Evaluation of in *vivo* anti-malarial activity of 80% methanol extract and solvent fractions of seeds of *Prunes persica (L)* Bastche against *Plasmodium berghei* in mice

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A Thesis Paper Submitted to the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University in partial fulfillment of the requirements for the Master of Science Degree in Pharmacology

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December, 2019

Addis Ababa, Ethiopia

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Addis Ababa University

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This is to certify that the thesis prepared by Halchaye Tsegaye, entitled "Evaluation of in *vivo* anti-malarial activity of 80% methanol and solvent fractions of seed of *Prunes persica (L)* Bastche against *Plasmodium berghei* in mice" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Acknowledgements

First, I praise the Lord Jesus Christ, who gave me power to finish this study. Then my gratitude goes to my advisors Dr. Solomon Mequanint and Dr. Teshome Nedi for their unreserved support by giving valuable scientific comments and guidance while I was working. Accordingly, I thank Mrs. Fantu Assefa and Mr. Molla Wale for providing me materials as well as guidance in handling of animals throughout my study. I am also grateful to Department of Pharmaceutical Chemistry and Pharmacognosy School of Pharmacy, College of Health Sciences, Addis Ababa University, which offered me the chance to conduct certain segment of the study in their laboratory. I would like to extend my appreciation to Ethiopian Public Health Institute, for giving me *P. berghei* infected donor and none infected mice.

Also, I am highly thankful to my mother Zewditu Robe for her support on medicinal plant collection and the support she provided; my father Tsegaye Obisse, my wife Selmawit Eyassu, my friend Desta Samuel for the financial support they provided and last but not least; I would like to thank Addis Ababa University, Department of Pharmacology and Clinical Pharmacy and Dilla University for funding my study.

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Abstract:

Evaluation of in *vivo* anti-malarial activity of *80%* methanol extract and solvent fractions of seeds of *Prunes persica (L)* Bastche against *Plasmodium berghei* in mice

Halchaye Tsegaye

Addis Ababa University, 2019

Malaria continues as major health problem in subtropical region of the world. The resistances of malaria parasites to available drugs endanger all the good result that has been found. Therefore, the development of novel drug is a must to do job. One of the key sources is traditional medicinal plant. *Prunes persica (L)* Bastche is a common fruit and belongs to the family Rosaceae (subfamily *Prunoideae).* The seed extract of *Prunes persica (L)* Bastche is traditionally used as anti-malarial agent in Shinasha society North West of Ethiopia. However, no scientific investigation has been carried out to substantiate this claim. This study was conducted to evaluate anti-*plasmodial* activity of 80% methanol seed extract and solvent fractions of the plant *Prunes persica (L)* Bastche in mice model. To assess the effect, various dose (200,400 and 800 mg/kg) of the seed extract and butanol (BF), chloroform (CF) and aqueous (AF) fractions of crude extract were administered to *Plasmodium berghei* infected mice using 4 day suppressive test scheme. The parameters, including parasitemia load, survival time, change in body weight, temperature and packed cell volume were then determined within 4 days. The result revealed that crude extract of seed has relatively good parasitemia suppression rate 34.59% at 200mg/kg, 51.75% at 400mg /kg, 76.04% at 800mg/kg, negative control DW10 ml/kg 0 % and positive control CQ25mg/kg 100%. From the solvent fractions BF scored highest parasitemia suppression rate 36.64% at 200mg/kg, 46.01% at 400mg/kg, 54.96% at 800mg/kg, negative control DW10 ml/kg 0 % and positive control CQ25mg/kg 100%. AF had second highest parasitemia suppuration rate 32.85% at 200mg/kg, 44.56% at 400mg/kg, 50.99% at 800mg/kg, negative control DW10 ml/kg 0 % and positive control CQ25mg/kg 100% and CF has lowest parasitemia suppuration rate29.49% at 200mg/kg, 34.15% at 400mg/kg, 44.00% at 800mg/kg, negative control DW10 ml/kg 0 % and positive control CQ25mg/kg 100%. All dose level of the seed crude extract prevented loss of weight, elevation in body temperature and anemia on early and established infection. BF and AF did also reverse reduction in body weight and packed cell volume. Lastly, phyto-chemical screening of the extracts showed the presence of different

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secondary metabolites such as tannins, alkaloids, anthocyanins, terpenoids, saponins, phenols, and flavonoids. In conclusion, the plant has anti-*plasmodial* activity.

Key words: In vivo, *Prunus persica (L).*Bastche, Anti-malarial, *Plasmodium berghei*, suppressive study.

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List of Abbreviations and Acronyms

AF	Aqueous Fraction
AMA	Apical Membrane Antigen
ANOVA	Analysis of Variance
BF	Butanol Fraction
CDC	Centers for Disease Control and Prevention
CF	Chloroform Fraction
CON	Control Group
CQ	Chloroquine
ICAM-1	Intercellular Adhesion Molecule-1
IRBCs	Infected Red Blood Cells
KAHRP	Knob-associated Histidine Rich Protein
MSP	Merozoite Surface Protein
MP	Membrane Protein
NIAID	National Institute of Allergy and Infectious Diseases
OECD	Organization of Economic Cooperation and Development
PP	*Prunes persica* crude
PPS	Percentage parasitemia suppuration
PCV	Packed Cell Volume
PECAM-1	Platelate Endothelial Cell Adhesion Molecule-1
Pf EMP	Plasmodium falciparum Erythrocyte Membrane Protein

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PRBC	Parasitized Red Blood Cell
RBC	Red Blood Cell
RDT	Rapid Diagnostic Test
SEM	Standard Error of the Mean
SPSS	Stastical Package for Social Science
UNICEF	United Nations International Children Emergence Fund
VAR2CSA	Variant Two Chondroitin Sulphate A Antigen
VCM-1	Vascular Cell Adhesion Molecule-1
WHO	World Health Organization

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1. INTRODUCTION

1.1. Background on Malaria

Malaria is a serious and life taking disease caused by a parasite *Plasmodium* [CDC, 2018]. The genus *Anopheles* mosquito, which embraces 537 documented species and most of which (87%) were officially named in the world [Harbach, 2013]. Out of all, *Anopheles gambiae* and *Anopheles funestus are* the two most hazardous malaria vectors in the world and they are principal malaria vectors in Africa. On the other hand *A. arabiensis* are co-dominant across much of the Africa continent, whereas in the Asian-Pacific region there is highly complex situation with multi-species coexistence and variable species dominance [Sinka *et al*, 2012; CDC, 2015; Alelign and Dejene, 2016]. The dominant vectors confirmed in Ethiopia include *Anopheles gambiae*, *Anopheles funestus*, *Anopheles nili* and *Anopheles pharoensis* [Adugna, 2011, FMoH, 2014].

Generally, in the world more than one million individuals die every year due to the *Anopheles mosquito* bite transmitted malaria infection, particularly the tropical and subtropical zones take the larger share [Mojarrab *et al*, 2014]. Malaria continues to persist as the top infectious ailment troubling billions of people globally [Mboowa, 2014]. The malaria extermination program had little success in numerous parts of Sub-Saharan Africa. Even, the number of people at risk of infected with malaria at the end of the 20th century rose to over 74% in this section of the world [Hay *et al.*, 2004]. Actually, Ethiopia, shares the huge burden of malaria like other sub Saharan African countries and it has become a prominent public health problem in the country [Deressa *et al.*, 2006; Alelign and Dejene, 2016].

1.2. Etiological Agents

The causative agent of malaria infection is classified under genus *Plasmodium* phylum apicomplexan [Dondorp, 2005]. Altogether, there are more than 200 diverse species of *Plasmodium* of which at least 13 are pathogenic to humans [Chavatte *et al.*, 2007; Liu *et al.*, 2010]. Nevertheless, other species infect other animals; comprising monkeys, rodents, and reptiles [Gueriard *et al*., 2010]. Prominently, there are about five *Plasmodium* species that cause malaria in humans which are *P. falciparum*, *P. vivax*, *P. malaria* and *P.ovale* and *P. knowleis* [William and Menon, 2014]. The species *P.falciparum* and *P.vivax* are the most dangerous

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species clinically in the world [Singh S, 2013]. In part of Africa Asia and South America, *P. malarae* causes periodic infections, while *P.ovale* is restricted to tropical Africa, New Guinea, and the Philippines. *P. knowlesi* is found in South East Asian countries [WHO, 2016]. In Ethiopia *P. falciparum* and *P.vivax* are the most dominant malaria parasites, the former causing greater burden in the country [FMoH, 2014].

1.3. Life Cycle of Plasmodium Parasites

The moment mosquito bites the skin probes for blood vessel the motile infectious form of *plasmodium* parasites is transmitted to human blood circulation. At that point thread-like sporozoites are passed to the liver by the circulatory system. Then in the liver a single *Plasmodium* sporozoite reproduces into tens of thousands of exoerythrocytic merozoites [krettli and miller, 2001; Manitoba, 2015]. Within 7-12 day of inoculation into liver cell, the sporozoites mature in to schizonts and can cultivate up to 30,000 merozoites, which burst out the hepatocytes [WHO, 2013a; Ricardo *et al*, 2014]. By chance, some *P.vivax* and *P.ovale* sporozoites transformed into hypnozoites, a form that can stay dormant in the liver for months or years and cause relapses in infected people [Greenwood *et al*, 2005; walker *et a*l, 2010].

Subsequently, the asexual erythrocyte cycle initiates with the merozoites surrounding the RBC to mature by consuming hemoglobin. After a while, within the host RBC, the parasite undergoes development from the early ring stage to late trophozoite stage and then, after mitotic divisions, to the schizonts stage, which contains 6–32 merozoites [UNICEF, 2000; Jiraprapa, 2002]. As time goes, the erythrocytes schizonts ruptures, then released merozoites continue the life cycle by invading other RBCs until it is brought under control.

