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Review on Malaria and Antimalarial Activity of Vernonia Amygdalina in Ethiopia: A Review Article

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Abstract
Malaria is a mosquito borne infectious disease caused by a protozoan of the genus Plasmodium. Humans are mainly infected by *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Around 44% of world's population is at risk from malaria. Malaria is one of the leading causes of morbidity and mortality in Ethiopia. The clinical features of malaria vary. The most characteristic symptom are fever, headache, lassitude, loss of appetite, muscle pain and chills, resulting in uncontrollable shivering with teeth chattering. Diagnosis of malaria is achieved by light microscopy, rapid diagnostic tests, polymerase chain reaction. Management of malaria includes general measures to be taken to save life of the person and prevention of recrudescence using drugs and other supportive measures. Parasite resistance to antimalarial medicines is a major threat to achieving malaria control and eventual elimination. The most important problem associated with the management of malaria are resistant to or is developing resistance to the most widely available, affordable and safest first line treatments. Prevention of malaria includes vector control to disrupt transmission from mosquito to human, prevention of infection and treatment after infection. *V. amygdalina* commonly called bitter leaf in English and 'Girawa' in Amharic. *V. amygdalina* has antimalarial properties and the aqueous extract of *Vernonia amygdalina* leaves exhibit antimalarial activity on *P.falcifarum, P.vivax, P. ovale* and *P. malariae*. Malaria is one of the life-threatening diseases. Moreover, *Vernonia amygdalina* can be used for the treatment of malaria in rural communities.
Keywords: Antimalarial activity, Ethiopia, Malaria, *V. amygdalina*.
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1. INTRODUCTION

Malaria is a mosquito borne infections disease caused by a protozoan of the genus Plasmodium (Odeh and Usman, 2014).Around 44% of world's population is at risk from malaria. An estimated 3.2 billion people were at risk of being infected with malaria and developing disease in 2013. Of this, 1.2 billion people are at high risk (>1 case per 1000 population) of malaria. Over half of all the countries in the world are affected by malaria (RBMP, 2015).

It is widespread in tropical Africa and Sub tropical regions including part of America, Asia and Africa (Odeh and Usman, 2014). In 2013, worldwide, there were an estimated 198 million cases of malaria. Most of these cases (82%) were in the WHO African Region, followed by the South-East Asia Region (12%) and the Eastern Mediterranean Region (5%). About 8% of estimated cases globally are due to *P. vivax*, although the proportion outside the African continent is 47% (WHO, 2014).

Of the estimated 584,000 malaria deaths that occurred worldwide in 2013. 528,000 of these deaths, or 90%, were in the African Region, with 7% in the South-East Asia Region, and 2% were in Eastern Mediterranean Region. About 0.27 % people die every day from malaria; more than 0.24% of those people are in Africa. About 453,000 malaria deaths were estimated to occur in children under 5 years of age, or 78% of the global total. Over 1,200 children die every day from malaria, which is equivalent to 50 children dying every hour (RBMP, 2015).

An estimated 437,000 of deaths occurred in children under 5 years of age in the WHO Africa Region, accounting for 83% of the total malaria deaths in the Africa Region, and 96% of total global under 5 malaria deaths. About 80% of malaria deaths in 2013 are estimated to occur in just 16 countries: Nigeria, Democratic Republic of the Congo, India, Angola, United Republic of Tanzania, Uganda, Ghana, Niger, Chad, Mozambique, Burkina Faso, Ethiopia, Côte d'Ivoire, Mali, Guinea, and Cameroon (RBMP, 2015).

The parasites are spread to people through the bites of infected Anopheles mosquitoes, called "malaria vectors"(Singh, 2011).There are four parasite species that cause malaria in humans which are *P.falciparum*, *P.vivax*, *P. malariae* and *P. ovale., P.falciparum* and *P. vivax* are the most common species clinically but *P. falciparum* is the most deadly leading to many fatal complications including cerebral malaria (Singh, 2011).

Malaria is one of the leading causes of morbidity and mortality in Ethiopia. An estimated 55.7 million people (68% of the population) are at risk for malaria and approximately 80% of the 736 districts in Ethiopia are considered "malarious". Protective immunity in Ethiopian populations is relatively low due to unstable transmission and, unlike large parts of sub-Saharan Africa; all age groups are at risk of infection and disease. *P. falciparum* accounts for 65-75% of infections, while *P. vivax* accounts for 25-35%. *P. ovale* and *P. malariae* are rare (The Carter Center's (TCC) Malaria Control Program (MCP), 2013).

