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In Vivo Antimalarial Activity Evaluation of 80% Methanol Crude Extract and Solvent Fractions of *Cucumis prophetarum* Roots (Cucurbitaceae) in *Plasmodium berghei* Infected Mice

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This is to certify that the thesis prepared by Melese Zenebe, entitled "*In Vivo* Antimalarial Activity of 80% Methanol Crude Extract and Solvent Fractions of *Cucumis prophetarum* Roots (Cucurbitaceae) in *Plasmodium berghei* Infected Mice" and submitted in partial fulfillment for the requirements of 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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DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university.

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Place and date of submission: Addis Ababa, Ethiopia, February, 2021

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ABSTRACT

In Vivo Antimalarial Activity Evaluation of 80% Methanol Crude Extract and Solvent Fractions of *Cucumis prophetarum* Roots in *Plasmodium berghei* Infected Mice

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Addis Ababa University, 2021

Malaria is one of the most important protozoan diseases in the world that cause illness for human. Resistance to currently available antimalarial drugs remains a major challenge and continued to cause an obstacle in the management of malaria. This calls for development and discovery of new antimalarial agents from alternative sources such as medicinal plants. As seen from history, most currently used antimalarial drugs were obtained from traditional medicinal plants. *Cucumis prophetarum* is one of the traditional medicinal plants that have been used traditionally in the treatment of malaria in different parts of Ethiopia. However, it lacks pharmacological investigations to confirm its antimalarial activity. Therefore, this study aimed to investigate *in vivo* antimalarial activity of 80ME and solvent fractions of root of *C. prophetarum* in *P. berghei* infected mice using 4-day suppressive and prophylactic test. Before evaluation of the antimalarial activity, oral acute toxicity study was carried out. To evaluate the antimalarial activity, three doses of the 80ME and solvent fractions (50mg/kg, 100mg/kg and 200mg/kg) were given to parasite infected mice orally. Parameters including parasitemia level, survival time, body weight, body temperature and packed cell volume were evaluated. In 4-day suppressive test, the chemosuppressive effects exerted by the crude extract, chloroform fraction, butanol fraction and aqueous fraction were in a range of 30.84-64.33%, 27.14-48.47%, 24.34-35.39% and 15.91-25.82%, respectively. In prophylactic test, the parasite suppression effect exerted by the 80ME was in a range of 24.58-39.70%. The highest suppression of parasitemia was displayed by the maximum dose (200mg/kg) of 80ME in 4-day suppression test. Furthermore, the 80ME and solvent fractions prevented loss of body weight, body temperature reduction and packed cell volume reduction when compared to the respective negative controls. In addition, they prolonged the survival time of infected mice. In conclusion, the results of this study collectively indicate that the root of *C. prophetarum* has *in vivo* antimalarial activity which supports the traditional claim.

Key words: Antimalarial drug, *Cucumis prophetarum*, *Plasmodium berghei*, Parasitemia.

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LIST OF ABBREVIATIONS AND ACRONYMS

80ME	80% Methanol Crude Extract
ACT	Artemisinin-Based Combination Therapy
ANOVA	Analysis of Variance
CDC	Centers for Disease Control and Prevention
CQ	Chloroquine
DDT	Dichlorodiphenayl Trichloroethane
FDA	Food and Drug Administration
INT	Insecticide Treated Nets
IRS	Indoor Residual Spraying
LD_{50}	Median Lethal Dose
MSP	Merozoite Surface Protein
MST	Mean Survival Time
PCV	Packed Cell Volume
RBCs	Red Blood Cells
SEM	Standard Error of Mean
SP	Sulfadoxine-Pyrimethamine
SPSS	Statistical Package for Social Science
WHO	World Health Organization

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1 INTRODUCTION

1.1 History and Definition of Malaria

Malaria is one of the oldest registered diseases in the world. In Hippocratic geological age, malaria was recognized just as 'the rise in the temperature of the body', acute burning heat' or by its recurring at regular intervals. In the modern age, malaria was recognized by shivering and periodic rise in body temperature (Cunha & Cunha, 2008). In the 18th century the Italians related malaria with 'bad air' – malaria from where the name malaria is originated. It is among the protozoan disease acquired through the parasites of the genus *Plasmodium* and transferred to human by peculiar species of infected female *Anopheline* mosquito (Kalra et al., 2006). The effectiveness of *anopheline* mosquitoes to transfer malaria to man varies greatly by species. More than 3300 different species of mosquitoes exist in the world. Among these different species of mosquitoes about 400 are under the genus *Anopheles*. Among *Anopheles species* around 70 species are possible transmitters for different diseases, but only about 36 are crucial malarial transmitters (Cunha & Cunha, 2008).

The *Plasmodium* species that produce malarial infections in human are *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*), *Plasmodium ovale* (*P. ovale*), *Plasmodium malariae* (*P. malariae*) and *Plasmodium knowlesi* (*P. knowlesi*). Among these *Plasmodium* species the most common causative agent of the disease malaria is *P. falciparum*. However, *P. vivax* malaria, which was previously thought benign, also causes life-threatening symptoms of the disease. *P. malariae* and *P. ovale* are less prevalent and cause less severe disease in humans, whereas *P. knowelsi* is primarily a pathogen of monkeys but has recently been recognized to cause illness, including severe disease, in humans in Asia (Aguiar et al., 2012).

1.2 Prevalence of Malaria

Malaria comprises one of the leading causes of death throughout the world, contempt decades of strategic interventions proposed at decreasing incidence and related mortality. This parasitic disease persists to greatly impact on community health and economies, with most severe results in countries with high poverty indices (Okombo and Chibale, 2018). According to the 2020, World Health Organization (WHO) Malaria Report, there were ~229 million malaria cases and ~409,000 related deaths globally in 2019. The majority of cases were from African Region with 94%, succeeded by the South-East Asia Region about

3% of the instances and the Eastern Mediterranean Region about 2% of the cases. Among the deaths due to malaria in 2019, 94% were happened from the African continent. Children less than 5 years old are the very susceptible group influenced by malaria. They accounted for 67% from all deaths due to malaria throughout the world in 2019 (WHO, 2020).

Among the five *Plasmodium species* that cause malaria, *P. falciparum* is the major causative with severe manifestation of clinical symptoms (Okombo and Chibale, 2018). In Africa, the utmost prevailing *Plasmodium* is *P. falciparum*. In 2017, *P. falciparum* accounted for 99.7%, 62.8%, 69% and 71.9% from estimated malaria cases in African regions, South East Asia, Eastern Mediterranean and Western Pacific, respectively. While *P. vivax* is the most prevalent parasite in the American Regions, representing 74.1% of malaria cases (WHO, 2018).

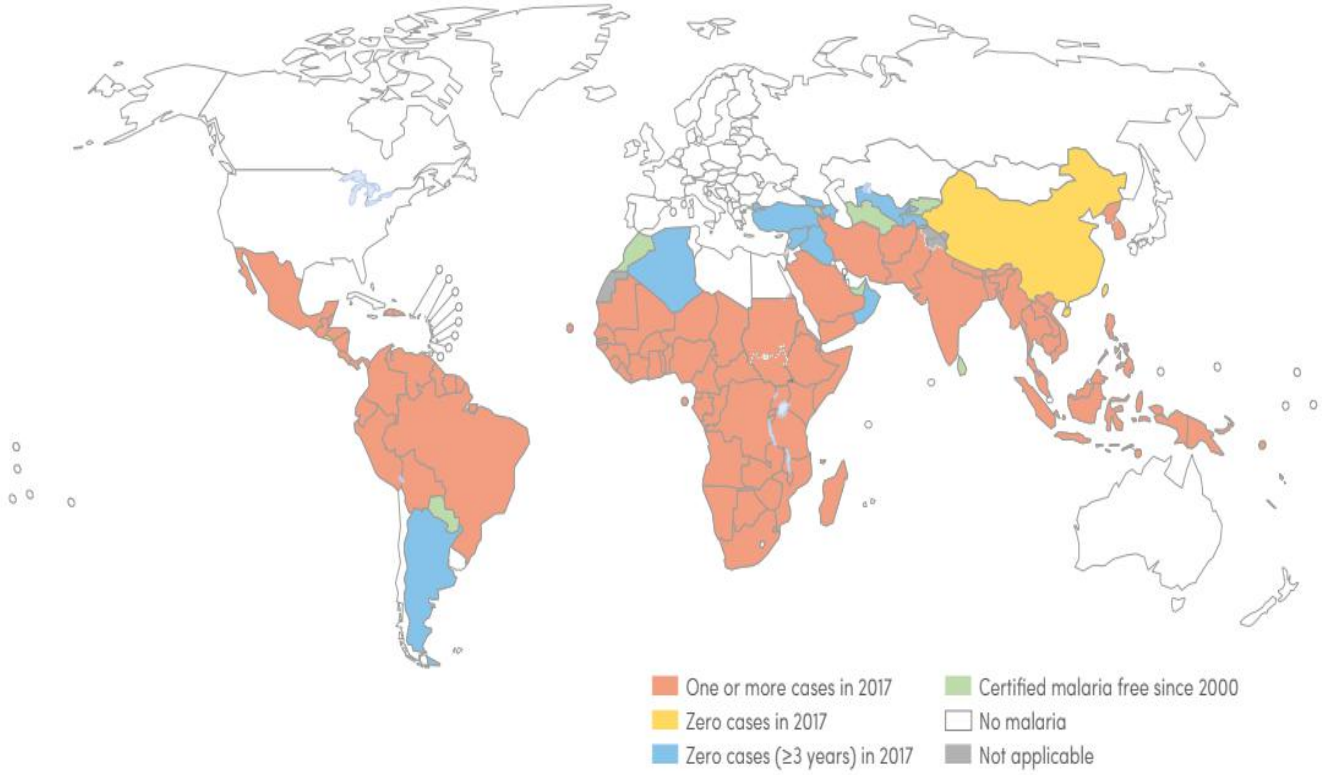

Figure 1: Countries with indigenous cases of malaria in 2000 and their status by 2017.*Countries with zero indigenous cases over at least the past 3 consecutive years are considered to be malaria free. All countries in the WHO European Region reported zero indigenous cases in 2016 and again in 2017. In 2017, both China and El Salvador reported zero indigenous cases* (WHO, 2018).

Ethiopia is one of the malaria-epidemic susceptible countries in Africa. It is at a high risk of epidemics of malaria because of climate and topography. Broad ranges of epidemics happen every 5–8 years in some areas due to climatic variations and drought-related nutritional emergencies. Malaria is prevailing in above 75% of the country's area, with 68% of the entire population being in danger. The disease was accountable for about 12% of outpatient consultations and 10% of health facility admissions, and represents the greatest single cause of morbidity in 2012. *P. falciparum* and *P. Vivax* are the two prevalent malaria parasites in the country, accounting for 60–70% and 30–40% of infections, respectively (FMOH, 2012; Tadesse et al., 2018).

1.3 Life Cycle of Malaria and Site of Antimalarial Drugs

Plasmodium species share the same life cycle, which occurs either in human host (asexual cycle) or in mosquito vector (sexual cycle), and the transmission to human is by peculiar species of infected *Anopheline* mosquito (Prato et al., 2012). Male mosquito does not feed on blood, hence only female serves as a vector (Giribaldi et al., 2015). Female *Anopheles* mosquitoes, whose saliva may carry one of the five species of the *Plasmodium* parasite, transmit malaria (Andrews et al., 2018). The malarial infection begins when the sporozoite stage of the parasite, which resides within the salivary gland of the mosquito, halts in the host liver. This happens when an infected female mosquito bites a healthy person and takes its feed, injecting a little amount of saliva into the skin wound (Prato et al., 2012). These *Anopheles* mosquitoes inoculate sporozoites, present in their salivary glands, into the host (Jiménez-Díaz et al., 2014). Generally, each infected bite comprises 5–200 sporozoites which continue to infect the human (Giribaldi et al., 2015). When the *Anopheles mosquitoes* injecte sporozoites into the skin of mammalian hosts, the sporozoites rapidly enter the blood circulation to succeed in liver hepatocytes (Aguiar et al., 2012).

Once within the human bloodstream, the sporozoites only mobilize for a matter of minutes ahead infecting liver cells. After circulating in the bloodstream, sporozoites move to the liver and finally infect a hepatocyte, after crossing several Kupffer's cell and hepatocytes (Prato et al., 2012). Upon invasion of hepatocytes, sporozoites differentiate and replicate to produce liver schizonts, which then rupture and release thousands of merozoites into the blood. A single schizont can produce thousands of merozoites. In a few species, including *P. vivax* and *P. ovale*, a fraction of parasites enters a quiescent stage (hypnozoite). Reactivated hypnozoites are thought to be the cause of the characteristic cyclic vivax and ovale malaria relapses that happen months after infection (Andrews et al., 2018; Jiménez-Díaz et al., 2014).

Released liver merozoites invade erythrocytes, mature and undergo endomitotic divisions to produce blood schizonts, which contain merozoites infective to new erythrocytes upon schizont rupture (Jiménez-díaz et al., 2014). The merozoites feed on erythrocytes, become rounded and modify into a trophozoite. Once the erythrocytic cycle is completed, thereafter, the merozoites burst from the red blood cell (RBC), and proceed to infect other erythrocytes. The parasite remains in the bloodstream for roughly 60 seconds before entering into another erythrocyte, restarting the process (Prato et al., 2012).

The erythrocyte stage causes the clinical symptoms and is the target of chemotherapy of malaria. The different *Plasmodium* species show marked differences in preference to infect reticulocytes or mature erythrocytes and on the erythrocytic cycle duration. For example, *P. vivax* selectively invades reticulocytes whereas *P. falciparum* is not selective. The erythrocytic cycle is of about 24 h in rodent malarial species and *P. knowlesi*, 48 h for *P. falciparum*, *P. vivax* and *P. ovale* whereas *P. malariae* shows a cycle of 72 h (Jiménez-díaz et al., 2014).

Merozoites infect erythrocytes and can either enter asexual reproduction or differentiate into gametocytes (Andrews et al., 2018). A low percentage of parasites per erythrocytic cycle differentiate into male (microgametocytes) and female (macrogametocytes) gametocytes (Jiménez-díaz et al., 2014). The gametocytes take roughly 8–10 days to succeed in full maturity and don't develop further until they get sucked by the acceptable species of mosquito. If this does not happen, they degenerate and die, because they require lower temperature for further development (Prato et al., 2012). Upon gametocyte activation and fertilization in the mid-gut of mosquitoes, new infective sporozoites that reach their salivary glands are produced (Jiménez-díaz et al., 2014).

Malaria therapy capable of achieving radical cure (i.e., eliminating all parasites from the body) is a critical component of efforts to eliminate malaria. The ideal attributes required to realize radical cure include (a) the ability to block transmission of gametocytes to mosquitoes, (b) the ability to block transmission by insect vectors, (c) activity against hypnozoites, (d) activity against parasites hepatic schizonts, and (e) the ability to clear the pathogenic asexual blood-stage parasites. Although it would be ideal to have one drug capable of achieving a radical cure, it is uncommon for one chemical entity to encompass all the necessary characteristics. Consequently, malaria therapeutics is given in combination to increase effectiveness and protect against the development of drug resistance (Andrews et al., 2018).