The cyclical fevers are typically happening shortly before or at the time of RBC lysise as schizonts rupture to release new infectious merozoites. Some merozoites differentiate into male and female sexual forms known as erythrocytic gametocytes, during this repeated cycle, with one nucleus and then awaiting the arrival of a blood seeking female anopheles mosquito [UNICEF, 2000; Jiraprapa, 2002]. The intake of gametocytes by the mosquito induces gametogenesis. The flagellated forms of microgametes, formed by a process known as ex-flagellation, penetrate or fertilize the macrogametes generating zygotes. Then zygotes changed into ookinetes and then become round oocytes. Lastly, inside the oocytes, the nucleus divides repeatedly, with the

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formation of a large number of sporozoites with 8–15 days and enlargement of the oocyte [WHO, 2013].

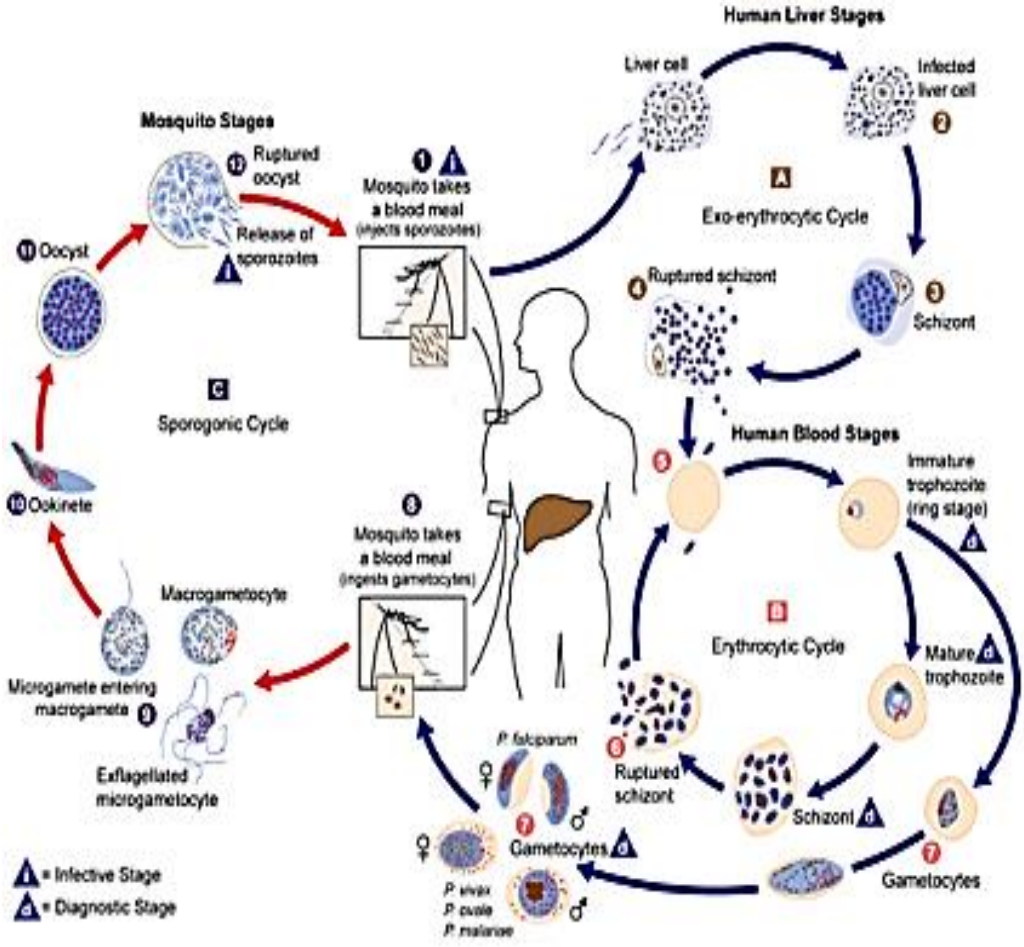

Figure 1. The life cycle of malaria parasites [CDC, 2017]

The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host **1**, sporozoites infect liver cells **2** and mature into schizonts **3** which rupture and release merozoites **4** of note, in *P.vivax* and *P.ovale* a dormant stage (hypnozoites) can persist in the liver and cause relapses by invading the blood stream weeks, or even years later. After this initial replication in the liver (exo-erythrocytic schizogany **A**. The parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogany **B**. The merozoites infect red blood cells **5**. The ring stage trophozoite mature into schizonts. Which rupture releasing merozoites **6**. Some parasites differentiate into sexual erythrocytic stages (gametocytes) **7**. Blood stage parasites is responsible for the clinical

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manifestations of the disease. The gametocytes are ingested by an anopheles mosquito during blood meal **8**. The parasites multiplication in the mosquito is known as the saprogenic cycle **C**. In the mosquitos, stomach, the micro gametes penetrates the macro gametes generating zygotes **9**. The zygote in turn become motile and elongated (ookinetes) **10**. Which invades the mid gut wall of the mosquito where they develop into oocytes' **11**. The oocytes' grow, rupture and release sporozoites**12**. Which make their way to the mosquito's salivary glands. Inoculation of the sporozoites1 into a new human host perpetuates the malaria life cycle.

1.4. Pathogenesis of Malaria Infection

The co-receptors on sporozoites mediate invasion out of three invasive forms on *Plasmodium* apical organelles [Dame *et al*, 1984; Robson *et al*, 1995 and Bannister *et al*, 2000]. Which in turn bind exactly to the heparin sulfate proteoglycans on hepatocytes and Kupffer cells [Frevert *et al*, 1993]. Consequently in hepatocytes, CD81 and CD68 are receptors for falciparum invasion [Cha *et al*., 2015]. After penetrating space of Disse in the liver, sporozoites migrate *via* several hepatocytes and involve in a final invasion, with the formation of a modified parasite phorous vacuolar membrane (PVM) [Silvie *et al*., 2003]. Meanwhile PVM is ruptured by *plasmodial* enzymes and merozoites drive out from the infected hepatocyte to access blood circulation [Cha *et al*.2015]. Once within the circulation, merozoites are swiftly and exactly enter RBCs, which are indicating receptor-ligand interactions. After binding to RBC, the merozoites reorient it using apical membrane antigen 1 (AMA-1) so that the apical end of the parasite will locate adjacent to the RBC membrane with a transient RBC deformation. The contents of apical organelles are going to be expelled as the parasite invades [Mitchell *et al*, 2004].

So, next to reorientation, a junction was formed between the parasite and host [Maier *et al*, 2003; Mayer *et al*, 2009; John, 2012]. These cell using microneme proteins, those distinguish and bind to receptors in the host cell proteins in the neck of rhoptry (RON) are inserted into the host membrane and bind to AMA-1 to form the tight junction [Tonkin *et al*, 2011]. Then, the contact area becomes free of RBC membrane proteins. After this, a merozoites enzyme results in a localized disruption of the sub membrane cytoskeleton and lipid architecture of RBC [Zuccala and Baum, 2011]. Formation of the junction triggers the release of rhoptry bulb, providing proteins and lipids required for the Parasito phorous vacuole (PV) [Riglar *et al*, 2011].

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So, an incipient PVM will be formed in the junction area [Sam-Yellowe, 1996]. The junction between the parasite and host becomes ring-like and the parasite appears to move via this annulus as it enters the expanding PV [Aikawa *et al*, 1978]. Following entry, the PVM and host cell membrane will need to be closed [Kappe *et al*, 2004; Besteiro, 2011]. Once inside the RBC; the parasite modifies the host cell to make it a more suitable environment and undergo major host cell changes which resulted in the pathogenesis of malaria.

Knob-associated histidine rich protein (KAHRP) and erythrocyte membrane protein-2 (PfEMP2) are two of several proteins which reorganize the host RBC sub membrane cytoskeleton and induce knob 5 formation [Ryan, 2001]. A polymorphic protein, PfEMP1, has been anchored to the knobs by KAHRP and is exposed on the host RBC surface [Craing and Scherf, 2001; Rasti *et al*, 2004].

On the other hand, IRBCs also adhere to uninfected RBCs to form red cell rosetting and to other parasitized RBCs to form agglutination [Rasti *et al*, 2004]. Since, *P.falciparum*, *P.vivax*, and *P.ovale* are all able to form rosettes but only those caused by *P.falciparum* have been associated with severe malaria [Doumbo *et al*, 2009]. The *P. vivax* and *P.ovale* show a marked penchant for young RBCs, while malarae prefers old cells. As a result, these parasites have low level of parasitemia in blood stream. However, *P. falciparum* can invade RBCs of all ages and produces very high levels of parasitemia [Mc Queen and McKenzie, 2003; Cowman, 2012].

If the above pathophysiologic process goes on uninhibited, it ultimately blocks the blood flow, limits the local oxygen supply, impedes mitochondrial ATP synthesis, and stimulates cytokine production – all these factors contributing to development of severe disease [Krishnegowda *et al*, 2005]. The systemic manifestations of malaria including fever have been largely attributed to the released cytokines and toxins [Coban *et al*., 2005; Ian *et al*, 2006]. The *plasmodial* DNA is presented by hemozoin to interact intracellular with the toll-like receptor-9, leading to release of pro-inflammatory cytokines that in turn induce cyclooxygenase (COX)-2-upregulating prostaglandins leading to induction of fever [Peggy *et al*, 2007; Ralf, 2007].

Also, hemozoin has been linked to induction of apoptosis in developing erythroid cells in the bone marrow, thereby causing anemia [Gordon *et al*, 2007; Lamikanra *et al*., 2009]. The

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pulmonary oedema which are directly correlated with the induction of strong pro-inflammatory immune responses [Schofield and Grau, 2005].

1.5. Epidemiology of Malaria

The latest WHO estimates, there are 30-50 million new clinical cases globally and 1 million deaths occur due to malaria each year [WHO, 2017]. As matter of fact, in the world, there are about 3.3 billion people at risk of being infected with malaria and developing disease of which, 1.2 billion are at high risk. However, the disease led to 584,000 deaths representing a decrease in malaria case incidence and mortality rates of 30% and 47% respectively [WHO,2015].

