

Antimalarial activity of 80% methanol extract of the stem bark of *Syzygium guineense* (Willd.) DC. (Myrtaceae) in mice infected with *Plasmodium berghei*

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This is to certify that the thesis prepared by Gemechu Zeleke, entitled: Antimalarial activity of 80% methanol extract of the stem bark of *Syzygium guineense* (Willd.) DC. (Myrtaceae) in mice infected with *Plasmodium berghei* and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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DEDICATION

To my Son, Na'ol and beloved Wife, Shashe Tesfaye

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List of Abbreviations

AAU:	Addis Ababa University
ACT:	Artemisinin-based Combination Therapy
ANOVA:	Analysis of Variance
CDC:	Centers for Disease Control and Prevention
COX:	Cyclooxygenase
GPI:	Glycosylphosphatidylinositol
HIV/AIDS:	Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome
IL:	Interleukin
IRBCs:	Infected Red Blood Cells
IRS:	Indoor Residual Spraying
ITNs:	Insecticide-treated Nets
LLINs:	Long Lasting Insecticide-treated Nets
OECD:	Organization for Economic Cooperation and Development
PCR:	Polymerase Chain Reaction
PCV:	Packed Cell Volume
Pfcr1:	<i>Plasmodium falciparum</i> Chloroquine Resistance Transporter
PfEMP1:	<i>Plasmodium falciparum</i> erythrocyte Membrane Protein 1
PfMDR1:	<i>Plasmodium falciparum</i> Multidrug Resistance 1
SERCA:	Sarco/Endoplasmic Reticulum Ca ²⁺ -ATPase
TNF:	Tumor Necrosis Factor
WHO:	World Health Organization

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ABSTRACT

Malaria is one of the world's most deadly infectious diseases. Spread and emergence of antimalarial drug resistance is the major challenge that endangers all the recent gains in malaria control. Medicinal plants are the key source of new effective antimalarials. *Syzygium guineense* is commonly used by traditional healers' in many parts of Africa including Ethiopia for treatment of malaria. Thus, this study attempted to evaluate *in vivo* antimalarial activity of 80% methanol extract of the stem bark of the plant in mice infected with *Plasmodium berghei* ANKA strain. To this effect various doses (100, 200, 400 mg/kg) of the extract were evaluated for the antimalarial effect using the four-day suppressive, curative and prophylactic tests. Parameters, including percent parasitemia, survival time, body weight, body temperature and packed cell volume (PCV) were determined using standard procedures. A significant ($p < 0.001$) parasite suppression of 11.20%, 34.04% and 45.05% was observed with 100, 200, and 400mg/kg of the extract, respectively, in early infection. On the other hand, a significant parasite suppression was observed by the extract in curative test with 400 mg/kg (36.3%, $p < 0.001$) being the highest. In the prophylactic test, 100, 200, and 400mg/kg of the extract produced 26.91%, 37.13%, 58.24% parasite suppression, respectively. In addition, the standard produced significant parasitemia suppression in all tests. In general, the extract of stem bark of *S. guineense* showed considerable *in vivo* antimalarial properties with outstanding prophylactic activity. The phytochemical screening of the extract showed the presence of alkaloids, anthraquinones, terpenoids, saponins, tannins, phenols and flavonoids. The findings suggest that *S. guineense* contain active phytochemicals that could potentially be a lead compound in the search for new antimalarials.

Key words: *Syzygium guineense*, *Plasmodium berghei*, Antimalarial activity

1 Introduction

Infectious diseases such as malaria are posing a major challenge for years and still today. Malaria, a severe and life-threatening disease, is the most prevalent and the main cause of morbidity and mortality in Africa (Mboowa, 2014). It is also a significant public health problem in 97 countries and territories (WHO, 2014). Even though there is a significant progress in malaria control during the last decade, the huge burden of malaria is still persisting especially in sub-Saharan Africa (Korenromp *et al.*, 2013).

1.1 Epidemiology of Malaria

According to the latest estimates of 2013, about 3.2 billion people are at risk of being infected with malaria and developing disease; of which 198 million cases and 584, 000 deaths of malaria occurred globally (WHO, 2014). An estimated 90% of all malaria deaths occur in Africa, particularly in children less than 5 years of age (WHO, 2014). In addition, malaria accounts for 40% of public health expenditure, 30-50% of inpatient admissions, and up to 50% of outpatient visits in areas with high malaria transmission (WHO, 2013). In high endemic areas, the disease burden is the greatest in infants and young children, while in areas of lower transmission many cases occur in older children and adults (Carneiro *et al.*, 2010). A number of factors account for the malaria burden in developing countries, which include climate change, lack of infrastructure, emerging drug and insecticide resistance, massive population and costs of containment and therapy (WHO, 2012). Malaria is also the major public health problem in Ethiopia. Approximately 4-5 million cases of malaria are reported annually. In Ethiopia, more than 75% of people live in malaria endemic areas, putting over 50 million people at risk of malaria. In addition, it accounts for 7% of outpatient visits, being the largest single cause of morbidity (WHO, 2013). Moreover, the problem is compounded by the increasing frequency and

magnitude of malaria epidemics in many areas of the country. Malaria is mainly seasonal in the highland fringe areas of Ethiopia and of relatively longer transmission duration in lowland areas, river basins and rift valleys of the country (Ayele *et al.*, 2013). Recently, malaria cases and deaths reduced by 26% and 54% respectively (WHO, 2014).

1.2 Etiology of Malaria

Malaria is caused by five species of the genus *Plasmodium*. *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi* are human malaria species that are spread from one person to another by female mosquitoes of the genus *Anopheles*. In Africa, malaria due to *P. falciparum* is the most predominant and deadly form (WHO, 2014). *Plasmodium vivax* has a wider distribution than *P. falciparum* because it is able to develop in the *Anopheles* mosquito vector at lower temperatures, and to survive at higher altitudes and in cooler climates. It also has a dormant liver stage (known as a hypnozoite) that enables it to survive during periods when *Anopheles* mosquitoes are not present to continue transmission, such as during winter months (Price *et al.*, 2007).

Plasmodium falciparum and *P. vivax* are the most dominant malaria parasites in Ethiopia, distributed all over the country and accounting for 60% and 40% of malaria cases, respectively. However, *P. malariae* accounts for less than 1% and *P. ovale* are rare in the country (Adugna, 2011). In many areas outside Africa, infections due to *P. vivax* are more common than those due to *P. falciparum*. In recent years, human cases of malaria have also been recorded due to *P. knowlesi*; a species that causes malaria among monkeys, and occurs in certain forested areas of South-East Asia (WHO, 2014).

Malaria is transmitted exclusively through the bites of female *Anopheles* mosquitoes. There are about 400 different species of *Anopheles* mosquitoes, but only 30 of these are vectors of major importance. *Anopheles gambiae* is the most widespread in Africa and the most difficult to control. In addition, *Anopheles arabiensis*, *Anopheles pharoensis*, *Anopheles funestus*, and *Anopheles nili* are the prominent *Anopheles* species in Ethiopia (WHO, 2014; Adugna, 2011).

1.3 Lifecycle of Malaria Parasite

After a bite, sporozoites from the mosquito salivary glands rapidly enter into the circulation and localize *via* specific recognition events in hepatocytes. In hepatocytes, each sporozoite undergoes extensive replication within a parasite-derived vacuole, essentially walling off the parasite from the liver cell cytoplasm. When sporozoites mature into schizonts, the infected liver cells rupture and release merozoites directly into the blood circulation (Sturm *et al.*, 2006). In the blood it invades new erythrocytes, and initiates the erythrocytic cycle. In *P. vivax* and *P. ovale* infections, some hypnozoites remain in the liver and produce relapses of erythrocytic infection months to years after the primary attack unlike *P. falciparum* and *P. malariae* infections. Schizont-containing erythrocytes rupture, each releasing 6 to 32 merozoites depending on the *Plasmodium* species. The merozoites invade more erythrocytes to continue the cycle, which proceeds until death of the host or modulation by drugs or acquired partial immunity. For *P. falciparum*, *P. vivax*, and *P. ovale*, it takes about 48 h to complete this process (Bannister & Sherman, 2009)

Some erythrocytic parasites differentiate into sexual forms known as *gametocytes*. After infected blood is ingested by a female mosquito, exflagellation of the male gametocyte is followed by fertilization of the female gametocyte in the insect gut. The resulting zygote, which develops as an oocyst in the gut wall, eventually gives rise to sporozoites, which invade the salivary gland of

the mosquito. The insect then can infect a human host by taking a blood meal (Bannister & Sherman, 2009)

1.4 Pathophysiology of Malaria

Lysis of infected red blood cells (iRBCs) resulted in release of newly developed merozoites and other toxic factors glycosylphosphatidylinositol (GPI) in to the blood. These products, particularly the GPI, activate macrophages and endothelial cells to secrete cytokines and inflammatory mediators such as tumor necrosis factor (TNF), interferon- γ , interleukin (IL)-1, IL-6, IL-8, macrophage colony-stimulating factor, and lymphotoxin, as well as superoxide and nitric oxide. The systemic manifestations of malaria such as headache, fever and rigors, nausea and vomiting, diarrhea, anorexia, tiredness, aching joints and muscles, thrombocytopenia, immunosuppression, coagulopathy, and central nervous system manifestations have been largely attributed to the various cytokines released in response to these parasite and red cell membrane products (Angulo & Fresno, 2002). In addition to these factors, the plasmodial DNA is also highly proinflammatory and can induce fever. The plasmodial DNA is presented by hemozoin to interact intracellularly with the Toll-like receptor-9. This leads to the release of proinflammatory cytokines that in turn induce cyclooxygenase (COX) mediated up regulation of prostaglandins, leading to the induction of fever. Hemozoin has also been linked to the induction of apoptosis in developing erythroid cells in the bone marrow, thereby causing anemia (Miller *et al.*, 2002).

