In vivo anti-malarial activities of the solvent fractions of the roots of *Euphorbia abyssinica* J.F.Gmel (Euphorbiaceae) against *Plasmodium berghei infection* in mice

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This is to certify that the thesis prepared by Muluemebet Fikadu, entitled "***In vivo* anti-malarial activities of the solvent fractions of the roots of *Euphorbia abyssinica* J.F.Gmel (Euphorbiaceae) against *Plasmodium berghei* infection in mice**" 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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Abstract

***In vivo* anti-malarial activities of the solvent fractions of the roots of *Euphorbia abyssinica* J.F.Gmel (Euphorbiaceae) against *Plasmodium berghei* infection in mice.**

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Despite all the advances in the health care system, malaria remains a devastating problem in sub-Saharan Africa. Moreover, resistance to the existing pharmacological agents is the major factor overrating the issue. Therefore, researches to discover newer antimalarial agents having better safety and efficacy are being held. Natural products particularly medicinal plants that are known sources of new drugs and/or lead compounds provide potential options. The root of *Euphorbia abyssinica* (Euphorbiaceae) is used to treat malaria in some parts of Ethiopia traditionally and the anti-malarial activity of the 80% hydroalcoholic crude extract of the roots has shown a very good antimalarial activity. However, no bioassay-guided fractionation study is conducted yet. Thus, the current study investigated, the *anti-plasmodial* activities of solvent fractions of the roots of *E. abyssinica* in mice infected with *Plasmodium berghei* using the four-day suppressive test. The antimalarial activities of ethyl acetate, methanol, and aqueous fractions of the crude extract of the roots of the plant were evaluated using parameters like; % parasitemia, mean survival time, body weight, rectal temperature and packed cell volume at doses of 100, 200 and 400 mg/kg/day administered orally. Windows SPSS version 25 was used for data analysis. One-way Analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare data between groups. All the three fractions tested exerted dose-dependent chemosupressive activity. The ethyl acetate fraction showed superior parasite suppression (78.87%) at the highest dose (400 mg/kg/day). The 100 and 200 mg/kg/day doses were also able to produce parasite suppression of 52.43% and 69.57%, respectively. Besides, all the three dose levels of ethyl acetate fraction produced a statistically significant mean survival time (MST) prolongation ($p<0.001$) as compared to the negative control. The methanol fraction also displayed parasite suppression of 50.49 and 68.50 % at 200 and 400 mg/kg/day, respectively. Likewise, at both doses, it prolonged MST significantly ($p<0.001$). However, the aqueous fraction exhibited weaker chemosupressive activity (43.92%)

and a statistically significant ($p<0.001$) prolongation in MST only at the dose of 400 mg/kg/day. A statistically significant ($p<0.001$) prevention of reduction in packed cell volume (PCV) was observed in mice treated with the ethyl acetate fraction at 200 and 400 mg/kg/day. Nevertheless, the methanol and the aqueous fractions failed to prevent reduction in PCV significantly. Except for the larger dose level of ethyl acetate fraction ($p<0.001$), all the dose levels of the three fractions failed to prevent a drop in temperature. Similarly, only the larger dose level of the methanol fraction prevented weight loss ($p<0.001$). From this study, it can be concluded that all three fractions showed promising antimalarial activity. The ethyl acetate fraction displayed a very good antiplasmodial activity. The finding of this study supports its traditional use and the previous study conducted on the crude extract of the plant. Thus, there is a need for further studies on the plant including evaluation of prophylactic activity and isolation of compounds responsible for bioactivity, particularly from the ethyl acetate fraction.

Keywords: *Euphorbia abyssinica,* solvent fractions, *in vivo* antimalarial, chemosupressive, *Plasmodium berghei*

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List of Abbreviations and/or Acronyms

ACT	Artemisinin-based combination therapies
ANOVA	Analysis of variance
CD36	Cluster of differentiation 36
CDC	Center of Disease Control
CR1	Complement receptor 1
CS	Citrate synthase
CSA	Chondroitin sulfate A
DDT	Dichlorodiphenyltrichloroethane
DHFR	Dihydrofolate reductase
DMSO	Dimethyl sulfoxide
EPHARM	Ethiopian pharmaceutical manufacturing share company
GPI	Glycosylphosphatidylinositol
HRP-2	Histidine-rich protein-2
HS- GAGs	Heparan sulfate-like glycosaminoglycans
ICAM1	Intercellular Adhesion Molecule 1
IgG	Immunoglobulin G
ILI	Interleukin I
IRS	Indoor residual spraying
ITN	Insecticide-treated nets
LDH	Lactate dehydrogenase
MST	Mean survival time
PBO	Pyrethroid- piperonylbutoxide
PCR	Polymerase chain reaction
Pfcsp	Plasmodium falciparum circumsporozoite protein
PfEMP1	Plasmodium falciparum erythrocyte membrane protein 1
PfKelch13	P. falciparum Kelch 13
Pfmdr1	Plasmodium falciparum multidrug resistance transporter 1
pfpI3K	Plasmodium falciparum phosphatidylinositol 3-kinase

POC	Point of care
pRBC	Parasitized red blood cell
RBC	Red blood cell
RDT	Rapid diagnostic test
TFC	Total flavonoid content
TNF- α	Tumor necrosis factor alpha
TPC	Total phenol content
TSP	Thrombospondin
WHO	World health organization

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1. Introduction

Malaria is named after the Italian term 'mal'aria', which means 'bad air' to represent the association of the disease with marshy areas [1]. It is an endemic vector-borne parasitic disease caused by protozoan parasites of the genus *Plasmodium* in tropical and subtropical regions worldwide [2]. *Plasmodium* consists of over 200 species, infecting mammals, birds, and reptiles, and malaria parasites generally tend to be host-specific [3]. *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale,* and *Plasmodium knowlesi* are the five known species of the genus *Plasmodium* that causes malaria in humans [4, 5]. Of the five *Plasmodium* species that cause malaria in humans, *P. falciparum* causes severe malaria [6].

The life cycle of the parasites is a complex process occurring in both vertebrate hosts and a mosquito vector. Besides, it undergoes both sexual and asexual reproduction mechanisms. This makes the development of drugs and vaccines challenging [7].

The first trial to treat malaria dated back to the 2nd century before Christ (168 BC) when China used Qinghai (Latin *Artemisia annua*) for the treatment of fever and chills [8]. The next documented trial was in the 16th century when the Spanish invaders in Peru discovered the *Cinchona* medications against malaria from the bark of the *Cinchona* tree (Latin *Cinchona succirubra*). The active ingredient of *Cinchona succirubra* that had been used for many years in chemoprophylaxis and treatment of malaria was isolated in 1820 by the French chemists Pierre Joseph, Pelletie, and Joseph Bienaimé Caventou. In 1970, a group of Chinese scientists led by Dr. Youyou Tu isolated an active substance Artemisinin, a compound that has proven activity against malaria, from the plant *Artemisia annua* [9].

Though artemisinin and its derivatives are potent treatments for malaria and are being widely used in combination therapies worldwide, resistance is emerging in some parts of the world. This calls for the need to discover new anti-malarial agents possessing high therapeutic value with minimal toxicity, and lower cost [10].

1.1. Epidemiology of malaria

Malaria is a global public health burden with an estimated 229 million cases reported worldwide (from 87 malaria-endemic countries) in 2019. About 94% of the reported cases (215 million cases) were recorded in Africa region. The Southeast Asia region accounted for about 3% of the burden of malaria cases globally. In the same year, an estimated 409,000 deaths from malaria have occurred worldwide of which 94% happened in Africa [11].

Malaria affects majorly children under the age of 5 years; with 67% death from the total death in 2019. Underdeveloped immunity is thought to be the major reason that makes children under five years of age vulnerable to malaria. Aftereffects of fever and illness like reduced appetite, limited social life, and restricted play contributes to meager growth [12].

In Ethiopia, 2.5 million malaria cases were reported in 2019. Over the past twenty years, Ethiopia has shown a significant reduction in the burden of malaria and able to transform from control to elimination strategy in some regions of the country. For instance, in the Harari region, the incidence of malaria cases decreased from 42.9 cases per 1000 population in 2013 to 6.7 cases in 2019 and mortality decreased from 4.7 deaths per 1,000,000 populations in 2013 to zero in 2015 [13].

P. falciparum and *P. vivax* are the two predominant *Plasmodium* species distributed all over the country, accounting for 60% and 40% of malaria cases, respectively. The primary malaria vector in the country is *Anopheles arabiensis* and the second vector is *Anopheles pharoensis* [11].

1.2. Etiology of malaria

Protozoan parasites of the genus *Plasmodium* originate from photosynthetic protozoa named *Dinoflagellates*. About 200 different species of the protozoa have been identified so far and among them at least 13 species are known to be pathogenic to humans [14]. Five of the parasites namely *P. falciparum, P. vivax, P. malariae, P. ovale* (*P. ovale curtisi* and *P.ovale wallikeri*), and *P. knowlesi* are well-known etiologies of malaria in humans [15].

In Africa, the most prevalent and pathogenic species is *P. falciparum*. However, malaria infection from most malaria-endemic regions of Africa shows the presence of multiple sympatric species

and co-infection within an individual human host or mosquito vector [16]. *P. malariae* is the species most commonly found in sympatry with *P. falciparum* in malaria-endemic regions of Africa [17].

In each endemic area, malaria is transmitted by a specific set of *Anopheles* species [18]. So far, more than 400 different species of *Anopheles* mosquitoes have been identified. But only 30 of them are known to transmit malaria. All vectors of malaria undergo the bite between dusk and dawn [19].

Stability is observed in the distribution pattern of the mosquito species in malaria-endemic regions of the Africa continent [18]. The two major vectors of malaria in Africa are two species complexes namely *Anopheles gambiae* and *Anopheles funestus* [19]. *Anopheles gambiae* comprises different species including *Anopheles coluzzii* and *Anopheles amharicus* [20]. *Anopheles coluzzii* is widely distributed in west-central Africa while Anopheles amharicus, named after the Ethiopian language Amharic, is the Ethiopian member of the *Anopheles gambiae* complex [21]. Complete disappearance of a given vector species from a region is unusual and when the non-indigenous vector is introduced to the area, it is a serious public health concern since it is known to result in devastating epidemics. Indigenous vectors are hard to eradicate with known vector eradication activities [18].

1.2.1. The life cycle of malaria parasite

The life cycle of the malaria parasite is a complex process comprising an *Anopheles* mosquito and a vertebrate host [22]. The first stage of the infection is the entrance of the sporozoites in mosquito saliva into the skin and bloodstream of the human host and then, it invades hepatocytes to undergo asexual replication [23]. During this phase (hepatic or pre-erythrocytic phase) the rupture of infected hepatocytes results in the release of thousands of merozoites [24]. In the case of *P. vivax* and *P. ovale* infections, some form dormant hypnozoites which remain within hepatocytes for periods of several months, and even as long as 4 years, before developing and multiplying to initiate a new episode of erythrocytic infection [25].

The erythrocytic infection involves the interaction of the merozoites with the red blood cells (RBC). The merozoites head orients and adjoin with erythrocytes membrane through deforming

the surface host cell. Then, through parasite-induced reorganization of the erythrocyte cytoskeleton, the parasite enters the erythrocyte to undergo the second asexual reproduction [26]. While younger erythrocytes are targeted favorably by *P. vivax* and *P. ovale,* erythrocytes of any age are invaded by *P. falciparum* and *P. knowlesi.* In contrast, *P. malariae* prefers senescent erythrocytes [23]. After invading RBC, merozoites reproduce to trophozoites and then to schizonts which erupt from the erythrocytes to release merozoites and reinvade new RBCs and continue the asexual replication cycle [26].

The sexual reproduction cycle of malaria starts when a portion of trophozoites matures to male and female sexual progeny or gametocytes [27]. The transmission of the malaria parasite from the mammalian host to the mosquito is mediated by these gametocytes. During the bite of an anopheles mosquito, the matured gametocytes will be taken to the midgut of the mosquito [28]. Inside the midgut, gametocytes get converted into fertile gametes and the next stage involves the conversion of zygotes into ookinetes which are motile and invasive [29]. The ookinetes in turn get converted into oocysts in midgut basal lamina. The oocyst then matures releasing sporozoites, which migrate to the salivary gland of the mosquito. The parasite is transmitted to another mammalian host through an infected mosquito bite [30].

