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SCHOOL OF PHARMACY

DEPARTMENT OF PHARMACOLOGY &CLINICALPHARMACY

Evaluation of anti-malarial activity of the aqueous root extract of *Euclea divinorum* Hiern (Ebenaceae) against *Plasmodium berghei* infected mice

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

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This is to certify that the thesis prepared by Fentaw Girmaw, entitled "Evaluation of antimalarial activity of the aqueous root extract of *Euclea divinorum* Hiern (Ebenacae) against *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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ABSTRACT

Drug resistance is a universal challenge to malaria control measures. As a result, development and discovery of new chemotherapeutic agents from medicinal plants having antimalarial traditional claims is very important. *Euclea divinorum* H. (Ebenaceae) is shown to have anti-plasmodial activity *in vitro* and the root of this plant is used traditionally to treat malaria in different countries, including Ethiopia. This work therefore attempted to evaluate the antimalarial activity of the root aqueous extract using rodent model of malaria. To this effect, the roots of *Euclea divinorum* were extracted by hot decoction using distilled water. Anti-malarial activity of various doses of the root aqueous extract was evaluated using the 4-day suppressive test as well as curative and repository test. The finding showed that there was a dose-related significant parasitemia chemo-suppression and increment in survival time as compared to the negative control ($p<0.001$) at 100 mg/kg, 200 mg/kg and 400 mg/kg in all tests. The chemo-suppression effect was higher at 400 mg/kg extract treated groups in the 4-day suppressive test followed by curative test. The lowest chemo-prophylaxis effect was observed at 100 mg/kg extract treated groups in the repository test. Considering other parameters, the extract prevented weight loss, temperature drop and hemolysis in all models but not in a consistent manner. The result of the current finding supports folkloric use of the plant against malarial infection and confirmed the antimalarial activity of the plant.

Keywords: Antimalarial activity, *Plasmodium berghei*, *Euclea devinorum*, Parasitemia, Mice

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Table of Contents

LIST OF ACRONYMS

ACT:	Artemisinin-based Combination Therapy
ANOVA:	Analysis of Variance
DW:	Distilled Water
IC50:	Inhibitory concentration of 50%
MST:	Mean Survival Time
OECD:	Organization for Economic Cooperation and Development
PCV:	Packed cell volume
PfEMP:	*Plasmodium falciparum* Erythrocyte Membrane Protein
RPM:	Revolutions per Minute
SEM:	Standard Error of the Mean
SPSS:	Statistical Package for the Social Sciences
WHO:	World Health Organization

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

"Malaria" is derived from the Italian word *mal'aria,* which stands for ''bad air" to describe mainly the swampy areas in Europe in which the occurrence of the disease was highly prevalent (Amorosa, *et al*., 2005). Later, the protozoan parasite *Plasmodium,* which is the causative agent for the disease was identified (Ridder *et al.*, 2008). *Plasmodium* parasite can infect many animal species such as reptiles, birds, rodents, monkeys and humans. There are more than 100 *Plasmodium* parasite species (WHO, 2015). The bite of infected female *Anopheles* mosquitoes of the genus *Plasmodium* and family Plasmodidae transmits the disease malaria. Malaria is a common protozoan disease in tropical and subtropical countries. Children and pregnant women are high risk groups for the disease (Srivastava *et al.*, 2015).

Globally malaria remains one of the critical public health problems and also continues to remain among the top three infectious diseases (malaria, tuberculosis and HIV) affecting billions of people (Mboowa, 2014). It is one of the most devastating parasitic infectious diseases in the world. Annually malaria kills more than one million individuals in the tropical and subtropical zones (Mojarrab *et al.*, 2014).

Africa faces the greatest economic impact of this disease, particularly in sub-Saharan countries with children and pregnant women are the main targets (Idowu *et al.*, 2010). In Ethiopia and other sub-Saharan countries, malaria is one of the leading causes of morbidity and mortality. As reported in the past years, the disease has been the first leading cause of outpatient visits, hospitalization and death in health facilities across the country (Karunamoorthi and Bekele, 2009).

1.1 Etiology of malaria

All malarial infections in humans are caused by *P. falciparum*, *P.vivax*, *P. ovale, P.malariae* and *P. knowelsi* (White *et al*.,2015). Among all the five causes of malaria *P. falciparum* is the most dominant and deadly form in Africa. In Ethiopia among *Plasmodium* species that infect humans, *P.falciparum* and *P. vivax* are the most common malaria parasites, which account for greater than 99% malaria cases. However, *P. ovale* is rare in the country and *P. malariae* accounts for

less than 1% (Woyessa *et al.*, 2012). *P. knowlesi* is one of the *Plasmodium* species that causes malaria among monkeys. Human cases of malaria due to *P. knowlesi* are reported from the forested regions of South-East Asia particularly the island of Moreno (WHO, 2015). Malaria is transmitted from one person to the other by the female *Anopheles* mosquitoes. From 400 different species of *Anopheles* mosquitoes, only 30 of these are vectors of major importance. In Ethiopia the most dominant species are *Anopheles arabiensis, Anopheles pharoensis, Anopheles funestus,* and *Anopheles nili* (Adugna, 2006).

1.2. Epidemiology of malaria

There were 228 million cases of malaria and 405,000 deaths worldwide and 94% of all malaria deaths affecting the WHO African region in 2018. Globally, nearly 85% of malaria deaths were found in the WHO African Region and India specifically in 20 different countries. From all global malaria deaths 52% were from six WHO African countries; Nigeria (24%), Democratic Republic of Congo (11%), United Republic of Tanzania (5%), Angola, Niger and Mozambique (4% each). Under five children accounted for 67% of all malaria death globally in 2018 (WHO, 2019). In high endemic areas, the disease burden is greater in infants and young children than older children and adults (Carneiro *et al.*, 2010).

The pattern of malaria transmission in Ethiopia is seasonal and unstable due to variations in topography and rainfall patterns. As a result, the transmission varies from place to place and season to season. During dry season people are highly vulnerable to malaria due to lack of acquired immunity that comes with frequent exposure to malaria infections (Graves *et al.*, 2009). The breeding of mosquito carriers is very temperature-sensitive and in many regions of the world altitude is significant in determining the distribution of malaria (Tesi, 2011). It is mainly seasonal in the highland fringe areas of Ethiopia and of relatively longer transmission duration in lowland areas, river basins and rift valleys of the country (Ayele *et al.*, 2013).

Malaria is prevalent in $3/4^{th}$of the land of Ethiopia in which more than 45 million people are at risk and above 4 million clinical cases are reported each year. *Plasmodium falciparum* and *Plasmodium vivax* contribute to malaria morbidity in the nation (FMOH, 2012). Malaria

accounts for 40% of public health expenditure, 30-50% of inpatient admissions, and up to 50% of outpatient visits in areas with high malaria transmission (WHO, 2013).

