainfall requirements estimated at 250 mm and maximum at over 3000 mm and a pH of 5.0–9.0. *Moringa oleifera* is a native of the western and sub-Himalayan tracts, India, Pakistan, Asia Minor, Africa and Arabia, but is now distributed in the Philippines, Cambodia, Central America, North and South America and the Caribbean Islands (Anwar *et al*. 2007). The plant is a small deciduous with pale grey bark and soft wood. It has crooked stem that often forks near the base. Its twigs and young shoots are densely hairy. The leaves are tripinnate usually with six pairs of pinnae, large and alternately arranged on the stem (Iwu 1993).

1.2.3.3.2 Ethnopharmacological Studies

Moringa oleifera forms an essential food component which has recently received a lot of attention. The leaves, fruit, flowers and immature pods of this tree are patronized for their highly nutritive components (Ferreira *et al.* 2008). These parts of the plant have been shown to contain a profile of important minerals, hence serving as good sources of proteins,

vitamins, β-carotene, amino acids including the essential sulphur amino acids, methionine and cysteine and various phenolics (Anwar *et al*. 2007; Ferreira *et al*. 2008; Idohou-dossou *et al*. 2011). The leaves have calcium equivalent of four times of milk, vitamin C content being seven times that of oranges, while the potassium levels is three times that of bananas. The iron levels are three times of spinach, four times the amount of vitamin A in carrots, and two times the protein in milk (Farooq *et al*. 2012).

In addition to the nutritional benefits from the different parts of the plant, most of the parts are considered to contain medicinal properties, hence their use in conditions like ascites, rheumatism, treatment of venomous bites and also for their cardiac and circulatory stimulant effects. Use is also made of in inflammatory conditions like sinusitis, bronchitis, stomatitis and asthma (Goyal *et al*. 2007). The root bark is as well employed in the treatment of eye infections, dyspepsia and spleen enlargement. The same, is also reported to exhibit abortifacient effects (Goyal *et al*. 2007). The leaves are used as antihelminthics, aphrodisiacs, antihallucinogens, and also used in the treatment of hiccups and asthma as well (Goyal *et al*. 2007).

In this regard, several ethnopharmacological studies have been carried out to confirm and validate the traditional use of this plant and also serve as an avenue to isolate and characterize compounds responsible for these effects. Thus, different authors have documented the *In-vitro* antimicrobial activities of aqueous and methanol extracts of the plant against microorganisms like *Pseudomonas aerogenosa, Staphylococcus albus, Staphylococcus aureus, Escherichia coli, Staphylococcus pyogenes* and *Enterobacter aerogenes* as well as fungi like *Trichophyton mentagrophyte, Pullarium sp, Aspergilus flav*us and *Penicillium sp.* (Thilza *et al*. 2010; Oluduro 2012; Walter *et al*. 2011). The wound healing effects of the aqueous extracts of the leaves have also been studied (Rathi *et al*. 2006) in culmination with the antioxidant properties which are implicated in the wound healing effects (Luqman *et al*.

2012; Iqbal & Bhanger 2006; Sreelatha & Padma 2009; Verma *et al*. 2012). The antioxidant effects are also shown to play a vital role in establishing the antiulcer activity of the leaves (Verma *et al*. 2012). The aqueous and methanol fraction of the leaves have shown antiulcerogenic and hepatoprotective effects in rats (Pal *et al*. 1995). The aqueous and alcohol extracts from the flowers were also established to elicit significant hepatoprotective effect (Ruckmani *et al*. 1998).

Following the traditional use of the plant in the treatment of ailments, the anti-inflammatory, antispasmodic and the diuretic effects of the different parts of the parts have also been evaluated (Gilani *et al*. 1992; Gilani *et al*. 1994; Dangi *et al*. 2002; Caceres *et al*. 1992; Ezeamuzie *et al*. 2008). *Moringa* leaf juice is known to have a stabilizing effect on blood pressure. The far-reaching blend of diuretic along with lipid and blood pressure lowering effects make this plant highly useful in cardiovascular disorders (Anwar *et al*. 2007; Fahey 2005). Studies commissioned on the leaves, the aqueous and ethanol extracts of the pods and other parts like pulp, seeds and coat have demonstrated the blood lowering effects of the plant amidst isolation of compounds responsible for these activities (Faizi *et al*. 1994; Faizi *et al*. 1998; Faizi *et al*. 1995). The crude extract of the leaves is shown to possess a significant cholesterol lowering action in the serum of high fat diet fed rats (Ghasi *et al*. 2000). The fruit has been established to decrease the serum cholesterol, phospholipids, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL) cholesterol to phospholipid ratio, atherogenic index lipid and also reduce the lipid profile of liver, heart and aorta in hypercholesteremic rabbits with increase in the excretion of faecal cholesterol (Mehta *et al*. 2003). In the field of parasitology, the oviposition stimulant and ovicidal effects of *Moringa oleifera* water soluble lectin on *Aedes aegypti*, which is a vector for yellow and dengue fevers, have been investigated (Moura *et al*. 2012). In addition to that, the larvicidal, pupicidal and repellent potential of the methanol extract of the seeds against malarial vector,

Anopheles stephensi Liston have also been carried out with satisfactory outcome implicating its use in the control of the insect (Prabhu *et al*. 2011). The plant formed part of samples that have been investigated for their *In-vitro* antiplasmodial activity using the microculture radioisotope technique (Köhler *et al*. 2002). The *In-vivo* antiplasmodial activity of the crude n-hexane and ethanol extracts of the plant seeds have recently been investigated against albino mice preinfected with chloroquine sensitive *Plasmodium berghei* ANKA strain (Olasehinde *et al*. 2012).

In addition to these effects are others like regulation of the thyroid hormone shown by the aqueous extract of the leaves (Pal *et al*. 1995; Tahiliani & Kar 2000), the significant protection of the bone marrow chromosomes in mice against radiation (Rao *et al*. 2001) and the prophylactic and therapeutic effects of the leaves against Herpes Simplex Virus type 1 (HSV1) (Lipipun *et al*. 2003).

1.2.3.3.3 Non-Medicinal Uses

Moringa seeds have been discovered to be one of the best natural coagulants. The crushed seeds have served as a viable replacement for synthetic coagulants (Kalogo *et al*. 2000). Crude extracts from the seeds have replaced alum in treating the highly turbid water for traditional use in some parts of Sudan, in the midst of fear of alum causing gastrointestinal disturbances and Alzheimer's disease (Crapper *et al*. 1973; Miller & Stober 1984; Martyn *et al*. 1989; Muyibi 1994). The seeds have also been studied and used as less expensive biosorbent for the removal of cadmium (Cd) from aqueous media (Sharma *et al*. 2006). The oil from the plant obtained by standard transesterification procedure with methanol and an alkali catalyst at 60 ^{o}C is shown to contain high content of oleic acid (>70%) with saturated fatty acids and this has proven to be a good feedstock for biodiesel, which is an alternative to petroleum-based conventional diesel fuel (Rashid *et al*. 2008).

1.2.3.3.4 Chemical constituents isolated with their bioactivity

Moringa oleifera is rich in compounds containing the sugar, rhamnose and a fairly distinctive class of compounds known as glucosinolates and isothiocyanates (Fahey 2005). The stem bark has been reported to contain the alkaloids, moringine and moringinine (Kerharo 1969). Vanillin, β-sitosterol [**71**], β-sitostenone, 4-hydroxymellin and octacosanoic acid have also been isolated from the stem bark (Faizi *et al*. 1994).

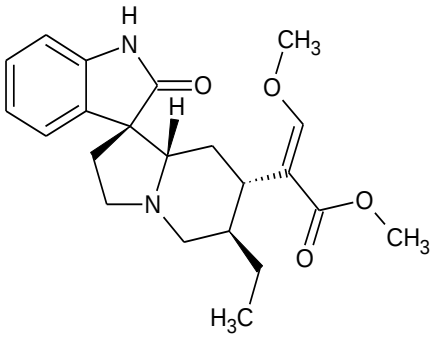
Moringa leaves serve as a vital source of natural antioxidants due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Anwar *et al*. 2005; Makkar & Becker 1996). The use of the leaves for purposes of dietary supplementation has stem out from the presence of constituents like ascorbic acid, oestrogenic substances and β-sitosterol, iron, calcium, phosphorus, copper, vitamins A, B and C, α-tocopherol, riboflavin, nicotinic acid, folic acid, pyridoxine, β-carotene, protein, and in particular essential amino acids such as methionine, cystine, tryptophan and lysine (Makkar & Becker 1996).

The composition of the sterols of *Moringa* seed oil principally include campesterol, stigmasterol, β-sitosterol, Δ^5-avenasterol and clerosterol accompanied by minute amounts of 24-methylenecholesterol, Δ^7-campestanol, stigmastanol and 28-isoavenasterol (Tsaknis *et al*. 1999; Anwar & Bhanger 2003; Anwar *et al*. 2005). The composition of the oil has been shown to be very different from conventional edible plant oils (Rossell & Pritchard 1991). From *Moringa* leaves have been isolated nitriles, mustard oil glycosides and thiocarbamate glycosides which are established to be responsible for the blood pressure lowering effect. Most of these compounds occur in their acetylated form and happen to be rare in nature (Faizi *et al*. 1995). An activity guided fractionation of the ethanol extract from the leaves led to the isolation of the following compounds, niazinin A [**66**], niazinin B, niazimicin [**67**] and niazinin (A + B) which mediate through calcium antagonism to elicit a blood pressure

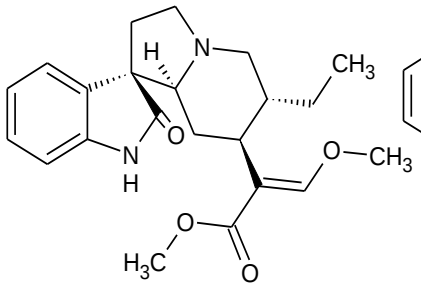
lowering effect in rats (Gilani *et al*. 1994). The study on the pods and the other parts like the pulp, coat and seed also led to the isolation of thiocarbamate and isothiocyanate glycosides known to show hypotensive effects (Faizi *et al*. 1995).

The presence of 4-[α-(L-rhamnosyloxy) benzyl]-O-methyl thiocarbamate in the ethanol extract of the leaves is shown to possess antispasmodic effect which has been linked to the use of the leaves in the treatment of diarrhoeal episodes (Gilani *et al*. 1992). The hepatoprotective effects offered by extracts of the flowers (Ruckmani *et al*. 1998) have been attributed to the presence of flavonoids, an example of which is quercitin. The antimicrobial effects of the roots of the plant have been attributed to the presence of pterygospermin, known to be both antibacterial and antifungal (Ruckmani *et al*. 1998). A similar compound with similar activity has also been identified in the extracts from the flowers (Das *et al*. 1954). Other isolated compounds known to elicit antibacterial activities are 4-α-L-rhamnosyloxybenzylisothiocyanate [**65**], from the root extracts (Eilert *et al*. 1981) and the aglycone of deoxy-niazimicine (N-benzyl, S-ethyl thioformate) [**69**], isolated from the chloroform fraction of an ethanol extract of the root (Nikkon *et al*. 2003).

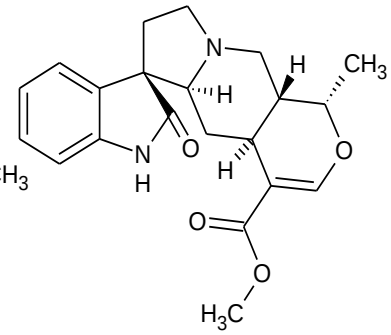
O-Ethyl-4-(α-L-rhamnosyloxy)-benzyl carbamate, 4(α-L-rhamnosyloxy)-benzylisothiocyanate, niazimicin [**67**] and 3-O-(6′-O-oleoyl-β-D-glucopyranosyl)-β-sitosterol [**64**] have been evaluated for *in-vitro* antitumor promoting activity and were also showed to exhibit significant inhibitory effects on Epstein–Barr virus-early antigen (Makonnen *et al*. 1997). Guevara *et al*. (1999) proposed that niazimicin is a potent chemopreventive agent in chemical carcinogenesis.



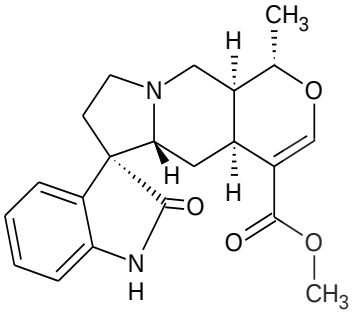
Isorhychophylline [**50**]



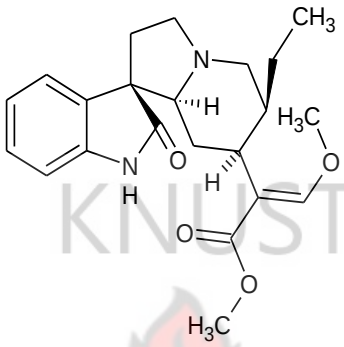
Rhychophylline [**51**]



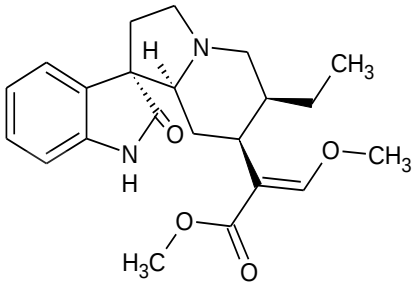
Mitraphylline [**52**]



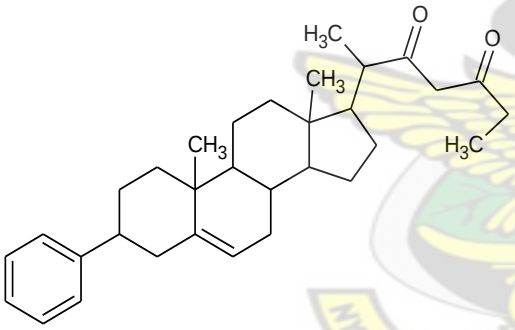
Uncarine D (Speciophylline) [**53**]



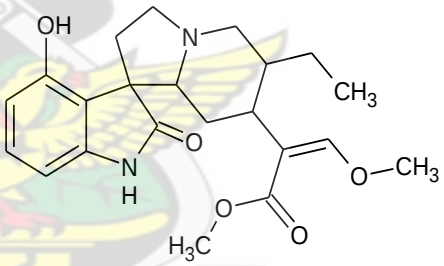
Corynoxine B [**54**]



Corynoxine A [**55**]



5-cholesten-3-phenyl-22, 24-β-diketone [**56**]



Rotundifoline [**57**]

Figure 1.12 - Some isolated compounds from Mitragyna inermis

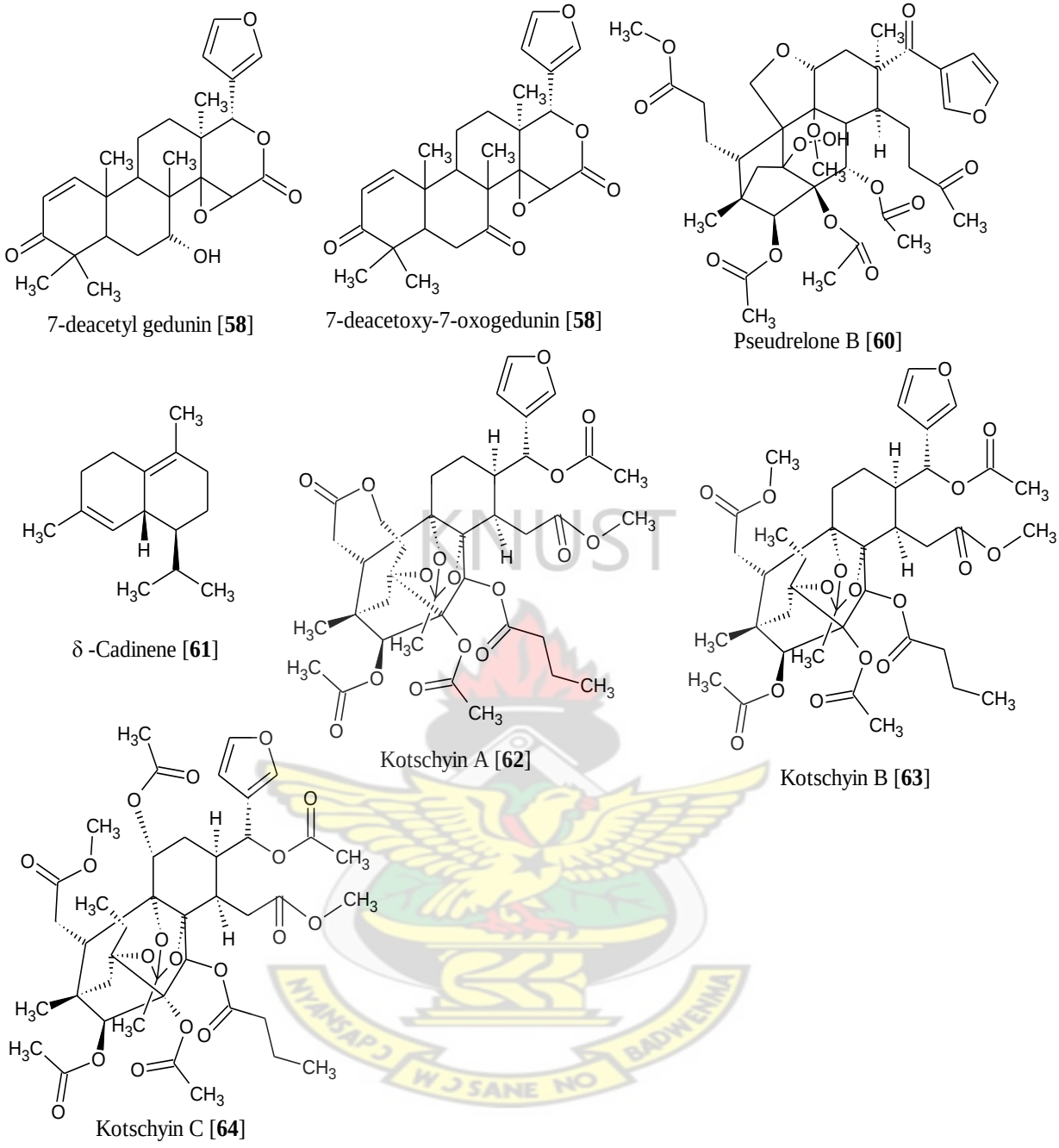
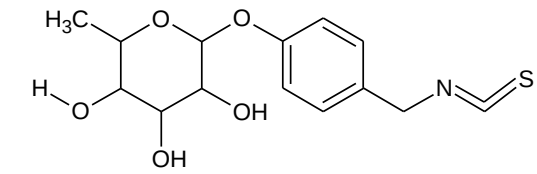
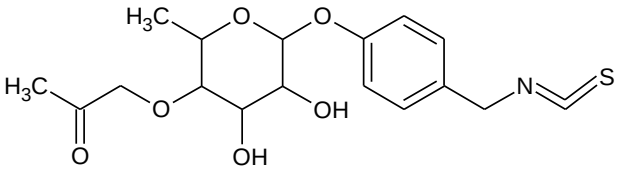


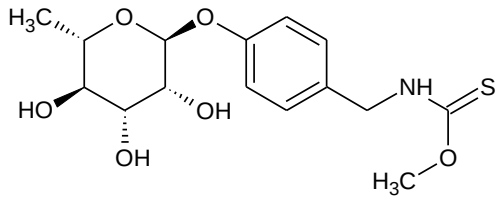
Figure 1.13 - Some isolated compounds from Pseudocedrela kotschyi



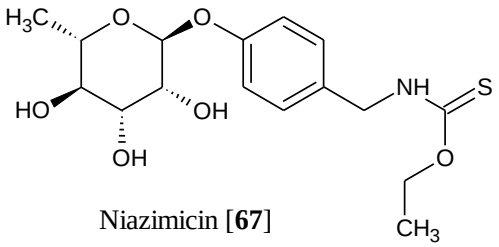
4-(α-L-rhamnosyloxy) benzyl isothiocyanate [**65**]



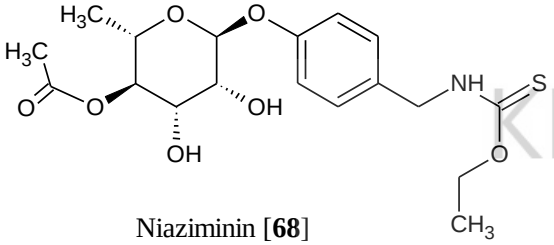
4-(4'-O-acetyl-α-L-rhamnosyloxy) benzyl isothiocyanate [**74**]



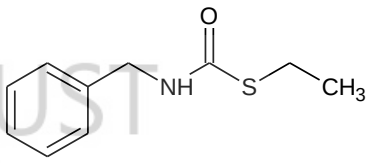
Niazinin A [**66**]



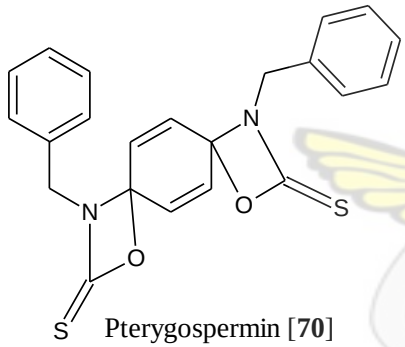
Niazimicin [**67**]



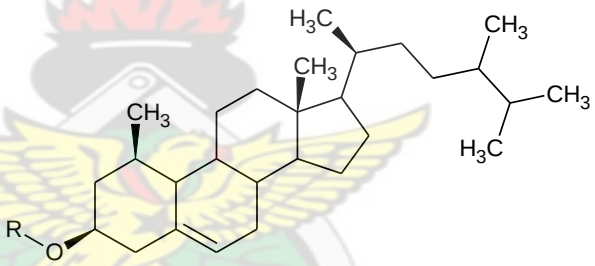
Niaziminin [**68**]



Deoxy-Niazimicine [**69**]



Pterygospermin [**70**]



R = H, β-Sitosterol [**71**]

R = 6'-O-oleoyl-β-D-glucopyranosyl, 3-O-(6'-O-oleoyl-β-D-glucopyranosyl)-β-sitosterol [**72**]

R = β-D-glucopyranosyl, β-sitosterol-3-O-β-D-glucopyranoside [**73**]

Figure 1.14 - Some isolated compounds from Moringa oleifera

CHAPTER TWO

PHYTOCHEMICAL INVESTIGATION

2.1 INTRODUCTION

Medicinal plant-derived substances or constituents recently have become of great interest due to the versatility in their applications. Of all natural sources, traditional medicinal plants are the richest in bio-resource of drugs for traditional systems of medicine, orthodox medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Tiwari *et al*. 2011). As a result of the recent insurgence of interest in the plant kingdom as a potential source of new drugs, strategies for the fractionation of plant extracts based on biological activity rather than on a particular class of compound, have been developed. Chemical examination follows the isolation of the active fraction (Evans 2002).

The phytochemical investigation of a plant may involve authentication and extraction of the plant material; separation and isolation of the constituents of interest: characterization of the isolated compounds; investigation of the biosynthetic pathways to particular compounds: and quantitative evaluations. Parallel to this may be the pharmacological assessment of the separated components (Evans 2002). The genesis of the investigation stems from the identification of certain constituents in the plants of interest known as secondary metabolites. These are plant constituents not considered to primarily contribute to the normal biochemical activities of the plant, but are thought by some scientists to aid in their survival in their environment (Evans 2002). Some of the secondary metabolites produced by plants include alkaloids, flavonoids, tannins, glycosides among others (Evans 2002).

Several studies have confirmed different biological activities of these secondary metabolites (Ferreira *et al*. 2008; Oluduro 2012; Bimlesh *et al*. 2011; Seyoum *et al*. 2006; Li *et al*. 2008;

Zongo *et al*. 2009; Traore *et al*. 2000; Faizi *et al*. 1998; Ara *et al*. 2004). Thus, an initial confirmation of these metabolites creates a good platform to link up their presence with the bioactive effects of the investigating plants.

In the current study, a phytochemical investigation was carried out on the dried powdered samples of the medicinal plants (see **Sec 3.2** below) and also on different extracts produced from them. As these compounds have different affinities for different solvents for extraction (Tiwari *et al*. 2011), it is expected that the different extracts produced from the medicinal plants would contain to a degree different secondary metabolites which would invariably affect their bioactivity; hence the need to carry out this investigation for the purposes of the study.

2.2 COLLECTION AND PREPARATION OF PLANT MATERIALS

Medicinal plant samples employed in the study included twigs of *Mitragyna inermis* (Willd) O. Kuntze (Rubiaceae), leaves of *Pseudocedrela kotschyi* (Schweinf.) Harms (Meliaceae) and leaves and stem bark of *Moringa oleifera* Lam. (Moringaceae). These samples, as shown to be employed in the traditional treatment of malaria (Asase *et al*. 2005; Köhler *et al*. 2002) in different parts of the country were collected, identified and authenticated in the Department of Herbal Medicine, KNUST before use and specimens placed in the Department Herbarium. *Moringa oleifera* was collected from the environs of Appiadu - Kokoben (Kumasi 3.6 km W), a suburb in the Kumasi metropolis. *Pseudocedrela kotschyi* samples were also collected from the KNUST environs (Kumasi 1.7 km NW) whiles *Mitragyna inermis* was collected from, Kwahu - Asakraka in the Eastern region ($6^{o}37'51"N\ 0^{o}41'28.1"W$).

Plant materials upon collection were dried at room temperature in the laboratory for 14 – 30 days and pulverized upon drying.

2.3 MATERIALS AND METHODS

2.3.1 MATERIALS

Unless otherwise specified, chemicals were of analytical grade and purchased from Sigma-Aldrich Co. Ltd. Irvine, UK. Organic solvents were of analytical grade and purchased from BDH Laboratory Supplies (England).

2.3.2 METHODS

2.3.2.1 Sample preparation and extraction

Initial preparation of samples involved simulation of the traditional modes of preparation of the plants for the purposes of malaria treatment. Thus, decoction of 1.000 kg of dried powdered twigs of *Mitragyna inermis* (Willd) O. Kuntze (Rubiaceae), 1.015 kg of dried powdered leaves *Pseudocedrela kotschyi* (Schweinf.) Harms (Meliaceae) and 1.000 kg of dried leaves and 2.000 kg of dried stem bark of *Moringa oleifera* Lam. (Moringaceae) were prepared with distilled water. The duration of extraction was 1 hour. The extracts were then filtered with sterile Whatman Number 1 filter papers and the marcs obtained, dried for re-extraction for similar period for two more times. The filtrates were concentrated on a Rotary evaporator at 40 ^{o}C and concentrates, freeze dried to obtain dried extracts. During the process of freeze drying, the extract was first frozen and the solid water converted to the gaseous state without passing through the liquid state. The gaseous water then escaped leaving behind the solid extract. The extracts were then stored in a frozen form at – 17 ^{o}C for future use.

For the organic extracts, methanol was the solvent employed for the extraction for a duration of 7 – 10 days. 1.5 kg of dried pulverized twigs of *Mitragyna inermis* (Willd) O. Kuntze (Rubiaceae) was extracted with 5 litres of methanol. 141.25 g of dried pulverized leaves of *Moringa oleifera* Lam. (Moringaceae) and 1.0148 kg of leaves of *Pseudocedrela kotschyi* (Schweinf.) Harms (Meliaceae) were also extracted with 2 litres and 5 litres of methanol respectively. The extracts obtained were filtered with sterile Whatman Number 1 filter papers

and marcs dried and re-extracted. The filtrates were concentrated on a Rotary evaporator at 40 ^{o}C and the concentrates evaporated on a water bath at 40 ^{o}C to dryness. Extracts were then kept under refrigeration at – 18 ^{o}C for future use. (See Table 3.1 in **Sec. 3.3.3.1** for a table showing the weights with the percentage yields of extracts obtained).

2.3.2.2 Phytochemical Analysis

In the quest to study the distribution of secondary metabolites in the different extracts obtained, a phytochemical analysis was conducted to test for either the presence or absence of them. Thus, phytochemical tests for the presence of alkaloids, sterols, coumarins, flavonoids, tannins, reducing sugars as general test for glycosides, saponin, cyanogenetic and anthracene glycosides were carried out.

Tests were first carried out on the dried powdered samples and later on the aqueous and the methanolic extracts. The standard methods employed for the tests are as described in literature (Sofowora 1993; Evans 2002; Harbone 1976).

2.3.2.2.1 Detection of alkaloids

Plant materials and extracts were extracted with ammoniacal alcohol, that is, strong Ammonia: 95% Ethanol (1:9). The extracts were filtered and solvents evaporated. 1% Sulphuric acid was then added and filtered to get off the undissolved constituents and also convert alkaloids to the soluble salt forms. The filtrates were then rendered alkaline with dilute ammonia and then shook gently with chloroform in a separating funnel. The chloroformic layer was then separated and solvent evaporated with the addition of 1% sulphuric acid to the residue. The following tests were then carried out on the acidified residue.

2.3.2.2.1.1 Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicated the presence of alkaloids.

2.3.2.2.1.2 Dragendorff's Test

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicated the presence of alkaloids.

2.3.2.2.2 Detection of Phytosterols

2.3.2.2.2.1 Salkowski's Test

Plant materials and extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shook and allowed to stand. Appearance of a golden yellow colour indicated the presence of triterpenes.

2.3.2.2.2.2 Libermann Burchard's test

Plant materials and extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring or blue green at the junction indicated the presence of sterols.

2.3.2.2.3 Detection of Coumarins

Plant materials and extracts were treated with chloroform and filtered. Evaporation was carried out and the residues extracted with hot water. Each solution obtained was divided into two, solutions A and B. Dilute ammonia was added to solution A and both observed under ultraviolet radiation. Solution A should give off blue green fluorescence to indicate the presence of coumarins whiles solution B should not.

2.3.2.2.4 Detection of Glycosides

2.3.2.2.4.1 Reducing sugars – Fehling's Test

Plant materials and extracts were hydrolysed with dil. Hydrochloric acid (HCl), neutralized with alkali (20% NaOH or KOH) and heated with Fehling's A & B solutions. Formation of brick- red precipitate indicated the presence of reducing sugars.

2.3.2.2.4.2 Test for Saponins (Froth Test)

Plant materials were extracted with hot water and the extracts were diluted with distilled water to 20ml. These were shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins.

2.3.2.2.4.3 Test for Anthracene glycosides

a) Borntrager's Test:

Plant materials and extracts were boiled with dilute Sulphuric acid, filtered whiles hot and allowed to cool. The filtrates were shaken with chloroform and the chloroformic layer separated and made alkaline with half its volume with dilute ammonia. The presence of anthracene glycosides was indicated by rose pink colouration.

b) Modified Borntrager's Test

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicated the presence of anthranol glycosides.

2.3.2.2.4.4 Test for Cyanogenetic glycosides

An amount of the powdered plant material and dried extract were placed in a dry test tube and a strip of sodium picrate paper suspended at the open end of the tube upon corking. The

test tubes containing them were warmed on a water bath and the release of hydrocyanic acid turned the paper reddish purple.

2.3.2.2.3 Detection of Flavonoids - Alkaline Reagent Test

The plant materials and the extracts were extracted with hot water and filtered. A strip of clean white paper was dipped into the filtrate and allowed to dry. Exposing the dried paper to fumes from concentrated ammonia turned paper to yellow if flavonoids were present and decolourisation took place upon exposure to fumes of concentrated HCl.

2.3.2.2.6 Detection of Tannins

2.3.2.2.6.1 Gelatin Test

To the aqueous extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins.

2.3.2.2.6.2 Lead Acetate Test

Extracts were treated with few drops of lead acetate solution. Formation of white coloured precipitate indicated the presence of tannins.

2.3.2.2.6.3 Ferric Chloride Test

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenolic compounds including tannins.

2.4 RESULTS

2.4.1 SAMPLE PREPARATION

Table 2.1 - Weights of extracts and percentage yields obtained in the study

Plant material	Voucher number	Weight of extract obtained (g)		Percentage yield (%)	
		Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract
Mitragyna inermis twigs	KNUST/HM1/2014/L030	16.736	33.9513	1.67	2.26
Pseudocedrela kotschyi leaves	KNUST/HM1/2014/L035	16.6736	60.019	3.33	5.91
Moringa oleifera leaves	KNUST/HM1/2014/L044	24.1123	16.4624	2.41	11.65
Moringa oleifera stem bark	KNUST/HM1/2014/S065	23.3355	-	1.17	-

2.4.2 PHYTOCHEMICAL INVESTIGATION

Table 2.2 – Phytochemical screening on the powdered samples

TEST	*MORINGA OLEIFERA*	*MITRAGYNA INERMIS*	*PSEUDOCEDRELA KOTSCHYI*
Alkaloids			
1. Dragendorff's test	+	+	+
2. Mayer's test	+	+	+
Phytosterols			
1. Salkowski's test (triterpenes)	+	+	+
2.Libermann's test (sterols)	+	+	+
Coumarins	–	+	+
Glycosides			
1. Fehling's test	+	+	–
2. Saponins	+	+	+
3. Anthracene	–	–	–
4. Cyanogenetic	–	–	–
Flavonoids	+	+	+
Tannins			
1. Gelatin test	+	+	+
2. Lead acetate test	+	+	+
3. Ferric chloride test	+	+	+

KEY: Present (+), Absent (-)

Table 2.3 - Phytochemical screening on the extracts produced from the medicinal plants

TEST	*M. OLEIFERA* LEAVES		*M. OLEIFERA* STEM BARK	*P. KOTSCHYI* LEAVES		*M. INERMIS* TWIGS	
	Aqueous Extract	Methanolic Extract	Aqueous Extract	Aqueous Extract	Methanolic Extract	Aqueous Extract	Methanolic Extract
ALKALOIDS							
Dragendorff's Test	+	+	+	+	+	+	+
Mayer's Test	+	+	+	+	+	+	+
PHYTOSTEROLS							
Salkowski's Test	–	+	–	–	+	–	+
Liebermann's Test	–	+	–	–	+	–	+
COUMARINS	–	–	–	–	+	–	+
GLYCOSIDES							
Fehling's Test (Reducing sugar)	+	+	+	–	–	+	+
Froth Test (Saponins)	+	–	+	+	+	+	–
Borntrager's Test (Anthracene)	–	–	–	–	–	–	–
Cyanogenetic glycosides	–	–	–	–	–	–	–
FLAVONOIDS	+	+	–	+	+	+	+
TANNINS							
Lead acetate Test	+	+	+	+	+	+	+
Gelatin Test	+	+	+	+	+	+	+
Ferric Chloride Test	+	+	+	+	+	+	+

KEY: Present (+), Absent (-)

2.4.3 SUMMARY OF RESULTS

Phytochemical investigation of the plant materials as well as the aqueous and methanolic extracts of the plants revealed the presence of secondary metabolites, some of which have been shown in several studies to be responsible for antiplasmodial activity. From table 2.2 above, it could be seen that the dried samples of all three medicinal plants contained alkaloids, phytosterols, saponins, flavonoids and tannins. Both *Mitragyna inermis* and *Moringa oleifera* contained reducing sugars whiles *Pseudocedrela kotschyi* did not. In addition to that also, *Mitragyna inermis* and *Pseudocedrela kotschyi* contained coumarins whiles *Moringa oleifera* did not. In the case of the extracts (table 2.3), only alkaloids and tannins were present in all the aqueous and the methanolic extracts. For phytosterols, which were present in the powdered plant materials, the investigation revealed its presence only in the methanolic extracts. Flavonoids, on the other hand were present in the leaves extracts (from the three plants), but were absent in the stem bark extract of *Moringa oleifera*. Reducing sugars, which were shown to be present in both *Mitragyna inermis* and *Moringa oleifera* plant samples, were also present in both aqueous and methanolic extracts of the same plants.