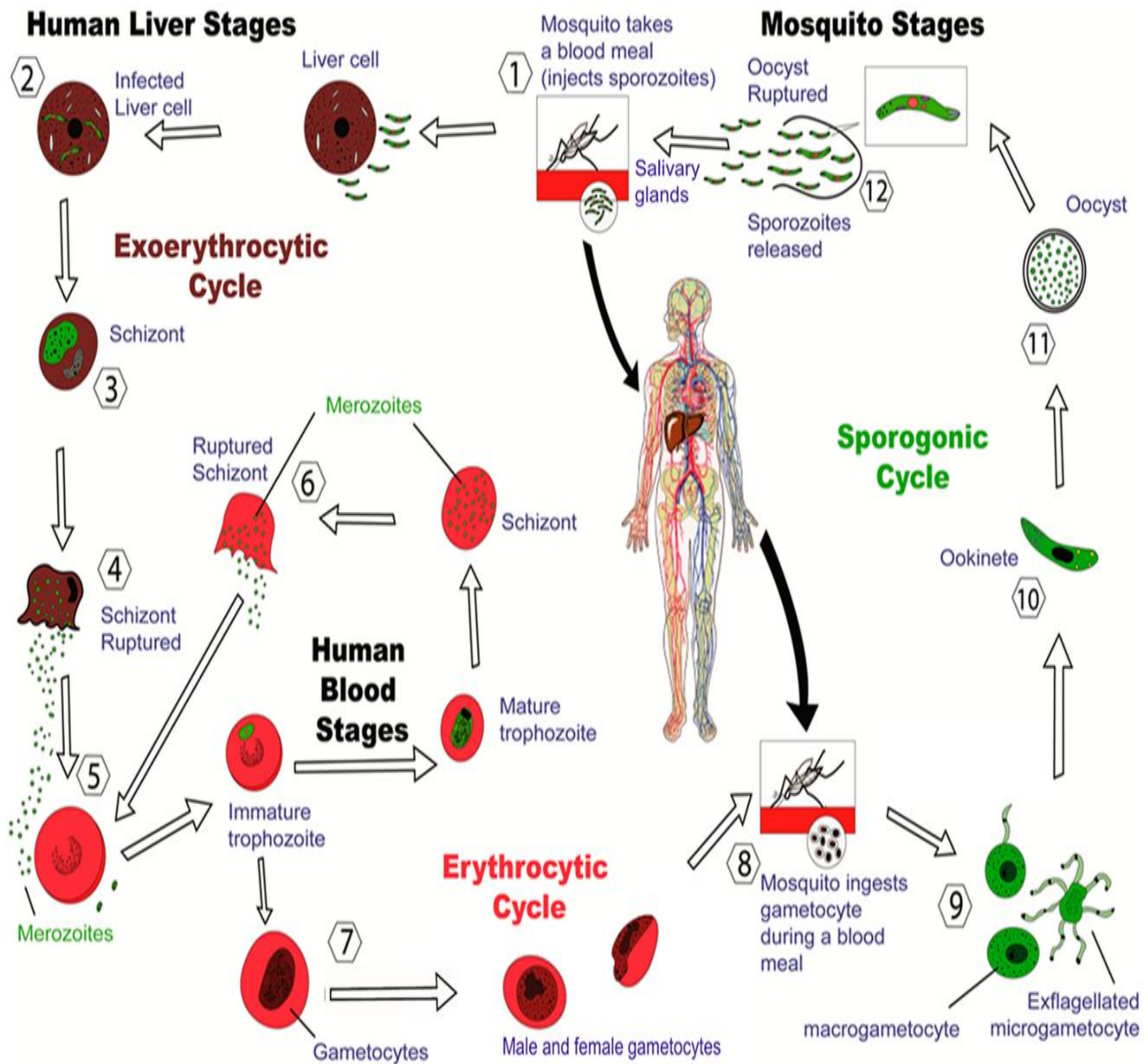

Figure 1: The life cycle of malaria [31]

1.3. Pathophysiology of malaria

The pathophysiology of uncomplicated malaria is characterized by fever [24] secondary to the rupture of erythrocytes, macrophage ingestion of merozoites, and/or the presence of antigen-presenting trophozoites in the circulation or spleen which mediates the release of tumor necrosis factor α (TNF-α) [31]. Fever associated with malaria infection is known by its periodicity which differs among different species of the parasite. Tertian fever ("tertian malaria") is expected in *P. vivax* and *P. ovale* malaria as a progeny of schizonts matures every 48 hours in these species. In contrast, *P. malariae* is attributed to quartan fever ("quartan malaria") which occurs every 72 hours. However, the fever in falciparum malaria may occur every 48 hours, but is usually irregular, showing no distinct periodicity [24].

The binding of matured infected RBC to host endothelial cells (cytoadherence) is the major player in the pathogenesis of severe malaria [32]. The expression of genes that encode proteins involved in cytoadherence and immune evasion explain the virulence of *P. falciparum* when compared with other species. The *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), rifin, and stevor proteins are encoded by members of the var, rif, and stevor gene families, respectively [33]. Var gene-encoded PfEMP1 is the best-characterized variant surface antigen which is expressed on the surface of infected erythrocytes where it mediates binding to endothelial receptors [34].

PfEMP1 family forms electron-dense protrusions named knobs on the membrane of parasitized RBC (pRBC) by getting inserted into and protruding from the erythrocyte membrane. Knobs serve as a site by which parasitized erythrocyte binds to other cell surfaces like normal RBC and endothelium [35].

The adhesion of parasitized erythrocyte to vascular endothelium leads to sequestration, the phenomenon by which infected RBCs get removed from the peripheral circulation through getting bound to the vascular endothelium, in the deep microvasculature of various tissues and organs [36]. Host molecules like Cluster of differentiation 36 (CD36), intercellular adhesion molecule-1 (ICAM1), thrombospondin (TSP), P-selectin, chondroitin sulfate A (CSA), and protein C receptor have been identified as a receptor binding for pRBC to the endothelium [37]. For instance, when PfEMP1 on infected RBCs binds to host receptors such as ICAM-1 and CD36 on brain endothelial cells, it mediates sequestration to cause cerebral malaria [38].

On the other hand, pRBCs can bind to uninfected RBC and impair microcirculation then cause hypoxia. The phenomenon is called rosettes, the spontaneous binding of normal RBCs to malaria-infected RBCs. Blood group antigens A and B, CD36, complement receptor 1 (CR1), and heparan sulfate-like glycosaminoglycans (HS-GAGs) are the five identified receptors on RBCs implicated in the process of rosettes [36].

Parasite-derived molecules called toxins are also implicated in the pathogenesis of severe malaria [39]. Glycolipids named glycosylphosphatidylinositol (GPI), coupled with protein or free form, induce overproduction of cytokines: TNF and interleukin I (ILI) by macrophages [40]. Although cytokines have a physiological role in defending microorganisms including malaria parasite when produced in lower amounts [41], overproduction causes high-grade fever, upregulation of endothelial receptor expression, and upregulation of nitric oxide production, this in turn may cause local damage and suppression of erythrocyte production in the bone marrow [43].

1.4. Diagnosis of malaria

Diagnosis of malaria can be done through clinical observation of the signs and symptoms of the disease. However, clinical diagnosis of malaria has poor accuracy due to the resemblance of the clinical symptoms with other tropical diseases and the possibility of incidence of coinfection [42]. Other diagnostic techniques used to diagnose malaria are microscopic detection of parasites from blood smears and antigen-based rapid diagnostic tests. The latter is based on immunologically detecting different malaria antigens such as lactate dehydrogenase (LDH), aldolase, and histidine-rich protein-2 (HRP-2) in a small amount of blood [43].

Though microscopy and rapid diagnostic test (RDTs) are being used widely, they are less sensitive and less selective to malaria parasites. The shortcomings of the conventional techniques necessitate the development of molecular and biosensing-based methods which are more accurate, easy to quantify and allow point of care (POC) application. Thus, newly developed techniques like dielectrophoretic and magnetophoretic detection are emerging [44]. However, polymerase chain reaction (PCR) based nucleic acid detection methods that are highly sensitive are applicable only in research laboratories because of their high running and maintenance costs [43].

1.5. Management of malaria

The management of malaria involves preventive and/or curative approaches. Since untreated uncomplicated malaria can progress to severe malaria, early diagnosis and effective rational treatment are the first core principles in the management of malaria. To prevent or delay the spread of antimalarial drug resistance, WHO recommends the use of combination therapy to all episodes of malaria with at least two effective antimalarial agents having a different mechanism of action [45].

1.5.1. Pharmacological treatment of malaria

Antimalarial agents can be grouped as quinoline derivatives, antifolates, and artemisinin derivatives based on their chemical structures and/or mechanism of action [46]. Quinoline derivatives that comprise chloroquine, amodiaquine, quinine, quinidine, mefloquine, primaquine, lumefantrine, and halofantrine are active against the erythrocytic stage of the parasite [47]. Among them, primaquine is active against the hepatic stage of the parasite and gametocytes [48].

The antimalarial mechanism of quinolone derivatives is proposed to be the result of the following two-step activities; the first step involves retarding deposition of heme onto the crystal surface by capping the growing hemozoin crystals and the second step involves complexing with free heme in the lumen of the digestive vacuole. The overall outcome of both steps is killing the parasite by halting heme crystalization after being released from the hemoglobin [49].

Antifolate antimalarial agents can be grouped as class I and class II, based on their mode of action. Class I antifolate agents act by inhibiting the production of dihydrofolic acid through inhibition of the enzyme dihydropteroate synthase (DHPS) and hence the synthesis of nucleic acid [50]. Class II antifolate agents block the reduction of dihydrofolate to tetrahydrofolate by inhibiting the enzyme DHFR in the parasite. Tetrahydrofolate is important for the production of nucleic acid and amino acid. Class II agents have schizonticidal activity and they act on the asexual form of the parasite [51]. Sulfadoxines are among the class I antifolate agents while Proguanil and pyrimethamine belong to Class II antifolate agents [50].

Artemisinin and its derivatives like artesunate, artemether, arteether, and dihydro-artemisinin are of natural origin [52]. Generation of free radical was the first suggested mechanism of action of artemisinin and its derivatives. The malaria parasite is known to be rich in heme since it causes proteolysis of the host cell hemoglobin. Therefore, artemisinin interacts with intraparasitic heme and gets activated into toxic free radicals through the process. A resulting carbon-centered free radical then kills the parasite by alkylating and denaturing one or more essential malarial proteins. The fact that artemisinin is selectively toxic to the parasite is attributed to its selective interaction with intraparasitic heme [53].

1.5.2. Prevention of malaria

1.5.2.1. Malaria chemoprophylaxis

Causal prophylaxis is administering a drug active against the pre-erythrocytic (liver stage) malaria parasite. These drugs can be discontinued after leaving the malaria-endemic area. Whereas, suppressive prophylaxis represents administering drugs that act against asexual blood-stage (erythrocytic) parasites. These drugs must be taken for at least 4 weeks after leaving the area to eliminate asexual parasites emerging from the liver weeks after exposure unlike causal prophylaxis [54]. In areas where *P. falciparum* malaria is prevalent, for instance in sub-Saharan Africa, suppressive prophylaxis is indicated. Whereas, in areas where *P. vivax* coexists with *P. falciparum* or alone causal prophylaxis is recommended [55].

Center for disease control emphasizes that there is no antimalarial agent that can prevent malaria 100%. Therefore prophylaxis shall be augmented with the use of personal protective measures. Currently, there are four drugs approved to be taken for chemoprophylaxis against malaria namely atovaquone/proguanil, chloroquine, doxycycline, and mefloquine. Selection is based on client factors (pregnancy, disease conditions like renal impairment and cardiac conduction abnormalities), cost, preference on the frequency of administration, tolerability, resistance profile of the area, and the like [56].

1.5.3. Vector control

Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are the two currently applicable malaria vector control methods recommended by WHO [57]. Whether treated with insecticide or

not, bed nets provide a physical impediment against insects. When treated with insecticide, it provides further protection by killing insects coming in contact with the net. Pyrethroids, like permethrin and deltamethrin, were the only insecticides used to impregnate bed nets [58].

The emergence of pyrethroid-resistant *anopheles* necessitates the discovery of pyrethroid-piperonyl butoxide (PBO). PBO works in synergy with pyrethroid by inhibiting parasitic metabolic enzymes like mixed-function oxidases that quench insecticides action by sequestering and detoxifying [59].

IRS is employed to prevent the entry of mosquitos by covering the walls and floors of a house with insecticide. The effect of insecticide lasts for several months [60]. According to WHO, five chemical classes that meet the safety and efficacy level stated by the WHO prequalification are advised to be used for IRS: pyrethroids, organochlorines, carbamates, organophosphates, and neonicotinoids. Nevertheless, the organochlorine insecticide, dichlorodiphenyltrichloroethane (DDT), is not included in the prequalified list [61].

1.5.4. Malaria vaccine

Resistance of the parasite to antimalarial agents and toxicity associated with chemoprophylaxis arose the need for the development of an effective vaccine against malaria. Recently, researchers are focusing on designing vaccines and so far, one candidate has emerged to reach a large phase-III trial. In addition, other promising candidates are also under investigation. In general, malaria vaccines can be grouped as pre-erythrocytic, erythrocytic, and transmission-blocking vaccines based on their target on the malaria parasite lifecycle [62].

RTS,S/AS01 is a monovalent recombinant protein vaccine successfully passed to advanced clinical trial and studied well in the blockage of *P. falciparum* sporozoite. It initiates an immune response against a protein covering the surface of sporozoite named circumsporozoite protein (PfCSP) [63]. Thus it promotes immunoglobulin G (IgG) antibody response towards the region of the citrate synthase (CS) protein and potent T-cell (CD4+) [62].

RTS,S/AS01 is currently recommended by WHO for use on children in sub-Saharan Africa and other regions of the world with moderate to high transmission of *P. falciparum*. It should be administered in a schedule of 4 doses in children starting from the age of 5 months. This decision

is made based on the result observed on the ongoing pilot program in Ghana, Kenya, and Malawi which covered 800,000 children since 2019. The pilot program in these three countries will continue to uncover the advantage of administering the 4th dose and the long-term outcome on child deaths [64]. Similarly, PAMAVAC is a promising blood-stage malaria vaccine among vaccines in the pipeline [65].

1.5.5. Resistance to anti-malaria agents

Though artemisinin and its derivatives are potent treatments for malaria and are being widely used in combination therapies worldwide, resistance is emerging in some parts of the world. This calls for the need to discover new anti-malarial agents possessing high therapeutic value, minimal toxicity, and low cost [66].

Antimalarial drug resistance has been defined as “the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject, given that the drug in question got access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action” [67].

In 1978 and 1995 the first case of chloroquine resistance by *P. falciparum* was observed in Africa and Ethiopia, respectively. Similarly, chloroquine treatment failure against *P. vivax* has been reported in Debre Zeit, Ethiopia in 1995 [68]. The mechanism of resistance to quinolones is primarily associated with an elevated level of drug efflux. Overexpression of p-glycoprotein transporter, *P. falciparum* multidrug resistance 1 (Pfmdr1) has been implicated in reduced responsiveness of the parasite to chloroquine and other quinolone antimalarial agents [69].

