
# EVALUATION OF ANTIMALARIAL ACTIVITY OF SEEDS OF *DODONAEA ANGUSTIFOLIA* AND LEAVES OF *ENTADA ABYSSINICA* AGAINST *PLASMODIUM BERGHEI IN* SWISS ALBINO MICE.

**BY**

**Andualem Getie**

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**Table of Contents** **Page**

**List of Tables** **Page**

**List of Figures**

Page

## Abbreviations

| | |
|---|---|
| ANOVA | analysis of variance |
| ASL | above sea level |
| CDER | Center for Drug Evaluation and Research |
| CQ | Chloroquine |
| $dH_2O$ | distilled water |
| D0 | day zero |
| D4 | day four |
| IP | Intraperitoneal |
| MeOH | methanol |
| ND | not determined |
| PCV | Packed Cell Volume |
| RBC | Red Blood Cell |
| SEM | Standard error of the mean |
| SPSS | Statistical Package for the Social Sciences |
| TMP | Traditional medicinal plants |
| WBC | White Blood Cell |
| WHO | World Health Organization |
| % Para | percentage of parasitaemia |
| % Supp | percentage of suppression |

**Abstract**

In the face of antimalarial drug resistance, there is need to develop more effective antimalarial drugs that are inexpensive. In this respect, traditional medicinal plant-based antimalarial products are more preferable candidates. In this study, the seeds of *Dodonaea angustifolia* and the leaves of *Entada abyssinica*, used in indigenous medicine to treat malaria in Ethiopia, were evaluated for an *in vivo* antimalarial activity against chloroquine sensitive *Plasmodium berghei* ANKA strain. Acute and sub acute toxicity studies of the extracts were carried out in Swiss albino mice prior to antimalarial activity test. Behavioral changes, mortality, weight loss, reduction in PCV, WBC and RBC count, in the mice were used as indicators of toxicity. All extracts revealed no obvious acute and subacute toxicities on mice up to the highest (1000/750mg/kg) dose given. To assess the antimalarial activity, male Swiss albino mice were infected by intraperitoneal inoculation of $10^6$ parasitized erythrocytes. The plant extracts were solubilized in Tween 80(3%) and experimental groups received once daily after 3 hours of infection for four days via gabage. CQ- phosphate was given for positive control and vehicle for negative control. Each extract was none toxic and showed significant level of parasite suppression compared to the negative control. The highest parasite suppressions (86.21%) at 600mg/kg and marginal efficacy were obtained form the methanol extracts of *D. angustifolia* at 100mg/kg. The results of this study provide support for the traditional therapeutic value and the reported antimalarial activity. Therefore, further investigation to determine the active ingredients of these plants as potential source of new antimalarial drugs should be carried out.

**Key words:** *antimalarial activity, Dodonaea anqustifolia, Entada abyssinica, in vivo, Plasmodium berghei.*

# 1. INTRODUCTION

## 1.1. Malaria and its Global Distribution

Malaria is a parasitic infectious tropical disease caused by protozoan parasites of the genus *Plasmodium* and transmitted by female *Anopheles* mosquitoes. Human malaria is caused by four species of the genus *Plasmodium* namely: *P. falciparum, P. vivax, P.ovalae* and *P. malariae.* The four species differ in microscopic appearance, clinical features, geographical distribution and the potential for development of resistance to antimalarial drugs. *P. falciparum* is the most dominant and pathogenic of the four human malaria parasites and is responsible for almost all malaria mortality in tropical and subtropical countries (Andare-Neto *et al*., 2004). In Africa the predominant malaria parasite species is *P. falciparum* (Bruce-Chwatt, *et al*., 1986). The two epidemiologically important species in Ethiopia are *P. falciparum* and *P. vivax* that contribute to almost 60% and 40% of all malaria cases, respectively (Bogale and Petros, 1996). *P. malariae* comprises less than 1% of all the cases and is most frequently reported in the Arba Minch area whereas no report about *P. ovale*. Ethiopia has the highest proportion of *P. vivax* malaria on the continent, as it accounts for approximately 40 % of all cases in the country (Tulu, 1993).

Malaria is a complex disease that varies widely in epidemiology and clinical manifestations in different parts of the world. This variability is the result of factors such as the species of malaria parasite, their susceptibility to antimalarial drugs, the distribution and efficiency of mosquito vectors, climate and other environmental conditions and level of acquired immunity of the exposed human population (Bloland, 2001).

Malaria causes an acute systemic disease. The repeated cycles of asexual reproduction in erythrocytes liberating new merozoites that invade and destroy new erythrocytes are responsible for the disease. In human malaria only asexual blood stages (late trophozoites and schizonts) result in pathology causing the disease symptoms which appear about two weeks after the sporozoite inoculation. The morbidity and mortality associated with malaria are derived solely from the erythrocytic stages (Phillips, 2001). Symptoms are mainly headache, periodically recurrent high fever (every 48 to 72 hours), shaking chills, nausea, vomiting, jaundice, anemia, hepatomegaly and splenomegaly. The sequestration of red blood cells infected with mature forms of *P. falciparum* parasites sticking to the endothelial cells of capillary vessels is responsible for severe cerebral and pulmonary malaria (Kappe *et al*., 2003).

Countries at risk of malaria are primarily in tropical and subtropical regions and include: Sub-Saharan Africa, Central and South America, the Caribbean islands of Hispaniola, the Middle East, the Indian Subcontinent, South-East Asia and Oceania. Highlands (>1500m asl) and arid area (< 1000mm rainfall/year) typically have less malaria (WMR, 2008; WHO, 2003) (Figure 1).

Half of the world's population living in 108 countries is at risk of malaria, and an estimated 243 million cases lead to nearly 863,000 deaths in 2008 (WMR,2009). The vast majority of cases (85%) were in African region, followed by the South-East Asia (10%) and Eastern Mediterranean regions (4%). Almost 89% of deaths occur in Africa, followed by Eastern Mediterranean regions (6%) and South-East Asia (5%) (WMR, 2009). According to 2008 WMR 91% of infection are due to *P. falciparum*. Almost 90% of the deaths occur in Africa and 85% of which are children under 5 years of age and is responsible for one in five childhood deaths in Africa (Ouattara *et al.,* 2006). Those at

highest risk are infants and young children (from six months to five years), pregnant women, non-immune people (travelers from non-endemic countries) and people living with HIV/ADIS (WHO, 2007). In addition, its economic effect is tremendous and includes direct cost of treatment and prevention, as well as indirect costs such as lost productivity from morbidity and mortality, and time spent seeking treatment (WHO, 1996). In Africa it is estimated that economic losses due to malaria amount to about $12 billion a year, which by far exceeds the resources needed for malaria control, estimated at about $3 billon (Muregi *et al*., 2007).

In Ethiopia, malaria is unstable (seasonal, causing frequent epidemics, lack of immunity and affect all age group). It usually occurs at altitude less than 2000 meter above sea level (asl). The two main malaria transmission seasons are September to December, after the heavy summer rains, and April to May, after the light rains. Most of the areas affected by epidemics are highland and highland fringe areas (mainly 1000–2000m asl), where the population lack protective immunity to malaria (Kiszewski and Teklehaimanot, 2004; Adhanom *et al*., 2006). Occasionally, transmission of malaria occurs in areas previously free, including areas greater than 2000m asl, in which the microclimate and weather conditions are not normally favorable to malaria. Recent reports have indicated that epidemics have expanded to areas up to 2400m asl (MOH, 2007). The disease occurs in different parts of Ethiopia in epidemic forms and about 75% of the total area is estimated to be malarious with 65% of the total population being at risk of infection (Hodes, 1996; WMR, 2009). The recent fall in cases coincides with the rapid expansion of control efforts (provision of insecticide- treated mosquito nets (ITNs), treatment with effective antimalarial drugs), reported in 2007(WMR, 2008).

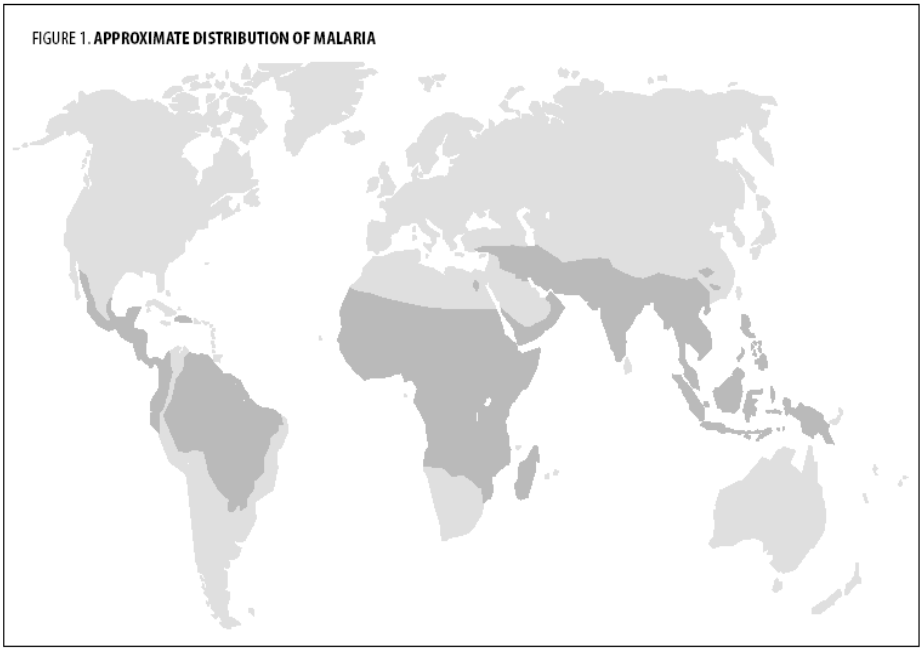


**Figure 1:** Malaria distribution in the world (Bloand, 2001); dark shaded refers to malarious regions and light shaded refers to non-malarious regions.

### 1.2. Life cycle

Knowledge of the life cycle of the malarial parasite is fundamental to understanding the methods of prevention, treatment and research pursuits. Although malaria parasites differ from each other in many respects, they have similar basic life cycle. *Plasmodium* species exhibit a heteroxenous life cycle involving vertebrate hosts and arthropod vectors (female Anopheles mosquito). *Plasmodium* species are quite host–specific and there is no zoonosis (Kakkilayos, 2006). The life cycle of *Plasmodium* species (Figure 2) starts when a malaria parasite infected female Anopheles mosquito bites a healthy individual and inoculates sporozoites (infective form) into the human host. These sporozoites infect liver cells (hepatocytes) within 30 to 40 minutes and mature into schizonts, which rapture the liver cells and are released as merozoites approximately from 6 to 15 days according to

species. Merozoites released from liver cells start to invade red blood cells within 15 to 20 seconds in the blood. However, in *P.vivax* and *P.ovale*, some of the sporozoites remain in the liver and become dormant hyponozoites. This form can remain for months and years until reactivated to complete its life cycle, responsible for secondary erythrocytic schizogony and relapses in patients.