2.5 DISCUSSION

From literature, *Moringa oleifera* has been shown to contain alkaloids (Kurmi *et al*. 2011), phytosterols (sterols and terpenes), tannins, flavonoids (Kasolo *et al*. 2010), glycosides, volatile oils and carbohydrates (Kurmi *et al*. 2011). The results obtained from the current study confirm those in literature, which also report of the absence of coumarins just like the current study (Kasolo *et al*. 2010). On the contrary, anthraquinones, which were reported to be present in one of the studies (Kasolo *et al*. 2010), was in this study, shown to be absent. This could be as a result of chemical races, in which case, similar plants exhibit different

compositions as a result of their geographical locations. Different geographical settings have different soil compositions and ecosystems, and these factors affect the nature of secondary metabolites synthesized in the plants. In addition to that also, the detection of anthraquinones is dependent on the hydrolysis of the bond between the sugar moiety and the aglycone. In the case of an O-glycoside, C – O bond, due to its polarity is easily hydrolysed in the presence of the hydrochloric acid (HCl). However, in a C-glycoside, due to similarities in the electronegativities of the carbons in the C – C bond, hydrolysis with HCl is difficult to achieve, hence, would test negative. For *Mitragyna inermis*, alkaloids, flavonoids, tannins, terpenoids and resins have been reported in the stem bark of the plant (Tor-Anyiin & Orokpo 2012). Polyphenols, flavonoids, catechic tannins, sterols, triterpenes and alkaloids have also been reported in the leaves (Konkon *et al*. 2008). The results from this study confirmed documented evidence. Carbohydrates, reducing sugars, glycosides, flavonoids, steroids, saponins, tannins and alkaloids have been reported to be present in the leaves of *Pseudocedrela kotschyi* (Ayo *et al*. 2010; Akuodor *et al*. 2013). Saponins, alkaloids and tannins have also been shown to be highly concentrated in the stem and root (Kolapo *et al*. 2009). From the investigation, it could be inferred that constituents shown to be present conformed to that reported in literature.

Bioactivities of most plants have been attributed to the presence of the phytochemicals. Thus, the confirmation of the presence of some classes of compounds in the selected medicinal plants could be inferred to be responsible for their bioactivity. Alkaloids, as a class of compounds have played a very key role in medicine; from the time in history when opium, derived from *Papaver somniferum* was being used medically, through to when cocaine extracted from *Erythroxylum coca* was used as a local anaesthetic in surgeries up to recent times (Osbaldeston & Wood 2004). Since then, quite a number of studies conducted have proved to associate some biological activities to this class of compounds. Some of these are

antimicrobial effects (Zongo *et al*. 2009; Asase *et al*. 2008; Goyal *et al*. 2007), antihypertensive effects (Dangi *et al*. 2002), anti-inflammatory effects (Barbosa-Filho *et al*. 2006), anticancer effects (Wilson *et al*. 2001), antimalarial effects (Carraz *et al*. 2008; Ancolio *et al*. 2002; Andrade-Neto *et al*. 2003) among others. The confirmation thus, of alkaloids in the selected plants for the study, could be said to play a key role in their investigating *In-vivo* antiplasmodial properties. Bero *et al* and Lopes and Nogueira, in their reviews have documented isolated alkaloidal compounds known to possess *In-vitro* and *In-vivo* antiplasmodial activity (Bero *et al*. 2009; Lopes & Nogueira 2011).

Flavonoids, another major class of compounds confirmed in the plant samples and the extracts, do also affect their bioactivities. Flavonoids, which are mostly found in the aerial parts of plants, are chiefly responsible for most antioxidant and antiradical effects of natural products (Rice-Evans *et al*. 1996; Sreelatha & Padma 2009; Verma *et al*. 2012; Pietta 2000; Burda & Oleszek 2001). Thus, they have been shown to possess anticancer effects (López-Lázaro 2002), anti-allergic effects (Cheong *et al*. 1998), wound healing effects (Rathi *et al*. 2006) and antimicrobial effects (Liu *et al*. 2010). One study draws a link between the antioxidant effect to the antiulcer effect of *Moringa oleifera* (Verma *et al*. 2012). In addition to these, some flavonoids have been shown to elicit antiplasmodial effects both *in-vitro* and *in-vivo* (Kraft *et al*. 2000; Awasthi *et al*. 2009; Bero *et al*. 2009).

Tannins (polyphenols) are another class of compounds with established biological activities. Polyphenols have been suggested to be anticarcinogenic and antimutagenic (Chung *et al*. 1998). They do also possess antimicrobial effects (Chung *et al*. 1998), metal chelating properties, antioxidant effects (Hagerman 2002), and antiplasmodial effects (Bero *et al*. 2009). The same could also be said about the terpenoids, especially the sesquiterpenes, which have in recent times yielded the artemisinin compound, changing the paradigm in malaria treatment and also saponins, coumarins, volatile oils among others.

It is therefore, proposed from the phytochemical investigations conducted, that the presence of alkaloids, phytosterols, saponins, flavonoids, tannins and glycosides in the plant samples and the extracts, could be responsible for their antiplasmodial activity. It stands to reason that the presence or absence of some of these constituents and their concentrations or proportions in the plant material and the extracts could also affect the degree of inhibition of *Plasmodium* parasite growth, hence, probable differences in their activity. In addition, synergism, potentiation and antagonism are likely to play critical roles in the antiplasmodial activity of the plants (Rasoanaivo *et al*. 2011; Bell 2005; Maranz 2012). Some authors propose that the presence of other compounds in *Artemisia annua*, especially the flavonoids, may synergistically act to enhance the action of Artemisinin (Liu *et al*. 1992; Liu *et al*. 1989; Elford *et al*. 1987). For example, the flavone known as Casticin, in *in-vitro* studies, was shown to enhance the activity of artemisinin by 3-5 fold (Elford *et al*. 1987). In this case, the flavonoid, which may possess either antiradical properties or antiplasmodial effect or both in itself, would act to increase the activity of the sesquiterpene, Artemisinin, which is the main antimalarial compound in the plant. Another beneficial effects has to do with the likely reversal of resistance by some plant constituents. For example, Chrysosplenol-D and Chrysosplenetin which are two flavonoids (flavones to be more specific) also present in *Artemisia annua*, have been shown to inhibit multi-drug resistant (*mdr*) pump in *Staphylococcus aureus* (Stermitz *et al*. 2002). These same compounds had earlier on been shown to potentiate the activity of Artemisinin against *P. falciparum* (Stermitz *et al*. 2002). Thus, it can be argued that, the potentiating effects of the two flavonoids when combined with Artemisinin, stem out from the inhibitory effects of the compounds on *P. falciparum* multi-drug resistance 1 (*pfmdr*-1) gene, which encode for p-glycoprotein pump. This pump is notable for antimalarial drug efflux resulting in resistance development. In the nutshell, the likeliness of similar interactions co-existing among the different classes of compounds identified to be present in the samples, cannot be overemphasized and this is likely to

influence the overall effects of the plants against the malaria parasites, which is the focus of the research.

2.6 CONCLUSION

Moringa oleifera, Mitragyna inermis and *Pseudocedrela kotschyi* plant materials upon examination have been shown to contain phytochemicals like alkaloids, phytosterols, saponins, flavonoids, tannins, coumarins and reducing sugars. The aqueous and methanolic extracts also contain similar constituents as in the dried plant materials although with some variations. Since some of these secondary metabolites have been linked to antiplasmodial activity, it stands to reason that, the medicinal plants under investigation may possess *In-vivo* antiplasmodial activity.

CHAPTER THREE

IN-VIVO ANTIPLASMODIAL ACTIVITY

3.1 INTRODUCTION

Malaria in human subjects is caused by five species in the *Plasmodium* genus. These parasites are known to be very specific in their infection, in that, they tend to cause infection only in human host (Fidock *et al*. 2004). They are essentially unable to infect non-primate animal models such as mice, rats, monkeys, birds, guinea pigs and hamsters (Collins *et al*. 2008; Fidock *et al*. 2004). With ethical concerns raised on clinical studies forbidding the use of human subjects in preliminary drug discovery studies among other limitations, such as the lack of access to relevant organs and tissue samples, and the inability to manipulate the immune response of humans for mechanistic studies (Langhorne *et al*. 2011), the use of animal models pave a way to unravel the mysteries surrounding pathogenesis, diagnosis and treatment of the disease condition (Janse *et al*. 2012). Thus, *Plasmodium* species well suited and scientifically documented for *In-vivo* studies are employed. Some of the organisms suited for murine models are *Plasmodium berghei*, *Plasmodium yoelii*, *Plasmodium chabaudi* and *Plasmodium vinckei* (Fidock *et al*. 2004) and these have been used extensively in drug discovery and early drug developments. There exist minor differences among these four rodent malaria parasites, for example differences in morphology, developmental time and size of different stages and isoenzymes (Carter & Diggs 1977). These variable characteristics influence the host-parasite interactions and are thus, responsible for differences in the course of infection, virulence and pathology (Carter & Diggs 1977). An established variation in terms of preference of invasion consists of the preference of *P. chabaudi* and *P. vinckei* for both immature and mature red blood cells whiles *P. berghei* and *P. yoelii* have a significant predilection for reticulocytes (Carter & Diggs 1977; Carlton *et al*. 2002). Other animal

models that have been used in malaria research include *P. coatneyi* in rhesus monkeys (Davison *et al*. 2000), *P. falciparum* in *Aotus* monkeys (Chang et al. 1996; Kumar et al. 1995; Glew et al. 1978; Andersen et al. 1995), *P. cynomolgi* in *Anopheles gambiae* (Kocken *et al*. 2009; Shortt & Garnham 1948; Galinski *et al*. 1987; Paskewitz *et al*. 1988; Zheng *et al*. 1997) and *P. relictum* in birds (Hayworth & van Riper 1987; Kilpatrick & LaPointe 2006; Fix & Waterhouse 1988; Atkinson et al. 1995).

Rodent models validation have come up by virtue of discovery of well notable antimalarial agents including mefloquine, halofantrine and more recently artemisinin derivatives (Fidock *et al*. 2004; Li *et al*. 2006). Other agents like quinine, proguanil, pamaquine, mepacrine, chloroquine, sulphadiazine, sulphanilamide, and 4:4'-diaminodiphenyl sulphone have been tested on these models (Thurston 1950). In view of their proven use in the prediction of treatment outcomes for human infections, these models remain a mainstay in drug discovery and development pathway. Recent literature have recorded new potential antimalarial agents through their use (Jimenez-Dıaz *et al*. 2013; Ene *et al*. 2009; Murakami *et al*. 2003; Oseni & Akwetey 2012; Melariri *et al*. 2011). In addition to that, *Plasmodium berghei* have been the subject of extensive research for many years by a group of researchers from University of Leiden headed by Dr. Chris J. Janse according to the institution's website (Janse *et al*. 2014). *Plasmodium berghei* presents in five strains, that is, K173, SP11, ANKA, LUKA and NK65 (Carter & Diggs 1977; Killick-Kendrick 1978).

Animal models are employed in malaria research because the entire life cycle of the rodent parasites as well as the morphology of the different developmental stages are conserved between mammalian parasites and the rodent parasite (Sinden 1978). Their life cycles are highly comparable, starting from their infection of the *Anopheles* sp., to the sexual sporozoites formation in the mosquito which then migrate to the liver to produce liver merozoites and schizonts upon infection and then to the erythrocytic stages and finally to the

gametocytes formation (Aikawa & Seed 1980; Sinden 1978). In addition to that, the genome organisation is conserved between rodent and human malaria parasites. The sequenced genome of *P. falciparum* and that of the four rodent parasites are all organized into 14 linear chromosomes with size ranging between 0.5 – 3.8 megabasepairs (Mb) (Janse *et al*. 1994). Also, the metabolic pathways are conserved between mammalian malaria parasites and that of the rodents. To date, there has not been any report of gross differences between the metabolic pathways of the two groups of parasites. Their similarities in terms of sensitivity to antimalarials and other specific inhibitors cannot be overemphasized (Barnwell *et al*. 1989). Notwithstanding these exceptional similarities championing the use of animal models, there are however, some slight differences that may pose questions. One of them is that, there are differences in the life cycle between the various mammalian malaria parasites and the rodent parasites, however these are mainly restricted to the duration of development and size of the different dividing stages (Janse *et al*. 2014). For example, there are significant variations in developmental time and size of liver schizonts, erythrocytic schizonts and oocysts, but these are the end result of multiple stages of mitotic division resulting in the production of large numbers of daughter parasites. However, ultra-structural study revealed similarities in the mitotic process but differences in the growth rate (G1-phase), mitotic rate (S- and M-phases) and the number of mitotic divisions per schizont of oocyst (Janse *et al*. 2014). Other fundamental differences include (a) the existence of the hypnozoite stage in *P. vivax* and some other primate malaria species but absent in the rodent species and (b) the distinct morphology and developmental time of the gametocytes of the human parasite *P. falciparum* when compared to the rodent parasite species (Janse *et al*. 2014).

3.1.1 FOUR-DAY SUPPRESSIVE TEST

This is the most widely used test for the initial screening of an agent suspected to possess antimalarial or antiplasmodial properties. The parasites commonly employed are *P. berghei* (mostly) and *P. chabaudi* (less frequently) (Fidock *et al*. 2004). In the test, the efficacy of four daily doses of the investigating agent, administered orally or intravenously, is measured by comparison of blood parasitemia after the four days of therapy (that is, on day-5 post infection) for both test and control groups. 'Mouse survival time' in treated and untreated mice can also be evaluated from such a study (Fidock *et al*. 2004).

Murine infection is typically initiated by needle passage from an infected rodent to a naïve rodent via the intraperitoneal or preferably the intravenous route, often using a small inoculum (typically in the range of 10^5 -10^8 infected rbcs/ml) (Janse *et al*. 2014). Chloroquine and the artemisinin derivatives normally serve as the control agents (Fidock *et al*. 2004).

Compounds identified as being active in four-day assays can subsequently be progressed through several secondary tests which are as follows:

3.1.2 FULL DOSE RANGING FOUR DAY SUPPRESSIVE TEST

In this test, different doses (say, 300 mg/kg, 100 mg/kg, 30 mg/kg and 10 mg/kg) are administered orally with a ball-ended needle or intravenously, and parasitemia is monitored after four days of therapy. ED_{50} and ED_{90} values are calculated by plotting the log dose against activity and these represent respectively, the drug concentrations at which 50% and 90% of suppression of parasitemia was achieved. It must be noted however, that due to the short half-life of some drugs, administration could be done twice daily. Other parameters that could also be measured in this study include relative potency when compared to an appropriate standard drug, and oral bioavailability (when different routes of administration are involved, for example, oral and parenteral) (Fidock *et al*. 2004).

3.1.3 ONSET OF ACTIVITY AND RECRUDESCENCE TEST

This test involves the use of a single dose (for example, 100 mg/kg) of the drug under investigation. The drug is administered on day-3 post-infection using the subcutaneous (s.c.) route. The control group only receive suspension vehicle (or diluent). Blood examination and parasitemia estimation starts 12 hours after the administration followed by subsequent determinations at 24 hours post infection and extending to day 33. Results are expressed as the rapidity of onset of activity (that is, disappearance of parasitemia), time point of recrudescence, increase of parasitemia and survival in number of days (Fidock *et al*. 2004).

3.1.4 CURATIVE TEST

Curative test involves a seven - day drug administration of either a single dose or different doses of the drug under investigation. In the test, therapy starts 3 days after infecting experimental mice and establishing parasitemia. The administration takes place for the next seven days during which period, blood smears are taken to determine the daily parasitemia and their respective percentage suppressions (Fidock *et al*. 2004).

3.1.5 PROPHYLACTIC TEST

Prophylactic activity is evaluated by administering the compound prior to infection, followed by daily examination of smears. The administration of the drug is done for three consecutive days before infection. Smears are then examined daily to assess for suppression of parasitemia. Survival times are also measured (Fidock *et al*. 2004).

3.2 MATERIALS AND METHODS

3.2.1 MATERIALS

3.2.1.1 Animal and diet

The study was approved by the Noguchi Memorial Institute for Medical Research Institutional Animal Care and Use Committee (NIACUC). All animal procedures were carried out in accordance with the suggested ethical guidelines for care of laboratory animals by NIACUC. The test animals employed in the study were specific pathogen free Imprinted Control Region (ICR) and BALB/c male mice. The mice were obtained from the Department of Animal Experimentation, NMIMR, of the University of Ghana and were between 8-10 weeks old. The weights ranged between 25-30 g for male ICR mice and 20-25 g for male BALB/c mice. They were maintained in an Animal Biosafety Level 2 facility (ABSL2) with controlled temperature (23 ± 2ºC) and illumination (12h; 6:00 am to 6:00 pm). They were housed in standard stainless steel cages (34×47×18 cm^3) with soft wood shavings as bedding, in groups of five, and fed with normal commercial pellet diet (AGRICARE, Kumasi) and, given autoclaved water *ad libitum*. The animals were transferred from the Barrier Breeding Facility to the Infectious Animal Experimentation Unit, for which reason they were allowed to acclimatize for 3 days prior to their randomization into the various experimental groups for experimentation.

3.2.1.2 Parasite strain

The parasites used for this experiment were cryopreserved *Plasmodium berghei* (NK 65) strain. This parasite strain was donated by the Department of Immunology of the Noguchi Memorial Institute for Medical Research, of the University of Ghana, Legon, Accra. Parasite stock was preserved in liquid nitrogen at -196 ºC. Parasite stock was sustained by serial passage of blood from infected mice to uninfected mice. Parasitemia was monitored regularly. At a desired parasitemia (for example, 25-30%), the mice were bled and euthanized

(Olfert *et al*. 1993; Garber 2011). Blood samples were collected into heparinized tubes and either frozen in cryotubes and stored in liquid nitrogen at -196 ºC for future use or injected intraperitoneally into an uninfected mouse to keep parasites alive.

3.2.1.3 Thawing and Inoculation of cryopreserved parasites

a) Materials needed
Thawing mix
Complete Parasite Medium (CPM)
Frozen stabilates of *Plasmodium berghei* NK 65 strain.
70% Ethanol
1ml sterile syringe for i.p injection

b) Equipment
Light microscope
Liquid N_2 freezer
Biological Safety Cabinet (BSC)
Pasteur micropipette
Water bath
Centrifuge

3.2.1.4 Inoculum preparation and Infection of Mice

a) Materials needed
Phosphate buffered saline (PBS)
Trypan Blue stain
Sterile cryotubes

b) Equipment
Haemocytometer (Neubeaur Counting Chamber)
Sterile syringe
Pasteur micropipette
Light microscope

3.2.2 METHODS

3.2.2.1 Thawing and Inoculation of cryopreserved parasites (Collins *et al*. 2008)

The vials containing frozen parasites were removed from the liquid nitrogen tank (-196 ^{o}C) and placed in 37 ^{o}C water bath for 1-2 minutes to thaw, shaking gently and taking care to

keep the screw cap on top of the vial above the water surface. The stabilized vial was then centrifuged at 1500 rpm for 10 mins. The exterior of the vial was carefully wiped with 70% ethanol and then placed aseptically in the Biological Safety Cabinet (BSC). The supernatant was removed with a Pasteur micropipette and equal volume of thawing mix added and mixed well. Centrifuging was carried out again at 1500 rpm for 10 minutes and aseptically placed back in the BSC after wiping the exterior of the vial with 70% ethanol. Once again, the supernatant was removed and about 500 µL Complete Parasite Medium (CPM) added and mixed well. A third centrifuging was carried out at 1500 rpm for 10mins and aseptically transferred into the hood. The supernatant was removed and about 1ml CPM was added and mixed well. 200 µL of the revived parasites now prepared for inoculation was injected intraperitoneally into a donor mouse and thin blood smears prepared daily to monitor parasite load, to establish parasitemia and confirm infection after 3-4 days.

Parasites normally appeared after 2 to 3 days, and after 8 to10 days, the parasitemia usually peaked around 30-50%. Mice succumbing to the infection usually died between days 9-12. Higher infectious doses give an earlier peak of parasitemia, while lower doses give a delayed peak; that is, a 10× concentrated dose gave a peak approximately 1 day earlier, while a dose diluted 1:10 caused a peak 1 day later. If injection had been by the intravenous (i.v) route, only one-tenth of the i.p. dose would be needed to obtain similar parasitemia (Fidock *et al*. 2004).

3.2.2.2 Inoculum preparation and Infection of Mice

Heparinized blood was taken from an infected donor mouse with approximately 30% parasitemia, (i.e. 30% of the erythrocytes are parasitized) through cardiac puncture after euthanasia. The total number of erythrocytes/ml of the whole blood collected was estimated and the blood was then diluted with phosphate buffered saline (PBS) to 10^8 parasitized

erythrocytes/ml. An aliquot of 0.2 ml or 200µL (= $2x10^7$ parasitized erythrocytes/ml) of this suspension was injected intraperitoneally (i.p.) into experimental mice (Fidock *et al*. 2004).

Estimation of the total erythrocytes/ml of the infected mouse was made with the help of the Neubeaur counting chamber or haemocytometer (fig. 3.1A) after diluting the whole heparinized blood from the euthanized mouse by 1000 fold. The diluted blood was then mixed in 1:1 ratio with Trypan Blue stain and allowed to stand for 10 minutes. The haemocytometer was then charged with the final solution obtained; counting of red blood cells was done under magnification of ×40 in all four big quadrants (fig. 3.1B) and the average number of cells per quadrant calculated to finally estimate the total number of erythrocytes/ml by factoring in the dilution factor. The total number of parasitized red blood cells was then estimated. Below is an illustration of a haemocytometer used to estimate the total number of parasitized red blood cells/ml (Bastidas 2014).

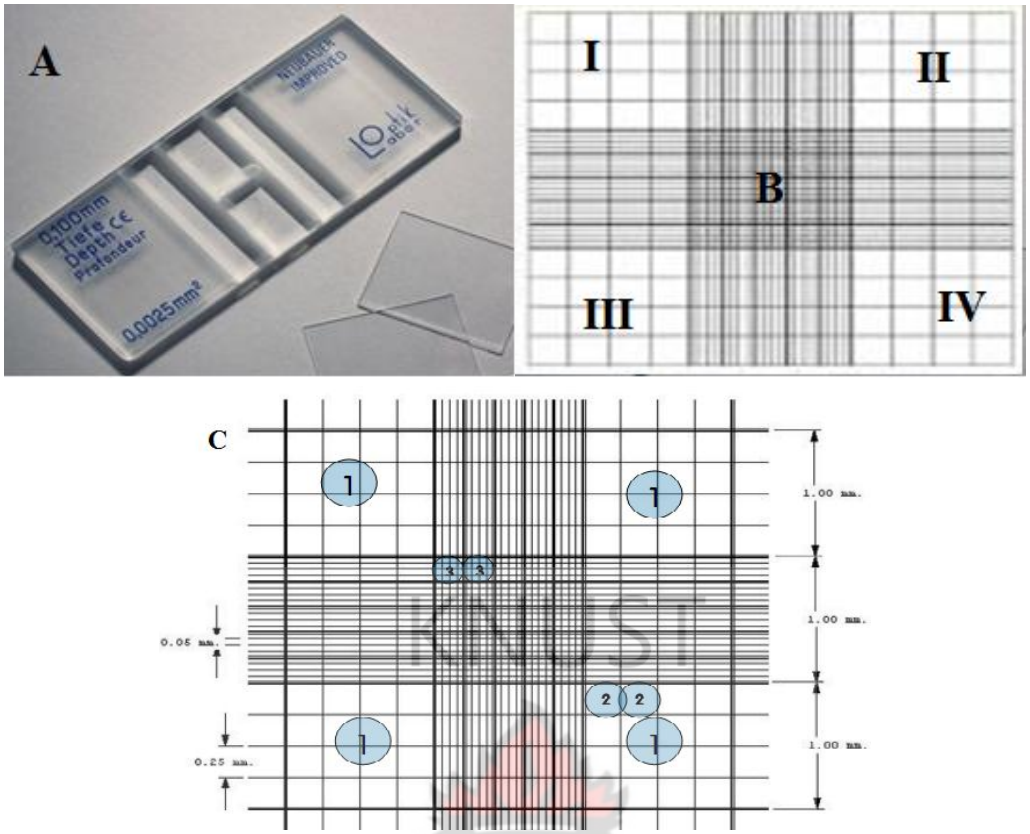


Figure 3.1 - Images of a haemocytometer with divisions under magnification (x40) used to estimate total RBCs

Calculations

Concentration (rbcs/ ml) = $\frac{\text{Average number of cells/quadrant}}{\text{Volume (ml)}}$

The number of cells equals the sum of all the counted cells in all squares counted and the volume is the total volume of all the squares counted.

Area of a big quadrant = 0.1 cm × 0.1 cm = 0.01 cm^2 (NB: 10 mm = 1 cm)

Volume of a quadrant = Area × depth of the chamber, where depth = 0.1 mm or 0.01 cm (from fig. 4.1A above)

Volume = 0.01 cm^2 × 0.01 cm = **0.0001 cm^3 (ml)** = **0.1 µL** (given that 1000 µL = 1 ml)

Therefore, concentration (rbcs/ml) = $\frac{\text{average number of cells/ quadrant}}{0.0001\ \text{ml}}$ = N × 10^4 cells/ml.

If dilution factor of the whole blood = DF, and N = average number of rbcs counted per quadrant, then, total number of rbcs/ml for the mouse = **$\underline{\mathbf{N \times 10^4 \times DF}}$**

Total number of parasitized rbcs/ml = total rbcs/ml × % parasitemia

Concentration of 1×10^8 parasitized rbcs/ml was prepared from the stock blood with Phosphate buffered saline and subsequent preparation of 2×10^7 parasitized cells/ml for intraperitoneal injection into experimental mice.

3.2.2.3 Estimation of Parasitemia

A thin blood film from tail blood was prepared and fixed with methanol for 2 min. The smear was then stained with Giemsa (dilute Giemsa stain is 1:10 in phosphate buffer) and allowed to stand for 10 -15 minutes before washing off gently with distilled water. Counting was done with the aid of a Leica DM 2500 M light microscope at a magnification of ×100 with oil of immersion and five fields counted each averaging 300-500 red blood cells. The estimation for parasitemia was done as follows:

Percentage parasitemia = $\frac{\text{number of infected red blood cells/field}}{\text{total number of red blood cells/field}} \times 100\%$

3.2.2.4 *In-vivo* Experimentation

Initial evaluation of the viability of the parasites after thawing and injection into a 'naïve' mouse was carried out. This was done to confirm the viability and regain of virulence of the parasites since they had been cryopreserved in liquid nitrogen at -196 ^{o}C for a long period of time. Regain of viability would be indicated by increasing parasite multiplication in the

blood. This was to be desired so that any inhibition in their multiplication upon treatment with an agent would signify antiplasmodial activity of the product administered.

In order to evaluate the antiplasmodial activity of the aqueous and methanolic extracts of the medicinal plants under investigation, a four – day suppressive and a seven - day curative *In-vivo* methods with some modifications were used. Using a murine model, that is, *P. berghei* infected Imprinted Control Region (ICR) mice, aqueous extracts of the medicinal plants, that is, ML, PK and MT at 500 mg/kg and MSB at 750 mg/kg, were initially screened for *In-vivo* antiplasmodial activity. Subsequent evaluation on the most effective extract from the screening, ML, was conducted on four doses (250, 500, 750 and 1000 mg/kg). These extracts were administered twice daily for a period of four or seven days, depending on the test, with estimation of parasitemia from thin blood smears. Methanolic extracts (that is, MOR, PSD and MIT) were given at a daily dose of 500 mg/kg for each extract. Estimation of the parasitemia was as follows:

$$\text{Percentage parasitemia} = \frac{\text{Infected Reb blood cells/field}}{\text{Total Red blood cells/ field}} \times 100\%$$

Upon estimation of the daily parasitemia of the individual experimental mice, percentage suppressions of the extracts were also estimated and compared to the positive control group, which is infected but not treated. Below is the relationship used to estimate the daily percentage suppression or activity of the extracts.

$$\text{Percentage suppression or activity} = \frac{\Delta(\text{mean parasitemia of positive control}) - \Delta(\text{mean parasitemia of treated group})}{(\Delta\text{mean parasitemia of positive control})} \times 100\%$$

3.2.2.4.1 Screening of the aqueous extracts for *In-vivo* antiplasmodial activity

A period of 7 days was employed for screening the aqueous extracts. The aqueous extracts from the twigs of *Mitragyna inermis* (Willd) O. Kuntze (Rubiaceae) (MT), leaves of *Pseudocedrela kotschyi* (Schweinf.) Harms (Meliaceae) (PK) and leaves (ML) and stem bark (MSB) of *Moringa oleifera* Lam. (Moringaceae) were screened for their antiplasmodial activity at a dose of 500 mg/kg for ML, PK and MT and 750 mg/kg for MSB. Experimental male ICR mice of weight 25-30 g and free from pathogens, were used for the study, after allowing for 3 days acclimatization. The animals were then weight matched into 3 groups for screening two extracts at a time. The groups for the first screening were;

- Group 1 – *Moringa oleifera* leaves aqueous extract (ML) – 500 mg/kg
- Group 2 – *Moringa oleifera* stem bark aqueous extract (MSB) – 750 mg/kg
- Group 3 – positive control (infected but not treated)

The second screening had the following groups for investigation;

- Group 1 – *Mitragyna inermis* twigs aqueous extract (MT) – 500 mg/kg
- Group 2 – *Pseudocedrela kotschyi* leaves aqueous extract (PK) – 500 mg/kg
- Group 3 – positive control (infected but not treated)

There were three mice in each group, making a total of nine for each screening process. Each group was housed in a different cage and fed regularly, morning and evening. All mice were infected with 200 µL of infected blood containing 2×10^7 parasitized cells/ml from the donor mouse after cardiac puncture on day 0. In the test, treatment was initiated on day 3 post-infection and continued through to day- 9 post-infection. Blood samples were taken from all experimental animals from day-3 to day-9. Three thin blood smears were prepared from each mouse and five fields counted for each slide. Daily estimation of parasitemia for each group was an average of nine values. The results obtained from the estimation were then

statistically analyzed using both One-Way and Two-way ANOVA followed by Newman-Keuls and Bonferroni post-tests respectively from GraphPad Prism version 5.

3.2.2.4.2 Screening of the organic extracts for *In-vivo* antiplasmodial activity

The method employed for the screening of the organic extracts was adopted and modified from a study by Jimenez-Diaz *et al* (Jimenez-Dıaz *et al*. 2013). Methanolic extracts from the twigs of *Mitragyna inermis* (Willd) O. Kuntze (Rubiaceae), leaves of *Pseudocedrela kotschyi* (Schweinf.) Harms (Meliaceae) and leaves of *Moringa oleifera* Lam. (Moringaceae) were evaluated for their antiplasmodial activity at a dose of 500 mg/kg for a period of 3 days. Extracts were suspended in 2% Tragacanth solution and administered once daily. Experimental BALB/c male mice free from pathogens (weight between 20-25 g) were used after 3 days acclimatization with laboratory conditions. They were weight matched into 7 groups. The groups for the investigation were;

- Group 1 – Treatment control (standard drug - Artemether -Lumefantrine at 4 mg/kg) (A/L)
- Group 2 – Positive control (Infected and untreated)
- Group 3 – Negative control (Uninfected and untreated)
- Group 4 – Vehicle only (2% Tragacanth suspension)
- Group 5 – *Moringa oleifera* leaves (MOR – 500 mg/kg)
- Group 6 – *Pseudocedrela kotschyi* leaves (PSD – 500 mg/kg)
- Group 7 – *Mitragyna inermis* twigs (MIT – 500 mg/kg)

Each experimental group had three mice, making a total of 21 mice for the screening process. Each group was housed in a different cage and fed regularly. All mice were infected with 200 µL of 2×10^7 parasitized cells/ml from the donor mouse after cardiac puncture on day 0. Treatment with the extracts, vehicle and the standard antimalarial agent for the treatment

control were initiated on day-2 post-infection through to day-4 post-infection. Blood samples were taken from all experimental animals from day-2 to day-5. Three thin blood smears were prepared from each mouse and five fields counted for each slide, to sum up to nine estimation for daily parasitemia for each group. Thus, daily estimation of parasitemia was an average of nine values. Parameters like the Percentage suppression (Fidock *et al*. 2004) and 'Parasite Reduction Ratio' (PRR) (Jimenez-Dıaz *et al*. 2013) were calculated as well as percentage change in the weights of the animals during the period of treatment. Results were statistically analyzed with both One-Way and Two-Way ANOVA followed with Newman-Keuls and Bonferroni post-tests respectively from GraphPad Prism v 5 at 95% confidence interval.

3.2.2.4.3 Dose ranging evaluation of the antiplasmodial effects of *Moringa oleifera* leaves

After initial screening of the aqueous extracts of the medicinal plants under investigation, the most effective of them, ML, was selected for full investigation into their *In-vivo* antiplasmodial activity in a dose dependent manner. The doses employed in the evaluation were 250, 500, 750 and 1000 mg/kg. Experimental male ICR mice of weight 25-30 g were used in 7 groups of five. The other three groups in addition were treatment control, positive and negative control groups. The standard antimalarial agent employed for the investigation was Artemether – Lumefantrine at a dose of 4mg/kg. Both 4-day suppressive, with some modifications and 7- day curative models were adopted for the investigation.