Resistance to antifolate antimalarial agents is found to be a result of a mutation in the target enzyme dihydrofolate reductase (DHFR). A study conducted by Sirawaraporn and Yuthavong [70], using a partially purified DHFR obtained from a cloned strain of pyrimethamine-sensitive *P. chabaudi* and its derived drug-resistant strain, showed a significant decrease in the affinity of binding of pyrimethamine to the enzyme from the resistant clone. Likewise, alterations in kinetic and other properties were also observed. This supports the claim that resistance is a result of the genetic change which further leads to a structurally different enzyme.

Artemisinin-resistant strains of malaria were first reported in 2008 and then spread in South East Asia (Greater Mekong) but not significantly in Africa so far [71]. In Africa, due to the high prevalence of *p. falciparum*, there is repeated exposure of the host to malaria resulting in a higher degree of acquired immunity which intern enable the host to defend against artemisinin-resistant infections. *P. falciparum* Kelch 13 (PfKelch13) is a molecular marker used to map the geographical distribution of artemisinin resistance. It is a substrate adapter for cullin E3 ligase, with a putative substrate of *P. falciparum* phosphatidylinositol 3-kinase (PfPI3K) and a redox sensor. Mutant K13 results in lowered artemisinin interactions with PfPI3K [72].

The emergence of resistant strains of malaria and the spread of the disease urges for a relentless search of antimalarial agents of a new mechanism of action with better safety and efficacy profile. Therefore, studies to develop new antimalarial agents having a distinct target from the conventional agents, with well-characterized safety, efficacy, and toxicity profile has to be one of the priorities of health science [73].

1.6. Traditional medicinal plants used in the management of malaria

Natural products are the direct or indirect sources of most of the drugs introduced in the past 30 years. Plants are a rich source of lead compounds for the development of new drugs against protozoan parasitic diseases such as malaria. Quinine and artemisinin are potent antimalarial natural products obtained from plants. Further development of these two drugs has also given rise to synthetic quinoline and artemisinin classes of antimalarials that form the basis of artemisinin-based combination therapies (ACT) [74].

About 80% of the population in developing countries depend on traditional medicine for their primary health care needs [75]. This could be attributed to different reasons which include poor access, unaffordability of modern medicine, and cultural acceptability of traditional medicines [76].

As part of developing countries, about 80% of the Ethiopian population as well uses traditional herbal medicines for various illnesses [77]. There are about 6,000 species of vascular plants in Ethiopia. Ten (10%) of them are endemic which could be due to the different topography and climatic conditions [78]. Ethiopia is a member of the top six plant-rich African countries. Among

the plants found in Ethiopia, 60% of them are said to be indigenous. In Ethiopia's traditional herbal medicine, plant species exceeding 1000 have been reported for use, and about 33 of these species are endemic to the country [79].

Many plants having medicinal value have been studied for their anti-malarial activity. Some of them showed a potent *in vitro* and *in vivo* activity [80]. Eighty-two studies identified a total of 200 different plant species used in traditional malaria treatments throughout Ethiopia [77]. Some of the plants found in Ethiopia and studied for antimalarial activity are presented in Table 1.

Table 1: Example of plants in Ethiopia studied for antimalarial activity

Plant name	Part studied	Study design	Result
Schinus molle (Anacardiaceae)	Seeds	Rane's Test and Repository Test	Active [81]
Bersama abyssinica Fresen. (Melianthaceae)	Leaves	Four-Day Suppressive Test, Rane's Test, and Repository Test	Active [82]
Cordia africana (Lam.) (Boraginaceae)	Leaves	Four-Day Suppressive Test, Rane's Test, and Repository Test	Active [83]
Salvadora persica L. (Salvadoracea)	Roots	Four-Day Suppressive Test	Active [84]
Balanites rotundifolia (Zygophyllaceae)	Leaves	Four-Day Suppressive Test	Active [84]

1.7. *Euphorbia abyssinica*

Euphorbia abyssinica J.F.Gmel, also known as "*kulkual in Amharic*" and "*Adami in Afan Oromo*" in Ethiopia [85], belongs to the family Euphorbiaceae, which includes about 300 genera and 7500 species of plants. The genus *Euphorbia* consists of over 2000 species of plants in all temperate and tropical regions [86].

Euphorbia abyssinica is a succulent, thorny tree, growing up to 10m in height. It grows in moist mountain forests, humid woodlands, and scrub savannahs and grows at an altitude of 1400 to 2400 meters, usually above 1900 meters. The plant is endemic to Eastern Africa particularly Ethiopia,

Eritrea, Somalia, and Sudan [85]. However, there is no documented evidence for the use of the plant as a folk medicine other than in Ethiopia, rather it is regarded as toxic and used for firewood and timber (roofing, matches, boxes, local tables, wooden saddles). The plant produces milky vesicant/poisonous latex which in part contributes to its traditionally claimed medicinal property [87]. It is taken in milk, or eaten with bread, to treat gonorrhoea and ascariasis and applied to wounds to facilitate healing [88].

The boiled fresh bark of the plant has also a traditional medicinal role in treating gastrointestinal complaints when taken orally [89]. Likewise, the root part of the plant is also used in the folk medicine of Ethiopia. For the treatment of malaria, both traditional healers and the community collect the fresh root, dry, chop, and mill it to be eaten with egg. Drinking milk after taking the prepared plant material is recommended [90].

Kothale and Pawade (2011) studied the chemical constituents of the family Euphorbiaceae by taking a sample, found a heterogeneous character. All genera studied are closely similar to each other due to the absence of saponin, tannins, and phlobatannins and the presence of alkaloids while iridoids, flavonols. steroids, cardenolides, anthraquinone, leucoanthocyaninm, and phenolics found in some genera and lack in others [91]. Phytochemical analysis of the stem-bark and latex extracts of *E. abyssinica* showed the presence of several compounds which include alkaloids, flavonoids, tannins, cardiac glycosides, carbohydrates, steroids, and saponins [92].

1.8. Significance of the study

Despite the tremendous advancements made to control malaria, the disease is still a global concern. In addition to the fact that WHO prequalified very few classes of insecticides, the emergence of resistant vectors and the spread to the newer area increased the transmission of malaria. The emergence and spread of resistance against effective anti-malarial agents and adverse drug reactions and drug interaction overrate the problem. As plants are known sources of a lead compound in drug discovery, the result of this study could provide an insight to acknowledge the antimalarial activity of specific solvent fractions of the study plant and it could be an input to

further isolate an active compound from and investigate the mechanism of action of a particular solvent fraction with better antimalarial activity.

Figure 2: Distribution of *E. abyssinica* [93]

Figure 3: *E. abyssinica* J.F.Gmel- Central Plateau, Somaliland photo taken by Helen Pickering [93]

2. Objectives

2.1. General objective

To evaluate *in vivo* anti-malarial activities of solvent fractions of the roots of *Euphorbia abyssinica* J.F.Gmel against *Plasmodium berghei* infection in mice

2.2. Specific objectives

- To determine effects of each solvent fraction on the level of parasitemia in mice infected with *P. berghei* in a four-day suppressive test
- To determine the effect of each solvent fraction on body weight, mean survival time, rectal temperature and packed cell volume of the mice infected with *P. berghei* in a four-day suppressive test
- To conduct qualitative and quantitative phytochemical analysis on the solvent fractions.

3. Materials and Methods

3.1. Materials

3.1.1. Plant Material

The roots of *E. abyssinica* (Euphorbiaceae) were collected on May 22/2020 from Ambo town located in the western Shewa zone of the Oromia region that is about 125km west of Addis Ababa. Identification and authentication of the plant specimen was done by a taxonomist, Mr. Melaku Wondafrash. Then voucher specimen (MF001) was deposited at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University.

3.1.2. Drugs, Chemicals, and Instruments

The main chemicals used in the study were acetic anhydride (Carlo erba, France), ammonia (CDH limited, India), chloroquine (EPHARM), chloroform (Loba chemei, India), diethyl ether (CDH limited, India), dimethyl sulfoxide (DMSO) (BDH chemicals LTD, England), distilled water (Physical chemistry laboratory, SOP, CHS, AAU), ethyl acetate (CarloErba, France), ferric chloride (BDH chemicals LTD, England), giemsa stain (10%) solution (BDH chemicals LTD, England), hydrochloric acid (Riedal de Haen, Germany), lead acetate (BDH chemicals LTD, England), methanol (Sisco research laboratories, India), n-hexane (Loba chemei, India), normal saline (Frensenius kabi, India), sulfuric acid (BDH chemicals LTD, England), sodium hydroxide (BDH chemicals LTD, England), trisodium citrate (BDH chemicals LTD, England), tween 80 (Lobe chemi, India) and oil immersion (Neolab, India). All the reagents used were analytical grade. The main equipment used for the study were; mortar and pestle, Whatman filter paper no.1 (whatman®, England), syringes, electrical balance, microscopic slides, soxhlet, rotary evaporator (Buchi rota vapor R-200, Switzerland), microscope (Carl-Zeiss, Germany), hematocrit centrifuge (Centurion scientific, UK), separatory funnel and lyophilizer (Operan, Korea vacuum limited, Korea).

3.1.3. Experimental Animals

Seventy-five healthy male Swiss albino mice (8–12 weeks, weighing 20–33 grams) were obtained from the animal house of the School of Pharmacy, Addis Ababa University for anti-malarial

activity testing. They were housed in a plastic cage under a natural 12/12 h light-dark cycle at room temperature. They had free access to a pellet diet and water *ad libitum*. All mice were acclimatized to the working environment for one week before the study. All experiments were conducted following the internationally accepted guidelines on laboratory animal use, care, and handling (guide for the care and use of laboratory animals, 2011) [94] and after obtaining ethical clearance from the ethical review committee of the School of Pharmacy, College of Health Sciences, Addis Ababa University.

3.1.4. Parasites

Chloroquine-sensitive *P. berghei* ANKA strain was used to induce malaria in the experimental mice. *P. berghei* infected donor mice were obtained from Mekelle University, Tigray Region, Ethiopia. Consecutive passage of blood was done from infected mice to non-infected ones weekly for the maintenance of the parasite.

3.2. Methods

3.2.1. Extraction

The collected fresh roots were cleaned, washed with tap water, and allowed to dry in the open air under shade at room temperature. Then, coarsely powdered dried plant material (600g) was extracted by cold maceration with 80% methanol (1.8 L) for three consecutive days at room temperature. Next, the marc obtained was remacerated twice using the same volume of 80% methanol. Occasional stirring was employed to facilitate the extraction process.

The resulting liquid extract was then filtered with filter paper (Watman number 1) & concentrated under reduced pressure using a Rota vapor and the concentrated extract was freeze-dried using a lyophilizer. The dried dark brown extract (58.48g; 9.75 %) was then transferred into a container and kept in a desiccator layered with silica gel until further use.

3.2.2. Fractionation

Solvent fractions of the root of *E. abyssinica* were obtained by sequential Soxhlet extraction with n-hexane, ethyl acetate, methanol, and water in increasing polarity. Fifty-eight point 48grams

(58.48g) of crude extract of *E. abyssinica* was weighed and placed in the extraction thimble of the Soxhlet apparatus. Then, about 250 ml of absolute n-hexane was added to the flask of the Soxhlet apparatus set up. Then, the n-hexane was heated at a temperature not exceeding 40°C to evaporate and condense into plant powder containing thimble. This extraction process was continued exhaustively but the solvent around the thimble siphoned into the solvent flask was clear all the time. The resultant solution was filtered with Whatman number 1 filter paper and the solvent was removed using a Rota evaporator at a temperature not exceeding 40°C at 60 rpm. However, no measurable yield was obtained when the solvent got removed.

The marc of the n-hexane based extraction was collected and dried at room temperature. The dried marc was extracted using ethyl-acetate and then the solvent was removed following the same procedures as described for n-hexane extraction. A yellowish 2.6g (4.45 %) ethyl acetate fraction was obtained.

Then, the marc of the ethyl acetate fraction was collected and dried at room temperature. With the same procedure used for n-hexane and ethyl acetate, the marc obtained after fractionation was further extracted with methanol and the solvent was removed using Rota vapor. It yielded a brown-colored extract weighing 26.12g (44.66 %). Finally, the whole dried marc was further extracted with water and freeze-dried using lyophilizer at -50°C and vacuum pressure of <2000 bar to obtain a light brown aqueous fraction of 27.76g (47.47%). Each of the dried fractions was separately packed in a plastic box and stored in a refrigerator until used for the study.

3.2.3. Inoculum preparation

The parasitemia level of the donor mice was determined by taking blood from the tail of the mice by making a small cut at the tip. A thin film of blood smear was prepared and investigated under a light microscope with 100x magnification. The number of parasitized RBCs was counted and the percentage of parasitemia was calculated. Then, blood was collected from auxiliary vessels of a donor mouse with a rising parasitemia of about 30-37% into a test tube having 0.5% trisodium citrate. Then, the blood was diluted in 0.9% normal saline and each mouse was infected by injecting 0.2 ml of 1×10^7 *P. berghei* infected red blood cells via the intra-peritoneal route [95].

3.2.4. Grouping and dosing of animals

Twenty-five *P. berghei* infected mice were randomly grouped into five groups each comprising 5 mice. The mice in the last group (positive control) were treated with chloroquine 25mg/kg, each. Group II-IV were treated with solvent fractions of *E. abyssinica* at a dose of 100, 200, and 400 mg/kg/day, respectively. Two percent (2%) tween 80 was used for reconstitution of methanol fraction, 10% DMSO for the ethyl acetate fraction, and distilled water for the aqueous fraction at a volume of 10 ml/kg. Accordingly, the mice in the first group (negative control) took 10 ml/kg of the vehicle used to reconstitute the fraction, each. Dose selection was done considering the result of an acute toxicity study done on the plant previously and empirical evidence. The administration was via oral route using oral gavage size 16.