1.3. Life cycle of the parasite

The life cycle of malaria parasites is complex which involves both human and mosquito hosts. The pattern of structural organization for the developmental stages in the life cycle of malaria parasites is distinct. There are different structural, biochemical and biological aspects throughout the erythrocytic schizogony, mosquito stages, and pre-erythrocytic schizogony of malaria parasite (Fujioka and Aikawa, 2002). Plasmodium alternates between vertebrate and mosquito hosts with its sexual phase in the mosquito. Infection of the human host occurs due to the bite of an infected *Anopheles* mosquito, which injects sporozoites from its salivary glands into the skin of a human host (Fig. 1).Then, the sporozoites enter into the blood stream and lymphatics to infect the liver, spleen, macrophages and endothelial cells (Bannister and Sherman, 2009).

After they invade liver hepatocytes, they undergo a phase of asexual multiplication (exoerythrocytic schizogony) resulting in the production of many uninucleate merozoite (Cox, 2010). In *P. vivax* and *P. ovale* infections, some hypnozoites remain in the liver and produce relapses of erythrocytic infection months to years after the primary attack unlike *P. falciparum* and *P. malariae* infections. Depending on the *Plasmodium* species, 6 to 32 merozoites are released per each rupture of schizonts containing erythrocytes. Until the death of the host or modulated by drugs or acquired partial immunity, the merozoites invade more erythrocytes to continue the cycle (Bannister and Sherman, 2009).

Some young merozoites develop into male and female gametocytes which in turn mature into male and female gametes. Then fertilization occurs and a motile zygote (ookinete) is formed that penetrates the gut wall and becomes a conspicuous oocyte within which another phase of multiplication occurs resulting in the formation of sporozoites. Afterwards, sporozoites migrate to the salivary glands of a mosquito and are injected when the mosquito feeds on a new host (Cox, 2010).

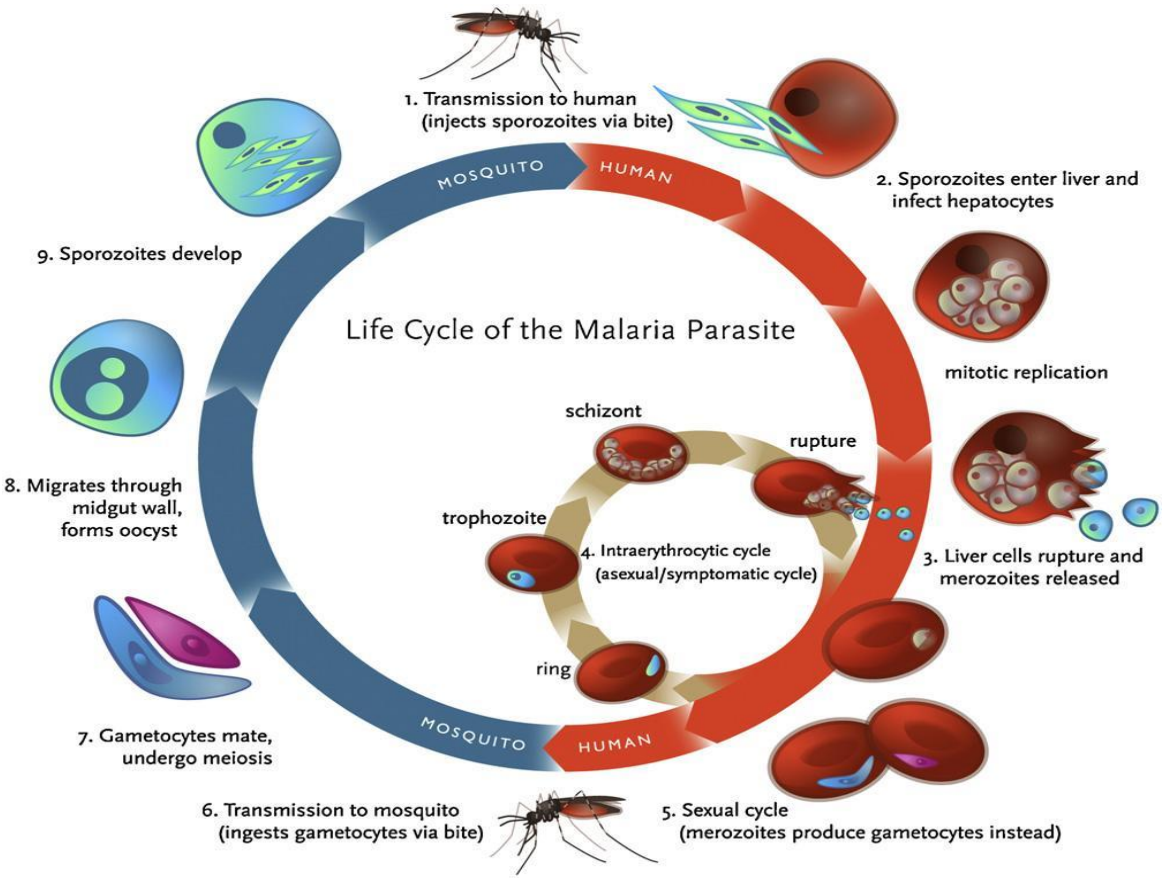

Figure 1: Life cycle of the malaria parasite(Klein, 2013): Transmission of malaria occurs through a vector, the mosquito, that inject sporozoites into a human during blood meal(s) (1), where they rapidly make their way to the liver and infect hepatocytes and begin asexual (mitotically) replication (2). After a period of ca. 6–15 days, the liver schizonts rupture, releasing thousands of merozoites into the blood, where they invade RBCs (3). Over the next ca. 48 h, the parasite begins replicating mitotically, progressing through a set of stages (ring, trophozoite and schizont), and produces an average of 16 new daughter merozoites per schizont. The schizonts then burst in near synchrony with other parasites, producing the characteristic fever cycle that embodies the clinical manifestations of the disease (4). Some of the merozoites produce gametocytes—the sexual form of the parasite (5), which is ingested by mosquito upon feeding on an infected human (6). Gametocytes, both male and female, mate within the gut of the mosquito, undergo meiosis (7), then migrate through the mid gut wall of the mosquito, and form an oocyst (8), within which thousands of sporozoites develop (9), that can then infect susceptible mosquitoes and bringing the transmission cycle full circle.

1.4 Pathophysiology of malaria

The correlation between clinical syndromes and the pathogenic processes of malaria is not as such simple one-to-one. Malaria parasites have intracellular signaling pathways which are mediated by phosphoinositide, cyclic AMP and calcium-dependent mechanisms. Malaria parasites sequester themselves into heart, lung, brain, liver, kidney, subcutaneous tissues and placenta (Miller *et al.*, 2002). Parasite infected red blood cells (iRBCs), after lysis, release newly

developed merozoites and other toxic factors like glycosylphosphatidylinositol (GPI) into the blood. This pathogenic factor has the ability to induce TNF-α and IL-1. Cytokines and inflammatory mediators, which are secreted by macrophages and endothelial cells, are also activated by GPI. The systemic manifestations of malaria are due to the various cytokines released in response to the parasite and red cell membrane products (Fresno and Angulo, 2002).