In the four-day suppressive test, four consecutive days treatment with extracts and standard drug were initiated after three days of infection. This was done to ensure parasitemia establishment in the experimental animals before treatment. Blood samples were taken for examination after the four days and parasitemia estimated. The results were analyzed using Two-Way ANOVA from GraphPad Prism v 5 at 95% confidence interval.

The curative test was as used for the initial screening (see **Sec. 4.2.2.4.1** above). All experimental mice were housed in different cages with adequate feed and drink and allowed to acclimatize for three days after which weight matching was carried out. Excluding the negative group, they were all then infected on day 0 with 200µL of 2×10^7 parasitized rbcs/ml intraperitoneally and monitored for any unusual behaviour.

3.2.2.4.4 Monitoring of clinical parameters from ML evaluation

Aside the daily parasitemia estimation, parameters such as weight distribution (Fidock *et al*. 2004) as the infection and treatment progressed as well as visual inspection of clinical changes (Basir *et al*. 2012) and mouse survival time (Fidock *et al*. 2004) were also monitored.

3.2.2.4.4.1 Weight distribution monitoring

The weights of the mice were taken daily in the morning before feeding and drug administration. The results obtained were then analyzed using One-Way ANOVA followed by Newman-Keuls post-tests from GraphPad Prism v 5.

3.2.2.4.4.2 Visual inspection of clinical changes

The changes in clinical conditions of the experimental animals throughout the duration of the investigation were also visually inspected. Parameters such as diarrhoea, lethargy, piloerection and locomotor activity were monitored. An arbitrary scale to grade to degree of clinical deterioration was adopted these are

Absent (-)
Mild (+)
Moderate (++)
Severe (+++)

3.2.2.4.4.3 Survival/Mortality Record

The survival or mortality of the animals as infection worsened and treatment progressed in the different groups was also monitored. Mice were monitored on a daily basis and any death

per day recorded and a survival curve plotted to evaluate the extent of toxicity of the doses on the lives of the mice in comparison to the treatment group and negative group. Results were analyzed using Mantel-Cox test and Log-rank test for trend from GraphPad Prism v 5.

3.3 RESULTS

3.3.1 EXPERIMENTATION ON AQUEOUS EXTRACTS

3.3.1.1 Confirmation of Virulence of *P. berghei* after thawing cryopreserved parasites

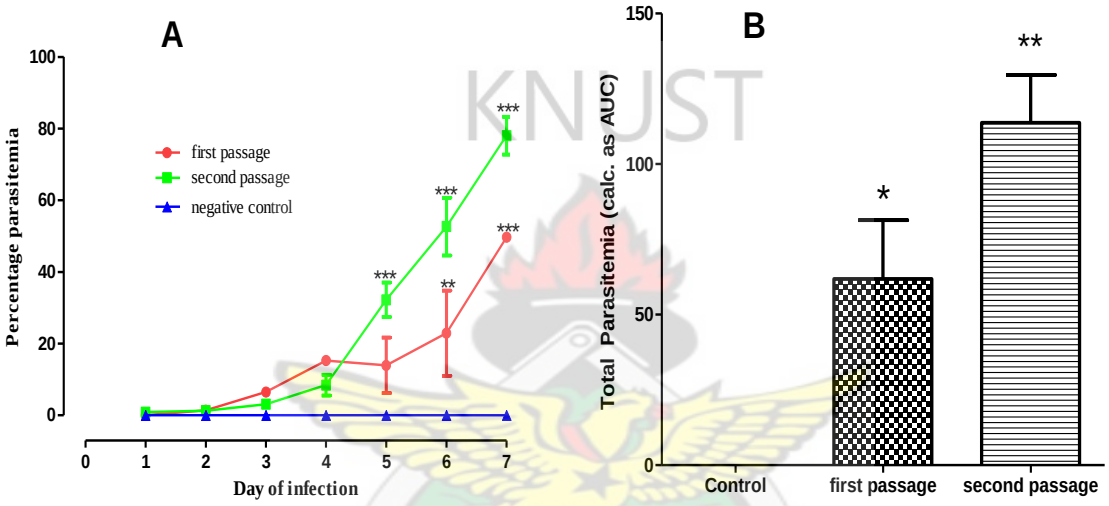


Figure 3.2 - Evaluation of the virulence of the thawed cryopreserved Plasmodium berghei NK- 65 strain parasites.

200µL of thawed cryopreserved parasites was injected intraperitoneally into a naive male ICR mouse after which parasitemia was daily monitored to confirm viability and regain of virulence. After 7 days of infection, 2 x10^7 parasitized rbcs/ml in 200 µL from the first mouse was also injected into a second mouse. Parasitemia was again monitored with light microscope using objective lens x100. [**A]**- Estimation of daily parasitemia. . Each point represents Mean ± SEM (N = 3). Results analyzed with Two-Way ANOVA followed by Bonferroni *post hoc* from GraphPad Prism v 5. * p<0.05, ** p<0.01 and *** p<0.001 denote significance levels in comparison to negative control. [**B]**- Total parasitemia calculated as Area under Curve (AUC) for both passages. Each bar represent Mean ± SEM. Results analyzed with One-Way ANOVA followed by Newman-Keuls *post hoc* from GraphPad Prism v 5. * p<0.05, ** p<0.01 and *** p<0.001 denote significance levels in comparison to negative control.

From figure 3.2(a), the parasites, after having been taken out of cryopreservation, were able to evade the host's immune system and multiply to cause an increase in their number in the blood, hence the increment in parasitemia. Due to the low virulence of the parasites

immediately after cryopreservation, the parasitemia registered for the first passage was significantly lower than that from second passage ($F_{2,6}$ = 15.28, p = 0.0044; fig. 3.2b), signifying regain of viability and virulence. Initial parasitemias (that is, from day 1- 4; fig. 3.2a) recorded however, showed relatively lower parasitemias from second passage as compared to the first. For example, on day 3, parasitemia from passage-1 was 15.29% ± 1.26%, whiles passage-2 registered 8.44% ± 2.90% but were not statistically different from each other (t = 1.216, p > 0.05). After day-4, parasitemia increased exponentially to contribute to the total parasitemia being higher for second passage as compared to first.

3.3.1.2 Curative Test for *Moringa oleifera* leaves and stem bark

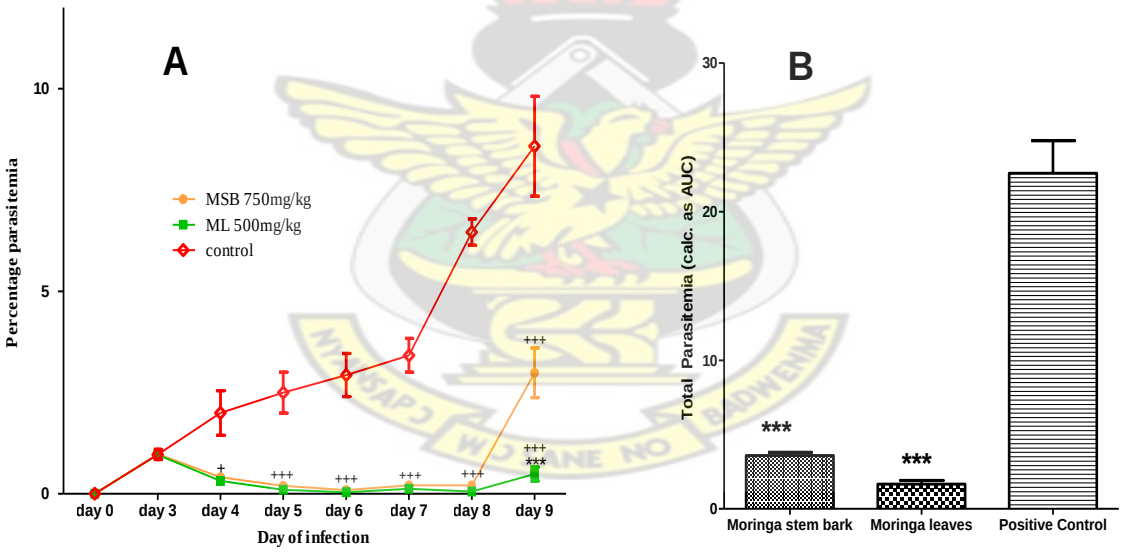


Figure 3.3 - Evaluation of the curative effects of aqueous extracts of both Moringa oleifera Lam. (Moringaceae) leaves and stem bark.

ML at 500 mg/kg and MSB at 750 mg/kg were screened using male ICR mice preinfected with 200µL of 2 ×10^7 parasitized rbcs/ml. N = 3. Drug treatment started on day 3 after infection and ended on day 9. Thin blood smears prepared from tail prick. [**A]**- Daily estimation of parasitemia for the experimental groups. Each point represents Mean ± SEM. Results obtained were analyzed using Two-Way ANOVA from GraphPad Prism v 5 followed by Bonferonni post-test at 95% confidence level. * p<0.05, ** p<0.01 and *** p<0.001 denote significance level in comparison to ML. + p<0.05, ++ p<0.01 and +++ p<0.001 denote significance levels in comparison to control. [**B**]- Estimation of the total parasitemia in each experimental group as the Area under Curve. Each bar represents Mean ± SEM. Results were analyzed using One-Way ANOVA followed by Newman-Keuls *post hoc*. *p<0.05, **p<0.01 and ***p<0.001 denote significance levels when compared to the positive control.

Initial screening of the aqueous extracts of the leaves and the stem bark of *Moringa oleifera* was conducted for a period of 7 days. From the investigation, it was observed that both stem bark (MSB) and leaves (ML) exhibited *In-vivo* antiplasmodial activity. The parasitemia in the positive control increased steadily right from the initiation (0% ± 0.0 on day 1, n = 3) of the therapy to the end (8.58% ± 1.236 on day 9, n = 3). On the other hand, the groups treated with 500 mg/kg of ML and 750 mg/kg of MSB exhibited suppression of parasite replication resulting in the reduction of their parasitemia as compared to the control group. The differences were statistically significant from Two-Way ANOVA analysis ($p < 0.0001$). It was also evident that, the effects of the two extracts at their stated doses were comparable ($p > 0.05$) from day 3-8 (fig. 3.3a) whiles the final day showed a difference ($t= 4.229$, $p < 0.05$). However, on the whole, there was no significant difference between ML and MSB from (fig. 3.3b). Another observation made was that ML, at the infection dose given to the mice, exhibited a plasmocidal activity. This was evident, when parasites failed to replicate on completion of the 7- day therapy. On the contrary, parasites replicated exponentially after withdrawal of MSB, signifying a potential plasmostatic activity.

3.3.1.3 Curative Test for *Pseudocedrela kotschyi* leaves and *Mitragyna inermis* twigs

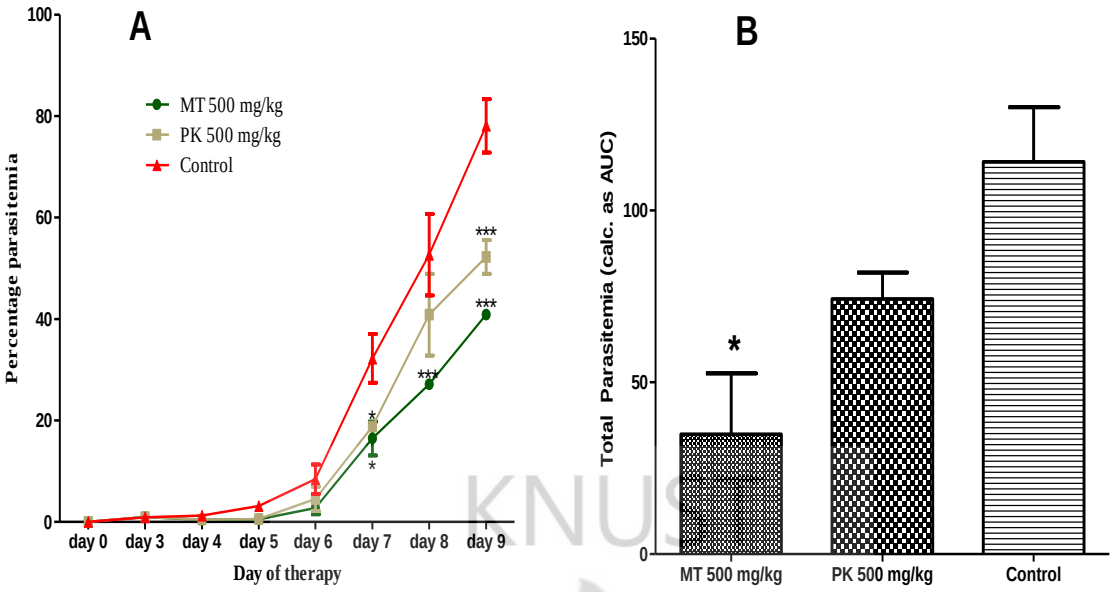


Figure 3.4 - Evaluation of the Curative effects of the aqueous leaves of Pseudocedrela kotschyi and twigs of Mitragyna inermis.

Treatment initiated at a dose of 500 mg/kg for both extracts on experimental ICR mice started on day 3 post infection. N = 3. Parasitemia estimated daily from thin blood smears. [**A**]- Daily estimation of parasitemia for the experimental groups. Each point represents Mean ± SEM. (Results obtained were analyzed with Two-Way ANOVA from GraphPad Prism v 5 followed by Bonferroni post-tests at a confidence level of 95%. *p<0.05, **p<0.01 and ***p<0.001 denote significance level when compared to control). [**B**]- Estimation of the total parasitemia in each experimental group as the Area under Curve. Each bar represents Mean ± SEM. (Results were analyzed using One-Way ANOVA followed by Newman-Keuls *post hoc*. *p<0.05, **p<0.01 and ***p<0.001 denote significance levels when compared to the positive control).

3.3.1.4 Percentage Suppression for aqueous extracts

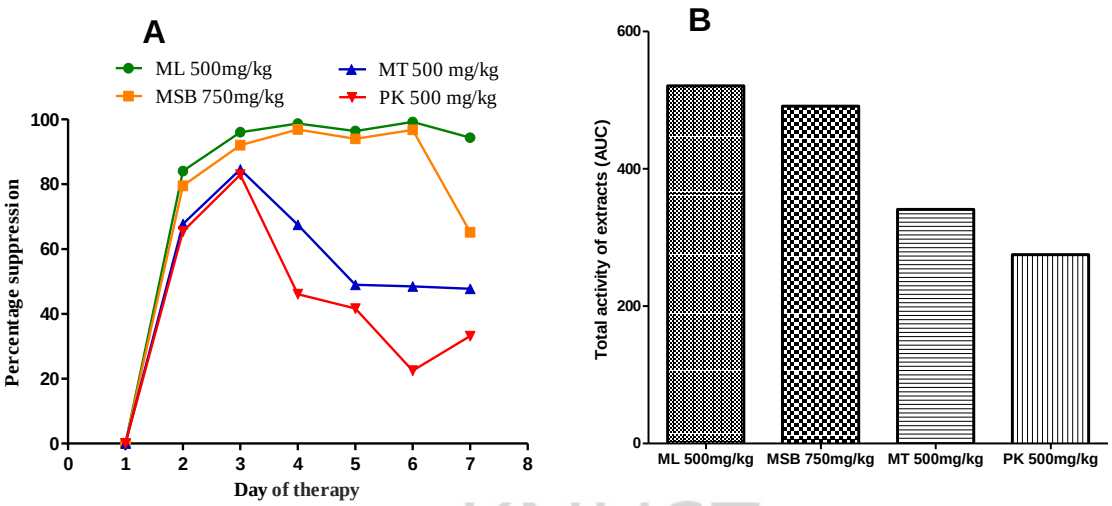


Figure 3.5 - Percentage suppression for the initially screened aqueous extracts of plants

ML, MT and PK at 500 mg/kg and MSB at 750 mg/kg were administered orally twice a day after infecting experimental mice with standard inoculum dose of *Plasmodium berghei* NK 65 strain intraperitoneally. N=3. [**A**]- Daily estimation of the percentage suppression of extracts. Each point represents Mean percentage suppression calculated from 9 values per group. [**B**]- Estimation of the total activity or suppression of the extracts as Area under Curve. Each bar represents Mean AUC for the extract after seven days of therapy.

The second screening was conducted on the aqueous extracts of *Pseudocedrela kotschyi* (PK) and *Mitragyna inermis* (MT) at a dose of 500 mg/kg. The results obtained revealed that both aqueous extracts possessed *In-vivo* antiplasmodial activity from One –Way ANOVA analysis ($F_{2,6}$ = 7.534, p = 0.0231; fig. 3.4A) but Newman-Keuls *post hoc* analysis showed that, it's only the activity of MT, that was significant as compared to the positive control group (q =5.489, $p < 0.05$) whiles that of PK was not (q = 2.763, $p > 0.05$). A closer look at the results obtained from the parasitemia estimation (fig. 3.4A) also showed that, although the extracts exhibited different degrees of suppression of parasite growth; significant reduction was only evident on day-7 post-infection for PK. In comparing the two extracts, there was no significant difference between the two (q = 2.726, $p > 0.05$; fig. 3.4B) under the experimental conditions employed for the evaluation. Notwithstanding these observations, the results from their percentage suppressions (fig. 3.5A) show that, generally, the activity of all the extracts

increased exponentially on the second day of therapy whiles ML and MSB experienced a plateaued activity or suppression afterwards, PK and MT had their activity declining after the third day of therapy, that is, from 84.49% to 67.42% for MT and from 82.91% to 46.09% for PK. Thus, the activity upon total estimation was highest for ML followed by MSB, MT and then PK (see fig. 3.5B).

3.3.2 EXPERIMENTATION ON ORGANIC EXTRACTS

3.3.2.1 Screening of organic extracts

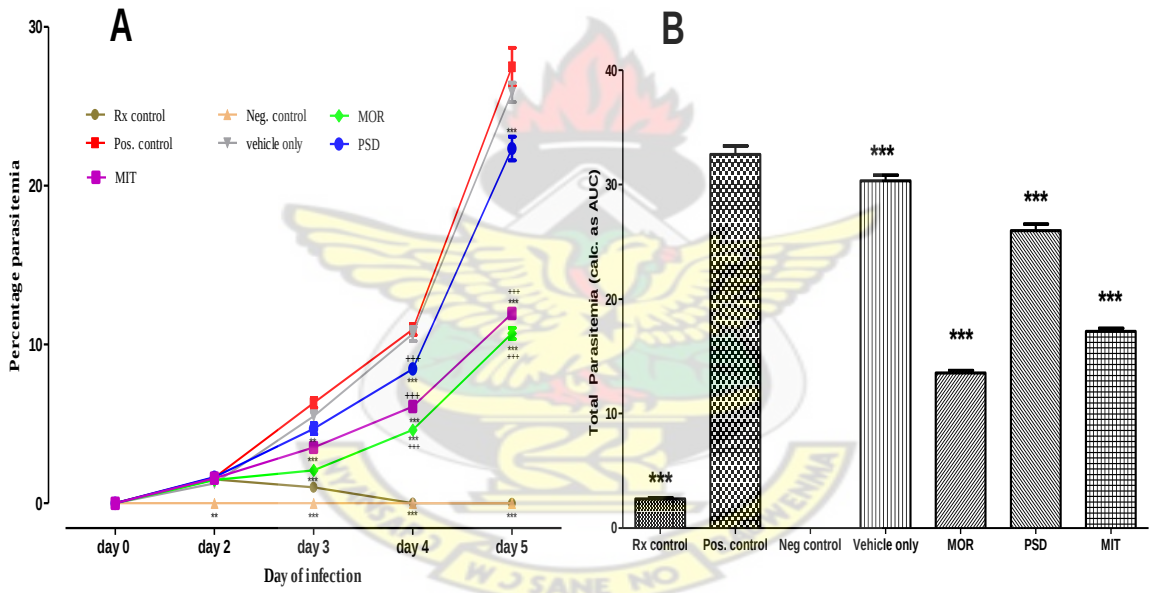


Figure 3.6 - Screening of methanolic extracts for In-vivo antiplasmodial activity.

[**A**]- Daily estimation of parasitemia from thin film smears. Extracts (500 mg/kg each) administered once daily orally and blood taken from tail vein. N = 3. Each point represents Mean ± SEM calculated from 9 values per group. Results analyzed using Two way ANOVA GraphPad Prism v 5 followed by Bonferroni post-tests.*p<0.05, **p<0.01 and ***p<0.001 denote significance levels in comparison to positive control. +p<0.05, ++p<0.01 and +++p<0.001 denote significance levels in comparison to treatment control. [**B**]- Estimation of total parasitemia of the experimental groups following 7 days of therapy with the extracts. Each bar represents Mean ± SEM calculated from 9 values per group. Results analyzed using One-Way ANOVA followed by Newman-Keuls *post-hoc*. .*p<0.05, **p<0.01 and ***p<0.001 denote significance levels in comparison to positive control.

Results from initial screening of the aqueous extracts signified that, the traditional medicinal plants employed for the studies possessed varying degrees of *In-vivo* antiplasmodial activity.

It was expedient thus, from deductions from the screening process, to carry out an evaluation on the methanolic extracts of the plant samples. From the three-day therapy employed [adopted with modification from Jimenez-Dıaz *et al*. (2013)], to mimic the number of days recommended for conventional uncomplicated malaria treatment, it was also shown that the methanolic extracts do possess *In-vivo* antiplasmodial activity ($F_{6,56}$ = 966, p <0.0001; fig. 3.6B). Parasitemia reduction was greatest for the treatment control (A/L) (q = 72, p < 0.05), followed by MOR (q = 45.72, p < 0.05), then MIT (q = 36.98, p < 0.05) and finally PSD (q = 15.91, p < 0.05). It was also evident from the investigation (fig. 3.6A) that, upon infecting experimental mice with 200µL containing 2×10^7 parasitized cells/ml, all the mice expressed average parasitemia around 1.5% on day-2 post infection (see table 3.1 below), corresponding to estimation from literature (Jimenez-Dıaz *et al*. 2013). For the treatment control, the three days of therapy was enough to totally clear the parasites from the blood, rendering them undetectable under the light microscope, whiles the extracts exhibited varying degrees of parasite reduction (see fig. 3.6A). Hence the treatment control could be termed as plasmocidal whiles the extracts, be termed as plasmostatic, as they did not clear the parasites but only suppressed their replication.

3.3.2.2 Evaluation of the organic extracts

Table 3.1 - Daily record of the parasitemia from experimental groups in the evaluation of the organic extracts

	Treatment control		**Pos. control**		**MOR**		**PSD**		**MIT**		
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	**N**
day 0	0	0	0	0	0	0	0	0	0	0	**9**
day 2	1.523	0.185	1.612	0.190	1.474	0.171	1.647	0.175	1.582	0.123	**9**
day 3	1.013	0.195	6.331	0.937	2.084	0.332	4.698	1.01	3.522	0.813	**9**
day 4	0.022	0.036	10.948	1.054	4.621	0.532	8.472	0.707	6.101	0.350	**9**

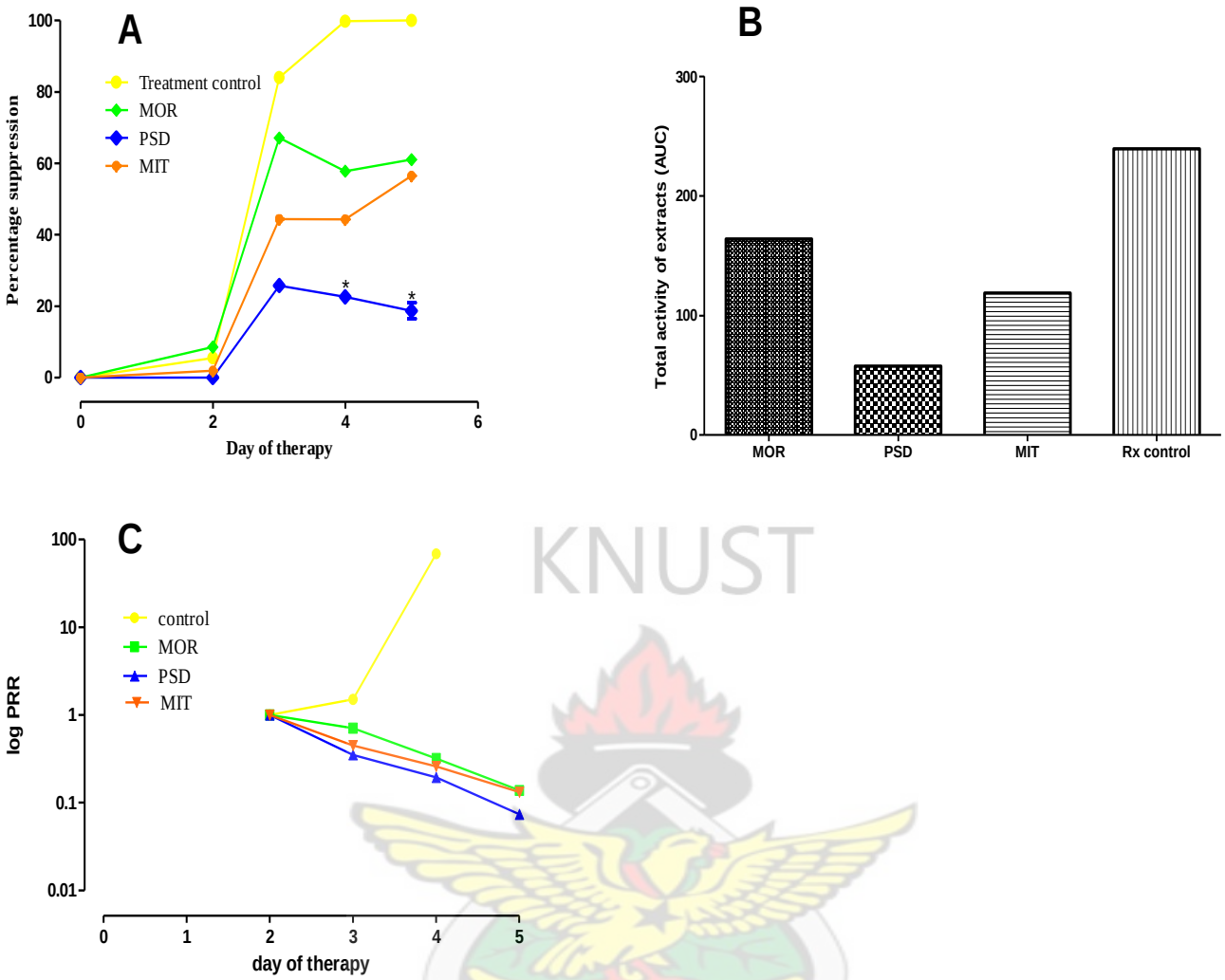


Figure 3.7 – Evaluation of the methanolic extracts of plants

[**A**]- Daily estimation of the percentage suppression of the methanolic extracts. N = 3. Each point represents Mean ± SEM calculated from 9 values per group. Results analyzed using Two-way ANOVA with Bonferroni post-tests. Statistical significance as compared to treatment control denoted by **p*<0.05, ***p*<0.01 and ****p*<0.001. [**B**]- Estimation of the total activity of the extracts employed in the evaluation, calculated as Area under Curve. Each bar represents Mean activity. [**C**]- Determination of the potency of the extracts using the Parasite Reduction Ratio (PRR). Each point represents Mean for the extract.

Table 3.2 - Percentage suppression of the organic extracts per day

Day of therapy	Treatment control			MOR			PSD			MIT		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
0	0	0	9	0	0	9	0	0	9	0	0	9
2	5.51	0.185	9	8.55	0.171	9	0	0.175	9	1.87	0.123	9
3	**83.99**	**0.195**	**9**	**67.08**	**0.332**	**9**	**25.79**	**1.010**	**9**	**44.37**	**0.813**	**9**
4	99.8	0.036	9	57.79	0.532	9	22.61	0.707	9	44.27	0.350	9
5	100	0	9	61.06	1.047	9	18.73	2.253	9	56.51	0.461	9

NB: Drugs or extracts with percentage suppression ≥ 40% by day 3 and onwards were deemed active (Jimenez-Dıaz *et al*. 2013).

Table 3.3 - Daily estimation of Parasite Reduction Ratio for the methanolic extracts

DAY	MOR	PSD	MIT	CONTROL
0	-	-	-	-
2	1	1	1	1
3	0.7074	0.3506	0.4492	1.5033
4	0.3191	0.1944	0.2593	68.62
5	0.1377	0.074	0.1323	∞

Potency of drug expressed as PRR = $\frac{Po}{Px}$, where P_o is the parasitemia before initiation of drug treatment and P_x is the parasitemia at a particular day of treatment and PRR is the Parasite Reduction Ratio. When Po > P_x, PRR > 1, this means net clearance; Po = P_x, PRR = 1, means maximum suppression of parasite growth; Po < P_x, PRR < 1, means different degree of suppression. While PRR for the control kept increasing with time, the opposite could be said generally for the extracts.

From the *In-vivo* screening of the methanolic extracts, some of the parameters determined were the percentage suppression of extracts (Fidock *et al*. 2004) and Parasite reduction ratio (PRR), to give an indication of their potency (Jimenez-Dıaz *et al*. 2013). Table 3.2 illustrates the percentage suppressions calculated for the extracts as treatment and infection days progressed. From the same Table 3.2 and (fig. 3.7A), it was evident that, the percentage suppressions generally increased along the days of therapy with the maximum peaking between days 3-5. In addition to that also, the values obtained showed that PSD exhibited the least parasite suppression with its maximum being 25.79% ± 1.010%, on day-3, as compared to 100% ± 0.00 for Rx control on day-5, 61.06% ± 1.047% on day-5 for MOR and 56.51% ± 0.461% also on day-5 for MIT (see Table 3.2). Generally, it was established that activity for PSD was the least in terms of the extracts and that of MOR was the greatest (fig. 3.7B). However, the activity of Rx control surpassed the three extracts. Upon estimating the PRR (see Table 3.3), it was shown that the potency of the treatment control kept increasing up to the point where it was determined to be infinite (that is, on day-5). On the contrary, the potency of the extracts declined as the days of infection progressed (see fig. 3.7C).

3.3.2.2 Weight distribution of mice tested with the organic extracts

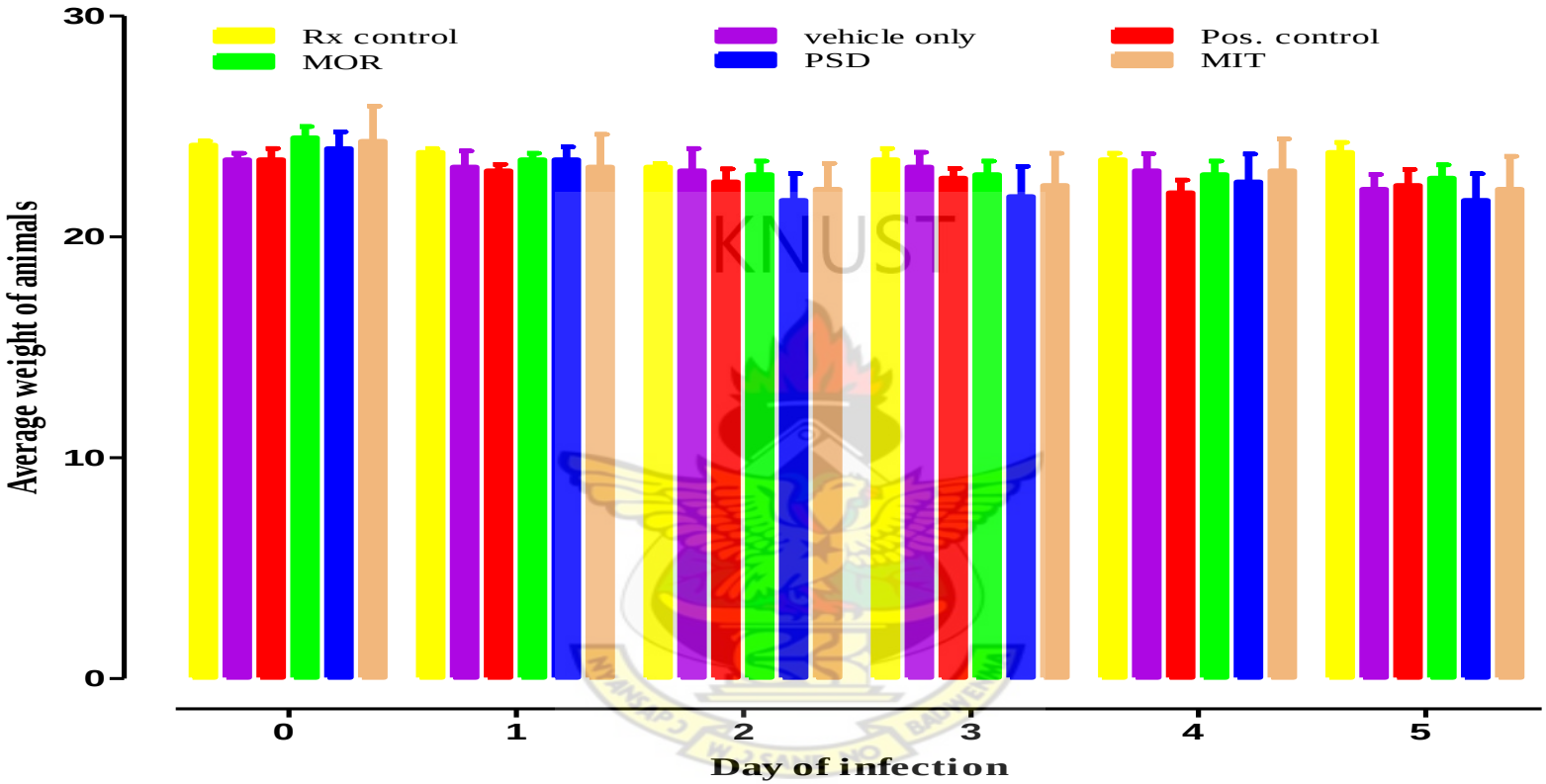


Figure 3.8 - Daily record of the weights of experimental ICR mice treated with organic extracts

Record taken during period of therapy with the organic extracts at a dose of 500 mg/kg for each. N = 3. Data expressed as mean ± SEM. Weights recording started on day of therapy initiation up to day 5 post-infection.