In *P. falciparum* infections, iRBCs become rigid and spherical losing their fluidity unlike the normal RBCs, which increases adhesiveness. Infected red blood cells (iRBCs) adhere to the capillary and post capillary venular endothelium in the deep microvasculature (Rowe *et al.*, 2009). Cytoadherence leads to sequestration of the parasites in the vital organs. This mediated by

the members of the *P. falciparum* erythrocyte membrane protein 1 (*PfEMP1*) family ((Baruch *et al.*, 1995). It ultimately blocks blood flow, limits the local oxygen supply, hampers mitochondrial ATP synthesis, and stimulates cytokine production - all these factors contribute to the development of severe complications of *P. falciparum* (inflammation, activation of platelets, anemia, microcirculatory dysfunction and tissue hypoxia, organ dysfunctions). The red cell deformability is increased in *P. vivax* infection. This enable *P. vivax* infected red cells to survive the passage through the splenic sinusoids; the accompanying increase in fragility of both infected and non-infected red cells that may contribute to severe anemia in *P. vivax* malaria (Storm & Craig, 2014; Miller *et al.*, 2002).

1.5 Diagnosis for Malaria

Diagnosis of malaria is based on clinical suspicion and on the detection of parasites in the blood. Persons of all age in any epidemiological setting with suspected malaria should receive a parasitological confirmation of diagnosis by either microscopy or rapid diagnostic tests. Blood microscopy is the gold standard for the diagnosis of malaria, but malaria rapid diagnostic tests are also becoming a primary diagnostic test in many areas, particularly where microscopy may not be available. Rapid diagnostic test is as specific as the blood microscopy and even appears more sensitive than microscopy (Azikiwe *et al.*, 2012). However, recent findings suggest that malaria diagnosis efficiency of microscopy is very low for species-specific and mixed infection of *P. falciparum* and *P. vivax* detection. In addition, parasite nucleic acids are also detected using polymerase chain reaction (PCR). Even though, real time PCR based assay is recommended to diagnose species-specific and mixed malaria infection, its high cost limits its use (Tajebe *et al.*, 2014). As a result, PCR is mostly used only for confirming the species of malaria parasite after

the diagnosis has been established by either smear microscopy or rapid diagnostic tests to prevent the advent of drug resistant strains due to misdiagnosis and mistreatment (Wilson, 2013).

1.6 Clinical presentations

Malaria symptoms can develop as early as 7 days after initial exposure in a malaria-endemic area and as late as several months or more after departure. Malaria is characterized by fever, chills, headache, myalgia, and malaise, and these symptoms can occur at intervals. If not treated within 24 h, *P. falciparum* malaria can progress to severe illness often leading to death. Children with severe malaria frequently develop one or more of the symptoms such as severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria. In adults, multi-organ involvement is also frequent. In *P. vivax* and *P. ovale*, clinical relapses may occur weeks to months after the first infection, even if the patient has left the malarious area. These new episodes arise from dormant liver forms and need special treatment with agents which targets the liver stages for a complete cure (CDC, 2014).

1.7 Management of malaria

Malaria is an entirely preventable and treatable disease, provided the currently suggested interventions are properly implemented. Strategies to control and eliminate malaria include vector control, chemoprevention for the most vulnerable populations, particularly pregnant women and infants; and timely treatment with appropriate antimalarial drugs. Malaria control relies on effective prevention and case management (WHO, 2013).

1.7.1 Non-pharmacological approaches

Over the years, the preventive measures have been very effective strategies in protecting the most vulnerable groups against vector contact and progression of malaria infection (Eisele *et al.*,

2012). Malaria vector controls reduce human-vector contact and protect individuals from mosquitoes that carry malaria-causing parasites. It also lowers the intensity of malaria transmission at community level by reducing the average lifespan of the local mosquito population. The novel or improved vector control strategies enhance control of adult vectors that enter houses to feed and/or rest by killing; or repelling them. To date, universal coverage of long lasting insecticide-treated nets (LLINs) or Indoor residual spraying (IRS) with behavioral change interventions is actively promoted as the main malaria prevention strategy (WHO, 2014; Killeen, 2014).

Insecticide-treated nets (ITNs) are the most prominent malaria preventive measure for large-scale deployment in highly endemic areas. These include both LLINs and conventional nets treated with an insecticide that protect the person sleeping under the net and the effect to an entire area (Lengeler, 2004; CDC, 2014).

The LLIN-IRS combination strategy is mostly suggested for accelerating malaria control in high transmission areas. In addition, Larviciding is the most widely used of larval source management approach that involves the regular application of a biological or chemical insecticide to water bodies to reduce the number of mosquito larvae and pupae (WHO, 2014).

Furthermore, the only malaria vaccine (RTS,S/AS01) is also now available as a prevention tool. RTS,S/AS01 targets *P. falciparum* and protected young children and infants from malaria up to 18 months after vaccination in large-scale clinical trial III. The vaccine comprises a fusion protein of a malaria antigen – the carboxy terminus of the *P. falciparum* circumsporozoite antigen, the most dominant surface antigen of the initial pre-erythrocytic phase, with hepatitis B surface antigen (The RTS,S Clinical Trials Partnership, 2014).

1.7.2 Conventional antimalarial agents

Chemotherapy of malaria

Nowadays, the ultimate aim of malaria chemotherapy is not only to treat symptomatic infection but also to reduce transmission potential. Artemisinin derivatives and 8-aminoquinolines are useful transmission-blocking antimalarials whose optimal actions are on different stages of gametocytes. Quinine remains important for treating complicated *P. falciparum* malaria despite its toxicity when used for extended period of time (WHO, 2013). Based on high-quality evidence of the efficacy superiority of artesunate over quinine in adults and children with severe malaria, the WHO guidelines have been revised. Chloroquine is highly effective against erythrocytic forms of *P. vivax*, *P. ovale*, *P. malariae*, and to chloroquine-sensitive strains of *P. falciparum*. In addition to malaria prophylactic use, Chloroquine is treatment of choice when these organisms are involved. Artemisinin and its semisynthetic derivatives, including artesunate, artemether and dihydroartemisinin, are short-acting antimalarial agents that kill parasites more rapidly than conventional antimalarials, and are active against both the sexual and asexual stages of the parasite cycle (Eckstein-Ludwig, 2003). The mode of artemisinin action involves multiple steps. The heme iron within the parasite catalyzes cleavage of endoperoxide bridge. This is followed by rearrangement to produce a carbon-centered radical that alkylates and damages macromolecules in the parasite, likely including sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) (O'Neill et al., 2010; Eckstein-Ludwig, 2003). Injectable artesunate is currently recommended as the first-line treatment for severe malaria (Noubiap, 2014). To delay or prevent emergence of resistance, artemisinins are combined with one of several longer-acting drugs (8-amodiaquine, mefloquine or lumefantrine), which permit elimination of the residual malarial parasites. Currently, artemisinin-based combination therapy (ACT) (artemether - lumefantrine) is used as first-line to

treat uncomplicated *P. falciparum* malaria. Chloroquine remains the currently recommended drug for the treatment of *P. vivax* in areas where the drug is still effective. However, ACTs are now recommended for the treatment of chloroquine-resistant *P. vivax*. Moreover, radical cure of *P. vivax* and *P. ovale* requires primaquine that prevents late relapses (WHO, 2014).

Chemoprophylaxis of malaria

Primary chemoprophylaxis involves taking a medicine before, during, and after travel to malaria endemic areas. In addition to primary prophylaxis, terminal prophylaxis is used after the exposure period (or immediately thereafter) to prevent relapses caused by hypnozoites (dormant liver stages) of *P. vivax* or *P. ovale*. Chloroquine phosphate or Hydroxychloroquine sulfate is used for prevention of malaria only in destinations where chloroquine resistance is not present. Prophylaxis should begin 1–2 weeks before travel to malarious areas and continued by taking the drug once a week during travel in malarious areas and for 4 weeks after a traveler leaves these areas. A fixed combination of atovaquone and proguanil is also used for prophylaxis malaria. In addition, primaquine phosphate is used for malaria prevention as primary and terminal prophylaxis in areas with primarily *P. vivax* prevalent (CDC, 2014; WHO, 2012).

1.7.3 Antimalarial drug resistance

Latest report showed that malaria case incidence and mortality rates decreased globally by 30% and 47% from 2000 to 2013, respectively (WHO, 2014). However, malaria control program was endangered by lack of accessibility to effective malaria control tools, emergence of resistance to insecticides and first-line drugs, artemisinin, and limited effective antimalarial drugs (Blayneh & Mohammed-Awel, 2014; Smith Gueye *et al.*, 2014). Antimalarial drug resistance occurs as a consequence of several factors, including poor treatment practices, inadequate patient adherence

to prescribed antimalarial regimens, artemisinin-based monotherapies and substandard forms of the drug (WHO, 2013).

Generally, mechanism of antimalarial drug resistance depends on the chemical class of the drug and its mode of action. Resistance to Quinine arises from mutations of genes encoding vacuolar trans-membrane proteins which regulate the influx/efflux of the drug at the target. Chloroquine resistance to *P. falciparum* is primarily attributable to single nucleotide polymorphisms in *pfcr1* (chloroquine resistance transporter). However, mutations in *P. falciparum* multidrug resistance 1 (PfMDR1) seem to be the main cause of resistance to mefloquine but are also implicated in Chloroquine resistance (Warhurst, 2007). Resistance to antifolates which results from stepwise accumulation of single point mutations of the genes, *pfdhps* and *pfdhfr*, that encode for the drug targets including dihydropteroate synthase and dihydrofolate reductase, respectively, is quite common worldwide.