The World Health Organization (WHO) estimates that approximately 80% of the world's inhabitants rely on

traditional or herbal medicines for their primary health care and plants have long formed the basis of sophisticated traditional medicine systems and purportedly provide excellent leads for new drug developments (Pravi, 2006; Akinjogunla *et al*., 2009). However, the increasing problems of multi-drug resistant (MDR) is of great concern to both the clinicians and pharmaceutical industries and this has made it significant to search for newer drugs that are highly effective, affordable, acceptable and available (Akinjogunla *et al*.. 2010).

In developing countries where modern medicine is expensive, most of the indigenes people rely on indigenous plants for the treatment of various ailments (Qureshi *et al*., 2009). Traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly spreading in ten industrilized countries (Kassaye *et al.,* 2006).

Phytochemicals differ from phytonutrients in that they are not a necessity for normal metabolism and absence will not result in deficiency disease. Phytochemicals are not required for the functioning of the body, but they are of benefit on health and play an active role in the treatment of diseases (Audu *et al*., 2012). Therefore, the objective of this review paper is to insight the burden of malaria and antimalarial activity of *V.amygdalina*.

2. LITERATURE REVIEW

2.1. Global epidemiology of malaria

Epidemiology of malaria is important for clear understanding of the distribution and transmission pattern of the disease. This is also relevant for the control of malaria at large. Several epidemiological studies have shown the degree, and intensity of transmission is varied within continents (Snow, 2005; Guerra *et al*., 2006).

An estimated 3.2 billion people were at risk of being infected with malaria and developing disease in 2013. Of this, 1.2 billion people are at high risk (>1 case per 1000 population) of malaria. In 2013, there were 97 countries and territories with ongoing malaria transmission, and 6 countries in the prevention of reintroduction phase, making a total of 103 countries and territories, 196 internationally recognized countries affected by malaria. There were 198 million cases of malaria worldwide in 2013, with 82% of these cases occurring in Africa. In 2013,584,000 people died from malaria worldwide, with 90% of these deaths occurring in Africa (WHO, 2014).

Malaria occurs mostly in poor tropical and subtropical areas of the world. In many of the countries affected by malaria, it is a leading cause of illness and death. In areas with high transmission, the most vulnerable groups are young children, who have not developed immunity to malaria yet and pregnant women, whose immunity has been decreased due to pregnancy (CDC, 2015).

P. vivax is regarded as the most cosmopolitan of the human malaria and the public health burden more significant that it causes severe morbidity and death. However, *P. falciparum* is the most serious intimidation to the world at a very scale. It causes more than 90% of death due to malaria. Four thousand years on, *P. falciparum* remains widespread in Africa. This may be due to optimal environmental conditions for Anopheline mosquito vectors, amid sustained poverty (Snow, 2015). The fluctuating pattern of malaria epidemiology throughout the world could be characteristics of complex interaction between environment, vector, the human host and the parasite species.

2.2. Epidemiology of malaria in Ethiopia

The 2011 Malaria Indicator Survey (MIS) shows that 1.3% of all age groups were positive for malaria using microscopy and 4.5% were positive for malaria using RDTs below 2,000 meters. *P. falciparum* constituted 77% of these infections (Malaria Operational Plan FY, 2014). According to the Federal Ministry of Health, malaria was the leading cause of outpatient visits and health facility admissions in 2010/2011, accounting for 15% of reported outpatient visits and nearly 15% of admissions. Malaria also was among the ten leading causes of inpatient deaths among children less than five years of age (Malaria Operational Plan Fiscal Year, 2014).

About 75% of the geographic area of the country has significant malaria transmission risk (defined as areas <2,000 m), with about 68% of the country's total population living in these areas. The FMOH estimates that there are about 12 million suspected malaria cases each year. The FMOH reported a total of 3,384,589 malaria cases from July 2011-June 2012, with1, 793, 832 (53.0%) of these laboratory confirmed, with 1,061,242 (59.2%) *P. falciparum* and 732,590 (40.8%) *P.vivax*. Ethiopia reported 936 malaria deaths in 2011, according to the 2012 World Malaria Report (Malaria Operational Plan FY, 2014).

2.3. Etiology

Malaria, the disease caused by protozoan parasites of the genus Plasmodium (Jennifer *et al*., 2005).All malaria is transmitted by female mosquitoes of the genus Anopheles. Humans are mainly infected by four species of Plasmodium: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*, although human infections with the monkey malaria parasite, *P. knowlesi* have also been reported recently in the forested regions of Southeast Asia (Kantele and Jokiranta,2011). The majority of all human malaria cases are caused by*P. falciparum* and *P. vivax* (Stainesl and Krishna, 2012).