3.3. Evaluation of *in vivo* antiplasmodial activity: Four-day suppressive test

For screening of *in vivo* antiplasmodial activity of the plant extract against early *P. berghei* infection, the standard four-day suppressive method was used as described by Peter *et al*. (1975) [96].

In determining the antiplasmodial activity of the methanol fraction, weight, packed cell volume (PCV), and temperature of each mouse were measured and recorded before the induction of malaria infection.

PCV was determined by drawing blood from the tail of each mice using heparinized capillary tubes pre (day zero) and post-infection (day four). Three fourth ($3/4^{th}$) of the length of the tubes were filled with blood and sealed with sealing clay. The tubes were then placed in a hematocrit centrifuge with the sealed end outwards and centrifuged for 15 mins at 1200 rpm. Then using the below formula (A) PCV was calculated.

$$Packed\ cell\ volume\ (PCV) = \frac{Volume\ of\ erythrocyte\ in\ a\ given\ volume\ of\ blood}{Total\ volume\ of\ blood}\ X\ 100 \qquad \text{(A)}$$

After the inoculation of the parasite, 25 mice were randomly grouped into five. Three hours after inoculation, the first group (negative control) received 10ml/kg of the vehicle (2% Tween 80). The last group (positive control) was treated with the standard drug 25mg/kg chloroquine. Group II-IV

were treated with 100, 200, and 400mg/kg of the methanol fraction, respectively on day zero. Then treatment continued daily for four consecutive days (i.e. from day 0 to day 3).

On the fifth day (D4), weight, rectal temperature, and PCV were recorded. Then, a thin blood film was prepared by taking blood from the tail of each mouse. After the blood got dried, the blood films were fixed with methanol for 10 minutes and stained with 10% Giemsa for 15 minutes. Then the slides were washed with distilled water and left to dry. Blood films were examined microscopically under a light microscope using the oil immersion objective (X100 objective) to determine the percentage of parasitemia and parasite suppression.

The percentage parasitemia was obtained by counting the number of parasitized RBCs and normal RBC and then calculating using the formula stated below (B). The percentage of parasite suppression was determined by counting parasitized RBC in the negative control and treatment group and calculating using the formula stated below (C). Two stained slides for each mouse and three fields with approximately 200-500 cells from each slide were examined. The mean parasitized and normal RBCs of the two slides and three fields were used for the calculation.

$$\%\ Parasitemia = \frac{Total\ number\ of\ parasitized\ red\ blood\ cell}{Total\ number\ of\ red\ blood\ cell}\ X\ 100 \quad \text{(B)}$$

$$\%\ Parasitemia\ supression = \frac{Parasitemia\ in\ control\ group - Parasitemia\ in\ study\ group}{Parasitemia\ in\ control\ group}\ X\ 100 \quad \text{(C)}$$

Besides, each mouse was observed for mean survival time (MST) each day. The mean survival time of each group was calculated using the following formula

$$Mean\ survival\ time\ (MST) = \frac{Sum\ of\ survival\ time\ of\ all\ mice\ in\ a\ group\ in\ days}{Total\ number\ of\ mice\ in\ the\ group} \quad \text{(D)}$$

Similar procedures were used for aqueous and ethyl acetate fractions except DMSO was used as a solvent for the ethyl acetate fraction and water was used for the aqueous fraction.

3.4. Phytochemical Screening

3.4.1. Qualitative phytochemical analysis

As the medicinal value of plants emanate from the presence of particular bioactive constituents having a physiological action [97], the solvent fractions of the roots of *E. abyssinica* were qualitatively screened for the presence or absence of phytochemical constituents (secondary metabolites) such as alkaloids, flavonoids, saponins, tannins, triterpenoids, and steroids. The stock solution was prepared by dissolving 500mg of the ethyl acetate, methanol, and aqueous extracts in 50ml of the mother solvent of each extract [98]. Then qualitative phytochemical screening was conducted following standard methods for the same.

3.4.1.1. Test for alkaloid

The test for alkaloids was done according to Madike et al., 2017. The 1g extract was macerated in 50 ml of 2% sulfuric acid. Then the solution was treated with diethyl ether. After that, the water portion was made alkaline by adding 10% ammonia solution. 100ml of chloroform was added and shaken gently to allow the layer to separate. The lower chloroform layer was then taken as an alkaloidal fraction. To 2 ml chloroform layer filtrate, 1 ml of Mayer’s reagent was added drop by drop. The formation of a cream precipitate indicates the presence of alkaloids [99].

3.4.1.2. Test for flavonoids

First, 1ml of stock solution of each solvent fraction was taken in a test tube and then a few drops of diluted NaOH (2N) were added. The appearance of intense yellow color was observed. Second, a few drops of dilute HCl was added. The disappearance of the yellow color, an indicator of the presence of flavonoids, was observed [98].

3.4.1.3. Test for triterpenoids

Dry solvent fractions of *E. abyssinica* (5mg) were taken in a test tube and dissolved with chloroform (2ml). Acetic anhydride (1ml) was added to the solution and then 1 ml of concentrated sulfuric acid was added looking for the formation of reddish-violet color, an indicator of the presence of triterpenoids [100].

3.4.1.4. Test for steroids

1mg of each solvent extract was taken in a test tube and dissolved with 10 ml of Chloroform. Next, 10 ml of concentrated sulfuric acid was added to the solution. The test tubes were then observed if red color appeared on the upper layer of the test tube and if yellow color with green fluorescence appeared on the sulfuric acid layer as an indicator of the presence of steroids [98].

3.4.1.5. Test for saponins

The stock solution of each solvent fraction (1ml) was added in separate test tubes and diluted with distilled water (20ml). Then each was shaken by hand for 5mins and allowed to stand for 30mins. The appearance and maintenance of the appeared foam were observed as an indicator of the presence of saponins for the length of the above specified time [101].

3.4.1.6. Test for tannins

1ml of stock solution of each solvent fraction was taken in test tubes and 4 drops of 5% ferric chloride solution were added to them. The test tube was observed for the formation of black or blue-green color indicated the presence of tannins [102].

3.4.1.7. Test for phenols

1ml of stock solution of each solvent fraction was taken in test tubes and 4 drops of 10% lead acetate solution were added. The test tube was observed for the formation of a white precipitate which is an indicator of the presence of phenolic compounds [103].

3.4.2. Quantitative phytochemical analysis

The total phenol content (TPC) and the total flavonoid content (TFC) of the two most active solvent fractions, the ethyl acetate and methanol fractions, was determined using the below stated procedures.

3.4.2.1. Total phenol content

Determination of the total phenol content was carried out using Folin–Ciocalteu's method. Serial dilutions of gallic acid were prepared in methanol with concentrations of 0.5, 0.25, 0.125, and 0.065 mg/ml to establish a calibration curve. One milliliter of the gallic acid solution was then transferred to test tubes. Then, 5ml of distilled water and 0.5ml of 2N Folin–Ciocalteu's reagent

(1:20) were added to the test tubes and incubated for 8 min. Afterward, 2 ml of 7.5 % Na2CO3 was added, and then distilled water was poured until the volume reached 10ml. The next step was incubating the solution for 30 min at an ambient temperature. Finally, the absorbance of the solution was recorded at 765 nm using a UV spectrophotometer (Jenway Model 6500, England). A similar procedure was carried out for the fractions (125 µg/ml) and the blank solutions. A linear calibration curve was plotted and TPC was read from the curve and expressed as mg of gallic acid equivalent per 1g of extracts. The experiment was conducted in triplicate and the average result was taken [104].

3.4.2.2. Total flavonoid content

An aluminum chloride complex-forming assay was employed to determine the total flavonoid content of the extracts. Serial dilutions of quercetin were prepared in methanol with concentrations of 0.5, 0.25, 0.125, and 0.065 mg/ml to establish a calibration curve. One milliliter of quercetin was then transferred into test tubes. $NaNO_2$ (0.3 ml 5%) was added and incubated for 5 min. afterwards 0.3 ml of 10% $AlCl_3$ was mixed with the solution and allowed to stand for 5 min. Next, 2 ml solution of 1M NaOH was added into the solution and then distilled water was added to make up the volume to 10 ml. Finally, the solution was incubated for 30 min at ambient temperature. The absorbance of the solution was recorded at 510 nm using a UV spectrophotometer (Jenway Model 6500, England). The same procedure was repeated with the fractions (125µg/ml) and the blank solutions. The total flavonoid content was expressed as mg of quercetin equivalent per 1g of extracts. The experiment was conducted in triplicate and the average result was taken [105].

3.5. Data analysis

All results of the study was expressed as mean + standard error of mean (SEM). All data were analyzed by windows SPSS version 25. One-way analysis of variance (ANOVA) was used to analyses differences among group. Sub group analysis was done by Tukey's multiple comparison tests. The analysis at 95% confidence interval and P-values of less than 0.05 was considered statistically significant.

4. Results

4.1. Percentage yield

Six hundred grams (600 g) of air-dried and powdered plant material subjected to cold maceration with 80% methanol yielded 58.48g crude extract. As summarized in Table 2, the highest yield obtained by fractionating the crude extract was from the aqueous fraction followed by the methanol fraction. The yield for the n-hexane fraction was insignificant.

Table 2: Yield of 80% methanol crude extract and solvent fractions of E. abyssinica

S. no	Description	Color of the extract	Actual yield (g)	% yield (actual yield/theoretical yield *100)
1	Crude extract	Dark brown	58.48	9.75
2	Methanol fraction	Brown	26.12	44.66
3	Ethyl acetate Fraction	Yellow	2.60	4.45
4	n-hexane fraction	-	-	-
5	Water Fraction	Light brown	27.76	47.47

4.2. Effect of solvent fractions in the four-day suppressive test

4.2.1. Effect of solvent fractions of the roots of *E. abyssinica* on parasite suppression and mean survival time

As demonstrated in Table 3, the solvent fractions of the root extract of *E. abyssinica* exhibited considerable chemosupressive activity *in vivo* against *P. berghei* infection in mice compared to the negative control. However, the activity was found to be less than the one produced by the standard drug, chloroquine as it caused chemosupression of 100%. The suppressive effect of the reference drug was statistically significant ($p<0.001$) as compared to the negative control and all dose levels of the three fractions.

All the fractions exhibited dose-dependent chemosuppresive response. The highest reduction in percentage parasitemia was produced by the ethyl acetate fraction. The percentage suppressions observed at doses 200 and 400mg/kg were 69.57 and 78.87%, respectively ($p<0.001$). The higher dose of the methanol fraction exhibited the third higher and statistically significant ($p<0.001$) parasite inhibition (68.5%) when compared with the vehicle-treated group.

The least chemosupressive activity was displayed by the lower dose of aqueous fraction (10.09%). At 100 mg/kg, the aqueous fraction failed to show a statistically significant chemosupressive effect in comparison with the negative control.

The effect of the solvent fractions of the root of *E. abyssinica* on the mean survival time of mice infected with *P. berghei* was also determined at different dose levels. As it can be seen from Table 2, the mice treated with vehicle survived only approximately a week while the mice treated with reference drug (chloroquine) survived for longer than the observation period (30 days). Statistically significant ($p<0.001$) prolongation of survival time was exhibited at all dose levels of ethyl acetate fraction compared to the negative control group.

The higher prolongation of survival time was produced by the higher dose of ethyl acetate fraction (MST=20.6±0.81) ($p<0.001$). In contrast, the lower survival time prolongation was observed at the lower dose of aqueous fraction (MST=7.2±0.37) which was not statistically significant when compared with the negative control. The methanol fraction produced statistically significant ($p<0.001$) prolongation of survival time when compared with the vehicle-treated group at the higher and middle doses. At these doses, the effect exhibited was mild when compared with all dose levels of ethyl acetate and aqueous fractions.

Table 3: Effect of the solvent fractions of roots of *E. abyssinica* on percentage parasitemia and survival time of *P. berghei* infected mice on a 4-day Peter's suppression test.