Although artemisinin derivatives represent the most efficacious class of antimalarial drugs, some cases of resistance have been detected in Cambodia, Myanmar, Thailand and Vietnam (WHO, 2014). Alterations in the expression of heat-shock proteins, a cell-cycle regulator and a DNA biosynthesis protein due to mutations or amplifications of the gene encoding a PfMDR1 or mutations in the gene encoding sarco-endoplasmic reticulum calcium ATPase6 have been reported to occur in artemisinin tolerant parasites (Witkowski *et al.*, 2010). Mutations in the Kelch 13 (K13)-propeller domain which associated with delayed parasite clearance is a molecular marker of artemisinin resistance (WHO, 2014).

1.7.4 Herbal medicine

Globally, over 1000 plants have been used as potential antimalarial remedies (Karunamoorthi *et al.*, 2013). In rural areas of Africa and elsewhere in developing countries, traditional medicines are widely used for treatment of malaria (Graz *et al.*, 2011). Historically, traditional medicinal plants have provided the source of the two major families of antimalarial drugs, Artemisinin from *Artemisia annua* and quinine from *Cinchona* bark, still in use today. Several research groups are working to screen plants for new antimalarial compound (Nwaka & Hudson, 2006).

Medicinal plants have been reported for their significant antimalarial activity against human and rodent malaria parasites. Previous studies suggest that *P. corymbosa*, *T. indica* (Koudouvo, 2011), *Dacryodes edulis*, *Vernonia amygdalina* (Zofou *et al.*, 2011), *Chrozophora oblongifolia*, *Ficus ingens*, *Lavandula dentata* and *Plectranthus barbatus* exhibited the greatest *in vitro* activity against *P. falciparum* (Al-Musayeib *et al.*, 2012). Besides, *Struchium parganophorum*, *Tithonia diversifolia*, *Premna angolensis* and *Morinda lucida* showed a promising *in vitro* and *in vivo* antiplasmodial activity (Madureira *et al.*, 2002).

In addition, *in vitro* antimalarial assay indicated *S. dagascariensis*, *C. glutinosum* and *T. bakis* were active against *P. falciparum* (Ouattaraa *et al.*, 2006). *In vivo* antimalarial activity showed that *A. maciverae* and *A. maritima* exhibited 73.0 and 63.0% parasitemia inhibition, respectively (Ene *et al.*, 2009). Likewise, *Ficus platyphylla* and *Acacia nilotica* showed markedly antimalarial activity against *P. berghei* in mice (Shittu *et al.*, 2011; Ali Jigam *et al.*, 2010). On the other hand aqueous leaf *Abrus precatorius* extract (25mg/ kg) is more effective than halofantrine (25mg/ kg) for acute malaria treatment (Saganuwan *et al.*, 2011).

In Ethiopia, *in vivo* antimalarial activity of *D. angustifolia* seed extracts significantly inhibited parasitemia and prevented packed cell volume reduction as showed by Mengiste *et al.* (2012). In

the study done by Deressa *et al.* (2010), crude extracts *Clerodendrum myricoides* and *Aloe debrana* of the plant showed strong activities against *P. berghei*. In study done on *in vivo* antimalarial activity of crude extracts of the leaves of *Adhathodas chimperiana* in Ethiopia, the hydro-alcoholic extracts of leaves of *A. schimperiana* significant reduced parasitaemia induced by chloroquine-sensitive *Plasmodium berghei* infection in suppressive, curative and prophylactic models in mice (Petros & Melaku, 2012). In addition, Dikasso *et al.* (2006) have reported the hydroalcoholic extracts from *Asparagus africanus* showed appreciable *in vivo* antimalarial activity against *Plasmodium berghei*.

Studies showed that secondary metabolites such as alkaloids (Cryptolepine, 2,7-dibromocryptolepine, isostrychnopentamine, ochrolifumamine, Strychnogucine B, Sungucine, Villalstonine A, Dihydrousambarensine, Macrocarpamine, Aspidocarpine, Nitidine, Skimmiamine, Aorborinine, Acronycine, Atallaphillinine, Normelicopicine) (Oliveira *et al.*, 2009), terpenoids (Nortopsentin A, Zambesiocolactone B, Turraeanin A, Aframodial, 16-oxolabda-8, 12(E)-dien- 15-oic acid, methyl-14,15-epoxylabda- 8, 12(E)-diene-16-oate, Ekeberin D4, Ekeberin D5, (+) *S*-nerolidol and turraeanin A (Amoa Onguéné *et al.*, 2013) and phenolic compounds (artoinin F, cycloartobiloxanthone, artocarpones A , artocarpones B, artoinin A, cycloheterophyllin, artoindonesianin R, heterophyllin, heteroflavanone C and artoindonesianin A-2, norcowanin, mangostin and tovophyllin A, chrobisiamone A, Gallagic acid and punicalagin) (Bero *et al.*, 2009; Monbrison *et al.*, 2006) detected and isolated from different plants are responsible for the antimalarial activities seen by medicinal plants.

Syzygium guineense is a medicinal plant common in Ethiopia. According to the ethnobotanical study done in southern Uganda (Segawa & Kasenene, 2007), D.R. Congo (Kasali *et al.*, 2014)

and Guinea (Traore *et al.*, 2013), decoctions of the stem bark of *Syzygium guineense* is traditionally used for treatment of malaria. This study was intended to evaluate the *in vivo* antimalarial activity of *S. guineense* since its antimalarial activity has not been reported yet.

1.7.5 Investigational antimalarial agents

The goal of developing new antimalarial drugs is to find a molecule that can target multiple stages of the parasite's life cycle, thus impacting prevention, treatment, and transmission of the disease. The 4(1*H*)-quinolone-3-diarylethers are highly active against the human malaria parasites *P. falciparum* and *P. vivax*. They target both the liver and blood stages of the parasite as well as the forms that are crucial for disease transmission, that is, the gametocytes, the zygote, the ookinete, and the oocyst (Nilsen *et al.*, 2013). Besides, the new antimalarial candidates (TCMDC29, GSK1057714, GNF156, KA609, MMV390048 and DSM265) exhibited a potent activity against blood stages of *P. falciparum* (Gamo, 2014).

A trioxolane, OZ439, now on clinical trial II, showed parasite killing activities similar to artemisinins, but with a longer half-life. OZ439 is capable of completely curing mice of malaria in a single dose of 20 mg/kg, a feat that artemisinin derivatives cannot achieve without the addition of a second drug. Furthermore, OZ439 has significant prophylactic activity, and a single 30 mg/kg oral dose of OZ439 administered 48 h prior to parasite inoculation is protective against malaria (Biamonte *et al.*, 2013; Wang *et al.*, 2013). The optimized 8, 8-dimethyl imidazolopiperazine analogues were also found to be more efficacious than the current antimalarials in a malaria mouse model (Nagle *et al.*, 2012).

Alkaloids such as benzophenanthridines (sanguinarine, chelerythrine), protoberberines (berberine) and protopines (protopine, allocryptopine) from aerial part of *Argemone mexicana* L.

(Papaveraceae) are also showed potent antimalarial activity. It is now on clinical trial I to investigate the pharmacokinetics, pharmacodynamics and tolerability of the decoction of the aerial parts of *A. mexicana*, administered in healthy volunteers (Rubio-Pina & Vazquez-Flota, 2013). A number of trials have also shown that Eurartesim® (dihydroartemisinin-piperaquine phosphate), an ACT, is highly effective in treating of uncomplicated *P. falciparum* malaria (Ubben & Poll, 2013).

In addition, Fosmidomycin-clindamycin (Borrmann *et al.*, 2004), Chlorproguanil-Dapsone-Artesunate (CDA) are also combinations that are shown to be effective in clinical studies against malaria parasites. Moreover, KAF156 showed a complete malaria protective activity as a single oral dose of 10 mg/kg in mice model. In addition, KAF156 displays potent Plasmodium transmission blocking activities both *in vitro* and *in vivo* (Kuhlen *et al.*, 2014).

1.8 The Experimental plant: *Syzygium guineense* (Willd.) DC.

Syzygium is a genus of flowering plants that belongs to the family Myrtaceae. Myrtaceae is a plant family widely used in folk medicine in different countries in which *Syzygium* is among its most important genera. The genus comprises about 1200 species, and has a native range that extends from Africa to Asia.

Members of the genus such as *Syzygium cumini* (Mohamed *et al.*, 2013; Thiengsusuk *et al.*, 2013; Gupta & Saxena, 2011; Kumar *et al.*, 2007), *Syzygium jambos* (Mohanty & Cock, 2010), *Syzygium cordatum* (Clarkson *et al.*, 2004), *Syzygium aromaticum* (Bagavan *et al.*, 2011) and *Syzygium fruticosum* (Islam *et al.*, 2013) have been shown to exhibit antimalarial, anti-diabetic, antibacterial, central nervous system depression antioxidant, anticancer properties. In addition, *Syzygium aromaticum*, *Syzygium cordatum* root and stem bark decoctions are used to treat of

malaria are traditionally used for treatment of malaria (Bhowmik *et al.*, 2012; Dahare & Jain, 2010).