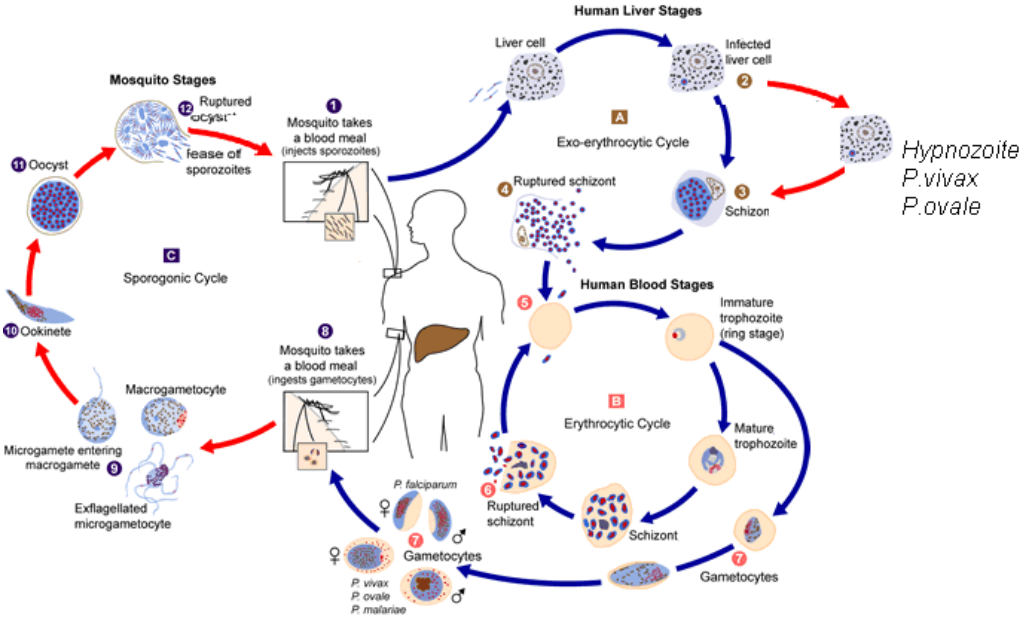


Fig.2 Malaria life cycle (Modified from CDC,2003)

In erythrocytes the ring stage trophozoites mature into schizonts which rapture to release merozoites, after 48- 72 hours depending on the species, which invade and destroy new erythrocytes. The asexual erythrocytic cycle usually continues until controlled by the immune response or chemotherapy or until the patient dies (in case *of P. falciparum*). According to Mendis *et al. (2001)* some merozoites differentiate into sexual erythrocytic stages (gametocytes). The male gametocytes (microgamete) and female (macrogamete) are ingested by an anopheles mosquito during a blood meal and the union of gametes

produces zygote (ookinate) develops to sporozoites. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle. The sexual process allows genetic recombination to occur, and with mutations the raw material upon which selective pressures such as antimalarial drugs resistance can work (http:// www.vivaxmalariacom/life cyclelarge.htm.).

### 1.3. Malaria Prevention and control

Despite over a century of effort and early optimism, a malaria- free world remain as much distant vision as ever (Elufioye and Agbedahunsi, 2004). Many malarial control strategies exist (such as the provision of prompt, effective malarial treatment, vector control and chemoprophylaxis), but none are appropriate and affordable in all contexts (Bloland, 2001). Some of the factors that contributed to this worst picture of malaria are high cost control programs, emergence of new insecticide resistant strains of the vector, creation of new mosquito breeding sites, the problem of drug resistance (*Plasmodium falciparum*) to almost all currently available antimalarial drugs, lack of organized health infrastructures and the migration behavior of people that are increase the incidence and spread of malaria (Talisuna, 2004; WHO, 1996). The present global situation indicates a recent resurgence in severity of disease and that malaria could still be described as one of the important communicable disease. Prevention and control of malaria is being approached on three fronts: Vector control, creating efficient vaccines and treatment with antimalarial drugs (Bloland, 20001).

Vector control is achieved by creating a barrier between the human host and mosquitoes, interrupting their life cycle and reducing vector density. Protect people from being bitten by infected mosquito though the use of insecticide-treated nets (ITNs), insect repellents

and indoor residual spraying of insecticides (DDT-Dichlorodiphenyltrichloroethane) to kill the adult or larval mosquitoes, using natural biological controls such as the introduction of fish which eat the mosquito's larvae or bacteria (*Bacillus thuringiesis*) which excrete larval toxins and reducing mosquito breeding sites (Talisuna *et al*., 2004). Vector manipulation (bioengineering) growing insect that is unable to transmit the malaria parasite (http: //ecology.cwru.edu/malaria).

Ideally, a protective vaccine would be the most effective approach to controlling malaria. However, attempts to develop a vaccine have been hindered by the great genetic diversity of the parasite, its multistage life cycle, as well as the complex and inefficient human immune response (White, 1999). A multicomponent *P. falciparum* vaccine, Spf66, showed only limited immunity when tested in a large scale human trail. With no vaccine on the immediate horizon, prevention and treatment of the disease relay mainly on antimalarial scizonticidal drugs (Talisuna *et al.*, 2004).

### 1.4. Malaria Treatment and Drug Resistance

Treatment of sick individuals using correct dose of antimalarial drugs interrupted the life cycle of the parasite. Early treatment of cases also reduces transmission by reducing the opportunities for mosquitoes to become infected. However, there are only a limited number of antimalarial drugs which can be used to treat or prevent malaria and *falciparum* malarial parasites develop resistance to almost all antimalarial drugs (Pillay, 2006). Antimalarial drugs fall into groups. The first are quinoline based antimalarials, which includes quinine and its derivatives chloroquine (CQ), amodiaquine, premaquine and mefloquine. Quinine has been used for more than three centuries and was the only

effective agent for the treatment of malaria until the 1930`s. Due to its undesirable side effect it is now used us an intravenous injection to treat severe malaria (Bloland, 2001).

The best compound Chloroquine, discovered in the 1940s, is a synthetic 4-aminoquinoline produced as less toxic and well tolerable effective antimalarial drug structurally similar to quinine. Since this drug was cheap, non–toxic and effective against all strains of the parasite, became the mainstay of prevention, until resistance was developed by *P. falciparum* to an extent that chloroquine has been rendered virtually useless in most endemic areas (Talisuna et *al.,* 2004).

The second class of common antimalarial is the Antifolate compounds that inhibit the synthesis of parasite pyrimidines and thus of parasitic Deoxyribonuclic acid (DNA) (Robert *et al*., 2001). There are two types of antifolates (that inhibit enzymes involved with folate synthesis): the dehydrofolate-reductase (DHFR) inhibitors (Proguanil, chlorproguanil, pyrimethamine and trimethoprim) and the dihydropteroate synthetase (DHPS) inhibitors, which include the sulphonamide drugs (dapsone, sulfalene, sulfamethoxazole and sulfadoxine). Due to marked synergistic effect, a drug of the first group is usually used in combination with a drug of the second one. Typical combinations include Pyrimethamine-sulphadoxine (SP) or Fansider (is the most widely used combination), sulfamethoxazole-trimethoprim(co-trimoxazole) and chlorproguanil-dapsone (Lap-dap) (Bloland, 2001; White, 1999).

The third class of antimalarial is based on the natural endoperoxide Artemisinin. Artemisinin and its semisynthetic derivatives (dehydroartemisinin (DHA), artemether, arteether, artesunate, artelinic acid) are the most rapidly acting and effective against multi-drug resistant strains of the parasite. However, the poor solubility of artemisinin, coupled with its short plasma half life led to a high rate of parasite recrudescence (Pillay,

2006). In Ethiopia CQ has been the first line treatment for uncomplicated malaria since 1950. Due to intense resistance of *P. falciparum* to CQ, Sulphadoxine- pyrimethamine was introduced as first line treatment for uncomplicated *falciparum* malaria in 1999 (Jima *et al*., 2005; MOH, 2004). Resistance of *P. falciparum* to SP caused a shift to artemisinin based combination therapies (ACT) five years later particularly artemether- lumefantrine or coartem, the first line treatment for uncomplicated *falciparum* malaria (MOH, 2007).

Antimalarial drug resistance has become as one of the greatest challenges against malaria control. Resistance to antimalarial drugs has been described for two of the four species of malaria parasites that naturally infect humans, *P. falciparum* and *P. vivax. P. falciparum* has developed resistance to nearly all antimalarials in current use (Bloland, 2001; Mendis *et al*., 2001). Quinine resistance was first documented in Brazil in 1880 and then in German railroad workers returning from Brazil (Peters, 1967). However, it remains important member of the antimalarial chemotherapy especially for treatment of severe *P. falciparum* cases where there is a resistance to CQ. Resistance of *P. falciparum* to CQ was first reported in the Thai- Kampuchian boarder areas and soon after in South America (Moore and Lassier, 1961) and gradually it spreads to South East Asia and appeared later in Africa (Cambell *et al*., 1979). Resistance to CQ in East Africa was reported in the late 1980`s (white, 1999). CQ resistance in *P. vivax* has reported the first cases from Papuo New Guinea in 1989 (Reickman *et al*., 1989). In Ethiopia CQ resistance of *P. falciparum* and *P.vivax* was first reported in1986 (Teklehaimanot, 1986). In Ethiopia CQ resistance of *P. falciparum* and *P.vivax* was reported in DebreZeit in1995 (Tulu, 1996).

Sulfadoxine-pyrimethamine (SP) resistance occurs frequently in South- East Asia and South America. SP resistance is becoming more prevalent in Africa as the drug is

increasingly being relied upon as a replacement for CQ (WHO, 1997). Schunk *et al.* (2006) reported high frequency of drug resistance mutations in both *P. falciparum* and *P.vivax*, which was observed in 69% and 31% of the patients, respectively and suggests that both CQ and SP are inadequate drugs in southern Ethiopia. Recently CQ resistant *P. vivax* was reported in DebreZeit (Teka *et al.*, 2008) and in Serbo town and its surrounding, Jimma Zone (Ketema, 2008). Resistance to proguanil and pyrimethamine was encountered during the America occupation of Vietnam (White, 1999). Artemisinin resistance was reported in 2003 (Pickard *et al.*, 2003). Although recurrence is associated with the monotherapy of artemisinin and its derivatives at a high rate, there is no solid data supporting the existence of resistance to artemisinin. However, reports showed that more than 5% of 65 isolate of the parasite from South East-Asia are resistant to artemisinin and artesunate (Abdulelah and Zainal-Abidin, 2007). Noedl *et al*. (2008) and Dondorp *et al*. (2009) reported that artemisinin is losing its potency in Cambodia. Resistance of *P.falciparum* to artemisinins has been reported at the Cambodia- Thailand boarder (WMR, 2009). The reasons for the development and spread of drug resistance involves the interaction of drug use patterns, characteristics of the drug , human host factors, parasite characteristics and vector and environmental factors. However, only gene mutation confers resistance to the parasite in nature (Pillay, 2006).