Parasite-induced RBCs alterations and microcirculatory abnormalities are accompanied by local and systemic immune reactions, resulting in multiple clinical forms of variable severity in pathogenesis of human *P. falciparum* infection. The major parasite adhesion, the variant *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) expressed on mature forms, is concentrated at electron-dense knobs on the iRBC membrane. Schizont rupture at the end of the cycle releases antigens and inflammatory components. This contributes to pathogenesis of malaria indirectly by stimulating expression of inducible endothelial receptors which further promotes cytoadherence (Buffet *et al.*, 2011). Parasite infection block blood flow, limits the local oxygen supply, hampers mitochondrial ATP synthesis, and stimulates cytokine production. This contributes to the development of severe complications of P. *falciparum*, resulting in inflammation, activation of platelets, anemia, microcirculatory dysfunction, tissue hypoxia and organ dysfunctions (Fresno and Angulo, 2002; WHO, 2013)

Cerebral malaria is related with sequestration of parasites in the brain and involvement of the intercellular adhesion molecule 1 (ICAM-1) receptor. An increase in the expression of ICAM-1 in brain endothelium may explain differences in parasite adhesion in cerebral malaria. Plasmodial DNA, which is pro-inflammatory and presented by hemozoin interact intracellularly with the Toll-like receptor-9, leading to the release of pro-inflammatory cytokines that in turn induce cyclooxygenase *(*COX*)* mediated up regulation of prostaglandins (Storm and Craig, 2014). Upregulation of the expression of ICAM-1 and vascular adhesion molecule-1 (VCAM-1) on endothelium cells by cytokines promote sequestration of parasitized erythrocytes in the brain, contributing to coma (Maitland and Marsh, 2004; Dondorp *et al.*, 2005).

1.5. Management of malaria

Since malaria is a preventable and treatable disease, there are several strategies to control and eliminate malaria. This includes vector control, chemoprevention and timely treatment with appropriate antimalarial. Most antimalarial agents, which are available in this time, can be classified according to their mechanism of action and chemical structures. Quinolone derivatives, antifolate combination drugs, antibiotics and artemisinin derivatives are the most commonly used antimalarial agents (Rosenthal, 1998). Patients having malaria can be diagnosed with either uncomplicated or severe malaria. Oral antimalarial agents are effective to treat uncomplicated malaria. The first-line therapy for uncomplicated *P. falciparum* malaria is artemisinin-based combination therapy (ACT) (artemether -lumefantrine) (WHO, 2013). ACT (artesunate plus amodiaquine or artemether plus lumefantrine or dihydroartemisinin plus piperaquine) or artesunate (plus clindamycin or doxycycline) or quinine (plus clindamycin or doxycycline) is preferred for the treatment of severe malaria (Singh *et al.*, 2013).

Globally, there are several malaria vaccine research projects. Most of the research on malaria vaccine has carried out on the *Plasmodium falciparum* strain. This is due to the ease of conducting *in vitro*/*in vivo* studies and greater mortality observed by the parasite. Throughout the parasite life cycle, there are many antigens that potentially could act as targets for the vaccine. The earliest vaccines attempted to use the most dominant surface antigen of the initial pre-erythrocytic phase (circumsporozoite protein) (Karunamoorthi *et al.*, 2014). Current malaria vaccine under preclinical incudes: pfs47, AMA1-RON2 and chemically attenuated parasites. GAP vaccine, CVac, pfRH5, pfs25 and pfs230 are under phase I clinical trial. Additionally, pfsPZ vaccine and RTS, S are under phase II and IV, respectively (Coelho *et al.*, 2018). Pilot implementation programs to assess safety and benefits of the first human malaria vaccine (RTS, S/AS01) were launched in 2019. Some new vaccine candidates seeks to improve the efficacy seen by RTS, S/AS0 so as to prevent clinical malaria in African children and pregnant women (Duffy and Gorres, 2020).

1.6. Antimalarial drug resistance

Resistance has been defined as the temporary or permanent ability of an organism and its progeny to survive or multiply despite the administration and absorption of a drug given in doses

within tolerance of the subject. Drug resistance may occur due to non-adherence, poor drug quality, interactions with other pharmaceuticals, poor absorption, misdiagnosis and incorrect dosing that lead to treatment failure. Even though treatment failure is not necessarily caused by drug resistance, it can assist its development (Cloete, 2003).

The most important cause of antimalarial drug resistance is gene mutation encoding for transporters, enzymes and receptors (Aminake and Pradel, 2013; Lo *et al.*, 2017). Drugs with long elimination half-lives are potentially more likely to develop resistance than short lived (rapidly eliminated) drugs (Guillermo *et al*., 2005). Lack of chemical diversity among the antimalarial drugs in use, which leads to cross resistance between drugs of the same class of compounds, further aggravate drug resistance (Olliaro and Yuthavong, 1999).

Antimalarial drug resistance has been observed in *P. falciparum*, *P. vivax*, and *P. malariae*. Almost all of the currently used antimalarial drugs have been developing resistance. The first chloroquine resistance in *P. falciparum* was detected in Thailand in 1962 and in India in 1973 (Mishra *et al.*, 2008; Kabri *et al.*, 2010). Single nucleotide polymorphism (SNP) in pfcrt (CQ resistance transporter) is implicated for chloroquine resistance and mutations in *P. falciparum* multidrug resistance 1 (PfMDR1) causes resistance to mefloquine and chloroquine (Warhurst *et al*., 2007). Mutations of genes encoding vacuolar trans-membrane proteins, which regulate the influx/efflux of 4-aminoquinolines, Quinine and highly hydrophobic arylaminoalcohols are the main cause of resistance.

Resistance of mosquitoes to insecticides and parasites to antimalarial drugs increase severe malaria, resurgence of malaria and complication for the eradication of the disease. The rapid rise of resistance to the current most effective and newest artemisinin derivatives is a great concern (Silva *et al.*, 2011). Mutations or amplifications of the gene encoding PfMDR1 or mutations in the gene encoding sarco-endoplasmic reticulum calcium ATPase6 that results in resistance to artemisinin derivatives have been recently detected (Marfurt *et al.*, 2010). The development of resistance to the artemisinin derivative can be prevented by combining an artemisinin derivative with a longer lasting partner drug, which provides a sustained antimalarial activity. Recently

artesunate-pyronaridine, arterolane-piperaquine, artemisinin-piperaquine and artemisinin-napththoquine combination have been introduced (WHO, 2015).

1.7. The experimental plant

Medicinal plants have a great importance for primary health care, income generation and livelihood improvement of millions of people living in the world. Plants have always been a source of drugs in the treatment of human ailments (Uprety *et al*., 2012). The families of plants with the most potent antimalarial effects include Annonaceae, Meliaceae, Rubiaceae, Aloaceae, Olacaceae, Fabaceae, Euphorbiaceae, Leguminosaere, Moraceae, Asphodelaceae and others (Chinsembu, 2015).