3.3.2.4 Percentage change in weight of animals

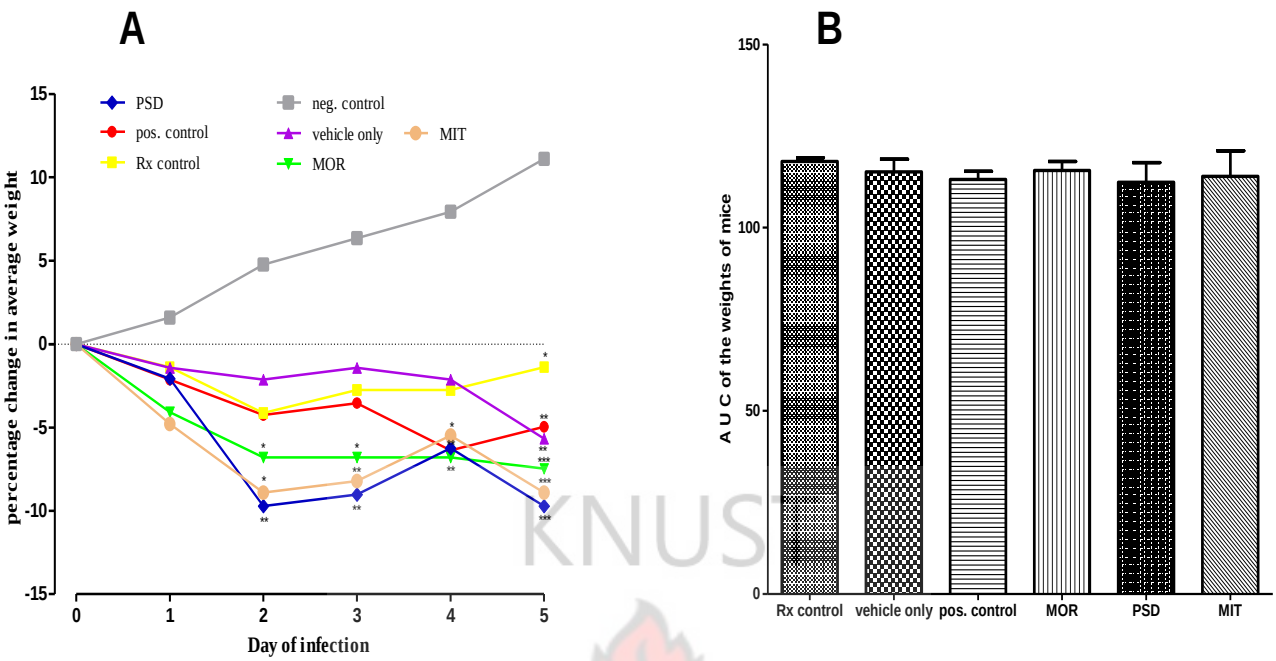


Figure 3.9 - Percentage change in the average weight of ICR mice preinfected with Plasmodium berghei NK65 strain and treated with methanolic extracts

Treatment with MOR, MIT and PSD extracts at 500 mg/kg each and A/L at 4 mg/kg. Agents administered orally. N=3. [**A**]- Percentage change in weight of animals estimated in relation to their weight before initiation of the infection. Each point represents Mean ± SEM for each experimental group. Results analyzed using Two-way ANOVA followed by Bonferroni post-tests from GraphPad Prism v 5. *p<0.05, **p<0.01 and ***p<0.001 denote significance levels when compared with negative control. [**B**]-Total estimation of the weight of each experimental group. Data expressed as Mean ± SEM. Results analyzed with One-Way ANOVA followed by Newman-Keuls post-test at a 95%CI.

In addition to the *In-vivo* antiplasmodial activity of the extracts being evaluated, the effect of the extracts on the weights of the animals was also observed. The effects on the weight could be proposed to be a balance between effect of the infection as well as the extracts. The weights of the experimental ICR mice ranged between 25 – 31 g (fig. 3.8). It was observed that whiles generally the weight of the negative control (that is, uninfected) group kept rising steadily (fig. 3.9A), that of the other experimental groups (which were infected) were declining notwithstanding the fact that these changes were shown not to be significant ($F_{5,12}$ = 0.245, p = 0.9346; fig. 3.9B) from One-Way ANOVA analysis. From fig. 3.9A, it is shown

that the greatest of percentage reductions was exhibited by PSD on day 2 post-infection (that is, -9.72%) whiles that of the positive control (infection without treatment) was -4.26% on the same day. Quite predictable for the treatment control group, the weights in the group declined a little and began to rise steadily from day 3 to day 5. However, for the three extracts, their percentages decline in weights were greater than that of the positive control group. On analysis using Two-Way ANOVA with Bonferroni post-tests, the percentage decline for the treatment control group was not significant for greater part of the days except on the 5th day of infection (t = 3.16, p < 0.05).

The group administered with only vehicle showed a better effect on the weights of the experimental animals when compared to groups treated with the extracts and the reference drug.

3.3.3 DOSE-DEPENDENT EVALUATION OF THE ANTIPLASMODIAL ACTIVITY OF AQUEOUS *MORINGA OLEIFERA* LEAVES EXTRACT (ML)

3.3.3.1 Four – day suppressive test

Table 3.4 - Percentage suppression for the aqueous Moringa oleifera leaves extract at different doses in Plasmodium berghei infected ICR mice.

	Treatment control	**250 mg/kg**	**500 mg/kg**	**750 mg/kg**	**1000 mg/kg**	**Positive control**
Parasitemia on initiation (%)	2.239 ± 0.584	1.571 ± 0.313	1.569 ± 0.318	1.637 ± 0.545	1.343 ± 0.438	2.000 ± 0.458
Parasitemia at end of therapy (%)	0 ***	14.010 ± 3.004 ***	10.787 ± 2.452 ***	31.926 ± 11.567 ***	38.992 ± 8.264	42.337 ± 4.099
Percentage suppression (%)	**105.52**	**69.31**	**77.26**	**25.28**	**07.12**	

Mice were infected with *P. berghei* and treated for four consecutive days with four different doses of ML (250-1000 mg/kg). N = 5. Data expressed as mean ± SEM (average of 15 readings from each group, that is, 3 readings from each mouse) and analyzed with Two-Way ANOVA at 95% Confidence level. *p<0.05, **p<0.01 and ***p<0.001 denote significance level in comparison with positive control.

3.3.3.2 Curative Test

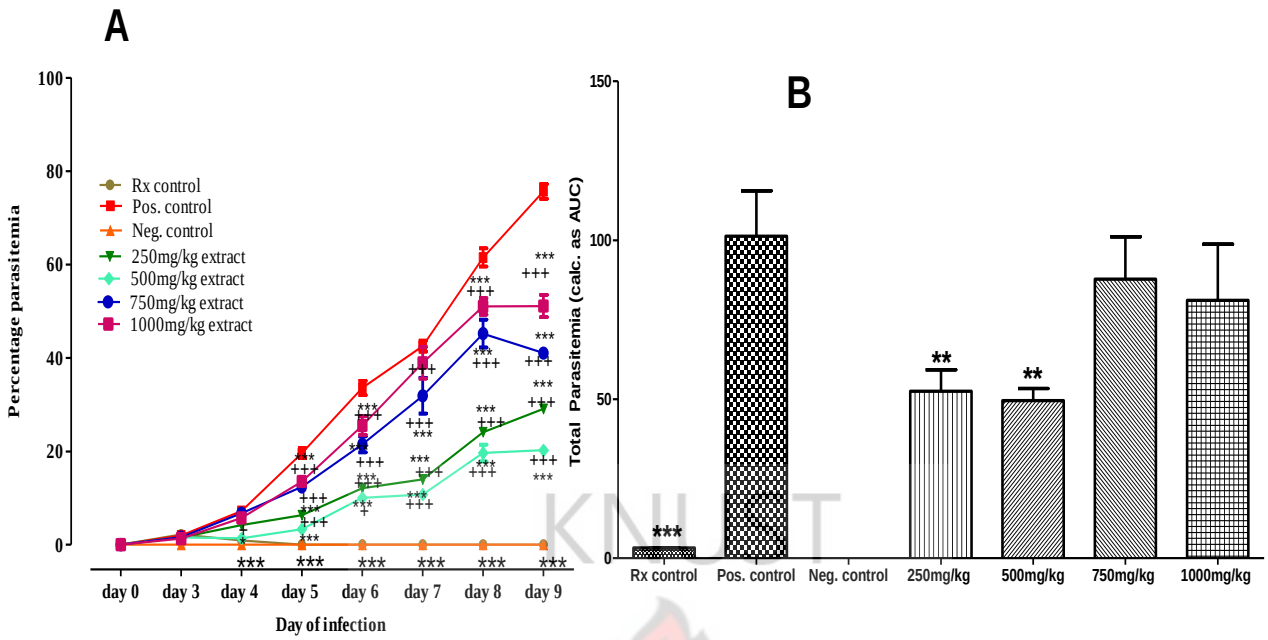


Figure 3.10 - Dose dependent curative antiplasmodial effects of the aqueous extract of Moringa oleifera.

[**A**]- Daily estimation of parasitemia from thin film smears. ML at doses 250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg administered twice daily orally. N = 5. Each point represented Mean ± SEM (calculated from 15 values per group, that is, estimation of parasitemia from three smears per animal in a group of five). Results analyzed with Two- way ANOVA followed by Bonferroni post-tests at 95% Confidence level from GraphPad Prism v5. **p*<0.05, ***p*<0.01 and ****p*<0.001 denoted significance levels in comparison to positive control. +*p*<0.05, ++*p*<0.01 and +++*p*<0.001 denoted significance levels in comparison to treatment control. [**B**]- Estimation of the parasitemia of each experimental group by the end of therapy. Each bar represented Mean ± SEM. Results analyzed using One-Way ANOVA followed by Newman-Keuls *post-hoc* from GraphPad Prism v5. **p*<0.05, ***p*<0.01 and ****p*<0.001 denoted significance levels in comparison to positive control.

The dose dependent investigation of the most effective of the aqueous extract from the initial screening, started with the evaluation for its suppressive properties in a four-day suppressive test. Table 3.4 above illustrates the results obtained from the four-day suppressive test. The percentage suppressions were determined in relation to their parasitemia on initiation of the therapy. The results showed that suppression was not entirely dose dependent as the two smaller doses, that is, 250 mg/kg (69.31%) and 500 mg/kg (77.26%), exhibited better suppression of parasite replication as compared to the two higher doses, that is, 750 mg/kg (25.28%) and 1000 mg/kg (7.12%). In addition to that also, statistical analysis revealed that

the suppression from 1000 mg/kg was insignificant (t = 2.453, p > 0.05) as compared to the positive control. Thus, the suppression increased from 250 mg/kg (69.31%) to 500 mg/kg (77.26%) and decreased with doses 750 mg/kg (25.28%) and ultimately 1000 mg/kg (7.12%). The treatment control exhibited the highest of suppressions with 105.52% as compared to the parasitemia of the group upon therapy initiation which was very significant (t = 38.00, p < 0.001).

In the curative test, One-Way ANOVA analysis showed significant reduction in the parasitemia of the treated groups ($F_{6,98}$ = 14.89, n = 7, p < 0.0001; fig. 3.10B). The story was no different from the four-day suppression test, with the total parasitemia (AUC) for the first two doses being significantly lower than the positive control, that is, 52.32 ± 6.732 (n = 15, q = 4.694, p < 0.01; fig. 3.10B) for 250 mg/kg and 49.62 ± 3.804 (n = 15, q = 4.974, p < 0.01; fig. 3.10B) for 500 mg/kg. That of 750 mg/kg (87.77 ± 13.36, n = 15, p > 0.05; fig. 3.10B) and 1000 mg/kg (81.16 ± 17.63, n = 15, q = 1.936, p > 0.05; fig. 3.10B) were shown not to be significantly different from the positive control (101.3 ± 14.32). The treatment control also showed significant reduction in parasitemia (3.201 ± 0.1569, n = 15, q = 9.446, p < 0.001) as compared to the positive control.

3.3.3.3 Percentage Suppression (activity) of the ML extracts

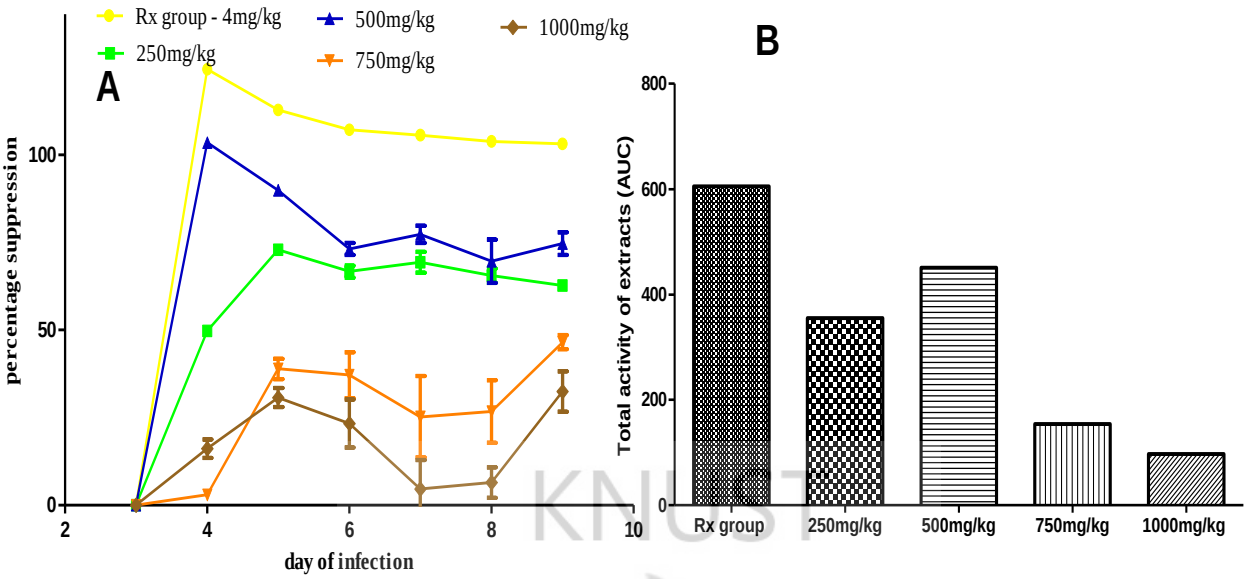


Figure 3.11 - Activity of the ML extracts (250 - 1000 mg/kg)

Determination of the activity of the four doses of ML extract. Drugs administered orally twice a day after infecting experimental mice with standard inoculum dose of *Plasmodium berghei* NK 65 strain intraperitoneally. N= 5. [**A**]- Daily estimation of the percentage suppression of extracts. Each point represented Mean ± SEM. [**B**] - Estimation of the total activity or suppression of the extracts from screening process as Area under Curve. Each bar represented Mean AUC for the extract after seven days of therapy

The four doses of ML extracts exhibited different patterns in their suppression of parasite growth (fig. 3.11A). For the treatment control group, treated with the standard drug (A/L), it was observed that the percentage suppression initially shot up to a maximum of 124.32% ± 0.246 on day 4, just a day after initiating the therapy. The activity however declined in the subsequent days, finally plateauing from day-6 (107.09% ± 0.0) to day-9 (103.04% ± 0.0). The next higher activity was exhibited by the 500 mg/kg group, which started with 103.43% ± 0.639 and declined finally to 74.65% ± 3.235. The 250 mg/kg group recorded 49.75% ± 0.756 initially on day-4 but the activity increased to 72.83% ± 1.218 on day-5 and then declined to 66.63% ± 1.676 on day-6. The group ended with 62.68 ± 1.148. For the 750 mg/kg and 1000 mg/kg groups, similar trends were observed from day-5 to day-9 with

slightly higher recordings for 750 mg/kg as compared to the 1000 mg/kg group. In addition to this, it is also shown from the average Area under curve calculations (see fig. 3.11B) that, activity was greatest for the treatment control (604.9) group followed by 500 mg/kg (456.0), 250 mg/kg (355.4), 750 mg/kg (154.2) and 1000 mg/kg (97.61) in that order.

3.3.3.4 Weight distribution of mice treated with aqueous *Moringa oleifera* leaves

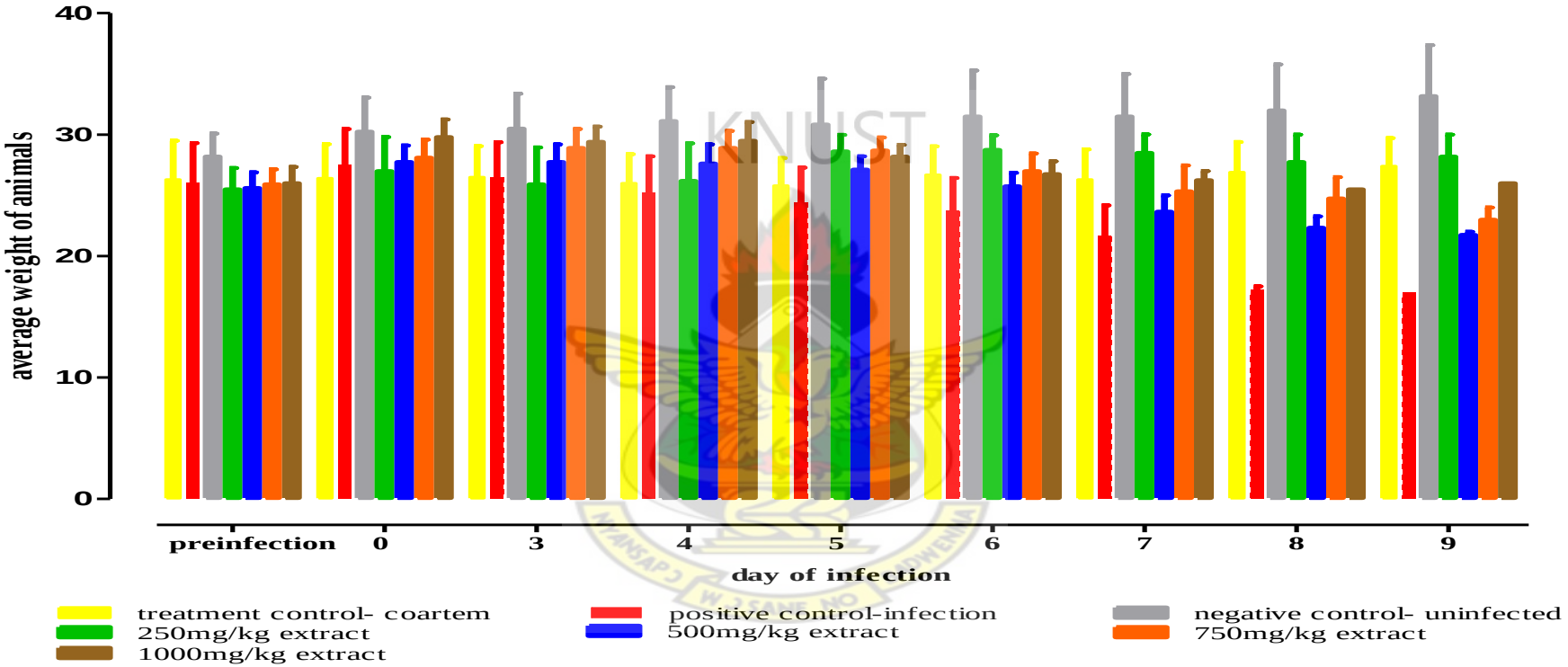


Figure 3.12- Daily record of the weights of experimental ICR mice treated with aqueous Moringa oleifera leaves

Recording done during period of therapy with the four doses of *Moringa oleifera*, that is, 250mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg. N = 5. Data expressed as mean ± SEM. Weights recording started 10 days before infection after acclimatisation and continued throughout to the end of the investigation.

3.3.3.5 Percentage change in weight during period of treatment

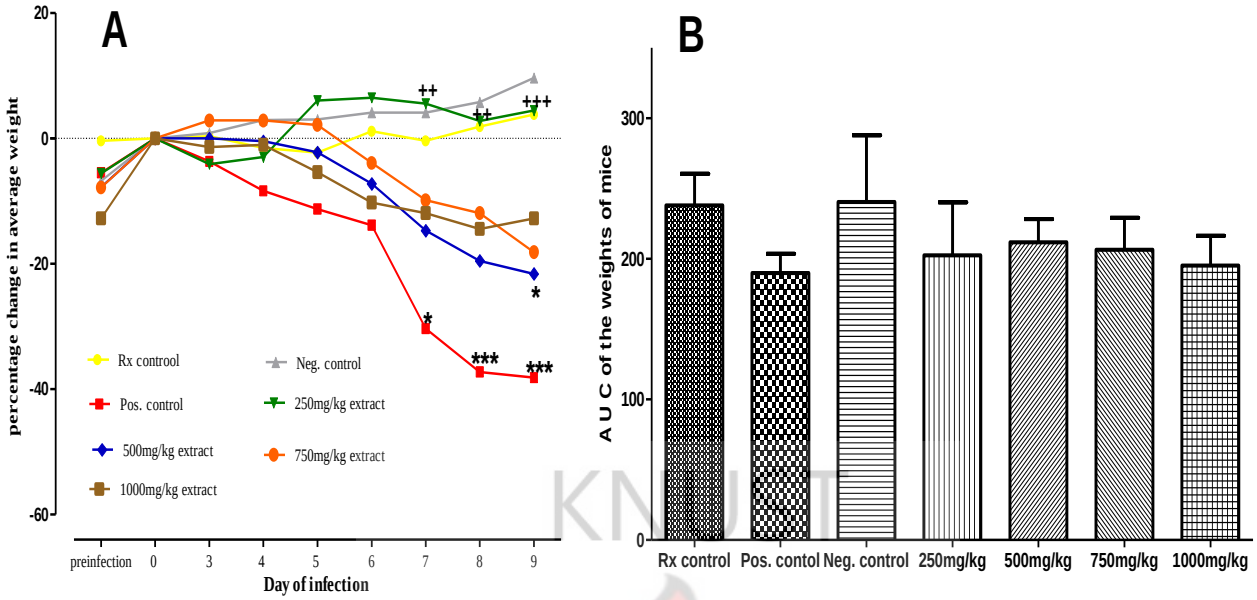


Figure 3.13 - Percentage change in the average weight of ICR mice preinfected with Plasmodium berghei NK65 strain

[**A**]- Estimation of the percentage change in weights of the infected mice treated with four different doses of ML. N=5. Each point represented Mean ± SEM. Statistical analysis using two-way ANOVA followed by Bonferroni post-tests. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ denoted significance levels when compared with negative control. +$p< 0.05$, ++$p< 0.01$ and +++$p<0.001$ denoted significance levels when compared to positive control. [**B**]- Total estimation of the weights of each experimental group by the end of the investigation. Each bar represented Mean ± SEM. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ represented significance level as compared to the negative control.

The weight distribution of the animals treated with the ML extracts were also monitored. From fig. 3.13A, the negative control group experienced a gradual increase in their weights throughout the duration of the investigation. This was predictable, as they were not infected and so were considered to be normal and healthy. For the treatment control group, there was a small decline in their weights on the 4th day (-1.52%) and 5th day (-2.27%) of infection. The weights then increased gradually afterwards, with a slight decline again on day 7 (-0.34%). In the case of the 250 mg/kg group, the weights declined on day 3 post-infection (-4.07%) and then increased to a peak of (+6.40%) on day 6 post-infection. For the other groups, 500 mg/kg, 750 mg/kg and 1000 mg/kg, they all experienced varying degrees of decline in their

weights. The best of them was the 750 mg/kg group. The weights of the animals were generally observed to decline from the Two-Way ANOVA analysis ($p < 0.0001$). The positive control group, experienced the greatest of percentage reductions from day 6 (-13.82%) to day 9 (-38.18%). This decline was significant as compared to the negative control group. On the contrary, these percentage changes in weights when summed together revealed no significant difference in the weights of all the groups in a One-Way ANOVA analysis, followed by the Newman-Keuls post-test ($F_{6,26} = 0.5253$, n = 7, $p = 0.7839$; fig. 3.13B).

3.3.3.6 Survival record of experimental mice

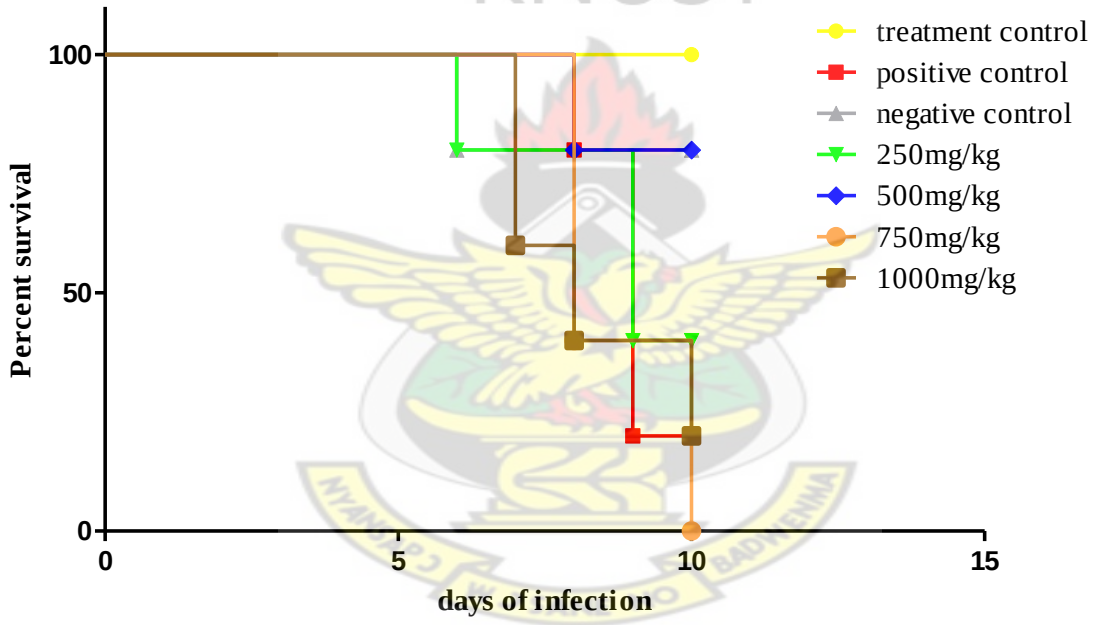
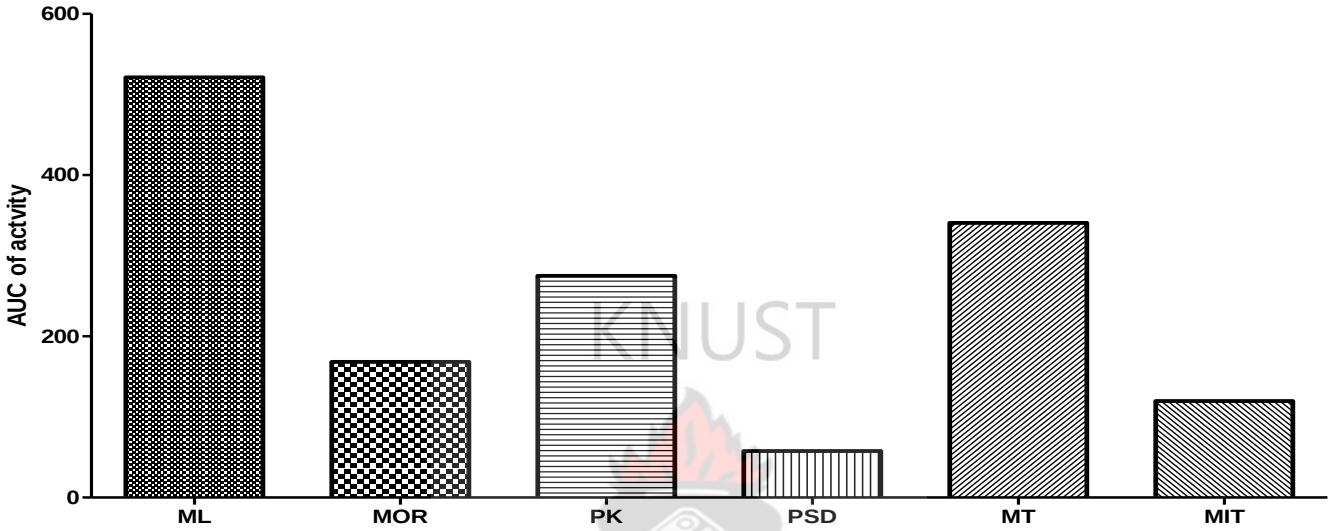


Figure 3.14 - Survival record for preinfected ICR mice in the 7-day curative test with four doses of ML extract.

Percentage survival of the experimental groups employed in the curative test. Each group started with 5 mice and depending on the extract, different curves were recorded from 10 days of observation. Each point represented percentage of survived animals on a given day of infection. Results were analyzed using Mantel-Cox test and Log-rank test for trend from GraphPad Prism v 5.

From the mortality and survival records obtained as the days of infection progressed, it was shown that none of the animals in the treatment control group was lost, thus, recording a 100% survival by the end of the investigation (fig. 3.14). The positive control group

maintained 100% survival up to the 8th day of infection, where it reduced to 80% with the loss of one animal. The next day saw a loss of three more animals in the same group, reducing survival to 20% and then maintained to the end of the study. In the case of the negative control, survival reduced to 80% on day 6 post-infection, with loss of one animal and this was then maintained throughout the rest of the days for the study. One animal was lost from the 250 mg/kg group on day 6 post-infection (that is, 80% survival). However, on day 9 post-infection, survival reduced to 40% and then maintained. For 500 mg/kg group, survival reduced to 80% on day 8 post-infection and was then maintained. For 750 mg/kg, survival reduced drastically on day 8 post-infection to 40% (that is, loss of three animals) and then on day 10 post-infection, none of the rest survived (0% survival). In the case of 1000 mg/kg group, survival reduced to 60% on day 7 post-infection and then to 40% on day 8 post-infection and finally to 20% on day 10 post-infection. Notwithstanding these observations, upon comparison of the survival curves from the different experimental groups, it was observed that the curves were not significantly different from each other according to the Mantel-Cox test analysis from GraphPad Prism ($\chi^2 = 12.48$, $df = 6$, $p = 0.0522$). In addition to that also, it was also shown that there was a significant linearity between the doses and the median survival from the Log-rank test for trend ($\chi^2 = 4.910$, $df = 1$, $p = 0.0267$). This meant that, the survival of the animals to a greater extent depended on the dose administered. It could therefore be inferred that, the higher the dose used in the experimentation, the more toxic it was to the animals, with the optimum dose offering a balance between the activity or efficacy, survival and toxicity and that was 500 mg/kg.



ML - aqueous extract of *Moringa leaves*
MOR - methanol extract of *Moringa leaves*
MT - aqueous extract of *Mitragyna inermis*
PK - aqueous extract of *Pseudocedrela kotschy*i
PSD - methanol extract of *Pseudocedrela kotschyi*
MIT - methanol extract of *Mitragyna inermis*

Figure 3.15 - Comparison of the activity of aqueous and methanolic extracts of the medicinal plants

3.3.3.7 Visual inspection of animal vitals during ML treatment

Table 3.5 - Physical signs of illness associated with the infection and therapy

DAY OF INFECTION	TREATMENT CONTROL				POSITIVE CONTROL				NEGATIVE CONTROL				250 MG/KG				500 MG/KG				750 MG/KG				1000 MG/KG			
	D	L	P	M	D	L	P	M	D	L	P	M	D	L	P	M	D	L	P	M	D	L	P	M	D	L	P	M
1	-	-	+	-	-	-	+	-	-	-	+	-	-	-/+	+	-/+	-	-	+	-	-	-	+	-	-	-	+	-
2	-	-/+	+	-	-	-/+	+	-	-	-`	+	-	-	-	+/++	-/+	-	-	+	-	-	-	+	-	-	-	+	-
3	-	-	+	-	-	-	++	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-
4	-	-	+	-	-	-	++	++	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-
5	-	+	+	+	-	+++	+++	+++	-	-	+	-	-	+	++	+	-	++	++	+	-	++	++	++	-	++	++	+++
6	-	-/+	+	-/+	++	+++	+++	+++	-	-	+	-	-	++/+++	++	++	-	++	++	++	-	+++	+++	+++	-	+++	+++	+++
7	-	-	+	-	++	+++	+++	+++	-	-	+	-	-	+++	++	+++	-	++	+++	++	-	+++	+++	+++	-	+++	+++	+++
8	-	-	+	-	++	+++	+++	+++	-	-	+	-	-	+++	+++	+++	-	+++	+++	++	-	+++	+++	+++	-	+++	+++	+++
9	-	-	+	-	++	+++	+++	+++	-	-	+	-	-	+++	+++	+++	-	+++	+++	+++	-	+++	+++	+++	-	+++	+++	+++

Physical signs of the experimental ICR mice were monitored to determine the effects of the infection on the animals and how these effects could be reversed with the administration of ML extracts. N = 5. KEY: (-) absent; (+) mild; (++) moderate and (+++) severe, signifying severity of illness; **D** –Diarrhoea; **L** – Lethargy; **P** – Piloerection and **M** – decreased Locomotion. (-), (+), (++) and (+++) assigned when 4-5 mice in the group exhibited similar signs; (-/+) and (+/++) assigned when at least 2 mice in the group exhibited one similar sign while the others also exhibited another similar sign.

From Table 3.5 above, it can be seen that progressively, the conditions of the infected experimental groups deteriorated with the exception of the negative control and the treatment control groups. Whiles the negative control group was generally considered to be well with just mild piloerection, because the animals were not infected, the treatment control group on the other hand, although being infected, didn't experience serious pathologic effects from the infection due to the complete parasite clearance from the blood (as was observed in the *In-vivo* studies, see sec. 3.3.2.1 above) resulting in remission after day-6 of infection (see Table 4.5 above). Due to the severity of the infection in the positive control group, there was progressive deterioration in signs associated with the infection. As early as on the third day, symptoms became paramount starting with a moderate piloerection and a mild decline in locomotion of the animals. By the fifth day of infection, lethargy, piloerection and decreased locomotion were all severe with the exception of diarrhoea but further deterioration hit the group on day 6 with diarrhoea becoming moderate. It was expected that these signs of illness exhibited in the positive control group would be ameliorated with the administration of ML extracts, however, this was not entirely the case. One key observation with the groups administered with the extracts was the one-day delay in the appearance of symptoms like piloerection and lethargy as compared to the positive control group. Notwithstanding this, symptoms in these treated groups also deteriorated in like manner as the positive control group with better profile by the 250 mg/kg and 500 mg/kg groups as compared to the 750 mg/kg and 1000 mg/kg groups.