The rapid emergence of drug resistant strains of the parasite is outpacing the development of new antimalarials (Kirby, 1996). Global malarial control program will require combined strategies that in addition to an effective vaccine. The methods to combat malaria includes providing long-lasting insecticidal nets, and had artemisinin-based combination therapy (ACT) as first- line treatment, supported by indoor residual spraying in high malaria transmission foci and intermittent preventive treatment in pregnancy (WMR, 2008). With the increase in cases of drugs resistance and absence of vaccine,

there is an urgent need for new antimalarial drugs with novel modes of action. One approach to this is the investigation of medicinal plants and natural products (Muregi *et al*., 2007; Rosenthal, 2003).

### 1.5. Antimalarial Drugs from Traditional Medicinal Plants

Traditional medicine is the embodiment of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that have been handed down from generation to generation. Traditional medicine has been described by the World Health Organization (WHO) as one of the surest means to achieve total health care coverage of the world's population (Conserve Africa organization, 2004). In the last decade, there has been a global upsurge in the use of traditional medicine (Pillay, 2006). The most widely used traditional medicinal therapies are herbal medicines and acupuncture. Medicinal plants have been used effectively for centuries in traditional medicine. It has been estimated by the WHO that approximately 80% of the world's inhabitants use plants for their primary health care (WHO, 1996). Nearly 80% of the population of Ethiopia use traditional medicines as their major health care system (Abebe, 2001). In Africa, the use of indigenous plants still plays an important role in malaria treatment. These plants might be an interesting source for the detection of novel antiplasmodial compounds (Hilou, 2006). The plant kingdom represents a virtually untapped reservoir of novel chemical compounds (new antimalarials) (Jayasinghe *et al.*, 2008; Bogale and Petros, 1996). Plants have for many years formed the basis of sophisticated traditional medicine systems and lately, natural products are proving to be a good source of lead compounds against various infective diseases (VanWyk, 2000). Natural products have made and continue to make an immense contribution to malaria chemotherapy either directly as antimalarial agents or as important lead compounds for

the discovery of more potent antimalarials. In the endemic area where malaria prevails, traditional herbal medicines are often used for antipyretic therapy. However, very little scientific information is available to assess the efficacy of these herbal remedies. Therefore, it is important to investigate the efficacy of antimalarial activities of medicinal plants in order to determine their potential as sources in the development of new antimalarial drugs.

Isolation of new lead compounds from plants is one of the strategies that can be followed in the search for new drugs. Historically the majority of the drugs have been derived from medicinal plants or structures modeled on plant lead compounds, more than 50% of all drugs in clinical use today contain certain substances of natural origin, most of these products are from plants (mcCorkle, 1995). For example, in 1819, the isolation of the analgesic morphine from the Opium poppy (*Papaver sobniferum*) laid the foundation for the purification of pharmacologically active compounds from medicinal plants ( Pillay, 2006 and Ruth, 2003), the anticancer drug, taxol, from the bark of *Taxus brevifolia*, the active ingredient in aspirin, salicylic acid originally isolated from the bark of the Willow tree (*Salix* species) and Ephedrine extracted from *Ephedra sinica*, relieves asthma and hay fever symptoms. These pharmaceuticals are considered as modern medicines, their history lies in medicinal plants (Ruth, 2003). Previous findings of antimalarial agents such as quinine obtained from *Cinchona* species and artemisinin obtained from *Artemisia annua* (Qing hao) from medicinal plants also encouraged the possibility of finding new antimalarial drugs from plant sources (Kim *et al.*,2004). Quinine the first effective antimalarial drug, isolated from the bark of *Cinchona* species in 1820, is still in clinical use today. The more recently discovered natural endoperoxide Artemisinin which was first extracted from the Chinese traditional medicinal plant *Artemisia annua* in 1972 (Pillay, 2006) and its derivatives for the treatment of resistant malaria has focused

attention on plants as a source of antimalarial drugs, and proved to be an incentive for further research into plants (Rosenthal, 2003). Hence it is possible that the investigation of traditional medicinal plants used in the treatment of malaria may lead to the discovery of new antimalarial compounds.

Traditional medicines include crude plant extracts, or combination of several medicinal plants, which contain numerous components that are thought to contribute to the overall therapeutic effect. Because the chemical compounds in the different plant components are often quite different, usually only a specified plant part is used medicinally such as the leaf, root, stem, flower, fruits, seeds, barks of stems and roots and in some cases the whole parts of the plant is used. Roots and leaves were found to be the most frequently sought plant parts in the preparation of remedies. The plants used for mainly depend on the practices of the local healer and accessibility of the plants (Giday *et al., 2*007). The method of preparation is crucial, which includes activities such as the addition of appropriate volumes of solvents ( water or alcohol) to a specified amount of fresh or dry plant material, boiling for a specified length of time or partial burning to achieve a desired color are important and can serve to neutralize certain toxins. Dosage forms (tinctures, extracts, ointments or enemas) as well as method of administration (orally, topically or nasally) are also critical and are conveyed by the healer (Giday *et al., 2*007; vanWyk, 2000).

Traditional knowledge can be a fundamental starting point for chemotherapeutic research. A principal approach to chemotherapeutic research against malaria consists of investigating the traditional medicinal plant based antimalarials (Sanon *et al*., 2003). The strategy of developing new drugs based on medicinal plants has an advantage over random screening, since it is guided by experience from a long history of clinical practice.

Plants investigated for pharmacologically active compounds are usually selected on the basis of ethnomedicinal information as there is a correlation between biological activity and the traditional use of the plant (Sanon *et al.*, 2003; Kim *et al*., 2004). In developing a drug from a plant attempts may be made to produce chemical analogues of the active principles with enhanced antiplasmodial activity and reduced host toxicity. Guides for conducting this work are often obtained from the original plant source since many plant compounds exists as groups of structurally related metabolites within a single species. The chemical diversity and stereo specificity of complex natural products are the main attraction of working with plants as opposed to synthetic chemistry approaches (David et *al*., 2004).

Before a compound can be used as a drug it is essential to know its likely effects when used in human. This is achieved after extensive laboratory testing followed by the application of clinical trials (Pillay, 2006). Biological testing for antimalarial activity in plants has progressed over the years. In the 1950‘s, the screening of crude plant extracts was based on avian malarias using *in vivo* tests against *P. gallinaceum* in chicks and against *P. cathemerium* and *P. lophurae* in ducklings (Jayasinghe *et al.*, 2008). In the 1970`s, *in vitro* procedures were developed using *P. falciparum* cultures in human red blood cells, a technique that enabled the development of a microdilution assay (Pillips *et al.*, 1972). This technique is useful to assess *in vitro* antimalarial activity of crude extracts prior to the isolation of active principles (Markler *et al.*, 1993). An *in vivo* screening is possible in mice using a natural infection with P. *berghei*. Detailed evaluation of antimalarial drugs is done in the Aotus monkey (*Aotus trivirgatus*) using *P. falciparum* infection or in the Rhesus monkey (*Macaca mulata*) using *P. cynomolgi* infection (Pillay, 2006). These extensive primary screens followed by the application of clinical trails helps to know its likely effects when used in humans.

On the other hand, plants which are frequently mentioned as antimalarials do not always show high activity *in vivo* tests. This can partly be explained by the fact that many plants are used in the treatment of malaria, not for their antiparasitic effects but because of other therapeutic effects. These include reducing fever, calming convulsion and headache, and possibly immuno-stimulatory effects. Another problem is that some plants are given in a mixture with few others and are possibly only active in these combinations due to synergistic effects (Sanon *et al.,* 2003). Also, an *in vitro* test cannot precisely reproduce the *in vivo* situation since certain plant extract components might only become active after specific metabolic processes *in vivo* (Gesseler *et al.,* 1994). Furthermore, crude extracts or compounds that show *in vitro* activity might be extremely toxic or totally fail to display *in vivo* activity (Kirby, 1996).

The urgency generated by drug resistant strains of malaria has accelerated antimalarial drug research over the last two decades (Rosenthal, 2003). The investigation of a range of plants from various countries used in traditional medicine for the treatment of malaria has led to the discovery of a large number of antimalarial compounds with significant structural variety. Examples of compounds with antimalarial activity isolated from traditional medicinal plants are quinines, triterpenes, sesquiterpenoids, quassinoids, liminoids, alkaloids, lignans and coumarins (Switch and Jarilla, 2004).

There are several reports on the antimalarial activities of medicinal plants. For example, Franssen *et al, (*1997) from Guatemala reported that methanol and dichloromethane extracts of *Simarouba glauca, Sansevieria guineensis, Croton guatemalensis* and *Neurolaena lobata* significantly reduced parasitemias in *Plasmodium berghei* infected mice and *P. falciparum in vitro*. Deharo *et al*., (2001) from Tecana reported that *Bowdichia virgilioides* was active *in vitro* ($IC_{50}$ = 1 µg/ml) and *in vivo* (51% at 100

mg/ml) and *Protium glabrescens* was active *in vivo* (61% at 100mg/ml). Jayasinghe *et al., (*2008) from Sri Lanka reported that aqueous root extract of *Barringtonia acutangula* significantly suppressed the parasitemias (800mg/kg = 60.88%) in *P. yoelii* murine model. Bhat and Surolia (2001) reported from India significant inhibitory effect of *Swertia chirata* and *Carica papaya on P. falciparum in vitro.* Abdulelah and Zainal-Abidin (2007) reported seed extracts of *Nigella sativa* showed suppression activity in mice infected with *P. berghei* in Malaysia.