Syzygium guineense (Willd.) DC. belongs to the family Myrtaceae and has synonyms including, among others *Calypttranthes guineense*, *Calypttranthes guineensis*, *Eugenia fourcadei*, *Eugenia guineensis*, *Syzygium fleuryi*, *Syzygium fourcadei* (Maroyi, 2008). *S. guineense* is commonly known as “water berry” in English, “Baddessa” in Afaan Oromo, “Dok’ma” in Amharic, “Ocha” in Gamo and “Karava” in Gurage. *S. guineense* is a medium-sized to large evergreen tree and usually grows up to 15–20m high. The bark is smooth and greyish-white to grey-brown in young trees, turning rough, flaky, creamy, light grey, dark brown or black in older trees. The leaf is broadly lanceolate, opposite, entire to the branch. The fruit is ellipsoid drupe, purplish in color and edible (Orwa *et al.*, 2009). *S. guineense* is native to Africa. It is found widely and abundantly in Ethiopia, Eritrea, Kenya, Lesotho, Mozambique, Namibia, Senegal, Somalia, South Africa, Swaziland, Tanzania, Uganda, Zambia, Botswana and Zimbabwe. The tree is used as a fire wood and shade in coffee plantations in Ethiopia. The plant is found in the altitude range 2,300 - 2,700 m. It has edible fruit with higher nutritional value (Maroyi, 2008).

Ethno-pharmacological studies revealed that *S. guineense* has a wide range of activities including spasmolytic, antidiarrheal, antibacterial, anti-Inflammatory and analgesic activities. The leave of *S. guineense* is also used as antiseptic against infection (Magassouba *et al.*, 2007). Antibacterial triterpenes (Arjunolic and Asiatic acids) isolated from *S. guineense* showed significant antibacterial activity against *Escherichia coli*, *Bacillus subtilis* and *Shigella sonnei* (Djoukeng *et al.*, 2005). Its dried twig, stem bark and fruits have also prominent spasmolytic, antitransit and against castor oil-induced antidiarrheal properties (Nigatu, 2004). In addition, the hydro-

alcoholic extract of the leaves of *S. guineense* is reported to have antihypertensive activity (Ayele *et al.*, 2010). Ethanolic extract of the leaves of *S. guineense* also has been shown to possess anti-inflammatory and analgesic properties (Ior *et al.*, 2012). In addition, the plant is endowed with compounds with immunomodulatory activities (Ghildyal *et al.*, 2010). The extract of stem bark of *S. guineense* also possesses strong antioxidant properties and act as free radical inhibitors or scavengers (Pieme *et al.*, 2014). The chemical composition of essential oil from *S. guineense* was also investigated. Caryophyllene oxide, δ -cadinene, viridiflorol, *epi*- α -cadinol, α -cadinol, *cis*-calamenen-10-ol, citronellyl pentanoate, β -caryophyllene and α -humulene are essential oils found abundantly in dried leaves of *S. guineense* (Noudogbessi *et al.*, 2008).



Figure 1: Photograph of *Syzygium guineense* (Willd.) DC.

Bark decoctions of *S. guineense* is used traditionally to treat stomachache, diarrhea and malaria in many counties. Stem bark of *Syzygium guineense* is used traditionally against HIV/AIDS opportunistic infections such as herpes zoster and chronic diarrhea in West Africa (Kisangau *et al.*, 2007). Root and stem bark of the plant also used to against internal parasite, stomachache, urinary problem in Ethiopia (Regassa, 2013; Amenu, 2007). *S. guineense* is also one of the medicinal plants utilized by traditional practitioners to control ectoparasites infection in livestock. Insecticidal activity study showed that *S. guineense* caused 47% mortality (Gemeda *et al.*, 2014).

On top of this, *S. guineense* is one of the African plant species that are traditionally used against malaria. Decoction of the stem bark and leaf of *S. guineense* is commonly used by traditional healers' in Ethiopia (Mesfin *et al.*, 2014), south Uganda (Segawa & Kasenene, 2007), D. R. Congo (Kasali *et al.*, 2014) and Guinea (Traore *et al.*, 2013) for treatment of malaria. Its antimalarial activity was not yet reported and as well no published evidence. This, traditional claims enforced to evaluate its *in vivo* antimalarial activity against *Plasmodium berghei* in mice model.

1.9 Rationale for the Study

The last decade has shown just how powerful our existing antimalarial drugs and tools are at saving lives; but still millions do not have access to them. The challenge now is to sustain and extend those gains to ensure that everyone at risk of malaria has access to prevention tools and first-line antimalarial drugs. Nonetheless, malaria continues as a leading public health problem and remains as a serious threat to humanity. This is due to the emergence of drug resistance (Smith Gueye *et al.*, 2014; Sá *et al.*, 2011), unaffordability of first-line antimalarial drugs, fragile health care facilities and absence of clinically proven vaccines (Mwangoka *et al.*, 2013); and poor efficacy of multi-target antimalarial chemotherapy (Marfurt *et al.*, 2010). Besides, the currently available indoor spraying with insecticides to reduce the transmission of malaria is hampered by insecticide resistance (Blayneh & Mohammed-Awel, 2014). Hence, if left unchecked it could threaten the incredible progress made during the past 13 years.

Indeed, Artemisinin resistance to a deadly *P. falciparum* now poses a threat to the control and elimination of malaria (Ashley *et al.*, 2014). Chloroquine resistant strains can be controlled by artemisinin and Artemisinin derivatives. But, currently there is no alternative approved

antimalarial drug to replace Artemisinin derivatives. Hence, it is extremely important to urgently intensify research in the development of new, cheap and effective antimalarial drugs. Medicinal plants remain the main focus by scientists and researcher as a noble source of lead compound in the search and development of new antimalarial agents. Screening of plants for antimalarial activity is of paramount importance to the effort made to develop new drugs. Hence, the present study attempted to evaluate *in vivo* malaria suppressive, curative and prophylactic activities of 80% methanol extract of stem bark of *S. guineense* on mice infected with chloroquine sensitive *P. berghei*.

2 OBJECTIVES

2.1 General Objective:

- To evaluate antimalarial activity of 80% methanol extract of the stem bark of *Syzygium guineense* against mice model of malaria

2.2 Specific Objectives:

- To evaluate acute oral toxicity of 80% methanol extract of the stem bark of *S. guineense*
- To evaluate antiplasmodial activity of 80% methanol extract of the stem bark of *S. guineense* in early infection using Peter's 4 day suppressive test,
- To evaluate antiplasmodial activity of 80% methanol extract of stem bark of *S. guineense* in established infection using curative/Rane's test,
- To evaluate the prophylactic activity of 80% methanol extract of the stem bark of *S. guineense* using peter's repository test, and
- To determine phytochemical constituents of 80% methanol extract of the stem bark of *S. guineense*

3 MATERIAL AND METHODS

3.1 Drug and chemicals

Chloroquine phosphate tablet and Distilled water (Ethiopian pharmaceutical manufacturer, Ethiopia), Normal saline (Fresenius kabi, India), Trisodium citrate (BDH chemicals Ltd poole, England), Tween 80 (Lobe chemi, India), Absolute methanol (Lobe chemi, India), Geimsa stain (BDH laboratory supplies Poole Gurr®, England), Dimethyl sulphoxide (UNI-CHEM chemical reagents, China), Dragendorff's reagent, Mayer reagent, concentrated sulfuric acid (Fisher Scientific, UK), Glacial acetic acid (Lobe chemi, India), microscope immersion oil (Neolab life science co, India), 5% ethanolic ferric chloride, 10% ethanolic ferric chloride, dilute ammonia, 1% aqueous hydrochloric acid and Chloroform (Finkem Laboratory Reagent, India) obtained from the respective vendors were used.

3.2 Plant material

Fresh stem bark of *S. guineense* (Figure 1) was collected from abay-chomen district of Oromia regional state, 310 km from Addis Ababa in May, 2014. The fresh stem bark was kept in plastic bag during transportation. The plant was identified as *S. guineense* by a taxonomist and a voucher specimen was deposited (No.GZ002) at the National Herbarium, College of Natural Sciences, Addis Ababa University for future reference.

3.3 Experimental Animals

Healthy male Swiss Albino mice (8-12 weeks, weighing 25-33 grams) bred and maintained at the Ethiopian public Health Institute was used. The animals were housed and maintained in a cage at room temperature with 12:12 hour light-dark cycle in the animal house facilities at School of Pharmacy, AAU. All mice were allowed to acclimatize for one week before commencement of the study and had free access to pellet diet and water *ad-libitum*. All

experiments were conducted in accordance with the internationally accepted guidelines on laboratory animal use, care and handling (National Research Council, 2011).

3.4 Preparation of crude extract

The stem bark of *Syzygium guineense* was sorted and cleaned to eliminate any dead matter or other unwanted particles and air dried under shade at room temperature for 14 days. The stem bark was pulverized to coarse powder using a grinding mill. Three hundred gram of air-dried and powdered stem bark of *S. guineense* (150 gram of dried stem bark in 600 ml of 80% methanol) was added in an Erlenmeyer flask and extracted by cold maceration technique on orbital shaker (Bibby scientific limited stone Staffordshire, SI150SA, UK) at 130 rotations per minute (rpm). After 72 h, the extract was filtered by Whatman filter paper No.1 (Schleicher & Schuell micro science GmbH, Germany). The residue was re-macerated for another 72 hours twice and methanol from the combined filtrates was removed under reduced pressure by rotary evaporator (Buchi labortechnik AG, Switzerland) at 45 rpm and 40°C to obtain the crude extract. The extract was further concentrated to dryness with a lyophilizer (Operan, Korea vacuum limited, Korea) at -40°C and vacuum pressure (200 mBar). A total of 26.6 grams (percentage yield of 17.4%) of dried extract was obtained and stored in a refrigerator at -4°C in air tight plastic containers until used.

3.5 Acute toxicity test

Acute oral toxicity of 80% methanol extract of the stem bark of *S. guineense* was evaluated in female mice aged of 6-8 weeks according to OECD guideline No 425 (OECD, 2008). Five female mice were fasted for three hours and orally administered with a limit dose of 2000 mg/kg

of the extract. The mice were observed for lacrimation, hair erection, and reduction in their motor and feeding activities for three hours and followed for 24 hours and/or 14 days.