3.4 DISCUSSION

Malaria infection as established from **Sec. 1.2.1** above, is made up of the sexual and the asexual stages; the asexual stage starting in the human host right after a mosquito bite to parasites developing in the erythrocytes, and the sexual stage, which starts from differentiated gametocytes in the host blood being taken up by the mosquito during another bite and going through development processes in the vector (Okwa 2012).

Infection of the host is only established when viable asexual forms of the parasite are able to overcome the host's immune responses and multiply in the blood. In a bigger picture, the balance between activation of both non-specific and specific host defensive mechanisms, the intrinsic susceptibility of the host red blood cells, and the ability of the parasites, present in substantial quantities (Thurston 1950) to multiply, due to their viability, are major factors that influence infection of the host (Chotivanich *et al*. 2000). Without viable forms of the asexual parasites, infection cannot be achieved. Thus, evaluation of agents on animals preinfected with non-viable forms, only flaws the entire study as absence of parasites upon examination of the blood smears may not be attributable to the activity of the agent but to non-viability of the parasites. It was therefore expedient to ascertain the viability of the parasites employed for the investigation. Hence the need for the initial investigation, to observe the increment in parasitemia after thawing (see fig. 3.2A; **Sec. 3.3.1.1** above). The inoculum size of 2×10^7 parasitized rbcs/ml employed, as informed from several studies (Thurston 1950; Janse *et al.* 2014; Fidock *et al*. 2004), was adequate to establish infection in the murine models. Increment in the parasitemia gave indication of regain of viability, as parasites were able to multiply. There was also an indication of virulence, by their ability to induce clinical complications in the host and cause death eventually after 7-10 days of infection. The higher total parasitemia (AUC) for second passage as compared to that from first passage (see **Sec. 3.3.3.1**; fig. 3.2B above) could be attributed to the increased virulence of the parasites after

several cycles of multiplication in the host (White 1997). Also, due to host immune responses, the initial parasitemias recorded after the second passage were relatively lower as compared to the first (see fig. 3.2a). As a result of increased viability after several cycles of multiplication in the first animal, the second passage was characterized by exponential or log increase in parasitemia, which is common with asynchronous infection (White 1997). Malaria infection can either be synchronous or asynchronous; the more synchronous a malaria infection, the more marked are the rises and falls in peripheral parasitemia, and this corresponds to merogony (development of merozoites in the blood) and sequestration, respectively. Asynchronous infections on the other hand have unpredictable cycles of merogony and sequestration. Thus, whiles synchronous infection leads to parasitemia rises like a step ladder, asynchronous infections are characterized by a log linear rise in parasitemia (White & Davis 1992).

The study kick started with screening of the aqueous extracts. These extracts were prepared, mimicking the traditional mode of preparation for the plant parts (see **Sec. 2.3.2.1** above). Exhibition of activity of the aqueous extracts prepared from these parts confirmed and justified the use of these parts for the treatment of malaria. The results obtained showed highest activity for ML followed by MSB, then MT and finally PK (see fig. 3.5b above). Considering the fact that bioactivity of these extracts have been attributed to the presence of secondary metabolites (see **Sec. 2.5** above), the difference in activity (see **Sec. 3.3.1.4**; fig. 3.5A & B above) for the extracts could be accounted for by the varying composition in the constituents. For example, ML and MSB contained almost similar constituents except for flavonoids, which were present in ML but absent in MSB (see Sec. **2.4.2**; table 2.2 above). Flavonoids, mostly found in the aerial parts of the plant and also known to possess antiplasmodial activity (Kraft *et al*. 2000; Bero *et al*. 2009; Tadigoppula *et al*. 2013; Bhattacharya *et al*. 2009), could be said to be responsible for the relatively higher activity of

ML as compared to MSB (fig.4.5b). Similar observations could be made of the varying activities observed for MT and PK as reducing sugars were present in MT, ML and MSB but not in the PK. In the evaluation of the organic extracts, MOR was shown to be more effective than MIT, which was in turn, also effective than PSD (see fig.3.7A & B). Once again, differences in bioactivity is hereby attributed to the varying compositions in the organic extracts. For example, coumarins, were shown to be absent in MOR but present in PSD and MIT (see Table 2.3 above). Also, saponins were present in PSD but not in MOR and MIT. The presence and/or absence of these compounds, impacting through potentiation, synergism and antagonism (Bell 2005; Rasoanaivo *et al*. 2011) influence the variation in bioactivity.

The exponential increment in the Parasite Reduction Ratio (PRR) for the reference drug (A/L), as compared to the three organic extracts (see fig. 3.7C above), may be attributed to the potency of the agent in fighting the infection, the pharmacodynamics of the agent and also the nature of infection (White 1997). The potent an agent is, the more effective it will be in clearing the parasites and thus, cause parasitemia at any point in time, to be lower than the initial parasitemia. Hence, PRR would be increasing with day (see Table 3.3 above). It stands to reason then, that A/L, being the reference drug, well-known for its rapid parasite clearance (Bosman *et al*. 2001), and causing a significant reduction in parasitemia after administration (see fig. 3.6A & B), is therefore potent. However, it may be premature to conclude then, that the extracts by virtue of their continuous decline in PRR, are not potent. This is because, the competing effects of the existing compounds in the extracts, may act to result in the net decline of their bioactivities. Isolation of specific compounds from the extracts may afford enhanced activity and increased potency. For example, history holds it that, the initial evaluation of the aqueous extract of *Artemisia annua* leaves, failed to show significant activity, which led to abandoning of project. However, through 'Program 523', *Artemisia annua* has yielded the artemisinins which have become a 'powerful force' to reckon with in

malaria treatment (Klayman 1985; Li *et al*. 2006; Faurant 2011). Thus, it may be inferred that, though the potencies of the extracts kept declining, isolation of specific compounds would offer a positive alternative to the currently used artemisinins.

Pharmacodynamics of an agent deals basically with how the drug acts in the host to cause inhibition (White 1997). The mode of action, the time of initiation of therapy, in relation to pre-existing parasitemia, as well as the competing effects of the compounds in the extracts, affect the pharmacodynamics of drug activity, and thus, PRR. In terms of mode of action, the effect exhibited by a plasmocidal agent would be different from that of a plasmostatic agent. A plasmocidal agent would invariably kill existing parasites and cause reduction of parasitemia whiles a plasmostatic agent would prevent the multiplication of parasites. Thus, PRR may seem to increase for a plasmocidal agent whereas PRR may either be maintained, increase slightly or reduce with time. This phenomenon could explain the continuous decline in PRR for the organic extracts. The efficacy of an agent in clearing the asexual forms or inhibiting them also largely depends on the time of introduction of the agent. Introducing the agent during when most of parasites are in the sequestered stage only means that the agent will not be accessible to all the parasites and the likelihood of infection proceeding a little further before clinical improvement, cannot be overlooked (Silamut & White. 1993).

The significantly higher activity for the two lower doses, 250 and 500 mg/kg as compared to 750 and 1000 mg/kg of ML extracts, in both the four-day suppressive and the curative models in the dose ranging tests, indicated a reversal of activity beyond a certain concentration of extract in the host's blood. The results obtained showed that, as the dose increased, selectivity to plasmodial parasites and specificity of antiplasmodial activity were reducing and this resulted in the declination of activity for the higher doses (that is, 750 and 1000 mg/kg) (see fig. 3.10B; fig. 3.11A & B above). Reversal of activity as a result of loss of selectivity to only parasites, could also explain for potential toxicity as was seen in the weight reductions and

survival plots (see **Sec. 3.3.3.5** above).The loss of activity could be attributed to functional antagonism, where increasing dose may lead to increasing the concentration of constituents, which act independently of each other but exhibit effects opposite to each other (Fleming 2004). It could also be competitive antagonism, where increasing the concentration of the compounds, allow for competition in occupation for the receptor site (Fleming 2004; Shapiro & Goldberg 2006). Thus, a relatively higher concentration of an antagonistically acting constituent would either prevent inhibition or rather enhance parasite growth. This could be the likely case for the 1000 mg/kg ML extract (see fig. 4.10a &b).

Contrary to the proposal that severity of the infection is dependent on the parasite biomass in the blood (Chotivanich *et al*. 2000), it has been shown from the dynamics of the malaria infection that, the relation is complex (White 1997; Thurston 1950). The asexual stage, which is responsible for the infection, and so the target of most currently used antimalarials, is shown to have a cycle of 48 hours in the erythrocytes. However, only half the period of this cycle is visible to the microscopist (merogony). The other half of the cycle involves asexual parasites sequestering in the microvasculature and this is responsible for the severity of the infection (White 1997). Since there is no antimalarial drug that act immediately after administration, even if administered intravenously (Bosman *et al*. 2001), the trend of parasitemia in the first few hours following the initiation of therapy, is usually the same as that which would have occurred without treatment (White 1997).

From the current study conducted, Artemisinin-Lumefantrine, which was given to the treatment control, exhibited properties expounded above; exhibiting rapid parasite clearance from the blood to cause a remission by the third day of administration in the treatment group. This could be attributed to the activity of the drug against the ring and the other asexual forms in the blood immediately after administration. This was also evident in the records from the signs and symptoms (Table 3.5; **Sec. 3.3.3.7** above) where clinical improvement was

observed in the same treatment group. For the extracts, it may be proposed that lack of activity against the early stages of the asexual cycle could account for the progressive rise in parasitemia even after therapy initiation (Hughes *et al*. 2010). Thus, the continuation of cytoadherence even after therapy initiation resulted in the severity of infection with attendant deteriorations in clinical signs observed in the infected groups treated with the ML extracts (Table 3.5; **Sec. 3.3.3.7** above) as well as the reduction in the weights of the experimental mice (see fig. 3.9A; **Sec. 3.3.2.4** and fig. 3.13A; **Sec. 3.3.3.5** above).

Piloerection, diarrhoea, lethargy, decreased locomotion (Table 3.5) and weight reduction (fig. 3.8 and 3.12) observed in the positive control and the other treated groups could be attributed to the release of the pro-inflammatory cytokines (eg. TNF-α) from the host's immune system (Kamada *et al*. 2000; Basir *et al*. 2012; Boampong *et al*. 2013; Hodges & Gill 2010). Basir *et al* also proposes hypothermia, as a homeostatic mechanism in adapting to the heat loss, to be one of the causative factors for piloerection and weight reduction to be as result of either lack of feeding due to the infection or consequences of disturbed metabolic function and hypoglycaemia associated with malaria infection or both (Basir *et al*. 2012).

It was observed that the aqueous extracts of the plants were more efficacious *In-vivo* as compared to the methanolic extracts from the same plants at comparable doses (see fig. 4.15 above). This could be attributed to the different constituents present in the aqueous and methanolic extracts as discussed in **Sec. 2.4** above.

Conventionally, a drug ought to be given at intervals corresponding to its biological half-life. In the case where the drug exerts its effects only at concentrations above a therapeutic level, then the biological half-life is said to correspond with the drug half-life and this would call for regular administration of the drug. On the other hand, if the drug exhibits a concentration-dependent killing, then biological effects would persist even when the drug's concentration

declines below the therapeutic level. Such a drug could be given less frequently than the stipulated regimen predicted from pharmacokinetic studies (White & Davis 1992; Drusano 1988). Available evidence depict that in spite of rapid absorption and elimination half-lives for the Artemisinins and their biologically active metabolite, Dihydroartemisinin, these compounds are equally effective when given once or twice daily due to this principle (Nosten *et al*. 1994; Looareesuwan 1994). This was also evident from the current study where the standard drug A/L given at 4 mg/kg once daily, in the evaluation of organic extracts (see fig. 4.6a above), was shown to exhibit similar response to the same drug at the same dose given twice daily in the dose-dependent evaluation of ML extracts (see fig. 3.10A). The results obtained from ML extracts (250 - 1000 mg/kg) in both four-day suppressive and the curative tests could also be explained by one of these two principles. Parasite inhibition by the extracts at lower doses (250 - 500 mg/kg) is proposed to be dose or concentration dependent. The higher the dose within this range, the better the suppression or activity (fig. 3.10B above). Above 500 mg/kg, the activity declines and this could mean that, optimum activity of ML from this investigation is 500 mg/kg.

From the survival plots, 250 and 500 mg/kg groups retained 80% of the starting animals but as the dose increased above that, there was a decline in the survival. However, groups treated with 750 and 1000 mg/kg recorded 100% and 80% mortality by the end of the experiment. Comparing the results from the survival plot to that from the weight distribution, it goes on to buttress the point that the most effective dose of ML from the investigations was 500 mg/kg. From (fig. 3.13b), it was shown that the calculated AUC of the weight for the 500 mg/kg group was 211.7 ± 16.53 which was higher than 202.3 ± 37.59 for the 250 mg/kg group, 206.5 ± 22.65 for 750 mg/kg and 195.2 ± 21.10 for the 1000 mg/kg group. One-Way ANOVA analysis however, revealed no significant difference ($F_{6,26} = 0.5253$, $p = 0.7839$).

3.5 CONCLUSION

The three medicinal plants employed in the study possessed *In-vivo* antiplasmodial activity. Both aqueous and methanolic extracts exhibited activity but the aqueous extracts have been shown to possess higher activity compared to the methanolic extracts. Also, aqueous *Moringa oleifera* extract (ML) on further investigation has been shown to be effective between the range 250 – 500 mg/kg. Above this range, likely issues of toxicity set in. In addition, ML administration has been shown not to significantly affect the weights or the survival of treated ICR mice from the statistical analysis conducted. ML also do not improve the clinical signs associated with the infection. Methanolic extracts from *Moringa oleifera* leaves (MOR) and *Mitragyna inermis* twigs (MIT), have been shown to be more effective *In-vivo* against *P. berghei* infected BALB/c mice when compared to the extract from *Pseudocedrela kotschyi* leaves (PSD).

CHAPTER FOUR

CHROMATOGRAPHIC FINGERPRINTING

4.1 INTRODUCTION

For several years, natural products, such as plants, animals and microorganisms have provided mankind with substrates for the treatment of diseases. Extensive studies on these substrates have yielded a number of compounds, some of which have seen developments into substantive drugs for clinical use (Butler 2004). The likes of aspirin, digitoxin, morphine, quinine, and pilocarpine are drugs that have been isolated and developed from plant sources. The famous class of antibiotics, the penicillins, have their origin from microbial sources and so are the cephalosporins (Butler 2004). Marine natural products have been a focus of research for anticancer agents, some of which having gone through clinical trials and with others still at different stages of it (Simmons *et al.* 2005). The process however, have suffered from setbacks such as being very long thereby making it expensive, issues about intellectual property, low success rate and the introduction of High Throughput Screening (HTS) with Combinatorial chemistry (Sanchez-Martin *et al.* 2004). Notwithstanding these challenges, recent developments in separation, spectroscopic and other instrumental techniques (Ovenden *et al.* 2008), coupled with natural products being a large source of chemical diversity (Harvey 2008) have induced renewed attention (Dinan 1998). Some of the motivating factors have been the recent breakthroughs in the likes of Paclitaxel (Taxol®) as an anticancer drug from *Taxus brevifolia* and the Artemisinins for malaria (Butler 2004). One study reveals that, 8 out of 29 drugs launched in the year 2000 were derived from natural products or hormones (Proudfoot 2002). It also concluded that HTS did not have a significant impact on the derivation of these drugs (Proudfoot 2002).

Embedded in drug development process from natural sources is dereplication, which involves the use of chemical techniques to eliminate extracts containing active constituents that have already been isolated and characterized. Dereplication deals with the chemical screening of bioactive extracts using procedures like chromatography, nuclear magnetic resonance (NMR), ultra-violet (UV) and visible (Vis) spectroscopy and mass spectrometry (MS), and comparing the results to a database to identify active compounds that have already been investigated (Thomas 2007). One technique that has played critical role in the process of dereplication is chemical fingerprinting. Chemical fingerprinting enables the detection of the chemical uniqueness of a series of extracts that have been identified to have bioactivity against specific biological targets of interest (Ovenden *et al*. 2008). Ovenden *et al*, argue that chemical fingerprinting serves as an indicator of the complexity of the extracts and allows for early identification of the types of chemistry (or chemical classes) present in the extracts (Ovenden *et al*. 2008).

In quality control of traditional medicines, chemical fingerprinting has found use in species identification and phytochemical profiling (Colegate & Molyneux 2007). In China, where traditional medicinal plants are highly patronized in their healthcare and also in the United States of America, where these plants are used as dietary supplements, this process has led to the developments of fingerprints for a number of extracts from plant samples, some of which are documented in pharmacopoeias (Schaneberg *et al*. 2003; Srivastava *et al*. 2004; Birk *et al*. 2005; Xie *et al*. 2006; Wu *et al*. 2006; Dong *et al*. 2008). This enables the detection of adulteration and assessment of quality of the preparations for human use (Colegate & Molyneux 2007).

Typically in chemical fingerprinting, chromatographic methods are employed in the separation of the complex extract matrices. Solid phase extraction (SPE), Countercurrent Chromatography (CC), Centrifugal Partition Chromatography (CPC), Thin Layer

Chromatography (TLC), High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) are some of the chromatographic methods reported to have been employed to cause the separations (Dinan 1998). Detection of the separated components have been achieved with UV/Vis detectors, MS, IR and NMR (Dinan 1998). Several methods employing these instrumental analysis have been developed; some available to be replicated for purposes of identification and confirmation as well as quality control (Srivastava *et al*. 2004; Lazarowych & Pekos 1998; van Elswijk *et al*. 2004; Staerk *et al*. 2009; Alali & Tawaha 2009). Application software programmes (for example, MS-Gold®) have also been developed to capture fingerprints of extracts to aid dereplication processes.

In this project, LC-UV would be used to obtain fingerprints for the bioactive extracts produced from the medicinal plants. This investigation seeks to augment results from the phytochemical analysis (see Chapter 3 above) in assessing the variation in composition of the extracts available. It also seeks to provide vital information on the nature of the constituents which would help in fractionation of the extracts using preparative HPLC after optimization of the chromatographic conditions.

4.2 MATERIALS AND METHODS

4.2.1 MATERIALS

The complete chromatograph was a Shimadzu UFLC that consisted of LC-10AT Shimadzu pump with programmable UV detector (783A Applied biosystems) and Shimadzu CR501 chromatopac. Column used was Agilent Zorbax SB C18 5µm (4.6 × 250 mm). Solvents for the mobile phase development were ethyl acetate, methanol and water.

4.2.2 METHOD

The mobile phase systems developed consisted of different proportions of ethyl acetate, methanol and water eluted isocratically at 0.8 ml/min and 1 ml/min. A composition of methanol: water (40:60) was developed for the aqueous extracts and ethyl acetate: methanol (50:50) for the methanol extracts. 20µl portions of 0.01%w/v of the extracts were loaded and injected unto the column after dissolving in suitable solvent amidst sonication at 40 ^{o}C. Elution was monitored at different wavelengths and chromatograms for each extract obtained for qualitative purposes and also for method development for fractionation and isolation.

4.3. RESULTS

4.3.1 FINGERPRINTS FOR AQUEOUS EXTRACTS

a) *Moringa oleifera* leaves (ML)

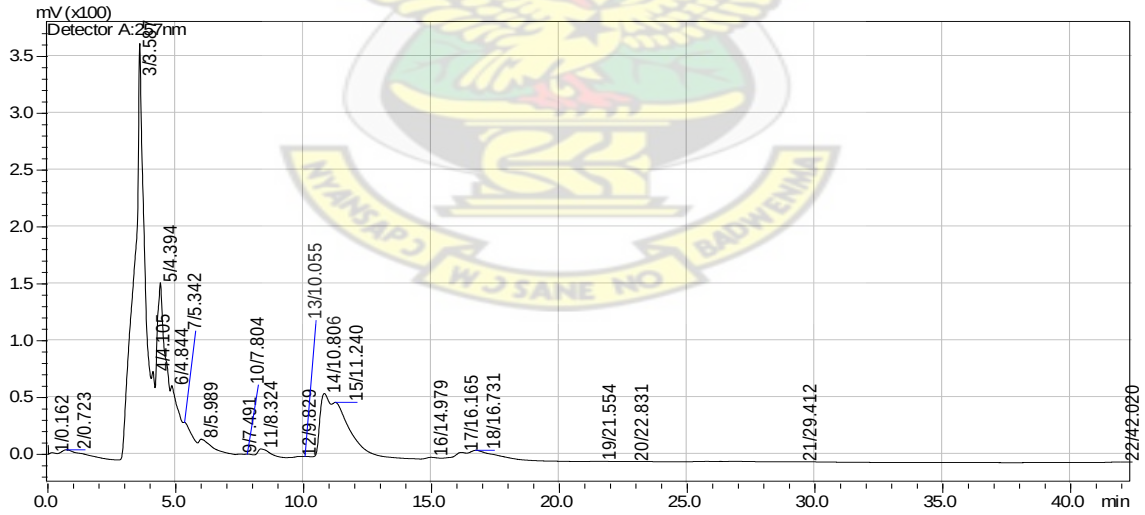


Figure 4.1 - Chromatogram for aqueous ML extract

Chromatogram showing peaks of compounds present in ML. Peaks remaining undetectable within the adopted scale of absorbance are components present in relatively smaller quantities in the extract. Conditions employed were methanol: water (40:60), flow rate of 1.2 ml/min and detection at 257 nm.

b) *Moringa oleifera* stem bark (MSB)

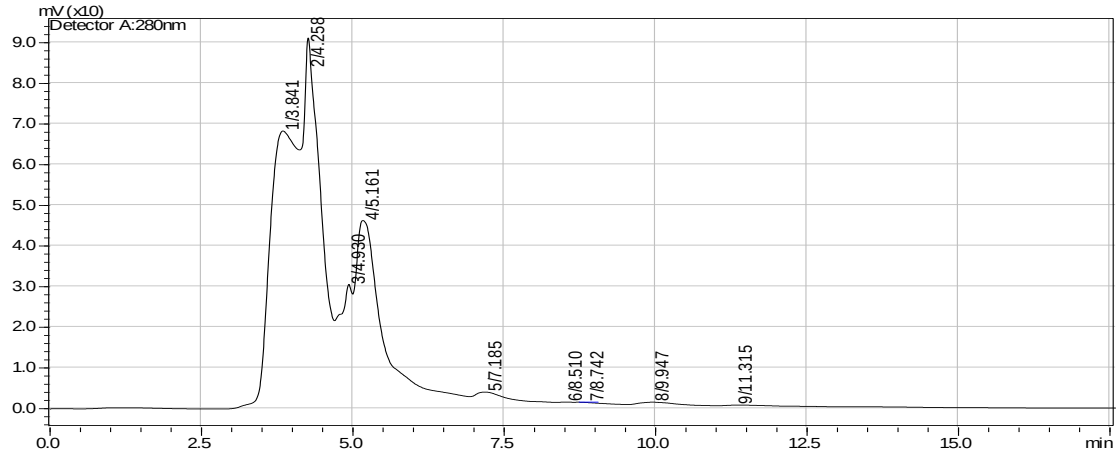


Figure 4.2 - Chromatogram for aqueous MSB extract

Chromatogram showing peaks of compounds in MSB. Presence of compounds indicated by the peaks. Other compounds in relatively smaller quantities remain not detected but retention times show on chromatogram. Conditions employed were methanol: water (40: 60), flow rate of 1 ml/min and detection at 280 nm.

c) *Pseudocedrela kotschyi* leaves (PK)

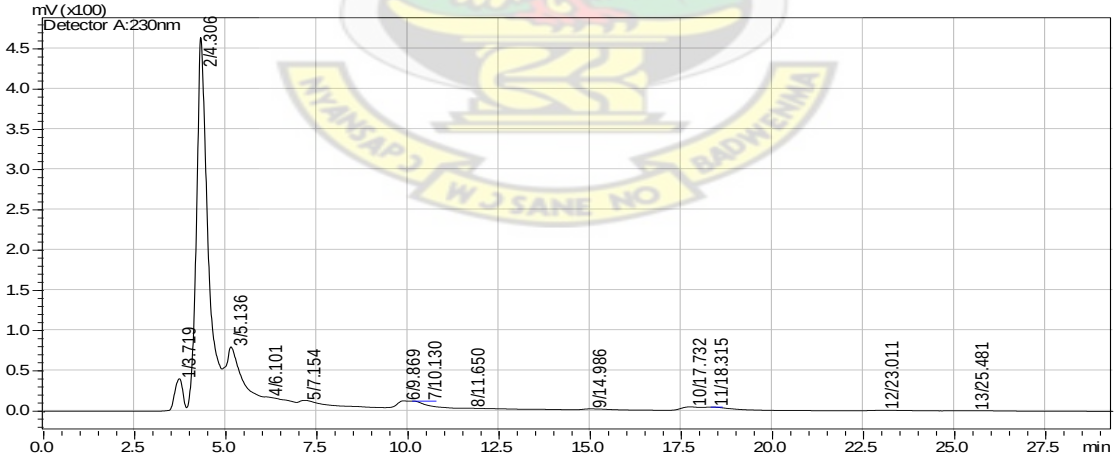


Figure 4.3 - Chromatogram for aqueous PK extract

Chromatogram showing peaks of compounds in PK. Presence of compounds indicated by the peaks. Compounds in relatively smaller quantities remain not detected but retention times show on chromatogram. Conditions employed were methanol: water (40: 60), flow rate of 1 ml/min and detection at 230 nm.

d) *Mitragyna inermis* twigs (MT)

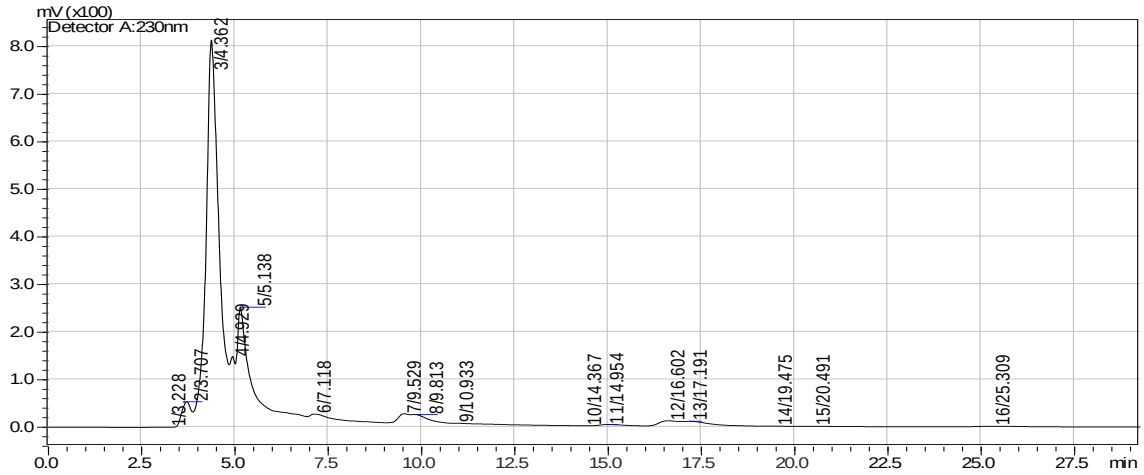


Figure 4.4 - Chromatogram for aqueous MT extract

Chromatogram showing peaks of compounds in MT. Presence of compounds indicated by the peaks. Compounds in relatively smaller quantities remain not detected but retention times show on chromatogram. Conditions employed were methanol: water (40: 60), flow rate of 1 ml/min and detection at 230 nm.

4.3.2 FINGERPRINTS FOR METHANOLIC EXTRACTS

a) *Moringa oleifera* leaves (MOR)

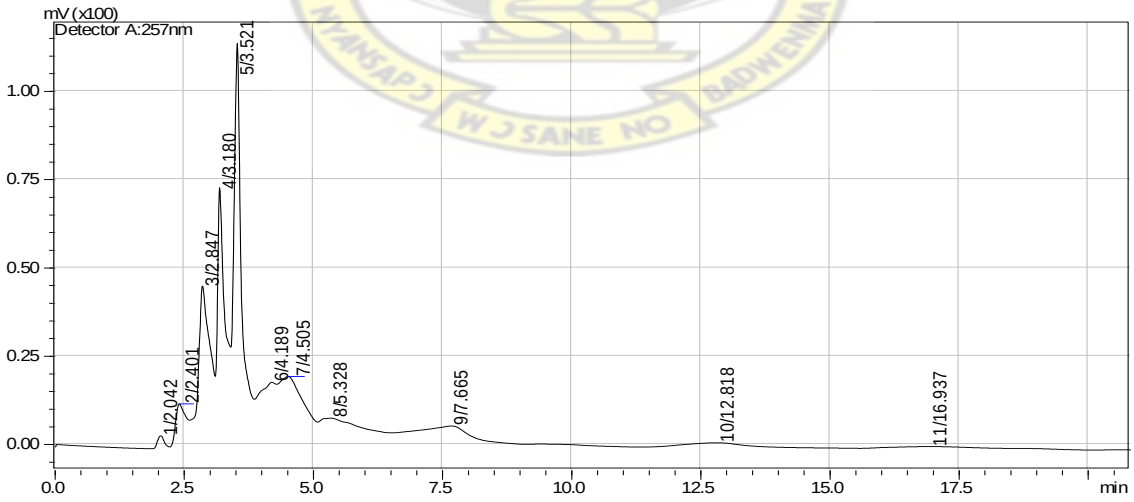


Figure 4.5 - Chromatogram for MOR extract

Chromatogram showing peaks of compounds in MOR. Presence of compounds indicated by the peaks. Compounds in relatively smaller quantities remain not detected but retention times show on chromatogram. Conditions employed were ethyl acetate: methanol (50: 50), flow rate of 0.8 ml/min and detection at 257 nm.

b) *Pseudocedrela kotschyi* leaves (PSD)

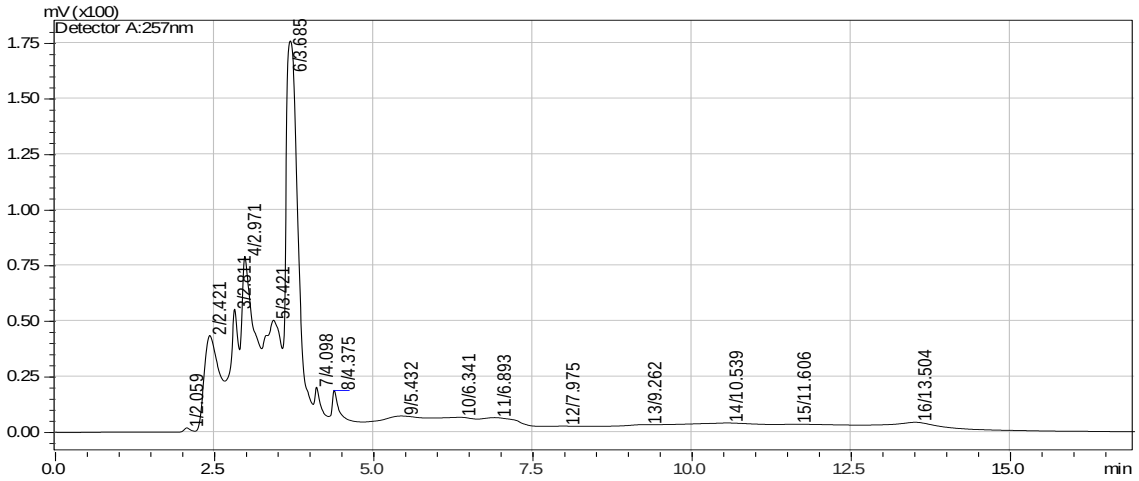


Figure 4.6 - Chromatogram for PSD extract

Chromatogram showing peaks of compounds in methanolic extract of PSD. Presence of compounds indicated by the peaks. Compounds in relatively smaller quantities remain not detected but retention times show on chromatogram. Conditions employed were ethyl acetate: methanol (50: 50), flow rate of 0.8 ml/min and detection at 257 nm.

c) *Mitragyna inermis* twigs (MIT)

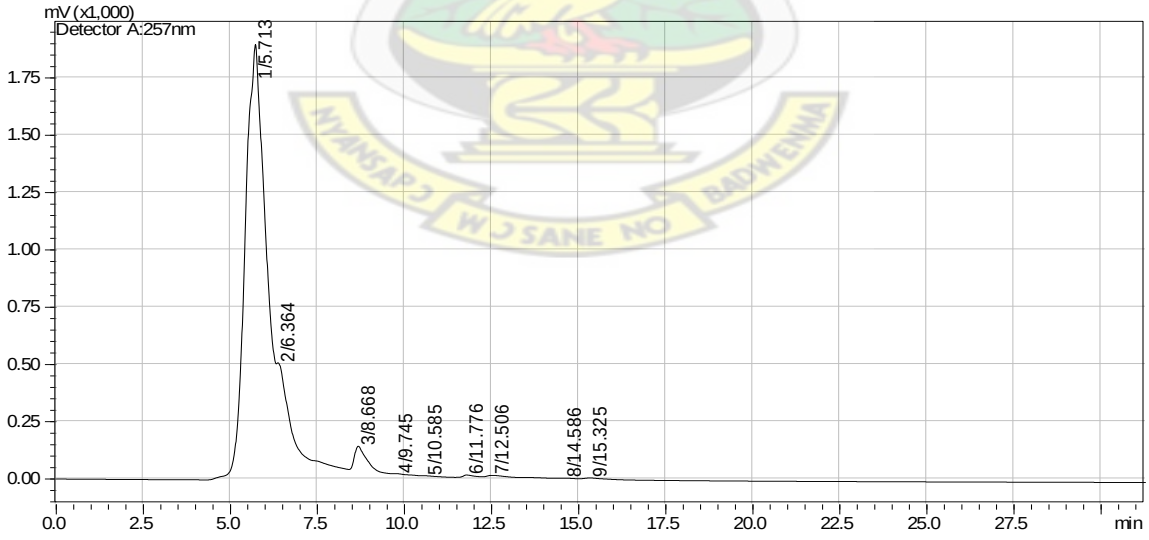


Figure 4.7 - Chromatogram for MIT extract

Chromatogram showing peaks of compounds in methanolic extract of MIT. Presence of compounds indicated by the peaks. Compounds in relatively smaller quantities remain not detected but retention times show on chromatogram. Conditions employed were ethyl acetate: methanol (50: 50), flow rate of 0.8 ml/min and detection at 257 nm.