There were also many African traditional plants evaluated for their safety and efficacy against malaria parasites. For example, Elufioye and Agbedahunsi, (2004) from Nigeria reported an *in vivo* antimalarial activity of *Tithonia diversifolia* and *Crossopteryx febrifuga* against *P.berghei*, the suppression of parasitemia by the extracts at the highest dose was similar to CQ and pyrimethamine. Hilou *et al*, (2006) reported betalain containing *Amaranthus spinosus* and *Boerhaavia erecta* show antimalarial activities $ED_{50}$ (effective dose) 789- 7564 mg/kg. Waako *et al*, (2007) reported ethyl acetate extracts from plants of East and central Africa *Emilia discifolia, Senecio stuhlmanni, Indigofera emarginella* and *Aspilia africana* have significant *in vitro* antiplasmodial activities. Ouattara *et al*, (2006) reported from Burkina Faso medicinal plants extracts of *Swartzia madagascariensis, Combretum glutinosum* and *Tinospora b*arkis *in vitro* screening against P. *falciparum* were active ($IC_{50}$ <5µg/ml). Muthaura *et al*, (2007) reported the potential for isolation of a lead compound from the extracts of the *Warburgia stuhlmanni,, Maytenus undata , Flueggea virosa , Maytenus putterlickiodes* and *Harungana madagascariensis* for antiplasmodial activities from Kenya, exhibited a high chemosuppression of parasitaemia >70%. Muregi *et al*, (2007) reported from Kenyan medicinal plants extracts of *Toddalia asiatica, Rhamnus prinoides* and *Vernonia casiopus*

showed high chemosuppression in the range of 51 – 75% *in vivo* activities against CQ resistant *P.berghei.*

Ethiopia is the home of many nationalities and rich for its diverse species of plants (but not well studied scientifically) that are being used to treat various ailments such as dysentery, flu, headache, stomachache, fever or malaria in the traditional health care system, including: *Haegenia abyssinica, Brucea antidysenterica, Glinus lotoidos, Taverniera abyssinica, Repidum sativum, Dodonaea angustifolia, Entada abyssinica, Plumbago Zeylanica, Croton macrostachyus and Phytolaca dodecandra* (Giday *et al*., 2007; Yineger and Yewhalaw, 2007; Seifu, 2004). A number of *in vivo* and *in vitro* studies have been conducted to evaluate antimalarial activities of Ethiopian medicinal plants, for instance, Sorsa (1992) reported that methanol extracts of *Croton macrostachyus* and seeds of *Dodonaea angustifolia* have shown the highest antimalarial activities against *P. falciparum in vitro* with $IC_{50}$ = 0.94µgm/ml and 7.06µgm/ml, respectively. Bogale and Petros (1996) reported that among nine Ethiopian medicinal plants that are used to treat malaria the antimalarial activities of *Withnia somnifera* and *Vernonia amygdalina* are substantial *in vitro* against *P. falciparum*. Asres and Balcha (1998) reported that the acetone and methanol extracts on the stem bark of *Comberatum molle* possessed remarkable schizont maturation inhibition value to *P. falciparum*. The ethanol extracts of *Artemisia afra, Artemisia rehan,* and *Artemisia remota* were found to have high antimalarial activities against *P. falciparum in vitro* (Kassa *et al.,* 1998). Animut (2002) showed that water extracts of *V. amygdalina* leaf and methanol extract of *Croton macrostachyus* fruits have significant suppressive effect against *P. berghei in vivo*. Teklemariam (2005) evaluated the antimalarial activities of *Moringa stenopetala, W. somnifera* and *V.amygdalina* against *P.berghei* in mice model showed valid results. According to Abate (1989) *Withania somnifera, Phytolaca dodecandra, Jasmincum*

*abyssicum, Combretum molle, and Cucumis prophetrum* are plants widely used in treatment of fever most likely caused by malaria, in different parts of Ethiopia. Finally, Dikasso *et a.* (2006) evaluated hydroalcoholic extracts from the roots and aerial parts of *Asparagus africanus* and showed significant antimalarial activity in the Swiss albino mice by 46.1`% and 40.7% respectively.

Although several plants that have potential antimalarial property have been studied, there still exist innumerable potentially useful medicinal plants waiting to be evaluated and exploited for therapeutic applications against various groups of pathogens (Krettli *et al.*, 2001). In addition, Ethiopia is rich for its diverse species of plants (but not well studied scientifically) that are being used to treat various ailments such as fever or malaria in the traditional health care system (Giday *et al.*, 2007; Yineger and Yewhalaw, 2007). Following this trend, this study evaluates the antimalarial activities of crude aqueous and methanol extracts of *Dodonaea angustifolia* and *Entada abyssinica*, plants commonly used in Ethiopian traditional medicine to treat malaria, bacteria and helminthes, against *P. berghei* in laboratory bread Swiss albino mice to further substantiated the traditional use for malarial treatment.

**Hypothesis**

- The seeds of *D.angustifolia* and the leaves of *E. abyssinica*, which are used in traditional medicine, possess antimalarial effects.

# 2. OBJECTIVES OF THE STUDY

## 2.1. General objective

- To evaluate the antimalarial effect of the crude aqueous and methanol extracts of the seeds and the leaves of two traditional medicinal plants against *P.berghei* in white albino mice.

## 2.2. Specific objectives

- To assess the crude extracts of the seeds of D. *angustifolia* and the leaves of *E.abyssinica* for toxicity in male Swiss albino mice.
- To test the antimalarial effects of crude extracts of the seeds of *D.angustifolia* and the leaves of *E. abyssinica* in male Swiss albino mice infected with *Plasmodium berghei*.

# 3. MATERIALS AND METHODS

## 3.1 Plant materials collection

Plant parts used in this study were collected from two different localities namely Gojeb, 427km southwest of Addis Ababa in the South Nation, Nationalities and Peoples Region (SNNPR) and Sekoru, 221km Southwest of Addis Ababa in the Oromiya Regional State of Ethiopia during the months of November and December, 2008. Leaves of *E.abyssinica* (Fabaceae) were collected 3 kms from Gojeb near Kaengham Road Camp South of Jimma and seeds of *D. angustifolia* (Sapindaceae) from Sekoru nearby forest. The identification and authentication of the plant specimens was done at National Herbarium, Department of Biology, Addis Ababa University. A voucher specimen of plant samples were deposited in the herbarium with voucher number of AG001 and AG002.

### 3.1.1 Description of the plant used in the study

1. *D. angustifolia* locally called Kitkita in Amharic, Hitacha in Oromifa, and Sarka in Gamogofa. *D. angustifolia* is an evergreen shrub growing up to three meter long; all parts are glabrous and resinous when young. Leaves of this plant are simple and petiolate (1-5mm) with narrowly attenuate lamina. The flower of this plant is dioeciously, found at the terminal and the axillary of the plant. Sepals are Ovate, yellowish green in colour and shortly connate. Ovary 2-3 locular 3mm long, style 4-7mm, and stigma 2-3mm. Fruit are circular in outline and yellowish with 3-6mm wide reddish tinged wings. Seeds are lenticular. The plant grows on the edge of upland forest, upland bush and grassland, in secondary forest and shrubs. It invades areas recently cleared of forest and overgrazed acacia-commiphora bush land (Thulin, 1989). *D. angustifolia* contains saponins, dodonic acid, hautriwaic acid, flavonoides, pinocembin, viscosal, santin and penduletin (Switch

and Jarilla, 2004). The plant part is traditionally used in sedating smooth muscle contractions, in limiting spasmodic diarrhea and stomach cramps. In addition, it is used in diminishing uterine cramps on a purely symptomatic level (Switch and Jarilla, 2004). Extracts from *D. angustifolia* was found to have a significant antimalarial activity against *P.falciparum in vitro* (Sorsa, 1992). In Ethiopia, leaf juice *of D. angustifolia* is traditionally used for the remedy of intestinal cestodes in human (Desta, 1995); leaves and twigs decoction is a remedy for stomach disturbance and diarrhea in human (Dharani. 2002) and used to treat malaria (Giday *et al.*, 2007)

2. *Entada abyssinica* Steud. Ex. A. Rich (Fabaceae) locally called kentefa (Amh.) Ambelta in Oromifa, and Gilo in Keficho is a tree which is tall up to 10m, young branches have glabrous or pubescent. The leaves are dark green compound with small leaflets, pinnate 4-22 pairs, leaflets 18-50 pairs which are linear-oblong, 4-16 x 1-3mm rounded at the apex pubescent on both surfaces. Racemes are 1-4 together, 7-16 cm long, axis pubescent, flower creamy white, and calyx glabrous 0.75-1mm long. The fruit is legume, long pod which contains 10 seeds. Woodland, wooded grassland and scrub grow from 1300-2050m above sea level, and they are wide spread in tropical Africa (Thulin, 1989). *E. abyssinica* leaf extracts reported to possess trypanocidal activity in Uganda (Freiburghaus *et al.,* 1998), its broad antibacterial activity reported from east Africa (Fabry *et al.,* 1998), its antimalarial activity (Kamanzi *et al.,* 2004) and its use to treat gonorrhea in Ethiopia(Yineger And Yewhalaw, 2007).

### 3.1.2 Preparation of Plant Crude Extracts

The collected plant parts specimens were dried in open air without exposure to sun light in the biomedical laboratory at the Department of Biology, AAU. The dried specimens were grounded to powder using electrical grinding mill (Straub, model 4E, Philadellphia,

USA). The crude extracts were prepared by cold maceration techniques as out lined by O`Neill *et al.* (1985). Considering the fact that organic substances either dissolves in water (polar) or alcohol (non-polar), aqueous and methanol extracts of each specimen were prepared as follows. The powder of plant parts in 1:10 (w/v) was mixed in distilled water or methanol in separate Erlenmeyer flasks and placed on orbital shaker (GFL, model 3020 Germany) at 120 rpm for 24hours of water and 72 hours of methanol at room temperature. The mixtures were first filtered by using cotton and then the filtrates were passed through Whatman filter paper (15cm size). The methanol extracts were concentrated in a rotary evaporator (Buchi type TRE121, Switzerland) at a temperature of $45^0$C whereas the water extracts were freeze-dried using centrifugal freeze drier (model 5 SP, England). All the extracts were stored in screw cap vials at $-20^0$C until used. The water extract were dissolved in distilled water and methanol extracts in less than 3% Tween 80 for use in the tests.

### 3.2 Experimental animal

Male Swiss albino mice, weighing 27-32g and age of 6-8 weeks, were used in the study. The mice were obtained from the animal house of Biology Department at the AAU. They were fed with standard commercial pellet food and tap water *ad libitum.* The test animals were put randomly into five groups each containing five mice.

### 3. 3 The Parasite, *Plasmodium berghei*

To test the antimalarial activity of the water and methanol extracts of each plant, the mouse-infective, CQ sensitive strains of *P. berghei* ANKA strain maintained at the animal house of Biology Department, AAU was used. The parasites were maintained by serial passage of blood from infected mice to the non-infected ones on weekly basis.

Blood samples taken from donor mouse with the growing parasitaemia of 30-40% was diluted with normal saline, so that each 0.2 ml of blood contained $10^6$-$10^7$ infected erythrocytes, the standard inoculums. These parasites were used to infect the experimental animals intraperitoneally (IP).

### 3.4. *In vivo* Toxicity Test of the Crude Plant Extracts

The crude water and methanol extracts of *D. angustifolia* and *E. abyssinica* intended for the antimalarial test against *P. berghei* were evaluated for their toxicity in non-infected male Swiss albino mice aged 6-8 weeks and weighing 27-32g. For each extract test, 20 mice were used by randomly dividing them into four groups of 5 mice per cage. Before oral administration of a single dose of each extract, the mice were deprived from food for 12 hours (WHO, 2000). For subacute toxicity studies weight and haematological parameters were measured before and after treatment. In haematological determinations, parameters such as packed cell volume (PCV), white blood cells (WBC) count and red blood cells (RBC) count were considered. The extracts in each case was administered orally in an increasing dose related manner for four days (i.e. D0 to D4) using gavage. Then, the mice in group one, two and three were given orally 500, 750 and 1000mg/kg body weight in single dose volume of 0.2 ml of each extract, respectively. The mice in the control groups received 0.2 ml of respective vehicle of each extract ($dH_2O$ or 3% Tween 80). The same procedure was followed for the remaining three extracts, except methanol extract of D. *angustifolia* (450, 600 and 750 mg/kg). Doses which can change the physical condition of the mice and significant differences on the parameters were not included in the efficacy test. Then, the mice were observed continuously for one hour after the treatment; intermittently for 4 hours, and thereafter over a period of 24 hours. The mice were also observed for gross behavioral changes such as feeding, lacrimation,

mortality and other signs of toxicity manifestations for 24 hours (Pillai and Santhakumari, 1984; Twaij *et al.,* 1983).