3.6 Grouping and dosing of animals

Pilot study was done, based on acute toxicity, with 50, 100, 200, and 400 mg/kg/day of 80% methanol extract of stem bark of *S. guineense* on early *P. berghei* infection using three mice per group. Results from the present preliminary work revealed that the extract had no antiparasmodial activity at 50mg/kg body weight of the extract. However, the extract was effective in suppressing parasitemia at the remaining doses (100, 200 and 400mg/kg). Therefore, from this preliminary work the doses 100, 200 and 400mg/kg/day of the extract were selected for the *in vivo* antimalarial study.

Accordingly, forty mice were randomly assigned into five groups (three treatment groups and two controls), eight mice per group. The negative control group was treated with the vehicle 1% dimethyl sulphoxide used as a reconstitution solvent of the extract, whereas positive control group was treated with the standard drug chloroquine 25 mg/kg/day (CQ25). The other groups received three different doses 100mg/kg/day (SG100), 200mg/kg/day (SG200) and 400mg/kg/day (SG400) of the plant extract. Dose selection was made based on safety and preliminary studies as described above. The doses were administered orally at a volume of 10 ml/kg.

3.7 Parasite inoculation

Chloroquine sensitive *P. berghei* ANKA strain obtained from Ethiopian Public Health Institute and maintained at animal house facilities of School of Pharmacy, AAU was used. The parasite was maintained by serial passage of blood from infected mice to non-infected ones on weekly

basis. A donor mouse infected with chloroquine sensitive *P. berghei* with a rising parasitemia of 20-30% was used for infecting mice for *in vivo* malaria suppressive, curative and prophylactic activities studies of the plant extract. After the parasitemia level of the donor mice was determined, the mice were sacrificed and total blood was collected in a petri-dish containing 2% trisodium citrate as anticoagulant. The blood was then diluted with 0.9% normal saline. Accordingly, each 0.2 ml of the aliquot would contain about 1×10^7 *P. berghei* infected RBCs and 1ml of the inoculums consisted of 5×10^7 *P. berghei* infected erythrocytes. Each mouse used in the experiment was inoculated with 0.2 ml of the aliquot intraperitoneally (Fidock *et al.*, 2004).

3.8. Activity testing

3.8.1 Chemo-suppressive test

In vivo antiplasmodial activity of the plant extract against early *P. berghei* infection was carried out according to the method described by Peter *et al.* (1975). Mice were inoculated on the first day (Day 0), intraperitoneally, with 0.2ml of infected blood containing about 1×10^7 *P. berghei* parasitized erythrocytes. The infected mice were randomly grouped as described earlier and the mice were treated accordingly after three hours of infection and continued for three days. Weight and rectal temperature were recorded for each mouse just before infection and daily throughout the experiment, while parasitaemia and packed cell volume (PCV) were recorded just before infection and on day four. On day four (96 hours after infection), a thin blood film was prepared from the tail blood of each mouse. The blood films were fixed with methanol and stained with 10% Giemsa for 15min and parasitaemia was examined microscopically to determine parasitemia level and percent parasite suppression. Moreover, each mouse was observed daily for determination of survival time.

3.8.2 Curative Test

In vivo antiparasmodial activity of the plant extract against established *P. berghei* infection was carried out using a method of Ryley and Peter (1970). Mice were inoculated with parasitized erythrocytes on the first day (Day 0). Seventy-two hours later (on day three), the mice were randomly grouped and treated (see section 3.6) after seventy-two hours of infection (day three) and then continued for four consecutive days from day three up to day six once daily. Parameters including parasitaemia level, rectal temperature (using digital rectal thermometer) and body weight were measured daily starting from day three (before treatment and also after treatment) till day seven. PCV were recorded on day three and day seven. Finally, survival time of the experimental mice in each treatment group were followed and determined.

3.8.3 Repository test

The prophylactic activity of the crude extracts was tested using the residual infection procedure as described by Peter's in 1965. Mice were grouped and treated as described previously for four days (Day 0-Day 3). On day four (on 96 hours after Day 0), all mice were inoculated intraperitoneally with 0.2ml of infected blood containing about 1×10^7 *P. berghei* parasitized erythrocytes and followed for 72 h (168 h from Day 0). Weight and rectal temperature were recorded for each mouse just before inoculation (96 h after Day 0) and throughout the experiment. However, PCV was recorded just before inoculation and at the end of the experiment (168 h from Day 0). In addition, parasitemia level was recorded on 168 h from Day 0) and all mice were followed for their survival time.

3.8.4 Determination of packed cell volume

Blood was drawn from the tail of the different group of mice using heparinized capillary tubes. The tubes were filled with blood up to $\frac{3}{4}$ th of their volume and sealed at the dry end with sealing clay. The tubes were then placed in hematocrit centrifuge (Centurion scientific, UK) with the sealed end outwards and centrifuged for 5 min at 11,000 rpm. PCV was measured before inoculating the parasite and after treatment as the proportion of RBCs to plasma using Wintrobe method described by Gilmour and Sykes (1951).

Packed Cell Volume = $100 \times (\text{Volume of erythrocytes in a given volume of blood}) / \text{Total blood volume}$

3.8.5 Parasitemia measurement

Thin smears of blood were made from the tail of each mouse. The smears were applied on microscope slides (76 × 26 mm), fixed with absolute methanol for 15 min and stained with 10% Geimsa stain at pH 7.2 for 15 min. The slides were taken out, washed with gentle passage of tap water and dried at room temperature. Two stained slides for each mouse were examined under light microscope (Olympus N-120 A, Philippines) with an oil immersion (Neolab life science co, India) nosepiece of 100 × magnifications. Five different fields on each slide were examined and average was taken and percentage parasitemia and parasite suppression were determined by using the formulas described below (Fidock *et al.*, 2004; Sanni *et al.*, 2002).

% Parasitemia = $\frac{\text{Number of parasitized RBC}}{\text{Total number of RBC}} \times 100$

Total number of RBC

% suppression = $\frac{\text{Mean parasitemia of negative control} - \text{mean parasitemia of treated group}}{\text{Mean parasitemia of negative control}} \times 100$

Mean parasitemia of negative control

3.9 Phytochemical Screening Test

Eighty percent methanolic extract of stem bark of *S. guineense* was screened for the presence of secondary metabolites to relate the antimalarial activity of the plant with the presence or absence of these constituents. Thus, tests for alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, anthraquinones and cardiac glycosides were performed using standard test procedures.

Test for Alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's and/or Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids (Yadav & Agarwala, 2011).

Test for Terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H₂SO₄ was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids (Yadav & Agarwala, 2011).

Test for Flavonoids

5ml of dilute NH₃ solution was added to 3ml aqueous filtrate of the sample followed by addition of 2ml concentrated H₂SO₄ and formation of yellow color indicates presence of flavonoids (Ayoola *et al*, 2008).

Test for Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration (Ayoola *et al*, 2008).

Test for saponins

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins (Yadav & Agarwala, 2011).

Test for phenols

The Solvent plant extract was treated with few drops of neutral ferric chloride solution 5%, intense colour developed indicates the presence of phenols (Yadav & Agarwala, 2011).

Test for anthraquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes (Ayoola *et al*, 2008).

Test for cardiac glycosides

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer (Ayoola *et al*, 2008).

4 Statistical analysis

Results of the study are presented as a mean plus or minus standard error of mean ($M \pm SEM$). Statistical significance was determined by one way analysis of variance (ANOVA) with multiple comparison tests (Tukey's test) to compare the measured parameters (parasitemia suppression, weight, rectal temperature and survival time) within and between groups using computer software spss version 16.0. In addition, two way repeated measures ANOVA was used to compare parasitemia level across time and between groups in curative test. The analysis was performed with 95% confidence interval and P-values less than 0.05 was considered to be statistically significant.

5 Results

5.1 Acute toxicity

The acute toxicity study indicated that eighty percent methanol extract of the stem bark of *S. guineense* caused no mortality at 2000mg/kg dose within the first 24h as well as on the following 14 days. Physical and behavioral observations of the experimental mice also revealed no visible signs of overt toxicity like lacrimation, loss of appetite, tremors, hair erection, salivation and diarrhea. The present study suggested that the oral median lethal dose (LD₅₀) of the extract could be greater than 2000 mg/kg body weight of the extract. The experimental determination of lack of acute toxicity at 2000 mg/kg body weight of the extract would justify the use of 80% methanol extract of the stem bark of *S. guineense* for malaria treatment.

5.2 Effect on early malaria infection

The results of the four-day suppressive test indicated that the extract had a considerable antiparasmodial activity against chloroquine sensitive *P. berghei* on early infection. The highest parasite suppression by the extract was observed at SG400, but mild suppression was seen at SG200 and SG100 (Table 1). At all dose levels evaluated, the extract caused a statistically significant ($p<0.001$) parasite reduction as compared to negative control in a dose dependent manner. When compared among the extract treated groups, SG400 ($p<0.001$) and SG200 ($p<0.001$) showed a statistically significant difference in reducing parasite level relative to SG100.

As regards to survival days, SG400 ($p<0.001$) and SG200 ($p<0.05$) of the extract showed a statistically significant prolongation of survival time when compared to negative control. When

compared among themselves, SG400 showed a significant ($p<0.001$) prolongation of survival time when compared to SG100 (Table 1).