4.4 DISCUSSION

Indication of *In-vivo* bioactivity for the medicinal plants in this study prompted the development of fingerprints using Reverse phase HPLC for both aqueous and methanolic extracts. The primary objective of this investigation was to develop fingerprints or profiles that give indication of the nature of constituents present in the extracts, which will facilitate preparative chromatographic fractionation leading to isolation of compounds with bioactivity from these extracts. From the chromatograms obtained, it was observed that generally, all the constituents in the extracts were eluted within 20 minutes of injection. Considering the aqueous extracts, it could be said that some peaks were recurrent in the extracts, signifying a likely similar component present in them. For example, the peak occurring in ML (t_R = 4.394s), MSB (t_R = 4.258s), PK (t_R = 4.306s) and MT (t_R = 4.362s) could be said to be a recurrent component. However, there were other peaks specific for some of the extracts, for example, a peak at t_R = 10.806s for ML. Recurrent peaks could also be said to be present in the chromatograms of the organic extracts but quite significantly, their hplc profiles were different from each other, especially for MIT.

The results obtained depicted that the diversity in composition of the extracts accounted for the differences in the chromatograms developed and this confirmed results from phytochemical analysis. Previous studies have shown that most of isolated compounds from plants, known to possess antiplasmodial activity usually originate from organic extracts (Bero *et al*. 2009; Caniato & Puricelli 2003; Lopes & Nogueira 2011). Having established from this project that the aqueous extracts are more active than the methanolic extracts at comparable doses, the current results provide a headway for developing and optimizing methods for fractionating and isolating compounds from the aqueous extracts, which are bioactive. Notwithstanding that, the chromatographic fingerprints developed could also serve as identification tools to confirm the presence of some of the extracts, especially when these

medicinal plants have been documented to be used traditionally in malaria treatment (Asase *et al*. 2005). The fingerprints of the aqueous extracts therefore could serve purposes in quality assessment for the traditional preparations involving their respective medicinal plants as is done in Traditional Chinese Medicine (TCM) (Xie *et al*. 2006; Dong *et al*. 2008; Wu *et al*. 2006).

4.5 CONCLUSION

Reverse HPLC with a UV detector has been used to develop chromatograms, giving indication of the complexity in the compositions of the aqueous and methanolic extracts. The methods developed required less than 20 minutes to elute all detectable constituents at the given conditions. Generally, chromatograms for the aqueous extracts were developed with a mobile system of methanol: water (40:60) and those for the methanolic extracts were ethyl acetate: methanol (50:50).

RECOMMENDATIONS

In view of the research findings from this study, the following recommendations have been made.

A. Toxicity studies be carried out on the extracts, especially the aqueous extracts, as they mimic traditional mode of preparation of the plants. Reporting the *In-vivo* antiplasmodial effects of the plants should be backed by the evidence of safety on long term administration. With concerns of toxicity springing up from the current study, it is highly recommended that an exhaustive toxicological study be carried out on the aqueous extracts to assess toxicity with administration. In that same regard, effects of the extracts on haematological parameters as well as on key organs such as spleen, kidney, liver and heart ought to be ascertained.

B. The current study concentrated on *Moringa oleifera* leaves (ML), which was the most effective of the aqueous extracts. It is therefore recommended that dose ranging studies be carried out on the other three aqueous extracts, that is, MSB, PK and MT.

C. For the purposes of drug discovery, it is recommended that a bioassay guided fractionation be carried out on the 'promising' organic extracts, that is, MOR and MIT which may lead to the isolation of bioactive compound(s).

D. Isolated compound(s) could then be evaluated in combination with some of the antimalarial drugs currently used for synergy or potentiation and better still for their ability to reverse resistance development.

E. It is also recommended that a computer aided drug design model be adopted on the isolated bioactive compound(s) through Quantitative Structural Activity Relationship (QSAR) studies to develop analogues; those of which, with suitable physicochemical parameters, would be synthesized and activity established both *In-vitro* and *In-vivo*.

F. Due to the numerous medicinal benefits of the investigating plant materials, it is recommended that, isolated compound(s) be also screened for other biological activities.

REFERENCES

Abdin, M.Z., Israr, M., Rehman, R.U. & Jain, S K 2003. Artemisinin, a novel antimalarial drug: biochemical and molecular approaches for enhanced production. *Planta medica*, 69(4), pp.289–99. Available at: https://www.thieme-connect. com/products/ ejournals/abstract/10.1055/s-2003-38871 [Accessed May 19, 2014].

Abiodun, O., Abiodun, O., Gbotosho, G., Ajaiyeoba, E., Happi, T., Falade, M., Wittlin, S., Sowunmi, A., Brun, R. & Oduola, A., 2011. *In-vitro* antiplasmodial activity and toxicity assessment of some plants from Nigerian ethnomedicine. *Pharmaceutical biology*, 49(1), pp.9–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20738218 [Accessed December 8, 2012].

Adeniyi, C.B.A., Odumosu, B.T., Aiyelaagbe, O.O. & Kolude, B., 2010. *In-vitro* Antimicrobial Activities of Methanol Extracts of *Zanthoxylum* xanthoxyloides and *Pseudocedrela kotschyi*. *African Journal of Biomedical Research*, 13(1), pp.61–68. Available at: www.ajbrui.net. [Accessed November 10, 2012].

Adoum, O.A., Nenge, H.P. & Chedi, B., 2012. The steroidal component and hypoglycaemic effect of stem bark extract of *Mitragyna inermis* (wild) o. Kundze (Rubiaceae) in alloxan induced diabetic wistar rats. *International Journal of Applied Biology and Pharmaceutical Technology*, 3(2), pp.169–174 [Accessed November 23, 2012].

Aikawa, M. & Seed, T.M., 1980. Morphology of Plasmodia. In J. P. Kreier, ed. *Malaria*. New York: Academic Press, Inc., pp. 285–344. Available at: http://www.cabdirect.org/abstracts/19810890011.html [Accessed May 28, 2014].

Akande, J.A. & Hayashi, Y., 1997. Potency of extract contents from selected tropical chewing sticks against *Staphylococcus aureus* and *Staphylococcus auricularis*. *World Journal of Microbiology and Biotechnology*, 14(2), pp.235–238. Available at: http://link.springer.com/article/10.1023/A:1008838331079 [Accessed April 21, 2014].

Akuodor, G. C., Essien, A. D., Essiet, G A., Essien D.O., Akpan, J. L. & Udoh, F.V., 2013. Evaluation of Antipyretic Potential of *Pseudocedrela kotschyi* Schweint . Harms. *European Journal of Medicinal Plants*, 3(1), pp.105–113 [Accessed April 13, 2013].

Alali, F.Q. & Tawaha, K., 2009. Dereplication of bioactive constituents of the genus *Hypericum* using LC-(+,-)-ESI-MS and LC-PDA techniques: *Hypericum triquterifolium* as a case study. *Saudi Pharmaceutical Journal*, 17(4), pp.269–74. Available at: http://www.sciencedirect.com/science/article/pii/S1319016409000292 [Accessed June 10, 2014].

Ancolio, C., Azas, N., Mahiou, V., Ollivier, E., Di Giorgio, C., Keita, A., Timon-David, P. & Balansard, G., 2002. Antimalarial activity of extracts and alkaloids isolated from six plants used in traditional medicine in Mali and Sao Tome. *Phytotherapy research*, 16(7), pp.646–649 [Accessed July 30, 2012].

Andersen, S. & Ager, A., 1994. Efficacy of azithromycin as a causal prophylactic agent against murine malaria. *Antimicrobial agents and chemotherapy*, 38(8), pp.1862–1863. Available at: http://aac.asm.org/content/38/8/1862.short [Accessed May 7, 2014].

Andersen, S. L., Ager, A., McGreevy, P., Schuster, B. G., Wesche, D., Kuschner, R., Ohrt, C., Ellis, W., Rossan, R. & Berman, J., 1995. Activity of azithromycin as a blood schizonticide against rodent and human plasmodia *in-vivo*. *The American journal of tropical medicine and hygiene*, 52(2), pp.159–61. Available at: http://europepmc.org/abstract/MED/7872444 [Accessed April 28, 2014].

Andersen, S. L., Oloo, A. J., Gordon, D. M., Ragama, O. B., Aleman, G. M., Berman, J. D., Tang, D. B., Dunne, M. W. & Shanks, G. D., 1998. Successful Double-Blinded, Randomized, Placebo-Controlled Field Trial of Azithromycin and Doxycycline as Prophylaxis for Malaria in Western Kenya. *Clinical Infectious Diseases*, 26(1), pp.146–150. Available at: http://cid.oxfordjournals.org/content/26/1/146.short [Accessed April 28, 2014].

Andrade-Neto, V. F., Brandão, M. G. L., Stehmann, J. R., Oliveira, L. A. & Krettli, A. U., 2003. Antimalarial activity of Cinchona-like plants used to treat fever and malaria in Brazil. *Journal of Ethnopharmacology*, 87(2-3), pp.253–256 [Accessed February 15, 2014].

Anuka, J., Yaro, A. & Otegbade, O., 2005. Some *in-vivo* studies of the aqueous leave extract of *Pseudocedrela kotschyi* (Meliaceae) on laboratory animals. In *23rd National Scientific Conference of the Nigerian Society of Pharmacognosy*. pp. 104–105. Available at: http://www.sciencedirect.com/science/article/pii/S0378874114002876 [Accessed April 21, 2014].

Anwar, F., Latif, S., Ashraf, M. & Gilani, A. H., 2007. *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytotherapy research : PTR*, 21(1), pp.17 – 25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17089328 [Accessed April 21, 2014].

Anwar, F., Ashraf, M. & Bhanger, M.I., 2005. Interprovenance variation in the composition of *Moringa oleifera* oilseeds from Pakistan. *Journal of the American Oil Chemists' Society*, 82(1), pp.45–51. Available at: http://link.springer.com/10.1007/s11746-005-1041-1 [Accessed May 6, 2014].

Anwar, F. & Bhanger, M.I., 2003. Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan. *Journal of agricultural and food chemistry*, 51(22), pp.6558–63. Available at: http://dx.doi.org/10.1021/jf0209894 [Accessed May 6, 2014].

Ara, K., Rahman, M. S., Rahman, A. H. M. M., Hasan, C. M. & Rashid, M. A., 2004. Terpenoids and coumarin from *Bursera serrata* Wall. *Methods*, 8(2), pp.2–6 [Accessed June 22, 2013].

Araújo, M., Bom, J. & Capela, R., 2005. Imidazolidin-4-one derivatives of primaquine as novel transmission-blocking antimalarials. *Journal of Medicinal Chemistry*, 48(1), pp.888–892. Available at: http://pubs.acs.org/doi/abs/10.1021/jm0494624 [Accessed May 29, 2014].

Asase, A., Kokubun, T., Grayer, R. J., Kite, G., Simmonds, M. S. J., Oteng-Yeboah, A. A. & Odamtten, G. T., 2008. Chemical constituents and antimicrobial activity of medicinal plants from Ghana: *Cassia sieberiana*, *Haematostaphis barteri*, *Mitragyna inermis* and

Pseudocedrela kotschyi. *Phytotherapy research*, 22(8), pp.1013–1016 [February 22, 2014].

Asase, A., Oteng-Yeboah, A. A., Odamtten, G. T. & Simmonds, M. S. J., 2005. Ethnobotanical study of some Ghanaian anti-malarial plants. *Journal of Ethnopharmacology*, 99(2), pp.273–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15894138 [Accessed September 19, 2012].

Asawamabasakda, W., Ittarat, I., Chang, C. C., McElroy, P. & Meshnick, S. R., 1994. Effects of antimalarials and protease inhibitors on plasmodial hemozoin production. *Molecular and Biochemical Parasitology*, 67(2), pp.183–191. Available at: http://www.sciencedirect.com/science/article/pii/0166685194001286 [Accessed May 17, 2014].

Atkinson, C., Woods, K. & Dusek, R., 1995. Wildlife disease and conservation in Hawaii: Pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected Iiwi (*Vestiaria coccinea*). *Parasitology*, 111, pp.s59–s69. Available at: http://journals.cambridge.org/abstract_S003118200007582X [Accessed May 28, 2014].

Awasthi, S. K., Mishra, N., Kumar, B., Sharma, M., Bhattacharya, A., Mishra, L. C. & Bhasin, V. K., 2009. Potent antimalarial activity of newly synthesized substituted chalcone analogs *in-vitro*. *Medicinal Chemistry Research*, 18(6), pp.407–420 [February 22, 2014].

Awe, S. O., Olajide, O. A., Oladiran, O. O. & Makinde, J. M., 1998. Antiplasmodial and Antipyretic Screening of *Mangifera indica* Extract. *Phytotherapy Research*, 12(1), pp.437–438 [Accessed June 25, 2013].

Ayo, R. G., Audu, O. T., Ndukwe, G. I. & Ogunshola, A. M., 2010. Antimicrobial activity of extracts of leaves of *Pseudocedrela kotschyi* (Schweinf.) Harms. *African Journal of Biotechnology*, 9(45), pp.7733–7737 [Accessed December 2, 2012].

Bannister, L. H., Hopkins, J. M., Fowler, R. E., Krishna, S. & Mitchell, G. H., 2000. A Brief Illustrated Guide to the Ultrastructure of *Plasmodium falciparum* Asexual Blood Stages. *Parasitology Today*, 16(10), pp.427–433 [Accessed November 19, 2013].

Barbosa-Filho, J. M., Piuvezam, M. R., Moura, Silva., M. D., Silva S. L., Da-Cunha K. V. B., Leitão, E. V., Fechine, I. M. & Takemura, O. S., 2006. Anti-inflammatory activity of alkaloids: a twenty-century review. *Revista Brasileira de Farmacognosia*, 16(1), pp.109–139. Available at: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-695X2006000100020&lng=en&nrm=iso&tlng=pt [Accessed June 4, 2014].

Barnes, K.I. & White, N.J., 2005. Population biology and antimalarial resistance: The transmission of antimalarial drug resistance in *Plasmodium falciparum*. *Acta Tropica*, 94(3), pp.230–240. Available at: http://www.sciencedirect.com/science/article/pii/S0001706X05000811 [Accessed November 5, 2013].

Barnwell, J. W., Asch, A. S., Nachman, R. L., Yamaya, M., Aikawa, M. & Ingravallo, P., 1989. A human 88-kD membrane glycoprotein (CD36) functions *in-vitro* as a receptor

for a cytoadherence ligand on *Plasmodium falciparum*-infected erythrocytes. *The Journal of Clinical Investigation*, 84(3), pp.765–72. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=329717&tool=pmcentrez&rendertype=abstract [Accessed May 29, 2014].

Barnwell, J. W., Wertheimer, S. P., Eaton, J.W., Meshnick, S.R. & Brewer, G.J., 1989. Plasmodium vivax merozoite antigens, the Duffy blood group, and erythrocyte invasion. In *Malaria and the red cell: 2. Proceedings of the second workshop on malaria and the red cell, held in Ann Arbor, Michigan, USA, October 24, 1988.* Alan R. Liss, Inc., pp. 1–11. Available at: http://www.cabdirect.org/abstracts/19900868292.html [Accessed May 28, 2014].

Baruch, D. I., Pasloske, B. L., Singh, H. B., Bi, X., Ma, X. C., Feldman, M., Taraschi, T. F. & Howard, R. J., 1995. Cloning the *P. falciparum* gene encoding *PfEMP1*, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. *Cell*, 82(1), pp.77–87. Available at: http://www.sciencedirect.com/science/article/pii/0092867495900543 [Accessed May 29, 2014].

Basilico, N., 1998. A microtitre-based method for measuring the haem polymerization inhibitory activity (HPIA) of antimalarial drugs. *Journal of Antimicrobial Chemotherapy*, 42(1), pp.55–60. Available at: http://jac.oxfordjournals.org/content/42/1/55.short [Accessed May 17, 2014].

Basir, R., Fazalul, R. S. S., Hasballah, K., Chong, W. C., Talib, H., Yam, M. F., Jabbarzare, M., Tie, T. H., Othman, F., Moklas, M.A.M., Abdullah, W. O. & Ahmad, Z., 2012. *Plasmodium berghei* ANKA Infection in ICR Mice as a Model of Cerebral Malaria. *Iranian J Parasitol*, 7(4), pp.62–74 [Accessed October 31, 2013].

Bastidas, O., 2014. Cell Counting with Neubauer Chamber (Basic Hemocytometer Usage)., pp.1–6. Available at: http://www.celeromics.com/cell-counting [Accessed April 4, 2014].

Bates, M. D., Meshnick, S. R., Sigler, C. I., Leland, P. & Hollingdale, M. R. 1990. *In-vitro* effects of primaquine and primaquine metabolites on exoerythrocytic stages of *Plasmodium berghei*. *The American journal of tropical medicine and hygiene*, 42(6), pp.532–7. Available at: http://europepmc.org/abstract/MED/2164790 [Accessed May 13, 2014].

Becker, K., Tilley, L., Vennerstrom, J. L., Roberts, D., Rogerson, S. & Ginsburg, H., 2004. Oxidative stress in malaria parasite-infected erythrocytes: host-parasite interactions. *International journal for parasitology*, 34(2), pp.163–89. Available at: http://www.sciencedirect.com/science/article/pii/S002075190300314X [Accessed May 11, 2014].

Bell, A., 2005. Antimalarial drug synergism and antagonism: mechanistic and clinical significance. *FEMS microbiology letters*, 253(2), pp.171–84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16243458 [Accessed December 2, 2012].

Berendt, A. R., Simmons, D. L., Tansey, J., Newbold, C. I. & Marsh, K., 1989. Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium*

falciparum. *Nature*, 341(6237), pp.57–9. Available at: http://dx.doi.org/10.1038/341057a0 [Accessed May 29, 2014].

Bero, J., Frédérich, M. & Quetin-Leclercq, J., 2009. Antimalarial compounds isolated from plants used in traditional medicine. *The Journal of Pharmacy and Pharmacology*, 61(11), pp.1401–1433. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19903367 [Accessed June 5, 2013].

Bhattacharya, A., Mishra, L. C., Sharma, M., Awasthi, S. K. & Bhasin, V. K., 2009. Antimalarial pharmacodynamics of chalcone derivatives in combination with artemisinin against *Plasmodium falciparum In-vitro*. *European Journal of Medicinal Chemistry*, 44(9), pp.3388–3393. Available at: http://dx.doi.org/10.1016/j.ejmech.2009.02.008 [Accessed November 20, 2013].

Bimlesh, K., Sandhar, H. K., Prasher, S., Tiwari, P., Salhan, M. & Pardeep, S., 2011. A Review of Phytochemistry and Pharmacology of Flavonoids. *Internationale Pharmaceutica Sciencia*, 1(1), pp.25 – 41. Available at: http://www.ipharmsciencia.com [Accessed Decemeber 8, 2012].

Birk, C. D., Provensi, G., Gosmann, G., Reginatto, F. H. & Schenkel, E. P., 2005. TLC fingerprint of flavonoids and saponins from *Passiflora* sp. *Journal of Liquid Chromatography & Related Technologies*, 28(14), pp.2285–2291. Available at: http://www.tandfonline.com/doi/abs/10.1081/JLC-200064212 [Accessed June 10, 2014].

Block, J.H., 2011. Antimalarials. In J. M. Beale & J. H. Block, eds. *Wilson and Gisvold's Textbook of Organic, Medicinal and Pharmaceutical Chemistry*. New York: Lippincott Williams and Wilkins, pp. 242–257 [Accessed May 14, 2014].

Boampong, J. N., Ameyaw, E. O., Aboagye, B., Asare, K., Kyei, S., Donfack, J. H. & Woode, E., 2013. The Curative and Prophylactic Effects of xylopic acid on *Plasmodium berghei* infection in Mice. *Journal of parasitology research*, 2013(Figure 1), p.356107. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3732589&tool=pmcentrez&rendertype=abstract [Accessed March 17, 2014].

Bosman, A., Delacollette, C., Olumese, P., Ridley, R. G., Rietveld, A., Shretta, R. & Teklehaimanot, A., 2001. *The use of antimalarial drugs. Report of a WHO Informal Consultation*, Geneva. Available at: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:the+use+of+antimalarial+drugs+Report+of+an+Informal+Consultation#0 [Accessed October 29, 2013].

Boyom, F. F., Fotio, D., Zollo, P. H. A., Agnaniet, H., Menut, C. & Bessière, J. M., 2004. Aromatic plants of tropical central Africa. Part XLIV.# Volatile components from *Pseudocedrela kotschyi* (Schweinf.) harms growing in Cameroon. *Flavour and Fragrance Journal*, 19(1), pp.9–11. Available at: http://dx.doi.org/10.1002/ffj.1254 [February 22, 2014].

Bray, P. G., Mungthin, M., Ridley, R. G. & Ward, S. A., 1998. Access to hematin: The basis of chloroquine resistance. *Molecular Pharmacology*, 54(1), pp.170–9. Available at: http://molpharm.aspetjournals.org/content/54/1/170.abstract [Accessed May 16, 2014].

Bray, P.G., 1999. Cellular uptake of chloroquine is dependent on binding to ferriprotoporphyrin IX and is independent of NHE activity in *Plasmodium falciparum*. *The Journal of Cell Biology*, 145(2), pp.363–376. Available at: http://jcb.rupress.org/content/145/2/363.abstract [Accessed May 16, 2014].

Bray, P.G., Ward, S.A. & O'Neill, P.M., 2005. Quinolines and Artemisinin : Chemistry, Biology and History. *CTMI*, 1(1), pp.3–38 [Accessed June 25, 2013].

Brown, G.D., 2010. The biosynthesis of artemisinin (Qinghaosu) and the phytochemistry of *Artemisia annua* L. (Qinghao). *Molecules (Basel, Switzerland)*, 15(11), pp.7603–98. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21030913 [Accessed May 28, 2013].

Bunnag, D., Harinasuta, T., Pinichpongse, S. & Suntharasami, P., 1980. Effect of primaquine on gametocytes of *Plasmodium falciparum* in Thailand. *Lancet*, 2, pp.8185–8191 [Accessed May 29, 2014].

Burda, S. & Oleszek, W., 2001. Antioxidant and antiradical activities of flavonoids. *Journal of Agricultural and Food Chemistry*, 49(6), pp.2774–2779. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11409965 [June 22, 2013].

Burkhill, H.M., 2004. *The Useful Plants of West Tropical Africa* 2nd Editio., Kew, UK: Royal Botanic Gardens [Accessed June 13, 2014].

Butler, A. R., Gilbert, B. C., Hulme, P. I., Lee R., Renton, L. & Whitwood, A. C., 1998. EPR evidence for the involvement of free radicals in the iron-catalysed decomposition of qinghaosu (artemisinin) and some derivatives; antimalarial action of some polycyclic endoperoxides. *Free Radical Biology and Medicine*, 28(5), pp.471–476. Available at: http://informahealthcare.com/doi/pdf/10.3109/10715769809066884 [Accessed May 17, 2014].

Butler, M.S., 2004. The role of natural product chemistry in drug discovery. *Journal of natural products*, 67(12), pp.2141–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15620274 [Accessed May 26, 2014].

Caceres, A., Saravia, A., Rizzo, S., Zabala, L., De Leon, E. & Nave, F., 1992. Pharmacologic properties of *Moringa oleifera*: Screening for antispasmodic, antiinflammatory and diuretic activity. *Journal of Ethnopharmacology*, 36(3), pp.233–237 [Accessed February 22, 2014].

Canfield, C.J. & Rozman, R.S., 1974. Clinical testing of new antimalarial compounds. *Bulletin of the World Health Organization*, 50(3-4), pp.203–12. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2481191&tool=pmcentrez&rendertype=abstract [Accessed May 13, 2014].

Caniato, R. & Puricelli, L., 2003. Review: Natural Antimalarial Agents (1995-2001). *Critical Reviews in Plant Sciences*, 22(1), pp.79–105. Available at: http://dx.doi.org/10.1080/713610851 [Accessed May 3, 2014].

Carlton, J. M., Angiuoli, S. V., Suh, B. B., Kooij, T. W., Pertea, M., Silva, J. C., Ermolaeva, M. D., Allen, J. E., Selengut, J. D., Koo, H. L., Peterson, J. D., Pop, M., Kosack, D. S.,

Shumway, M. F., Bidwell, S. L., Shallom, S. J., van Aken, S. E., Riedmuller, S. B., Feldblyum, T. V., Cho, J. K., Quackenbush, J., Sedegah, M., Shoaibi, A., Cummings, L. M., Florens, L., Yates, J. R., Raine, J. D., Sinden, R. E., Harris, M. A., Cunningham, D. A., Preiser, P. R., Bergman, L. W., Vaidya, A. B., van Lin, L. H., Janse, C. J., Waters, A. P., Smith, H. O., White, O. R., Salzberg, S. L., Venter, J. C., Fraser, C. M., Hoffman, S. L., Gardner, M. J. & Carucci, D. J., 2002. Genome sequence and comparative analysis of the model rodent malaria parasite *Plasmodium yoelii yoelii*. *Nature*, 419(6906), pp.512–519. Available at: http://dx.doi.org/10.1038/nature01099 [Accessed May 28, 2014].

Carraz, M., Jossang, A., Rasoanaivo, P., Mazier, D. & Frappier, F., 2008. Isolation and antimalarial activity of new morphinan alkaloids on *Plasmodium yoelii* liver stage. *Bioorganic & Medicinal Chemistry*, 16(11), pp.6186–92. Available at: http://www.sciencedirect.com/science/article/pii/S0968089608003611 [Accessed May 13, 2014].

Carter, R. & Diggs, C.L., 1977. Plamodia of rodents. In *Parasitic Protozoa*. pp. 359–465 [Accessed May 28, 2014].

CDC, 2014. CDC - Malaria - About Malaria - Biology. Available at: http://www.cdc.gov/malaria/about/biology/ [Accessed March 31, 2014].

CDC, 2004. Treatment of Malaria (Guidelines for Clinicians). , (CDC), pp.1–9. Available at: www.cdc.gov/malaria [Accessed March 22, 2014].

Chang, S. P., Case, S. E., Gosnell, W. L., Hashimoto, A., Kramer, K. J., Tam, L. Q., Hashiro, C. Q., Nikaido, C. M., Gibson, H. L., Lee-Ng, C. T., Barr, P. J., Yokota, B. T. & Hui, G. S., 1996. A recombinant baculovirus 42-kilodalton C-terminal fragment of *Plasmodium falciparum* merozoite surface protein 1 protects *Aotus* monkeys against malaria. *Infection and immunity*, 64(1), pp.253–261. Available at: http://iai.asm.org/content/64/1/253.short [Accessed May 28, 2014].

Chen, P.Q., Li, G.Q., Guo, X.B., He, K.R., Fu, Y.X., Fu, L.C. & Song, Y.Z., 1994. The infectivity of gametocytes of *Plasmodium falciparum* from patients treated with artemisinin. *Chinese Medical Journal*, 107(9), pp.709–711 [Accessed May 29, 2014].

Cheng, Z., Yu, B. & Yang, X., 2002. 27-Nor-triterpenoid glycosides from *Mitragyna inermis*. *Phytochemistry*, 61, pp.379–382 [Accessed June 25, 2013].

Cheong, H., Ryu, S. Y., Oak, M. H., Cheon, S. H., Yoo, G. S. & Kim, K. M., 1998. Studies of structure activity relationship of flavonoids for the anti-allergic actions. *Archives of pharmacal research*, 21(4), pp.478–480 [Accessed June 22, 2013].

Chotivanich, K., Udomsangpetch, R., Simpson, J. A., Newton, P., Pukrittayakamee, S., Looareesuwan, S. & White, N. J., 2000. Parasite multiplication potential and the severity of falciparum malaria. *Journal of Infectious Diseases*, 181, pp.1206–1209 [Accessed June 6, 2014].

Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W. & Lin, Y., 1998. Tannins and human health : A review. *Critical Reviews in Food Science and Nutrition*, 38(6), pp.421–464 [Accessed March 18, 2014].

Clark, I.A. & Cowden, W.B., 2003. The pathophysiology of falciparum malaria. *Pharmacology and Therapeutics*, 99(1), pp.221–260 [Accessed November 19, 2013].

Clark, I.A. & Schofield, L., 2000. Pathogenesis of Malaria. *Parasitology Today*, 16(10), pp.451–454 [Accessed November 19, 2013].

Clough, B., Strath, M., Preiser, P., Denny, P. & Wilson, I., 1997. Thiostrepton binds to malarial plastid rRNA. *FEBS Letters*, 406(1-2), pp.123–125. Available at: http://www.sciencedirect.com/science/article/pii/S001457939700241X [Accessed May 19, 2014].

Coatney, G. R., Cooper, W. C., Eddy, N. B. & Greenbeeg, J., 1953. *Survey of Antimalarial Agents: Chemotherapy of Plasmodium gallinaceum Infections; Toxicity; Correlation of Structure and Action.*, Public HealthServices Publications. Available at: http://www.cabdirect.org/abstracts/19542900622.html;jsessionid=E8509AF6C19B703B30F621608995DA61 [Accessed May 15, 2014].

Colegate, S.M. & Molyneux, R.J., 2007. *Bioactive Natural Products: Detection, Isolation, and Structural Determination, Second Edition* Secong Edi., CRC Press. Available at: http://books.google.com/books?id=In8kaHnX5gUC&pgis=1 [Accessed June 10, 2014].

Collins, W. E., Helmby, H., Souza, B., Blazquez, S., Thiberge, S., Amino, R. & Menard, R., 2008. Animal Models. In Moll, K., Ljungström, I., Perlmann, H., Scherf, A. & Wahlgren, M., eds. *Methods in Malaria Research*. Sweden: Malaria Research and Reference Reagents Resource Center (MR4), pp. 141 –149. Available at: www.malaria..mr4.org/publication.html [Accessed March 17, 2014].

Cowman, A.F. & Crabb, B.S., 2006. Invasion of Red Blood Cells by Malaria Parasites. *Cell*, 124(1), pp.755–766 [Accessed November 19, 2013].

Cox-Singh, J. et al., 2008. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clinical infectious diseases : An official publication of the Infectious Diseases Society of America*, 46(2), pp.165–71. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2533694&tool=pmcentrez&rendertype=abstract [Accessed March 31, 2014].

Crapper, D.R., Krishnan, S.S. & Dalton, A.J., 1973. Brain Aluminum Distribution in Alzheimer's Disease and Experimental Neurofibrillary Degeneration. *Science*, 180(4085), pp.511–513. Available at: http://www.sciencemag.org/content/180/4085/511.abstract [Accessed May 6, 2014].

Croft, A. & Garner, P., 1997. Mefloquine to prevent malaria: a systematic review of trials. *British Medical Journal*, 315 (1), pp.1412-1416. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2127902/ [Accessed May 7, 2014].

Dahl, E.L. & Rosenthal, P.J., 2007. Multiple antibiotics exert delayed effects against the *Plasmodium falciparum* apicoplast. *Antimicrobial agents and chemotherapy*, 51(10), pp.3485–90. Available at: http://aac.asm.org/content/51/10/3485.short [Accessed May 19, 2014].

Dangi, S.Y., Jolly, C.I. & Narayanan, S., 2002. Antihypertensive Activity of the Total Alkaloids from the Leaves of *Moringa oleifera*. *Pharmaceutical Biology*, 40(2), pp.144–148. Available at: http://informahealthcare.com/doi/abs/10.1076/phbi.40.2.144.5847 [Accessed May 7, 2014].

Das, B.R., Kurup, P.A. & Narasimha Rao, P.L., 1954. Antibiotic principle from moringa pterygosperma. *Die Naturwissenschaften*, 41(3), pp.66–66. Available at: http://link.springer.com/10.1007/BF00634183 [Accessed May 8, 2014].

Davison, B. B., Cogswell, F.B., Baskin, G.B., Falkenstein, K. P., Henson, E. W., Krogstad, D. J., 2000. Placental changes associated with fetal outcome in the *Plasmodium coatneyi*/rhesus monkey model of malaria in pregnancy. *Am J Trop Med Hyg*, 63(3), pp.158–173. Available at: http://www.ajtmh.org/content/63/3/158.short [Accessed May 28, 2014].

Delves, M., Plouffe, D., Scheurer, C., Meister, S., Wittlin, S., Winzeler, E. A., Sinden, R. E. & Leroy, D., 2012. The activities of current antimalarial drugs on the life cycle stages of *Plasmodium*: a comparative study with human and rodent parasites. *PLoS medicine*, 9(2), p.e1001169. Available at: http://www.plosmedicine.org/article/info:doi/10.1371/journal.pmed.1001169#pmed-1001169-g005 [Accessed July 18, 2012].

Dinan, L., 1998. Dereplication and partial identification of natural products. In S. D. Saker, Z. Latif, & A. I. Gray, eds. *Natural Product Isolation*. Totowa, NJ: Humana Press, pp. 279–327 [Accessed June 20, 2014].

Dondorp, A. et al., 2005. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet*, 366(9487), pp.717–25. Available at: http://europepmc.org/abstract/MED/16125588 [Accessed May 6, 2014].

Dondorp, A. M., Fanello, C. I., Hendriksen, I. C. E., Gomes, E., Seni, A., Chhaganlal, K. D., Bojang, K., Olaosebikan, R., Anunobi, N., Maitland, K., Kivaya, E., Agbenyega, T., Nguah, S. B., Evans, J., Gesase, S., Kahabuka, C., Mtove, G., Nadjm, B., Deen, J., Mwanga-Amumpaire, J., Nansumba, M., Karema, C., Umulisa, N., Uwimana, A., Mokuolu, O. A., Adedoyin, O. T., Johnson, W. B. R., Tshefu, A. K., Onyamboko, M. A., Sakulthaew, T., Ngum, W. P., Silamut, K., Stepniewska, K., Woodrow, C. J., Bethell, D., Wills, B., Oneko, M., Peto, T. E., von Seidlein, L., Day, N. P. J. & White, N. J., 2010. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet*, 376(9753), pp.1647–57. Available at: http://www.sciencedirect.com/science/article/pii/S0140673610619241 [Accessed May 2, 2014].

Dong, J., Jia, L. & Sun, Q., 2008. HPLC Fingerprint of Compound Oral Liquid of *Rhodiola*. *Lishizhen Medicine and Materia Medica Research*, 7(1) [Accessed June 10, 2014].

Dong, Y., 2002. Synthesis and antimalarial activity of 1,2,4,5-tetraoxanes. *Mini reviews in medicinal chemistry*, 2(2), pp.113–123 [Accessed February 22, 2014].

Dong, Y., Wittlin, S., Sriraghavan, K., Chollet, J., Charman, S. A., Charman, W. N., Scheurer, C., Urwyler, H., Santo Tomas, J., Snyder, C., Creek, D. J., Morizzi, J., Koltun,

M., Matile, H., Wang, X., Padmanilayam, M., Tang, Y., Dorn, A., Brun, R. & Vennerstrom, J. L., 2010. The structure-activity relationship of the antimalarial ozonide arterolane (OZ277). *Journal of medicinal chemistry*, 53(1), pp.481–491 [Accessed February 22, 2014].