In subacute toxicity studies, the mice were closely observed for a week and data were recorded on day 0 and day 4 (after 12hours of the last dose given). The subacute toxicity of each extracts was evaluated in terms of gross behavioral changes, mortality, weight loss, and reduction in PCV, WBC and RBC. The body weight of each mouse was determined in grams using weighing balance. The haematological parameters white blood cells and red blood cells count were enumerated using haemocytometer (Neubauer improved, Germany) and the packed cell volume was determined by microhaematocrit reader. Data on weight, packed cell volume, white blood cell count and red blood cell count of the mice were obtained on day zero ($D_0$) and day four ($D_4$). For all experimental mice, the averages of all parameters before treatment ($D_0$) and after treatment ($D_4$) were compared with control group and among different dose levels (Doull *et al.,* 1980; Twaij *et al.,* 1986; Pillai and Santhakumari, 1984; CDER, 1996).

### 3.5. *In vivo* Evaluation of the Antimalarial Activity of Plant Extracts

For an *in vivo* evaluation of each extract the Peter`s 4 day suppressive test against *P. berghei* infection in mice was employed (Peter *et al.,* 1975). Forty male Swiss albino mice weighing 27-32g were infected with *P. berghei* and randomly divided into eight groups of five mice per cage: six test groups (for *D.angustifolia* methanol extract four test groups) G2, G3, G4, G5, G6, and G7 and two control groups (G8-CQ a standard drugs as a positive control and G1-vehicles, $dH_20$ or 3% Tween 80, as a negative control). To infect the mice, blood sample was collected by heart puncture from donor mouse, anaesthetic with chloroform/ spinal displacement, with a rising parasitaemia of about 30-40%. Then the blood was diluted with normal saline so that each 0.2 ml contained

approximately $10^6$-$10^7$ parasite infected erythrocytes. Each mouse received standard inoculums of about $10^6$-$10^7$ parasite infected red cells per gram body weight through the intraperitoneal route in a saline suspension of 0.2ml on day zero (D0) to produce a steadily rising infection in mice. The stock of aqueous extracts were made dissolved in distilled water ($dH_2O$) and methanol extracts were dissolved in less than 3% Tween 80 according to the weight of mice in each group. The extracts were given via intragastric route by using gavage in six different doses (100, 200, 400,600,800 and 1000 mg/kg/day), except methanol extract of *D. angustifolia* (four dose 100, 200, 400 and 600mg/kg), while the positive control mice received chloroquine (CQ) at 25mg/kg/day and the negative control received vehicles with in a volume of 0.2ml. Each treatment was administered as a single dose per day and was started after 3 hours of infection on day zero continued daily for four days. On the fourth day drops of blood sample was collected from the tail snip of each mouse. Thin smears were prepared and stained with 10% Giemsa solution. Then, each stained slide was examined under microscope with an oil immersion objective of 1000 magnification (1000x) power to evaluate the percentage of parasitaemia and suppression of each extract with respect to the control groups. The percentage parasitaemia and suppression was calculated using the formula indicated in Li *et al.* (2003) and Devi *et al.* (2001), respectively.

$$\% \text{ Parasitaemia} = \frac{\text{Number of infected RBCs}}{\text{Number of infected RBCs + Number of non-infected RBCs}} \times 100$$

$$\% \text{ chemosuppression} = \frac{\text{parasitaemia in negative control- parasitaemia in test group}}{\text{Parasitaemia in negative control}} \times 100$$

### 3.6. Data Analysis

Results of the study were presented as a mean plus or minus standard error of the mean (M ± SEM). Statistical significance was determined by one way analysis of variance (ANOVA) using computer software spss version 15. Students paired t-test was used to compare parameters with in groups. The data obtained from subacute toxicity and suppressive studies of plants extracts were analyzed among different groups corresponding to each dose levels and vehicle control group at fixed time and overtime (D0 and D4). To observe any significance differences in the parameters across the two time periods, the average of both parameters was calculated and compared using one way ANOVA Post hoc HSD. All the data were analyzed at a 95% confidence interval (P=0.05).

# 4. RESULTS

## 4.1. Toxicity Tests

Aqueous and methanol crude extracts of the seeds of *D.angustifolia* and leaves of *E. abyssinica* were tested against laboratory breed Swiss albino mice. When administered orally in a single dose up to 1000mg/kg body weight for each plant extract showed no lethal effect within 24 hours of observation. Gross physical and behavioral observation of experimental mice also revealed no visible signs of acute toxicity such as urination, hair erection, lacrimation, reduction in motor activity and reduction in feeding activity. In general, they were physically active.

In subacute toxicity study, the four extracts administered orally daily for three days (D0-D3). Aqueous and methanol extracts of *E. abyssinica* and aqueous extracts of *D.angustifolia* did not produce death of the mice within a week up to 1000mg/kg body weight, the remaining methanol extracts of *D. angustifolia* treated mice were safe up to 750mg/kg body weight. Three haematological parameters (PCV, WBC, and RBC) and body weight were also compared at fixed time among the groups and overtime in each group. Subacute toxicity study results of aqueous extract of the seeds of *D. angustifolia* treated mice (Table1) did not show statistically significant difference ($P > 0.05$) when the parameters compared in each group between pre-treatment (D0) and post-treatment (D4), and also between treatment and negative control groups mice. However, all parameter values of mice in group 3 were relatively lower on day four than on day zero. Similarly, overtime reductions in PCV and WBC values of mice were observed in control group. The analysis of variance between treatment groups in comparison to control group of the aqueous extract of *D. angustifolia* on day four was not statistically significant in all parameters measured.

**Table 1:** Subacute toxicity test of aqueous extract of seeds of *D. angustifolia* in Swiss albino mice

| Group | Dose (mg/kg/day) | Parameters | Pre-treatment (D0) | Post-treatment (D4) | P value |
|---|---|---|---|---|---|
| | | Weight (g) | 29.58 ±0.77 | 30.38±0.92 | 0.065 |
| | | PCV (%) | 49.9±1.26 | 50.72±1.08 | 0.143 |
| 1 | 500 | WBC /$mm^3$ | 7408 ±571.83 | 7925.6±590.56 | 0.613 |
| | | RBC /$mm^3$ | $8.84x10^6$ ±$5.6x10^5$ | $9.13x10^6$±$3.47x10^5$ | 0.324 |
| | | Weight (g) | 29.52±0.95 | 30.88±1.51 | 0.056 |
| | | PCV (%) | 50.76±0.64 | 51.56±0.66 | 0.802 |
| 2 | 750 | WBC /$mm^3$ | 8629.6±534.61 | 8964.2±838.2 | 0.202 |
| | | RBC /$mm^3$ | $8.1x10^6$±$7.5x10^4$ | $8.51x10^6$±$9.81x10^5$ | 0.619 |
| | | Weight (g) | 30.36±0.83 | 30.02±0.62 | 0.198 |
| | | PCV (%) | 51.14±1.77 | 50.46±0.79 | 0.125 |
| 3 | 1000 | WBC /$mm^3$ | 8050.4±781.63 | 7277.6±554.66 | 0.227 |
| | | RBC /$mm^3$ | $8.59x10^6$±$8.48x10^5$ | $8.33x10^6$±$5.67x10^5$ | 0.571 |
| | | Weight (g) | 29.26±1.27 | 29.84±1.43 | 0.185 |
| | | PCV (%) | 51.9±0.93 | 50.6±0.82 | 0.211 |
| 4 | NC | WBC /$mm^3$ | 8441.8±740.11 | 8378.4±650.67 | 0.325 |
| | | RBC /$mm^3$ | $8.86x10^6$±$6.92x10^5$ | $9.04x10^6$±$5.23x10^5$ | 0.299 |

**Key:** Values are presented as mean ± SEM: n =5; NC=negative control (0.2ml of dH2O); D0 =day zero; D4 =day four; PCV = packed cell volume: WBC = white blood cell; RBC =Red blood cell; P>0.05.

Methanol extract of *D.angustifolia* seeds treated mice (Table 2) overtime analysis indicated that almost all parameters measured did not show statistically significant difference (P > 0.05) in group 1, 2 and 3 mice administered with 450, 600 and 750 mg/kg, except WBC count of group 3. Such significant reduction in overtime analysis was also

**Table 2:** Subacute toxicity test of methanol extract of seeds of *D. angustifolia* in Swiss albino mice.

| Group | Dose (mg/kg/day) | Parameters | Pre-treatment (D0) | Post-treatment (D4) | P value |
|---|---|---|---|---|---|
| 1 | 450 | Weight (g) | 29.84±1.37 | 29.36±1.47 | 0.120 |
| | | PCV (%) | 50.76±1.02 | 51.72±1.13 | 0.632 |
| | | WBC /$mm^3$ | 7639.6±491.27 | 8194.8±454.69 | 0.052 |
| | | RBC /$mm^3$ | $8.46x10^6$±$6.54x10^5$ | $8.17x10^6$±$5.28x10^5$ | 0.142 |
| 2 | 600 | Weight (g) | 28.96±1.09 | 28.5±1.28 | 0.073 |
| | | PCV (%) | 50.98±1.44 | 51.48±1.2 | 0.059 |
| | | WBC /$mm^3$ | 6967.2±354.2 | 7086±383.12 | 0.113 |
| | | RBC /$mm^3$ | $8.91x10^6$±$3.03x10^5$ | $8.05x10^6$±$4.17x10^5$ | 0.403 |
| 3 | 750 | Weight (g) | 29.76±1.51 | 28.14±1 | 0.069 |
| | | PCV (%) | 51.82±1.41 | 49.36±1.82 | 0.108 |
| | | WBC /$mm^3$ | 7611.8±425.27* | 5484.2±652.25[b] | 0.029 |
| | | RBC /$mm^3$ | $8.42x10^6$±$5.32x10^5$ | $7.65x10^6$±$4.63x10^5$ | 0.206 |
| 4 | NC | Weight (g) | 29.72±1.3 | 30.06±0.72 | 0.509 |
| | | PCV (%) | 52.68±0.65 | 53.08±.0.52 | 0.509 |
| | | WBC /$mm^3$ | 7931±545.18* | 5904.1±506.62 | 0.045 |
| | | RBC /$mm^3$ | $8.32x10^6$±$4.84x10^5$ | $7.78x10^6$±$5.77x10^5$ | 0.093 |

**Key:** Values are presented as mean ± SEM: n =5; NC=negative control (0.2mlof 3% Tween 80); D0 =day zero; D4 = day four; PCV = packed cell volume: WBC = white blood cell; RBC =Red blood cell; P>0.05; *significance difference (P< 0.05).

observed in the RBC count of the control group. Although statistically insignificant, the RBC count of mice treated with the extract at all dose levels; weight of group 2 and 3 and PCV of group 3 were relatively lower on day four than on day zero. In addition, the analysis of variance of treatment groups with the negative control on day four post–infection was not statistically significant in all parameters measured.