Anopheles gambiae the most aggressive among the more than 60 mosquito species that transmit malaria to

people in sub-Saharan Africa and bears partial responsibility for millions of human deaths per year (Vizioli *et al.*, 2000; Dunavan, 2005 and Legoff *et al*., 2006).*P. falciparum* and *P. vivax* are the two dominant parasite species with relative frequency of 60% and 40%, respectively in Ethiopia (Gezahegn, 2004).

2.4. Life cycle

The malaria parasite has a complex, multistage life cycle occurring within two living beings, the vector mosquitoes and the vertebrate hosts (Fakhreldin *et al*., 2003; Brian *et al*., 2008).The parasite passes through several stages of development such as the sporozoites, merozoites, trophozoites and gametocytes (sexual stages) and all these stages have their own unique shapes and structures and protein complements. The surface proteins and metabolic pathways keep changing during these different stages that help the parasite evade the immune clearance, while also creating problems for the development of drugs and vaccines (Fakhreldin *et al*., 2003).

The sporozoite form of the parasite is inoculated into humans when bitten by an infected female Anopholes mosquito (Figure 1). The parasites go through several host cells by breaching their plasma membrane before infecting a final hepatocyte (Dunavan, 2005). Sporozoites rapidly enter the liver cells where they multiply to form thousands of merozoites. These then enter the blood stream where they invade red blood cells and multiply to form new merozoites. Infected red blood cells burst, releasing merozoites that infect new red blood cells. This is referred to as the asexual blood stage, the stage of the plasmodial life cycle that causes the clinical signs and symptoms of malaria. Some merozoites that invade the red blood cells develop into gametocytes, the sexual stages of the parasite. Gametocytes are ingested by the mosquito when it takes a blood meal. In the mosquito gut, the gametocytes develop into gametes and fuse to form a zygote. After fertilization, the zygote transforms into a motile ookinete, which penetrates the mosquito stomach wall and becomes an oocyst. The oocyst divides to produce sporozoites, which move into the salivary glands, from where another human can be infected when the mosquito takes a blood meal from (Stainesl and Krishna, 2012).

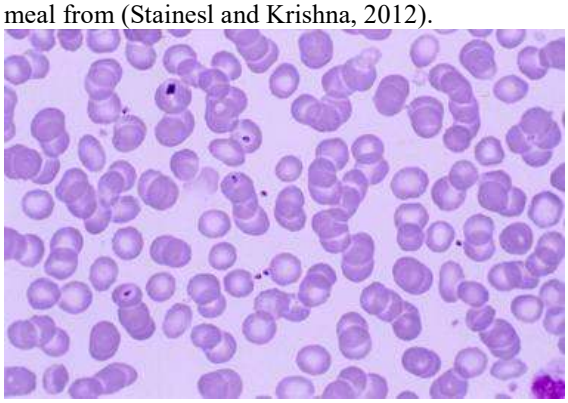

Figure 1.Malarial merozoites in the peripheral blood.

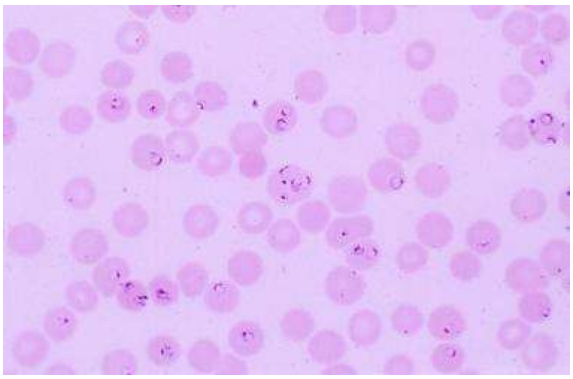

Figure 2. Trophozoite form of the malarial parasite within peripheral erythrocytes (Thomas, 2018).