Euclea divinorum Heirn belongs to the genus *Euclea* and family Ebenaceae. It is an evergreen, shrub or small tree up to 9-15 m tall (Fig. 2) and widespread from Ethiopia and Sudan to South Africa. The Ebenaceae family, with about 500-600 species, are pantropical in distribution and encompass the genera *Diospyros* and *Euclea*. Main centers of diversity are in South East-Asia, Madagascar, tropical Africa, and South America. The genus *Euclea* is well presented in Eastern and Southern Africa and Southern Arabia (Mebe *et al.*,1998; Wallnöfer, 2001). There are two types of *Euclea* species in Ethiopia namely; *Euclea divinorum* (Fig. 2) and *Euclea racemosa,* which is mainly distributed in hot dry regions (Friis and White, 2003). The most widely distributed compounds in Ebenaceae family are naphthoquinones, terpenoids (especially lupanes, ursanes, oleananes, and taraxeranes), benzopyrones, polyphenols, and tannins. Beyond the aforementioned compounds steroids, naphtalene-based aromatics, hydrocarbons, lipids, amino acids, carotenoids, and sugars are also found (Mallavadhani *et al.*,1998). Preliminary phytochemical investigations of methanol and aqueous root extracts of *E. divinorum* identified secondary bioactive metabolites, including cardiac glycosides, saponins, tannins, flavonoids, steroids and terpenoids (Woldemedhin *et al*., 2017).

The root bark of *Euclea divinorum* H. in Soqotra Island is used mostly as a toothbrush or as powder for cleaning by rubbing on the teeth. It is also a famous plant in this area in which the root is used to color the lips and mouth red by chewing or rubbing. The wood is used as firewood and carving. Similar traditional uses of *Euclea divinorum* root were found in the ethno botany of

Namibia and Kenya (Olliaro and Yuthavong, 1999; Bussmann *et al.*, 2006). It is one of the most important medicinal plants in Kenya in which the roots and stems are used as tooth brushes as well as for treatment of chest pain, pneumonia and internal body swelling (Ngari *et al.*, 2013).

In Zambia, the root decoction of *E.divinorum* is used to treat different genital and oral diseases in HIV/AIDS-related diseases (Chinsembu, 2016). In Zimbabwe the root bark is used to treat diarrhea, cancer and dermal ailments (Mebe *et al.*, 1998). In South Africa, the leaves are used for management of noisy stomach, headache, general cleansing, and tooth ache (Samie *et al.*, 2010). In Ethiopia, the plant is known, by its vernacular name, as Dedeho (Amharic) and Mi'essa (Oromiffa) and used for some kidney problems (Wondimu *et al.*, 2007). Indeed, studies showed that the crude extract and solvent fraction of the leaves of *E. divinorum* to have renoprotective effect in rats (Feyissa, et al., 2013) and the crud extract to be endowed with antimicrobial effect against several bacterial strains and *Candida* maltose (More *et al.*, 2008; Mothana *et al.*, 2009). The hot root decoction is drunk for the treatment of malaria, fever and anaplasmosis in Kenya (Nanyingi *et al.*, 2008). In Ethiopia the root of *E. divinorum* concoction with water can be taken to treat malaria (Meragiaw and Asfaw, 2014).

An ethno-botanical survey done in Jabitehnan district, West Gojjam (Berhan et al, 2006) and a KAP study on the Konso community (South-west Ethiopia) related to the use of the root of *Euclea divinorum* for malaria(Dori *et al.*, 2012) also reported that this plant is used to treat malaria. More importantly, a previous study showed that methanolic root extract of *Euclea divinorum* showed moderate *in vitro* antiplasmodial activity against chloroquine resistant and sensitive strain of P. falciparum (IC_{50}=37.5**)**, suggesting that the potential antimalarial activity of this plant (Mothana *et al.*, 2012). However, the antimalarial activity of this plant is not yet scientifically studied *in vivo.* Thus, this study was initiated to investigate the plant's antimalarial activity using an *in vitro* paradigm.

Figure 2: Photograh of *Euclea divinorum*

1.8. Rationale for the study

The major challenge in the current malaria control measure is the spread of resistance to the available frontline antimalarial drugs (Ashley *et al.*, 2014). Resistance of the parasites to antimalarial drugs and the limited therapeutic efficacy that ensues provided the impetus to find new antimalarial drugs from medicinal plants *(*Karunamoorthi *et al.*, 2014; Shelton *et al.*, 2015). The major threat in controlling and eliminating malaria is resistance of *Plasmodium falciparum* to artemisinin (Ashley *et al.*, 2014). Globally, there is an increase in cost to control malarial infection due to a rapid rise in resistance to the available antimalarial drugs. There is a consensus in the scientific society that medicinal plants have a great role as a source of novel drug development for the treatment of human disease like malarial infection. There are some exemplary antimalarial drugs derived from medicinal plants such as quinine from cinchona, artemisinin from *Artemisia annua* (Oliveira *et al.*, 2009). Noble and safe antimalarial agents with wide margin of safety and new mechanism of action are a great concern. Treating malaria with plant derived bioactive compounds, which possess fewer side effects, highly attractive, accessible, cost effective and convenient for use, is of paramount importance.

The current finding helps the scientific society to isolate and identify the antimalarial compound which could be an alternative for the development of new antimalarial agent. The search for

new antimalarial drugs from plants and new drug development using their chemical structures as templates continues due to the increase in multi-drug resistant malaria. As a result, it is reasonable that studies like the current one should be conducted to screen medicinal plants for their antimalarial activity which further provide a clue for novel drug development Ethno botanical studies and *in vitro* activities showed that *E. divinorum* (Dori *et al.*, 2012; Mothana *et al.*, 2012) has anti-malarial activity. Therefore, this study would attempt to validate the *in vitro* activity and traditional use of the plant, to further ascertain in which extract the constituents responsible for anti-malarial activity are concentrated so as to provide a clue about the nature of the phytochemical constituents responsible for its action. In addition, the results of this study will help to further investigate on molecular mechanisms and formulation of plant source drugs by identifying the specific agent responsible for the anti-malarial effect

2. OBJECTIVES

2.1. General objective

To evaluate *in vivo* anti-malarial activities of aqueous root extracts of *Euclea divinorum* against *P. berghei* infected mice.

2.2. Specific objectives

- ✓ To evaluate the chemo-suppressive activity of aqueous root extract using the 4-day suppressive test
- ✓ To evaluate the curative potential of the aqueous root extract using the curative test
- ✓ To evaluate the prophylactic effect of the aqueous root extract using the repository test

3. MATERIAL AND METHODS

3.1. Drugs and chemicals

Chloroquine (Epharm, Ethiopia), normal isotonic saline, absolute methanol (Reagent Chemical Services Limited, USA), 2% tween 80 (Research-lab Fine Chem Industries, Mumbai), trisodium citrate (BDH Chemicals Ltd, England), giemsa stain (BDH Chemicals Ltd, England), oil immersion (Neolab, India) and distilled water (Epharm, Ethiopia) obtained from the respective vendors were used. All chemicals and reagents were of analytical grade.

3.2. Plant collection and identification

The fresh root of *Euclea divinorum* was collected from Gedeba Kebele, Raya Kobo Woreda, North Wollo Zone, Amhara Region (North, Ethiopia) in December, 2019. The fresh root was wrapped with plastic sheets during transportation. The specimen of the plant was identified as *Euclea divinorum* Hiern by a taxonomist and a specimen of the plant material was deposited, voucher number (No.FG 001), in the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University for future reference.