**Table 3:** Subacute toxicity test of aqueous extract of leaves of *E. abyssinica* in Swiss albino mice.

| Group | Dose (mg/kg/day) | Parameters | Pre-treatment (D0) | Post-treatment (D4) | P value |
|---|---|---|---|---|---|
| 1 | 500 | Weight (g) | 28.18±0.97 | 28.91±0.97 | 0.261 |
| | | PCV (%) | 51.32±0.63 | 51.9±0.69 | 0.129 |
| | | WBC /$mm^3$ | 9171±253.81 | 8138.6±453.47 | 0.289 |
| | | RBC /$mm^3$ | $8.32x10^6$±$4.96x10^5$ | $8.69x10^6$±$4.6x10^5$ | 0.183 |
| 2 | 750 | Weight (g) | 28.58±1.15 | 29.3±1.13 | 0.402 |
| | | PCV (%) | 51.44±0.59 | 52.46±0.46 | 0.239 |
| | | WBC /$mm^3$ | 8064.6±225 | 8872.4±396.69 | 0.972 |
| | | RBC /$mm^3$ | $7.85x10^6$±$3.05x10^5$ | $8.71x10^6$±$4.57x10^5$ | 0.635 |
| 3 | 1000 | Weight (g) | 29.16±0.67 | 30.08±0.61 | 0.479 |
| | | PCV (%) | 51.24±0.98 | 57.1±0.95 | 0.182 |
| | | WBC /$mm^3$ | 8579±498.67 | 7973.4±413.29 | 0.062 |
| | | RBC /$mm^3$ | $8.52x10^6$±$6.23x10^5$ | $8.4x10^6$±$5.41x10^4$ | 0.069 |
| 4 | NC | Weight (g) | 29.92±1.33 | 30.22±0.9 | 0.102 |
| | | PCV (%) | 51.81±0.72 | 52.18±0.31 | 0.130 |
| | | WBC /$mm^3$ | 8110.1±388.49 | 7674.4±271.25 | 0.067 |
| | | RBC /$mm^3$ | $8.69x10^6$±$4.76x10^5$ | $8.89x10^6$±$7.3x10^5$ | 0.609 |

Key: Values are presented as mean ± SEM: n =5; NC=negative control (0.2ml of dH2O); D0 =day zero; D4 =day four; PCV = packed cell volume: WBC = white blood cell; RBC =Red blood cell; P>0.05.

The subacute toxicity study of aqueous leaves extract of *E. abyssinica* (Table 3) displayed insignificant difference (P>0.05) between the treatment groups and negative control in the weight, PCV, WBC count and RBC count on day four. Similarly, comparison of pre-treatment and post-treatment of all parameter values of mice showed no significant

**Table 4:** Subacute toxicity test of methanol extract of leaves of *E. abyssinica* in Swiss albino mice

| Group | Dose (mg/kg/day) | Parameters | Pre-treatment (D0) | Post-treatment (D4) | P value |
|---|---|---|---|---|---|
| 1 | 500 | Weight (g) | 28.62±0.83 | 31.18±0.71* | 0.041 |
| | | PCV (%) | 50.68±0.6 | 51.36±0.65 | 0.094 |
| | | WBC /$mm^3$ | 7574±464.45 | 8027.8±133.28 | 0.052 |
| | | RBC /$mm^3$ | $8.71x10^6$±$3.94x10^5$ | $9.12x10^6$±$2.48x10^5$ | 0.134 |
| 2 | 750 | Weight (g) | 29.82±0.54 | 28.42±0.42 | 0.101 |
| | | PCV (%) | 50.06±0.47 | 50.88±0.49 | 0.813 |
| | | WBC /$mm^3$ | 6979.2±344.73 | 7747±300.46 | 0.169 |
| | | RBC /$mm^3$ | $8.02x10^6$±$2.73x10^5$ | $7.65x10^6$±$3.87x10^5$ | 0.206 |
| 3 | 1000 | Weight (g) | 29.18±0.72 | 28.8±0.46 | 0.207 |
| | | PCV (%) | 51.1±0.62 | 50.18±0.84 | 0.091 |
| | | WBC /$mm^3$ | 7840.2±501.7 | 9237.2±608.68* | 0.032 |
| | | RBC /$mm^3$ | $8.71x10^6$±$4.2x10^5$ | $8.51x10^6$±$4.66x10^5$ | 0.758 |
| 4 | NC | Weight (g) | 29.16±0.88 | 30.36±0.5 | 0.302 |
| | | PCV (%) | 51.22±0.44 | 50.92±0.5 | 0.662 |
| | | WBC /$mm^3$ | 8213.6±360.63 | 8196.6±366.89 | 0.505 |
| | | RBC /$mm^3$ | $8.3x10^6$±$3.07x10^5$ | $8.75x10^6$±$3.92x10^5$ | 0.114 |

**Key:** Values are presented as mean ± SEM: n =5; NC=negative control (0.2mlof 3% Tween 80); D0 =day zero; D4 = day four; PCV = packed cell volume: WBC = white blood cell; RBC =Red blood cell; P>0.05; *significance difference (P< 0.05).

differences (P > 0.05). However, the mice treated with 1000mg/kg were observed lower WBC and RBC count on day 4. Similarly, the PCV values of mice in group 2 decreased on day 4. In this study, comparison of pre-treatment and post- treatment of all parameter values of mice showed no significance difference (P >0.05).

The toxicity study of methanol leaves extract of *E. abyssinica* (Table 4) showed no significance difference (P>0.05) between the treatment groups of mice and the mice in the negative control in all parameters on day four. The comparison of all parameter values of mice showed no significant difference (P>0.05) between pre-treatment and post-treatment except weight at dose of 500mg/kg and WBC count at dose of 1000mg/kg. However, RBC counts of group 2 and 3 and PCV of group 3 revealed the reduction in values. The highest doses of the four extracts which had caused death and/or significant negative impact on gross behaviors in any one of the experimental mice within a week were not included in suppressive test against *P. berghei* in mice.

**4.2. Antimalarial Tests**

Antimalarial suppressive test of crude methanol and aqueous extracts of *D.angustifolia* and *E. abyssinica* against drug sensitive parasites, *P.berghei* (ANKA strain), in mouse results were shown in table 5-8. The multiple comparison tests indicated that all the mice treated with the four extracts resulted in reduced parasite load as compared to their respective negative control groups. The extracts did not clear the parasite completely, where as positive control groups treated with CQ phosphate, used as a standard antimalarial drug, at daily dose of 25mg/kg body weight totally cleared the parasite on day four under identical condition. Moreover, mice treated with the four extracts survived longer than mice in the corresponding negative control groups.

Water extract of seeds of *D. angustifolia* (Table 5) showed statistically significant (P<0.05) chemosuppression against *P.berghei* at all dose levels (100-1000mg/kg) tested mice compared to the mice in the negative control group on day 4. The mean parasitemia in *P.berghei* infected mice ranged from 5.06 ± 0.59% to 14.3± 1.28%, where as the corresponding figure in the negative control group was 19.85±0.9%. This reveals that the

parasitemia in the experimental mice were lower than that of the negative control groups. The mice treated with CQ were completely cleared the parasites on day 4, but not the highest dose of the extract. Hence, the extract was less potent than CQ phosphate. Treatment with the highest dose, 1000 mg/kg dose of this extract exerted 79.45± 3.02% chemosuppression, not totally cleared the parasites unlike CQ. The mean survival time of mice of the given extract was ranged from 8.65±0.65% to 12.1±1.01% days (increased as the dose increases to 1000mg/kg), which was statistically significant, except the lowest dose (100mg/kg) compared to the negative control group mice (7.45± 0.84 days). Furthermore, although statistically not significant, the mean survival time of mice treated at dose of 100mg/kg was relatively longer than the negative control.

**Table 5:** Antimalalarial activities of aqueous extract of seeds of *D. angustifolia* in male Swiss albino mice infected with *P. berghei*.

| Group | Dose (mg/kg/day) | Antimalarial activity of extract on D4 post- infection | | Survival time (days) |
|---|---|---|---|---|
| | | % Parasitemia± SEM | % Suppression±SEM | |
| 1 | NC | 19.85 ±0.9 | 00.00 | 7.45±0.84 |
| 2 | 100 | 14.3±1.28$^{*a}$ | 27.66±9.19$^{*a}$ | 8.65±0.65 |
| 3 | 200 | 11.45±0.5$^{*a}$ | 43.63±3.14$^{*a}$ | 9.75±0.69$^{*a}$ |
| 4 | 400 | 9.78±0.34$^{*a}$ | 50.73±2.81$^{*a}$ | 10.38±1.11$^{*a}$ |
| 5 | 600 | 7.53±0.59$^{*a}$ | 62.07±3.32$^{*a}$ | 11.3±1.12$^{*a}$ |
| 6 | 800 | 5.3±0.58$^{*a}$ | 72.67±3.91$^{*a}$ | 11.85±1.25$^{*a}$ |
| 7 | 1000 | 5.06±0.59$^{*a}$ | 79.45±3.02$^{*a}$ | 12.1±1.01$^{*a}$ |
| 8 | CQ | 00.00 | 100.00 | ND |

**Key:** Values are presented as M±SEM; n=5; CQ= Chloroquine Phosphate; NC = negative control (0.2ml of dH2O); ND = not done;; D4 = day four; *=P>0.05;
a = comparison against negative control.

The methanol extract of the *D. angustifolia seeds* (Table 6) revealed significant ($P<0.05$) suppressive effect on parasitemia at the oral dose range of 100-600mg/kg per day compared to the untreated control. The average parasitemia of the mice treated with the highest dose (600mg/kg) was 2.85±0.75% and untreated control have 20.67±0.93%,which is by far greater than the treatment groups. The suppressive effect of the extract was also found to be dose dependent, which was increases with increasing in the concentration of the extract, the highest (86.21±3.87%) being recorded at 600mg/kg dose. The methanol extract of D.*angustifolia* seed showed the highest antimalarial activity compared to the other extracts. However, it was 13.79% less potent than CQ phosphate (25mg/kg), which totally cleared the parasite, *P. berghei*, in mice. The mice treated with the extracts had a survival time ranging from 8.75±0.73 to 11.25± 0.69 days, while the corresponding value of the untreated control group was 7.65±0.65 days. The mice treated with this extract, except the lowest dose (100mg/kg), survived significantly longer than mice in the negative control group. However, although statistically not significant, the mean survival time of the mice treated at dose of 100mg/kg was relatively longer than the negative control. The extract showed significant effect on the mean survival time of the treatment groups compared to the untreated control, which is increased as dose the increases (600mg/kg).