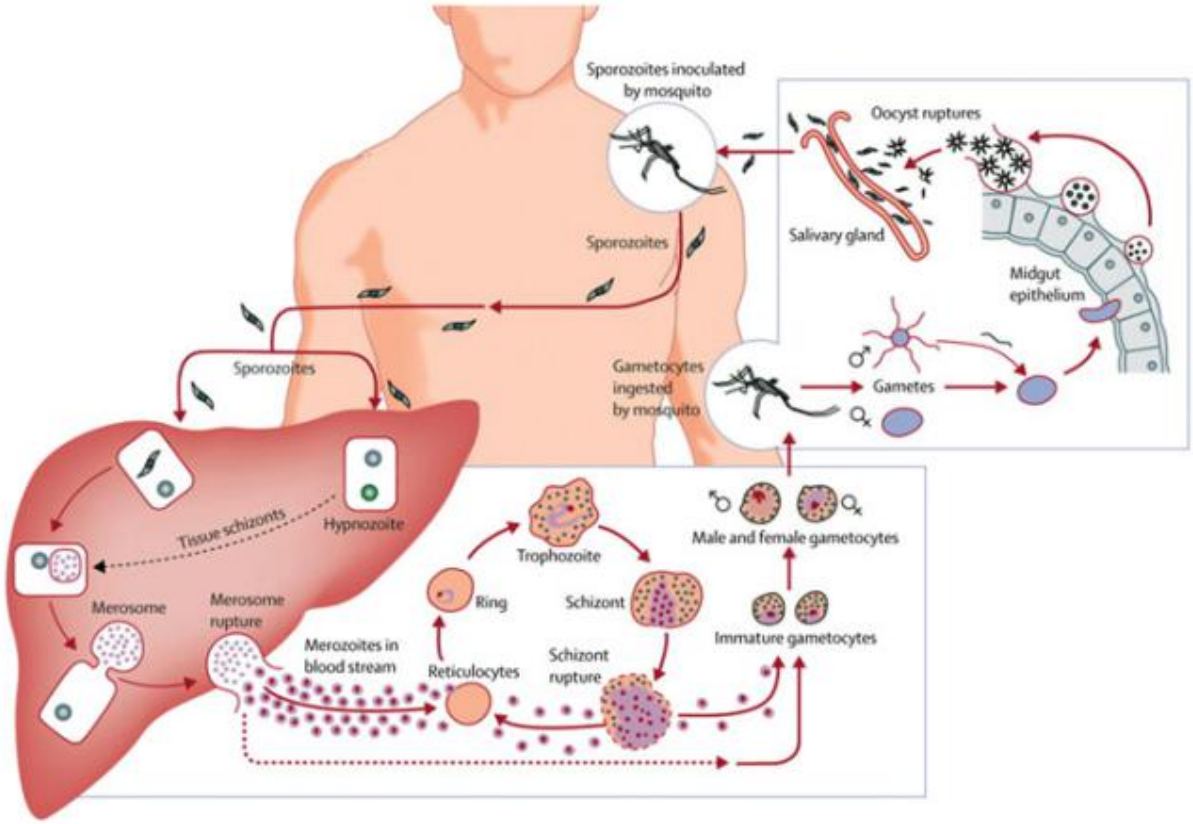

Figure 2: The lifecycle of *Plasmodium*: from mosquito to human and back (Kumar, 2017).

1.4 Clinical Features and Pathology

Cyclical fever has always been conceived as a distinctive symptom of malaria, long before the identification of *Plasmodium* parasites as the etiological agents of the disease (Giribaldi et al., 2015). Malaria illnesses are in general related to periodic fever, chills, shivering, headache, nausea, vomiting, and lots of other clinical conditions. Clinical symptoms of malarial infections are seen shortly after the initiation of the blood stage infection, in which merozoite forms of parasites invade RBCs (Dayananda et al., 2018).

Even though the pathophysiology of malaria is multifactorial and only partially understood, there are some crucial interactions among the parasite and the host that can influence the clinical outcome of the disease. The invasion of erythrocytes by merozoites involves some proteins arising from both parasites and RBCs. In peculiar, major merozoite surface proteins (MSP)-1 and -9 bind to erythrocyte band 3 proteins, the merozoite orients its apical end towards the erythrocyte surface through merozoite apical membrane antigen-1 transmembrane protein and penetrates in the RBC involving erythrocyte binding antigens and

the *P. falciparum* reticulocyte- binding homologs, which binds glycophorins and other unknown receptors. In *P. vivax*, reticulocyte invasion occurs after interaction with the Duffy blood type antigen, the erythrocyte receptor for the chemokine Interleukin-8/CXCL8 (Giribaldi et al., 2015).

The parasite by its cytostome feeds on hemoglobin and other nutrient taken from the plasma. Some digestive enzymes, secreted by the food vacuole of the parasite, break down hemoglobin into proteins and hematin. Proteins are used by the parasite as nutriment source, where hematin is changed into a waste product called haemozoin (Das et al., 1994). Haemozoin avidly phagocytosed by host immune cells. Because of phagocytosis, various monocyte functions are afflicted, such as oxidative burst, bacterial killing, antigen presentation, coordination of erythropoiesis. Furthermore, the product of several pro-inflammatory molecules, including cytokines, chemokines and matrix metalloproteinases, in addition to the production of anti-apoptotic molecules, such as heat shockprotein-27, is enhanced. Too much production of these host molecules as a reply to a parasite product has been suggested to play an important role in clinical progress towards complicated malaria, including cerebral malaria and respiratory distress (Prato et al., 2012).

The toxicants are liberated into the blood beside the release of merozoites then deposed into the liver and the spleen or under the skin, so that the host gets a sallow color. The cumulated toxicants cause malaria fever: the patient endures from chills, shivering, sweating, high temperature headache, abdominal and back pain, nausea, diarrhea, and sometimes vomiting. The fever lasts for 6–10 h and then it comes again after every 48 h with the liberation of a new generation of merozoites (Prato et al., 2012).

1.5 Management of Malaria

The multiple life cycle stages of *Plasmodium* species allow for a number of targets for antimalarial chemotherapy. Nevertheless, all drugs in clinical use for the management of malaria work primarily against the intraerythrocytic development of *Plasmodium* parasites (Wilson et al., 2013). Current antimalarial drugs can be grouped into multiple categories namely: 4-aminoquinolines (chloroquine, Piperaquine), 8-aminoquinolines (Primaquine), aryl-amino alcohols (Quinine, mefloquine, lumefantrine, halofantrine), antifolates (sulfadoxine and pyrimethamine), artemisinins (artesunate, artemether and dihydroartemisinin), antibiotics (doxycycline or clindamycin) and inhibitors of the cytochrome bc1 complex in the parasitic electron transport chain (Atovaquone) (Okombo and Chibale, 2018).

Quinine was for a long time the drug of choice for management of malaria, but later it has ran into resistance by the parasite in some areas of the world, this prompting change to other therapies. Chloroquine (CQ) was a favorable drug against *Plasmodia* used for several decades after quinine, but its use was also arrested by exploitation of resistance by the parasite. As a result, artemisinin-based combination therapy (ACT) was accepted as the first-line regimen against falciparum malaria (Sifuna et al., 2019). ACTs constitute the current WHO-recommended treatment option for uncomplicated malaria. Artemisinins act on both early and late stages of the malaria parasite's lifecycle in the human host. They are fast-acting, efficacious and safe, and commonly combined with other longer-acting antimalarial in a strategy aimed at delaying the onset of resistance (Okombo and Chibale, 2018).

The artemisinin constituent quickly clears parasites from the blood and it is also active against the sexual stages of parasite that mediate onward transmission to mosquitos. The long-acting partner drug of artemisinin clears the remaining parasites and provides protection against development of resistance to the artemisinin derivative (WHO, 2015). The antifolate sulfadoxine and pyrimethamine inhibit enzymes within the folate pathway, and their combination (which is synergistic) constitutes a WHO-recommended intermittent preventive therapy for pregnant women. Antibiotic antimalarial, like doxycycline or clindamycin, inhibit protein synthesis and due to their slow onset of action, are often partnered with faster-acting antimalarial such as the artemisinins. Mefloquine and lumefantrine constitute ACT partner drugs in combination with artesunate and artemether, respectively, while halofantrine is not widely used due to concerns of severe cardiotoxicity (Okombo and Chibale, 2018). The ACTs recommended by the WHO for management of uncomplicated *P. falciparum* malaria are: (i) artemether + lumefantrine, (ii) artesunate + amodiaquine, (iii) artesunate + mefloquine, (iv) artesunate + sulfadoxine–pyrimethamine (SP), (v) dihydroartemisinin + piperaquine and (vi) artesunate + pyronaridine (WHO, 2015).

Atovaquone is mostly used for chemoprevention in combination with the antifolate proguanil, and for treatment of uncomplicated *P. falciparum* malaria. Primaquine is the drug of choice against *P. vivax* and other relapsing forms of malaria. Chloroquine - currently used to treat uncomplicated vivax malaria. Following widespread clinical resistance to chloroquine, amodiaquine was designed as an alternative, although its prolonged use on its own has since been shown to result in an unacceptably high risk of agranulocytosis and hepatitis. Piperaquine, another 4- aminoquinoline, is currently part of an ACT treatment regimen with dihydroartemisininin a fixed-dose combination, which has shown excellent tolerability and high malaria cure rates (Okombo and Chibale, 2018). Quinidine, a stereoisomer of quinine,

is the FDA approved drug for treatment of severe malaria. The CDC can provide intravenous artesunate as an alternative drug for the treatment of severe malaria. However, it is important to recognize that a late hemolytic complication can occur after the use of intravenous artesunate (Daily, 2017).

1.6 Drug Resistance

Raising antimalarial drug-resistance endangers effective antimalarial drug treatment, malaria control, and elimination. Antimalarial drug-resistance of *P. falciparum* tends to emerge in low transmission settings, in particular in Southeast Asia or South America, before expanding to high-transmission settings in sub-Saharan Africa (Menard & Dondorp, 2017). After the discovery of quinine in 1820, several other natural and synthetic compounds have been developed. Nevertheless, as time passed, strains of the parasite started to indicate signs of resistance for these drugs, rendering them less effective. Consequently, their use has quitted or limited to peculiar conditions (Tse et al., 2019).

Quinine resistance case was first reported in South America in the early 1900s and later in the Thai-Cambodian border in the mid-1960s (Kumar, 2017). Quinine is no longer used as a front-line treatment for malaria because of resistance, but is still on the WHO's Model List of Essential Medicines for the treatment of severe malaria in cases where artemisinins are not available (Tse et al., 2019). Chloroquine has been introduced in the late 1940s and it was the gold standard drug for the treatment of uncomplicated malaria for many years, but it is no longer appropriate for the treatment of *P. falciparum* malaria in nearly all areas because of drug resistance (Cui et al., 2015). Its resistant parasites emerged approximately 10 years after introduction, in the late 1950s (Kumar, 2017). Mefloquine was originally introduced in the mid-1970s for the treatment of chloroquine-resistant malaria, and it has been used as both a curative and a prophylactic drug but its resistance was first reported in 1986 (Tse et al., 2019). The combined drug of SP was introduced in the late 1970s as a drug of choice for the treatment of chloroquine-resistant malaria. Unfortunately, resistance to SP occurred rapidly with the first resistance case reported from the Thai-Cambodian border in the early 1980s (Kumar, 2017).

Other drugs used in antimalarial therapy, such as amodiaquine, piperaquine, lumefantrine, primaquine and atovaquone that are quinine derivatives, have been used in the treatment of human malaria over the years with different ranges of success. Nevertheless, since the broad mechanism of action of these quinine-derived drugs is similar, resistant parasite strains have emerged readily with the polymorphisms in the

genes multidrug-resistant transporter (pfmdr-1) and chloroquine-resistant transporter (pfcrt) that encode efflux pumps (Kumar, 2017).

Because of all the mainstream drugs in the management of malaria have ensued in the development of resistant parasites, the ACTs become the mainstay in the treatment of malaria worldwide (Kumar, 2017). Artemisinin was first isolated in 1971, and it has been shown to be effective against all multi-drug resistant forms of *P. falciparum*. Even though slow to develop, the first resistance case to artemisinin was reported in western Cambodia in 2008 (Tse et al., 2019). The emergence of parasites resistant to artemisinins in South East Asia and altered sensitivities to artemisinin partner drugs pose great threats to efforts to control and, eventually, eradicate malaria (Cui et al., 2015).

Resistance has been described for nearly all available drugs. For many drugs the extent of resistance is uncertain and mechanisms of resistance are unknown (Cui et al., 2015). Therefore, tracking drug sensitivities of clinical infections is critical to inform treatment strategy as well as strategies of new drug development that aim to eliminate malaria (Haldar et al., 2018).

1.7 Prevention and Control of Malaria

Many malarial prevention and control schemes exist such as the provision of prompt, effective malarial treatment, vector control and chemoprophylaxis, but none are appropriate and affordable in all contexts (Bloland, 2001). Some of the factors that contributed to this worst picture of malaria are high cost control programs, emergence of new insecticide resistant strains of the vector, creation of new mosquito breeding sites, the problem of drug resistance to almost all currently available antimalarial drugs, lack of organized health infrastructures and the migration behavior of people that increase the incidence and spread of malaria (WHO, 1996). In most countries, especially those in Africa with the highest burden, two approaches to reduce mortality and morbidity based on effective and low cost interventions can be applied to give full coverage (Mendis et al., 2009).

1.7.1 Vector Control

The historical successful elimination of malaria in various parts of the world has been achieved mainly by vector control. In order to control vector-borne diseases, control of mosquitoes is the most important aspect. It is accomplished by application of chemical pesticides against adult-stage mosquitoes. The most prominent classes of insecticides are organochlorines (OCs), organophosphates (OPs), carbamates (Cs), and pyrethroids (PYs). In general, they act by poisoning the nervous system of insects, which is

fundamentally similar to that of mammals (Prato et al., 2012). There are two primary options for this: insecticide treated nets (ITNs) and indoor residual spraying (IRS) (WHO, 2011).

Application of insecticides remains the primary control tool in the majority of vector control programs throughout the world since early nineteenth century. Based on the increasing scientific evidences, WHO gave a clean bill to use of DDT (dichlorodiphenyltrichloroethane) to fight against malaria in Africa and other areas where the vectors are still susceptible to DDT (Prato et al., 2012).

1.7.2 Chemoprophylaxis

In the past, WHO recommended that pregnant women and young children in malaria-endemic areas should receive a full antimalarial treatment followed by weekly chemoprophylaxis with chloroquine. However, the implementation of this policy was limited by a number of factors, including the spread of drug resistance, poor compliance, adverse drug effects, contraindication and cost. Chemoprophylaxis is now only recommended, as a short term measure for international travelers to malaria endemic areas and for soldiers, police and labor forces serving in highly endemic areas (WHO, 2011).

The present priority antimalarials for prophylaxis are atovaquone/proguanil, doxycycline, and mefloquine. Chloroquine is infrequently used as a priority prophylaxis because of widespread resistance. The choice of drug will be determined by tolerability, efficaciousness, adherence, and cost conditions and must also take into account traveler comorbidities and co-medications. The ideal prophylactic agent is effective, safe, cheap, and has action against liver stage and blood stage parasites (Schlagenhauf et al., 2018).

1.8 Medicinal Plants for Malaria Treatment

Man's symbiotic relationship over time with plants has given the world many invaluable benefits. The most crucial plant products are medicines, cosmetic and flavor products, and other pharmaceuticals, in addition to their used as raw materials to form our variety of foods (Sofowora, 1996). Plants are utilized as medicine in different communities of the world, and are origin of potent and powerful drugs. A wide range of medicinal plant components are used as raw drugs as they have various medicinal properties thus herbal drugs constitute a major part in all traditional systems of medicines. Above all other agents, plants have been utilized for medicine from time immemorial as they have suited the prompt personal need, easily accessible and inexpensive (Shrivastava & Roy, 2013).

WHO estimates that up to 80% of the rural population in the developing world still relies on herbal medicine. The long tradition of herbal medicine continues to the present day in China, India, and many countries in Africa and South American. In Africa, traditional medicine is part of the culture, and is practiced by a variety of traditional medicine practitioners, as it is less expensive, accessible and acceptable (Jin-ming et al., 2003; Tadele, 2017).

Natural resources, especially plants, are a potential source of new antimalarial drugs, as they comprise a quantity of metabolites with a great variety of structures and pharmacological activities. Traditional preparations, such as macerations, concoctions and decoctions from plant materials, have been the principal source of treatment of malaria in Africa and other continents where the disease is endemic (Ntie-Kang et al., 2014). Throughout history, medicinal plants have been the focus of numerous studies designed at finding alternative antimalarial drugs in different parts of the world. This has resulted to the finding of numerous antimalarial compounds with important structural varieties, including quinines, triterpenes, sesquiterpenoids, quassinoids, limnoids, alkaloids, lignans, and coumarins (Alebie et al., 2017).

The finding of the first antimalarial treatment nearly 400 years ago resulted from observations that acutely ill patients were cured of malaria after treatment with infusions of bark obtained from *Cinchona calisaya* and *Cinchona succirubra* plants. Such activity in *Cinchona calisaya* and *Cinchona succirubra* plants was later attributed to the alkaloid quinine, which was characterized by French chemists in 1820 (Aguiar et al., 2012). Moreover, artemisinin was first discovered in 1971 from the plant *Artemisia annua,* a plant that has ordinarily been utilized in Chinese Traditional Medicine (Tse et al., 2019). The importance of quinine (and of synthetic drugs derived from quinine such as chloroquine and mefloquine), and more recently of artemisinin and its semi-synthetic derivatives as plant derived antimalarial, has encouraged the proceeding search for new natural product-derived antimalarial (Wright, 2005).