The incidence of malaria was estimated to account about 216 million cases of malaria across the globe in 2016, which showed a relative increment when compared with 211 million in 2015 and a considerable reduction relative to the 237 million in 2010 [WHO, 2016]. Looking at trends in malaria incidence between 2010 and 2016, the WHO reports that the incidence rate of malaria is estimated to be decreased by 18 percent globally, from 76 to 63 cases per 1,000 population at risk [WHO, 2018]. Overall, all WHO regions saw a reduction in recorded mortality in the period from 2010 to 2016, apart from the eastern Mediterranean region, which remained virtually un-changed [WHO, 2018].

1.6. Clinical Manifestations of Malaria

Malarial signs can develop within 8–25 days following infection [Olasunkanmi *et al*, 2013]. It is similar to the symptoms of a minor systemic viral illness and enteric fever or other acute febrile infections [Bartoloni and Zammarchi, 2012; Singhet *et al*, 2013; WHO, 2015]. Symptoms includes headache, fatigue, abdominal discomfort, muscle and joint aches, usually followed by fever, chills, perspiration, anorexia, vomiting and worsening malaise.

Additionally, splenomegaly and hepatomegaly can also be manifested in patients having malaria [Idro *et al*., 2005; WHO, 2015; Hermansyah *et al*, 2017]. On the other hand, when conditions worsen severe malaria manifestations takes place like, severe anemia, metabolic acidosis, hypoglycemia, acute renal failure, acute pulmonary oedema, cerebral malaria, coma and death [Schofield and Grau, 2005; Bartoloni and Zammarchi, 2012; Hermansyah *et al*, 2017]. As a consequence of that, fatal outcome will result [Weatherall *et al*, 2002; Trampuz *et al*, 2003; Rasti

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et al, 2004; Idro *et al*, 2005]. In children with malaria infection frequently exhibit abnormal posture sign indicating severe brain damage and cognitive impairment [Ekwebene, 2012].

1.7. Diagnosis of Malaria

According to the World Health Organization recommendation, clinical diagnosis of malaria should be suspected in any patient presenting with a history of fever, shaking, chills or temperature greater than 37.5^0C and the clinical diagnosis of malaria is based on the signs and symptoms obtained from physical examination [Ryan,2001; Wongsrichanalai *et al*, 2007]. In the absence of other obvious cause's examination is done using light microscopy and rapid diagnostic tests [CDC, 2016]. Rapid diagnostic tests (RDTs) have been available, in kit form, with all necessary reagents and are rapid when compared to microscopy and can be performed easily by any medical stuff without the need of laboratory technician [Mohammed *et al*, 2012]. RDTs are lateral-flow immuno-chromatographic tests that detect specific antigens produced by malaria parasites [Azikiwe *et al*, 2012].

However, microscopy remains the golden standard of choice since, RDTs are incapable to quantify parasite density and differentiate between *P. vivax*, *P. ovale* and *P.malareae* [Mekonnen *et al*, 2014]. Polymerase chain reaction (PCR) is a most useful method for definitively identifying the species of malaria parasite and detecting mixed infections [CDC, 2014]. PCR is not routinely used for the clinical confirmation of malaria, instead it could be employed for epidemiological identification of the type of malaria parasites [CDC, 2016; Snounou *et al*, 1993; WHO, 2015]. So far, light microscopy and RDTs are routinely employed methods for parasitological diagnosis of malaria [FMoH, 2012]. All of suspected malaria should be confirmed with a parasitological diagnosis in all settings [WHO, 2015]. Rolling circle enhanced enzyme activity detection (REEAD) and micro magnetic resonance reflaxometric (MMR) tests are also amenable to deployment in field conditions [Kumar and Renu, 2015].

1.8. Management of Malaria

1.8.1. Antimalarial Drugs

Antimalarial drugs are classified as 4-aminoquinolines such as chloroquine, amodiaquine, piperaquine and 8-aminoquinolones such as primaquine. Other antimalarial drugs group aryl amino alcohols include quinine, mefloquine, halofantrine, lumefantrine. In addition to that

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antimetabolites such as pyrimethamine, dapson, proguanil, and sulfadoxine. Moreover artemisinins such as arteether, artesunate, dihydroarthemisinin and artemisone the last group hydroxyl naphtha quinone such as atovaquone and clindamycin are some of them functional drug in use [Peter W, 1973; Saifi *et al*, 2013].

The quinolone anti-malarials work by inhibiting polymerization of haem to hemozoin, which is toxic to the parasite. On the other hand, the antimetabolites inhibit the synthesis of folic acid by blocking the dihydrofolate reductase and dihydropteroate syntheses enzymes of the parasite. Besides, the antibiotic antimalarial inhibit protein synthesis while artemisinins are supposed to work by interfering with the parasite sarco-endoplasmic calcium ATPase and production of free radical [Santos *et al*, 2013].

Most of today's drugs like artemisinins target both the asexual blood stages and inhibit the development of early gametocytes, artemisinin derivatives and 8-aminoquinolines are useful transmission-blocking antimalarial whose optimal actions are on different stages of gametocytes. The drug primaquine targets both the pre erythrocytic stages and mature gametocytes of *P. falciparum*, yet atovaquone and anti-folates inhibit sporozoite formation [Saifi *et al*, 2013; WHO, 2015]. Following initial parenteral treatment, once the patient can tolerate oral therapy, it is essential to continue and complete treatment with an effective oral antimalarial using a full course of an effective ACT (artesunate plus amodiaquine or artemether plus lumefantrine or dihydro artemisinin plus piperaquine) or artesunate (plus clindamycin or doxycycline) or quinine (plus clindamycin or doxycycline) [Singh *et al*, 2013]. Injectable artesunate is recommended in the case of severe malaria followed by standard regimens of ACTs as indicated for uncomplicated malaria at least after 24h of treatment with artesunate [Eckstein-Ludwig, 2003; Noubiap, 2014]. In the absence of artemisinins, quinine or quinidine plus doxycycline or clindamycin or tetracycline for seven days is recommended based on the patient conditions, costs, availability and safety profile of the drugs [CDC, 2013;WHO, 2015].

Quinine remains important for treating complicated *P. falciparum* malaria despite its toxicity when used for extended period of time [WHO, 2013]. Chloroquine remains the currently recommended drug for the treatment of *P.vivax* in areas where the drug is still effective. However, in areas where *P. vivax* is known to be chloroquine-sensitive, the WHO recommends three days of chloroquine plus two weeks of primaquine (provided the affected individual is not

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severely G6PD deficient [Douglas *et al*., 2012]. Moreover, radical cure of *P.vivax* and *P.ovale* requires primaquine that prevents late relapses [WHO, 2014].In general, the treatment strategy of malaria plans to end the acute blood infection, to cure the clinical symptom to clear hypnozoites from the liver to prevent future relapse and to prevent the spread of infections [CDC, 2016].

1.8.2. Vector Control

There are many of such apparatuses available for vector control; several of these methods are targeted at inhibiting the bite of mosquitoes [Karunamoorthi, 2011; Naseem *et al*, 2016]. Properly covering the whole body with clothes early evening and when mosquitoes biting are high, the use of insect repellents, appropriately closing the doors [Karunamoorthi, 2011; WHO, 2017]. Moreover insecticide treated nets (ITNs), long lasting insecticide impregnated nets (LLINs), larviciding and indoor residual spraying (IRS) are widely used by the governments of malaria endemic countries as components of malaria control and elimination tools [WHO, 2011].

1.8.3. Emerging Anti-malarial Agents

New drug are in pipeline. Those candidates under investigation include: (TCMDC29, GSK1057714, GNF156, KA609, MMV390048 and DSM265) exhibited potent activity against blood stages of *P.falciparum* [Gamo, 2014]. Another candidate KAF156 displays potent *Plasmodium* transmission blocking activities both *in vitro* and *in vivo* and OZ439, acting the same way to artemisinins is under clinical trial II [Kuhen *et al*, 2014; Opsenica and Šolaja, 2009]. Moreover a second-generation artemisinin derivative, artemisone, a drug in Phase II trial, provides a single-dose cure when combined with mefloquine [Phillips *et al*, 2015]. Moreover, multiple novel combination therapies, including azithromycin-chloroquine [Chandra *et al*,2013], pediatric pyronaridine-artesunate, pediatric dihydroarthemisinin in piperaquine [Bvgh, 2015] and trimethoprim-sulfamethoxazole are in phase III trial [Janet *et al*, 2016]. Apparently, the primaquine analog Tafenoquine is has recently shown great promise in Phase II trial and is currently being tested in pivotal Phase III trial and has proven activity against hypnozoites [Rajapakse *et al*., 2015]. The anti-helminthes, levamisole and ivermectin are also under clinical trials [Rowe *et al*., 2009; Smit *et al*, 2018]. Alkaloids such as benzophenanthridines (sanguinarine, chelerythrine), protoberberines (bebeerine) and protopines (protopines, allocryptopine) from aerial part of *Argemone mexicana L. Papaveraceae*) are also now on clinical trial I [Rubio-Pina and Vazquez-Flota, 2013].

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1.8.4. Malaria Vaccine

The current malaria tools are not sufficient to effectively control and eradicate the disease [WHO, 2017]. Moreover, the emergence and spread of drug and insecticide resistance has been the major obstacles to control the disease. As a result, safe and effective malaria vaccine is needed to aid the eradication of malaria from the globe. The malaria life cycle is very complex involving multiple stages that involve many antigenic molecules that could be a target for malaria vaccine [Fujiokaa and Aikawab, 2002; Verma *et al*, 2013]. Accordingly, vaccines targeting the different stages of the malaria life had been extensively investigated including pre-erythrocytic vaccine, erythrocytic vaccine and transmission blocking vaccine [Lennartz *et al*, 2017].