**Table 6**: Antimalarial activities of methanol extract of seeds of *D. angustifolia* in male Swiss albino mice infected with *P. berghei*

| Group | Dose (mg/kg/day) | Antimalarial activity of extract on D4 post-infection | | Survival time (days) |
|---|---|---|---|---|
| | | % Parasitemia± SEM | % Suppression±SEM | |
| 1 | NC | 20.67±0.93 | 00.00 | 7.65±0.65 |
| 2 | 100 | 9.63±1.11$^{*a}$ | 53.5±4.78$^{*a}$ | 8.75±0.73 |
| 3 | 200 | 8.08±0.42$^{*a}$ | 62.04±4.12$^{*a}$ | 9.25±0.88$^{*a}$ |
| 4 | 400 | 4.61±0.6$^{*a}$ | 77.71±3.33$^{*a}$ | 10.1±0.86$^{*a}$ |
| 5 | 600 | 2.85±0.75$^{*a}$ | 86.21±3.87$^{*a}$ | 11.25±0.69$^{*a}$ |
| 6 | CQ | 00.00 | 100.00 | ND |

**Key:** Values are presented as M±SEM; n=5; CQ= Chloroquine Phosphate; NC = negative control (0.2mlof vehicle); ND = not done;; D4 = day four; *=P>0.05; a = comparison against negative control

The mean parasitemia in *P.berghei* infected mice ranged from 7.75±0.62% to 13.38±0.15% after four days treatment with the aqueous extract of *E.abyssinica*, where as the corresponding value in the control group was 15.92±0.72% (Table 7). Significant reduction of parasitemia (P<0.05) was observed in all groups of mice treated with aqueous extract of the leaves of *E. abyssinica* compared to that of the negative control.

**Table 7:** Antimalarial activities of aqueous extract of leaves of *E. abyssinica* in male Swiss albino mice infected with *P. berghei*.

| Group | Dose (mg/kg/day) | Antimalarial activity of extract on D4 post-infection | | Survival time (days) |
|---|---|---|---|---|
| | | % Parasitemia± SEM | % Suppression ± SEM | |
| 1 | NC | 15.92±0.72 | 00.00 | 7.75±0.65 |
| 2 | 100 | 13.38±0.15$^{*a}$ | 15.96±2.09$^{*a}$ | 8.2±0.76 |
| 3 | 200 | 11.91±0.64$^{*a}$ | 25.08±4.19$^{*a}$ | 8.95±1.2 |
| 4 | 400 | 10.35±1.17$^{*a}$ | 34.95±7.11$^{*a}$ | 9.25±0.69 |
| 5 | 600 | 9.98±0.39$^{*a}$ | 39.2±2.12$^{*a}$ | 11.45±0.6$^{*a}$ |
| 6 | 800 | 8.71±0.86$^{*a}$ | 45.18±5.26$^{*a}$ | 12.1±0.99$^{*a}$ |
| 7 | 1000 | 7.75±0.62$^{*a}$ | 51.32±4.68$^{*a}$ | 13.05±1.15$^{*a}$ |
| 8 | CQ | 00.00 | 100.00 | ND |

**Key**: Values are presented as M±SEM; n=5; CQ= Chloroquine Phosphate; NC = negative control (0.2mlof vehicle); ND = not done; D4 = day four; *=P>0.05;
a= comparison against negative control

The percent suppression of the mice received the lowest dose (100mg/kg) of the extract was 15.96±2.09% and the mice received the highest dose (1000mg/kg) was 51.32±4.68%. The suppression was found to be dose dependent. In comparison to the other extracts the aqueous extract of *E.abyssinica* revealed the lowest antimalarial activity, which was 48.68% less potent than CQ phosphate. The mean survival time of the mice received the lowest dose (100mg/kg) of the extract was 8.2±0.76 days and the mice received the highest dose (1000mg/kg) was 13.05±1.15 days; where as the negative control was 7.75±0.65 days. The mean survival time of mice treated with 100, 200, and 400mg/kg

were not statistically significant compared to the negative control. Although statistically not significant, the mean survival time of mice treated at doses of 100,200, and 400mg/kg were relatively longer than that observed in the negative control. This indicates that the average parasitemia and survival time of the mice treated with the extract was better than the negative control.

Methanol extract of the leaves of *E. abyssinica* caused statistically significant ($P<0.05$) reduction of parasitemia of *P. berghei* on day four at all dose levels compared to the negative control groups (Table 8). The mice treated with the highest dose (1000mg/kg) of the methanol extract of *E.abyssinica l*eaves had a mean parasitemia of 3.2±0.57% and that of the mice received the lowest dose (100mg/kg) was 9.93±0.81%, while the parasitemia of untreated mice was 13.84±0.87%. Percent suppression of the parasite was 28.25±7.02%, 39.33±2.34%, 52.9±5.77%, 66.4 ± 4.11%, 75.15 ±5.91% and 76.45±5.61% of the 100, 200, 400,600, 800, 1000mg/kg, respectively; compared to the negative control caused statistically significant ($P< 0.05$) effect. Moreover, as indicated in table 8 the mice treated with the extract survived significantly longer than mice in the negative control. The mean survival time of the mice treated with the extract increases as the dose increase.

**Table 8:** Antimalarial activities of methanol extract of leaves of *E. abyssinica* in male Swiss albino mice infected with *P. berghei*.

| Group | Dose (mg/kg/day) | Antimalarial activity of extract on D4 post-infection | | Survival time (days) |
|---|---|---|---|---|
| | | % Parasitemia± SEM | % Suppression±SEM | |
| 1 | NC | 13.84±0.87 | 00.00 | 7.8±0.76 |
| 2 | 100 | 9.93±0.81$^{*a}$ | 28.25±7.02$^{*a}$ | 8.75±1.05$^{*a}$ |
| 3 | 200 | 8.39±0.51$^{*a}$ | 39.33±2.34$^{*a}$ | 10.1±0.84$^{*a}$ |
| 4 | 400 | 6.5±0.68$^{*a}$ | 52.9±5.77$^{*a}$ | 10.95±1.04$^{*a}$ |
| 5 | 600 | 4.65±0.56$^{*a}$ | 66.4±4.11$^{*a}$ | 11.65±0.65$^{*a}$ |
| 6 | 800 | 3.44±0.67$^{*a}$ | 75.15±5.91$^{*a}$ | 12.05±0.89$^{*a}$ |
| 7 | 1000 | 3.2±0.57$^{*a}$ | 76.45±5.61$^{*a}$ | 12.8±0.76$^{*a}$ |
| 8 | CQ | 00.00 | 100.00 | ND |

**Key:** Values are presented as M±SEM; n=5; CQ= Chloroquine Phosphate; NC = negative control (0.2mlof vehicle); ND = not done; D4 = day four*=P>0.05;
a= comparison against negative control

Figure 3 and 4 summarize the results of the antimalarial suppressive effects of the four extracts at 600 mg/kg and 100mg/kg, respectively. Comparison of the suppressive rates at 600mg/kg showed methanol extract of the seeds of *D. angustifolia* to have the highest (86.21%) followed by methanol extract of the leaves of *E. abyssinica* (66.4%) and the aqueous extract of the seeds of *D. angustifolia* (62.07%). Similarly, the comparison of the suppressive effect at 100mg/kg only methanol extract of the seeds of *D. angustifolia* displayed a marginal efficacy (53.21% ) where as other extracts showed insignificant suppression.

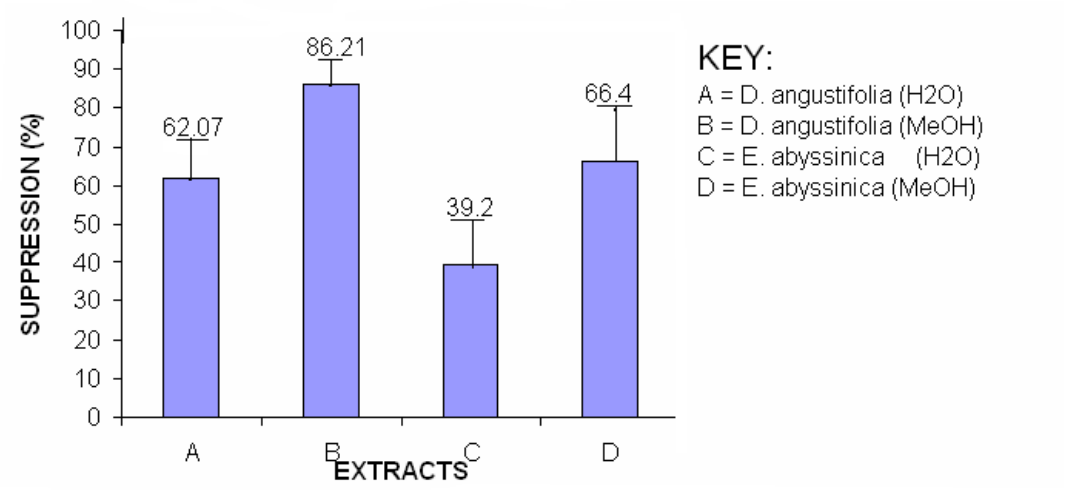


**Figure 3**: Comparison of the antimalarial suppressive effect of the extracts at 600mg/kg in the four day suppressive assay.

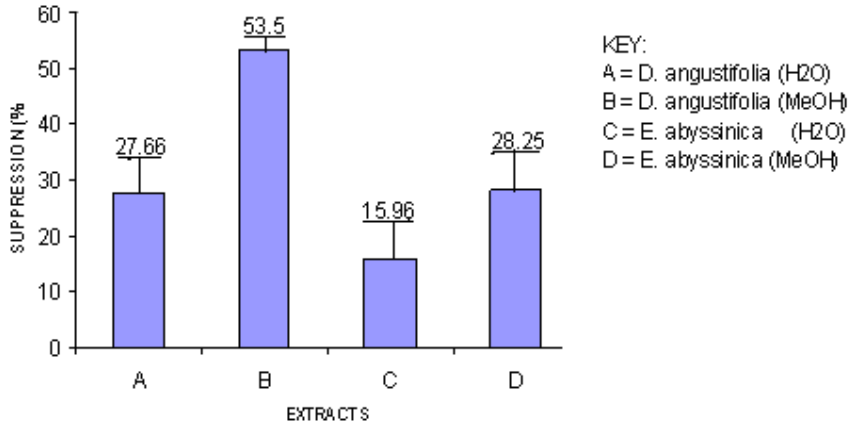


**Figure 4**: Comparison of the antimalarial suppressive effect of the extracts at 100mg/kg in the four day suppressive assay.