In Africa, the use of native plants still plays key role in malaria treatment. These plants might be interesting sources for the discovery of new antimalarial drugs (Hilou et al., 2006). Approximately 80% of peoples of Ethiopia (especially rural societies) still rely on traditional medicinal plants to treat numerous diseases. This was because of high price of conventional drugs, scarcity and inaccessibility of modern health services, and cultural suitability of folkloric medicine. A number of medicinal plants are used as traditional malaria therapy in different parts of Ethiopia (Alebie et al., 2017).

Proper documentation of traditional medicine and plants used in the treatment of malaria makes an important task not only in maintaining precious indigenous knowledge and biodiversity but also in stimulating future research on safety and efficacy of medicinal plants and identification of chemical entities that could be developed into new standardized phytomedicines (Debela H. et al, 2006). Now a day, most of the researchers are focusing on medicinal plants via researches and WHO has encouraged and recommended the use of them as an alternative therapy for malaria (Ansarullah et al., 2011).

1.9 Family Cucurbitaceae

Cucurbitaceae families, also called vine family, are mostly known prostrate or climbing annuals plants. It is known to be the largest family of vegetable crops. The origin of Cucurbitaceae is tropical and to some extent in temperate areas, and most of its genera originated from Asia, America and largely Africa (Fapohunda et al., 2018). Cucurbitaceae family consists 825 species under 125 genera (Jamuna et al., 2015). The important genera belonging to the family are *Trichosanthes, Lagenaria, Luffa, Benincasa, Momordica, Cucumis, Citrullus, Cucurbita, Bryonopsis* and *Corallocarpus* (Fapohunda et al., 2018).

Cucurbitaceae are known for their nutritive and medicinal values (Fapohunda et al., 2018). In human food conception, this family contributes highest rank among the plant families (Jamuna et al., 2015). Cucurbits are vegetable crops, belonging to the family cucurbitaceae, which primarily comprised species consumed as food worldwide. Cucurbits are excellent in nature having composition of all the essential constituents required for good health of humans (Shrivastava & Roy, 2013). They contain vitamins, large amount of other nutrients and water (Jamuna et al., 2015).

In addition to nutritional use, Cucurbit plants are used actively as traditional herbal remedies for various diseases. Members of this family have always been considered as a subject of research because of the fact that they have many of biological activities like anti-fungal, anti-bacterial, anti-viral, anti-diabetic and anti-tumor. They have also demonstrated anti-inflammatory, hepatoprotective, cardiovascular and immune-regulatory activities (Fapohunda et al., 2018). Cucurbit plants are also recognized to have numerous bioactive compounds such as cucurbitacins, triterpenes, sterols and alkaloids (Rajasree et al., 2016).

Phytochemical screening of the plants under the cucurbitaceae family confirms the existence of various phytochemicals, like tannins, glycosides, terpenoides, steroids, saponins, carotenoids, and resins, that have various biological activities (Rajasree et al., 2016).

1.9.1 Genus Cucumis

Cucumis, one of the major Genus of Cucurbitaceae family, botanically highly specialized genus mainly climbing, hairy and both wild and cultivated plants. It is well represented in the moist and moderately dry tropics of the world, particularly in grass and bush land areas of Africa including Ethiopia. It is a genus of more than 52 species. A lot of works that have been done by the researchers throughout the world on various plants of this genus which showed the presence of triterpene like compounds namely cucurbitacins (Abebe, 2016; Rajasree et al., 2016).

Phytochemical screening of the plants under the genus *Cucumis* confirms the existence of various phytochemicals, for example the ethanolic and chloroform extract of leaves and stems of *Cucumis sativus* possessed phytoconstituents such as alkaloid, glycoside, steroid, saponin, tannin and flavonoid. A number of phenolic glycosides also have been isolated from the seeds of *Cucumis melo* (Rajasree et al., 2016).

This genus are used actively as traditional herbal remedies as anti-inflammatory, antitumor, hepato-protective, cardio vascular and immune-regulatory activities and also reported to possess purgatives and anti-helminthic properties due to its secondary metabolite cucurbitacin content (Saboo et al., 2013). Cucurbitacins have received a great deal of attention because of their cytotoxic, anti-proliferative, anti-inflammatory, analgesic, antimicrobial, anti-helminthic, hepatoprotective, cardiovascular, and antidiabetic effects, antioxidant activity and anticancer effects in vitro and in vivo (Tamiru et al., 2019).

1.9.2 *Cucumis prophetarum*

Cucumis prophetarum is a species of tendril bearing herb in the Cucurbitaceae family. It is mostly a prostrate or climbing, monoecious herb. It has variously colored fruits. The fruits are ellipsoid and green with white strips. It is widely grown in Africa, Asia and Australia (Kavishankar & Lakshmidevi, 2014). In Ethiopia, it is distributed in different parts of the country including Afar, Tigray, Wello, North Shewa Zone, Sidamo and Jimma Zone. It is commonly known by the local name "**Yemdir embuay**" (Amharic) (Meragiaw & Asfaw, 2014; Suleman et al., 2017).

Traditional healers use *C. prophetarum* in different part of the world to treat a number of diseases. For example, in Saudi folk medicine it is used for the treatment of liver disorders. Moreover, as cited by Abdulrhman Alsayari et al., 2018 the antidiabetic and antioxidant activity of the different fractions of fruits of *C. prophetarum* has been reported (Alsayari et al, 2018).

Ethiopian folkloric medicine use *C. prophetarum* as a medicine for numerous conditions including coughing, rabies in dogs, stomach pain, to induce abortion, toothache, bad breath, diarrhea, eye diseases, gonorrhea, TB, skin cancer and malaria. While different parts of the plant are used to treat the different situation, for skin cancer, pulverized roots made as pastes/ointments are reported to be applied directly onto affected areas. Consistent with the reported anticancer claim in Ethiopia, scientific studies have also demonstrated potent antineoplastic activity for some compounds isolated from the fruits of the plant. Moreover, compounds obtained from the related species, *C. ficifolius* (cucurbitacin D and E), have been found to be potent cytotoxic and antineoplastic agents (Abebe, 2016; Meragiaw & Asfaw, 2014; Suleman et al., 2017).

According to the report by ethnobotanical study of the Ethiopian Traditional Herbal Medicine (Meragiaw & Asfaw, 2014), and Treatment of Malaria and Related Symptoms Using Traditional Herbal Medicine in Ethiopia (Suleman et al., 2017), the root of *C. prophetarum* is used traditionally for the treatment of malaria. Despite the extensive traditional use of this plant part there is no prior scientifically evaluated report on the antimalarial activities of the root extract of this plant. Therefore, it is justifiable to scientifically evaluate its anti-malarial activity in rodent malarial model to support or deject its folk use in the treatment of malaria. Hence, in the present study, efforts was made to evaluate the antimalarial activity of the root extract of *C. prophetarum* against *Plasmodium berghei* infected mice.

Figure 3: *C. prophetarum* plant

1.10 Justification of the Study

Malaria is one of the major global health problems. The burden of malarial disease is greatest in sub-Saharan Africa. The main problem in controlling the disease has been resistance to the antiplasmodial drugs. Besides the development of resistance to currently available drugs, there is also the absence of new drugs coming to the market which leads to lack of alternative safe and effective drugs. The parasite resistances for the available antiplasmodial drugs and absence of new drugs coming to the market have allowed the necessity to discover novel antiplasmodial drugs. New and free from harm antiplasmodial drugs with broad boundary of safety and novel mechanism of action are an important pertains. One of the areas for the search for new antimalarial is the traditionally claimed antimalarial plants. Medicinal plants play an important role in the treatment of malaria especially in developing countries where resources are limited. In sub-Saharan Africa, malaria is endemic and in other parts of the world, plants are extensively used for treating periodic fevers and malaria.

Studies have been conducted on traditionally claimed medicinal plants in Ethiopia and elsewhere for scientific validation. This is because they have been part of human life since time immemorial; and a number of plant products have been in extensive use in ethno-medicine. It has been claimed that about 80% of the Ethiopian population rely on medicinal plants for treating various illnesses including malaria. Plants and their extracts have enormous potential for treatment of malaria. However, there is a need for scientific confirmation, standardization and safety evaluation of traditionally used medicinal plants before they are recommended for treatment of malaria.

An ethnobotanical study of the Ethiopian Traditional Herbal Medicine (Meragiaw & Asfaw, 2014) and Treatment of Malaria and Related Symptoms Using Traditional Herbal Medicine in Ethiopia (Suleman et al., 2017) for the root of *C. prophetarum* reported that this plant is used to treat malaria. Therefore, this study was attempted to validate the traditional use of the plant. Furthermore, the outcomes of this work could aid the scientific community to further investigate the plant by initiating advanced studies on molecular mechanisms and preparation of plant origin medicines by isolating the particular agent accountable for the antiplasmodial action.

2 OBJECTIVE OF THE STUDY

2.1 General Objective

- ✓ To evaluate *in vivo* antimalarial activities of 80% methanol crude extract (80ME) and solvent fractions of *C. prophetarum* roots against *P. berghei* infected mice.

2.2 Specific Objectives

- ✓ To extract the roots of *C. prophetarum* using the 80% methanol.
- ✓ To fractionate the 80ME using three different solvents.
- ✓ To determine acute toxicity of the extract of the root of *C. prophetarum.*
- ✓ To evaluate the antimalarial activity of the 80ME of root of *C. prophetarum* using the 4-day suppressive test.
- ✓ To assess the antimalarial activity of the solvent fractions of the root of *C. prophetarum* using the 4-day suppressive test.
- ✓ To evaluate the antimalarial activity of the root extract of *C. prophetarum* using the prophylactic test.

3 MATERIALS AND METHODS

3.1 Plant Materials

The roots of *C. prophetarum* were collected in December, 2019 GC, from Kora Tamo kebele, Merhabete District, North Shewa Zone, Amhara Region, Ethiopia which is around 200km away from North of Addis Ababa. Authentication and identification of the plant was done by the National Herbarium, Addis Ababa University, and a specimen of the plant material was preserved for future reference, where voucher specimen of MZ001.

3.2 Chemicals and Reagents

In this study the following chemicals and reagents were used: Chloroquine phosphate (Ethiopian Pharmaceutical Manufacturing, Ethiopia), normal saline (Fresenius Kabi, India), methanol (Carlo Erba, France), n-butanol (Fischer Scientific, England), chloroform (Carlo Erba, France), tween 80 (Lobe Chemi, India), trisodium citrate, Giemsa stain, distilled water (Medicinal Chemistry Laboratory, School of Pharmacy, CHS, AAU), and oil immersion (Neolab, India). Analytical grade chemicals were used for the experiment.

3.3 Experimental Animals

Swiss albino mice of either sex weighing 24-30 g and age 6-8 weeks were used. The mice were obtained from the animal house of the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Science, Addis Ababa University. The mice were held at room temperature in 12 hour light- dark interval and allowed free feeding to standard pellet laboratory food and water. Mice were adapted to the laboratory environments for a week before the initiation of the experiment. All the experiments were carried out in agreement with the internationally consented laboratory animal use, care and guideline (Clark et al., 1997; National Academy of Sciences, 2011).

3.4 The Rodent Malaria Parasite

Plasmodium berghei ANKA strain, which is sensitive for chloroquine, was obtained from Department of Pharmacology and Toxicology, School of Pharmacy, College of Health Sciences, Mekelle University, Mekelle, Ethiopia. Then the parasite was maintained in the laboratory by consecutive passage of blood from the mice that had parasite in its blood to the mice that had no parasite in its blood on weekly basis till 30% parasites were present in the blood (Fidock et al., 2004).

3.5 Preparation of Crude Extracts

The roots of the *C. prophetarum* were washed with tap water to remove dirt and then shade dried at room temperature. The dried roots were crushed into coarse powder. The coarse powder was divided into four parts, 150gm each, by weighing sensitive digital balance. Then, the weighed coarse powdered plant material was macerated in 80% methanol in Erlenmeyer flasks for 72 hours, by macerating each 150gm coarse powder in 1500ml of 80% methanol. The extraction process was facilitated with occasional shaking and stirring using stirrer. After 72 hours, the ensuing mixture having the 80% methanol crude extract was separated from the marc with guaze, followed by filtration via Whatman filter paper number 1 under suction filtration. Then the same procedure was repeated two times by adding fresh solvent (80% methanol) into the marc, macerated for 72 hours.

The filtrate obtained from the consecutive maceration were pulled together and mixed in a flask. Then the methanol was allowed to remove from the filtrates by using the rotary evaporator (Buchi Rota vapor R-200, Switzerland) under reduced pressure with a temperature of not exceeding 40°C. Then the resulting extract obtained after evaporation of the methanol using reduced pressure was frozen in deep freezer (-18 ^{0}C) overnight and then allowed to dry in the lyophilizer (Operan, Korea Vacuum Limited, Korea) to remove the water from the extract. Finally, the concentrated dried extract was transferred into vials and kept at -20^{0}C until used for the experiment (Bantie et al., 2014; Endale et al., 2013).

3.6 Fractionation of 80% Methanol Crude Extracts

The 80ME was undergone successive extraction using solvents with different polarity (water, n-butanol and chloroform). Thirty grams of 80ME was suspended in 200ml of distilled water in a separatory funnel. Then, the suspension was shaken with 100ml of chloroform. After a few minutes, a layer was formed between the aqueous and chloroform suspension. Then, the chloroform layer was collected in a beaker by careful opening of the regulator located near to the bottom of the separatory funnel. Then the regulator of the funnel was closed after collecting all the chloroform and the aqueous layer was shaken again twice by adding the same amount of chloroform to that of the previously added and then the chloroform fractions was collected and combined to the previous one. The chloroform fraction was dried in an oven with temperature not exceeding 40°C.

The remaining aqueous layer was again shaken with 100ml of n-butanol. Then, the aqueous layer was first collected into a beaker by careful opening of the regulator of the separatory funnel. Then, the remaining n-

butanol layer in the separatory funnel was collected into another beaker. The collected aqueous layer was then transferred into the separatory funnel and then was shaken twice with the same amount of n-butanol, and the n-butanol fraction was collected and combined to the previous one. The combined n-butanol fraction was concentrated by rotary evaporator (Buchi Rota vapor R-200, Switzerland) at temperature not exceeding 40°C, and finally dried in an oven with temperature 40°C.

The last remaining aqueous residue in the separatory funnel was transferred into a beaker, frozen in deep freezer (-18 ^{0}C) overnight and then freeze dried by a lyophilizer to eliminate water and got the aqueous fraction. Finally, dried chloroform fraction, n-butanol fraction and aqueous fraction were collected and the yield were calculated, and then were kept in separate vials and stored in a refrigerator at -20°C until used for the experiment (Bantie et al., 2014; Eyasu et al., 2013; Zeleke et al., 2017).

3.7 Acute Oral Toxicity Study

Before the evaluation of the antimalarial activity, oral acute toxicity test and determination of median lethal dose (LD_{50}) of 80ME was carried out according to the Lorke's Method (Lorke, 1983) using female Swiss albino mice, aged 6-8 weeks that weighed 26-30g. The mice were acclimatized to the experimental laboratory condition for one week before the administration of the dose of the extract. Then, at the last day of a week the mice were deprived of food but not water for 4 hour. Following the period of fasting, the mice were weighed and given a single dose of the extract by oral gavage for each group. Food was then withheld for further 2 hours.

The test was conducted in two phases. In the first phase of the study, 9 mice were randomized into 3 groups with 3 mice in each group (n=3) and were given a dose of 10mg/kg for group 1 (G1), 100mg/kg for group 2 (G2) and 1000mg/kg for group 3 (G3) of 80ME orally. Then, the mice were watched for gross behavioral alterations, such as loss of food intake, hair erection, lachrymation, involuntary movement, seizures, death and the like which are signs of toxicity, with special attention given during the first 4 hours and subsequently daily for 7 days. Based on the results obtained from phase one test, phase two test was carried out by using another new set of 4 mice randomized into 4 groups with 1 mouse in each group (n=1), and were given a single dose of 600mg/kg for G1, 1000mg/kg for G2, 1600mg/kg for G3 and 2900mg/kg for G4. These were also watched for signs of toxicity and mortality with special attention given during the first 4 hours and thereafter daily for 7 days.

Then oral LD_{50} was calculated as the geometric mean of the least dose that kills a mouse and the highest dose that does not kill any mouse, as shown the equation below (Lorke, 1983; Madara et al., 2010; Ngulde et al., 2013; Uraku et al., 2015).