Drakeley, C. J., Secka, I., Correa, S., Greenwood, B. M. & Targett, G. A. T., 1999. Host haematological factors influencing the transmission of *Plasmodium falciparum* gametocytes to Anopheles gambiae s.s. mosquitoes. *Tropical Medicine and International Health*, 4(2), pp.131–138. Available at: http://doi.wiley.com/10.1046/j.1365-3156.1999.00361.x [Accessed June 10, 2014].

Drusano, G.L., 1988. Role of pharmacokinetics in the outcome of infections. *Antimicrob. Agents Chemother.*, 32, pp.289–297 [June 7, 2014].

Egan, T.J., 2006. Interactions of quinoline antimalarials with hematin in solution. *Journal of Inorganic Biochemistry*, 100, pp.916–926 [Accessed November 20, 2013].

Eichner, M., Diebner, H. H., Molineaux, L., Collins, W. E., Jeffery, G. M. & Dietz, K., 2001. Genesis, sequestration and survival of *Plasmodium falciparum* gametocytes: parameter estimates from fitting a model to malariatherapy data. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 95(5), pp.497–501. Available at: http://trstmh.oxfordjournals.org/content/95/5/497.short [Accessed May 29, 2014].

Eilert, U., Wolters, B. & Nahrstedt, A., 1981. The antibiotic principle of seeds of *Moringa oleifera* and Moringa stenopetala. *Planta medica*. Available at: http://www.researchgate.net/publication/15942669_The_antibiotic_principle_of_seeds_of_Moringa_oleifera_and_Moringa_stenopetala/file/9c960528099a21c727.pdf [Accessed May 8, 2014].

Ekong, D.E.U. & Olagbemi, E.O., 1967. Novel meliacins (limonoids) from the wood of. *Tetrahedron Letters*, 8(36), pp.3525–3527. Available at: http://www.sciencedirect.com/science/article/pii/S004040390189835X [Accessed April 21, 2014].

Elford, B. C., Roberts, M. F., Phillipson, J. D. & Wilson, R. J.M., 1987. Potentiation of the antimalarial activity of qinghaosu by methoxylated flavones. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 81(3), pp.434–436. Available at: http://trstmh.oxfordjournals.org/content/81/3/434.short [Accessed June 5, 2014].

van Elswijk, D. A., Schobel, U. P., Lansky, E. P., Irth, H. & van der Greef, J., 2004. Rapid dereplication of estrogenic compounds in pomegranate (*Punica granatum*) using on-line biochemical detection coupled to mass spectrometry. *Phytochemistry*, 65(2), pp.233–241. Available at: http://www.sciencedirect.com/science/article/pii/S0031942203006241 [Accessed June 10, 2014].

Ene, A. C., Atawodi, S. E., Ameh, D. A., Ndukwe, G. I. & Kwanashie, H. O., 2009. Bioassay-guided fractionation and *in-vivo* antiplasmodial effect of fractions of chloroform extract of *Artemisia maciverae* Linn. *Acta Tropica* , 112, pp.288–294 [Accessed March 17, 2014].

Evans, W.C., 2002. Trease and Evans Pharmacognosy. In W. B. Saunders, ed. *Trease and Evans Pharmacognosy*. London: Elsevier Limited, pp. 137–149 [Accessed March 18, 2014].

Ezeamuzie, I. C., Ambakederemo, A. W., Shode, F. O. & Ekwebelem, S. C., 2008. Antiinflammatory effects of *Moringa oleifera* Root Extract. *Pharmaceutical Biology*, 34(3), pp.207 – 212. Available at: http://informahealthcare.com/doi/abs/10.1076/phbi.34.3.207.13211 [Accessed May 3, 2014].

Fahey, J., 2005. *Moringa oleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1. *Phytochemistry*. Available at: http://stipulae.johnvanhulst.com/DOCS/PDF/*Moringa oleifera* medisch.pdf [Accessed May 3, 2014].

Faizi, S., Siddiqui, B. S., Saleem, R., Siddiqui, S., Aftab, K. & Gilani, A. H., 1995. Fully acetylated carbamate and hypotensive thiocarbamate glycosides from *Moringa oleifera*. *Phytochemistry*, 38(4), pp.957–963. Available at: http://www.sciencedirect.com/science/article/pii/003194229400729D [Accessed May 6, 2014].

Faizi, S., Siddiqui, B S., Saleem, R., Aftab, K., Shaheen, F. & Gilani, A. H. 1998. Hypotensive constituents from the pods of *Moringa oleifera*. *Planta medica*, 64(3), pp.225–8. Available at: https://www.thieme-connect.com/ejournals/abstract/10.1055/s-2006-957414 [Accessed October 29, 2013].

Faizi, S., Siddiqui, B. S., Saleem, R., Siddiqui, S., Aftab, K. & Gilani, A. H., 1994. Isolation and Structure Elucidation of New Nitrile and Mustard Oil Glycosides from *Moringa oleifera* and Their Effect on Blood Pressure. *Journal of Natural Products*, 57(9), pp.1256–1261. Available at: http://dx.doi.org/10.1021/np50111a011 [Accessed May 5, 2014].

Farooq, F., Rai, M., Tiwari, A., Khan, A. A. & Farooq, S., 2012. Medicinal properties of *Moringa oleifera* : An overview of promising healer. *Journal of Medicinal Plants Research*, 6(27), pp.4368–4374. Available at: http://www.academicjournals.org/JMPR [Accessed September 19, 2012].

Faurant, C., 2011. From bark to weed: the history of artemisinin. *Parasite (Paris, France)*, 18(3), pp.215–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3671478&tool=pmcentrez&rendertype=abstract [Accessed May 27, 2014].

Ferreira, P. P. M., Farias, D. F., Oliveira, J. T. A. & Carvalho, A. F., 2008. *Moringa oleifera* : Bioactive compounds and nutritional potential. *Revista de Nutricao*, 21(4), pp.431–437 [Accessed February 22, 2014].

Fidock, D. A., Rosenthal, P. J., Croft, S. L., Brun, R. & Nwaka, S., 2004. Antimalarial drug discovery: efficacy models for compound screening. *Nature reviews. Drug discovery*, 3(6), pp.509–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15173840 [Accessed November 1, 2012].

Fitch, C D., Yunis, N G., Chevli, R. & Gonzalez, Y., 1974. High-affinity accumulation of chloroquine by mouse erythrocytes infected with *Plasmodium berghei*. *The Journal of clinical investigation*, 54(1), pp.24–33. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=301521&tool=pmcentrez&rendertype=abstract [Accessed May 16, 2014].

Fix, A. & Waterhouse, C., 1988. Plasmodium relictum as a cause of avian malaria in wild-caught Magellanic penguins (*Spheniscus magellanicus*). *Journal of Wildlife*. Available at: http://www.jwildlifedis.org/doi/abs/10.7589/0090-3558-24.4.610 [Accessed May 28, 2014].

Fleming, W.W., 2004. Mechanisms of Drug Action. In C. R. Craig & R. E. Stitzel, eds. *Modern Pharmacology with clinical applications*. Lippincott Williams and Wilkins, pp. 10–19 [August 8, 2014].

Foley, M. & Tilley, L., 1997. Quinoline Antimalarials : Mechanisms of Action and Resistance. *International Journal for Parasitology*, 27(2), pp.231–240 [Accessed November 20, 2013].

Foley, M. & Tilley, L., 1998. Quinoline Antimalarials : Mechanisms of Action and Resistance and Prospects for New Agents. *Pharmacology and Therapeutics*, 79(1), pp.55–87 [Accessed November 20, 2013].

Francis, S.E., Sullivan, D.J. & Goldberg, D.E., 1997. Hemoglobin metabolism in the malaria parasite *Plasmodium falciparum*. *Annual review of microbiology*, 51, pp.97–123. Available at: http://www.annualreviews.org/doi/abs/10.1146/annurev.micro.51.1.97 [Accessed May 12, 2014].

Frederich, M., Tits, M. & Angenot, L., 2008. Potential antimalarial activity of indole alkaloids. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 102(1), pp.11–19 [Accessed February 22, 2014].

Galinski, M., Arnot, D. & Cochrane, A., 1987. The circumsporozoite gene of the Plasmodium cynomolgi complex. *Cell*. Available at: http://www.sciencedirect.com/science/article/pii/009286748790434X [Accessed May 28, 2014].

Garber, J.C., 2011. *Guide for the care and use of laboratory animals* Eighth Edi., Washington: The National Academies Press, 500 5th Street. Available at: www.nap.edu [Accessed April 1, 2014].

Ghasi, S., Nwobodo, E. & Ofili, J.O., 2000. Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed wistar rats. *Journal of Ethnopharmacology*, 69(1), pp.21–25 [February 22, 2014].

Gilani, A. H., Aftab, K., Shaheen, F. & Siddiqui, B.S., 1992. Antispasmodic activity of active principle from *Moringa oleifera*. *Natural Drugs and the Digestive Tract*, 1(1), pp.60–63. Available at: http://scholar.google.com/scholar?q=Antispasmodic+activity+of+active+principle+from+Moringa+oleifera&btnG=&hl=en&as_sdt=0,5#4 [Accessed May 7, 2014].

Gilani, A. H., Aftab, K. & Suria, A., 1994. Pharmacological studies on hypotensive and spasmolytic activities of pure compounds from *Moringa oleifera*. *Phytotherapy*. Available at: http://onlinelibrary.wiley.com/doi/10.1002/ptr.2650080207/abstract [Accessed May 3, 2014].

Glew, R., Collins, W. & Miller, L., 1978. Selection of increased quinine resistance in *Plasmodium falciparum* in Aotus monkeys. *The American journal of tropical medicine and hygiene*, 27(1), pp.9–13. Available at: http://europepmc.org/abstract/MED/415628 [Accessed May 22, 2014].

Goldberg, D. E., Slater, A. F. G., Beavis, R., Chait, B., Cerami, A. & Henderson, B., 1991. Hemoglobin Degradation in the Human Malaria Pathogen *Plasmodium falciparum* : A Catabolic Pathway Initiated by a Specific Aspartic Protease. *J. Exp. Med.*, 173(April), pp.961 – 969 [Accessed May 12, 2014].

Goyal, B. R., Agrawal, B. B., Goyal, R. K. & Mehta, A. A., 2007. Phyto-pharmacology of *Moringa oleifera* Lam . ó An overview. *Natural Product Radiance*, 6(4), pp.347–353 [Accessed July 12, 2012].

Grimberg, B.T. & Mehlotra, R.K., 2011. Expanding the Antimalarial Drug Arsenal-Now, But How? *Pharmaceuticals (Basel, Switzerland)*, 4(5), pp.681–712. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3102560&tool=pmcentrez&rendertype=abstract [Accessed August 21, 2012].

Guevara, A. P., Vargas, C., Sakurai, H., Fujiwara, Y., Hashimoto, K., Maoka, T., Kozuka, M., Ito, Y., Tokuda, H. & Nishino, H., 1999. An antitumor promoter from *Moringa oleifera* Lam. *Genetic Toxicology and Environmental Mutagenesis*, 440(1), pp.181–188. Available at: http://www.sciencedirect.com/science/article/pii/S138357189900025X [Accessed May 3, 2014].

Hagerman, A.E., 2002. Tannin Chemistry. , p.electronic copy [Accessed March 18, 2014].

Harbone, J.R., 1976. Phytochemical methods. In *A guide to modern technique of plant analysis*. London: Charpan and Hall, p. 78 [Accessed May 29, 2014].

Harvey, A.L., 2008. Natural products in drug discovery. *Drug discovery today*, 13(19-20), pp.894–901. Available at: http://www.sciencedirect.com/science/article/pii/S1359644608002651 [Accessed May 23, 2014].

Hastings, I.M., 2006. Gametocytocidal activity in antimalarial drugs speeds the spread of drug resistance. *Tropical medicine & International health*, 11(8), pp.1206–1217 [Accessed February 22, 2014].

Hay, A. E., Ioset, J. R., Ahua, K. M., Diallo, D., Brun, R. & Hostettmann, K., 2007. Limonoid orthoacetates and antiprotozoal compounds from the roots of *Pseudocedrela kotschyi*. *Journal of natural products*, 70(1), pp.9–13. Available at: http://dx.doi.org/10.1021/np0680230 [Accessed June 22, 2013].

Haynes, R. K., Monti, D., Taramelli, D., Basilico, N., Parapini, S. & Olliaro, P., 2003. Artemisinin antimalarials do not inhibit hemozoin formation. *Antimicrobial agents and*

chemotherapy, 47(3), p.1175. Available at: http://aac.asm.org/content/47/3/1175.short [Accessed May 12, 2014].

Haynes, R.K., Pai, H.H.O. & Voerste, A., 1999. Ring opening of artemisinin (qinghaosu) and dihydroartemisinin and interception of the open hydroperoxides with Formation of N-oxides- a chemical model for antimalarial mode of action. *Tetrahedron Letters*, 40(25), pp.4715–4718. Available at: http://www.sciencedirect.com/science/article/pii/S0040403999008308 [Accessed May 17, 2014].

Hayworth, A. & van Riper, C., 1987. Effects of *Plasmodium relictum* on the metabolic rate and body temperature in canaries (*Serinus canarius*). *The Journal of parasitology*, 73(4), pp.850–853. Available at: http://www.jstor.org/stable/3282431 [Accessed May 28, 2014].

Held, J., Westerman, R., Kremsner, P. G. & Mordmüller, B., 2010. *In-vitro* activity of mirincamycin (U24729A) against *Plasmodium falciparum* isolates from Gabon. *Antimicrobial agents and chemotherapy*, 54(1), pp.540–2. Available at: http://aac.asm.org/content/54/1/540.short [Accessed May 19, 2014].

Heseltine, E., 2010. *Global Report on Antimalarial Drug Efficacy and Drug Resistance: 2000-2010*, Geneva [Accessed December 2, 2012].

Hodges, K. & Gill, R., 2010. Infectious diarrhoea. *Gut Microbes*, 1(1), pp.4–21 [Accessed June 7, 2014].

Hutchison, J. & Dalziel, J.M., 1958. *Flora of West Tropical Africa. Volume 1, Part 2*. 2nd Editio., Crown Agents for the Colonies for overseas governments and administration. Available at: http://www.cabdirect.org/abstracts/19551700340.html [Accessed April 21, 2014].

Idohou-dossou, N., Diouf, A., Gueye, A L., Guiro, A T. & Wade, S., 2011. Impact of daily consumption of *Moringa* dry leaf powder on iron status of Senegalese lactating women. *African Journal of Food, Agriculture, Nutrition and Development*, 11(4), pp.4985–4999 [Accessed September 19, 2012].

Idowu, O A., Soniran, O T., Ajana, O. & Aworinde, D O., 2010. Ethnobotanical survey of antimalarial plants used in Ogun State, Southwest Nigeria. *African Journal of Pharmacy and Pharmacology*, 4(2), pp.55–60 [Accessed December 2, 2012].

Iqbal, S. & Bhanger, M.I., 2006. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *Journal of Food Composition and Analysis*, 19(6-7), pp.544–551. Available at: http://www.sciencedirect.com/science/article/pii/S0889157505000670 [Accessed May 2, 2014].

Isaka, M., Punya, J., Lertwerawat, Y., Tanticharoen, M., Thebtaranonth, Y., 1999. Antimalarial activity of macrocyclic trichothecenes isolated from the fungus *Myrothecium verrucaria*. *Journal of natural products*, 62(2), pp.329–331.

Ito, J., Ghosh, A., Moreira, L. A., Wimmer, E. A. & Jacobs-Lorena, M., 2002. Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Letters to Nature*, 417(6887), pp.452–5. Available at: http://dx.doi.org/10.1038/417452a [Accessed May 15, 2014].

Iwu, M.M., 1993. *Handbook of Medicinal plants* 2nd Editio., CRC Press, Taylor and Francis group, 6000 Broken Sound Park, NW Suite 300, Boca Raton,FL 33487-2742. Available at: www.crcpress.com [Accessed April 21, 2014].

Jain, M., Vangapandu, S., Sachdeva, S., Singh, S., Singh, P. P., Jena, G. B., Tikoo, K., Ramarao, P., Kaul, C. L. & Jain, R., 2004. Discovery of a bulky 2-tert-butyl group containing primaquine analogue that exhibits potent blood-schizontocidal antimalarial activities and complete elimination of methemoglobin toxicity. *Journal of medicinal chemistry*, 47(2), pp.285–7. Available at: http://dx.doi.org/10.1021/jm0304562 [Accessed May 13, 2014].

Janse, C., Franke-Fayard, B. & Khan, S.M., 2014. *P. berghei In-vivo - Model of Malaria*. Leids Universitair Medisch Centrum. Available at: https://www.lumc.nl/con/1040/81028091348221/810281121192556/811070740182556/811070749352556/ [Accessed March 20, 2014].

Janse, C. J., Carlton, J. M.R., Walliker, D. & Waters, A. P., 1994. Conserved location of genes on polymorphic chromosomes of four species of malaria parasites. *Molecular and Biochemical Parasitology*, 68(2), pp.285–296. Available at: http://www.sciencedirect.com/science/article/pii/0166685194901732 [Accessed May 28, 2014].

Janse, C.J., Frank-Fayad, B. & Khan, S.B., 2012. The role of animal models for research on severe malaria. *PLoS pathogens*, 8(2), p.e1002401. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3271056&tool=pmcentrez&rendertype=abstract [Accessed March 20, 2014].

Jimenez-Dıaz, M. B., Viera, S., Ibanez, J., Mulet, T., Noemı, M. M., Garuti, H., Gomez, V., Cortes-Gil, L., Martınez, A., Ferrer, S., Fraile, M. T., Caldero, F., Fernandez, E., Shultz, L. D., Leroy, D., Wilson, D. M., Jose Francisco Garcıa-Bustos, F. J. G. & Angulo-Barturen, I., 2013. A New *In-vivo* Screening Paradigm to Accelerate Antimalarial Drug Discovery. *PloS ONE*, 8(6), p.e66967 [Accessed November 16, 2-13].

Kalogo, Y., Rosillon, F., Hammes, F. & Verstraete, W., 2000. Effect of a water extract of *Moringa oleifera* seeds on the hydrolytic microbial species diversity of a UASB reactor treating domestic wastewater. *Letters in Applied Microbiology*, 31(3), pp.259–264. Available at: http://doi.wiley.com/10.1046/j.1365-2672.2000.00814.x [Accessed May 6, 2014].

Karou, S. D., Tchacondo, T., Iiboudo, D. P. & Simpore, J., 2011. Sub-saharan Rubiaceae: A Review of their Traditional Uses, Phytochemistry and Biological activities. *Pakistan Journal of Biological Sciences*, 14(3), pp.149–169 [Accessed December 2, 2012].

Kasolo, J. N., Bimenya, G. S., Ojok, L., Ochieng, J. & Ogwal-okeng, J. W., 2010. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities.

Journal of Medicinal Plants Research, 4(9), pp.753–757 [Accessed September 19, 2012].

Kassim, O O., Loyevsky, M., Amonoo, H., Lashley, L., Ako-Nai, K. A. & Gordeuk, V. R., 2009. Inhibition of *In-vitro* growth of *Plasmodium falciparum* by *Pseudocedrela kotschyi* extract alone and in combination with *Fagara zanthoxyloides* extract. . *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 103(7), pp.698–702. Available at: http://dx.doi.org/10.1016/j.trstmh.2009.02.018 [Accessed February 22, 2014].

Kerharo, P., 1969. Un remede populaire Sengalais: Le "Nebreday"(*Moringa oleifera* lann.) employs therapeutiques en milieu Africain chimie et pharmacologie. *Plantes Med Phytother*, 3, pp.14 – 219. Available at: /citations?view_op=view_citation&continue=/scholar?hl=en&as_sdt=0,5&scilib=1&scioq=Kerharo+PJ.+1969.+Un+remede+populaire+Sengalais:+Le+%E2%80%98Nebreday%E2%80%99+(Moringa+oleifera++lann.)+employs+therapeutiques+en+milieu+Africain+chimie+et+pharmacologie.+Plantes+Med+Phytother+3:+14%E2%80%93219&citilm=1&citation_for_view=QPCpw6MAAAAJ:d1gkVwhDpl0C&hl=en&oi=p [Accessed May 5, 2014].

Killick-Kendrick, R., 1978. Taxonomy, Zoography and Evolution. In R. Killick-Kendrick & W. Peters, eds. *Rodent Malaria*. London: Academic Press, pp. 1–52 [Accessed May 28, 2014].

Kilpatrick, A. & LaPointe, D., 2006. Effects of chronic avian malaria (*Plasmodium relictum*) infection on reproductive success of Hawaii Amakihi (*Hemignathus virens*). *The Auk*. Available at: http://www.bioone.org/doi/abs/10.1642/0004-8038(2006)123[764:EOCAMP]2.0.CO;2 [Accessed May 28, 2014].

Klayman, D., 1985. Qinghaosu (artemisinin): an antimalarial drug from China. *Science*, 228(4703), pp.1049–1055. Available at: http://www.sciencemag.org/content/228/4703/1049.short [Accessed May 19, 2014].

Kocisko, D.A. & Caughey, B., 2006. Mefloquine, an antimalaria drug with antiprion activity *In-vitro*, lacks activity *In-vivo*. *Journal of Virology*, 80(2), pp.1044–1046. Available at: http://jvi.asm.org/cgi/content/abstract/80/2/1044 [Accessed June 22, 2013].

Köhler, I., Jenett-Siems, K., Siems, K., Hernández, M. A., Ibarra, R. A., Berendsohn, W. G., Bienzle, U. & Eich, E., 2002. Herbal remedies traditionally used against malaria in Ghana: bioassay-guided fractionation of *Microglossa pyrifolia* (Asteraceae). *Zeitschrift für Naturforschung. C, Journal of biosciences*, 57(11-12), pp.1022–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12562088 [Accessed July 12, 2012].

Koné, W M., Atindehou, K. K., Terreaux, C., Hostettmann, K., Traoré, D. & Dosso, M., 2004. Traditional medicine in North Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. *Journal of ethnopharmacology*, 93(1), pp.43–9. Available at: http://www.sciencedirect.com/science/article/pii/S0378874104001084 [Accessed March 24, 2014].

Konkon, N. G., Adjoungoua, A. L., Manda, P., Simaga, D., N'Guessan, K. E. & Kone, B. D., 2008. Toxicological and phytochemical screening study of *Mitragyna inermis* (willd .)

O ktze (Rubiaceae), anti diabetic plant. *Journal of Medicinal Plants Research*, 2(10), pp.279–284 [Accessed December 2, 2012].

Kouznetsov, V. V & Gomez-Barrio, A., 2009. Recent developments in the design and synthesis of hybrid molecules based on aminoquinoline ring and their antiplasmodial evaluation. *European Journal of Medicinal Chemistry*, 44(1), pp.3091–3113 [Accessed November 20, 2013].

Krishna, S., Uhlemann, A. & Haynes, R., 2004. Artemisinins: mechanisms of action and potential for resistance. *Drug Resistance Updates*, 7, pp.233–244. Available at: http://www.sciencedirect.com/science/article/pii/S1368764604000494 [Accessed May 17, 2014].

Krudsood, S., Wilairatana, P., Tangpukdee, N., Chalermrut, K., Srivilairit, S., Thanachartwet, V., Muangnoicharoen, S., Luplertlop, N., Brittenham, G. M. & Looareesuwan, S., 2006. Safety and tolerability of elubaquine (bulaquine, CDRI 80/53) for treatment of *Plasmodium vivax* malaria in Thailand. *The Korean Journal of Parasitology*, 44(3), p.221. Available at: http://synapse.koreamed.org/DOIx.php?id=10.3347/kjp.2006.44.3.221 [Accessed May 13, 2014].

ter Kuile, F., White, N.J., Holloway, P., Pasvol, G. & Krishna, S., 1993. *Plasmodium falciparum*: *In-vitro* studies of the pharmacodynamic properties of drugs used for the treatment of malaria. *Experimental parasitology*, 76(1), pp.85–95.

Kurmi, S., Yadav, P. & Dwivedi, S., 2011. Phytochemical screening of leaves of Moringa *olifera* Lam . (Munga) -A traditional medicine. *International Journal of Drug discovery and Herbal research*, 1(3), pp.164–167 [Accessed June 25, 2013].

Langhorne, J., Buffet, P., Galinski, M., Good, M., Harty, J., Leroy, D., Mota, M. M., Pasini, E., Renia, L., Riley, E., Stins, M. & Duffy, P., 2011. The relevance of non-human primate and rodent malaria models for humans. *Malaria journal*, 10(1), p.23. Available at: http://www.malariajournal.com/content/10/1/23 [Accessed May 28, 2014].

Lazarowych, N.J. & Pekos, P., 1998. Use of Fingerprinting and Marker Compounds for Identification and Standardization of Botanical Drugs: Strategies for Applying Pharmaceutical HPLC Analysis to Herbal Products. *Therapeutic Innovation & Regulatory Science*, 32(2), pp.497–512. Available at: http://dij.sagepub.com/content/32/2/497.short [Accessed June 10, 2014].

Lederberg, J., 2000. Infectious history. *Science Margazine*, p.2. Available at: http://ftp.columbia.edu/itc/hs/pubhealth/p8475/readings/lederberg.pdf [Accessed May 27, 2014].

Lell, B., Ruangweerayut, R., Wiesner, J., Missinou, M. A., Schindler, A., Baranek, T., Hintz, M., Hutchinson, D., Jomaa, H. & Kremsner, P. G., 2003. Fosmidomycin, a Novel Chemotherapeutic Agent for Malaria. *Antimicrobial Agents and Chemotherapy*, 47(2), pp.735–738. Available at: http://aac.asm.org/content/47/2/735.short [Accessed May 13, 2014].

Lell, B. & Kremsner, P.G., 2002. Clindamycin as an Antimalarial Drug : Review of Clinical Trials. *Antimicrob. Agents Chemother.*, 46(8), pp.2315–2320 [Accessed March 22, 2014].

Lemke, T.L., 2007. Antiparasitic Agents. In T. L. Lemke & D. A. Williams, eds. *Foye's Principles of Medicinal Chemistry*. Lippincott Williams and Wilkins, pp. 1084 – 1110 [Accessed May 15, 2014].

Lemmens, R.H.M.J., 2008. *Pseudocedrela kotschyi* (Schweinf.) Harms. In D. Louppe, A. A. Oteng-Amoako, & M. Brink, eds. *Prota : Timbers/Bois d'œuvre*. PROTA, Wageningen, Netherlands, p. CD ROM. Available at: http://database.prota.org/PROTAhtml/*Pseudocedrela kotschyi*_En.htm [Accessed April 21, 2014].

Li, Na., Liu, J, Zhang, J. & Yu, B.Y., 2008. Comparative evaluation of cytotoxicity and antioxidative activity of 20 flavonoids. *Journal of Agricultural and Food Chemistry*, 56(10), pp.3876–3883. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18433100 [Accessed June 22, 2013].

Li, Y., Huang, H. & Wu, Y.-L., 2006. Qinghaosu (Artemisinin)-A fantastic antimalarial drug from a traditional Chinese medicine. In X.-T. Liang & W.S. Fang, eds. *Medicinal Chemistry of Bioactive Natural Products*. Hoboken, NJ, USA: John Wiley & Sons, Inc., pp. 183–239 [Accessed October 29, 2013].

Liu, H., Mou, Y., Zhao, J., Wang, J., Zhou, L., Wang, M., Wang, D., Han, J., Yu, Z & Yang, F., 2010. Flavonoids from *Halostachys caspica* and their antimicrobial and antioxidant activities. *Molecules*, 15(11), pp.7933–7945. Available at: http://www.mdpi.com/1420-3049/15/11/7933/ [Accessed June 25, 2013].

Liu, K C., Yang, S L., Roberts, M F., Elford, B C. & Phillipson, J D., 1989. The contribution of flavonoids to the antimalarial activity of *Artemisia annua* Pl. *Med*. 55, pp.654-655. Available at: http://scholar.google.com/scholar?q=The+contribution+of+flavonoids+to+the+antimalarial+activity+of+Artemisia+annua&btnG=&hl=en&as_sdt=0,5#0 [Accessed June 5, 2014].

Liu, K C., Yang, S L., Roberts, M F., Elford, B C. & Phillipson, J D., 1992. Antimalarial activity of Artemisia annua flavonoids from whole plants and cell cultures. *Plant Cell Reports*, 11, pp.637–640 [Accessed June 5, 2014].

Looareesuwan, S. 1994., 1994. Overview of clinical studies on artemisinin derivatives in Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 88(suppl. 1), pp.9–12 [Accessed June 7, 2014].

Lopes, L.M.X. & Nogueira, C.R., 2011. Antiplasmodial Natural Products. *Molecules*, 16(3), pp.2146–2190 [Accessed October 29, 2013].

López-Lázaro, M., 2002. Flavonoids as anticancer agents: structure-activity relationship study. *Current medicinal chemistry Anticancer agents*, 2(6), pp.691–714 [Accessed June 22, 2013].

Loria, P., Miller, S., Foley, M. & Tilley, L., 1999. Inhibition of the peroxidative degradation of haem as the basis of action of chloroquine and other quinoline antimalarials. *Biochemical Journal*, 339, pp.363–370. Available at: http://www.biochemj.org/bj/339/bj3390363.htm [Accessed May 13, 2014].

Lubell, Y., Riewpaiboon, A., Dondorp, A. M., Seidlein, L., Mokuolu, O. A., Nansumba, M., Samwel G., Alison K., George M., Olaosebikan, R., Ngum, W. P., Fanello, C. I, Hendriksen, I., Day, N PJ., White, N. J. & Shunmay Y., 2011. Cost-effectiveness of parenteral artesunate for treating children with severe malaria in sub-Saharan Africa. *Bulletin of the World Health Organization*, 89, pp.504–512. Available at: http://www.who.int/bulletin/volumes/89/7/11-085878/en/ [Accessed May 17, 2014].

Luqman, S., Srivastava, S., Kumar, R., Maurya, A. K. & Chanda, D., 2012. Experimental Assessment of *Moringa oleifera* Leaf and Fruit for Its Antistress, Antioxidant, and Scavenging Potential Using *In-vitro* and *In-vivo* Assays. *Evidence-based complementary and alternative medicine : eCAM*, 2012, p.519084. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3247066&tool=pmcentrez&rendertype=abstract [Accessed May 21, 2013].

Makkar, H.P.S. & Becker, K., 1996. Nutrional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Animal Feed Science and Technology*, 63(1), pp.211–228. Available at: http://www.animalfeedscience.com/article/S0377-8401(96)01023-1/abstract [Accessed May 6, 2014].

Makonnen, E., Hunde, A. & Damecha, G., 1997. Hypoglycaemic Effect ofMoringa stenopetala Aqueous Extract in Rabbits. *Phytotherapy Research*, 11(2), pp.147–148. Available at: http://doi.wiley.com/10.1002/(SICI)1099-1573(199703)11:2<147::AID-PTR41>3.0.CO;2-V [Accessed May 8, 2014].

Maranz, S., 2012. An alternative paradigm for the role of antimalarial plants in Africa. *The Scientific World Journal*, 2012, pp.1–9. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3346345&tool=pmcentrez&rendertype=abstract [Accessed November 5, 2012].

Martiney, J., Cerami, A. & Slater, A., 1996. Inhibition of hemozoin formation in *Plasmodium falciparum* trophozoite extracts by heme analogs: possible implication in the resistance to malaria conferred by the B -Thalassemia Trait. *Molecular Medicine*, 2(2), pp.236 – 246. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2230121/ [Accessed May 12, 2014].

McConkey, G.A., Rogers, M.J. & McCutchan, T.F., 1997. Inhibition of *Plasmodium falciparum* Protein Synthesis: Targeting the plastid-like organelle with thiostrepton. *J. Biol. Chem.*, 272(4), pp.2046–2049. Available at: http://www.jbc.org/content/272/4/2046.short [Accessed May 19, 2014].

Mehta, K., Balaraman, R., Amin, A.H., Bafna, P.A. & Gulati, O.D., 2003. Effect of fruits of *Moringa oleifera* on the lipid profile of normal and hypercholesterolaemic rabbits. *Journal of Ethnopharmacology*, 86(2-3), pp.191–195. Available at: http://www.sciencedirect.com/science/article/pii/S0378874103000758 [Accessed May 7, 2014].

Miller, L. H., Ackerman, H. C., Su, X., Wellems, T. E. 2013. Malaria biology and disease pathogenesis: insights for new treatments. *Nature medicine*, 19(2), pp.156–67. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23389616 [Accessed May 28, 2013].

Miller, L. H., Baruch, D. I., Marsh, K., Doumbo, O. K., 2002. The pathogenic basis of malaria. *Nature*, 415(6872), pp.673–9. Available at: http://dx.doi.org/10.1038/415673a [Accessed March 25, 2014].

Missinou, M. A., Borrmann, S., Schindler, A., Issifou, S., Adegnika, A. A., Matsiegui, P. B.,
Binder, R., Lell, B., Wiesner, J., Baranek, T., Jomaa, H. & Kremsner, P. G., 2002. Fosmidomycin for malaria. *Lancet*, 360(9349), pp.1941–2. Available at: http://www.sciencedirect.com/science/article/pii/S0140673602118605 [Accessed May 19, 2014].

MOH, 2009. *Antimalaria Drug Policy for Ghana* [Accessed May 19, 2014].

Moura, S. D., Napolea, T. H., Santos, N., Cassandra, L., Barroso, B., Maria, D. & Paiva, G., 2012. Oviposition-Stimulant and Ovicidal Activities of *Moringa oleifera* Lectin on *Aedes aegypti*. *PloS One*, 7(9), p.e48840 [Accessed September 19, 2012].

Muhammad, I., Khan, I. A., Fischer, N. H. & Fronczek, F. R., 2001. Two stereoisomeric pentacyclic oxindole alkaloids from *Uncaria tomentosa*: uncarine C and uncarine E. *Acta Crystallographica*, 57(4), pp.480–482. Available at: http://journals.iucr.org/c/issues/2001/04/00/bj1025/hklsup.html [Accessed May 2, 2014].

Musa, Y.M., Haruna, A.K., Ilyas, A., Yaro, A.H., Ahmadu, A.A. & Usman, H., 2005. Analgesic and anti-inflammatory activities of the leaves of *Pseudocedrela kotschyi*i Harms (Meliaceae). In *23rd National Scientific Conference of the Nigerian Society of Pharmacognosy*. pp. 88–89 [Accessed April 21, 2014].