## 5. DISCUSSION

*D. angustifolia* and *E. abyssinica* are used to treat malaria traditionally in Ethiopia (Giday *et al.*, 2007). The antiparasitic activities of the plant part extracts demonstrated against *P. berghei* in mice in the present study could be an indication that the extracts possibly are effective against human malaria parasites. Acute and subacute toxicity, which is the main concern of indigenous therapeutic preparations (Jayasinghe *et al*., 2008) were addressed by the demonstrated lack of toxicity of the extracts in mice, in the present study. The fact that changes in general behavior, effect on body weight and mortality, which are critical for the evaluation of adverse effects of a compound on test animals, were not evident on the test animals is good evidence for the absence of toxicity. This fulfills the criteria set for lack of acute toxicity by CDER (1996). Therefore, these extracts were found to be non-toxic to mice, as they did not show signs of acute toxicity within 24 hours at the dose levels up to 1000 / 750 mg/kg body weight.

Statistical comparison of the effect of the extracts on the four toxicity parameters (weight, PCV, WBC and RBC) among groups at fixed time and overtime, have shown the parameters to be within the normal range of values established for mice by Flecknell (1987) (adult body weight (g) = 25 - 40; PCV (%) = 32-54; WBC ($mm^3$) = 5.4 - 16.0 x $10^3$; and RBC ($mm^3$) = 6.7 - 12.5 x $10^6$). Therefore, the present findings indicated that the aqueous extract of seeds of *D.angustifolia* was non-toxic to the test mice at doses less than or equal to1000mg/kg body weight.

In subacute toxicity studies, statistical comparison of the effects of the extracts on the four toxicity parameters (weight, PCV, WBC and RBC)among groups at fixed time and overtime , have shown the parameters to be with in the normal range of values established

for mice by Flecknell (1987) (adult body weight (g)= 25-40; PCV (%)=32-54: WBC ($mm^3$)= 5.4-16.0x $10^3$ and RBC ($mm^3$)= 6.7-12.5x$10^6$).

Since no significant change in the test parameters used in the study of aqueous extracts of the plant parts were evident and neither changes in animal behavior nor other signs of toxicity were recorded during post-treatment evaluation of the experimental mice, it can be assumed that, the extracts can be safe at the dose levels used in the study. Furthermore, treatment with aqueous extracts of the leaves of the plant parts showed no significant toxic effect in mice compared with the negative control in all test parameters. Similarly, since no gross behavioral changes and mortality were evident on post-treatment observations on the mice for a week. Therefore, the aqueous extract of the plant parts can be considered safe at the dose levels used in the experiment.

Subacute toxicity study with methanol extract of *D.angustifolia* seeds up to a dose of 750mg/kg depicted insignificant effect, except on WBC count on day four. Palmerio *et al*, (2003) has suggested that when the control group values are significantly different from the experimental, the values of the experimental mice would be comparable with the normal values. According to him, the WBC count in mice would be considered normal. In addition, the WBC count (5.48x$10^3$cells/$mm^3$) in mice, which show significant difference, lies within the normal range of Flecknell (5.4-16.0 x $10^3$ cells/$mm^3$). Hence, the comparison of the WBC count at the indicated values showed that the extract did not affect the normal value.

The fact that significant increments overtime in weight at the dose of 500mg/kg and WBC count at the dose of 1000mg/kg of the tested mice was observed is good indication that the methanol extract of *E.abyssinica* leaves was not toxic. Since the parameters

measured were within the range of normal values set by Flecknell (1987) no significant difference in other haematological parameters was also observed. In addition, comparison of haematological values with that of the negative controls revealed no significant differences. Therefore, the present finding indicated that the aqueous and methanol extracts of the two plant parts were non-toxic to the test mice at doses less than or equal to 1000 (750 methanol extracts of *D.angustifolia*) mg/kg body weight.

Thus, since *D.angustifolia* and *E.abyssinica* are believed to have several traditional medicinal uses, including malaria treatment by different traditional healers (Giday *et al.*, 2007; Deharo *et al.,* 2001) the experimental determination of lack of acute and subacute toxicity would justify the use of the plant extracts for malaria treatment at primary health care level. The present determination of anti-malarial effect of the leaf extracts of E.*abyssinica* and seed extracts of *D.angustifolia* are additional proof of its medicinal values.

According to Deharo *et al*, (2001) and Munoz *et al, (*2000) an *in vivo* antiplasmodial activity can be classified as moderate, good, and very good if an extract displayed a percent parasite suppression equal to or greater than 50% at a dose of 500, 250 and 100mg/kg body weight per day, respectively. Based on this classification, the aqueous seed extract of *D. angustifolia* exhibited a moderate antiplamodial activity. Previous study on the leaf aqueous extract of this plant has shown moderated (50.99%) parasite suppression at 500mg/kg while its seed extract at 400mg/kg showed a relatively higher (50.73%) suppression against *P. berghei* in mice (Legesse, 2009). This indicates that the seeds of *D. angustifolia* contain a relatively more potent antimalarial compounds compared to its leaf aqueous extracts.

The life prolonging potential of the water extract of *D.angustifolia* in malaria infected mice could be seen from its prolongation of the mean survival time. The mice treated with are extract had significantly longer survival time than the negative control mice, which did not survive longer than eight days. This effect has value in patient care whereby the extracts could be used until such time that curative medication can be found for proper treatment of malaria patients. The presence of remarkable suppression is an indication of the values of the seeds of *D. angustifolia* as a possible source of antimalarial compounds. Previous studies have shown methanol extract of the seeds of *D.angustifolia* to have a high antimalarial activity against *P. falciparum in vitro* (Sorsa, 1992). Similarly, the methanol extract of the root of *D. angustifolia* has been shown to have high *in vivo* suppressive (84.52%) effect at a dose of 600mg/kg against *P.berghei* (Deressa, 2007) and Legesse (2009)has been reported 76.67% at 500mg/kg suppression effect for the methanol extracts of leaves against *P. berghei in vivo*. Thus, the result of this study is consistent with the above mentioned results and also the traditional use of the plant for antimalarial therapy in different parts of Ethiopia (Giday et al., 2007). According to Munoz *et al,* (2000) and Deharo *et al,* (2001) the methanol extract of the plant can be considered to have a very good antiplasmodial activity, since it has significant suppression (53.5%) at the lowest dose (100mg/kg).

The high degree of parasite suppression might be explained by mechanisms of drug action such as having an indirect effect on the immune system or by other pathways that are not yet fully understood (Muthaura *et al*., 2007). The higher parasite inhibitory effect of the extract with unknown compound can be the result of the presence of specific compound that has antiplasmodial effect (Koch *et al.*, 2005). According to Switch and Jarilla (2004) *D. angustifolia* possesses compounds such as alkaloids, saponins,

terpenoids, and flavonoids that could probably be responsible for the antiplasmodial effect of the plant.

In accordance with the rating of Munoz *et al.*(2000) and Deharo *et al.*(2001) the antimalarial effect of *E.abyssinica* aqueous leaf extract falls below moderate since more than 50% suppression was obtained at concentration of 600mg/kg. While its methanol extract was observed to cause a moderate suppressive effect on the parasitaemia. On the other hand, the extract of stem bark of *E. abyssinica* has been reported to possess high antiplasmodial activity against *P. falciparum in vitro* (Kamanzi *et al.,* 2004) and in vivo (Legesse, 2009) against *P. berghei*. Thus, the result of this study is not consistent with the above mentioned and also the traditional use of the plant for antimalarial therapy. On the other hand, the improvement seen in the survival time of mice treated with the water and methanol extracts of *E. abyssinica* was an additional evidence that its potential as a possible source of an antimalarial drug cannot be totally discounted. The antimalarial activity of *E. abyssinica* might be attributed to the presence of diterpens (Alembert *et al*., 2006). However, the active compound known to give the observed activity need to be identified.

The difference between the antimalarial activities of the water and methanol extracts of *D. angustifolia* and *E. abyssinica* may indicate the differences in the presence of active compounds responsible for the observed results. Additionally, the difference possibly is due to the presence of heterogeneous mixture of compounds in the extracts (Pillay, 2006; murgei *et al.,* 2003). However, the water extracts of both plants resulted in lower antimalarial activity compared to its methanol extract, which probably is due to lack of extraction of the active lipophilic constituents into the water (Muthaura *et al*., 2007). The observed low antimalarial activity of plant part extracts tested could be partly explained

by the fact that many antimalarial traditional medicinal plants may lack direct antiplasmodial activity to cure the disease but their beneficial role could be in their antipyretic, analgestic and immune stimulatory effect as demonstrated in other studies (Wanyoki *et al.*, 2004; Murgei *et al.,* 2003; Krettli *et al.*, 2001). Furthermore, in traditional medicinal practice, the healers are known to prescribe concoctions made from different species of plants that might have high synergetic effects. Also, the effectiveness of a given extract can also be influenced by the rate of gastrointestinal uptake and the half life in plasma metabolism of the active compounds (Franssen *et al.,* 1997).

The extracts of the two plant parts did not totally cleared the parasitaemia up to the highest tested doses. Most probably, complete clearance of the parasites may be obtained within the toxic range of the extracts. These findings provide information on the antimalarial effect of the plant extract tested. The safety record demonstrated through toxicity studies further suggests their possible ethnopharmacological usefulness as antimalarials.

# 6. CONCLUSIONS

1. Acute and subacute toxicity studies showed the safety of water and methanol extracts of leaves of *E. abyssinica* and the seeds of *D. andustifolia* in mice.
2. The aqueous and methanol extracts of the seeds of *D.andustifolia* and leaves of *E.abyssinica* possess antimalarial activity as seen in their ability to suppress *P. berghei* infection in Swiss albino mice in a dose related manner.
3. Antimalarial activities as well as the lack of toxicity of the extracts suggest their ethnopharmacological usefulness as antimalarials.
4. Therefore, low antimalarial activity of the aqueous extract, in the present *in vivo* study may not totally rule out the value of the plant as the source of drugs or its traditional use for the treatment of malaria. In particular, the methanol extracts of both plant parts, especially the seeds of *D.angustifolia,* displayed higher chemosuppression and may serve as a potential source for isolation of lead antimalarial compounds.

# 7. RECOMMENDATIONS

7.1 The active components of the plant extracts need to be purified by bioassay-guided isolation method and further work must be undertaken to isolate the active compounds responsible for the observed antimalarial activity of the extracts.

7.2 Further investigation as a potential antimalarial in the Atotus Monkey (*Aotus trivirgatus*) against *P. falciparum* infection will be justifiable.

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## 9. Annex: Photographs during the plant materials collection

Photograph -1: Leave of *E. abyssinica* collection ( near Gojab River)

Photograph -2: Leave of *E. abyssinica* collection (near Gojab river)

Photograph -3: Seeds of *D. angustifolia* collection (near Sekoru )

Photograph -4: Seeds of *D. angustifolia* collection (near Sekoru)