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where, D_0 = Maximum dose that presented no death

D_{100} = Minimum dose that produced death

3.8 *In vivo* Antimalarial Activity Test

3.8.1 Dosing and Grouping of Animals

To evaluate the *in vivo* antiplasmodial activity of 80ME and solvent fractions of root of *C. prophetarum*, *P. berghei* infected mice were randomly divided into 5 groups with 6 mice (n=6) in each group. For each test, three groups (II, III, and IV) were treated with three different doses (50mg/kg, 100mg/kg and 200mg/kg, respectively) of the plant extract. The remaining two groups (I and V) served as negative and positive controls, respectively. The negative control groups in all tests received solvents for reconstitution; 10ml/kg of distilled water for aqueous fraction, 10ml/kg of 2% tween 80 in case of the other three tests (80ME, chloroform fraction and n-butanol fraction). The positive control groups in all tests received standard drug, chloroquine phosphate 25mg/kg (CQ). Dose selection for the plant extract was made according to the result of oral acute toxicity study conducted on the extracts. Each dose was reconstituted by appropriate vehicle. Each dose of the crude extracts, fractions and standard drug after reconstitution by the respective solvents, and the solvents for reconstitution in negative control groups were administered via the oral route using gavage. For all animals, volume of administration was 10ml/kg and calculated based on individual mouse body weight. Duration of administration was based on the type of test performed (Fidock et al., 2004; Nureye et al., 2018; OECD, 2008).

3.8.2 Parasite Inoculation

For induction of malaria in the experimental mice, *Plasmodium berghei* ANKA strain was used. Swiss albino mice previously infected with *P. berghei* were used as donor. The parasite was preserved by consecutive passage of blood from the mice that had the parasite in its blood to the mice that had no parasite in its blood previously on weekly basis until approximately 30% parasitemia level was reached. First, the parasitemia level of the contributor mice were ascertained from the blood taken by cutting at the tip of tail of the mice with scissor. Then, to infect the test mice, the donor mice were anesthetized by

placing in closed chamber that contain chloroform as anesthetic and infected blood with a rising parasitemia of approximately 30% was obtained by cardiac puncture and collected in a tube having 2% trisodium citrate as prevention of blood clotting. The blood that collected from the donor mice was then diluted in normal saline so that the final suspension contained about $1x10^7$ infected RBCs in every 0.2ml suspension. The dilution was formed based on parasitemia level of the contributor mice and RBC count of the healthy mice in such a way that 1ml blood contains $5x10^7$ parasitized RBCs. All mice used in the study were infected intraperitoneally with 0.2ml infected blood having about 1×10^7 *P. berghei* infected RBCs. The antiplasmodial action of the extracts were tested using 4-day suppressive test and prophylactic test (Bantie et al., 2014; Birru et al., 2017; Fidock et al., 2004; Nureye et al., 2018).

3.8.3 The 4-Days Suppressive Test

The standard four-day suppressive method was used to assess the antimalarial action of the 80ME and solvent fractions of *C. prophetarum* roots. On day 0 (D0); each mouse, that were used in this model, was given 0.2ml of blood having about $1x10^7$ *P. berghei* parasitized RBCs via intraperitoneal route. Subsequently, 3 hour post-infection, treatments were started with a single dose as mentioned above in the dosing and grouping section. On day 1 to 3; 24 hour, 48 hour and 72 hour post-infection, the treatments were continued with the same dose and by the same route as on D0. On day 4 (D4); 24 hour after the last treatment (i.e. 96 hour post-infection), blood smears from all mice were prepared.

On D4, blood was taken from the tail of every mouse using clean slides, and thin films were prepared and allowed to air dry. The films were then fixed with few drops of methanol, left for about 15 min to air dry. Then the slides were stained with freshly prepared 10% Giemsa for 15 min. Then after, the stained slides were washed out with distilled water and the slides were left to air dry. Then the slides were viewed under the light microscope using the oil immersion and parasites were counted using the X100 objective. The percentage (%) of parasitemia was found by counting the number of parasitized RBCs out of erythrocytes in random fields of the microscope. Three fields with approximately 300 cells were counted for each slide. Then, % parasitemia and % suppression were determined (Birru et al., 2017; Fidock et al., 2004; Peters & Robinson, 1999).

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

The mean % parasitemia of the groups in each crude extract and solvent fractions, and the respective negative controls were determined. Then, the % suppression was calculated using the formula (Fidock et al., 2004).

$$\% \text{ Suppression } = \frac{\text{Mean parasitemia of negative control} - \text{Mean parasitaemia of treatment group}}{\text{Mean parasitemia of negative control}} \text{X } 100$$

To evaluate the effect of crude extract and solvent fractions on survival time, mice were supervised daily and the number of days from the time of injection up to death was documented for every mouse in treatment and control groups throughout the follow up period (30 days). Then, the mean survival time (MST) for each group was determined as:

$$MST = \frac{Sum\ of\ survival\ time\ of\ all\ mice\ in\ a\ group(days)}{Total\ number\ of\ mice\ in\ that\ group}$$

3.8.4 Determination of Body Weight, Temperature and Packed Cell Volume

The body weights of the mice were measured to observe whether the root extracts or solvent fractions prevent weight loss. Rectal temperature was also measured. Weight and rectal temperature of each mouse in each group was recorded just before treatment on D0, and after treatment on D4 using sensitive digital weighing balance and digital rectal thermometer, respectively. The mean percentage changes prior to treatment and after treatment was then calculated and analyzed for each group (Fentahun et al., 2017; Gebrehiwot et al., 2019; Satish & Sunita, 2017).

The packed cell volume (PCV) was determined to anticipate the efficacy of the extracts in preventing hemolysis resulting from increasing parasitemia related with malaria. In the same way to that of weight and temperature measurement, PCV was measured just before treatment on D0 and at the end of D4 using micro-hematocrit centrifuge. Blood was collected from the tail of every mouse in heparinized micro-hematocrit capillary tubes by filling three-quarters of its volume. Then the capillary tubes were sealed at one side by sealant and placed in a micro-hematocrit centrifuge with the sealed ends towards the outside. The blood was then centrifuged at 12,000 rpm for 15 min. Then, the capillary tubes were taken out from the centrifuge and PCV was ascertained using a standard Micro-Hematocrit Reader (Birru et al., 2017; Moseley & Bull, 1982; Gilmour.& Sykes, 1951; Mekonnen, 2015).

$$\text{PCV } = \frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}} \text{ x}100$$

3.8.5 Prophylactic Test

Evaluation of the preventive effect of the 80ME of the *C. prophetarum* roots was done according to the method reported by Peters (Peters, 1965). Thirty mice were weighed and randomly divided into 5 groups with 6 mice in each group, and were treated for four consecutive days (D0-D3) as depicted earlier in animal grouping and dosing section. On the 5^{th} day (D4), all mice in each group were infected with inoculum of 1×10^7 parasitized RBCs. Then, blood smears were made 72 h post-infection from every mouse to determine parasitemia level. Other parameters like weight change, temperature change, and PCV change also were determined. Then, the mice were monitored for 30 days post infection for their existence to calculate MST (Nureye et al., 2018).

4 DATA ANALYSIS

The collected data were organized and entered into windows statistical package for social science (SPSS) version 25 and then were analyzed. The data were presented as mean ± standard error of the mean (SEM). Data obtained from all tests were analyzed with one-way ANOVA followed by Tuckey post hoc test to compare the levels of parasitemia, survival time, body weight change, rectal temperature change and PCV change of the parasitized mice between control and experimental groups, as well as between treatment groups. The analysis at 95% confidence interval and p-value less than 0.05 was considered to be statistically significant.

5 RESULTS

5.1 Percentage Yield of 80ME and Solvent Fractions

The weight by weight percentage yield of 80ME and solvent fractions of the *C. prophetarum* roots is presented in Table 1 below. As seen from the Table, from 600g coarse powder of root of *C. prophetarum,* 47g (7.83%) of crude extract was obtained. Among the solvent fractions of the 80% methanol crude extract of the *C. prophetarum* roots, the highest percentage of yield was obtained from the aqueous fraction (59.90%), whereas the lowest percentage yield was obtained from n-butanol fraction (12.10%).

Table 1: Percentage yield of 80ME and solvent fractions of *C. prophetarum* roots

Crude Extract/Solvent Fraction	Weight	Percentage Yield (%)
80ME	47g of 600g	7.83
Aqueous fraction	17.97g of 30g	59.90
Chloroform fraction	6.45g of 30g	21.50
Butanol fraction	3.63g of 30g	12.10

5.2 Acute Oral Toxicity Study

The result of the acute oral toxicity test of the 80ME of root of *C. prophetarum* using the Lorke's method is presented in Table 2 below. There were gross physical and behavioral signs of toxicity (these include: loss of appetite, hair erection, reduced activity and depression), and finally death during the observation period in the experimental mice that was given a dose of 1600mg/kg and above of the extract. According to this test to determine the safety of the extract, LD_{50} was calculated using the formula shown below and found to be an estimated LD_{50} of 1265mg/kg.

$$LD_{50} = \sqrt{D_0 \times D_{100}} = \sqrt{1000 \times 1600}$$

LD_{50} = 1265mg/kg

D_0 = Highest dose that gave no mortality- for phase-two

D_{100} = Lowest dose that produced mortality- for phase-two

It is known that 10% of the LD_{50} is the moderate dose of the tested therapeutic dose. Accordingly 10% of 1265 is 126.5, which approximate to 100. Then, half of the moderate dose (50mg/kg), moderate dose (100mg/kg) and double of the moderate dose (200mg/kg) were used, because in most *in vivo* studies that

has done previously follow this method of dose selection for the activity test. This was the reason for why did choose 50, 100 and 200mg/kg dose for the antimalarial activity.

Table 2: The result of acute oral toxicity test of the 80ME of root of *C. prophetarum*

Phase-One				Phase-Two			
Group	Mice no. /group	Dose(mg/kg)	death/group & time of death	Group	Mice no. /group	Dose(mg/kg)	death/group & time of death
1	3	10	0	1	1	600	0
2	3	100	0	2	1	1000	0
3	3	1000	1, at day 1	3	1	1600	1, at day 1
				4	1	2900	1, at day 1

5.3 Four-Day Suppressive Test

5.3.1 Chemosuppressive Effect of the Plant Extract in 4-Day Suppressive test

The results of the effects of 80ME and the three solvent fractions of root of *C. prophetarum* at different dose levels on parasitemia and survival time of Swiss albino mice infected with chloroquine sensitive *P. berghei* are presented in Table 3. The results are expressed as mean percentage parasitemia, suppression, and mean survival time in days in comparison with the respective negative control group treated with the vehicle. The 4-day suppressive test indicated that the crude extract as well as the solvent fractions of root of *C. prophetarum* displayed a significant reduction of parasitemia ($p<0.05$) when compared to the respective negative control. Different dose levels in crude extract and in each solvent fractions exhibited statistically significant ($p<0.05$) difference in suppressing parasite load at all dose levels evaluated in the test. The higher the dose of each extract given, the higher the percentage suppression of the parasite displayed. Furthermore, all the solvent fractions and the crude extract reduced parasitemia at different level. From all the doses, the highest parasitemia suppression (64.33%) was displayed by the 80ME at a dose of 200mg/kg/day while the lowest parasitemia suppression (15.91%) was displayed by the aqueous fraction at a dose of 50mg/kg/day.

When doses of 80ME and each fraction were compared among each other, exhibited statistically significant ($p<0.05$) difference in reducing parasite load at all dose levels evaluated in the study. Among the solvent fractions, chloroform fraction at a dose of 200mg/kg/day produced the highest parasitemia suppression (48.47%) when compared to the same dose of butanol fraction (35.39%) and aqueous fraction

(25.82%), whereas the lowest parasitemia suppression (15.91%) was exhibited by the aqueous fraction. More specifically, the increase in effect was consistent as the dose was increased from lower to higher level at all dose levels evaluated regardless of the fraction tested. The chemosuppressive effects of all the solvent fractions were lower than the chemosuppressive effect of 80ME. However, the effect produced by all doses of the crude extract as well as the solvent fractions was less than that of the standard drug, CQ, which suppresses the parasitemia to undetectable level.

The mice treated with different doses of the 80ME and solvent fractions of root of *C. prophetarum* survived for more days than the respective negative control group that was treated with the vehicle (Table 3). The MST of mice treated with the 80ME and solvent fractions was increased when the dose increased. The longest MST (14.16 days) was exhibited at the highest dose (200mg/kg/day) of 80ME. However, the highest effect displayed by the highest dose of 80ME was lower than that of the standard drug, CQ, in which none of the mice exhibited death in the follow-up period.

When compared among the solvent fractions, the highest dose (200mg/kg/day) of chloroform fraction exhibited the longest MST (10.67 days), whereas the shortest MST (6.50 days) was exhibited by the lowest dose of aqueous fraction. However, the maximum MST exerted by the highest dose of chloroform fraction was lower than that of the MST (>30 days) exerted by the standard drug, CQ, in which none of the mice showed death in the period of follow up. When compared to the respective negative control group, the aqueous fraction was not significant ($p<0.05$) in prolongation of MST. When compared the effects of 80ME to the effects of all the solvent fractions on MST, the 80ME exhibited better survival time.

When doses of 80ME and each fraction were compared among each other, all doses of the 80ME showed significant ($p<0.05$) difference in prolonging the survival time. However, the lowest and middle doses of the chloroform and butanol fraction did not show significant ($p<0.05$) difference in prolonging the survival time. The aqueous fraction, which showed no significant in prolonging survival time as compared to the negative control, did not also show any significant difference among each of its doses ($p<0.05$).

Table 3: Effect of 80ME and solvent fractions of *C. prophetarum* roots on Parasitemia and Survival Time in 4-day suppressive test

Group	% Parasitemia	% Suppression	Survival Time (days)
NC	51.33±0.88	0.00	6.17±0.31
CP50	35.50±0.34	30.84^{a*,b*,d*,e*}	8.83±0.30 ^{a*,b*,d*,e*}
CP100	25.83±0.54	49.68^{a*,b*,c*,e*}	10.33±0.42 ^{a*,b*,c*,e*}
CP200	18.00±0.26	64.33^{a*,b*,c*,d*}	14.16±0.47 ^{a*,b*,c*,d*}
CQ25	0.00±0.00	100.00^{a*}	>30.00±0.00^{a*}
NC	54.67±0.33	0.00	6.17±0.16
CF50	39.83±0.48	27.14 ^{a*,b*,d*,e*}	7.67±0.21 ^{a*,b*,e*}
CF100	35.83±0.31	34.46 ^{a*,b*,c*,e*}	8.83±0.30 ^{a*,b*,e*}
CF200	28.17±0.40	48.47 ^{a*,b*,c*,d*}	10.67±0.49 ^{a*,b*,c*,d*}
CQ25	0.00±0.00	100.00 ^{a*}	>30.00±0.00 ^{a*}
NC	52.50±0.43	0.00	6.17±0.16
BF50	39.72±0.26	24.34 ^{a*,b*,d*,e*}	6.83±0.30 ^{b*,e*}
BF100	36.91±0.28	29.69 ^{a*,b*,c*,e*}	7.67±0.33 ^{a*,b*}
BF200	33.92±0.49	35.39 ^{a*,b*,c*,d*}	8.33±0.21 ^{a*,b*,c*}
CQ25	0.00±0.00	100.00 ^{a*}	>30.00±0.00 ^{a*}
DW	51.17±0.70	0.00	6.33±0.21
AF50	43.03±0.46	15.91 ^{a*,b*,d*,e*}	6.50±0.22 ^{b*}
AF100	40.75±0.39	20.36 ^{a*,b*,c*,e*}	6.83±0.30 ^{b*}
AF200	37.96±0.20	25.82 ^{a*,b*,c*,d*}	7.17±0.30 ^{b*}
CQ25	0.00±0.00	100.00 ^{a*}	>30.00±0.00 ^{a*}

Data are expressed as mean ± SEM (n=6); a, compared to NC; b, compared to CQ25; c, compared to 50mg/kg; d, compared to 100mg/kg; e, compared to 200mg/kg; *, $p<0.05$. NC= negative control, received vehicle 10ml/kg; DW = negative control, received distilled water; CP = crude extract of *Cucumis prophetarum*; CF = chloroform fraction; BF = butanol fraction; AF = aqueous fraction; CQ = chloroquine. Numbers afterward letters in the first column refer to dose in mg/kg.

5.3.2 Effects on Body Weight and Rectal Temperature in 4-Day Suppressive Test

The results of the effects of 80ME and the solvent fractions of root of *C. prophetarum* at different dose levels on body weight and rectal temperature of mice that were infected with CQ sensitive *P. berghei* are summarized in Table 4 below. The results are expressed as the change in mean body weight and rectal temperature that were between before treatment (D0) and after treatment (D4) for each group of mice.