3.3. Experimental animals and parasites

Healthy Swiss albino mice of either sex with weight (20-35 g) or age (6–8 weeks) were used in the study. The mice were obtained from the animal house of the School of Pharmacy, Addis Ababa University. The animals were kept in 12 h light- dark cycle and had free access to standard pellet laboratory diet and water *ad libitum*. Animals were acclimatized to the laboratory conditions for 1 week before initiation of the experiment. The care and handling was according to international guidelines for the use and maintenance of experimental animals (OECD, 2001) and the protocol was approved by the School of Pharmacy Ethics Committee.

Chloroquine sensitive *Plasmodium berghei* ANKA strain was obtained from Department of Pharmacology, Mekelle University.

3.4. Methods

3.4.1. Preparation of crude extract

The root of the plant was thoroughly washed to remove any dead matter or other unwanted particles and then dried under shade at room temperature (25–27^{o}C) with optimal ventilation for 1 month. The dried root plant material was pulverized to coarse powder using a grinding mill. About 400g of air-dried and powdered root of *E. divinorum* (100 g dried root in 600 ml of DW) was extracted by hot decoction technique and 40gm (9.1%) aqueous extract was obtained. Of note, a pilot study carried out on 80% methanol root extract showed that it is less effective than the aqueous extract, which lead to the use of the latter extract for the study.

3.4. 2. Parasite inoculation

Experimental mice were infected with *P. berghei* ANKA strain parasite to induce malaria. The parasites were maintained by intraperitoneal serial passage of blood (Fidock *et al.*, 2004). *P. berghei* infected mice having parasitemia level of 20-30% was used as a donor and donor mouse was then sacrificed. Blood was collected by heparinized tube containing 0.5% tri-sodium citrate. The blood was then diluted with normal saline (0.9%) based on parasitemia level of the donor mice and the red blood cell (RBC) count of normal mice in 1 ml blood contains 5×10^7 infected RBCs . Then, each mouse was inoculated with 0.2ml of blood suspension containing 1×10^7 *P. berghei* parasitized erythrocytes intraperitonially (Gurmu *et al*., 2017).

3.4.3. Dosing and grouping of animals

The mice were divided to five groups randomly (n=6). Group I (negative control) was treated with D/W; Group II, III and IV was treated with 100 mg/kg, 200 mg/kg and 400 mg/kg of the aqueous extract respectively and Group V was treated with the standard drug, chloroquine (25 mg/kg) (OECD, 2001). The doses for the aqueous extract were chosen after performing a pilot study. For all animals, oral rout of administration was used and the maximum volume administered was 10 ml/1kg (OECD, 2008).

3.4.4. The 4-days suppressive test

The 4-day suppressive test was employed to test the chemo-suppressive activity of the aqueous extract against mice infected with chloroquine sensitive *P. berghei*. Thirty mice were infected on the first day. The mice were then randomly distributed into five groups two-hour after inducing infection and treatment was started immediately as described in animal grouping and dosing section at day 0. The treatment continued for additional three consecutive days. On the 5^{th} day of the experiment, blood was collected from the tail of each mouse. After that thin smear was prepared on a microscope slides to determine level of parasitemia. Additionally, mice weight, temperature and packed cell volume (PCV) were measured before infection and at the end of the experiment. To determine the mean survival time (MST) each group of mice was followed for 30 (day 0-day 29) days. Percent parasitemia and percent parasitemia suppression (% PS) were calculated using the modified Peters and Robinson formula (Peters and Robinson, 1992):

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

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

$$\text{MST} = \frac{\text{Total number of days mice survived}}{\text{Total number of mice}} \times 100$$

3.4.5. Curative test

The curative test was carried out for the aqueous extract to evaluate its curative potential according to the method describe in Reyley and Peteres(1970). On the first day, mice were injected intraperitonially with standard inoculum of $1x\ 10^7$ *P. berghei* infected erythrocytes. After seventy-two hours, the mice were divided into five groups randomly (n=6) and treated with the respective agents, as described in grouping and dosing section once daily for 3 days. Thin blood films were prepared from tail blood of each mouse daily to monitor the levels of parasitemia and mean survival time for each group was followed for 30 days. Similarly, as described in the four-day suppressive test section, other parameters were also determined before the 1^{st} dose and at the 7^{th} day of experiment.

3.4.6. Repository test

To observe the prophylactic potential of aqueous extract repository test was done according to the method described by Peters (Peters, 1965). Thirty mice were randomly distributed into five groups of six mice each and treated as described in animal grouping and dosing section for 4days (D1-D4). On the 5^{th} day all the groups were infected with inoculum of 1x 10^7 *P. berghei* infected erythrocytes. Blood smears were drawn 72 h post infection from each mouse to determine parasitemia level. Other parameters also determined as described in the four-day suppressive test section pre and post treatment.

3.4.7. Packed cell volume measurement

The packed cell volume (PCV) was calculated to determine the potential of test extracts to prevent the occurrence of hemolysis in *P. berghei* infected mice. Blood was collected from the tail of each mouse in heparinized micro-hematocrit capillary tubes by filling three-quarters of its volume. The tubes were sealed by sealant and placed in a micro hematocrit centrifuge with the sealed ends outwards. The blood was then centrifuged at 12, 000 rpm for 15 min. The tubes were then taken out of the centrifuge and PCV was determined as follows (Gurmu et al., 2017).

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

3.4.8. Determination of body weight and temperature changes

The body weight of each mouse was measured before infection (day 0) and on day 4 using a sensitive digital weighing balance to observe whether the aqueous extract prevented weight loss for 4-day suppressive test. In addition, rectal temperature was also measured by a digital thermometer before infection and 4 h after infection and then daily to check whether the aqueous extract prevents the reduction in rectal temperature. For curative test, body weight and temperature was measured before infection and from day 3–7 after infection to see the effect of aqueous extract on these parameters.

3.5. Statistical analysis

The data was expressed as mean ± standard error of the mean. Means of all parameters among groups and within a group were compared using one-way ANOVA followed by Tuckey's post hoc multiple comparison test. In the Rane's test, two-way ANOVA was also used to analyze the development of parasitemia across days of treatment. P-values < 0.05 were considered statistically significant. SPSS Version 23 Software was used for statistical analysis.

4. RESULTS

4.1 Effect of aqueous extract in the four-day suppressive test

The aqueous root extract of *Euclea divinorum* in the 4-day suppressive test showed dose dependent reduction in parasitemia ($p<0.001$ in all case) compared to the negative control. The percentage parasitemia suppression of the extract at 100 mg/kg, 200 mg/kg and 400 mg/kg was 33.49, 47.46 and 62.41, respectively (Table 1). When comparing with positive control (standard drug), the aqueous extract had lower parasitemia suppression at all doses ($p<0.001$). In addition, the aqueous extract exhibited significant ($p<0.001$) increment in survival time at 200 mg/kg and 400mg/kg as compared to negative control but it was lower than the standard drug. Both parasitemia suppression and survival date was significantly higher at 400mg/kg compared with the lower dose and middle dose but lower than positive control.