Fractions	Dose (mg/kg) and treatment groups	Parasitemia level	% Suppression	Survival period (days)
Ethyl acetate fraction	100	25.2±0.93 $^{a1\ c3\ d1\ e1}$	52.43	13.2±0.58 $^{a1\ c1\ d1\ e1}$
	200	16.12±0.69 $^{a1\ b3\ e1}$	69.57	17.6±0.74 $^{a1\ b1\ d3\ e1}$
	400	11.18±0.37 $^{a1\ b1\ e2}$	78.87	20.6±0.81 $^{a1\ b1\ c3\ e1}$
	NC	52.9±4.3	0.00	6.8±0.37
	CQ	0.00±0.00 $^{a1\ b1\ c1\ d2}$	100	>30.00 $^{a1\ b1\ c1\ d1}$
Methanol fraction	100	45.35±3.17 $^{a2\ c1\ d1\ e1}$	19.31	7.40±0.60 $^{c1\ d1\ e1}$
	200	27.83±1.89 $^{a1\ b1\ d2\ e1}$	50.49	12.60±0.74 $^{a1\ b1\ e1}$
	400	17.70±0.64 $^{a1\ b1\ c2\ e1}$	68.50	14.6±1.36 $^{a1\ b1\ e1}$
	NC	56.2±1.43	0.00	6.2±0.37
	CQ	0.00±0.00 $^{a1\ b1\ c1\ d1}$	100.00	>30.00 $^{a1\ b1\ c1\ d1}$
Aqueous fraction	100	50.98±1.10 $^{c3\ d1\ e1}$	10.09	7.2±0.37 $^{d1\ e1}$
	200	39.22±1.56 $^{a1\ b3\ c3\ e1}$	29.07	9.60±0.24 ^{e1}
	400	30.09±0.59 $^{a1\ b1\ e1}$	43.92	12.6±0.50 $^{a1\ b1\ e1}$
	NC	57.3±0.8	0.00	6.4±0.24
	CQ	0.00±0.00 $^{a1\ b1\ c1\ d1}$	100.00	>30.00 $^{a1\ b1\ c1\ d1}$

Data are expressed as mean ± SEM; [n=5]. ^{a} Compared to negative control; ^{b} Compared to 100 mg/kg; ^{c} Compared to 200mg/kg; ^{d} Compared to 400mg/kg; ^{e} Compared to CQ 25mg/kg. $^{1}p<0.001$, $^{2}p<0.01$, $^{3}p<0.05$. Numbers = mg/kg/day of extract administered, CQ = Chloroquine, NC= Negative control

4.2.2. Effect of the solvent fractions of the roots of *E. abyssinica* on packed cell volume (PCV)

It is apparent from Table 4 that the middle and higher doses of the ethyl acetate fraction significantly ($p<0.001$) prevented the decrease in packed cell volume as compared to the negative control. Mice treated with 400mg/kg/day of methanol as well maintained PCV but the effect was not statistically significant. Mice treated with lower and middle doses of the methanol fraction, the lowest dose of ethyl acetate fraction, and all the dose levels of an aqueous fraction didn't show effects against PCV reduction. Mice treated with the reference drug (chloroquine) didn't show a notable reduction in PCV.

Table 4: Effects of the solvent fractions of roots of *E. abyssinica* on PCV of *P. berghei* infected mice on 4-day Peter's suppression test.

Fractions	Animal group	Packed cell volume (%)		% Change
		Day 0 (%)	Day 4 (%)	
Ethyl acetate fraction	100	50.60±2.11	47.00±2.12	-3.60±0.51 $^{c2\,d1\,e1}$
	200	49.78±1.46	50.20±1.50	0.42±0.37 $^{a1\,b2}$
	400	48.66±0.77	49.40±0.74	0.74±0.26 $^{a1\,b2}$
	NC	53.8±1.2	49.2±1.60	-4.60±1.25
	CQ	48.70±1.01	49.70±1.01	1.00±0.06 $^{a1\,b1}$
Methanol fraction	100	57.90±0.89	57.03±2.97	-0.87±2.86
	200	54.95±1.63	54.48±2.38	-0.43±2.55 $^{a2\,b3}$
	400	53.48±1.48	53.88±2.12	0.39±3.52
	NC	50.71±2.40	45.08±1.88	-4.90±0.52
	CQ	53.38±1.04	54.46±1.50	1.08±0.54 ^{a1}
Aqueous fraction	100	44.80±3.07	39.00±3.11	-5.80±0.37 ^{e1}
	200	51.00±0.89	47.20±1.32	-3.80±0.66 ^{e1}
	400	50.60±1.44	47.20±1.5	-3.40±0.24 $^{a3\,b3\,e1}$
	NC	47.60±3.37	41.60±3.56	-6.00±0.63
	CQ	50.20±1.20	50.80±1.11	0.60±0.68 $^{a1\,b1\,c1\,d1}$

Data are expressed as mean ± SEM; [n=5]. a Compared to negative control; b Compared to 100 mg/kg; c Compared to 200mg/kg; d Compared to 400mg/kg; e Compared to CQ 25mg/kg. [1]$p<0.001$, [2]$p<0.01$, [3]$p<0.05$. Numbers = mg/kg/day of extract administered, CQ = Chloroquine, NC= Negative control

4.2.3. Effect of the solvent fractions of the roots of *E. abyssinica* on rectal temperature

The effects of the solvent fractions of *E. abyssinica* on the rectal temperature of *P. berghei* infected mice in 4-day Peter's suppression test is summarized in Table 5. Closer inspection of the table reveals that a statistically significant ($p<0.001$) prevention in rectal temperature reduction was observed in mice treated with 400mg/kg/day of ethyl acetate fraction.

Compared to the negative control, all doses of the methanol fraction ($p<0.001$) and middle and higher doses of aqueous fraction ($p<0.01$) prevented a reduction in rectal temperature significantly. However, a considerable reduction in rectal temperature was recorded when compared with the rectal temperature measured before infection.

Table 5: Effects of the solvent fractions of roots of *E. abyssinica* on the rectal temperature of *P. berghei* infected mice on 4-day Peter's suppression test.

Fractions	Animal group	Rectal Temperature		Change (°C)
		Day 0 (°C)	Day 4 (°C)	
Ethyl Acetate fraction	100	37.30±0.22	36.70±0.28	-0.60±0.14 $^{a2\ d2\ e1}$
	200	37.14±0.22	36.90±0.22	-0.24±0.02 $^{a1\ e3}$
	400	36.74±0.21	37.12±0.16	0.38±0.08 $^{a1\ b2}$
	NC	37.54±0.18	35.96±0.17	-1.58±0.26
	CQ	36.62±0.26	37.16±0.12	0.54±0.16 $^{a1\ b1\ c3}$
Methanol fraction	100	37.36±0.29	36.64±0.29	-0.72±0.04 $^{a1\ d2\ e1}$
	200	37.34±0.32	37.16±0.31	-0.18±0.16 $^{a1\ e2}$
	400	37.14±0.25	37.28±0.25	-0.14±0.81 $^{a1\ b2}$
	NC	37.26±0.11	35.38±0.24	-1.88±0.26
	CQ	36.56±0.22	37.24±0.21	0.68±0.06 $^{a1\ b1\ c2}$
Aqueous fraction	100	36.26±0.39	35.06±0.25	-1.20±0.28 $^{c3\ e1}$
	200	36.72±0.26	36.00±0.29	-0.72±0.67 $^{a2\ e2}$
	400	36.38±0.51	36.00±0.47	-0.38±0.67 ^{a2}
	NC	36.96±0.26	35.34±0.29	-1.60±0.31
	CQ	36.60±0.22	36.94±0.20	0.34±0.08 $^{a1\ b1\ c2}$

Data are expressed as mean ± SEM; [n=5]. a Compared to negative control; b Compared to 100 mg/kg; c Compared to 200mg/kg; d Compared to 400mg/kg; e Compared to CQ 25mg/kg. $^{1}p<0.001$, $^{2}p<0.01$, $^{3}p<0.05$. Numbers = mg/kg/day of extract administered, CQ = Chloroquine, NC= Negative control

4.2.4. Effect of the solvent fractions of the roots of *E. abyssinica* on body weight of *P.berghei* infected mice

As can be seen from the data in Table 6, the higher dose of the methanol fraction produced a significant (p<0.001) and superior effect in preventing weight loss. Although it's not statistically significant, the higher dose of ethyl acetate fraction also prevented body weight loss considerably.

Mice treated with the aqueous fraction and lower and middle doses of methanol and ethyl acetate fractions showed a loss in body weight but not to the extent of mice treated with a vehicle.

Table 6: Effects of the solvent fractions of roots of *E. abyssinica* on body weight of *P. berghei* infected mice on 4-day Peter's suppression test.

Fraction	Animal group	Bodyweight (g)		Change (g)
		Day 0 (g)	Day 4 (g)	
Ethyl Acetate fraction	100	28.35±1.60	26.88±1.38	-1.47±0.54
	200	25.71±0.66	25.70±1.26	-0.006±1.11
	400	26.62±1.11	26.91±0.94	0.30±1.11
	NC	23.56±1.80	21.34±0.95	-2.22±0.89
	CQ	25.10±0.74	27.19±1.34	2.10±0.70 [a3]
Methanol fraction	100	22.63±2.02	21.76±1.92	-0.87±0.55 [a1 d2 e1]
	200	30.59±1.11	31.38±0.48	-0.79±0.91 [a1 e2]
	400	30.38±0.80	31.33±0.55	0.95±0.87 [a1 b2]
	NC	22.31±0.49	19.33±0.42	-2.98±0.13
	CQ	23.87±0.89	24.94±0.10	1.06±0.34 [a1 b1 c2]
Aqueous fraction	100	26.14±0.50	23.60±0.31	-2.53±0.35 [e3]
	200	27.70±1.53	26.01±1.30	-1.70±2.06
	400	22.85±1.80	21.86±1.19	-0.99±0.66
	NC	27.00±0.97	24.40±1.80	-2.60±2.06
	CQ	24.00±0.80	27.46±0.78	3.46±0.30 [a3 b3]

Data are expressed as mean ± SEM; [n=5]. a Compared to negative control; b Compared to 100 mg/kg; c Compared to 200mg/kg; d Compared to 400mg/kg; e Compared to CQ 25mg/kg. $^{1}p<0.001$, $^{2}p<0.01$, $^{3}p<0.05$. Numbers = mg/kg/day of extract administered, CQ = Chloroquine, NC= Negative control

4.2.5. Results of the Preliminary Phytochemical Screening Tests

4.2.5.1. Qualitative phytochemical analysis

The results of phytochemical screenings conducted on the solvent fractions are presented in Table 7.

Table 7: Results of the qualitative phytochemical screening tests of the solvent fractions of the root of *E. abyssinica.*

Secondary metabolites	**Solvent fractions of the root of *E. abyssinica***		
	Ethyl acetate	**Methanol**	**Aqueous**
Alkaloids	+	+	-
Flavonoids	+	+	+
Triterpenoids	+	+	-
Steroids	-	-	-
Saponins	-	+	+
Tannins	+	+	-
Phenols	+	+	-

+ indicates the presence and − indicates absence of particular secondary metabolites

4.2.5.2. Quantitative phytochemical analysis

4.2.5.2.1. Total phenols content

TPC content was estimated by Follin - Ciocalteu method using Gallic acid as a standard. Based on the calibration curve (Fig 4), an equation ($Y = 0.2194X + 0.9391$) with a correlation coefficient (R2) of 0.9391 was used to calculate the concentration of TPC. Accordingly, it was found to be 2.56 mgGAE/g and 1.51 mgGAE/g in the ethyl acetate and methanol fractions, respectively.

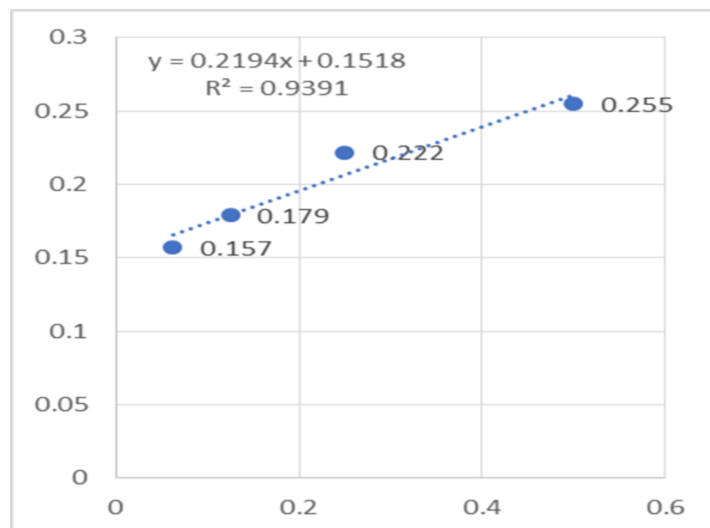

Figure 4: Calibration curve for Concentration of gallic acid (μg/ml)

4.2.5.2.2. Total flavonoids content

TFC content was estimated by Aluminum chloride colorimetric method using quercetin as a standard using an equation (Y = 0.054X + 0.9379) with a correlation coefficient (R2) of 0.9379 (Fig. 5). Accordingly, TFC was found to be 1.68 mgQE/g and 0.35 mgQE/g in the ethyl acetate and methanol fractions, respectively.

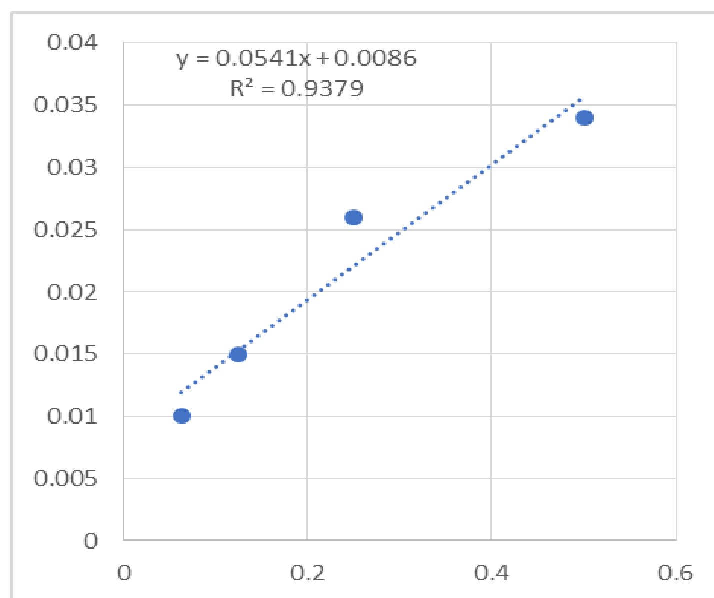

Figure 5: Calibration curve for Concentration of gallic acid (μg/ml)

5. Discussion

In this study, in *vivo* antimalarial activity of the solvent fractions of the root of *E. abyssinica* was investigated using the standard four-day suppressive test. As the results of an acute toxicity study done by Muluye *et al*., [90] on the 80% methanol extract of the roots of *E. abyssinica* at a limit dose of 2000mg/kg reported no signs of toxicity within 24 hours as well as no death within 14 days, in the present study 100, 200 and 400 mg/kg/day dose levels were selected based on empirical and literature evidence to investigate the antimalarial activity of the solvent fractions.