The rate of decline of body weight and rectal temperature were low in the groups of mice treated with 80ME and solvent fractions of root of *C. prophetarum* when compared to their respective vehicle treated groups of mice. Furthermore, the 80ME and solvent fractions improved body weight and rectal temperature to different levels when compared to the respective negative control groups. The highest increment in body weight and rectal temperature was displayed by the 80ME at a dose of 200mg/kg/day while the lowest increment in both cases was displayed by the aqueous fraction at a dose of 50mg/kg/day (Table 4). When compared among themselves, different dose levels of 80ME, displayed statistically significant ($p<0.05$) difference in protection from parasite induced body weight loss and rectal temperature reduction. Nevertheless, the effect produced by the extracts was less than that of the standard drug, CQ, which displayed the highest protection from parasite induced body weight loss and rectal temperature reduction.

When compared among the fractions, the highest increment in both, body weight and rectal temperature, was displayed by the chloroform fraction at the dose of 200mg/kg/day, whereas the minimum increment was displayed by the aqueous fraction at the dose of 50mg/kg/day. However, the effects displayed by all the fractions were less than that of the standard drug, CQ, which showed the highest protection from the parasite induced body weight loss and rectal temperature reduction. Again when compared among themselves, different dose levels in each fraction, displayed statistically significant ($p<0.05$) difference in protection from parasite induced body weight loss and rectal temperature reduction, except the aqueous fraction. Moreover, the higher the dose of each fraction of root of *C. prophetarum* given, the better the prevention from parasite induced body weight loss and rectal temperature reduction. When compared the effects of 80ME to the effects of all the solvent fractions on body weight loss and rectal temperature reduction, the 80ME was exhibited better protection from parasite induced body weight loss and rectal temperature reduction.

Table 4: Effect of 80ME and solvent fractions of root of *C. prophetarum* on Body weight and rectal temperature in 4-day suppressive test

Group	Weight (g)			Temperature (°C)		
	D_0	D4	Change	D_0	D4	Change
NC	27.03±0.61	23.03±0.65	-4.00±0.19	36.32±0.15	34.05±0.10	-2.26±0.18
CP50	25.86±0.71	22.75±0.73	-3.11±0.10^{a*,b*,d*,e*}	36.30±0.13	34.56±0.14	-1.73±0.11 ^{a*,b*,d*,e*}
CP100	26.30±1.15	24.46±1.05	-1.83±0.30 ^{a*,b*,c*,e*}	36.40±0.16	35.33±0.13	-1.06±0.06 ^{a*,b*,c*}
CP200	27.48±1.05	26.43±1.03	-1.05±0.08 ^{a*,b*,c*,d*}	36.43±0.13	35.55±0.14	-0.88±0.06 ^{a*,b*,c*}
CQ25	25.40±1.30	25.55±1.24	0.15±0.12^{a*}	36.42±0.12	36.78±0.12	0.37±0.16^{a*}
NC	28.30±0.51	24.10±0.49	-4.23±0.08	36.83±0.14	34.57±0.19	-2.26±0.08
CF50	28.72±0.50	24.76±0.48	-3.95±0.12 ^{b*,d*,e*}	36.38±0.18	34.46±0.13	-1.92±0.12 ^{b*,e*}
CF100	25.58±1.17	22.35±1.22	-3.23±0.07 ^{a*,b*,c*,e*}	35.87±0.33	34.42±0.26	-1.45±0.12 ^{a*,b*}
CF200	25.13±0.80	22.72±0.84	-2.41±0.12 ^{a*,b*,c*,d*}	36.43±0.23	35.25±0.18	-1.18±0.12 ^{a*,b*,c*}
CQ25	24.58±1.26	24.83±1.26	0.25±0.06^{a*}	35.67±0.37	35.87±0.26	0.20±0.17 ^{a*}
NC	26.92±0.68	22.65±0.72	-4.26±0.05	36.72±0.17	34.45±0.19	-2.27±0.06
BF50	26.73±0.49	22.63±0.49	-4.10±0.11^{b*,e*}	36.52±0.19	34.43±0.15	-2.08±0.11^{b*}
BF100	27.82±0.37	24.13±0.33	-3.68±0.17 ^{a*,b*,e*}	36.41±0.20	34.41±0.29	-1.98±0.13^{b*}
BF200	25.98±0.36	22.95±0.39	-3.03±0.10 ^{a*,b*,c*,d*}	36.27±0.15	34.56±0.16	-1.70±0.08 ^{a*,b*}
CQ25	24.80±0.75	25.00±0.71	0.20±0.10 ^{a*}	36.15±0.34	36.06±0.35	-0.08±0.09 ^{a*}
DW	26.76±0.48	22.70±0.44	-4.06±0.08	36.53±0.14	34.28±0.09	-2.25±0.15
AF50	27.08±0.76	23.06±0.73	-4.02±0.06 ^{b*}	36.75±0.12	34.71±0.14	-2.03±0.06 ^{b*}
AF100	27.31±0.29	23.33±0.36	-3.98±0.12 ^{b*}	36.41±0.10	34.31±0.08	-2.10±0.07 ^{b*}
AF200	27.80±0.46	23.85±0.43	-3.85±0.30 ^{b*}	36.38±0.11	34.53±0.12	-1.85±0.11 ^{a*,b*}
CQ25	26.61±0.84	26.75±0.83	0.13±0.05 ^{a*}	36.36±0.15	36.40±0.16	0.03±0.03^{a*}

Data are expressed as mean ± SEM (n = 6); a, compared to NC; b, compared to CQ25; c, compared to 50mg/kg; d, compared to 100mg/kg; e, compared to 200mg/kg; *, $p<0.05$. NC= negative control, received vehicle 10ml/kg; DW = negative control, received distilled water; CP = crude extract of *Cucumis prophetarum*; CF = chloroform fraction; BF = butanol fraction; AF = aqueous fraction; CQ = chloroquine. Numbers after letters in the first column refer to dose in mg/kg.

5.3.3 Effects on Packed Cell Volume in 4-Day Suppressive Test

The effects of 80ME and three solvent fractions of root of *C. prophetarum* at different dose levels on PCV of mice infected with CQ sensitive *P. berghei* are presented in Figure 4-7 below. Moreover, the results are expressed as the change in mean PCV between before treatment (D0) and after treatment (D4) for each group in reference to the change in mean PCV of the respective negative control groups that were treated with the vehicle.

The mean value of the PCV displayed reduction in group of mice treated with 80ME and solvent fractions and, in those groups of mice treated with the vehicle on D4, when compared to the respective positive control groups of mice. Nevertheless, there was significant ($p<0.05$) prevention from parasite induced reduction of PCV in groups of mice treated with 80ME as well as the chloroform and butanol fractions when compared to their respective negative control group of mice. Furthermore, the extract protection against parasite induced PCV reduction was at different levels. Among all the extracts the highest protection from parasite induced PCV reduction was displayed by 80ME at the maximum dose given, (200mg/kg/day), whereas the least prevention was displayed by the aqueous fraction at the least dose given, (50mg/kg/day). However, the effect produced by 80ME and the solvent fractions was less than the effect produced by the standard drug, CQ, which displayed the highest protection from parasite induced reduction in PCV.

When compared among each solvent fraction, different dose levels in each fraction of root of *C. prophetarum* were exhibited difference in prevention from parasites induced reduction in PCV. Moreover, the higher the dose of each fraction given, the better the prevention from parasites induced reduction in PCV. The effect of 80ME of *C. prophetarum* roots in prevention of parasites induced PCV reduction was higher when compared to the effects produced by all the solvent fractions. The highest prevention displayed by the chloroform fraction was lower than the effect displayed by the 80ME.

Although the effects were lower than the standard drug, all doses of the 80ME, the middle and highest dose of chloroform fraction, and highest dose of butanol fraction treated groups significantly protected reduction of PCV ($p<0.05$) when compared to the respective vehicle treated groups.

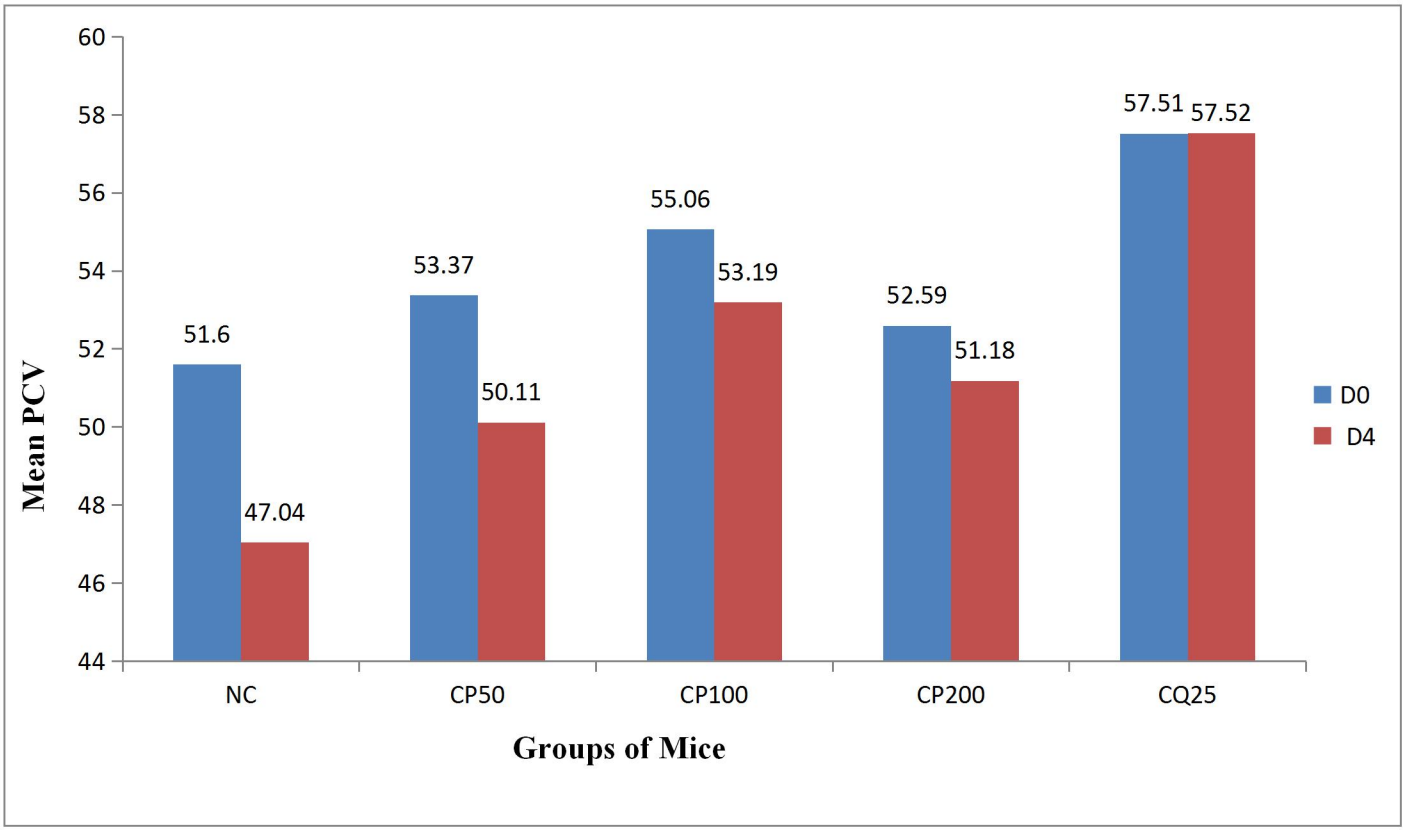

Figure 4: Effect of 80ME of *C. prophetarum* roots on PCV in 4-day suppressive test. NC= negative control, received vehicle 10ml/kg; CP = crude extract of *Cucumis prophetarum*; D0, pre-treatment value on day 0; D4, post-treatment value on day four. Numbers after letters in the x-axis refer to dose in mg/kg.

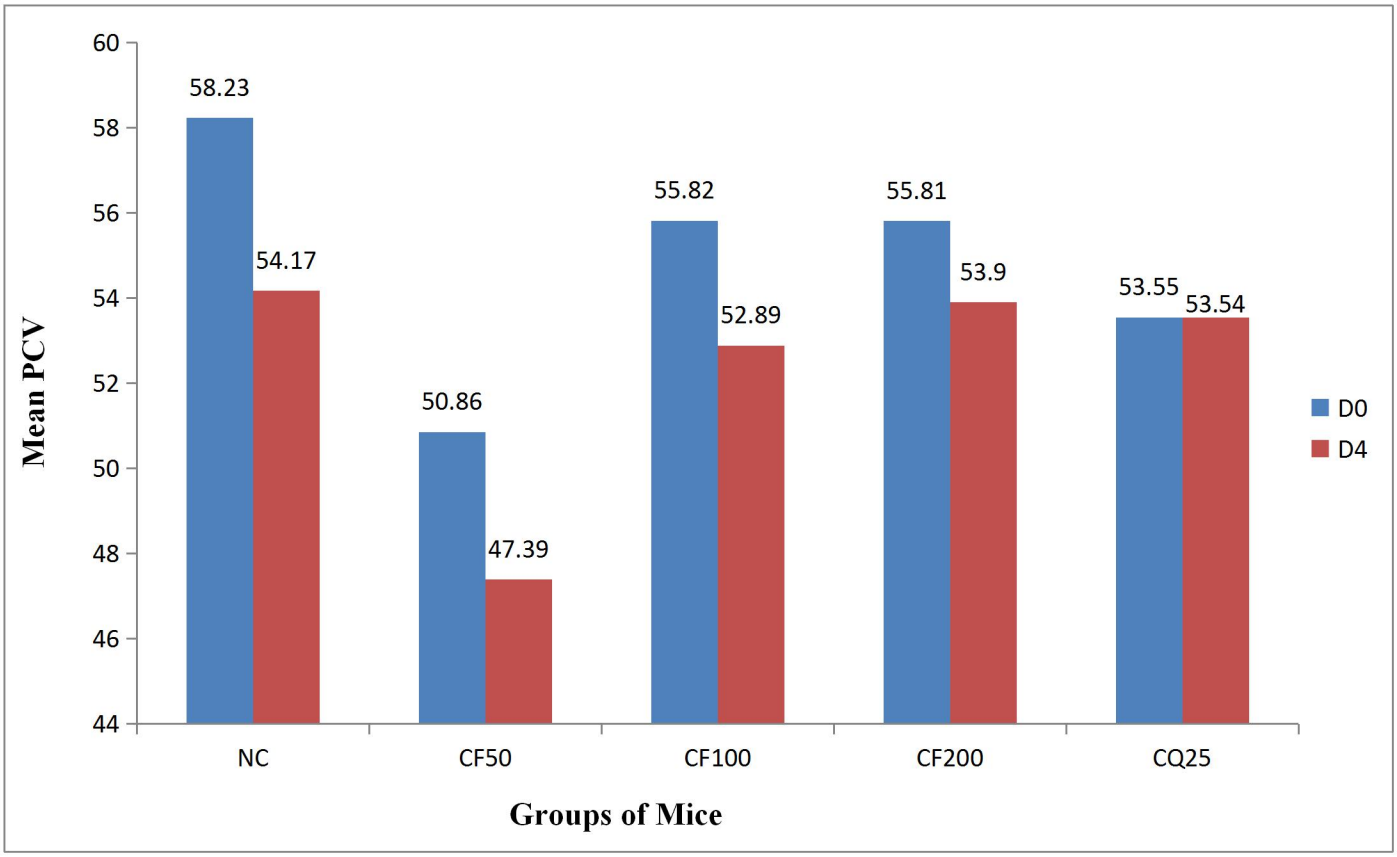

Figure 5: Effect of Chloroform fraction of *C. prophetarum* roots on PCV in 4-day suppressive test. NC= negative control, received vehicle 10ml/kg; CF = chloroform fraction; D0, pre-treatment value on day 0; D4, post-treatment value on day four. Numbers after letters in the x-axis refer to dose in mg/kg.