Table 1: Parasitemia and survival time of *Plasmodium berghei* infected mice treated with 80% methanol extract of the stem bark of *S. guineense* in early infection

Treatment	% Parasitaemia	% suppression	Survival time(days)
Negative control	48.76 $\pm$ 4.36	0.00	6.87 $\pm$ 0.64
CQ25	0.00	100 ^{a3}	30.00 $\pm$ 0.00 ^{a3}
SG100	43.3 $\pm$ 3.45	11.20 ^{a2, b3, d3, e2}	7.25 $\pm$ 0.71 ^{b3, e2}
SG200	32.16 $\pm$ 2.22	34.04 ^{a3, b3, c3, e2}	8.00 $\pm$ 0.53 ^{a1, b3}
SG400	26.79 $\pm$ 1.5	45.05 ^{b3, c3, d2}	8.62 $\pm$ 1.30 ^{a3, b3}

Data are expressed as mean $\pm$ SEM; n = 8; a = compared to negative control(vehicle; 1% dimethyl sulphoxide), b = compared to chloroquine 25 mg/kg/day, c = compared to 100 mg/kg/day of the extract, d =compared to 200 mg/kg/day of the extract, e = compared to 400 mg/kg/day of the extract; 1= $p< 0.05$; 2= $p< 0.01$; 3= $p< 0.001$; CQ = chloroquine, SG = 80% methanol extract of stem bark of *Syzygium guineense*, numbers refer to dose in mg/kg/day.

The effect of the extract on body temperature of *P. berghei* infected mice is presented in Table 2. SG200 and SG400 considerably attenuated the reduction in body temperature compared to negative control. Analysis of percent body temperature change, between day 0 and 4, indicated that *P. berghei* infected mice treated with SG200 ($p<0.01$) and SG400 ($p<0.001$) showed a statistically significant difference when compared to negative control. However, only those *P. berghei* infected mice treated with SG400 ($p<0.05$) showed a statistically significant attenuation of body temperature reduction when compared to those treated with SG100 on early infection.

Percent PCV change analysis, between days 0 and 4, revealed that *P. berghei* infected mice treated with SG200 ($p<0.01$) and SG400 ($p<0.001$) showed a statistically significant difference when compared to negative control (Table 2). In addition, this change was significantly smaller ($p<0.01$) in SG400 when compared to SG100. On the other hand, the PCV change in SG400 and SG200 mice was not statistically significant to that observed with CQ25. Accordingly, the PCV protection effect observed by the extract was comparable with the standard drug.

Analysis of percent change in body weight, between days 0 and 4, revealed that SG400 ($p<0.01$) and CQ25 ($p<0.001$) showed significantly greater protection against parasite induced body weight reduction when compared to negative control. However, this change was significantly ($p<0.001$) greater when compared with CQ25 (Table 2).

Table 2: Temperature, weight and packed cell volume of *Plasmodium berghei* infected mice treated with 80% methanol extract of the stem bark of *Syzygium guineense* in early infection

Treatment	Temperature (°C)			Weight (gram)			Packed cell volume (%)		
	Day 0	Day 4	% change	Day 0	Day 4	% change	Day 0	Day 4	% change
Negative control	37.26±0.18	35.95±0.2	-3.65	28.85±1.37	26.92±1.19	-7.14	58.45±1.44	47.04±3.3	-24.81
CQ25	37.25±0.15	37.39±0.15	0.37 ^{a3}	26.3±2.6	27.45±2.6	4.13 ^{a3}	55.8±2.38	54.09±3.26	-3.43 ^{a3}
SG100	37.10±0.24	36.00±0.41	-3.06 ^{b3}	32.16±1.64	31.15±2.46	-3.49 ^{b3}	57.95±2.87	48.69±5.23	-20.27 ^{e2, b2}
SG200	37.06±0.09	36.19±0.3	-2.42 ^{a2, b3}	28.03±2.04	27.02±2.14	-3.80 ^{b3}	57.85±2.73	53.1±2.4	-8.86 ^{a2}
SG400	37.06±0.12	36.31±0.14	-2.07 ^{a3, b3, c1}	28.05±1.41	27.55±1.26	-1.81 ^{a1, b3}	56.22±2.24	53.37±1.86	-5.38 ^{a3, c2}

Data are expressed as mean ± SEM; n = 8; a = compared to negative control (vehicle; 1% dimethyl sulphoxide), b = compared to chloroquine 25 mg/kg, c = compared to compared to 100 mg/kg/day of the extract, d = compared to 200 mg/kg/day of the extract, e = compared to compared to 400 mg/kg/day of the extract; 1p< 0.05; 2p< 0.01; 3p< 0.001; Day 0 = pre-treatment value on day 0, Day 4 = post-treatment value on day four, CQ = chloroquine, SG, 80% methanol extract of stem bark of *S. guineense*, numbers refer to dose in mg/kg/day.

5.3 Effect on established malaria infection

In the curative test, a daily increase in parasitaemia was observed in all treatment groups except in CQ25 treated once (Figure 2). Two way repeated measures ANOVA analysis of parasitemia across days of treatment showed a statistically significant ($p<0.001$) difference in parasitemia level between the three doses of the extract and control groups. The average percentage parasite suppression of the extract treated groups on day seven was 16.47%, 19.41% and 36.27% in mice treated with SG100, SG200 and SG400, respectively (Table 3). But, CQ25 completely eradicated the parasite to undetectable level. In addition, SG400 ($p<0.001$) and CQ25 ($p<0.001$) prolonged survival time of *P. berghei* infected mice significantly when compared negative control.

Table 3: Parasitemia and survival time of *Plasmodium berghei* infected mice treated with of 80% methanol extract of the stem bark of *S. guineense* from in established infection

Treatment	% Parasitemia	% suppression	Survival time (days)
Negative control	73.47±5.23	0.00	6.62±0.52
CQ25	0.00±0.00	100	30±0.00
SG100	61.37±6.88	16.47 ^{a3, b3, e3}	7.5±0.93
SG200	59.21±4.84	19.41 ^{a3, b3, d3}	7.00±0.53
SG400	46.82±5.02	36.27 ^{a3, b3, c3, d3}	8.37±0.92 ^{a3, b3}

Data are expressed as mean ± SEM; n = 8; a = compared to negative control (vehicle; 1% dimethyl sulphoxide), b = compared to chloroquine 25 mg/kg/day, c = compared to 100 mg/kg/day of the extract, d = compared to 200 mg/kg/day of the extract, e = compared to 400 mg/kg/day of the extract; 1= $p<0.05$; 2= $p<0.01$; 3= $p<0.001$; CQ = chloroquine, SG, 80% methanol extract of stem bark of *S. guineense*, numbers refer to dose in mg/kg/day.

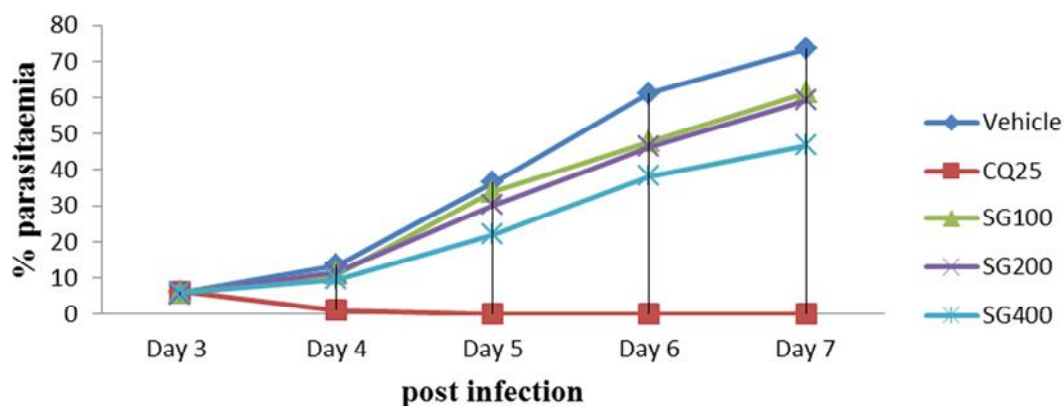


Figure 2: Parasitemia level of *P. berghei* infected mice treated with of 80% methanol extract of the stem bark of *S. guineense* from day three to day seven in established infection. Data are mean $\pm$ SEM; n=8; CQ = chloroquine, SG, 80% methanol extract of stem bark of *S. guineense*, numbers refer to dose in mg/kg/day

The effect of the extract on body temperature of *P. berghei* infected mice is presented in Table 4. Body temperature change analysis, between days 3 and 7, indicated that SG400 ($p<0.01$) and SG200 ($p<0.05$) of the extract caused a significant attenuation of the reduction in body temperature when compared to negative control. This change was significantly ($p<0.001$) different when compared to CQ25. However, CQ25 completely prevented the mice from body temperature reduction.

The extract did not significantly ameliorated PCV reduction on established infection compared to negative control except SG400 ($p<0.05$). By contrast, a considerable protection was noted with CQ25 and SG400 with no significant difference to each other.

Analysis of percent change in body weight, between days 3 and 7, showed the extract had not significantly protected the mice from parasite induced body weight reduction compared to negative control (Table 4). However, CQ25 ($p<0.01$) caused a statistically significant protection of body weight loss when compared to negative control.