The pre-erythrocytic vaccine targets the infective sporozoite proteins. One such vaccine is the RTS, vaccine that is in phase III clinical trial and it is the most promising vaccine [WHO, 2015; WHO, 2017]. The blood stage vaccines that target MSP were also investigated, where MSP-l and MSP-2 has been found to be promising [Jepsen *et al*, 2013]. VAR2CSA, variant of pfEMP1 that target the receptors on placenta has been studied and shown promising protection against malaria complications occurring during pregnancy [Rogerson *et al*, 2007]. In general, different parasitic antigenic molecules are involved in the pathogenesis of malaria which made challenge to the development of effective malaria vaccine to be licensed [Lennartz *et al*, 2017; Ouattara and Laurens, 2015].

1.8.5. Antimalarial Drug Resistance

Antimalarial drug resistance occur as a consequence of several factors, including poor treatment practices, inadequate patient adherence to prescribed antimalarial regimens, and substandard forms of the drug [WHO, 2013]. The mechanism of antimalarial drug resistance is different for different classes of antimalarial drugs [Antony and Parija, 2016; WHO, 2015]. Antimalarial drug resistance to quinolones and arylaminoalcohols involves the mutations of the genes encoding vacuolar trans membrane proteins that regulate the transport of these drugs at the target, CQ resistance in *P.falciparum* is mainly because of single nucleotide polymorphisms in the genes encoding pfcrt which is CQ transporter while polymorphisms in the genes encoding for the *P. falciparum* multidrug resistance (pfMDR1) is involved in mefloquine resistance [Farooq and Mahajan, 2004; White, 2004; WHO,2015]. Mutations in *P. falciparum* multidrug resistance 1

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(PfMDR1) seem to be the main cause of resistance to mefloquine but are also implicated in Chloroquine resistance [Warhurst, 2007]. Atovaquone resistance is associated with single point mutations in the cytochrome b gene of *P.falciparum* [WHO, 2010].

Even artemisinin is, the most active regimens in the current antimalarial drugs reduced sensitivity in parts of Africa, South East Asia with artemisinin-resistant *P. falciparum* [Krishnaa *et al*, 2004, White, 2004; Alelign and Dejene, 2016]. This may be due to alterations in the expression of heat-shock proteins, a cell-cycle regulator and a DNA biosynthesis protein mutations or amplifications of the gene encoding a PfMDR1 or mutations in the gene encoding sarco-endoplasmic reticulum calcium ATPase6 have been reported to occur in artemisinin tolerant parasites [Witkowski *et al*, 2010]. The emergence of antimalarial drug resistant parasites will not only obscure the malaria control efforts achieved but, also alarms to look for new treatment regimens to treat and control the disease [Antony and Parija,2016].

1.9. Traditional Medicinal Plants

Apart from anti-folate antimalarial drugs, all the other commonly used antimalarial molecules are based upon plant-derived compounds [Giday, *et al*, 2006]. Since, artemisinin is sesquiterpene endo peroxide that has been isolated as the active principle from the Chinese antimalarial herb *Artemisia annua* and quinine from *Cinchona* bark by the French scientists Caventou and Pelletier [Nwaka & Hudson, 2006]. Several research groups are working to screen plants for new antimalarial compound [Nwaka & Hudson, 2006]. In *vivo* antimalarial activity testing showed that *A. maciverae* and *A. maritima* exhibited 73.0 and 63.0% parasitemia inhibition, respectively [Ene *et al*, 2009]. Similarly, *Ficus platyphylla* and *Acacia nilotica* showed markedly antimalarial activity against *P. berghei* in mice [Ali Jigam *et al*, 2010; Shittu *et al*, 2011]. Hence, it is rational to conduct research on traditionally used medicinal plants on the way to discover new antimalarial drugs [Kazembe *et al*, 2012; Kassaye *et al*, 2006].

1.10. The Experimental Plant

The experimental plant *Prunus persica (L)* Bastsch, commonly called peach, is a common fruit, and belongs to the family *Rosaceae* (subfamily Prunoideae), in many Ethiopian languages such as Amharic, Shinashgna, Geddeuffa, Afaan Oromo,it is vernacularly known as ’’ *koke”* which is known for its nutritional values and therapeutic properties and has more than 2,000 varieties found in various parts of the world, it is a small deciduous tree 6 to 10 ft. tall, with a spreading

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canopy and is cultivated in plains and hills [Kirtikar and Basu, 2001; Aziz 2013; Kumar N and Chaudhary, 2017]. The fruit part of *Prunus persica (L.)* Bastsch has yellow or whitish flesh, which is very delicate and easily bruised in some cultivators but is fairly firm in some varieties.

Consequently, *Prunus persica (L)* Bastsch fruit has a single large seed which is red-brown having oval shape [Kirtikar and Basu, 2001]. According to many researches the leave, bark, flower and seed part of *Prunus persica (L)* Bastsch has very important medicinal use [Edward *et al*, 1994]. It has been reported that the seeds have anti-inflammatory and anti-tumor activity and the nucleus in the seed can improve blood circulation [Wei *et al*, 2014]. In addition, this study plant possesses in vitro anti-tumor activity [Wei Han *et al*, 2015]. The bark is used in leprosy and jaundice [Kirtikar and Basu, 2001]. The increased consumption of the fruit showed significant increase in antioxidant in blood [Cao *et al*, 1998].

In Ethiopia, the seed extract of *Prunus persica (L.)* Bastsch is traditionally used as anti-malaria in Shinasha society North West of Ethiopia [Giday, *et al* 2006] bioassay guided chromatographic separation of the crude extracts showed different phytochemical constituent like, *amygdalin, prunasin, Amygdalinicacid, madalinic acid* [Fukuda *et al*, 2003].

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Figure2: The plant of *Prunus persica (L)* Bastche

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1.11. Rationale for the Study

Resistance to antimalarial drugs has been among the major challenges in controlling the disease [Dippmann *et al*, 2008; Dondorp *et al*, 2009]. So far control measure includes prevention and treatment is undertaken [WHO, 2015]. In case of treatment failure with known anti-malarial drugs the traditional medicinal plant certainly, is good source of lead compound for the anti-malaria drug [Willcox and Bodeker, 2004; Adams *et al*, 2011; Liu *et al*, 2013]. However, most traditional medicinal plants used in folk medicine luck pharmacological investigations for their antimalarial activity. *Prunus persica (L),* Bastche previously had been reported to have been used for malaria treatment traditionally [Gidey *et al*, 2006].

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2. OBJECTIVE

2.1. General Objective

- To evaluate anti-plasmodial activity of 80% methanol and solvent fractions of seed of *Prunus persica (L)* Bastche against *P.berghei* using *in vivo* mice model.

2.2. Specific Objectives

- To determine acute toxicity of 80% methanol extract of seed and solvent fractions of *Prunus persica (L)* Bastsch.
- *To* evaluate blood schizonticidal activity of 80%of *Prunus persica (L)* Bastsch extracts *in vivo* using peters 4-day suppressive test.
- To evaluate anti-plasmodial activities ((blood schizonticidal activity or suppressive effects) of solvent fractions of seed extract of *Prunus persica (L)* Bastsch extracts *in vivo* using peters 4-day suppressive test.
- To conduct preliminary phytochemical screening on the 80% methanol seed extract and solvent fraction of *Prunus persica (L)* Bastsch.

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3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Drug, Reagent and Chemicals

The following chemicals, reagents and drug were used in the present study: distilled water (Ethiopian Pharmaceutical Manufacturing, Ethiopia), n-hexane (BDH Chemicals Ltd,England Absolutmethano(CarloErba,France),nbutanol(FischerScientific,England),normalsaline (Fresenius Kabi, India), Chloroform (CarloErba,France), trisodium citrate (BDH Chemicals Ltd,England), Geimsastain (BDHChemicalsLtd,England), Chloroquine phosphate (Ethiopian Pharmaceuticals Manufacturing, Ethiopia), glacial acetic acid (Lobe Chemi, India), ferric chloride (Fisher Scientific, England), hydrochloric acid (Riedal de Haen, Germany) and tween 80 (Lobe Chemi, India), All the chemicals and reagents used in this study were of analytical grade.

3.1.2. Collection and Authentication of Plant Material

The ripened fruit with seed of *Prunus persica (L)* Bastche were collected in November, 2018 from forest around Asosa, Benshangul Gumuz region, North West Ethiopia, which is about 590 km away from Addis Ababa, the capital city of Ethiopia. Identification and authentication of the plant was done by carefully observing the leaves, fruit of the plant by taxonomist at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University, where a voucher specimen was coded and deposited for future reference with voucher number of HT001.

3.1.3. Experimental Animals

Healthy Swiss albino mice (22-31g), aged 4- 6 weeks, female for toxicity and male for the antimalarial activity study were purchased from Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia and maintained in the animal house at School of Pharmacy, Addis Ababa University. Animals were kept for a week for the purpose of acclimatization, and they were housed in stainless steel cage at room temperature with 12 h light-dark cycle and provided with a commercial obtained food and water *ad libtum*. All procedures and techniques used in this study were in accordance with the Guide for care and use of laboratory animals [National Institute of Health Guidelines for Care and Use of Laboratory Animals, 1996].

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3.1.4. Source of Rodent Parasite

For *in vivo* antimalarial assays of plant extract, chloroquine sensitive *P. berghei* (NK65 Strain) infected donor mice was obtained from Ethiopian Public Health Institute Addis Ababa. The parasites were maintained by serial passage of blood from infected donor mice to non-infected ones on weekly basis until 30-37% parasitemia level was attained [Bantie *et al*, 2014].

3.2. Methods

3.2.1. Extraction and Fractionation

a.Extraction

Fresh seeds of *Prunus persica (L)* Bastche plant where thoroughly washed with tap water to remove dirt and cleaned with gauze. After that, it was broken into pieces manually and dried under shade and then, pulverized using a mortar and pestle to get a coarse powder used for the extraction. The air-dried and powdered(400gram) plant material was cold macerated with 1200ml of n- hexane for 3 days to defat, and again macerated with 1200ml of 80% methanol i.e. in the ratio of 1:3 in an erlenmeyer flask for three consecutive days at room temperature. The extraction process was facilitated with occasional shaking at 120 rpm using a mechanical shaker (Bibby Scientific Limited Stone Staffo Reshire, UK). Then in another erlenmeyer flask (400gram) plant material was also cold macerated with 1200ml of n-hexane for 3-day to defat, and again macerated with1200ml of 80% methanol as before in the ratio of 1:3 for three consecutive days at room temperature.