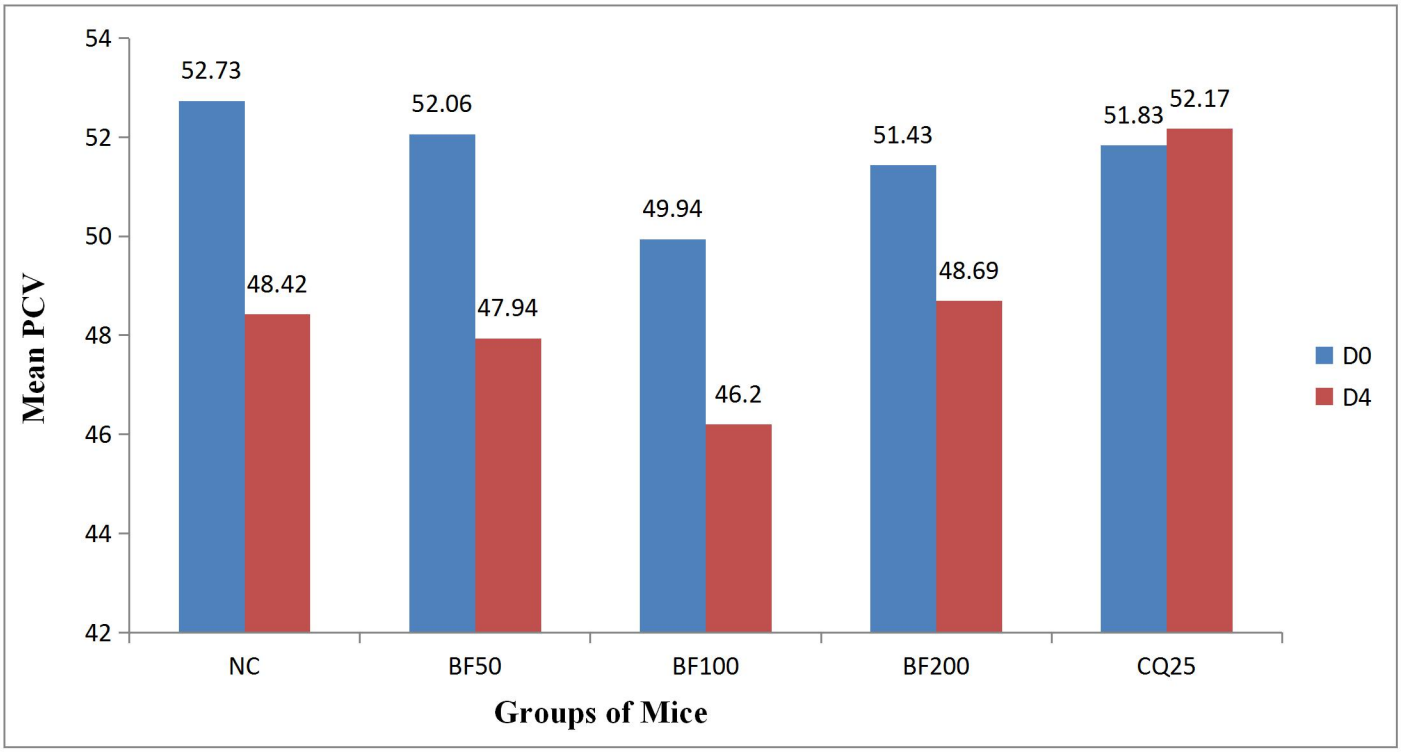

Figure 6: Effect of Butanol fraction of *C. prophetarum* roots on PCV in 4-day suppressive test. NC= negative control, received vehicle 10ml/kg; BF = butanol fraction; D0, pre-treatment value on day 0; D4, post-treatment value on day four. Numbers after letters in the x-axis refer to dose in mg/kg.

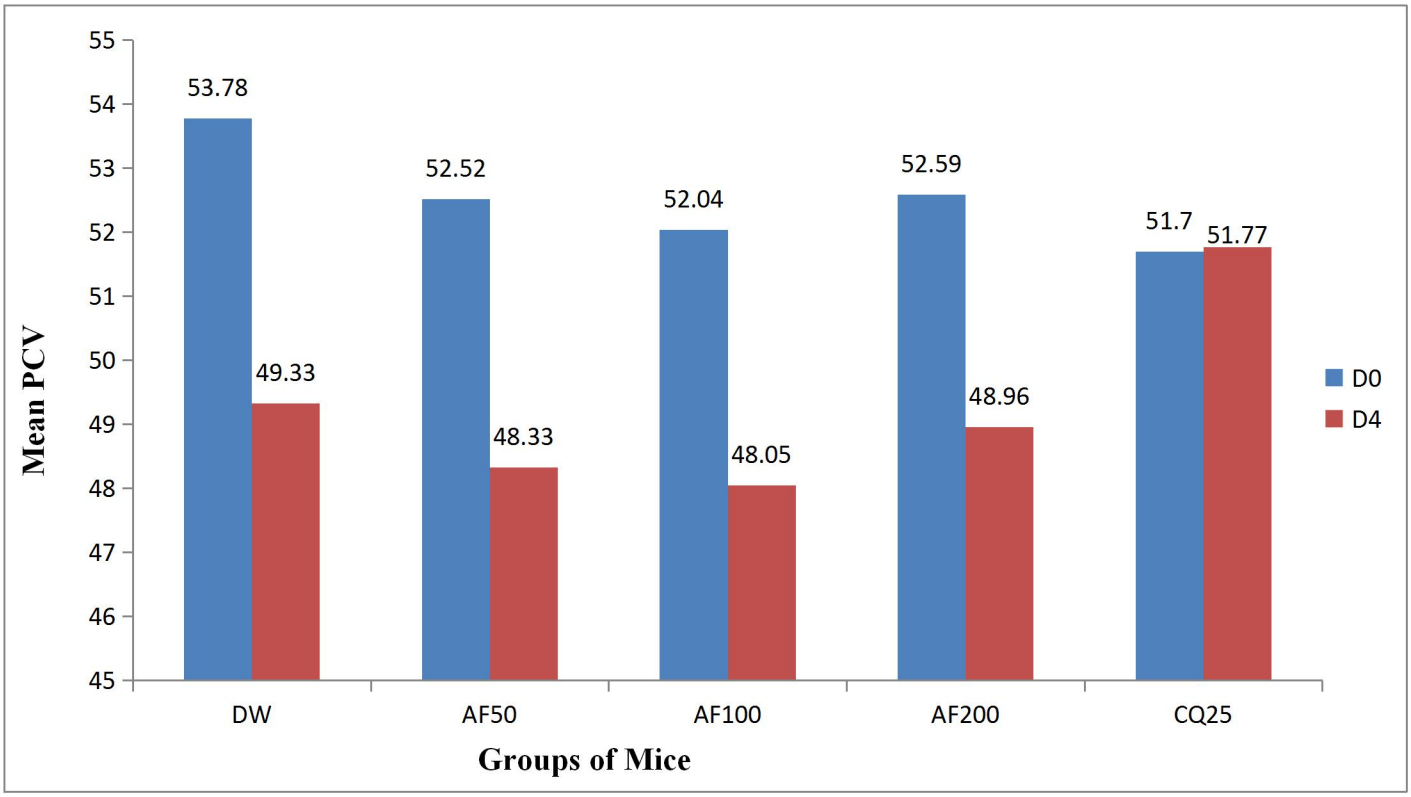

Figure 7: Effect of Aqueous fraction of *C. prophetarum* roots on PCV in 4-day suppressive test. DW= negative control, received distilled water 10ml/kg; AF = aqueous fraction; D0, pre-treatment value on day 0; D4, post-treatment value on day four. Numbers after letters in the x-axis refer to dose in mg/kg.

5.4 Prophylactic Test

5.4.1 Effects on Parasitemia and Survival Time in Prophylactic Test

The result of the effect of 80ME of root of *C. prophetarum* at different dose levels on parasitemia and survival time of Swiss albino mice, infected with CQ sensitive *P. berghei,* using the prophylactic test are summarized in Table 5 below. The results are expressed as mean percentage parasitemia and MST in days in reference to the respective negative control group that treated with the vehicle. All doses of 80ME suppressed the *P. berghei* parasite load significantly ($p<0.05$) when compared to the respective negative control group. The highest percentage parasitemia suppression (39.70%) of the crude extract was exhibited at the dose of 200mg/kg/day, whereas the least suppression (24.58%) of parasitemia was displayed at minimum dose given, 50mg/kg/day. However, the results displayed by the crude extract were less than that of the result produced by standard drug, CQ. Even though complete obliteration of the parasite was not attained, maximum inhibition (94.49%) of parasitemia was observed by the positive control group. When compared among each dose level of crude extract, different dose levels exhibited

statistically significant ($p<0.05$) difference in preventing parasite load. Furthermore, the higher the dose of crude extract given, the higher the percentage suppression of parasitemia was exhibited.

The groups of mice treated with different doses of 80ME of *C. prophetarum* roots survived more days than the respective negative control group of mice treated with the vehicle, as showed in Table 5 below. Similar to the parasitemia suppression, from all the doses of 80ME the longest MST (10.33 days) was showed in group of mice that received the highest dose of the 80ME. However, the maximum result displayed by the highest dose of the 80ME was lower than that of the effect produced by standard drug, CQ, in which prolong survival time (26.50 days) was noted. When compared among each dose level, the lowest and the middle doses showed no significantly different in prolonging survival time. But the highest dose of the crude extract showed significance difference in prolonging survival time when compared to the middle and lowest doses.

Table 5: Percent of parasitemia, suppression and survival time of *P. berghei* infected mice treated with 80ME of *C. prophetarum* roots in prophylactic test.

Group	% Parasitemia	%Suppression	Survival Time(days)
NC	50.35±0.86	0.00	6.33±0.21
CP50	37.97±0.40	24.58 ^{a*,b*,d*,e*}	7.16±0.40 ^{b*,e*}
CP100	35.34±0.58	29.81 ^{a*,b*,c*,e*}	8.33±0.21 ^{a*,b*,e*}
CP200	30.36±0.55	39.70 ^{a*,b*,c*,d*}	10.33±0.21 ^{a*,b*,c*,d*}
CQ25	2.77±0.21	94.49 ^{a*}	26.50±0.62 ^{a*}

Data are expressed as mean ± SEM (n = 6); a, compared to NC; b, compared to CQ25; c, compared to 50mg/kg; d, compared to 100mg/kg; e, compared to 200mg/kg; *, $p<0.05$. NC= negative control, received vehicle 10ml/kg; CP = crude extract of *Cucumis prophetarum*; CQ = chloroquine. Numbers after letters in the first column refer to dose in mg/kg.

5.4.2 Effects on Body Weight and Rectal Temperature in Prophylactic Test

The results of the effects of 80ME of root of *C. prophetarum* on body weight and rectal temperature of Swiss albino mice infected with CQ sensitive *P. berghei* using prophylactic test are showed in Table 6 below. The results are expressed as the change in mean body weight and rectal temperature between before treatment at D0 and after treatment at D7 for each group of mice in reference to the change in mean value of the respective negative control group of mice that treated with the vehicle.

All doses of the 80ME showed a preventive effect in body weight loss and rectal temperature reduction when compared to the respective vehicle treated group of mice. Furthermore, groups of mice that treated different doses of the 80ME improved body weight and rectal temperature to different levels between D0 and D7 when compared to the vehicle treated group of mice. From all the three doses of the 80ME, the highest increment of body weight and rectal temperature was displayed by the maximum dose (200mg/kg/day) given, whereas the lowest increment was displayed by the minimum dose (50mg/kg/day) given. The higher the dose of the 80ME given, the better the prevention from parasite induced body weight loss and rectal temperature reduction. However, the effects displayed by all the doses 80ME was less than that of the standard drug, CQ, which displayed the highest prevention from *P. berghei* parasite induced body weight loss and rectal temperature reduction.

Table 6: Effects of 80ME of *C. prophetarum* root on body weight and rectal temperature in prophylactic test.

Group	**Weight (g)**			**Temperature(°C)**		
	D0	**D7**	**Change**	**D0**	**D7**	**Change**
NC	28.23±0.88	23.95±0.87	-4.28±0.03	36.50±0.12	34.10±0.14	-2.40±0.19
CP50	27.56±0.52	24.13±0.36	-3.43±0.18 ^{a*,b*,d*,e*}	36.18±0.17	34.13±0.18	-2.05±0.05 ^{b*}
CP100	29.11±0.46	26.48±0.62	-2.63±0.18 ^{a*,b*,c*}	36.38±0.24	34.55±0.16	-1.83±0.14 ^{b*}
CP200	27.67±0.67	25.73±0.67	-1.93±0.21 ^{a*,b*,c*}	36.36±0.18	34.67±0.27	-1.70±0.19 ^{a*,b*}
CQ25	26.35±0.55	25.43±0.58	-0.92±0.22 ^{a*}	36.00±0.17	35.40±0.29	-0.60±0.14 ^{a*}

Data are expressed as mean ± SEM (n = 6); a, compared to NC; b, compared to CQ25; c, compared to 50mg/kg; d, compared to 100mg/kg; e, compared to 200mg/kg; *, $p<0.05$. NC= negative control, received vehicle 10ml/kg; CP = crude extract of *Cucumis prophetarum*; CQ = chloroquine. Numbers after letters in the first column refer to dose in mg/kg.

5.4.3 Effects on Packed Cell Volume in Prophylactic Test

The effects of the different dose levels of 80ME of root of *C. prophetarum* on PCV of Swiss albino mice infected with CQ sensitive *P. berghei* parasite using prophylactic test are summarized in Figure 8 below. The results are expressed as the change in mean PCV between before treatment at D0 and after treatment at D7 for each group in reference to the respective vehicle treated negative control group.

All doses of the 80ME were showed a preventive effect in PCV reduction when compared to the respective vehicle treated negative control group. The different doses of crude extract improved PCV of

parasite infected mice to different levels. From all doses of the crud extract, the highest prevention of PCV reduction was displayed by the maximum dose (200mg/kg/day) of the 80ME given, while the lowest prevention of PCV reduction was displayed by the minimum dose (50mg/kg/day) of the 80ME given.

Furthermore, the higher the dose of the 80ME given, the better the prevention from parasite induced reduction of PCV. However, the effects displayed by all doses of the 80ME were less than that of the standard drug, CQ, which showed the highest prevention from parasite induced PCV reduction.

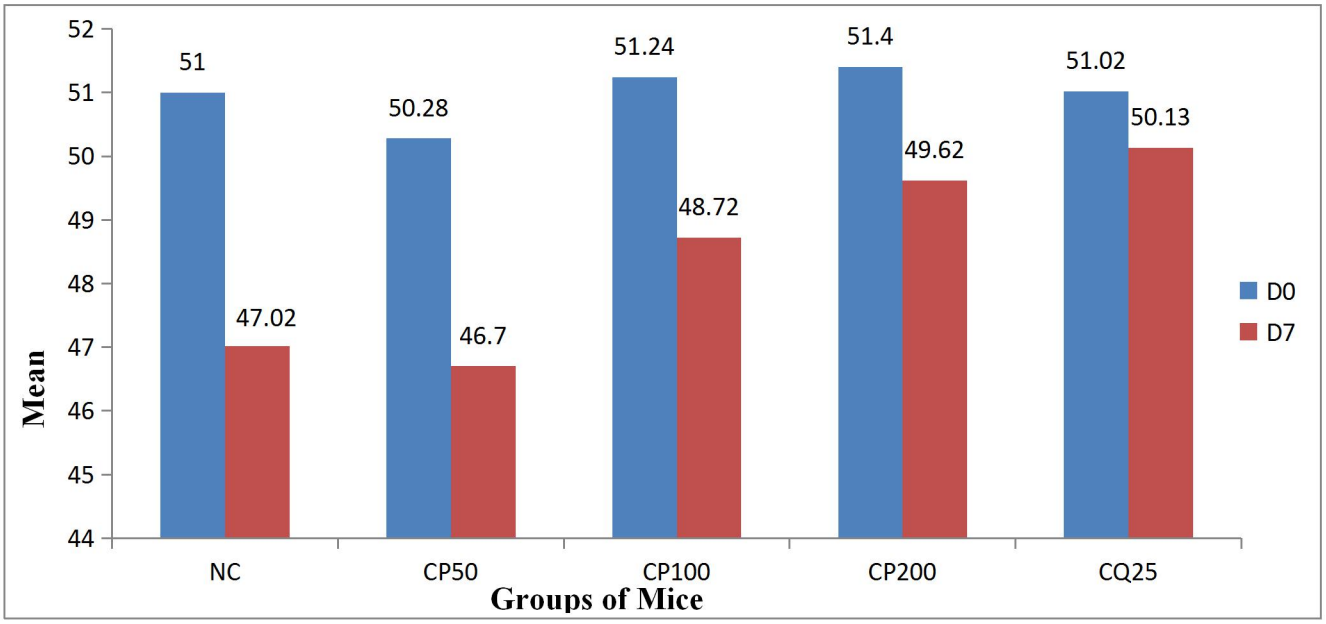

Figure 8: Effects of 80ME of *C. prophetarum* root on PCV in prophylactic test. NC= negative control, received vehicle 10ml/kg; CP = crude extract of *Cucumis prophetarum*; D0, pre-treatment value on day 0; D4, post-treatment value on day four. The numbers in rectangles before graphs show the change in mean PCV between D0 and D4. Numbers after letters in the x-axis refer to dose in mg/kg.