Evidence suggests that an extract that displays percentage parasite suppression of greater than or equal to 50% at a dose level of 500, 250, and 100mg/kg/day in *in vivo* antiplasmodial activity test is considered to have moderate, good, and very good antiplasmodial activity, respectively [106]. The result of this study (Table 3) showed that the ethyl acetate fraction exhibited a very good antiplasmodial activity as the percent parasite suppression achieved by the lower dose level of ethyl acetate fraction was 52.43%. In contrast, the methanol fraction exerted good antiplasmodial activity by achieving 50.49 percent parasite suppression at the middle dose level.

The highest percent parasite suppression achieved by the aqueous fraction was 43.92 at a dose of 400mg/kg/day. Though the activity is below the intermediate level, it can be considered as active against malaria since an extract producing percentage suppression of parasitemia ≥30% can be considered as active [107]. All three fractions exerted dose-dependent antiplasmodial activities in this study.

From the results of this study, it is noted that the ethyl acetate fraction exhibited statistically significant and superior parasite suppression compared to the other fractions and the negative control. This finding is concordant with the study conducted by Nureye *et al.,* [108] on the antiplasmodial activity of hydroalcoholic crude extract and solvent fractions of Z*ehneria scabra* where the ethyl acetate fraction has shown superior antimalarial activity compared to the other solvent fractions.

Similarly, a previous study conducted by Vadalia *et al.,* [109] on the antiplasmodial activities of two plants from the Euphorbiaceae family, *E. hirta* and *E. thymifolia* demonstrated an anti-malarial activity which could suggest that members of the family have significant activity against malaria.

The results of the preliminary phytochemical study revealed the presence of different secondary metabolites such as alkaloids, flavonoids, and tannins which could justify the antimalarial activity of the solvent fractions of *E. abyssinca*.

All three solvent fractions of *E. abysinica* contain flavonoids. Monbrison et al., [110] and Bilia et al., [111] reported the antimalarial activity of flavonoids. Even if, the mode of antimalarial action of flavonoids is not known, it is speculated that they work by inhibiting the fatty acid biosynthesis and the influx of L-glutamine and myoinositol into infected erythrocytes during the intraerythrocytic phase of the *Plasmodium* life cycle [112]. Thus, the antiplasmodial activity exerted by the solvent fractions of *E. abysinica* might be due to the presence of flavonoids.

Alkaloids, triterpenoids, tannins, and phenols were the other secondary metabolites identified in the ethyl acetate and methanol fractions of *E. abysinica*. The selective localization of these constituents can probably be the reason why the methanol and the ethyl acetate fractions exhibited better antimalarial activity than the aqueous fraction.

Researchers explored the antimalarial activities of alkaloids [101, 102] but how they bring such effect is not fully elucidated yet [113]. Inhibition of parasite enzyme, phosphodiesterase, and inhibition of the fatty acids biosynthesis pathway which is essential for parasite growth are suggested mechanisms of action [114]. Phenolic compounds are also studied to have an antiplasmodial effect and positive results were reported by different researchers [115]. Their antioxidant property emanated from their redox ability is thought to play a major role in their antimalarial activity by adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [116].

Whereas, the parasite suppressive effect of terpenes could be associated with endoperoxidation [117] while tannins probably work by interfering with important pathways like hemozoin crystallization, protein synthesis, or DNA fragmentation [118]. The antiplasmodial activity exerted by the ethyl acetate fraction might be attributed to these secondary metabolites. The methanol and the aqueous fractions of *E. abysinica* were found to comprise saponins. Research conducted by Nafiu *et al.* [119] showed the effect of saponins against malaria parasites sharing a similar mechanism of action with phenolic compounds, anti-oxidant property.

The aqueous fraction exhibited lower parasite suppression when compared with the methanol and ethyl acetate fractions. This finding is consistent with the result obtained from the study conducted on the antimalarial activity of *Croton macrostachyus* Hocsht. (Euphorbiaceae) [120] and *Olea europaea* L. cuspidata (Oleaceae) [121] where the aqueous fraction brought a minimal activity.

Results of quantitative phytochemical analysis showed that the ethyl acetate fraction contains relatively larger amount of phenols and flavonoids when compared with the methanol fraction. This could be one of the reasons for the superior antimalarial activity produced by the ethyl acetate fraction.

Another important finding was that chemosupressive activity produced by each solvent fraction was lower than the activity produced by the 80% methanol crude extract (93.69%) of the root of *E. abyssinica* [90]. This result goes in line with studies conducted on the anti-malarial activity of *Dodonaea angustifolia* [122] *and Schinus molle* L (Anacardiaceae) [123] where the crude extract has shown superior activity. The better activity produced by the crude extract than the solvent fractions could be justified by the possibility of synergistic or additive activity of different constituents in the crude extract which got fractioned in different solvents during fractionation. The other possible explanation could be the larger dose level (600 mg/kg/day) of the crude extract used during the study [90] than the dose used for the solvent fractions (400 mg/kg/day).

One of the features of *P. berghei* infection in mice is the development of anemia as a result of red blood cell destruction [124]. Infected RBC destruction is associated with parasite maturation and recognition by macrophages. Whereas, uninfected RBC destruction is a result of increased activity and number of macrophages [125]. A test substance that is active against malaria is ought to prevent hemolytic anemia and maintain PCV. In this study, the middle and the higher dose levels of the ethyl acetate fraction and the higher dose of methanol fraction have shown statistically significant prevention in the reduction of PCV as compared to the vehicle-treated group. This could be a result of a reduction in the level of parasitemia which further reduces the number of infected RBC and spleen activity in addition to clearance of the parasite from the infected RBC.

Another possible reason could be the availability of antioxidant constituents in the fractions which could scavenge reactive oxygen species liberated as a result of the immune system activation [126] In contrast, the aqueous fraction failed to prevent a reduction in PCV at all the dose levels. It could

be probably justified by the presence and concentration of saponins which are known to have hemolytic activity [127].

Mean survival time is the other parameter studied in the evaluation of the anti-malarial activity of a given test substance. An extract that prolongs survival time to 12 days or longer is regarded as active [128]. In this study, all the doses of ethyl acetate fraction, the middle and higher doses of methanol fraction, and the higher dose of the aqueous fraction produced a statistically significant mean survival time prolongation beyond 12 days in contrast with the negative control. This can be associated with the ability of the extracts to suppress the level of parasitemia and overall pathologic conditions resulting from the parasitic infection [129].

Another parameter studied to predict the effectiveness of the test substances against malaria was a rectal temperature. Malaria infection leads to hypothermia in rodents instead of pyrexia opposite to the human host. Infected mice develop a significant fall in rectal temperature (up to 9 °C from the normal). The reason why the malaria parasite lacks pyrogenic effect in rodents and other similar small animals has mainly been attributed to a higher degree of loss of heat resulting from the larger surface area-to-body mass ratio in small animals [130] [131].

Loss of weight due to general pathologic conditions associated with the infection could be a cause of an increase in surface area-to-body mass ratio and hence heat loss [131]. Decrease in internal body temperature results in a decrease in metabolic rate and finally death [132]. Therefore, a test substance preventing reduction in rectal temperature could be regarded as active against malaria [133].

In this study, statistically significant prevention in reduction of rectal temperature was observed in a group treated with 400mg/kg/day dose of ethyl acetate fraction. Superior parasite clearance by this specific dose level of the fraction might underline the result as a drop in temperature is a consequence of escalation on the level of parasitemia [132].

Even though there was a significant decrease in parasitemia, all the rest doses of the three solvent fractions were unable to prevent a reduction in temperature as compared to the temperature recorded on day zero. This finding is comparable with the findings of other antimalarial studies conducted on *Bersama abyssinica Fresen.* (Melianthaceae) [82] and *Zehneria scabra* [106] where

extracts with a statistically significant parasite level suppressive activity failed to prevent a drop in rectal temperature. This could be associated with a possible anti-fever activity of the plant extracts which is mounted by results reported from studies conducted on the antipyretic activity of plant extracts of *E. granulata* [135] and *E. peplis L* [134] which belongs to the same family, Euphorbiaceae, with *E. abyssinica.*

Reduction in body weight is also one of the clinical features of malaria infection in mice [124]. It could be a result of appetite suppression, altered metabolic function, and hypoglycemia associated with an increase in the level of parasitemia, and prevention in a reduction in body weight is expected from a test substance active against malaria. [132].

The current study reported statistically significant prevention in bodyweight reduction by the higher dose level of the methanol fraction of the test substance when compared with the negative control. Though it's not statistically significant, the larger dose level of the ethyl acetate fraction also prevented weight loss. A possible explanation for this might be the maximum parasite clearance exhibited by the higher dose of the two fractions resulted in improvement in feeding [137]. However, irrespective of the ability to suppress the parasite level, the rest dose levels of all the fractions couldn't prevent weight loss. This finding is in line with researches conducted on the crude extract of the same plant [90] and studies conducted on other plants including *B. abyssinica* Fresen. (Melianthaceae) [82], *Z. scabra* [104], and *D. angustifolia* [122] in which failure to prevent body weight loss was reported despite a statistically significant parasite suppression.

This could probably be justified by the inferior parasite suppression exhibited by the lower dose levels of the ethyl acetate and methanol fractions and all dose ranges of the aqueous fraction when compared with the higher dose levels of the ethyl acetate and methanol fractions [137]. Furthermore, it could be associated with the presence of other means of weight loss like localization of metabolites with appetite depressant activity in the aqueous fraction.

But the activity produced by all the fractions was below the effect produced by the standard drug chloroquine. This finding is concordant with other similar researches. This could probably be a result of the fastest clearance of the extracts [121].

6. Conclusion

This study has shown that the three solvent fractions of *E. abyssinica* possessed significant antiplasmodial activity in varying degrees. The ethyl acetate fraction was found to be the most active fraction that produced a very good antiplasmodial activity at the highest dose level followed by the methanol fraction which exhibited good antimalarial activity. Statistically significant mean survival time prolongation and prevention in reduction of packed cell volume was also reported. The preliminary phytochemical study reveals the presence of different secondary metabolites in each solvent fraction which possibly explains the antimalarial activity of each solvent fraction. Furthermore, the superior activity reported by the ethyl acetate and methanol fractions could be associated with a possible localization of bioactive secondary metabolites in the nonpolar to a semi-polar fraction which supports the traditional use of the plant to treat malaria.

7. Recommendation

Based on the findings of this study, future researchers could conduct:-

- ✓ Prophylactic activity test on the plant,
- ✓ Compound isolation from the ethyl acetate fraction and further studying the antimalarial activity.
- ✓ *In vitro* antimalarial activity test to investigate the effect against the most virulent species of *plasmodium* to humans, *P. falciparum* and
- ✓ Studying the possible mechanisms of action of the plant in the treatment of malaria.

8. References

1. Tuteja R. Malaria − an overview: FEBS J. 2007, Sep;274(18):4670–4679.
2. Escalante AA, Pacheco MA. Malaria Molecular Epidemiology: An Evolutionary Genetics Perspective. Microbiol Spectr. 2019, Jul; 7(4): 1-17.
3. Singh B, Daneshvar C. Human Infections and Detection of *Plasmodium knowlesi*. Clin Microbiol Rev. 2013, Apr;26 (2):165–184.
4. Antony HA, Parija SC. Antimalarial drug resistance: An overview. Trop Parasitol. 2016, Jun;6 (1):30–41.
5. White NJ. *Plasmodium knowlesi*: The Fifth Human Malaria Parasite. Clin Infect Dis. 2008, Jan;46(2):172–173.
6. WHO. Severe Malaria. Trop Med Int Health. 2014, Sep; 19 (Suppl. 1):7–131.
7. Guttery DS, Holder AA, Tewari R. Sexual Development in *Plasmodium*: Lessons from Functional Analyses. PLOS Pathog. 2012, Jan;8(1): 1-4.
8. Premji ZG. Coartem®: the journey to the clinic. Malar J. 2009, Oct;8(1):1-6.
9. Talapko J, Škrlec I, Alebić T, Jukić M, Včev A. Malaria: The Past and the Present. Microorganisms. 2019, Jun;7 (6): 1-17.
10. Sharma C, Awasthi SK. Recent Advances in Antimalarial Drug Discovery-Challenges and Opportunities. In: Samie A, editor. An Overview of Tropical Diseases. InTech; 2015. p. 39-60.
11. World Health Organization. World malaria report 2020. Regional Office for South-East Asia New Delhi: World Health Organization; 2021.
12. Ethiopian public health institute. Ethiopia National Malaria Indicator Survey 2015. EPHI; 2016, May.
13. Al-Awadhi M, Ahmad S, Iqbal J. Current Status and the Epidemiology of Malaria in the Middle East Region and Beyond. Microorganisms. 2021, Feb;9(2):1-20.
14. Nureye D, Assefa S. Old and Recent Advances in Life Cycle, Pathogenesis, Diagnosis, Prevention, and Treatment of Malaria Including Perspectives in Ethiopia. Sci World J. 2020, Feb;2020 (1): 1-17.