The resulting crude extract was collected together and was separated from the marc with gauze and further filtered by Whatman filter paper No. 1 (Whatman®, England). This procedure was repeated two times by adding another fresh solvent into the marc. The filtrates were combined and the solvent was removed by evaporation under reduced pressure using rotary evaporator (Buchi Rota vapor R-200, Switzerland) with temperature not exceeding 40°C. The extract was then freeze dried using lypholizer (Operan, Korea Vacuum Limited, Korea) to remove water. Finally, the extract was transferred into vials and kept at −20°C until used for the proposed experiment.

b. Fractionation

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The 80% methanol crude seed extract was subjected to fractionation using solvents with differing polarity, i.e., chloroform, n-butanol and distilled water. Around 200g of the crude extract was suspended in 200 ml of distilled water in a separatory funnel. Then, the suspension was shaken with 100ml of chloroform. The chloroform layer was collected in a beaker and the aqueous layer was further shaken twice with 100ml of chloroform. The chloroform fraction was collected and combined to the previous one. The combined chloroform fraction was concentrated in Rotavapour and then dried in dry oven with temperature not exceeding 40^{o}C. The remaining aqueous layer was again shaken with 100ml of n-butanol. Then, the aqueous layer was first collected in a beaker. Then, the remaining n-butanol layer in the separatory funnel was collected using a separate beaker. The aqueous layer was then transferred to the separatory funnel and shaken twice with 100ml of n-butanol, and the n-butanol fraction was collected and combined to the previous one. Then, combined n-butanol layers were transferred to a round bottom flask and concentrated in rotary evaporator (Buchi Rota vapor R-200, Switzerland) at 45 rpm and temperature not exceeding 40^{o}C. The remaining aqueous residue in the separatory funnel was transferred to a round bottom flask, frozen in deep freezer overnight and then freeze dried with a lyophilizer (Operan, Korea Vacuum Limited, Korea) to remove water at - 50 ^{0}C and vacuum pressure of <2000 mbar to obtain the aqueous fraction. At last, the fractions were kept in an amber glass bottle and stored in a refrigerator (−20°C) for use at the time of treatment.

3.2.2. Acute Toxicity Test

Acute toxicity test was performed according to the Organization of Economic Cooperation and Development (OECD) 425 guideline for crude extract (OECD, 2008). Non pregnant female Swiss albino mice, age 6-8 weeks and weight 25-31g, were used for the toxicity study. The mice were fasted for three hours before the test. Following the period of fasting, the animals were weighed and 2000mg/kg of the crude extracts and fractions were administered by oral gavage as a single dose. Food was then withheld for further 2 hours. First, 5 female mice, one for each crude extract and fraction, was given 2000mg/kg of each of the extracts and fractions as a single dose by oral gavage one take distilled water. Then, each mouse was observed over a period of 24 hours. Since no death was observed, another four female mice for each crude extract and fraction, were given the same dose and observed for gross behavioral changes such as loss of appetite, hair erection, lacrimation, tremors, convulsions, mortality and the like which are signs of toxicity over a period of 14 days.

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3.2.3. Parasite Inoculation

Parasitemia level of the donor mice were determined from the blood collected by cutting a 0.5 to 1mm section from tail of the donor mice with scissor. The parasites were then maintained by serial passage of blood from infected mice to non-infected ones every week until 30-37% parasitemia level was attained. Then the donor mice were euthanized with cervical dislocation and infected blood obtained by cardiac puncture was collected in a falcon tube containing 2% trisodium citrate (BDH Chemicals, England) as anticoagulant [Bantie *et al*., 2014; Nureye *et al*., 2018]. Then, the blood was diluted in normal saline up on addition of few drops (0.5 ml) of trisodium citrate as anticoagulant so that the final suspension would contain about $1x10^7$ infected RBCs in every 0.2 ml suspension. The dilution was made based on the parasitemia of the donor mice & the RBC count of the normal mice ($4.5x10^9$ RBC/ml) in such a way that 1ml blood contains $5x\ 10^7$ infected erythrocytes. Therefore, each mouse used in the study was infected intra-peritoneal with 0.2 ml infected blood containing about $1x10^7$*P. berghei* parasitized RBCs [Bantie *et al*., 2014; Birhanu *et al*, 2015]. On day 4 (d4, 96h from d01), parameters detailed below were determined and the mice were monitored daily for 30 days to assess survival time [Peter *et al*, 1975].

3.2.4. Grouping and Dosing of Animals

To evaluate the crude extract of *Pernus persica* (L) Batsch (PP) and solvent fractions (BF=butanol fraction, CF=chloroform fraction and AF=aqueous fraction) for antimalarial activity, infected mice were randomly divided into five groups of 6 mice per group. Three groups (I-III) were treated with the extracts at 200 mg/kg, 400 mg/kg and 800 mg/kg, respectively. The remaining two groups served as negative (CON) and positive controls and received 10 ml/kg of distilled water used for reconstitution and chloroquine (CQ) 25 mg/kg, respectively. Dose selection was made based on the result of acute toxicity study conducted on the extracts with different dose. Each dose was reconstituted by distilled water and administered via the oral route using gavages. The solvent for reconstitution volume administered was calculated based on individual mouse body weight.

3.2.6. Determination of Suppressive Activity of Extract on Early Infection (4 - day test)

The percentage parasitemia was obtained by counting the number of parasitized red blood cells out of 500 erythrocytes in random fields of the microscope. Weight & rectal temperature was

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recorded for each mouse just before infection & daily throughout the experiment, while packed cell volume (PCV) was only be recorded just before infection & at the end of the 4th day. After that, the mice were followed for 28 days for their survival [Peter W, etal. 1975]. On day 4, blood was collected from the tail of each mouse using clean, non-greasy slides and thin films made and allowed to air dry. The films were then fixed with few drops of methanol, left for about 15 minutes to air dry and transferred to slide box. Then, the slides were stained with freshly prepared 10% Giemsa at a pH of 7.2 for 15 minutes. The stain was then washed off with distilled water and slides were left to air dry. Then, the slides were viewed under the light microscope using the oil immersion objective and parasites were counted using the X100 objective. The percentage parasitemia (PP) was obtained by counting the number of PRBCs out of erythrocytes in random fields of the microscope. Two stained slides for each mouse were examined. Three fields [Bantie *et al*., 2014] with approximately 200-500 cells were counted for each slide and Percentage parasitemia for each mouse was determined using the following formula [Adumanya *et al*, 2014; Fidock *et al*, 2004; Mengiste *et al*, 2012].

Percentage of parasitemia=Number of PRBC/Total number of RBCs

The mean Percentage parasitemia of the groups in each crude extract and fraction, and the respective negative controls was determined. Then, the mean percentage parasitemia suppression (PPS) was calculated using the formula described below [Adumanya *et al*., 2014; Fidock *et al*., 2004].

To assess the extracts' and fractions' effect on survival time, of any mice were monitored daily and the number of days from the time of inoculation up to death was recorded for each mouse in treatment and control groups throughout the follow up period (30 days). Then, the mean survival time (MST) for each group was calculated as follows [Amelo *etal*, 2014].

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

3.2.7. Packed Cell Volume Measurements

Packed cell volume (PCV) was measured just before treatment and at the end of day 4 using micro hematocrit centrifuge (Centurion Scientific, UK). Blood was collected from the tail of each mouse in heparinized micro hematocrit capillary tubes. The capillary tubes were filled to

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3/4th of their height with blood and sealed with sealing clay at their dry end. The tubes were then placed on a micro-hematocrit centrifuge (Centurion Scientific, UK) with the sealed end facing the periphery and centrifuged at 11,000 rpm for 5 minutes [Bantie *et al*, 2014]. Finally, the tubes were taken out of the centrifuge and PCV was determined using the standard hematocrit reader (Hawksley and Sons, England). PCV is a measure of the proportion of RBCs to plasma in the whole blood and determined using the formula shown below [Gilmour and Sykes 1951; Dikasso *et al*, 2006; Asnake *et al*, 2015; Mengiste *et al*, 2012].

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

3.2.8. Parasitemia &Survival Time Measurement

The number of parasitized red blood cells (PRBC) was counted using a microscope. Six fields were counted for each slide, average was taken and percentage parasitemia was determined using the following formula [Mengiste *et al*, 2012].

Percentage of parasitemia=Number of PRBC/Total number of RBCs

Percent parasitemia suppression of the extracts was compared with respect to the controls. Percent parasitemia suppression was calculated using the following formula [Mengiste *et al*, 2012].

$$\text{Average \% of Parasitemia Suppression} = \frac{\text{Parasitemia in negative control} - \text{Parasitemia in treatment group}}{\text{Parasitemia in negative control}}$$

The mean survival time (MST) for each group was calculated as follows [Amelo *et al*, 2014].

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

3.2.9. Phytochemical Screening

Preliminary phytochemical screening was performed using standard procedures to identify different phytochemical constituents in 80% methanol seed extract and solvent fractions using standard procedures.

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Test for alkaloids

Five hundred milligrams of the extract was treated in a test tube with 10 ml of 1% HCl for 30 minutes in a water bath and then filtered through cotton in to a test tube. Small portion of the extract was transferred into two test tubes and to one of the test tubes, five drops of Mayer's reagent and to the second five drops of Wagner's reagent were added and the formation of whitish opalescence (Mayer's reagent) or reddish brown precipitate (Wagner's reagent) was inspected [Tiwari *et al*, 2011].

Test for anthocyanins

Two ml of the extract is prepared using distilled water then it is added to 2 ml of 2N HCl & NH_3, the appearance of pink red turns blue violet indicates presence of Anthocyanin [Godghate and Rajaram, 2013].

Test for flavonoids

The dried crude extract and fractions were dissolved in a solvent. To 2 ml of the solution, three to five drops of 2% ammonia solution were added. Then, it was observed whether it developed yellow or orange color, which indicates the presence of flavonoids [Jones and Kinghorn, 2006].