6 DISCUSSION

Malaria is one of the utmost important protozoan diseases in the world that cause illness for human. Resistance to currently available antimalarial drugs remains a major challenge and upheld to cause an obstacle in the management of malaria. Therefore, searching new alternative antimalarial drugs is crucial importance to fight and control this disease. As seen from history, most currently used antimalarial drugs (such as; artemisinin from *Artemisia annua*, quinine from *Cinchona* bark) were obtained from traditional medicinal plants (Tse et al., 2019). Accordingly, the present study searched the *in vivo* antimalarial activity of roots of *C. prophetarum* using the 4-day suppressive test and prophylactic test, as well as its oral acute toxicity.

Hydroalcoholic (80% methanol) solvent was used for the extraction of the experimental plant, because such solvent is well for extracting broad variety of polar and moderately polar compounds (Otsuka, 2006). The *in vivo* model was used for this study since it takes into account the possible prodrug effect of the extract and probable participation of the immune system in obliteration of the parasite. *Plasmodium berghei ANKA* parasite was used in the anticipation of treatment outcomes as such parasite was appropriate for such type of study. Furthermore, various conventional antiplasmodial drugs such as chloroquine, halofantrine, mefloquine and artemisinin derivatives have been discovered by using rodent model of malaria (Bantie et al., 2014; Fidock et al., 2004). The 4-day suppressive test is a standard test commonly applied for antiplasmodial activity screening of a substance, and the determination of percentage suppression of parasitemia is the most reliable parameter (Fidock et al., 2004).

As shown by the results, the oral acute toxicity test of 80ME of root of *C. prophetarum* has LD_{50} of 1265mg/kg in Swiss albino mice. Any substance that has oral LD_{50} greater than 1000mg/kg is considered as being of low toxicity or relatively safe (Osibemhe & Onoagbe, 2016). The LD_{50} (1265mg/kg) of this experimental plant extract was 25 times greater than the minimum effective dose (50mg/kg) used in this study. According to the previous studies, if the LD_{50} of the experimental substance is 3 times greater than the lowest effective dose used in that study, then the substance is considered a good candidate for further study. Therefore, the results of the oral acute toxicity test of the 80ME of root of *C. prophetarum* could justify the relatively safe traditional use for the management of malaria disease by the local people.

As noted from the above mentioned results of this study, there was significant inhibition of parasitemia in 80ME as well as in the solvent fractions of root of *C. prophetarum* treated groups of mice when compared

to the respective negative control groups of mice that received the vehicle. Furthermore, the crude extract and the solvent fractions decreased parasitemia to different level. Among the crude extract and the three solvent fractions, the maximum suppression effect (64.33%) of parasitemia was exhibited by the 80ME at the maximum dose given (200mg/kg). However, the highest parasite suppression effect produced by the crude extract of the experimental plant was less than that of the effect produced by the standard drug, CQ (100%).

Phytochemical screening of the plants under the genus *Cucumis* confirms the existence of various phytochemicals, like alkaloid, glycoside, steroid, saponin, tannin and flavonoid (Rajasree et al., 2016). According to the previous studies, common antiplasmodial plants used to treat malaria infection in folklore medicine contain different secondary metabolites such as alkaloids, terpenoids, coumarins, flavonoids, chalcones, quinones and xanthones (Dharani et al., 2010). In addition to this, other secondary metabolites such as tannins and phenols also have antimalarial activity (Eyasu et al., 2013). The secondary metabolites present in this experimental plant extract could be responsible for its antimalarial activity which might act synergistically to inhibit the parasite.

When compared among the solvent fractions that evaluated in 4-day suppressive test, the maximum parasite suppression was displayed by the chloroform fraction at the maximum dose given, and this might be ascribed to the localization of active secondary metabolites in high concentration in this fraction. The butanol fraction also showed chemosuppressive effect next to the chloroform fraction. The lowest chemosuppressive effect was exhibited by the aqueous fractions at the lowest dose given. Nevertheless, none of the fractions totally cleared the parasite from the mice blood after the consecutive treatment in the 4-day suppressive test. The high parasite suppression effect of the chloroform and butanol fractions indicate that the possible localization of active components in these two fractions when compared to the aqueous fraction. This study indicates that non-polar to semi-polar compounds present in these two fractions that act individually or synergistically could be responsible for the chemosuppression activity of the experimental plant. The lower parasitemia inhibition displayed by the aqueous fraction may probably because of the lack of most bioactive phytoconstituents from this fraction.

If the plant extract displayed percentage parasite suppression greater than or equal to 50% at a dose of 500-250, 250-100 and <100mg/kg of body weight, then the *in vivo* antimalarial activity of the plant extract can be classified as moderate, good and very good, respectively (Asrade et al., 2017; Mzena et al., 2018). Therefore, the current finding of the root extract of *C. prophetarum* has good antimalarial activity in 4-day

suppressive test at a dose of 200mg/kg/day. The effects of the present study was also in line with the same genus *Cucumis metuliferus* conducted by Mzena *et al*. (Mzena et al., 2018) in which the extract showed antimalarial activity.

Even though the mechanism of parasite suppression activity of the crude extract and solvent fractions of root of *C. prophetarum* is not proven, the parasite load reduction in those extract treated mice might be due to the presence of active secondary metabolites that have antimalarial activity. Previous studies have indicated the potential of these secondary metabolites for antiplasmodial drug development (Mebrahtu et al., 2013; Nureye et al., 2018). Moreover, secondary metabolites present in the plant extract might be target the previously distinguished targets in the pathogenesis and life cycles of malaria parasite or unique mechanism of action at molecular level (Bantie et al., 2014). Nevertheless, it has to be standardized by scientific community by carrying out advance investigation on this plant.

MST is the other parameter used to evaluate the antiplasmodial activity of the experimental plant extract as presented in the results section above. Plant extracts that can prolong the survival time of parasite infected experimental groups of animals when compared to the respective negative control groups of animals are considered as active agents against malaria (Mzena et al., 2018). The survival time of mice treated with different doses of 80ME and solvent fractions of root of *C. prophetarum* were longer than the respective negative control groups that treated with the vehicle. All doses of the crude extract and fractions were capable of increasing survival time at different significant levels when compared to the respective negative controls. When doses of the extract are compared among each other, the middle and lowest doses did not indicate significant difference in prolonging the survival time except the crude extract. Differently, the highest dose was displayed significant prolongation of survival time as compared to the lowest doses except the aqueous fraction. These differences might be due to difference in amount of phytoconstituents in each dose.

The group of mice treated with a maximum of dose given, 200mg/kg, of the 80ME was the one that exhibited the longest survival time in 4-day suppressive test, which supports that the experimental plant contain antiplasmodial compound that reduces the number of parasite load and thus prolongs the survival time. Moreover, the highest prolongation of survival time displayed by the maximum dose given might be due to localization of the active compounds in high amounts and also because of its better parasite inhibition effect. This is in line with the studies conducted by Nureye *et al*. and Bantie *et al*. (Bantie et al.,

2014; Nureye et al., 2018). However, the longest survival time effect displayed by the maximum dose of the 80ME was lower than that of the effect produced by standard drug, CQ.

When compared among solvent fractions of the root of *C. prophetarum*, the chloroform fraction was exhibited the longest MST at the maximum dose given, this might be due to the better parasite inhibition activity of this fraction and its difference in secondary metabolites contents from the other two fractions. Nevertheless, the longest survival time effect displayed by the maximum dose of chloroform fraction was lower than the effect produced by the 80ME and the standard drug, CQ. Significant prolongation of survival time was not displayed by all given doses of the aqueous fraction when compared to the respective negative control group of mice. This might be due to the absence and reduction of active secondary metabolites with antimalarial activity in this fraction. This finding was in line with another study on *Gardenia ternifolia* root bark in which the aqueous fraction was found to be not significant in prolonging survival time (Nureye et al., 2018).

Body weight loss, reduction in body temperature and reduction in PCV are general features of malaria parasite infected mice (Asrade et al., 2017). So, an ideal antiplasmodial agent obtained from plants should prevent body weight loss, body temperature reduction and PCV reduction due to increment of parasitemia (Zeleke et al., 2017). In the present study, the change in mean body weight of experimental group of mice before treatment at D0 and after treatment at D4 or D7 was determined. There were significant increments of body weight in group of mice treated with crude extracts, chloroform fraction (100 and 200mg/kg) and butanol fraction (100 and 200mg/kg) of the experimental plant when compared to the respective negative control group of mice that treated with the vehicle. This improvement of body weight by the crude extract and fractions might be because of the extracts or fractions effect on parasitemia that counteract other aspects of malaria illness such as fever, immunosuppression and pain, improvement in PCV, rectal temperature and parasite clearance (Fentahun et al., 2017; Nureye et al., 2018).

Furthermore, when compared to the respective negative control mice, the crude extract and the fractions treated mice showed body weight increment to different level between D0 and D4 or D7. The maximum increment was displayed by the 80ME of the experimental plant at the maximum dose given while the lowest increment of body weight was displayed by the aqueous fraction. The reason behind the differences in increment of body weight might be related to the differences in the type and amount of secondary metabolites, where the crude extract could be the richest while the aqueous fraction could be the poorest. This result is in line with the study conducted on *Gardenia ternifolia* root bark in which the 80ME

displayed better increment in body weight, while the aqueous fraction displayed minimal increment when compared to the respective negative controls (Nureye et al., 2018). However, the effect produced by this study experimental plant was less than the effect produced by the standard drug, CQ, in which the highest prevention from parasite induced body weight loss was exhibited. These might be ascribed to the incapability of the extracts and fractions of *C. prophetarum* roots to entirely remove the parasites from the body of mice otherwise reducing to different level. It could also be because of the appetite suppressants present in the crude extracts and fractions of the experimental plant (Asrade et al., 2017; Mzena et al., 2018).

A reduction in the metabolic rate of *P. berghei* parasite infected mice happens before death and is accompanied by a corresponding reduction in body temperature. Ideally, the rectal temperature of parasite infected mice decrease as parasite levels increases. Active compounds should protect the quick reducing of body temperature (Bantie et al., 2014; Nureye et al., 2018). In this study, the change in mean rectal temperature of experimental groups of mice before treatment at D0 and after treatment at D4 or D7 was determined. The rate of decline of temperature was low in group of mice treated with crude extracts and solvent fractions of the experimental plant to different levels when compared to the respective negative control group of mice that treated with the vehicle. Better protection from reduction in rectal temperature was exhibited in group of mice treated with higher doses of 80ME and, chloroform and butanol fractions. This might be attributed to the fewer in amounts of phytoconstituents in the lower doses, and the absence of these phytoconstituents in the aqueous fraction. This finding is in line with the study reported on *Gardenia ternifolia* root bark in which the higher doses improved rectal temperature as compared to the lower doses (Nureye et al., 2018).

The maximum increment was displayed by the 80ME of the root of *C. prophetarum* while the lowest was displayed by the aqueous fraction in the same way to the findings of the body weight, survival time and parasite inhibition that were presented in the above. Nevertheless, the effect displayed by the crude extract and solvent fractions were less than the effect produced by the standard drug, CQ, which displayed the highest prevention from parasite induced rectal temperature reduction. These might be ascribed to the incapability of the 80ME and solvent fractions of *C. prophetarum* roots to entirely remove the parasites from the body of mice otherwise decreasing to different level. In addition, it might be because of the hypothermic effect of the components present in the crude extract and fractions of the experimental plant (Bantie et al., 2014; Mebrahtu et al., 2013).

The underlying causes of anemia in humans and mice include destroying and/or clearance of infected RBCs, suppression of RBCs formation and dyserythropoiesis. In mice that did not treat, the parasite count escalate and the PCV reduced significantly from day to day until the day of death of mice which was observed in other previous studies (Asrade et al., 2017). The PCV of mice infected with *P. berghei* was measured to assess the activities of the 80ME and solvent fractions in protecting hemolysis because of a increasing parasitemia level.

The crude extract and fractions of the root of *C. prophetarum* improved change in mean PCV to different levels where the higher doses displayed better prevention from reduction in PCV. The maximum protection from parasite induced decrease in mean PCV was displayed by the crude extract of the experimental plant while the lowest protection was displayed by the aqueous fraction similar to the finding of body weight, rectal temperature, survival time, and parasite suppression as presented above. This finding is in line with other findings in which the higher doses better protected PCV from decreasing when compared to the lower doses (Asrade et al., 2017). In this study showed that the crude extract and fractions of the experimental plant improve PCV and loss of RBCs. This might be due to the decrease in parasite load in the course of infection in group of mice treated with the crude extract and solvent fractions.

However, the mean value of the PCV displayed reduction in group of mice treated with the crude extract and fractions and in those that were treated with the vehicles when compared to the standard drug, CQ, treated groups of mice. This might be ascribed to the incapability of the 80ME and solvent fractions to entirely remove the parasites from the body of the mice other than decreasing to different levels. It might also be because of the secondary metabolites present in the extract of the experimental plant that cause hemolysis like saponins, as some studies showed that saponins are known to cause hemolysis by increasing the permeability of plasma membrane of the RBCs (Bantie et al., 2014; Mebrahtu et al., 2013).

As noted from results of the prophylactic test, there was significant ($p<0.05$) inhibition of parasitemia in 80ME of *C. prophetarum* roots treated groups of mice when compared to the respective negative control groups of mice that received the vehicle. There was also significant difference between each dose of 80ME in suppression of parasitemia in the prophylactic test. The highest suppression effect (39.70%) of parasitemia was exhibited at the maximum dose given (200mg/kg) of 80ME. However, the highest parasite suppression effect produced by the crude extract of the experimental plant was less than that of the effect produced by the standard drug, CQ (94.49%). Treatment of mice infected with *P. berghei* with

80ME of *C. prophetarum* roots exhibited prophylactic activity, though the effect produced was not comparable to that of the effect produced by the 80ME in the 4-day suppressive test.

Overall, the crude extract and fractions treated groups of mice in the case of 4-day suppressive test and prophylactic test had lower parasite count and survived better than those the respective negative control groups of mice. This could largely be due to the decrease in parasitemia level or the prevention of cerebral malaria caused by the rising parasitemia in mice. Even though the active compound is yet to be identified, the antiplasmodial activity of *C. prophetarum* roots might be ascribed to a single or combination phytoconstituents that could present in the extract of this experimental plant. The secondary metabolites such as alkaloids, terpenoids, tannins and phenolic compounds have been reported to have different extent of antiplasmodial effects in the studies (Asrade et al., 2017; Bantie et al., 2014).

7 CONCLUSION

The present study indicated that the crude extract and solvent fractions of root of *C. prophetarum* possess antimalarial activity. The maximum antimalarial activity was displayed by the 80ME at the maximum dose given, 200mg/kg. Among the solvent fractions evaluated, the most active in inhibiting the parasite was the chloroform fraction, indicating the possible localization of the active phytoconstituents in this fraction. The findings indicate that the secondary metabolites responsible for antimalarial activity of the extract of *C. prophetarum* roots could be non-polar to semi-polar in nature. According to the oral acute toxicity test of the crude extract using the Lorke's method (Lorke, 1983), it can be assumed that the extract can be safe at a dose level used in the study with an estimated oral LD_{50} of 1,265mg/kg. Moreover, the data obtained from this study give an evidence to support the folkloric use of the plant by the traditional medicine practitioners. Therefore, the root of *C. prophetarum* could be used as a new source for the advance of new plant based antiplasmodial agent.

8 RECOMMENDATIONS

As root of *C. prophetarum* possesses antimalarial activity and the overall results of this study provide a basis for further studies on the plant, the following further investigations are recommended:

- Sub-acute and chronic toxicity studies should be conducted to prove the better safety status of the plant.
- Evaluating the antimalarial activity of the plant in *in vitro* against *P. falciparum* to better simulate the actual human malaria infection.
- Isolation and characterization of the bioactive principles of the chloroform fractions that responsible for the antimalarial activity of the plant.
- Elucidating the structure and the mechanism of action of antimalarial activity.

9 REFERENCES

Alsayari, A., Kopel, L., Ahmed, S.M., Soliman, M.S. H., Annadurai, S. & Halaweish T. F. (2018). Isolation of anticancer constituents from Cucumis prophetarum var . prophetarum through bioassay-guided fractionation. *BMC Complementary and Alternative Medicine*, *18*(274), 1–12.

Abebe, W. (2016). An Overview of Ethiopian Traditional Medicinal Plants Used for Cancer Treatment. *European Journal of Medicinal Plants*, *14*(4), 1–16.