Table 1: Parasitemia and survival time of infected mice treated with aqueous root extract of *Euclea divinorum* in the 4-day suppressive test

Animal group	Parasitemia level	% suppression	Survival time
CON	34.58±0.71	-	7.5±0.76
CQ 25mg/kg	0.00±0.00	100.00 ± 0.00^{a3}	29.33 ± 0.33^{a3}
100mg/kg	23±0.73	33.49 ± 2.03^{a3b3d1e3}	9.5 ± 0.76^{b3d2e3}
200mg/kg	18.17±1.82	47.46 ± 5.044^{a3b3e3}	13.33 ± 0.88^{a3b3e1}
400mg/kg	10.33±0.67	62.41 ± 1.85^{a3b3}	17.67 ± 0.677^{a3b3}

Data are expressed as mean ± SEM; n = 6; a, compared to negative control; b, to CQ25 mg/kg; c, to 100 mg/kg; d, to 200 mg/kg; e, to 400 mg/kg; 1, $p < 0.05$; 2, $p < 0.01$; 3, $p < 0.001$; CON, control; CQ, chloroquine.

Considering the body weight, the extract significantly prevented weight loss at all dose ($p<0.001$ for 200 mg/kg and 400 mg/kg but $p<0.05$ for 100 mg/kg) compared to negative control. Even though there was no statistically significance difference between 100 mg/kg and 200 mg/kg treated group in preventing weight loss, there was significant difference in between higher dose and lower dose treated group ($p<0.05$) (Table 2). However, the effect of the extract in prevention of weight reduction was less ($p<0.05$) in lower dose as compared to positive control.

The higher and middle dose extract treated groups significantly ($p<0.01$) prevented the reduction in rectal temperature of infected mice compared to the negative control. The lower dose had no significant detectable difference as compared with the negative control in rectal temperature stabilization but the difference was significant ($p<0.01$) compared to the positive control (Table 2).

Analysis of the PCV revealed that the higher and middle dose treated groups showed statistically significant effect ($p<0.001$) in attenuating PCV decline compared to the negative control. However, the effect in prevention of PCV reduction was less ($p<0.05$) in lower dose treated compared to higher dose and standard treated groups (Table2).

Table 2: Effect of aqueous root extract of *Euclea divinorum* on body weight, temperature and packed cell volume of *P. berghei* infected mice in the 4-day suppressive test

	Body weight			Rectal temperature			Packed cell volume		
Groups	D0	D4	%change	D0	D4	%change	D0	D4	%change
CON	28.54±0.97	26.44±1.15	-7.34	36.67±0.18	35.55±0.21	-3.05	53.29±1.34	50.07±1.37	-6.04
CQ25mg/kg	29.86±1.04	31.06±0.85	4.02^{a3}	36.68±0.27	37.12±0.19	1.20^{a3}	50.83±1.65	50.72±1.59	-0.22^{a3}
100mg/kg	28.86±1.76	28.12±1.85	-2.56^{a1b1e1}	36.60±0.18	35.97±0.51	-1.72^{b2}	56.02±3.18	53.86±3.41	-3.86^{b2e1}
200mg/kg	29.24±2.62	29.58±2.70	1.16^{a3}	37.03±0.26	37.10±0.25	0.19^{a2}	58.02±1.63	56.83±1.66	-2.05^{a3}
400mg/kg	29.43±1.67	30.65±1.65	4.15^{a3}	37.08±0.13	37.28±0.87	0.54^{a2}	53.73±1.07	53.03±1.34	-1.3^{a3}

Data are expressed as mean ±SEM; n=6; a, compared to negative control; b, to CQ25 mg/kg; c, to 100 mg/kg; d, to 200 mg/kg; e, to 400 mg/kg; 1, $p<0.05$; 2, $p<0.01$; 3, $p<0.001$; CON, control; CQ, chloroquine; D0, pre-treatment value on day 0; D4, post- treatment value on day 4.

4.2 Effect of aqueous extract in the curative test

Throughout the course of treatment, there was a gradual decline in parasaitemia level at all doses of the extract and standard drug as compared to the negative control. Only the positive control decreased the parasitemia level to undetectable level at day 7. In addition, two way repeated measures ANOVA analysis of parasitemia across the course of treatment showed significant ($p<0.001$) difference in parasite development. The percentage suppression of the aqueous root extract in Rane's test at 100 mg/kg, 200 mg/kg and 400 mg/kg was 26.4%, 41%, and 57.79%, ($p<0.001$), respectively compared to the negative control. However, all test doses had lower ($p<0.001$) suppression potential compared to the positive control. Besides, there were also significantly different ($p<0.001$ in all dose) effect in parasitemia suppression when comparison was made among the different doses of the extract suggesting a dose-dependent suppression effect of the extract (Table 3).

All doses of the extract prolonged the mean survival time significantly ($p<0.001$) as compared to the negative control but lower than the standard drug. Additionally, there was significant difference ($p<0.05$) among different doses of the extract in prolonging survival time (Table 3).

Table 3: Parasitemia, percentage suppression survival time of infected mice treated with aqueous extract of root of *Euclea divinorum* in the Rane'se test.

Groups	D3	D4	D5	D6	D7	%suppression	Survival date
CON	20.80±0.56	22.63±0.73	26.09±0.42	33.12±0.33	38.09±0.30	-	8.5±0.22
CQ25mg/kg	19.37±0.89	11.09±0.75	4.93±0.64	1.32±0.37	0.00±0.00	100 ± 0.00^{a3}	28 ± 0.58^{a3}
100mg/kg	19.12±0.72	22.05±0.77	24.35±0.42	26.26±0.36	28.03±0.36	26.4 ± 0.94^{a3b3d3e3}	11.83 ± 0.46^{a3b3d1e3}
200mg/kg	21.32±0.47	21.70±0.40	21.99±0.45	22.28±0.41	22.47±0.52	41 ± 1.36^{a3b3e3}	14 ± 0.63^{a3b3e3}
400mg/kg	19.65±0.44	19.40±0.28	19.02±0.25	18.81±0.26	18.36±0.43	51.79 ± 1.13^{a3b3}	16 ± 0.36^{a3b3}

Data are expressed as mean ± SEM; n = 6; a, compared to negative control; b, to CQ 25 mg/kg; c, to 100 mg/kg; d, to 200 mg/kg; e, to 400 mg/kg; 1, $p < 0.05$; 2, $p < 0.01$; 3, $p < 0.001$; D/W, distilled water; CQ, chloroquine

In Rane's test, the middle and the higher doses prevented weight loss compared to the negative control ($p<0.01$) but no significant detectable change was observed in lower dose. Likewise, there were no significant changes among the test doses as well as between all test doses of the extract positive control in preventing weight reduction

All doses of the extract significantly attenuated the reduction in rectal temperature ($p<0.01$ for 200 mg/kg and 400 mg/kg, $p<0.05$ for 100 mg/kg). However, there was no statistically significant difference among all test doses of the extract as well as between the extract and standard drug in preventing rectal temperature drops.

As shown in Table 4, all test doses of the extract significantly ($p<0.001$) prevented PCV reduction as compared to the negative control. The extract at a higher dose (400mg/kg) halted PCV dropping significantly ($p<0.05$) compared to lower dose (100mg/kg). Similarly the standard drug significantly ($p<0.01$) prevented PCV decline compared to lower dose treated group.