Test for terpenoids

Five ml of the crude extract and fractions dissolved in the solvent was mixed with 2 ml of chloroform, and 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration at the interface was formed to show positive result for the presence of terpenoids [Trease and Evans, 1989].

Test for saponins

Half gram of the sample in 10 ml of distilled water was shaken in a test tube and the formation of honeycomb froth that persists for 30 minutes was considered as positive for saponins [Debella, 2002].

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Test for phenols

To 2 ml of filtered solutions of the samples with distilled water , freshly prepared three drops of a mixture of 1 ml of 1% $FeCl_3$ and 1 ml of 1% $KFe(CN)_6$ were added and the formation of a green blue color was examined [Yadav et al., 2010].

Test for cardiac glycosides

Two ml of each extract was dissolved in 2 ml of glacial acetic acid containing one drop of $FeCl_3$ solution. The mixture was then poured into a test tube containing 1 ml of concentrated H_2SO_4. A brown ring at the interphase indicates the presence of a deoxy sugar, characteristic of cardenolides [Njoku and Obi, 2009].

Test for steroids

One gram of extract was weighed and placed in a test tube. This was dissolved in 2 ml of acetic anhydride, followed by the addition of 4 drops of chloroform. Two drops of concentrated sulphuric acid were then added by means of a pipette at the side of the test tube. The development of a brownish ring at the interface of the two liquids and the appearance of violet color in the supernatant layer were indicative of the presence of steroids [Yadav *et al*, 2010; Ranjit *et al*, 2012].

Test for tannins

About 2 ml of solid extract was stirred with 2 ml of distilled water and few drops of $FeCl_3$solution were added. The formation of a green precipitate was an indication for the presence of tannins [Njoku and Obi, 2009].

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3.3. Data Analysis

Data obtained from the 4 day suppressive study were organized and entered into windows statistical package for social science (SPSS) version 22 and then analyzed by one way analysis of variance (ANOVA) followed by Tuckey (Post-hoc test) to determine Stastical significance for comparison of parasitemia % suppression, body weight, rectal temperature, PCV and survival time among groups. The analysis was performed with 95% confidence interval and the significance was set at P-values less than 0.05 was considered to be statically significant. Results of the study were expressed as mean $\pm$ standard error of mean (M$\pm$ SEM)

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4. RESULTS

4.1. Acute Toxicity Study

The acute toxicity test conducted to determine the safety level of the crude extracts and solvent fractions of the seeds of *Prunus persica (L.)* Batsch and all the fractions were found to be safe up to 2000 mg/kg. The test substances that were administered orally in a single dose of 2000 mg/kg to the laboratory bred Swiss albino mice caused no mortality within24 h and the next 14 days of the observation period. Moreover, the gross behavioral and physical observation of the experimental mice revealed that the substances caused no visible signs of acute toxicity such as lacrimation, loss of appetite, hair erection, salivation, and diarrhea, reduction in motor and feeding activities.

4.2. Effect of the Crude Seed Extract and Fractions on Parasitemia and Survival Time

Effects of 80% methanol crude seed extract and solvent fractions, at different dose levels, on parasitemia and survival time measurements in mice infected with *P. berghei* are summarized in Table1. The suppressive study revealed that 80% methanol crude extract as well as fractions exhibited a significant inhibition of parasitemia ($p<0.001$) compared to negative controls. However, the effect produced was smaller than that of the standard drug. The standard drug, CQ, cleared the parasite to undetectable level. Comparable suppression was also seen when both the lower dose and middle doses of chloroform fraction were compared, but the higher dose of chloroform fraction displayed greater suppression compared to both the low ($p<0.001$) and middle dose ($p<0.05$)chloroform fraction.

Whereas, the butanol fraction produced the highest parasitemia inhibition than aqueous fraction but lower than that of crude extract. But chloroform fraction showed the lowest parasitic suppression rate. All doses of the crude extract were capable of significantly increasing survival time compared to negative control. Survival time was also significantly prolonged by the high ($p<0.001$) and middle doses ($p<0.01$) of aqueous fraction, but all doses of butanol fraction was significantly ($p<0.001$) for middle and larger dose, $p<0.05$ for lower dose improved survival time compared to CON mice. CQ-treated groups had a long ($p<0.001$) mean survival time when compared to the respective control and treatment groups. Comparable difference in survival time among doses of the crude extract was noted. With fractions, however, only the higher dose had a significantly greater reduction than the low and middle doses in both butanol fraction and

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aqueous fraction. By contrast, suppression was comparable between the different doses of the chloroform fraction as well as between the doses and the negative control.

Table 1: Effect of the 80% methanol seed extract and solvent fractions of *Prunus persica (L)* Bastche on parasitemia and survival time in *P. berghei* infected mice

Group	% Parasitemia	% Suppression	Survival time
CON	56.38±0.86	0.00	5.83±0.54
PP200	36.88±0.93	$34.59^{a3b3d3e3}$	$8.50±25^{a2b3d3e3}$
PP400	27.20±0.49	51.75^{a3b3e3}	$11.50±0.22^{a3b3e3}$
PP800	13.51±0.76	76.04^{a3b3}	$13.63±0.37^{a3b3}$
CQ25	0.00+0.00	100^{a3}	$28±00^{a3}$
CON	54.66±0.45	0.00	6.33±0.24
BF200	37.63±0.29	$31.15^{a3b3d3e3}$	$7.67±0.51^{a1b3e3}$
BF400	29.51±0.27	46.01^{a3b3e3}	$9.66±0.31^{a3b3e1}$
BF800	24.92±0.38	54.96^{a3b3}	$12.50±0.43^{a3b3}$
CQ25	0.00±0.00	100^{a3}	$28.00±00^{a3}$
CON	54.95±0.70	0.00	6.16±0.47
CF200	38.74±0.57	$29.49^{a3b3d3e}$	$7.16±0.60^{b3e3}$
CF400	36.18±0.33	34.15^{a3b3e3}	$7.50±0.33^{a3b3e3}$
CF800	30.77±0.38	44.00^{a3b3}	$9±0.45^{a3b3}$
CQ25	0.00±0.00	100^{a3}	$28±00^{a3}$
CON	53.81±0.27	0.00	6.5±0.37
AF200	38.13±0,95	29.13^{a3b3e3}	$7.3±0.43^{b3}$
AF400	29.83±0.17	44.56^{a3b3e3}	$7.8±0.21^{b3}$
AF800	26.37±0.23	50.99^{a3b3}	10.25±0.40
CQ25	0.00±0.00	100^{a3}	$28±00^{a3}$

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Data are expressed as mean +SEM (n=6); a, compared to CON; b, to CQ 25; c, to 200 mg/kg; d, to 400 mg/kg; e, 800 mg/kg: $^{1}p<0.05$, $^{2}p<0.01$, $^{3}p<0.001$; CON, negative control, received distilled water 10 ml/kg; PP = crude extract of *Prnus persica*, BF = n-butanol fraction, CF = Chloroform fraction, AF = aqueous fraction and CQ = chloroquine (positive control). Numbers refer to dose in mg/kg

4.3. Effect of the Crude Seed Extract and Fractions on Body Weight

The findings of the effect 80% methanol seed extracts of *Prunus persica (L)* Bastche and fractions at different dose levels on body weight are summarized in Table 2. The results are expressed as the change in mean body weight between the pre-treatment value (day 0) and the post- treatment value (day 4) for each group in reference to the change in mean values of the respective negative control mice. Significant protection from weight loss was exhibited in mice treated with 80% methanol crude seed extracts compared to their respective negative controls (Table2).

The 80% methanol extracts improved body weight increment to different levels between the day 0 and the day 4 compared to the respective negative control mice highest weight loss protection was exhibited by 80% methanol crude seed extract at 800 mg/kg/day while the lowest weight loss protection was exhibited by chloroform crude seed extract at 200 mg/kg/day (Table 2). However, the effect produced was less than that of the standard drug, CQ which showed the highest protection from parasite-induced body weight loss.

When compared among themselves, different dose levels in each 80% methanol seed extract dose level, exhibited statistically significant ($p<0.05$) difference in protection from parasite induced body weight loss evaluated in this study. Moreover, the higher the dose of both crude extract and fractions given, the better the protection from infection induced body weight loss.

Table 2: Effect of the crude seed extract and solvent fractions of *Prunus persica (L)* Bastche on body weight in *P.berghei* infected mice

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Group	Weight(gram)		%Change
Dose/Kg	D0	D4	
CON	29.54±0.68	25.48±0.63	-4.06
PP200	29.57±0.65	28.01±0.61	-1.54^{a1b1e1}
PP400	29.39±0.76	28.25±0.79	-1.14^{a2b3}
PP800	28.56±0.91	28.19±0.85	-0.37^{a3b2}
CQ25	29.27±0.90	30.81±0.73	1.54^{a3}
CON	28.84±0.76	24.75±0.82	-4.09
BF200	30.46±0.60	28.20±0.63	-2.26^{b3e1}
BF400	29.55±0.67	28.46±0.62	-1.09^{a2b3}
BF800	27.18±0.1.2	26.88±0.98	-0.3^{a3b3}
CQ25	26.63±0.99	30.34±0.15	3.71^{a3}
CON	28.75±0.53	25.36±0.70	-3.39
CF200	29.10±0.81	27.10±0.48	-2^{b3}
CF400	30.54±0.94	28.61±0.31	-1.93^{b3}
CF800	29.77±0.43	27.75±0.49	-2.02^{a3b3}
CQ25	29.71±48	32.11±0.56	2.4^{a3}
CON	30.83±0.65	26.78±0.74	-4.05
AF200	28.59±0.49	26.69±0.31	-1.9^{b3}
AF400	29.57±0.62	27.45±0.24	-2.12^{b3}
AF800	30.97±0.85	29.65±0.76	-1.32^{b3}
CQ25	29.92±0.11	31.84±0.45	1.92^{a3}

Data are expressed as mean ± SEM (n=6); a, compared to CON; b, compared to CQ 25 mg/kg; c, compared to 200 mg/kg; d, compared to 400 mg/kg; e, compared to 800 mg/kg; 1p< 0.05;

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2p<0.01, 3p<0.001; CON, negative control; Pp. = crude extract, BF = n-butanol fraction, CF = chloroform fraction, AF = aqueous fraction and CQ = chloroquine (positive control); D0 = pre-Rx value on day zero, D4 = post-Rx value on day four.