Aguiar, A. C. C., Mm, E., Souza, N. B. De, França, T. C. C., & Krettli, A. U. (2012). New approaches in antimalarial drug discovery and development - A Review. *Memorias Instituto Oswaldo Cruz, Rio de Janeiro*, *107*(7), 831–845.

Alebie, G., Urga, B., & Worku, A. (2017). Systematic review on traditional medicinal plants used for the treatment of malaria in Ethiopia: Trends and perspectives. *Malaria Journal,16*(1), 1-13.

Andrews, K. A., Wesche, D., McCarthy, J., Möhrle, J. J., Tarning, J., Phillips, L., Kern, S., & Grasela, T. (2018). Model-Informed Drug Development for Malaria Therapeutics. *Annual Review of Pharmacology and Toxicology*, *58*(1), 23.1-23.16.

Ansarullah, Bharucha, B., Dwivedi, M., Laddha, N. C., Begum, R., Hardikar, A. A., & Ramachandran, A. V. (2011). Antioxidant rich flavonoids from Oreocnide integrifolia enhance glucose uptake and insulin secretion and protects pancreatic β-cells from streptozotocin insult. *BMC Complementary and Alternative Medicine*, *11*(126), 1–13.

Asrade, S., Mengesha, Y., Moges, G., & Gelayee, D. A. (2017). In vivo antiplasmodial activity evaluation of the leaves of Balanites rotundifolia (Van Tiegh.) Blatter (Balanitaceae) against Plasmodium berghei. *Journal of Experimental Pharmacology*, *9*, 59–66.

Bantie, L., Assefa, S., Teklehaimanot, T., & Engidawork, E. (2014). In vivo antimalarial activity of the crude leaf extract and solvent fractions of Croton macrostachyus Hocsht . (Euphorbiaceae) against Plasmodium berghei in mice. *BMC Complementary and Alternative Medicine*, *14*(79), 1–10.

Birru, E. M., Geta, M., & Gurmu, A. E. (2017). Antiplasmodial activity of Indigofera spicata root extract against Plasmodium berghei infection in mice. *Malaria Journal*, *16*(198), 1–7.

Bloland, P. B. (2001). Drug resistance in malaria. *WHO/CDS/CSR/DRS/2001.4 1.,* 1–32.

Chung, S. O., Kim, Y. J., & Park, S. U. (2015). An updated review of cucurbitacins and their biological and pharmacological activities. *Experimental and Clinical Sciences Journal,14*, 562–566.

Clark, J. D., Gebhart, G. F., Gonder, J. C., Keeling, M. E., & Kohn, D. F. (1997). The 1996 Guide for the Care and Use of Laboratory Animals. *Institute for Laboratory Animal Research Journal*, *38*(1), 3073–3077.

Cui, L., Mharakurwa, S., Ndiaye, D., Rathod, P. K., & Rosenthal, P. J. (2015). Antimalarial drug resistance: Literature review and activities and findings of the ICEMR network. *American Journal of Tropical Medicine and Hygiene*, *93*(3), 57–68.

Cunha, C. B., & Cunha, B. A. (2008). Brief history of the clinical diagnosis of malaria : from Hippocrates to Osler. *Journal of Vector Borne Disease*, *45*(1), 194–199.

Moseley, L.D. & Bull, S. B. (1982). A comparison of the Wintrobe, the Westergren and the ZSR erythrocyte sedimentation rate (ESR) methods to a candidate reference method. *Clinical & Laboratory Haematology*, *4*, 169–178.

Daily, J. P. (2017). Malaria 2017: Update on the Clinical Literature and Management. *Current Infectious Disease Reports,19*(8), 1–7.

Das, A., Elmendorf, H. G., & Haldar, K. (1994). Biosynthesis , export and processing of a 45 kDa protein detected in membrane clefts of erythrocytes infected with Plasmodium falciparum. *Biochemical Journal*, *496*(1), 487–496.

Gilmour, D. & Sykes, J. A. (1951). Westergren and wintrobe methods of estimating E.S.R. compared. *British Medical Journal*, *1*, 1496–1497.

Dayananda, K. K., Achur, R. N., & Gowda, D. C. (2018). Epidemiology, drug resistance, and pathophysiology of Plasmodium vivax malaria. *Journal of Vector Borne Disease, 55*(1), 1–8.

Dharani N, Rukunga G, Yenesew A, Mbora A, Mwaura L, Dawson I, J. R. (2010). *Common antimalarial trees and shrubs of East Africa : a description of species and a guide to cultivation and conservation through use* (1st ed.). The World Agroforestry Centre (ICRAF).

Endale, A., Bisrat, D., Animut, A., Bucar, F., & Asres, K. (2013). In vivo antimalarial activity of a labdane diterpenoid from the leaves of otostegia integrifolia benth. *Phytotherapy Research*, *27*(12), 1805–1809.

Fapohunda, S. O., Adewumi, A. A., & Jegede, D. O. (2018). Cucurbitaceae - the family that nourishes and heals. *MicroMedicine*, *6*(2), 85–93.

Fentahun, S., Makonnen, E., Awas, T., & Giday, M. (2017). In vivo antimalarial activity of crude extracts and solvent fractions of leaves of Strychnos mitis in Plasmodium berghei infected mice. *BMC Complementary and Alternative Medicine*, *17*(13), 1–12.

Fidock, D. A., Rosenthal, P. J., Croft, S. L., Brun, R., & Nwaka, S. (2004). Antimalarial drug discovery: efficacy models for compound screening. *Nature Reviews Drug Discovery*, *3*, 1–9.

FMOH. (2012). *Ethiopia National Malaria Indicator Survey Technical Summary*. 1–12.

Gebrehiwot, S., Shumbahri, M., Eyado, A., & Yohannes, T. (2019). Phytochemical Screening and in Vivo Antimalarial Activity of Two Traditionally Used Medicinal Plants of Afar Region, Ethiopia, against Plasmodium berghei in Swiss Albino Mice. *Journal of Parasitology Research*, 1–8.

Giribaldi, G., D'Alessandro, S., Prato, M., & Basilico, N. (2015). Etiopathogenesis and pathophysiology of malaria. In *Human and Mosquito Lysozymes: Old Molecules for New Approaches Against Malaria*, 1–18.

Haldar, K., Bhattacharjee, S., & Safeukui, I. (2018). Drug resistance in Plasmodium. *Nature Reviews Microbiology*, *16*(3), 156–170.

Hilou, A., Nacoulma, O. G., & Guiguemde, T. R. (2006). In vivo antimalarial activities of extracts from Amaranthus spinosus L. and Boerhaavia erecta L. in mice. *Journal of Ethnopharmacology*, *103*(2), 236–240.

Hunde, D., Asfaw, Z. and Kelbessa, E. (2006). Use of traditional medicinal plants by people of "Boosat" sub district, central Eastern Ethiopia. *Ethiopian Journal of Health Sciences*, *16*(2), 141–155.

Jamuna S, K. K. and P. S. (2015). Phytochemical and pharmacological properties of certain medicinally important species of Cucurbitaceae family – a review. *Journal of Research in Biology*, *5*(6), 1835–

1849.

Jiménez-díaz, M. B., Viera, S., Fernández-alvaro, E., & Angulo-barturen, I. (2014). Animal models of e ffi cacy to accelerate drug discovery in malaria. *Parasitology*, *141*(1), 93–103.

Jin-ming, K., Ngoh-khang, G. O. H., Lian-sai, C., & Tet-fatt, C. (2003). Recent advances in traditional plant drugs and orchids. *Acta Pharmacologica Sinica*, *24*(1), 7–21.

Kalra, B. S., Chawla, S., Gupta, P., & Valecha, N. (2006). Screening of antimalarial drugs : An overview overview. *Indian Journal of Pharmacology*, *38*(1), 5–12.

Kavishankar, G. B., & Lakshmidevi, N. (2014). Anti-diabetic effect of a novel N-Trisaccharide isolated from Cucumis prophetarum on streptozotocin-nicotinamide induced type 2 diabetic rats. *Phytomedicine*, *21*(5), 624–630.

Kumar, S. C. M. (2017). Drug Resistance in Malaria. *Springer International Publishing*, 429–447.

Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. *Archives of Toxicology*, *54*, 275–287.

Madara, A. A., Ajayi, J. A., Salawu, O. A., & Tijani, A. Y. (2010). Anti-malarial activity of ethanolic leaf extract of Piliostigma thonningii Schum . (Caesalpiniacea) in mice infected with Plasmodium berghei. *African Journal of Biotechnology*, *9*(23), 3475–3480.

Mebrahtu, E.; Workineh, S.; Mirutse, G. (2013). In vivo antimalarial activity of hydromethanolic leaf extract of Calpurnia aurea (Fabaceae) in Mice infected with chloroquine sensitive Plasmodium berghei. *International Journal of Pharmacology*, *2*(9), 131–142.

Mekonnen, L. B. (2015). In vivo antimalarial activity of the crude root and fruit extracts of Croton macrostachyus (Euphorbiaceae) against Plasmodium berghei in mice. *Journal of Traditional and Complementary Medicine*, *5*(3), 168–173.

Menard, D., & Dondorp, A. (2017). Antimalarial drug resistance: a threat to malaria elimination. *Cold Spring Harbor Perspectives in Medicine*, *7*(7), 1–24.

Mendis, K., Rietveld, A., Warsame, M., Bosman, A., Greenwood, B., & Wernsdorfer, W. H. (2009). From malaria control to eradication: The WHO perspective. *Tropical Medicine and International Health*,

14(7), 802–809.

Meragiaw, M., & Asfaw, Z. (2014). Review of Antimalarial , Pesticidal and Repellent Plants in The Ethiopian Traditional Herbal Medicine. *Journal of Herbal Science*, *3*(3), 21–45.

Mzena, T., Swai, H., & Chacha, M. (2018). Antimalarial activity of Cucumis metuliferus and Lippia kituiensis against Plasmodium berghei infection in mice. *Research and Reports in Tropical Medicine*, *9*, 81–88.

National Academy of Sciences. (2011). *Guide for the care and use of laboratory animals* (8th ed.).

Ngulde, S. I., Sandabe, U. K., & Tijjani, M. B. (2013). Phytochemical constituents, antimicrobial screening and acute toxicity studies of the ethanol extract of Carissa edulis Vahl. root bark in rats and mice. *American Journal of Research Communication*, *1*(9), 99–110.

Ntie-Kang, F., Onguene, P. A., Lifongo, L. L., Ndom, J. C., Sippl, W., & Mbaze, L. M. (2014). The potential of anti-malarial compounds derived from African medicinal plants , part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. *Malaria Journal*, *13*(1), 1–20.

Nureye, D., Assefa, S., Nedi, T., & Engidawork, E. (2018). In Vivo Antimalarial Activity of the 80% Methanolic Root Bark Extract and Solvent Fractions of Gardenia ternifolia Schumach. & Thonn. (Rubiaceae) against Plasmodium berghei. *Evidence-Based Complementary and Alternative Medicine*, *20*(18), 1–10.

OECD. (2008). Organization of Economic Co-operation and Development (OECD), "Guidelines for Testing of Chemicals: Guideline 425: Acute Oral Toxicity. In *Organization of Economic Co-operation and Development (OECD), "Guidelines for Testing of Chemicals,* 1–27.

Okombo, J. and Chibale, K. (2018). Recent updates in the discovery and development of novel antimalarial candidates. *Medicinal Chemistry Communications*, *1*(1), 1–53.

Osibemhe, M., & Onoagbe, I. (2016). Acute Toxicity of Aqueous and Ethanolic Extracts of Strophanthus hispidus Stem Bark. *International Journal of Biochemistry Research & Review*, *9*(1), 1–5.

Otsuka, H. (2006). Purification by Solvent Extraction Using Partition Coefficient. *Natural Products Isolation*, *20*, 269–273.

Peters, W. (1965). Drug resistance in Plasmodium berghei Vincke and Lips, 1948. I. Chloroquine resistance. *Experimental Parasitology*, *17*(1), 80–89.

Peters, W., & Robinson, B. L. (1999). Malaria. *Malaria in human,* 757–773.

Prato, M., Khadjavi, A., Mandili, G., G., V., & Giribaldi, G. (2012). Insecticides as Strategic Weapons for Malaria Vector Control. *Insecticides - Advances in Integrated Pest Management,* 91–114.

Rajasree, R. S., Francis, F., & William, H. (2016). Phytochemicals of Cucurbitaceae Family-A Review. *International Journal of Pharmacognosy and Phytochemical Research*, *8*(1), 113–123.

Saboo, S. S., Thorat, P. K., Tapadiya, G. G., & Khadabadi, S. S. (2013). Ancient and recent medicinal uses of cucurbitaceae family. *International Journal of Therapeutic Applications*, *9*, 11-19.

Satish, P. V. V., & Sunita, K. (2017). Antimalarial efficacy of Pongamia pinnata (L) Pierre against Plasmodium falciparum (3D7 strain) and Plasmodium berghei (ANKA). *BMC Complementary and Alternative Medicine*, *17*(458), 1–26.

Schlagenhauf, P., Wilson, M. E., Petersen, E., Mccarthy, A., & Chen, L. H. (2018). Malaria Chemoprophylaxis. *Travel Medicine* (Fourth Edi), 145–167.

Shrivastava, A., & Roy, S. (2013). *Cucurbitaceae : A Ethnomedicinally Important Vegetable*. *1*(4), 16–20.

Suleman, S., Tufa, T. B., Kebebe, D., Belew, S., Mekonnen, Y., Gashe, F., Musa, S., Wynendaele, E., Duchateau, L., & Spiegeleer, B. De. (2017). Treatment of Malaria and Related Symptoms Using Traditional Herbal Medicine in Ethiopia. *Journal of Ethnopharmacology*, 1–50.

Tadele, A. (2017). Ethiopian Herbal Medicine Research Article Profile Part 1. 1-343

Tadesse, F., Fogarty, A. W., & Deressa, W. (2018). Prevalence and associated risk factors of malaria among adults in East Shewa Zone of Oromia Regional State , Ethiopia : a cross-sectional study. *BMC Public Health*, *18*(25), 1–8.

Tamiru, E., Temesegen, A., & Demise, D. (2019). Phytochemical Investigation on the Root Extraction of Cucumis Prophetarum L. *Chemistry Africa*, *2*(3), 351–360.

Tse, E. G., Korsik, M., & Todd, M. H. (2019). The past , present and future of anti - malarial medicines.

Malaria Journal, *18*(93), 1–21.

Uraku, A. J., Okaka, A. N. C., & Ibiam, U. A. (2015). Antiplasmodial Activity of Ethanolic Leaf Extracts of Spilanthes uliginosa, Ocimum basilicum (Sweet Basil), Hyptis spicigera and Cymbopogon citratus on Mice Exposed to Plasmodium berghei. *International Journal of Biochemistry Research & Review*, *6*(1), 28–36.

Wekesa Sifuna, M., Wambui, M., Kang'Ethe Nganga, J., Wainaina Kariuki, D., Kimani, F. T., & Muregi, F. W. (2019). Antiplasmodial Activity Assay of 3-Chloro-4-(4-chlorophenoxy) Aniline Combinations with Artesunate or Chloroquine in Vitro and in a Mouse Model. *BioMed Research International*, *2019*(1), 1–7.

WHO. (1996). Assessment of Therapeutic Efficacy of Antimalarial Drugs For Uncomplicated Falciparum Malaria in Areas with Intense Transmission. Geneva, Switzerland.

WHO. (2011). World Malaria Report. World Health Organization.Geneva, Switzerland.

WHO. (2015). Guidelines for the treatment of malaria, 3rd ed. Geneva, Switzerland.

WHO. (2018). World malaria report. World Health Organization. Geneva, Switzerland.

WHO. (2020). World malaria report. World Health Organization. Geneva, Switzerland.

Wilson, D. W., Langer, C., Goodman, C. D., McFadden, G. I., & Beeson, J. G. (2013). Defining the timing of action of antimalarial drugs against plasmodium falciparum. *Antimicrobial Agents and Chemotherapy*, *57*(3), 1455–1467.

Wright, C. W. (2005). Plant derived antimalarial agents: New leads and challenges. *Phytochemistry Reviews*, *4*(1), 55–61.

Zeleke, G., Kebebe, D., Mulisa, E., & Gashe, F. (2017). In Vivo Antimalarial Activity of the Solvent Fractions of Fruit Rind and Root of Carica papaya Linn (Caricaceae) against Plasmodium berghei in Mice. *Journal of Parasitology Research*, *20*(17), 1–10.