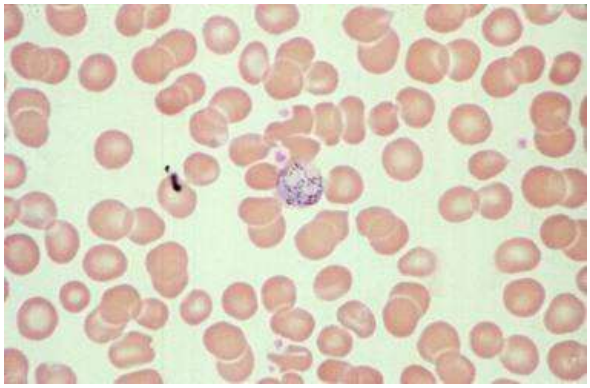

Figure 3. A mature schizont within an erythrocyte (Thomas, 2018).

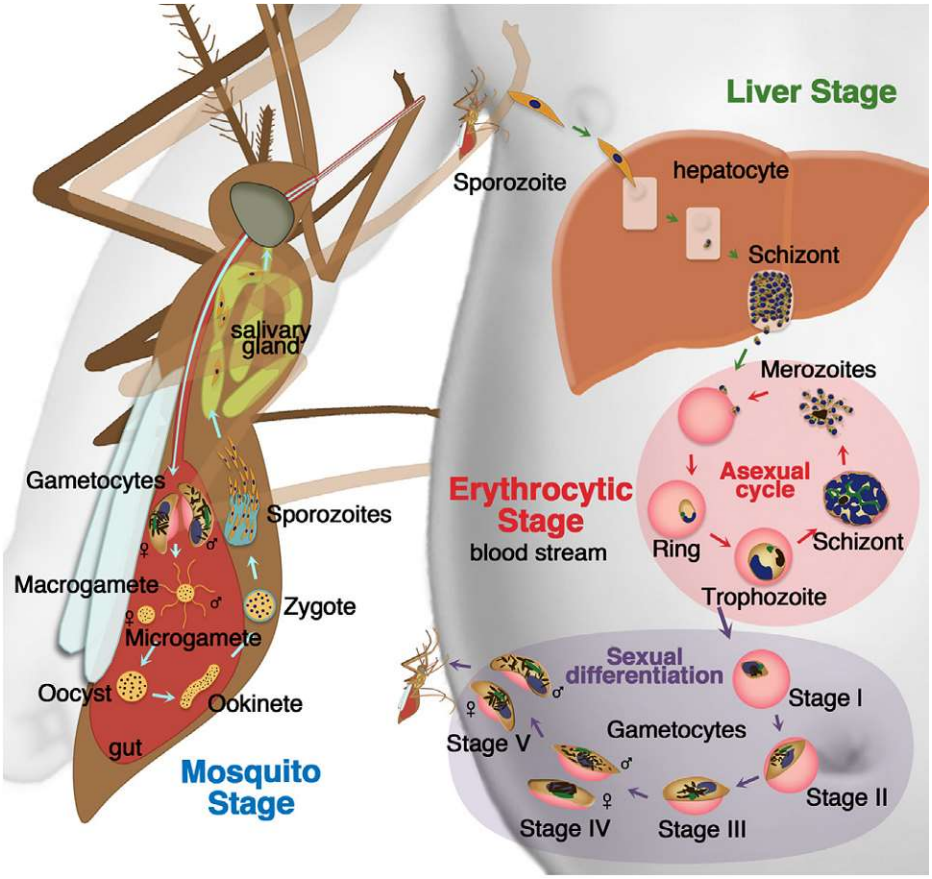

Figure 4. Plasmodium life cycle (Biamonte *et al*., 2013).

2.5. Pathogenesis

Proliferation of the parasite within the host's erythrocyte takes place using hemoglobin as predominant source of nutrition. The malaria parasite digests hemoglobin within the digestive vacuole through the sequential metabolic processes involving multiple proteases (Tekwani and Walker, 2005).Malaria pathogenesis could be explained by *Plasmodium falciparum* erythrocyte membrane protein mediated sequestration of parasitized erythrocytes. This protein helps the parasite infected RBC adhere to blood elements including non infected erythrocytes, leukocytes, and wall of endothelial cells of microcirculation. These binding events enable parasitized erythrocytes to sequester and avoid clearance by the spleen and also contribute to disease by causing microvascular inflammation and obstruction (Rogerson *et al*., 2004; Fairhurst and Wellems, 2006). Cytoadherance of IRBCs on human dermal microvascular endothelial cells (HDMECs) is responsible for pathogenesis of malaria. IRBCs were observed to tether, roll, and adhere on resting HDMECs, rossets will be formed causing clogging of capillaries. IRBCs interact synergistically with multiple adhesion molecules on vascular endothelium (Bryan *et al*., 2000).