4.4. Effect of the Crude Seed Extract and Fractions on Rectal temperature.

The findings of the effect 80% methanol crude seed extracts of *Prunus persica (L)* Bastche and fractions at different dose levels on rectal temperature increment preventation are summarized in Table 3. Significant protection of rectal temperature increment was exhibited in mice treated with 80% methanol seed extracts compared to their respective negative controls (Table3).

Moreover, from the fractions of *Prunus persica (L)* Bastche two fractions were able to significantly prevent the increase in rectal temperature caused by P. *berghei* infection, 80% methanol crude seed extract at 200 mg/kg/day (p<0.001) and middle 400 mg/kg/day (p<0.01) doses of the extract had less effect compared to the standard, while the higher 800 mg/kg/day dose had a comparable effect.

In addition, the 800 mg/kg/day dose also produced a significant protection (p<0.01) compared to the 200 mg/kg/day dose. Whilst no apparent difference was observed between the chloroform fraction and vehicle treated animals, middle dose 400 mg/kg/day and high doses 800 mg/kg/day of both butanol fraction (p<0.01 & p<0.001) and aqueous fraction (p<0.05 & p<0.01) protected a significant increment of temperature compared to controls to maintain the rectal temperature at its physiological level..

On the other hand, the effect attained by chloroquine was higher than of the activity of all fractions (p<0.001).

Table 3: Effect of the crude seed extract and solvent fractions of *Prunes persica (L)* Bastche on rectal temperature in *Plasmodium berghei* infected mice

Group	**Temperature(^{0}c)**		**%Change**
Dose/Kg	**D0**	**D4**	
CON	36.99±0.23	33.50±0.35	-3.49
PP200	36.51±0.43	35.14±0.15	-1.37^{a1b3e2}

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PP400	36.20±0.34	34.94±0.17	-1.26^{a3b2}
PP800	36.56±0.34	36.52±0.48	-0.04^{a3}
CQ25	36.58±0.50	36.99±0.25	0.41^{a3}
CON	36.8±0.26	33.65±0.40	-3.15
BF200	36.72±0.40	34.55±0.41	-2.17^{b3e1}
BF400	36.26±0.43	34.99±0.33	-1.27^{a2b3}
BF800	36.49±0.48	36.20±0.40	0.29^{a3b}
CQ25	36.42±0.33	36.96±0.53	0.54^{a3}
CON	36.53±0.21	33.56±0.43	-2.97
CF200	36.60±0.33	34.33±0.24	-2.27^{b3}
CF400	36.86±0.30	35.60±0.21	-1.26^{a1b3}
CF800	36.21±0.16	35.85±0.28	-0.36^{a2b3}
CQ25	36.70±0.26	36.89±0.23	0.19^{a3}
CON	36.86±0.25	33.67±0.34	-3.19
AF200	36.36±0.48	34.07±0.23	-2.29^{b3}
AF400	36.52±0.17	34.89±0.28	-1.63^{b3}
AF800	36.43±0.15	35.36±0.34	-1.07
CQ25	36.22±0.33	36.43±0.28	0.21^{a3}

Data are expressed as mean ± SEM (n=6); a, compared to CON; b, compared to CQ 25 mg/kg; c, compared to 200 mg/kg; d, compared to 400 mg/kg; e, compared to 800 mg/kg; $^{1}p< 0.05$; $^{2}p<0.01$, $^{3}p<0.001$; CON, negative control; Pp. = crude extract, BF = n-butanol fraction, CF = chloroform fraction, AF = aqueous fraction and CQ = chloroquine (positive control); D0 = pre-Rx value on day zero, D4 = post-Rx value on day four.

4.5 Effect of the Crude Seed Extract and Fractions on Packed Cell Volume

The findings of the effect of 80% methanol crude seed extracts of *Prunus persica (L)* Bastche at different levels on PCV of mice infected with CQ sensitive *P. berghei* are summarized in (Table 4). The mean value of PCV showed attenuation from hemolysis in mice treated with crude

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extracts and in those that were treated with the vehicle on day-4 as compared to day-0 (Table 4). For that matter, significant protection against reduction of PCV ($p<0.05$) was exhibited in 80% methanol crude extracts compared to their respective negative controls.

Moreover, the crude extracts improved mean PCV to different levels. The highest protection against reduction of PCV was exhibited by 80% methanol seed extract at 800 mg/kg/day (Table 4) while the lowest protection was exhibited at200 mg/kg/day crude seed extract (table 4). Nevertheless, the effect produced was less than that of the standard drug, CQ which showed the highest protection from infection induced reduction in PCV. When compared among themselves, different dose levels in 80% methanol crude seed extract, exhibited statistically significant ($p<0.05$) difference in protection from infection induced reduction in PCV in this study. Moreover, the higher the dose of crude extract given, the better the protection from infection induced reduction in PCV exhibited.

The effect of the three fractions of 80% methanol crude seed extract of *Prunus persica (L)* Bastche at different dose levels on PCV of Swiss albino mice infected with CQ sensitive *P. berghei* are summarized in Table 5-7.The mean value of the PCV showed reduction in mice treated with solvent fractions and in those that were treated with the vehicle on day 4 as compared to day 0. However, significant protection from infection induced reduction in PCV ($p<0.05$) was exhibited in all the three solvent fractions compared to their respective negative controls. Additionally, all the fractions protected against infection induced reduction in PCV to different levels.

The highest protection was exhibited by the butanol fraction of the 80% methanol seed extract at the highest dose given, 800 mg/kg/day (table 5) while the least protection among the three fractions was exhibited by the chloroform fraction at the least dose given, 200 mg/kg/day (Table 5-7). Yet, the effect produced was less than that of the standard drug, CQP which showed the highest protection from infection induced reduction in PCV. When compared among themselves, different dose levels in each fraction, exhibited statistically significant ($p<0.05$) difference in protection from reduction in PCV. Furthermore, the higher the dose of each fraction given, the better the protection from infection induced reduction in PCV exhibited.

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Comparison of the results of the effects of fractions of 80% methanol crude seed extract of *Prunus persica (L)* Bastche on PCV of mice infected with *P. bergie* to the crude extracts showed difference in the level of protection from infection induced reduction in PCV. Accordingly, the highest protection exhibited by butanol fraction is lower than the effect produced by the 80% methanol extract which was mentioned earlier. Except the chloroform fraction, the other two fractions also showed greater level of protection compared to aqueous crude seed extract fractions. Hence, the chloroform fraction showed the least protection from infection induced reduction in PCV compared to any other fraction or crude extracts at any dose given.

Table 4: Effect of the 80% methanol crude seed extract on packed cell volume in *P. berghei* infected mice

Group	%Packed cell volume		Change (%)
Dose/Kg	D0 PCV	D4 PCV	
Dw10ml/kg	61.35±1.02	56.06±2.43	-5.29
Pp200mg/kg	62.19±2.45	59.72±2.87	-2.47
Pp400mg/kg	61.82±2.34	61.64±2.31	-0.18
Pp800mg/kg	61.92±1.89	62.55±1.98	0.63
CQ25mg/kg	61.31±2.78	63.44±1.56	2.13

Data are expressed as mean ± SEM (n=6); compared to the negative control and among the doses, the difference in mean change in PCV was significant at $p<0.05$; TW, negative control, 2% tween 80; CQ, positive control, chloroquine base;, 80% methanol crude seed extract; PCV, packed cell volume; d0, pre-treatment value on day 0; d4, post-treatment value on day four. The numbers in square of graphs show the in mean PCV between D0 and D4.

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Table 5: Effect of the butanol solvent fractions on packed cell volume in *P. berghei* infected mice

Group	Packedcellvolume		% change
Dose/Kg	D0 PCV	D4PCV	
Dw10ml/kg	61.88±2.16	56.71±2.24	-5.17
BF200mg/kg	61.45±2.36	57.95±1.79	-3.5
BF400mg/kg	61.63±2.03	60.23±2.34	-1.4
BF800mg/kg	61.99±2.56	61.03±2.56	-0.96
CQ25mg/kg	62.16±2.23	64.17±2.26	2.01

Data are expressed as mean ± SEM (n=6); compared to the negative control and among the doses, the difference in mean change in PCV was significant at $p<0.05$; TW, negative control, 2% tween 80; CQ, positive control, chloroquine base; BF, butanol fraction; PCV, packed cell volume; d0, pre-treatment value on day 0; d4, post-treatment value on day four. The numbers in square of graphs show the mean PCV between D0 and D4

Table 6: Effect of the aqueous solvent fractions on packed cell volume in *P. berghei* infected mice

Group	Packedcellvolume		% change
Dose/Kg	D0PCV	D4PCV	
Dw10ml/kg	61.37±2.56	56.19±1.97	-5.18
AF200mg/kg	61.48±2.76	57.48±2.04	-4.00
AF400mg/kg	62.20±2.02	59.97±2.36	-2.23
AF800mg/kg	61.44±1.89	59.34±2.07	-2.1
CQ25mg/kg	61.44±2.07	63.28±2.64	1.84

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Data are expressed as mean ± SEM (n=6); compared to the negative control and among the doses, the difference in mean change in PCV was significant at $p<0.05$; TW, negative control, 2% tween 80; CQ, positive control, chloroquine base; AF, aqueous fraction; PCV, packed cell volume; D0, pre-treatment value on day 0; D4, post-treatment value on day four. The numbers in square of graphs show the mean PCV between d0 and d4

Table 7: Effect of the chloroform solvent fractions on packed cell volume in *P. berghei* infected mice

Group	Packed Cell Volume	(%)	%change
Dose/Kg	D0 PCV	D4PCV	
Dw10ml/kg	61.56±1.44	56.47±3.34	-5.09
CF200mg/kg	61.91±2.38	56.79±3.26	-5.12
CF400mg/kg	61.97±2.87	57.73±2.48	-4.24
CF800mg/kg	61.80±2.73	59.97±2.46	-1.83
CQ25mg/kg	61.76±2.24	63.92±1.86	2.16

Data are expressed as mean ± SEM (n=6); compared to the negative control and among the doses, the difference in mean change in PCV was significant at $p<0.05$; TW, negative control, 2% tween 80; CQ, positive control, chloroquine base; CF, chloroform fraction; PCV, packed cell volume; D0, pre-treatment value on day 0; D4, post-treatment value on day four. The numbers in square of graphs show the mean PCV between D0 and D4.