Table 4: Rectal temperature, weight and packed cell volume of *Plasmodium berghei* infected mice before and after treatment with 80% methanol extract of the stem bark of *S. guineense* in established infection (curative / Rane's test)

Treatment	Temperature (^o c)			Weight (gram)			Packed cell volume (%)		
	Day 3	Day 7	% change	Day 3	Day 7	% change	Day 3	Day 7	% change
Negative control	37.21±0.25	32.35±0.37	-15.05	29.05±1.11	25.84±1.38	-12.5	53.42±2.54	37.29±3.39	-44.35
CQ25	37.19±0.17	37.35±0.15	0.43 ^{a3}	27.42±1.52	30.16±2.04	9.00 ^{a3}	53.93±1.33	52.3±1.75	-3.22 ^{a3}
SG100	37.11±0.15	32.82±0.43	-13.08 ^{b3}	28.83±1.52	25.03±1.64	-15.35 ^{b3}	53.31±2.55	38.36±5.45	-42.1 ^{b3}
SG200	37.14±0.21	33.05±0.5	-12.38 ^{a1, b3}	28.75±2.54	25.95±2.5	-10.90 ^{b3}	52.27±1.49	40.29±7.09	-32.95 ^{b1}
SG400	37.19±0.24	33.11±0.59	-12.34 ^{a2, b3}	28.16±2.45	25.41±2.21	-10.83 ^{b3}	54.11±2.43	46.5±3.49	-16.78 ^{a1}

Data are expressed as mean ± SEM; n = 8; a = compared to negative control (vehicle; 1% dimethyl sulphoxide), b = compared to chloroquine 25 mg/kg/day, c = compared to 100 mg/kg/day of the extract, d = compared to 200 mg/kg/day of the extract, e = compared to 400 mg/kg/day of the extract; 1= p< 0.05; 2= p< 0.01; 3= p< 0.001; Day 3= pre-treatment value on day three after infection, Day 7 = post-treatment value on day seven; CQ = chloroquine, SG, 80% methanol extract of stem bark of *S. guineense*, numbers refer to dose in mg/kg/day

5.4 Prophylactic Effect

The prophylactic test result indicated that, the extract had a magnificent prophylactic activity against chloroquine sensitive *P. berghei* (Table 5). The extract had prophylactic parasite suppression of 26.91%, 37.13% and 58.24% in mice pre-treated with SG100, SG200 and SG400, respectively. CQ25 suppressed the parasite by 91.15%. However, neither the extract nor CQ25 prevented the infection. At the three dose levels evaluated, the extract showed statistically significant ($p<0.001$) difference in reducing parasite level when compared to negative control. Besides, SG400 ($p<0.001$) and SG200 ($p<0.01$) dose levels showed a statistically significant difference in parasitemia reduction as compared to SG100. The parasite reduction showed by SG400 was also statistically significant ($p<0.01$) when with compared to SG200.

In addition, survival time of *P. berghei* infected mice pre-treated with the extract on prophylactic study was also recorded (Table 5). Those mice pre-treated with SG400 (9.0 ± 1.41 days, $p<0.01$) and SG200 (8.87 ± 1.55 days, $p<0.01$) showed statistically significant difference in prolongation of survival time when compared to negative control (6.5 ± 0.53 days). When compared among themselves, those mice pre-treated with SG400 ($p<0.001$) showed a significant prolongation of survival time as compared to SG100 (7.25 ± 0.71 days). Likewise, SG400 and SG200 had a comparable effect to CQ25 in prolongation of survival time with no significant difference unlike SG100 (7.37 ± 1.06 , $p<0.01$).

Table 5: Parasitemia and survival time of *Plasmodium berghei* infected mice pre-treated with 80% methanol extract of the stem bark of *S. guineense* in prophylactic test

Treatment	% Parasitaemia	% suppression	Survival time (days)
Negative control	34.58±1.54	0.00	6.5±0.53
CQ25	3.06±0.9	91.15 ^{a3}	9.62±1.93
SG100	25.27±2.38	26.91 ^{a3, b3, e3}	7.37±1.06 ^{b2}
SG200	21.74±3.15	37.13 ^{a3, b3, e2}	8.87±1.55 ^{a2}
SG400	14.44±4.04	58.24 ^{a3, b3, c3, d2}	9.00±1.41 ^{a2}

Data are expressed as mean ± SEM; n = 8; a = compared to negative control (vehicle; 1% dimethyl sulphoxide), b = compared to chloroquine 25 mg/kg/day, c = compared to 100 mg/kg/day of the extract, d = compared to 200 mg/kg/day of the extract, e = compared to 400 mg/kg/day of the extract; 1= p< 0.05; 2= p< 0.01; 3= p< 0.001; CQ = chloroquine, SG, 80% methanol extract of stem bark of *S. guineense*, numbers refer to dose in mg/kg/day.

The effect of 80% methanol extract of the stem bark of *S. guineense* on rectal temperature in prophylactic study was also observed (Table 6). Rectal temperature change analysis, between days 0 and 3, indicated that SG400 (-0.07%, P <0.001) and SG200 (-0.13%, P <0.01) of the extract had a significant and dose-dependent attenuation of the reduction in temperature compared to negative control. Both SG400 and SG200 had a comparable effect to CQ25 with no significant difference, while SG100 (-0.75%, p<0.001) showed statistically significant difference compared to CQ25. However, only those mice pre-treated with SG400 (p<0.05) significantly lessened the reduction in rectal temperature as compared to those pre-treated with SG100.

In addition, SG400 of the extract and the standard drug CQ25 protected the mice from PCV reduction (Figure 5). PCV change analysis, between days 0 and 3, revealed that SG400

($P < 0.001$) and CQ25 ($P < 0.001$) showed a statistically significant difference as compared to negative control. However, the standard drug (CQ25) and the extract (SG400) treated groups

The extract had protected the mice from parasite induced body weight reduction compared to negative control (Table 6). Analysis of percent change in body weight, between days 0 and 3, revealed that SG400 ($p < 0.01$) and SG200 (-0.13%, $p < 0.05$) showed a statistically significant difference as compared to negative control.

Table 6: Temperature, weight and packed cell volume of *Plasmodium berghei* infected mice pre-treated with 80% methanol extract of the stem bark of *S. guineense* in prophylactic test

Treatment	Temperature (^o c)			Weight (gram)			Packed cell volume (%)		
	Day 0	Day 3	% change	Day 0	Day 3	% change	Day 0	Day 3	% change
Negative control	37.11±0.14	36.76±0.13	-0.95	28.72±1.28	28.37±1.3	-1.24	56.03±1.47	51.16±1.6	-9.56
CQ25	37.14±0.14	37.21±0.12	0.2 ^{a3}	29.74±1.15	31.34±1.53	5.04 ^{a3}	56.07±1.96	55.39±2.32	-1.26 ^{b2, a3}
SG100	37.12±0.07	36.85±0.27	-0.75 ^{e1, b2}	30.3±1.23	30.31±1.15	0.00	56.24±3.81	53.18±3.68	-6.13
SG200	37.12±0.15	37.07±0.17	-0.13 ^{a2}	31.96±2.56	32.52±2.41	1.76 ^{a1, b2}	56.17±3.96	54.22±4.13	-3.64
SG400	37.15±0.11	37.12±0.09	-0.07 ^{a3}	32.42±1.41	33.04±1.76	1.8 ^{a2, b2}	55.38±1.16	54.39±1.06	-1.82 ^{a1}

Data are expressed as mean ± SEM; n = 8; a = compared to negative control (vehicle; 1% dimethyl sulphoxide), b = compared to chloroquine 25 mg/kg, c = compared to 100 mg/kg/day of the extract, d = compared to 200 mg/kg/day of the extract, e = compared to 400 mg/kg/day of the extract; 1= p< 0.05; 2= p< 0.01; 3= p< 0.001; Day 0 = pre-infection value on day 0, Day 3 = post-infection value on day three, CQ = chloroquine, SG, 80% methanol extract of stem bark of *S. guineense*, numbers refer to dose in mg/kg/day.

5.5 Phytochemical screening

The results of the phytochemical analysis of 80% methanol extract of stem bark of *S.guineense* showed the presence of some secondary metabolites (Table 7).

Table 7: Phytochemical screening results for 80% methanol extract of the stem bark of *S. guineense*

Investigated class of compounds	Test results
Alkaloids	+
Anthraquinones	+
Cardiac glycosides	-
Flavonoids	+
Phenols	+
Saponins	+
Tannins	+
Terpenoids	+

Note: (+) indicates the presence and (-) indicates absence of particular metabolites

6 Discussion

Malaria is one of the world's most deadly infectious diseases. Spread and emergence of resistance to front line antimalarial drugs including artemisinin is the major challenge that endanger all the recent gains in malaria control and have major implications for public health (Ashley *et al.*, 2014; Mubjer *et al.*, 2011; Marfurt *et al.*, 2010). The scientific community is combating this problem by searching and screening for new, cheap and effective antimalarial agents from medicinal plants and other sources (Gamo, 2014). The present study attempted to investigate the *in vivo* antimalarial activity of 80% methanol extract of the stem bark of *S. guineense* using four-day suppressive (early infection), curative (established infection) and prophylactic models in mice infected with chloroquine sensitive *P. berghei* ANKA strain. *In vivo* evaluations of antimalarial activity begin with the use of the rodent malaria parasites *P. berghei*, *P. yoelli*, *P. chabaudi* and *P. vinckei*. *In vivo* studies take into account any prodrug effect or the role of the immune system in controlling malaria infection unlike *in vitro* ones. The most widely used model for initial drug evaluation is the *P. berghei*-infected mouse model. Accordingly, the rodent model of malaria has been employed in the present study. Even though, the rodent malaria model, *P. berghei*, is not exactly similar to that of the human *Plasmodium* parasites, it is the first step to screen most of the *in vivo* antimalarial activities of compounds (Fidock *et al.*, 2004).

The observation that no death with an oral dose of 2000mg/kg body weight of the extract could indicate that the extract is safe for use. This was consistent with previous toxicity study done on the stem bark of the plant by Nigatu (2004).