Mustofa, A. V., Benoit-Vical, F., Pelissier, Y., Kone- Bamba, D. & Mallie, M., 2000. Antiplasmodial activity of plant extracts used in west African traditional medicine. *Journal of Ethnopharmacology*, 73, pp.145–151 [Accessed June 25, 2013].

Na-Bangchang, K. & Kanda, T., 1996. Activity of artemether-azithromycin versus artemether-doxycycline in the treatment of multiple drug resistant falciparum malaria. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 27(3), pp.522–525. Available at: http://europepmc.org/abstract/MED/9185262 [Accessed May 7, 2014].

Nikkon, F., Saud, Z. A., Rahman, H. & Haque, E., 2003. *In-vitro* Antimicrobial Activity of the Compound Isolated from Chloroform Extract of *Moringa oleifera* Lam. *Pakistan Journal of Biological Sciences*, 6(22), pp.1888–1890. Available at: http://www.docsdrive.com/pdfs/ansinet/pjbs/2003/1888-1890.pdf [Accessed May 8, 2014].

Niven, M.L. & Taylor, D.A.H., 1988. Revision of the structure of the limonoid pseudrelone B from *Pseudocedrela kotschyi*i. *Phytochemistry*, 27(5), p.1542. Available at: http://www.sciencedirect.com/science/article/pii/0031942288802371 [Accessed April 21, 2014].

Nosten, F., Luxemburger, C., TerKuile, F. O., Woodrow, C., Eh, J. P., Chongsuphajaisiddhi, T. & White, N. J., 1994. Treatment of multi-drug resistant *Plasmodium falciparum* malaria with 3 day artesunate-mefloquine combination. *Journal of Infectious Diseases*, 170, pp.971–977 [Accessed June 7, 2014].

Ohrt, C., 1997. Mefloquine Compared with Doxycycline for the Prophylaxis of Malaria in Indonesian Soldiers. *Annals of Internal Medicine*, 126(12), p.963. Available at: http://annals.org/article.aspx?articleid=710611 [Accessed May 7, 2014].

Okunade, M. & Adejumobi, J., 2007. Chemical, Phytochemical compositions and antimicrobial activities of some local chewing sticks used in South Western Nigeria. *Journal of phytopharmacotherapy and Natural products*, 1(1), pp.49–52. Available at: /citations?view_op=view_citation&continue=/scholar?hl=en&as_sdt=0,5&scilib=1&scioq=mb+okunade,+ja+adejumobi,+mo+ogundiya,+al+kolapo+-+%e2%80%a6+and+natural+products,+2007,chemical,+phytochemical+compositions+and+antimicrobial+activities+of++some+local+chewing+sticks+used+in+south+western+nigeria&citilm=1&citation_for_view=qpcpw6maaaaj:u-x6o8ysg0sc&hl=en&oi=p [Accessed April 21, 2014].

Okwa, O.O., 2012. *Malaria Parasites* 1st Editio., In Tech, Janeza Trdine, Croatia. Available at: www.intechopen.com [Accessed March 17, 2014].

Olasehinde, G I., Ayanda, O I., Ajayi, A A. & Nwabueze, A P., 2012. *In-vivo* antiplasmodial activity of crude n-hexane and ethanolic extracts of *Moringa oleifera* (LAM .) seeds on *Plasmodium berghei*. *International Journal of Medicinal Plant Research*, 1(5), pp.50–54 [Accessed March 17, 2014].

Olfert, E.D., Cross, B.M. & Mcwilliam, A.A., 1993. *Guide to the care and use of experimental* Volume 1., Canadian Council on Animal Care, pp.1-298 [Accessed March 24, 2014].

Oliver-Bever, B., 1986. *Medicinal Plants in Tropical West Africa* First Edit., New York: Cambridge University Press, New York, pp. 125-186 Available at: http://books.google.com/books?hl=en&lr=&id=e1I9AAAAIAAJ&pgis=1 [Accessed April 21, 2014].

Olliaro, P., 2001. Mode of action and mechanisms of resistance for antimalarial drugs. *Pharmacology & Therapeutics*, 89(2), pp.207–219. Available at: http://www.sciencedirect.com/science/article/pii/S0163725800001157 [Accessed May 8, 2014].

Ollivier, E., Fiot, J., Baghdikian, B., Boyer, L., Mahiou, V., Azas, N., Gasquet, M., Timon-david, P. & Balansard, G., 2005. HPLC Quantification of uncarine d and the anti-plasmodial activity of alkaloids from leaves of *Mitragyna inermis* (Willd .) O . Kuntze. *Phytochemical*, 33(March 2004), pp.30–33 [Accessed June 25, 2013].

Oluduro, A.O., 2012. Evaluation of Antimicrobial properties and nutritional potentials of *Moringa oleifera*. *Malaysian Journal of Microbiology*, 8(2), pp.59–67 [Accessed September 19, 2012].

Omoregie, E.S. & Sisodia, B.S., 2012. *In-vitro* antiplasmodial activity and cytotoxicity of leaf extracts of Jatropha T. *Bayero Journal of Pure and Applied Sciences*, 5(1), pp.90–97 [Accessed June 25, 2013].

Osbaldeston, T.A. & Wood, R.P.A., 2004. Dioscorides. In *De Materia Medica*. Johannesburg: IBIDIS Press, South Africa, pp.337-580 [Accessed June 4, 2014].

Oseni, L.A. & Akwetey, G.M., 2012. An *in-vivo* evaluation of antiplasmodial activity of aqueous and ethanolic leaf extracts of *Azadirachta indica* in *Plasmodium berghei* infected BALB/c mice. *International Journal of Pharmaceutical Sciences and Research*, 3(5), pp.1406–1410. Available at: http://academia.edu/1830860/an_*in-vivo*_evaluation_of_antiplasmodial_activity_of_aqueous_and_ethanolic_leaf_extracts_of_azadirachta_indica_in_plasmodium_ [Accessed July 11, 2013].

Ouédraogo, S., Ralay Ranaivo, H., Ndiaye, M., Kaboré, Z. I., Guissou, I. P., Bucher, B. & Andriantsitohaina, R., 2004. Cardiovascular properties of aqueous extract from *Mitragyna inermis* (wild). *Journal of ethnopharmacology*, 93(2-3), pp.345–50. Available at: http://www.sciencedirect.com/science/article/pii/S0378874104001679 [Accessed March 7, 2014].

Ovenden, S. P. B., Chavchich, M., Cobbe, M., Pigott, E. J., Laws, M. J. & Edstein, M. D., 2008. Preparation of a Natural Product Extract Library for Investigation Against Disease States Specific to Defence Health: A Mini Long Range Research Project. , pp.1–20 [Accessed June 10, 2014].

Pal, S.K., Mukherjee, P.K. & Saha, B.P., 1995. Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. *Phytotherapy Research*, 9(6), pp.463–465. Available at: http://doi.wiley.com/10.1002/ptr.2650090618 [Accessed May 7, 2014].

Pandey, A. V., Tekwani, B. L., Singh, R. L. & Chauhan, V. S., 1999. Artemisinin, an Endoperoxide Antimalarial, Disrupts the Hemoglobin Catabolism and Heme Detoxification Systems in Malarial Parasite. *J. Biol. Chem.*, 274(27), pp.19383–19388. Available at: http://www.jbc.org/content/274/27/19383.short [Accessed May 17, 2014].

Pang, L.W., Boudreau, E. F., Limsomwong, N. & Singharaj, P., 1987. Doxycycline prophylaxis for falciparum malaria. *The Lancet*, 329(8543), pp.1161–1164. Available at: http://www.sciencedirect.com/science/article/pii/S0140673687921416 [Accessed May 7, 2014].

Paskewitz, S.M., Brown, M.R., Lea, A.O. & Collins, F.H., 1988. Ultrastructure of the encapsulation of *Plasmodium cynomolgi* (B strain) on the midgut of a refractory strain of *Anopheles gambiae*. *The Journal of parasitology*. 74(3), pp. 432-439. Available at: http://www.jstor.org/stable/3282053 [Accessed May 28, 2014].

Pietta, P.G., 2000. Flavonoids as antioxidants. *Journal of Natural Products*, 63(7), pp.1035–42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10924197 [Accessed June 22, 2013].

PMI, 2013. *President's Malaria Initiative, Ghana: Malaria Operational Plan FY 2013*, Available at: http://pmi.gov/countries/mops/fy13/ghana_mop_fy13.pdf [Accessed November 5, 2013].

Ponnudurai, T., Lensen, A. H. W., Van Gemert, G. J. A., Bensink, M. P. E., Bolmer, M. & Meuwissen, J. H., 1989. Infectivity of cultured *Plasmodium falciparum* gametocytes to mosquitoes. *Parasitology*, 98(02), pp.165–173. Available at: http://journals.cambridge.org/abstract_S0031182000062065 [Accessed May 29, 2014].

Posner, G. H., Wang, D., Cumming, J. N., Oh, C. H., French, A. N., Bodley, A. L. & Shapiro, T. A., 1995. Evidence for Fe (IV): O in the molecular mechanism of action of the trioxane antimalarial artemisinin. *Journal of the American Chemical Society*, 117, pp.5885–5886. Available at: http://pubs.acs.org/doi/pdf/10.1021/ja00126a042 [Accessed May 17, 2014].

Posner, G.H., Park, S. B., González, L., Wang, D., Cumming, J. N., Klinedinst, D., Shapiro, T. A. & Bachi, M. D., 1996. Evidence for the Importance of High-Valent Fe O and of a Diketone in the Molecular Mechanism of Action of Antimalarial Trioxane Analogs of Artemisinin. *Journal of the American Chemical Society*, 118(14), pp.3537–3538. Available at: http://pubs.acs.org/doi/pdf/10.1021/ja954131p [Accessed May 17, 2014].

Prabhu, K., Murugan, K., Nareshkumar, A., Ramasubramanian, N. & Bragadeeswaran, S., 2011. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine*, 1(2), pp.124–129. Available at: http://linkinghub.elsevier.com/retrieve/pii/S2221169111600099 [Accessed April 2, 2012].

Price, R. N., Nosten, F., Luxemburger, C., ter Kuile, F. O., Paiphun, L., Chonsuphajaisiddhi, T. & White, N.J., 1996. Effects of artemisinin derivatives on malaria transmissibility. *The Lancet*, 347(9016), pp.1654–1658 [Accessed May 29, 2014].

Proudfoot, J.R., 2002. Drugs, leads, and drug-likeness: an analysis of some recently launched drugs. *Bioorganic & Medicinal Chemistry Letters*, 12(12), pp.1647–1650. Available at: http://www.sciencedirect.com/science/article/pii/S0960894X02002445 [Accessed June 10, 2014].

Prousek, J., 2007. Fenton chemistry in biology and medicine. *Pure and Applied Chemistry*, 79(12), pp.2325–2338. Available at: http://www.degruyter.com/view/j/pac.2007.79.issue-12/pac200779122325/pac200779122325.xml [Accessed May 13, 2014].

Pullman, T. N., Eichelberger, L., Alving, A. L. F. S., Jones, R., Craige, B. & Whorton, C. M., 1948. The use of SN-10,275 in the prophylaxis and treatment of sporozoite-induced vivax Malaria (Chesson Strain). *J Clin Invest*, 27(3), pp.12–16 [Accessed May 15, 2014].

Rao, A. V, Devi, P.U. & Kamath, R., 2001. *In-vivo* radioprotective effect of *Moringa oleifera* leaves. *Indian Journal of Experimental Biology*, 39(9), pp.858 – 863. Available at: http://nopr.niscair.res.in/handle/123456789/24000 [Accessed May 7, 2014].

Rashid, U., Anwar, F., Moser, B. R. & Knothe, G., 2008. *Moringa oleifera* oil: A possible source of biodiesel. *Bioresource Technology*, 99(17), pp.8175–8179. Available at: http://www.sciencedirect.com/science/article/pii/S0960852408003039 [Accessed May 3, 2014].

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 Suppl 1(Suppl 1), p.S4. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3059462&tool=pmcentrez&rendertype=abstract [Accessed October 29, 2012].

Rathi, B.S., Bodhankar, S.L. & Baheti, A.M., 2006. Evaluation of aqueous leaves extract of *Moringa oleifera* Linn for wound healing in albino rats. *Indian journal of experimental biology*, 44(11), pp.898–901. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17205710 [Accessed February 24, 2014].

Raynes, K., 1998. Bisquinoline antimalarials their role in malaria chemotherapy. *International Journal of Parasitology*, 29, pp.367 – 379 [Accessed November 20, 2013].

Raynes, K., Galatis, D., Cowman, A. F., Tilley, L. & Deady, L. W., 1995. Synthesis and activity of some antimalarial bisquinolines. *Journal of medicinal chemistry*, 38(1), pp.204–206 [Accessed February 22, 2014].

Rice-Evans, C.A., Miller, N.J. & Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, 20(7), pp.933–956. Available at: http://linkinghub.elsevier.com/retrieve/pii/0891584995022279 [Accessed June 22, 2013].

Robert, V., Awono-Ambene, H.P., Le Hesran, J.Y. & Trape, J.F., 2000. Gametocytemia and infectivity to mosquitoes of patients with uncomplicated *Plasmodium falciparum* malaria attacks treated with Chloroquine or Sulphadoxine plus Pyrimethamine. *Am. J. Trop. Med. Hyg.*, 62(1), pp.210–216 [Accessed May 29, 2014].

Rogers, M J., Bukhman, Y V., McCutchan, T F. & Draper, D E., 1997. Interaction of thiostrepton with an RNA fragment derived from the plastid-encoded ribosomal RNA of the malaria parasite. *RNA (New York, N.Y.)*, 3(8), pp.815–20. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1369527&tool=pmcentrez&rendertype=abstract [Accessed May 19, 2014].

Rossell, J.B. & Pritchard, J.L.R., 1991. Analysis of oilseeds, fats and fatty foods. In *Analysis of oilseeds, fats and fatty foods*. Elsevier Science Publishers Ltd., p. 558. Available at: http://www.cabdirect.org/abstracts/19931467003.html [Accessed May 6, 2014].

Sam, G.H., Mensah, M.L.K., Annan, K. & Zahree, S., 2011. Plants traditionally used in treating malaria , typhoid fever and piles in the Wa Municipality and Wa East (Funsi) District of the Upper West Region of Ghana. *Advances in Environmental Biology*, 5(10), pp.3352–3358 [Accessed September 19, 2012].

Sanchez-Martin, R.M., Mittoo, S. & Bradley, M., 2004. The Impact of Combinatorial Methodologies on Medicinal Chemistry. *Current Topics in Medicinal Chemistry*, 4(7), pp.653–669 [Accessed June 10, 2014].

Sato, A., Hiramoto, A., Morita, M., Matsumoto, M., Komich, Y., Nakase, Y., Tanigawa, N., Hiraoka, O., Hiramoto, K., Hayatsu, H., Higaki, K., Kawai, S., Masuyama, A., Nojima, M., Wataya, Y. & Kim, H. S., 2011. Antimalarial activity of endoperoxide compound 6-(1,2,6,7-tetraoxaspiro[7.11]nonadec-4-yl)hexan-1-ol. *Parasitology International*, 60(3), pp.270–273 Accessed February 22, 2014].

Schaneberg, B. T., Crockett, S., Bedir, E. & Khan, I. A., 2003. The role of chemical fingerprinting: application to *Ephedra*. *Phytochemistry*, 62(6), pp. 911–918. Available at: http://www.sciencedirect.com/science/article/pii/S0031942202007161 [Accessed June 10, 2014].

Schlagenhauf, P., 1999. Mefloquine for malaria chemoprophylaxis 1992–1998: a review. *Journal of travel medicine*. 6(2), pp. 122-133, Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1708-8305.1999.tb00843.x/abstract [Accessed May 7, 2014].

Schlitzer, M., 2008. Antimalarial drugs - what is in use and what is in the pipeline. *Archiv der Pharmazie*, 341(3), pp. 149–63. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18297679 [Accessed April 28, 2014].

Schuhwerk, M. & Behrens, R., 1998. Doxycycline as first line malarial prophylaxis: how safe is it? *Journal of travel medicine*, 5(2), pp. 102, Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1708-8305.1998.tb00476.x/abstract [Accessed May 7, 2014].

Seaberg, L. S., Parquette, A. R., Gluzman, I. Y., Phillips, G. W., Brodasky, T. F. & Krogstad, D. J., 1984. Clindamycin activity against chloroquine-resistant *Plasmodium falciparum*. *Journal of Infectious Diseases*, 150(6), pp.904–911. Available at: http://jid.oxfordjournals.org/content/150/6/904.short [Accessed May 19, 2014].

Seyoum, A., Asres, K. & El-Fiky, F.K., 2006. Structure-radical scavenging activity relationships of flavonoids. *Phytochemistry*, 67(18), pp.2058–2070. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16919302 [Accessed June 22, 2013].

Shahina, G., 1989. *Savanna Plants: An illustrated Guide*, London: Macmillan Publishers Ltd. pp. 105, Available at: http://scholar.google.com/scholar?q=Savanna+Plants.+An+illustrated+Guide.&btnG=&hl=en&as_sdt=0,5#1 [Accessed April 21, 2014].

Shanks, G., Oloo, A. & Aleman, G. M., 2001. A new primaquine analogue, tafenoquine (WR 238605), for prophylaxis against *Plasmodium falciparum* malaria. *Clinical infectious Disease* Available at: http://cid.oxfordjournals.org/content/33/12/1968.short [Accessed May 7, 2014].

Shapiro, T.A. & Goldberg, D.E., 2006. Chemotherapy of protozoal infections: Malaria. In L. L. Brunton, J. S. Lazo, & K. L. Parker, eds. *Goodman & Gilman's The Pharmacological*

Basis of Therapeutics. New York: McGraw-Hill Medical Publishing Division, p. electronic copy [Accessed October 30, 2013].

Sharma, P., Kumari, P., Srivastava, M M. & Srivastava, S., 2006. Removal of cadmium from aqueous system by shelled *Moringa oleifera* Lam. seed powder. *Bioresource technology*, 97(2), pp.299–305. Available at: http://www.sciencedirect.com/science/article/pii/S096085240500132X [Accessed May 6, 2014].

Shellard, E.J., Phillipson, J.D. & Sarpong, K., 1971. N-Oxides of the Oxindole Alkaloids Rhynchophylline and Isorhynchophylline. *Xenobiotica*, 1(4-5), pp.455–456. Available at: http://informahealthcare.com/doi/abs/10.3109/00498257109041510 [Accessed June 25, 2013].

Shellard, E.J. & Sarpong, K., 1970. The alkaloidal pattern in the leaves, stem-bark and root-bark of *Mitragyna* species from Ghana. *Journal of Pharmacy and Pharmacology*, 22(S1), p.34S–39S. Available at: http://doi.wiley.com/10.1111/j.2042-7158.1970.tb08577.x [Accessed April 21, 2014].

Shellard, E.J. & Sarpong, K., 1969. The alkaloids of the leaves of *Mitragyna inermis* (Willd.) O. Kuntze. *Journal of Pharmacy and Pharmacology*, 21(S1), p.113S–117S. Available at: http://doi.wiley.com/10.1111/j.2042-7158.1969.tb08359.x [Accessed April 21, 2014].

Sherman, I.W., 1979. Biochemistry of Plasmodium (malarial parasites). *Microbiological reviews*, 43(4), pp.453–95. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=281489&tool=pmcentrez&rendertype=abstract [Accessed May 10, 2014].

Sherman, I.W., Eda, S. & Winograd, E., 2003. Cytoadherence and sequestration in *Plasmodium falciparum*: defining the ties that bind. *Microbes and Infection*, 5(10), pp.897–909. Available at: http://www.sciencedirect.com/science/article/pii/S128645790300162X [Accessed May 29, 2014].

Sibley, L.D., 2004. Intracellular parasite invasion strategies. *Science (New York, N.Y.)*, 304(5668), pp.248–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15073368 [Accessed March 21, 2014].

Silamut, K. & White., N.J., 1993. Relation of the stage of parasite development in the peripheral blood to prognosis in severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 87, pp.436–443 [Accessed June 6, 2014].

Sinclair, D., Donegan, S. & Lalloo, D., 2011. Artesunate versus quinine for treating severe malaria (Review). *Cochrane Database of Systematic Reviews*, Issue 3. Art. No. CD005967. DOI: 10.1002/14651858.CD005967.pub3 [March 22, 2014].

Sinden, R., 1978. Cell Biology. In R. Killick-Kendrick & W. Peters, eds. *Rodent malaria*. London: Academic Press, pp. 85–168. Available at: http://scholar.google.com/scholar?q=Sinden,+R.E.+(1978)+Cell+Biology.+In:+Rodent+Malaria+&btnG=&hl=en&as_sdt=0,5#1 [Accessed May 28, 2014].

Slater, A.F.G., 1993. Chloroquine: Mechanism of drug action and resistance in *Plasmodium falciparum*. *Pharmacology & Therapeutics*, 57(2-3), pp.203–235. Available at: http://www.sciencedirect.com/science/article/pii/016372589390056J [Accessed May 15, 2014].

Smalley, M.E., Abdalla, S. & Brown, J., 1981. The distribution of *Plasmodium falciparum* in the peripheral blood and bone marrow of Gambian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 75(1), pp.103–105. Available at: http://www.sciencedirect.com/science/article/pii/0035920381900195 [Accessed May 29, 2014].

Sofowora, A., 1993. *Medicinal Plants and Traditional Medicine in Africa.* Second Edi., New York: John Wiley [Accessed May 29, 2014].

Soh, P.N. & Benoit-Vical, F., 2007. Are West African plants a source of future antimalarial drugs? *Journal of Ethnopharmacology*, 114(2), pp.130–140 [Accessed February 22, 2014].

Sory, E., Amofah, G., Amankwah, J., Faried, K., Bart-Plange, C., Allotey, N. K., Ansah-Mante, O., Appiah-Kusi, J., Amable, E., Agbemodzi, F., Badu, S., Owusu-Antwi, F. & Psychas, P., 2009. Guidelines for case management of malaria in Ghana, *Ghana Health Service Training Manual*, Accra, Ministry of Health, pp. 2-8 [Accessed October 30, 2013].

Sreelatha, S. & Padma, P.R., 2009. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant foods for human nutrition (Dordrecht, Netherlands)*, 64(4), pp.303–11. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19904611 [Accessed May 2, 2014].

Srivastava, A., Misra, H., Verma, R. K., Gupta & Madan M., 2004. Chemical fingerprinting of *Andrographis paniculata* using HPLC, HPTLC and densitometry. *Phytochemical analysis*, 15(5), pp. 280–285. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15508831 [Accessed June 10, 2014].

Staerk, D., Kesting, J. R., Sairafianpour, M., Witt, M., Asili, J., Emami, S. A. & Jaroszewski, J. W., 2009. Accelerated dereplication of crude extracts using HPLC-PDA-MS-SPE-NMR: quinolinone alkaloids of *Haplophyllum acutifolium*. *Phytochemistry*, 70(8), pp.1055–61. Available at: http://www.sciencedirect.com/science/article/pii/S003194220900199X [Accessed June 10, 2014].

Stermitz, F. R., Scriven, L. N., Tegos, G. & Lewis, K., 2002. Two flavonols from *Artemisa annua* which potentiate the activity of berberine and norfloxacin against a resistant strain of *Staphylococcus aureus*. *Planta medica*, 68(12), pp.1140–1. Available at: https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-2002-36347 [Accessed June 5, 2014].

STG, 2010. *Standard Treatment Guidelines* 6th Editio., Accra: Ghana National Drugs Programme. Available at: www.ghndp.org [Accessed April 4, 2014].

Tahiliani, P. & Kar, A., 2000. Role of *Moringa oleifera* leaf extract in the regulation of thyroid hormone status in adult male and female rats. *Pharmacological research : the official journal of the Italian Pharmacological Society*, 41(3), pp.319–23. Available at: http://www.sciencedirect.com/science/article/pii/S104366189990587X [Accessed May 7, 2014].

Talman, A. M., Domarle, O., McKenzie, F. E., Ariey, F. & Robert, V., 2004. Gametocytogenesis: the puberty of *Plasmodium falciparum*. *Malaria journal*, 3(1), p.24. Available at: http://www.malariajournal.com/content/3/1/24 [Accessed May 27, 2014].

Tang, Y., Dong, Y. & Vennerstrom, J.L., 2004. Synthetic peroxides as antimalarials. *Medicinal research reviews*, 24(4), pp.425–48. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15170591 [Accessed May 19, 2014].

Tapsoba, H. & Deschamps, J. P., 2006. Use of medicinal plants for the treatment of oral diseases in Burkina Faso. *Journal of ethnopharmacology*, 104(1-2), pp.68–78. Available at: http://www.sciencedirect.com/science/article/pii/S0378874105005866 [Accessed April 8, 2014].

Targett, G., Drakeley, C., Jawara, M., von Seidlein, L., Coleman, R., Deen, J., Pinder, M., Doherty, T., Sutherland, C., Walraven, G. & Milligan, P., 2001. Artesunate reduces but does not prevent post treatment transmission of *Plasmodium falciparum* to *Anopheles gambiae*. *Journal of Infectious Diseases*, 183(8), pp.1254–1259 [Accessed May 29, 2014].

Taylor, D.A.H., 1979. A limonoid, pseudrelone B, from *Pseudocedrela kotschyi*i. *Phytochemistry*, 18(9), pp.1574–1576. Available at: http://www.sciencedirect.com/science/article/pii/S0031942200985054 [Accessed April 21, 2014].

Taylor, D. K., Avery, T. D., Greatrex, B. W., Tiekink, E. R. T., Macreadie, I. G., Macreadie, P. I., Humphries, A. D., Kalkanidis, M., Fox, E. N., Klonis, N. & Tilley, L., 2004. Novel endoperoxide antimalarials: synthesis, heme binding, and antimalarial activity. *Journal of Medicinal Chemistry*, 47(7), pp.1833–1839.

Taylor, W. & Richie, T., 1999. Malaria prophylaxis using azithromycin: a double-blind, placebo-controlled trial in Irian Jaya, Indonesia. *Clinical infectious Diseases*, 28(1), pp. 74-81. Available at: http://cid.oxfordjournals.org/content/28/1/74.short [Accessed May 7, 2014].

Thilza, I B., Sanni, S., Isah, Z. A., Sanni, F S., Talle, M. & Joseph, M. B., 2010. *In-vitro* antimicrobial activity of water extract of *Moringa oleifera* leaf stalk on bacteria normally implicated in eye diseases. *Academia Arena*, 2(6), pp.80–82. Available at: http://www.sciencepub.net/academia 80 [Accessed July 12, 2012].

Thomas, G., 2007. *Medicinal Chemistry: An Introduction* Second edi., West Sussex: John Wiley & Sons, Ltd. Available at: www.wileyeurope.com [Accessed October 31, 2013].

Thurston, J.P., 1950. The action of antimalarial drugs in mice infected with *Plasmodium berghei*. *Brit. J. Pharmacol.*, 5, pp.409–416 [Accessed March 17, 2014].

Tilley, L., Dixon, M.W.A. & Kirk, K., 2011. The *Plasmodium falciparum* infected red blood cell. *International Journal of Biochemistry and Cell Biology*, 43(6), pp.839–842. Available at: http://dx.doi.org/10.1016/j.biocel.2011.03.012 [Accessed November 19, 2013].

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. & Kaur, H., 2011. Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia*, 1(1), pp.98 – 106. Available at: http://www.ipharmsciencia.com [Accessed June 25, 2013].

Tor-Anyiin, T. & Orokpo, A., 2012. Phytochemical screening, chromatographic evaluation and antibacterial activity on stem bark extracts of *Mitragyna inermis*. *International Journal of Natural Products Science*, 2(3), pp.45–52 [Accessed December 2, 2012].

Toure, H., Balansar, G., Pauli, M. & Scotto, M., 1996. Pharmacological investigation of alkaloids from leaves of *Mitragyna inermis* (Rubiaceae). *Journal of Ethnopharmacology*, 54, pp.59–62 [Accessed June 25, 2013].

Traore, F., Gasquet, M., Laget, M., Guiraud, H., Giorgio, C D., Azas, N. & Doumbo, O., 2000. Toxicity and Genotoxicity of Antimalarial Alkaloid Rich Extracts Derived from *Mitragyna inermis* O . Kuntze and *Nauclea latifolia*., *Phytotherapy Research*,14, pp.608–611 [Accessed March 17, 2014].

Tsaknis, J., Lalas, S., Gergis, V., Dourtoglou, V. & Spiliotis, V., 1999. Characterization of *Moringa oleifera* Variety Mbololo Seed Oil of Kenya. *Journal of Agricultural and Food Chemistry*, 47(11), pp.4495–4499. Available at: http://dx.doi.org/10.1021/jf9904214 [Accessed May 6, 2014].

USEPA, 2014. Mosquito Control. Available at: http://www2.epa.gov/mosquitocontrol [Accessed June 12, 2014].

Valecha, N., Adak, T., Bagga, A K., Asthana, O P., Srivastava, J S., Joshi, H. & Sharma, V P., 2001. Comparative antirelapse efficacy of CDRI compound 80/53 (Bulaquine) vs primaquine in double blind clinical trail. *Current Science*, 80, pp.561–563. Available at: http://14.139.230.5:8080/dspace/handle/123456789/508 [Accessed May 13, 2014].

Vennerstrom, J. L., Arbe-Barnes, S., Brun, R., Charman, S. A., Chiu, F. C. K., Chollet, J., Dong, Y., Dorn, A., Hunziker, D., Matile, H., McIntosh, K., Padmanilayam, M., Santo Tomas, J., Scheurer, C., Scorneaux, B., Tang, Y., Urwyler, H., Wittlin, S. & Charman, W. N., 2004. Identification of an antimalarial synthetic trioxolane drug development candidate. *Nature*, 430(7002), pp.900–904 [Accessed February 22, 2014].

Verma, V. K., Singh, N., Saxena, P. & Singh, R., 2012. Anti-Ulcer and Antioxidant Activity of *Moringa oleifera* (Lam) Leaves against Aspirin and Ethanol Induced Gastric Ulcer in Rats. *International Journal of Pharmaceuticals*, 02(02), pp.46–57 [Accessed October 31, 2013].

Wallace, D., 1996. The use of chloroquine and hydroxychloroquine for non-infectious conditions other than rheumatoid arthritis or lupus: a critical review. *Lupus*, 5(1 suppl), pp.S59–S64. Available at: http://lup.sagepub.com/content/5/1_suppl/S59.short [Accessed May 14, 2014].

Wang, J., Huang, L., Li, J., Fan, Q., Long, Y., Li, Y. & Zhou, B., 2010. Artemisinin directly targets malarial mitochondria through its specific mitochondrial activation. *PloS one*, 5(3), pp.1–12. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2833198&tool=pmcentrez&rendertype=abstract [Accessed May 23, 2013].

Wells, T.N.C., Burrows, J.N. & Baird, J.K., 2010. Targeting the hypnozoite reservoir of Plasmodium vivax : the hidden obstacle to malaria elimination. *Cell*, 26(3), pp.145–151 [Accessed November 19, 2013].

White, N., 2008. *Plasmodium knowlesi*: The Fifth Human Malaria Parasite. *Clin Infect Dis.*, 46(2), pp.172–173 [Accessed November 7, 2013].

White, N.J., 1997. Assessment of the pharmacodynamic properties of antimalarial drugs *In-vivo*. *Antimicrobial agents and chemotherapy*, 41(7), pp.1413–22. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=163932&tool=pmcentrez&rendertype=abstract [Accessed May 10, 2014].

White, N.J. & Davis, T.M.E., 1992. Anti-infective drugs. In C. van Boxtall, N. H. G. Holford, & M. Danhof, eds. *The In-vivo study of drug action: principles and applications of kinetic-dynamic Modelling*. Amsterdam: Elsevier Limited, pp. 313–336 [Accessed June 6, 2014].

WHO, 2013a. Country's profiles: Ghana. In *World Malaria Report 2012*. p. 131. Available at: http://www.who.int/malaria/publications/world_malaria_report_2012/wmr2012_country_profiles.pdf [Accessed November 5, 2013].

WHO, 2010. *Guidelines for the treatment of malaria* 2nd Editio. P. Olumese, ed., Geneva: World Health Organization, 20, avenue Appia, 1211 Geneva 27, Switzerland (tel. +41 22 791 3264; fax: +41 22 791 4857; e-mail: bookorders@who.int) [Accessed October 30, 2013].

WHO, 2013b. *World Malaria Report 2012 -Summary*, Geneva. Available at: http://www.who.int/malaria/publications/world_malaria_report_2012/wmr2012_summary_en.pdf [Accessed November 5, 2014].

Wilson, C.R., Sauer, J.M. & Hooser, S.B., 2001. Taxines: a review of the mechanism and toxicity of yew (Taxus spp.) alkaloids. *Toxicon*, 39(2), pp.175–185. Available at: http://www.sciencedirect.com/science/article/pii/S004101010000146X [Accessed June 4, 2014].

Wu, Q., An, Y., Gao, J. H., Lu, Y. C., Bao, J.Y., Hu, S. Q. & Yu, J., 2006. Study on fingerprint of *Rhodiola rosea* from the Qingzang Plateau by high performance liquid chromatography. *Journal of Southwest University for Nationalities (Natural Science Edition)*, 1(1) [Accessed June 10, 2014].

Xie, P., Chen, S., Liang, Y., Wang, X., Tian, R. & Upton, R., 2006. Chromatographic fingerprint analysis--a rational approach for quality assessment of traditional Chinese herbal medicine. *Journal of chromatography. A*, 1112(1-2), pp.171–80. Available at:

http://www.sciencedirect.com/science/article/pii/S0021967306000306 [Accessed June 1, 2014].

Yayon, A., Cabantchik, Z.I. & Ginsburg, H., 1984. Identification of the acidic compartment of *Plasmodium falciparum*-infected human erythrocytes as the target of the antimalarial drug chloroquine. *The EMBO journal*, 3(11), pp.2695–700. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=557751&tool=pmcentrez&rendertype=abstract [Accessed May 16, 2014].

Zongo, C., Akomo, E.F.O., Savadogo, A., Obame, L. C., Koudou, J. & Traore, A. S., 2009. *In-vitro* Antibacterial Properties of Total Alkaloids Extract from *Mitragyna inermis* (Willd.) O. Kuntze, a West African Traditional Medicinal Plant. *Asian Journal of Plant Sciences*, 8(2), pp.172–177. Available at: http://www.cabdirect.org/abstracts/20093127042.html;jsessionid=8AC2CDFF8493F359A279D4BEED0E7C65 [Accessed October 29, 2013].