4.6. Preliminary Phytochemical Screening

Phytochemical screening of 80%methanol extracts and solvent fractions of seed of *Prunes persica (L)* Bastche shown in table 8 below

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Table 8: Phyto chemical screening of 80% methanolic crude extract of seeds and solvent fractions of *Prunes persica (L)* Bastche

Phyto Constituents	80%methanol Extract	Butanol Fraction	Chloroform Fraction	Aqueous Fraction
Alkaloids	+	+	+	+
Anthocyanins	+	+	_	_
Flavonoids	+	+	+	+
Cardiac glycosides	+	+	_	_
Phenols	+	+	_	_
Saponins	+	+	+	+
Steroids	_	_	_	_
Tannins	+	+	+	+
Terpenoides	+	_	_	+

(-), absent ;(+), present

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5. DISCUSSION

Malaria is one of the world's most deadly diseases. Spread and emergency of resistance to the front line anti-malarial drugs including artemisinin is the major challenge [Ashley *et a*l, 2014]. The scientific community is now underway to combat this problem by searching for new, affordable and effective antimalarial agents from medicinal plants and other sources [Gamo, 2014]. The present study was aimed to determine the *in vivo* anti plasmodial activity of crude extract and solvent fraction of seeds of *Prunes persica (L)* Bastche using 4-day suppressive test. As it was observed none of the test mouse died or showed signs of toxicity within 24 h and the next 14 days of treatment with 2000 mg/kg of the test extract during acute toxicity study. The oral dose of greater than 2000 mg/kg body weight is 10 times greater than the minimum effective dose of the extract [Dikasso *et al*, 2006]. The acceptable justification for oral dosing of the extract and fractions was used, to replicate the ethno-medical method of administration and the likely route during clinical evaluation [Giday *et al*, 2006]

Based on the results, one could see that percentage parasitemia measured in the four-day test was reduced by the 80% methanol crude extract in infected mice. As it was also revealed in phytochemical analysis, alkaloids, flavonoids, saponins, phenols and terpenoids present in the 80% methanol crude extract of the study plant could be responsible for its antimalarial activity. The highest effect by butanol fraction next to the80% methanol crude extract might from presence of the important phyto-constituents. Also in this fraction the reason behind a closer effect to the extract by butanol fraction than other fractions might have also been instigated from the alcoholic nature of butanol and the active ingredient(s) might be concentrated in butanol fraction. This finding is also consistent with other studies in which butanol fraction had high activity than chloroform and aqueous fraction [Kwaghe and Ambali, 2009; Mengiste *et al*, 2012; Asnake *et al*, 2015].

Concurrently, aqueous fraction was found to have moderate antimalarial activities compared to fractions. This effect could have been associated with the existence of alkaloids, flavonoids, saponins and terpenoids as in butanol fraction. An Aqueous fraction lack phenols, which probably explain why it had lower activity than butanol fraction. However, the lowest dose was unable to suppress parasitemia significantly. This might be due to the absence of sufficient concentration of active constituent(s) since; high levels of chemo suppression were produced at

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high doses of the 80% methanol extract and fractions, indicating the presence of good concentrations of active compound(s) in higher doses.

Generally, the parasite suppressive effect of the 80% methanol seed extract and solvent fractions might be *via* indirect boosting of immune system or by inhibition of other target pathways which are not fully realized. The therapeutic benefits of traditional remedies are often attributed to the presence of non-nutritive bioactive constituents. The steroids, anthocyanins, flavonoids and saponins noticed in this plant have been proved to possess potential immune modulatory effects in other plants [Miguel, 2011; Saxena *et al*, 2013].

As observed from the results of this study, significant inhibition of parasitemia was exhibited in the 80% methanol crude seed extracts and all the three fractions of the seed. But, the suppressive activity of all three fractions is lower than that of the crude extract. This finding is in agreement with another study where the plants with different fractions have lower activity than from the crude [Asnake *et al*, 2015]. Activity reduction in the fractions could be explained by the loss of synergistic action among the chemical compounds or differential distribution of secondary metabolites within the fractions. The crude extract prolonged survival time in early parasite infection, which is concordant with the studies conducted on ethanolic leaf extract of *Chromolaena odorata* [Ukpi and Amaechi, 2012], hexane extract of *Pluchea lanceolata* [Mequanint, 2014] and methanolic extract of *Phytolacca dodecandra* leaves in the four-day test, the crude extract has greater survival time than fractions in line with [Bantie *et al*, 2014]. From fractions, all dose levels of butanol fraction and two higher doses of chloroform fraction significantly improved the survival time of the study mice on early infection [Amelo *et al*, 2014 and Bihonegn, 2016].

Consequently, body weight loss, hypoglycemia and reduction in PCV and body temperature are cardinal signs of malaria-infected mice [Langhorne *et al*, 2012]. Weight loss protection by the extract on early infection was, also experienced in other observations of different plant, and might have been determined by nutritional components of the plant [Amelo *et al*, 2014]. More than other detrimental factors the increase in weight was consistent with increase in dose respective to negative group. The higher doses of butanol fraction and the higher dose of aqueous fraction showed remarkable increase in body weight when compared with the infected untreated group.

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The observed changes in temperature were correlated to weight changes measured during the experiment rather than parasite suppression since weight loss is indirectly influenced with malaria fever [Kabiru *et al*, 2012]. This could also be attributed to the extract as it may have less amount of hypothermic effect on the treated mice [Mebrahtu *et al*, 2013; Adugna *et al*., 2014].

Anemia is caused by destruction of RBCs, either by parasite multiplication or by spleen reticulo endothelial cell action as the presence of many abnormal RBC stimulates the spleen to produce many phagocytes, erythropoietic suppression and dyserythropoiesis and oxidative stress, which increases erythrocyte RBC membrane fragility [Chinchilla *et al*, 1998, Lamikanra *et al*, 2007,Iribhogbe *et al*, 2012]. As we can see in chemo suppressive study, the extract and fractions of *Prunus persica (L)* Bastche prevented significant PCV reduction. The effect is in line with the outcome produced by crude extract of *Clerodendrum myricoides* leaves [Asnake *et al*, 2015].

The PCV reduction preventation effect might have been resulted from significant parasite suppression induced by active constituent(s) in the administered doses of the extract since the increase in blood parameters corresponded to the decreased parasites load [Mequanint, 2014]. Anthocyanins detected in this study and organic acids identified in other study from this plant could also be responsible for the protection of anemia as these metabolites have been proved to possess the ability to interact with proteins and stabilize erythrocytes membrane by preventing the oxidation of membranes phospholipids [Mpiana *et al*, 2011; Ngbolua *et al*, 2015].

As indicated in the results section, all treated groups brought about reduction of parasitemia after the second dose, however, the standard drug started its activity right after the first dose. This delay of activity may be indicative of the need for a loading dose or the extract might have a slower onset of action compared to chloroquine [Balogun *et al*, 2009]. The suppressive effect on established infection was higher than the four-day suppressive test probably due to non-selectivity of the extract against the proliferative processes of the parasite. The presence of the parasite alone in the blood does not induce disorder, but the response of the host immune system against foreign pathogenic organism via free radical generation, activation of a phospholipase cascade and generation of prostaglandin [Tijani *etal*, 2010]. The pronounced anti-malarial activity observed in the established infection test may be due inhibitory effect of the extract on generation of free radicals and hemolytic principles resulting from high parasitemia level [Calvalho *et al*, 1991].

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Moreover, anthocyanins, flavonoids which also exist in the plant, are generally known to have antioxidant, anti-proliferation and anti-inflammatory activity [Miguel, 2011]. Agents with such properties are known to produce additional remedy to malaria patients [Adzu and Salawu, 2009]. In summary, tannins, flavonoids, alkaloids, saponins, terpenoids, steroids and phenols detected in seed of this study plant might have been responsible for its antiplasmodial activity as per suggested in other plants [Kaur *et al*, 2009; Oliveira *et al*, 2009; Soh *et al*, 2012; Arise *et al*, 2012; Saxena *et al*, 2013]. On the other hand, the plant may contain antiprotozoal active compound that could serve as alternative antimalarial lead drugs. Additionally, presence of more than one class of phytochemicals in a given plant extract determines the nature and extent of its biological activity [Musila, 2013].

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6. CONCLUSION

In *vivo* antimalarial test results indicated that 80% methanol crude extract and fractions of the seed *Prunes persica (L)* Bastche plant materials possess antimalarial activity; the findings confirmed that the study plant has significant parasitemia suppressive effect suggesting the anti-plasmodial activity of the 80% methanol crude seed extract. On other hand, butanol fraction was found to be more active among the fractions and might contain a potential plant constitute for the development of new drug to treat malaria. The antimalarial action of the extract and solvent fractions had been attributed to the presence of ingredients in the 80% methanol crude seed extract of the plant. In addition *in vivo* antimalarial test data would provide evidence to uphold the claims made by the Ethiopian traditional medicine healers.

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7. RECOMMENDATION

From the present study, the following works are suggested for further investigation on the plant.

- ✓ Further quantitative phytochemical investigation to identify and quantify the antiplasmodial active components from the plant.
- ✓ Sub-acute and chronic toxicity study should be conducted to better establish the safety statues of the plant.
- ✓ Elucidating mechanism of action of the active principle(s).

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