Table 4: Effect of aqueous root extract of *Euclea divinorum* on body weight, temperature and packed cell volume of *P. berghei* infected mice in the Rane's test

	Body weight			Rectal temperature			Packed cell volume		
Groups	D3	D7	%change	D3	D7	%change	D3	D7	%change
CON	29.59±1.46	26.50±1.65	-10.44	36.00±0.36	34.92±0.31	-3.00	53.04±1.69	47.99±.1.89	-9.52
CQ25mg/kg	30.18±1.41	29.67±1.55	-1.69^{a3}	35.43±0.54	35.78±0.25	0.99^{a2}	51.14±1.62	51.23±1.52	0.18^{a3}
100mg/kg	28.17±1.63	26.33±1.66	-6.53	36.67±0.23	36.53±0.19	-0.38^{a1}	52.71±2.66	50. ±842.5	-3.55^{a3b2e1}
200mg/kg	27.66±0.45	26.58±0.44	-3.90^{a2}	36.33±0.38	36.53±0.38	0.55^{a2}	53.99±2.95	53.01±2.94	-1.82^{a3}
400mg/kg	30.02±1.40	29.12±1.20	-2.99^{a2}	36.62±0.28	36.88±0.23	0.71^{a2}	53.08±3.26	52.67±3.27	-0.77^{a3}

Data are expressed as mean ±SEM; n=6; a, compared to negative control; b, to CQ25 mg/kg; c, to 100 mg/kg; d, to 200 mg/kg; e, to 400 mg/kg; 1, $p<0.05$; 2,; $p<0.01$; 3, $p<0.001$; CON, control; CQ, chloroquine ;D3, pre-treatment value on day 0; D7, post- treatment value on day 4.

4.3. Effect of aqueous extract in the repository test

Although the overall parasitemia suppression effect was much lower than the positive control in repository test, the entire test dose resulted in a significant chemo-prophylactic effect ($p<0.001$ in all dose) in a dose-dependent manner as compared to negative control.

All treatment group increased the mean survival time significantly ($p<0.001$ in all dose) compared to negative control but lower than the standard drug. There were also significant changes in survival time between treated groups as shown in Table 5.

Table 5: Parasitemia and survival time of infected mice treated with aqueous root extract of *Euclea divinorum* in the repository test

Animal group	Parasitemial level	% suppression	Survival date
CON	23.01±0.41	-	5.5
CQ 25mg/kg	6.33±0.23	72.5 ± 1.15^{a3}	16.17^{a3}
100mg/kg	19.78±0.28	18.4 ± 1.74^{a3b3d3e3}	$8.17^{a3b3d1e3}$
200mg/kg	15.14±0.22	31.4 ± 1.52^{a3b3e3}	9.67^{a3b3e3}
400mg/kg	12.61±0.43	42.13 ± 1.04^{a3b3}	11.83^{a3b3}

Data are expressed as mean ± SEM; n = 6; a, compared to negative control; b, to CQ25 mg/kg; c, to 100 mg/kg; d, to 200 mg/kg; e, to 400 mg/kg; 1, $p < 0.05$; 2, $p < 0.01$; 3, $p < 0.001$; CON, control; CQ, chloroquine.

The middle and the higher dose treated group significantly ($p<0.01$) prevented weight loss as compared to negative control. Indeed, there was no statistically significant difference observed among these doses and the positive control in preventing weight reduction. However, the prevention of weight loss brought about by the standard was significantly higher ($p<0.05$) than that of 100 mg/kg of the extract (Table 6).

Only the higher dose treated group attenuated temperature drop significantly ($p<0.05$) as compared to the negative of control. Although the lower and middle dose were unable to prevent the drop in temperature compared to the control, no apparent difference was observed when compared to the higher dose as well as the positive control (Table 6).

As regards to PCV, the same pattern observed with that of the rectal temperature was observed except for higher dose treated group significantly ($p<0.05$) halted PCV decline as compared to the negative control. The lower dose treated group had statistically significance ($p<0.01$) lower potential in preventing PCV reduction compared to positive control. Besides, there was no significantly detectable change between negative control and lower dose as well as middle dose treated groups in preventing PCV reduction.

Table 6: Effect of aqueous root extract of *Euclea divinorum* on body weight, temperature and packed cell volume of *P. berghei* infected mice in the repository test

Groups	Body weight			Rectal temperature			Packed cell volume		
	D0	D3	%change	D0	D3	%change	D0	D3	%change
CON	24.96±0.83	23.31±1.20	-6.61	35.6±0.41	34.67±0.43	-2.61	54.45±2.04	52.87±1.98	-2.9
CQ25mg/kg	24.34±1.12	24.8±1.21	1.89^{a3}	35.7±0.40	35.87±0.25	0.47^{a2}	55.84±3.35	56.45±3.10	1.09^{a2}
100mg/kg	24.20±0.88	23.30±0.76	-3.72^{b1}	36.0±0.35	35.42±0.19	-1.16	55.14±0.86	54.03±1.10	-2.01^{b2}
200mg/kg	29.37±1.07	29.35±1.27	-0.07^{a2}	36.03±0.29	35.67±0.28	-1.00	51.69±2.10	51.28±2.01	-0.79
400mg/kg	27.17±1.13	27.32±1.12	0.56^{a2}	36.17±0.39	36.13±0.47	-0.11^{a1}	54.24±1.02	54.13±0.87	-0.20^{a1}

Data are expressed as mean ±SEM; n=6; a, compared to negative control; b, to CQ25 mg/kg; c, to 100 mg/kg; d, to 200 mg/kg; e, to 400 mg/kg; 1, $p<0.05$; 2, $p<0.01$; 3, $p<0.001$; CON, control; CQ, chloroquine; D0, pre-inoculation value on day 0; D3, post- treatment value on day 3 after inoculation.

5. DISCUSSION

In this study, an *in vivo* model, which accounts for the involvement of immune system to combat infection and possible pro-drug effect, was employed. The rodent parasite, *P. berghei* ANKA was an appropriate parasite for studying the *in vivo* antimalarial activity of plant extract (Waako *et al.*, 2005). Chloroquine was used as the standard treatment drug during this study since *P. berghei* is sensitive to it (Pierrot *et al.*, 2003). The most reliable parameters in *in vivo* antimalarial models were percentage parasitemia suppression and survival time (Peter, 1998).

The aqueous extract of *E. divinorum* was investigated for its antimalarial activity using the 4-day suppressive, Rane's and repository tests. These tests were employed to evaluate schizontocidal activity against early infection, curative ability against established infection and prophylactic activity of the extract against residual infection in *P. berghei* infected mice, respectively.