In the four-day suppression test, the parasite suppression exhibited by the extract was comparable with similar studies done on *A. debrana* (Deressa *et al.*, 2010); *Asparagus africanus*

(Dikasso *et al.*, 2006); *D. Angustifolia* (Mengiste *et al.*, 2012); *Toddalia asiatica* (Naghbi *et al.*, 2013); *Parkia biglobosa* (Builders *et al.*, 2011); and *Lophira alata* (Falade *et al.*, 2014). The extract also prolonged the mean survival time of *P. berghei* infected mice on early infection. Particularly, the higher two doses (SG200 and SG400) of the extract prolonged the mean survival time of mice significantly ($p<0.05$) when compared to negative control. The survival time prolongation effect could be directly associated with the low parasite level in the extract treated groups. This observation is in agreement with similar studies done on *Croton macrostachys* (Bantie *et al.*, 2014); *D. Angustifolia* (Mengiste *et al.*, 2012); *C. aurea* (Eyasu *et al.*, 2013); *A. africanus* (Yared *et al.*, 2012); and *N. sativa* (Abdulelah & Zainal, 2007).

In the curative test, the extract had shown a significant ($p<0.001$) parasite suppression with maximum of 36.27% at SG400 which is in agreement with the previous study done on methanol extract of the stem bark of *Anthocleista grandiflora* (Odeghe *et al.*, 2012). Even though the extract failed to cure the infection, it caused a progressive reduction in parasitaemia level compared to negative control starting from the third dose. The extract exhibited a low schizontocidal activity on established infection. This observation suggests that the crude plant extract appears to have better plasmodistatic than plasmodicidal effects in which its bioactive components may require initial conversions that would provide time lag for parasite proliferation. As a result, a loading dose might be needed to induce better curative capability.

Likewise, the extract showed significant ($p<0.001$) prophylactic activity against *P. berghei* with a parasite suppression of 26.91%, 37.13% and 58.24% by SG100, SG200, SG400, respectively, whereas CQ25 caused 91.15% parasite suppression when compared to negative control. Furthermore, SG200, SG400 prolonged the mean survival time comparably with the standard.

This revealed that the extract possesses a better blood schizontocidal activity in the prophylactic test. The parasite suppression exhibited by the extract in this test was greater relatively than the four-day suppression effect. Previous study (Ghildyal *et al.*, 2010) revealed that the plant was also endowed with compounds having immunomodulatory activities. The extract immunomodulatory property might have contributed for the superior antiparasmodial activity seen in this test. On other hand, prodrug effects of the extract metabolites might have also accounted for the observed effect. The extract parasite suppression effect demonstrated in the present malaria prophylactic study was in line with previous reports on *P. thoningii* (Madara *et al.*, 2010); *Alstonia booni* (Iyiola *et al.*, 2011); *Ajuga bracteosa* (Chandel & Bagai, 2010). In addition, the antiparasitic effect displayed by the stem bark of *S. guineense* in the present study coupled with the reported *in vitro* antiparasmodial activity of *Syzygium cordatum* (Clarkson *et al.*, 2004) and *Syzygium aromaticum* (Bagavan *et al.*, 2011) suggests that plants that belong to the genus could be endowed with antiparasmodial activity.

Fever is one of the key manifestations of human malaria. However, *P. berghei* infected mouse model of malaria was associated with hypothermia rather than pyrexia. The mechanism(s) underlying the fall in temperature may include a general debilitating effect of the infection, such as anaemia, on heat production and/or heat conservation in small animals. This is associated with an increased turnover of serotonin in the brain (Dascombe & Sidara, 1994). Generally, mice body temperature declines as the parasitaemia level increases. Extract containing active constituents should prevent the rapid falling of body temperature. In the present study, the extract (SG200, SG400) prevented the mice from body temperature reduction significantly compared to negative control in all the tests. This protection could be directly linked to the significant parasite suppression effect of the extract at the administered dose. In addition to this, the body

temperature shielding effect was comparable with the standard with no significant difference. This reveals that the active phytochemical constituents responsible for the observed effect were likely found in the administered dose of the extract in sufficient concentration. Although SG100 caused significant parasite suppression, this effect was not sufficient enough to significantly protect the mice from body temperature reduction when compared with the control and the standard. This might be due to the presence of active constituents in trace amount in the administered dose of the extract.

After malaria infection the host (human or mouse) suffer from anaemia (Chinchilla *et al.*, 1998). The underlying cause of anemia includes; loss of infected erythrocytes through parasite maturation, destruction of uninfected red blood cells in the spleen and liver by macrophages activation and/or enhanced phagocytosis, reduced erythropoiesis and dyserythropoiesis (Lamikanra *et al.*, 2007). Each of these mechanisms has been implicated in both human and mouse malarial anemia. This necessitates hematocrit PCV analysis to evaluate the effectiveness of the extract in preventing hemolysis caused by the parasite. In the present study, the extract (SG200 and SG400) significantly protected PCV reduction when compared to negative control. On top of this, PCV protection effect of the extract was also comparable to CQ25 with no significant difference. This observation was in line with similar studies done on *D. Angustifolia* (Mengiste *et al.*, 2012); *Croton macrostachys* (Bantie *et al.*, 2014). This protection effect could be as a result of the significant parasite suppression effect induced by active constituents in the administered doses of the extract. However, the low dose of the extract was not effective in ameliorating PCV reduction in all the tests. This show that the extract parasite suppression effect was not sufficient enough to prevent/delay RBC hemolysis that could be linked to the small concentration active constituents in the administered dose.

Furthermore, an ideal antimalarial agents should prevent body weight loss resulted from a rise in parasitemia. A significant body weight loss attenuation effect was observed at highest tested (SG400) dose level of the extract when compared to negative control on early infection. However, SG100 and SG200 not significantly protected the animals from body weight loss. This showed that the significant parasite suppression effect observed at the administered low dose was not sufficient enough to significantly attenuate the weight loss. This result is in agreement with that of the previous studies done on *D. Angustifolia* (Mengiste *et al.*, 2012); *C. aurea* (Eyasu *et al.*, 2013). Conversely, the results obtained from the prophylactic test suggest that the extract caused a gain in body weight unlike in other tests. This is strongly linked to the extensive parasite suppression effect showed by the extract which is capable to prevent parasite induced body weight loss.

In vivo antiplasmodial activity can be categorized as moderate, good, and very good if an extract displayed percentage parasitemia suppression equal to or greater than 50% at a dose of 500, 250 and 100 mg/kg body weight of mice per day, respectively (Deharo *et al.*, 2001; Muñoz *et al.*, 2000). Accordingly, 80% methanol extract of the stem bark of *S. guineense* had a moderate antiplasmodial activity against *P. berghei* infection in mice on prophylactic and four-day suppression tests. The moderate antiplasmodial effect exhibited by the extract with the longest survival time compared to negative control could be linked to the presence of active secondary metabolites in sufficient amount.

The phytochemical screening of the extract showed the presence of alkaloids, anthraquinones, terpenoids, saponins, tannins, phenols and flavonoids. Alkaloids (Oliveira *et al.*, 2009), flavonoids (Ferreira *et al.*, 2010; Monbrison *et al.*, 2006) and terpenoids (Amoa Onguéné *et al.*,

2013) are major antimalarial compounds. Although the mechanism of antimalarial action was not clearly indicated, these metabolites have been proved to possess potential antimalarial activity. Some alkaloids such as quinine inhibit malarial potassium channels and prevent polymerization of heme in haemozoin in the digestive vacuole (Sullivan *et al.*, 1998). In addition, terpenoids generate free radicals inside the parasite that alkylate macromolecule and disrupt intracellular transport process (O'Neill *et al.*, 2010). There is an increased peroxidative activity with free radical production during malaria infection.

Phenolic compounds act as antioxidant or free radical scavenger to prevent/reduce oxidative stress induced by the parasite (Iribhogbe *et al.*, 2012). Antioxidants can inhibit also heme polymerization and the unpolymerised heme is very toxic for the parasite (Taramelli *et al.*, 1999). Phenolic compounds (tannins, phenols and flavonoids) detected in the stem bark of *S. guineense* also reported for their antioxidant properties (Pieme *et al.*, 2014). Despite, the active compound responsible for the antimalarial activity was not identified in the present study, the antimalarial activity of *S. guineense* attributed to a single or combined effect of secondary metabolites including alkaloids, flavonoids, terpenoids, phenolic compounds and anthraquinones detected in this plant.

7 Conclusions

The finding of the present study revealed that the extract caused a significant parasite suppression activity in all the three tests at all doses evaluated. On top of this, the higher doses of the extract exhibited a moderate antiplasmodial effect with the longest survival time compared to negative control in prophylactic and four-day suppression test. The extract also caused a significant protection against parasite induced PCV and body temperature reduction. In addition, the extract significantly prevented parasite induced body weight loss especially in the prophylactic test. From this finding, it seems reasonable to use the plant for malaria prophylaxis rather than for treatment.

Phytochemical screening of *S. guineense* stem bark showed the presence of flavonoids, saponins, alkaloids, tannins, terpenoids, anthraquinones and phenols. The findings suggest that *S. guineense* contain active phytochemicals that could potentially be a lead compound in the search for new antimalarial agents.

8 Suggestion for future works

The 80% methanol extract of stem bark of *S. guineense* have a promising malaria prophylactic activity against chloroquine sensitive *P. berghei* ANKA strain in mice model; therefore, the following recommendations are suggested to further investigate the experimental plant.

- ✓ *In vitro* antimalarial activity of stem bark of *S. guineense* against *P. falciparum* should be done
- ✓ Further fractionation of the crude extract should be done to identify its active antimalarial constituents
- ✓ Isolation and identification of the active constituents of stem bark of *S. guineense* should be done
- ✓ Toxicological studies (sub-acute and chronic toxicity) should be done to know the safety of *S. guineense* stem bark.

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