15. Lalremruata A, Jeyaraj S, Engleitner T, Joanny F, Lang A, Bélard S, et al. Species and genotype diversity of Plasmodium in malaria patients from Gabon analysed by next generation sequencing. Malar J. 2017, Oct;16(1):1-11.
16. Gneme A, Guelbeogo WM, Riehle MM, Tiono AB, Diarra A, Kabre GB, et al. *Plasmodium* species occurrence, temporal distribution and interaction in a child-aged population in rural Burkina Faso. Malar J. 2013, Feb;12(1):1-9.
17. Bruce MC, Macheso A, McConnachie A, Molyneux ME. Comparative population structure of *Plasmodium malariae* and *Plasmodium falciparum* under different transmission settings in Malawi. Malar J. 2011, Feb;10(1):1-12.
18. Oaks S. Malaria: Obstacles and Opportunities. Washington DC: Nat. Acad. Pr.; 1991.
19. WHO. Fact sheet about malaria [Internet]. Geneva: WHO;1 April 2021.
20. Maureen C, Richard H, Richard W, Alessandra D T, Mamadou B C, Nora J B. Anopheles coluzzii and Anopheles amharicus, new members of the Anopheles gambiae complex. Zootaxa. 2013, Feb; 3619 (3): 246 –274.
21. Melina C, Mark H, Hans G, Travis C, Yoosook L, Anthony J, Joao P, Diego A, Herodes R, and Gregory C. The origin of island populations of the African malaria mosquito, Anopheles coluzzii. Commun Biol. 2021, May; 4 (1): 1-9.
22. Siciliano G, Alano P. Enlightening the malaria parasite life cycle: bioluminescent *Plasmodium* in fundamental and applied research. Front Microbiol. 2015;6(1):1-8.
23. Ansong D, Seydel KB, Taylor TE. Malaria. In: Hunter's Tropical Medicine and Emerging Infectious Diseases. *Elsevier;* 2020. p. 734–754.
24. Crutcher JM, Hoffman SL. Malaria. In: Baron S, editor. Medical Microbiology. 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston; 1996.
25. Jong EC. Malaria. In: Netter's Infectious Diseases. Elsevier; 2012 .p. 373–82.
26. Zuccala ES, Baum J. Cytoskeletal and membrane remodeling during malaria parasite invasion of the human erythrocyte: Review. Br J Haematol. 2011, Sep;154(6):680–689.
27. Venugopal K, Hentzschel F, Valkiūnas G, Marti M. *Plasmodium* asexual growth and sexual development in the hematopoietic niche of the host. Nat Rev Microbiol. 2020, Mar;18 (3):177–189.
28. Ngwa CJ, Rosa TF de A, Pradel G. The Biology of Malaria Gametocytes. In: Rodriguez-Morales AJ, editor. Current Topics in Malaria. InTech; 2016.

29. Bennink S, Kiesow MJ, Pradel G. The development of malaria parasites in the mosquito midgut: Malaria parasite development in the mosquito midgut. Cell Microbiol. 2016, Jul; 18(7):905–918.
30. Smith RC, Barillas-Mury C. *Plasmodium* Oocysts: Overlooked Targets of Mosquito Immunity. Trends Parasitol. 2016, Dec;32(12):979–990.
31. Milner DA. Malaria Pathogenesis. Cold Spring Harb Perspect Med. 2018, Jan 2;8 (1):1-11.
32. Alister GC, Mohd Fadzli Mustaffa K, Pradeep RP. Cytoadherence and Severe Malaria. Malays J Med Sci MJMS. 2012;19(2):5–18.
33. Crabb BS, Cowman AF. Plasmodium falciparum virulence determinants unveiled. Genome Biol. 2002;3(11):1-4.
34. Lavstsen T, Magistrado P, Hermsen CC, Salanti A, Jensen AT, Sauerwein R, et al. Expression of *Plasmodium falciparum* erythrocyte membrane protein 1 in experimentally infected humans. Malar J. 2005, Apr 27; 4(1):1-9.
35. Roberts DJ, Pain A, Chitnis CE. Molecular pathogenesis of malaria. In: Provan D, Gribben J, editors. Molecular Hematology. 1st ed. Wiley; 2019. p. 193–206.
36. Chen Q, Schlichtherle M, Wahlgren M. Molecular Aspects of Severe Malaria. Clin Microbiol Rev. 2000, Jul 1;13(3):439–450.
37. Hermand P, Cicéron L, Pionneau C, Vaquero C, Combadière C, Deterre P. *Plasmodium falciparum* proteins involved in cytoadherence of infected erythrocytes to chemokine CX3CL1. Sci Rep. 2016, Sep 22;6 (1):1-12.
38. Wah ST, Hananantachai H, Kerdpin U, Plabplueng C, Prachayasittikul V, Nuchnoi P. Molecular basis of human cerebral malaria development. Trop Med Health. 2016, Dec;44(1):1-7.
39. Jakobsen PH, Bate CAW, Taverne J, Playfair JHL. Malaria: toxins, cytokines and disease. Parasite Immunol. 1995, May;17(5):223–231.
40. Schofield L, Hackett F. Signal transduction in host cells by a glycosylphosphatidylinositol toxin of malaria parasites. J Exp Med. 1993, Jan;177(1):145–153.
41. Dunst J, Kamena F, Matuschewski K. Cytokines and Chemokines in Cerebral Malaria Pathogenesis. Front Cell Infect Microbiol. 201, Jul 20;7(1):1-16.
42. Murray CK, Bennett JW. Rapid Diagnosis of Malaria. Interdiscip Perspect Infect Dis. 2009; 2009(1):1-7.

43. Kasetsirikul S, Buranapong J, Srituravanich W, Kaewthamasorn M, Pimpin A. The development of malaria diagnostic techniques: a review of the approaches with focus on dielectrophoretic and magnetophoretic methods. Malar J. 2016, Jul 12;15(1):1-14.
44. Krampa FD, Aniweh Y, Awandare GA, Kanyong P. Recent Progress in the Development of Diagnostic Tests for Malaria. Diagnostics. 2017, Sep;7(3):1-12.
45. WHO. WHO Guidelines for malaria (approved by the Guidelines Review Committee). Geneva: World Health Organization; 2021.
46. Belete TM. Recent Progress in the Development of New Antimalarial Drugs with Novel Targets. Drug Des Devel Ther. 2020, Sep;14:3875–3889.
47. Renslo AR. Antimalarial Drug Discovery: From Quinine to the Dream of Eradication. ACS Med Chem Lett. 2013, Nov;4 (12):1126–1128.
48. Wampfler R, Hofmann NE, Karl S, Betuela I, Kinboro B, Lorry L, et al. Effects of liver-stage clearance by Primaquine on gametocyte carriage of *P. vivax* and P. falciparum. PLoS Negl Trop Dis. 2017, Jul;11 (7):1-15.
49. Kapishnikov S, Staalsø T, Yang Y, Lee J, Pérez-Berná AJ, Pereiro E, et al. Mode of action of quinoline antimalarial drugs in red blood cells infected by *Plasmodium falciparum* revealed *in vivo.* Proc Natl Acad Sci U S A. 2019, Nov;116 (46):22946–22952.
50. Pinheiro LCS, Feitosa LM, Silveira FFD, Boechat N. Current Antimalarial Therapies and Advances in the Development of Semi-Synthetic Artemisinin Derivatives. An Acad Bras Ciênc. 2018;90 (2):1251–1271.
51. Nzila A. The past, present and future of antifolates in the treatment of *Plasmodium falciparum* infection. J Antimicrob Chemother. 2006, Apr 4;57(6):1043–1054.
52. Meshnick SR. Artemisinin and Its Derivatives. In: Rosenthal PJ, editor. Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery. Totowa, NJ: Humana Press; 2001. p. 191–201.
53. Meshnick SR. Artemisinin: mechanisms of action, resistance and toxicity. Int J Parasitol. 2002, Dec;32(13):1655–1660.
54. Wold health organization. WHO guidelines for malaria, 13 July 2021. World health organization.2021.
55. Castelli F, Odolini S, Autino B, Foca E, Russo R. Malaria Prophylaxis: A Comprehensive Review. Pharmaceuticals. 2010, Oct;3(10):3212–3239.

56. Center for disease control. Malaria - Travelers - Choosing a Drug to Prevent Malaria. CDC. November 18, 2019.
57. World Health Organization. Insecticide-treated nets for malaria transmission control in areas with insecticide-resistant mosquito populations: preferred product characteristics. Geneva: World Health Organization; 2021.
58. Lindsay SW, Thomas MB, and Kleinschmidt I. Threats to the effectiveness of insecticide-treated bednets for malaria control: thinking beyond insecticide resistance. Lancet Glob Health. 2021, Sep;9(9):1325–1331.
59. Martin JL, Mosha FW, Lukole E, Rowland M, Todd J, Charlwood JD, et al. Personal protection with PBO-pyrethroid synergist-treated nets after 2 years of household use against pyrethroid-resistant *Anopheles* in Tanzania. *Parasit Vectors*. 2021, Mar;14(1):1-8.
60. CDC - Malaria - Malaria Worldwide - How Can Malaria Cases and Deaths Be Reduced? - Indoor Residual Spraying [Internet]. Cdc.gov. 2019.
61. Tangena J-AA, Hendriks CMJ, Devine M, Tammaro M, Trett AE, Williams I, et al. Indoor residual spraying for malaria control in sub-Saharan Africa 1997 to 2017: an adjusted retrospective analysis. Malar J. 2020;19(1):1-15.
62. Arora N, Anbalagan LC, Pannu AK. Towards Eradication of Malaria: Is the WHO’s RTS,S/AS01 Vaccination Effective Enough? Risk Manag Healthc Policy. 2021, Mar; 14:1033–1039.
63. Draper SJ, Sack BK, King CR, Nielsen CM, Rayner JC, Higgins MK, et al. Malaria Vaccines: Recent Advances and New Horizons. Cell Host Microbe. 2018, Jul;24(1):43–56.
64. World health organization. WHO recommends groundbreaking malaria vaccine for children at risk. Geneva; 6 October 2021.
65. Bonam SR, Rénia L, Tadepalli G, Bayry J, Kumar HMS. *Plasmodium falciparum* Malaria Vaccines and Vaccine Adjuvants. Vaccines. 2021, Oct;9(10):1-35.
66. Ouji M, Augereau J-M, Paloque L, Benoit-Vical F. *Plasmodium falciparum* resistance to artemisinin-based combination therapies: A sword of Damocles in the path toward malaria elimination. Parasite. 2018, Apr;25(1):1-12.
67. World Health Organization. World health organization technical report series 375. Geneva World Health Organization; 1967.

68. Shibeshi MA, Kifle ZD, Atnafie SA. Antimalarial Drug Resistance and Novel Targets for Antimalarial Drug Discovery. Infect Drug Resist. 2020, Nov;13(1):4047–4060.
69. Foley M. Quinoline Antimalarials Mechanisms of Action and Resistance and Prospects for New Agents. Pharmacol Ther. 1998, Jul;79 (1):55–87.
70. Sirawaraporn W, Yuthavong Y. Kinetic and molecular properties of dihydrofolate reductase from pyrimethamine-sensitive and pyrimethamine-resistant *Plasmodium chabaudi*. Mol Biochem Parasitol. 1984, Mar;10(3):355–367.
71. World health organization. Tackling antimalarial drug resistance Launch of the WHO Report on antimalarial drug efficacy, resistance and response: 10 years of surveillance (2010–2019). WHO. 19 November 2020.
72. Blasco B, Leroy D, Fidock D. Antimalarial drug resistance: linking *Plasmodium falciparum* parasite biology to the clinic. Nature Medicine. 2017, Aug;23(8):917-928.
73. Tang Y-Q, Ye Q, Huang H, Zheng W-Y. An Overview of Available Antimalarials: Discovery, Mode of Action and Drug Resistance. Curr Mol Med. 2020, Feb;20(8):583-592.
74. Pereira T, Rocha e Silva L, Amorim R, Melo M, Zacardi de Souza R, Eberlin M et al. In vitro and in vivo anti-malarial activity of limonoids isolated from the residual seed biomass from *Carapa guianensis* (andiroba) oil production. Malaria Journal. 2014, Aug;13(1):1-8.
75. Oyebode O, Kandala N-B, Chilton PJ, Lilford RJ. Use of traditional medicine in middle-income countries: a WHO-SAGE study. Health Policy Plan. 2016, Oct;31(8):984–991.
76. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014, Jan;4(1):1-10.
77. Alebie G, Urga B, Worku A. Systematic review on traditional medicinal plants used for the treatment of malaria in Ethiopia: trends and perspectives. Malar J. 2017, Aug;16 (1):1-13.
78. Jima TT, Megersa M. Ethnobotanical Study of Medicinal Plants Used to Treat Human Diseases in Berbere District, Bale Zone of Oromia Regional State, South East Ethiopia. Evid Based Complement Alternat Med. 2018, Jul 17;2018(1):1-17.
79. Tuasha N, Petros B, Asfaw Z. Medicinal plants used by traditional healers to treat malignancies and other human ailments in Dalle District, Sidama Zone, Ethiopia. J Ethnobiol Ethnomedicine. 2018, Dec;14 (1):1-21.