The standard test commonly used for antimalarial screening of plant extract is the 4-day suppressive test (Peters, 1965). In the 4-day suppressive test, the aqueous crude extract reduced the level of parasitemia in a dose-dependent manner, suggesting that the plant extract potentially mitigated early malaria infection. The highest parasite suppression (62.41%) was recorded in 400 mg/kg extract treated groups. The extract prolonged mean survival time in the three tests but lower than the standard drug. As a result, the survival time of standard drug treated group was higher than extract treated group. This may be due to the crude nature of the extract, as it contains a mixture of compounds, compared to the pure compound used as a standard. This finding is in line with the previous report on *Olea europaea* (Misganaw *et al.*, 2019). The chemo-suppressive potential of the extract was also lower than the standard drug. The middle and higher doses (200 mg/kg and 400 mg/kg) of the aqueous extract treated groups showed 47.46%, 62.41% chemo-suppression, respectively, as compared to negative control in the 4-day suppressive test. The suppression effect of the extract in the current study is comparable with other reports on *Brassica nigra* (Muluye *et al*., 2015) and *Phytolacca dodecandra* (Adinew, 2014), which showed 50%, 53.13 % and 50.93%, 55.24 % at a dose of 200 mg/kg and 400 mg/kg, respectively.

Although there was significant parasitemia suppression in Rane's test at all doses' showing its curative potential, chemo-suppressive potential of the aqueous extract was lower than the 4-day suppressive test, indicating that the extract had greater effect in early infection than established infection, in which the parasite was exponentially growing. This was possibly related to the metabolic process of *E. divinorum* extract by mice and reduction of its concentration in the body associated with rapid multiplication of the parasite in established infection. This finding is in agreement with other report on *Artemisia turanica* (Taherkhani *et al.*, 2013), in which the crude extract had greater schizontocidal activity in early infection than established one.

Even if all treated groups in the repository test compared with negative control had significantly suppress parasitemia, the percentage parasitemia suppression and survival time were lower than the other two tests. Metabolic inactivation of active components in extract before inoculation of parasite may hinder suppression potential in repository test (Alli *et al.*, 2011). A rapid metabolism of administered extract (before inoculation) to inactive products may be due to the high parasite count in the repository test (Dahanukar *et al*., 2009). This result is in agreement with other report on *Olea europaea* (Misganaw *et al*., 2019) and *Morinda lucida* (Unekwuojo, 2011), which showed that the extract chemo-suppression and survival time in repository test were lower that Rane's test and 4-day suppressive test.

The general features of malaria-infected mice are: anemia, body weight loss and body temperature reduction (Langhorne *et al*., 2002). Some of these clinical features are linked with the level of parasitemia directly (Cross *et al*., 1998). The plant extract having antimalarial activity in traditional claim report were expected to control some of these manifestations. Ideally, there is a reduction in body weight, temperature and PCV in *P. berghei* infected mice due to rapid increase in parasitemia level. The aqueous extract of *E. divinorum* prevented weight loss, temperature drop and PCV decline in *P. berghei* infected mice. This finding is in agreement with other studies (Misganaw *et al*., 2020)

Body weight loss manifestation of infected mice was due to appetite suppression, disturbed metabolism and hypoglycemic potential of the parasite. The aqueous root extract of *E. divinorum* prevented the weight loss in higher and middle dose treated group as compared to the negative

control in all three tests. This is probably due to reduction in the level of parasitemia by the extract in infected mice to continue metabolizing and growing without serious hindrances. The result of the present study on body weight is in agreement with other studies (Melese *et al*., 2015). However, the finding is not in agreement with previous study on other plants (Mengiste *et al.*, 2012). This inconsistency may be due to variation in the concentration of appetite suppressing components and nutrient contents of the plants.

Ideally, there is a decrease in metabolic rate and rectal temperature in *P. berghei* infected mice. The extract having active compound could ameliorate a decrease in rectal temperature. The temperature stabilization effect of the extract was statistically significant as compared to the negative control in both the 4-day suppressive and curative tests but not in the repository test. This could probably be related to rapid inactivation of the active component in the extract due to increment in the level of parasitemia in the repository test. The presence of secondary metabolites which have a tendency to stabilize temperature may contribute for the prevention in parasite induced temperature decline (Mengiste *et al*.,2012). This result in line with other study plants conducted previously (Misganaw *et al*., 2019).

There is a parasite induced PCV reduction to a haematocrit of 43-44% within 48 h post-infection by rodent malaria (Taylor and Hurd, 2001). Analysis of PCV in the entire three tests has a great importance to evaluate the effectiveness of the extract in preventing hemolysis in *P. berghei* infected mice. In the curative and 4-day suppressive test, there was a significant preventive role of the extract to PCV drop at all doses but in repository test only the higher dose treated group significantly prevented PCV reduction as compared to the negative control. The finding showed that the extract could ameliorate anemia by halting parasite induced RBC destruction. The presence of some secondary bioactive metabolites like tannins and flavonoid having antioxidant activity in the extract may play an important role for preventing RBC from oxidative stress. This is in agreement with other studies (Mengiste *et al*., 2012).

Cardiac glycosides, saponin, terpenoids, steroids, flavonoids and tannins have been reported in aqueous root extract of *E.divinorum* (Woldemedhin *et al*., 2017) and these secondary metabolites could be responsible for the antimalarial activity, as terpenoids (Okokon and Nwafor, 2009) and

tannins (Asres *et al.*, 2001) showed antimalarial activities. The proposed mechanism of this secondary metabolite includes: inhibiting the growth and multiplication of the parasite, block entry of essential nutrients into the RBCs, cytotoxic effect on the parasites, stimulating natural and adaptive defense mechanisms of the host (Misganaw *et al*., 2019). The current finding is concordant with others studies (Ayoola *et al.*, 2008), which suggest that the presence of tannin and flavonoid can counteract the oxidative damage induced by the malaria parasite due to their antioxidant activity which prolonged the survival date of infected mice. As a result the antimalarial activity of the aqueous root extract of *E. divinorum* may be due to the individual or combined effect of the aforementioned bioactive secondary metabolites.

If the reduction in parasitemia is ≥ 30%, then the agent is considered as active in standard screening studies (Krettli *et al*., 2009). As the extract meets this criterion, particularly in the middle and higher dose, it can be considered as an active agent worthy of further investigation. Depending on percent parasite suppression, an extract can be classified as having good, moderate, very good *in vivo* antimalarial activity, if percentage suppression is equal to or greater than 50% at a dose of 500, 250 and 100 mg/kg, respectively (Deharo *et al*., 2001). Based on this classification, the aqueous root extract of *E. divinorum* exhibited good antimalarial activity, with a dose-dependent inhibition against *P. berghei* infection in mice. The current finding is in agreement with other report on *Brassica nigra* (L.) Koch which showed good antimalarial activity based on the above classification (Muluye *et al*., 2015).

6. CONCLUSION

The current study showed that aqueous root extract of *E. divinorum* possessed varying degree of antimalarial activity in all the three tests, with greater parasitemia suppression observed in the 4-day suppressive test. The extract produced higher parasitemia chemo-suppression and longer survival time in early infections followed by established and then by residual infection. Additionally, the findings also provide an evidence to support the *in vitro* study as well as the traditional claims made by the traditional medicine practitioners.

7. RECOMMENDATION

Since the plant *E. divinorum* has good antimalarial activity, further investigations should be conducted:

- To isolate and identify the active component responsible for the antimalarial activity.
- To know the mechanism of action of the plant extract for antimalarial activity.
- To determine the sub-acute, sub-chronic and chronic toxicities profile of the extract

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