80. Oteng Mintah S, Asafo-Agyei T, Archer M-A, Atta-Adjei Junior P, Boamah D, Kumadoh D, et al. Medicinal Plants for Treatment of Prevalent Diseases. In: Perveen S, Al-Taweel A, editors. Pharmacognosy - Medicinal Plants. Intech Open; 2019.
81. Mekuria AB, Geta M, Birru EM, Gelayee DA. Antimalarial Activity of Seed Extracts of Schinus molle Against *Plasmodium berghei* in Mice. J Evid-Based Integr Med. 2021, Dec;26(1):1-10.
82. Alehegn AA, Yesuf JS, Birru EM. Antimalarial Activity of Crude Extract and Solvent Fractions of the Leaves of *Bersama abyssinica Fresen*. (Melianthaceae) against *Plasmodium berghei* Infection in Swiss Albino Mice. Evid-Based Complement Altern Med ECAM. 2020, Jun;2020(1).1-14.
83. Wondafrash DZ, Bhoumik D, Altaye BM, Tareke HB, Assefa BT. Antimalarial Activity of *Cordia africana* (Lam.) (Boraginaceae) Leaf Extracts and Solvent Fractions in *Plasmodium berghei*-Infected Mice. Evid Based Complement Alternat Med. 2019, Aug;2019(1):1-14.
84. Gebrehiwot S, Shumbahri M, Eyado A, Yohannes T. Phytochemical Screening and In Vivo Antimalarial Activity of Two Traditionally Used Medicinal Plants of Afar Region, Ethiopia, against *Plasmodium berghei* in Swiss Albino Mice. J Parasitol Res. 2019, Jun;2019(1):1–8.
85. Azene Bekele-Tesemma, Tengnäs B. Useful trees and shrubs of Ethiopia: identification, propagation, and management for 17 agroclimatic zones. Nairobi: RELMA in ICRAF Project, World Agroforestry Centre, Eastern Africa Region; 2007. p.550.
86. Ebob T, Ukwuoma I. In- vitro Evaluation of Antifungal Effects of *Euphorbia abyssinica* (Desert Candle) Extract. J Adv Med Pharm Sci. 2017, Jan;13(1):1–10.
87. Abebe W. An Overview of Ethiopian Traditional Medicinal Plants Used for Cancer Treatment. Eur J Med Plants. 2016, May;14(4):1–16.
88. *Euphorbia abyssinica* - Useful Tropical Plants [Internet]. Tropical plants database, 2021.
89. Siraj J, Belew S, Suleman S. Ethnobotanical Assessment and Physicochemical Properties of Commonly Used Medicinal Plants in Jimma Zone, Southwest Ethiopia: Traditional Healers Based Cross-Sectional Study. J Exp Pharmacol. 2020, Dec;12(1):665–681.
90. Muluye AB, Desta AG, Abate SK, Dano GT. Anti-malarial activity of the root extract of *Euphorbia abyssinica* (Euphorbiaceae) against *Plasmodium berghei* infection in mice. Malar J. 2019 Dec;18 (1):1-8.

91. Kothale KV, Rothe SP, Pawade PN. Phytochemical screening of some Euphorbiaceae members. J Phytol. 2011, Dec;3(12): 60-62.
92. Tarh JE, Iroegbu CU. In-vitro Anti-Bacterial Activity of Extracts of *Euphorbia abyssinica* (Desert Candle) Stem-Bark and Latex. J Adv Microbiol. 2017, May;2(4): 1-13.
93. *Euphorbia abyssinica* J.F.Gmel. Plants of the World Online. Kew Science. Plants of the World Online. Available from: http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:345491-1.
94. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th ed. Washington (DC): National Academies Press (US) (The National Academies Collection: Reports funded by National Institutes of Health); 2011.
95. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: efficacy models for compound screening. Nat Rev Drug Discov. 2004, Jun;3(6):509-520.
96. Peters W, Portus JH, Robinson BL. The chemotherapy of rodent malaria, XXII: The value of drug-resistant strains of *P. berghei* in screening for blood schizontocidal activity. Ann Trop Med Parasitol. 1975, Jun;69 (2):155–171.
97. Mumtaz F, Raza SM, Ahmad Z, Iftikhar A, Hussain M. Qualitative phytochemical analysis of some selected medicinal plants occurring in local area of Faisalabad, Pakistan. J Pharm Altern Med. 2014;3(3):17-21.
98. Hossain MA, AL-Raqmi KAS, AL-Mijizy ZH, Weli AM, Al-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris.* Asian Pac J Trop Biomed. 2013, Sep;3(9):705–710.
99. Madike LN, Takaidza S, Pillay M. Preliminary Phytochemical Screening of Crude Extracts from the Leaves, Stems, and Roots of *Tulbaghia violacea.* Int J Pharmacogn Phytochem Res. 2017, Oct;9(10):1300-1308.
100. Alabri THA, Al Musalami AHS, Hossain MA, Weli AM, Al-Riyami Q. Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. J King Saud Univ - Sci. 2014, Jul;26(3):237–243.
101. Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A, Ibrahim A. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). Vet Res Forum Int Q J. 2014;5(2):95–100.

102. Gonfa T, Teketle S, Kiros T. Effect of extraction solvent on qualitative and quantitative analysis of major phyto-constituents and *in-vitro* antioxidant activity evaluation of *Cadaba rotundifolia* Forssk leaf extracts. Cogent Food Agric. 2020, Jan; 6(1):1-12.

103. Mamta S, Jyoti S. Phytochemical screening of *acorus calamus* and *lantana camara*. IRJP. 2012, May;3(5):324-326.

104. Maria R, Shirley M, Xavier C, Jaime S, Davi V, Rosa S & Jodie d. 2018. Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. Journal of King Saud University-Science, 30, 500-505.

105. NIGATU, H., BELAY, A., AYALEW, H., ABEBE, B., TADESSE, A., TEWABE, Y. & DEGU, A. 2021. In vitro Antileishmanial Activity of Some Ethiopian Medicinal Plants. Journal of Experimental Pharmacology, 13, 15.

106. Shibeshi MA, Enyew EF, Adinew GM, Aragaw TJ. Antimalarial Activity of Methanolic Extracts and Solvent Fractions of *Combretum molle* Leaves in *Plasmodium berghei* Infected Mice. J Exp Pharmacol. 2021, Feb;13(1):69–89.

107. Alehegn AA, Yesuf JS, Birru EM. Antimalarial Activity of Crude Extract and Solvent Fractions of the Leaves of *Bersama abyssinica Fresen*. (Melianthaceae) against *Plasmodium berghei* Infection in Swiss Albino Mice. Evid Based Complement Alternat Med. 2020, Jun;2020(1):1-14.

108. Nureye D, Tekalign E, Fisseha N, Tesfaye T, Hammeso WW. Evaluation of Antiplasmodial Activity of Hydroalcoholic Crude Extract and Solvent Fractions of *Zehneria scabra* Roots Against *Plasmodium berghei* in Swiss Albino Mice. Infect Drug Resist. 2021, Jul;14:2583–2596.

109. Vadalia J, Sanandia J, Bhatt V, Sheth N. Antiplasmodial and cytotoxic activities of two Euphorbiaceae family plants, *Euphorbia hirta* and *Euphorbia thymifolia*. Curr Bioact Compd. 2021, Mar;18(1):13-17.

110. Monbrison F, Maitrejean M, Latour C, Bugnazet F, Peyron F, Barron D et al. In vitro antimalarial activity of flavonoid derivatives dehydrosilybin and 8-(1; 1)-DMA-kaempferide. Acta Trop. 2006, Jan;97(1): 102-107.

111. Bilia AR, Sannella AR, Vincieri FF, Messori L, Casini A, Gabbiani C, et al. Antiplasmodial Effects of a few Selected Natural Flavonoids and their Modulation of Artemisinin Activity. Nat Prod Commun. 2008, Dec;3(12):1999-2002.

112. Rudrapal M, Chetia D. Plant Flavonoids as Potential Source of Future Antimalarial leads. Syst Rev Pharm. 2016, Nov;8(1):13–18.

113. Uzor PF. Alkaloids from Plants with Antimalarial Activity: A Review of Recent Studies. Evid Based Complement Alternat Med. 2020, Feb;2020(1):1-17.

114. Gounoue R, Nangap J, Yamthe L, Ngueguim F, Tsouh Fokou PV, Tali MB, et al. Antimalarial activity of the aqueous extract of *Euphorbia cordifolia Elliot* in *Plasmodium berghei*-infected mice. Asian Pac J Trop Med. 2020, Jan;13(4):176-184.

115. Anatole Guy Blaise Azebaze JEMT. Antiplasmodial activity of some phenolic compounds from *Cameroonians Allanblackia*. Afr Health Sci. 2015, Sep; 15(3):835-840.

116. Builders M. Antimalarial phenolic compounds in Africa: a review. eJbps. 2019, Mar;6(4):6-11.

117. Okokon JE, Antia BS, Mohanakrishnan D, Sahal D. Antimalarial and antiplasmodial activity of husk extract and fractions of *Zea mays*. Pharm Biol. 2017, Mar;55(1):1394–1400.

118. Keita S, Wélé M, cissé C, Diarra N, Kirkman L, Baba-Moussa L. Antibacterial and Antiplasmodial Activities of Tannins Extracted from *Zizyphus mauritiana* in Mali. Int J Biochem Res Rev. 2018, Dec;24(1):1–8.

119. Nafiu MO, Adewuyi AI, Abdulsalam TA, Ashafa AOT. Antimalarial activity and biochemical effects of saponin-rich extract of *Dianthus basuticus Burtt Davy* in *Plasmodium berghei*-infected mice. Adv Tradit Med. 2021, Apr;1-17.

120. Bantie L, Assefa S, Teklehaimanot T, Engidawork E. In vivo antimalarial activity of the crude leaf extract and solvent fractions of *Croton macrostachyus Hocsht*. (Euphorbiaceae) against Plasmodium berghei in mice. BMC Complement Altern Med. 2014, Mar;14(1):1-10.

121. Hailesilase GG, Rajeshwar Y, Hailu GS, Sibhat GG, Bitew H. In Vivo Antimalarial Evaluation of Crude Extract, Solvent Fractions, and TLC-Isolated Compounds from *Olea europaea Linn subsp*. *cuspidata* (Oleaceae). Evid Based Complement Alternat Med. 2020, May 14;2020(1):1-12.

122. Amelo W, Nagpal P, Makonnen E. Antiplasmodial activity of solvent fractions of methanolic root extract of *Dodonaea angustifolia* in *Plasmodium berghei* infected mice. BMC Complement Altern Med. 2014, Dec;14(1):1-7.

123. Habte G, Nedi T, Assefa S. Antimalarial Activity of Aqueous and 80% Methanol Crude Seed Extracts and Solvent Fractions of *Schinus molle Linnaeus* (Anacardiaceae) in Plasmodiumberghei-Infected Mice. J Trop Med. 2020, Feb;2020(1):1-9.

124. Chinchilla M, Guerrero OM, Abarca G, Barrios M, Castro O. An in vivo model to study the anti-malaric capacity of plant extracts. Rev Biol Trop. 1998, Mar;46(1):35-39.

125. Lamikanra AA, Brown D, Potocnik A, Casals-Pascual C, Langhorne J, Roberts DJ. Malarial anemia: of mice and men. Blood. 2007, Jul;110(1):18–28.

126. Misganaw D, Engidawork E, Nedi T. Evaluation of the anti-malarial activity of crude extract and solvent fractions of the leaves of *Olea europapaea* (Oleaceae) in mice. BMC Complement Altern Med. 2019, Jul;19 (1):1-12.

127. Yang Z-G, Sun H-X, Fang W-H. Haemolytic activities and adjuvant effect of *Astragalus membranaceus saponins* (AMS) on the immune responses to ovalbumin in mice. Vaccine. 2005, Oct;23(44):5196–5203.

128. Ural I, Kayalar H, Durmuskahya C, Cavus I, Ozbilgin A. *In vivo* Antimalarial Activity of Methanol and Water Extracts of Eryngium thorifolium Boiss (Apiaceae Family) against *P. berghei* in Infected Mice. Trop J Pharm Res. 2014, Sep;13 (8):1313-1317.

129. Fentahun S, Makonnen E, Awas T, Giday M. In vivo antimalarial activity of crude extracts and solvent fractions of leaves of *Strychnos mitis* in *Plasmodium berghei* infected mice. BMC Complement Altern Med. 2017, Dec;17(1):1-12.

130. Havenith G. Human surface to mass ratio and body core temperature in exercise heat stress - A concept revisited. J Therm Biol. 2001, Sep;26:387–393.

131. Basir R, Rahiman SF, Hasballah K, Chong W, Talib H, Yam M, et al. *Plasmodium berghei* ANKA Infection in ICR Mice as a Model of Cerebral Malaria. Iran J Parasitol. 2012, Oct;7 (4):62–74.

132. Bihonegn T, Giday M, Yimer G, Animut A, Sisay M. Antimalarial activity of hydromethanolic extract and its solvent fractions of *Vernonia amygdalina* leaves in mice infected with *Plasmodium berghei:* SAGE Open Med. 2019, May;7(1): 1 –10

133. Zeleke G, Kebebe D, Mulisa E, Gashe F. In Vivo Antimalarial Activity of the Solvent Fractions of Fruit Rind and Root of *Carica papaya Linn* (Caricaceae) against *Plasmodium berghei* in Mice. J Parasitol Res. 2017, Dec;2017(1):1-9.

134. Abdela J, Shibeshi W. In Vivo Antimalarial Activity of Solvent Fractions of the Leaves of *Justicia schimperiana* Hochst. Ex Nees against *Plasmodium berghei* in Mice. Ethiop Pharm J. 2014, Jul ;30(2):95–108.

135. Ghauri MA, Iqbal L, Raza A, Hayat U, Atif N, Javeed A. In vivo anti-inflammatory, antipyretic, analgesic activity and in vitro anti-proliferative activity of aqueous methanolic extract of *Euphorbia granulata Forssk*. Future J Pharm Sci. 2021, Feb 4;7(1):1-10.

136. Parmar NS, Mossa JS, Al-Yahya MA, Al-Said MS, Tariq M, Ageel AM. Anti-inflammatory and Antipyretic Activity of *Euphorbia peplis* L. Int J Crude Drug Res. 1989, Jan;27(1):9–13.

137. Nureye D, Assefa S, Nedi T, Engidawork E. In Vivo Antimalarial Activity of the 80 Methanolic Root Bark Extract and Solvent Fractions of *Gardenia ternifolia Schumach. & Thonn.* (Rubiaceae) against *Plasmodium berghei*. Evid Based Complement Alternat Med. 2018, Jun 12;2018(1):1-10.