Different factors participate in the neuro-pathogenesis of malaria. They seem to include abnormally high production of cell-derived cytokines such as tumor necrosis factor (TNF) and IFN- induced by infected erythrocytes. These cytokines may play an important role in causing certain pathological changes, by up-regulating the expression of cell surface markers like ICAM-1 and chondroitin sulfate A, thus leading to the sequestration of infected erythrocytes, leukocytes and monocytes in the cerebral capillaries. The pathogenesis of severe malaria therefore involves a cascading interaction between parasite and red cell membrane products, cytokines and endothelial receptors, leading to inflammation, activation of platelets, hemostasis, a procoagulant state, microcirculatory dysfunction and tissue hypoxia, resulting in various organ dysfunctions manifesting in severe malaria (Henri *et al*., 2006).

2.6. Clinical manifestations

The clinical features of malaria vary. The most characteristic symptom is the occurrence of paroxysm which include fever with temperatures up to 40 - 41ºC at regular intervals –every 48 or 72 hours (tertian or quartan), alternating with good periods of no fever. This is preceded by headache, lassitude, loss of appetite, muscle pain and chills, resulting in uncontrollable shivering with teeth chattering. This is accompanied by thirst, nausea, vomiting. Severe and complicated malaria causing renal failure, hypoglycemia, anemia, pulmonary oedema, shock

and coma can have fatal consequences, leading to death (Singh, 2011).

Nonimmune people, children and pregnant women who live in endemic regions are at highest risk of complications from malaria. Complications generally involve the central nervous, pulmonary, renal and hematopoietic systems. Hypoglycemia occurs because of parasite consumption of glucose and treatment with quinine. Acidosis is another common metabolic derangement. Severe anemia, acute renal failure, respiratory failure, intravascular hemolysis, coagulopathies, and shock may also develop (Kathryn *et al*., 2004).

One of the most serious complications is cerebral malaria, manifested by altered level of consciousness, focal neurologic findings and seizures (Kathryn *et al*., 2004). Mortality is high (15% to 25%), and survivors may have residual neurologic deficits. Although semi-immune people and those living in endemic regions tend not to experience severe malaria, they may still experience complications from recurrent infections. In children, severe anemia is the most common complication of chronic malaria, with hematocrits approaching 15% (Kathryn *et al*., 2004). The predominant manifestations of severe malaria in African children are cerebral malaria and severe anemia. *P.falciparum* slightly damages blood brain barrier (Gitau and Newton, 2005). Malaria is not only a direct cause of death but also contributes indirectly to death due to respiratory infections, diarrhea and malnutrition by impairing immunity (Kager, 2002).

2.7. Diagnosis

2.7.1. Light microscopy

In addition to providing a diagnosis with a high degree of sensitivity and specificity when performed well, microscopy allows quantification of malaria parasites and identification of the infecting species. It is considered to be the "field standard" against which the sensitivity and specificity of other methods must be assessed. A skilled microscopist is able to detect asexual parasites at densities of fewer than 10 per µl of blood, but under typical field conditions, the limit of sensitivity is approximately 100 parasites per µl (WHO, 2010).

A high degree of suspicion and rapid diagnosisare essential to optimize outcome. Thick and thin peripheral blood smears, stained with Giemsa stain (or, alternatively, Wright's or Field's stains), remain the "gold standard" for routine clinical diagnosis. Malaria smears permit both species identification and quantification (expressed as a percentage of erythrocytes infected or as parasites per microlitre) of parasites but malaria should not be excluded until at least 3 negative blood smears have been obtained within 48 hours (Kathryn et al., 2004).

2.7.2. Rapid diagnostic tests

Rapid diagnostic tests are immunochromatographic tests that detect parasite-specific antigens in a finger-prick blood sample. Some tests detect only one species (*P. falciparum*), others detect one or more of the other species of human malaria parasites (*P. vivax*, *P. malariae* and *P. ovale*). They are available commercially in different formats, e.g. dipsticks, cassettes or cards. Cassettes and cards are easier to use in difficult conditions outside health facilities. Plasmodium lactate dehydrogenase *(pLDH) or pan-specific aldolase.* These antigens have different characteristics, which may affect suitability for use in different situations, and these should be taken into account when developing RDT policy (WHO, 2010).

Current tests are based on the detection of histidine-rich protein 2 (HRP2), which are specific for *P.falciparum*, pan specific or species specific Plasmodium lactate dehydrogenase (pLDH) or pan-specific aldolase. These antigens have different characteristics, which may affect suitability for use in different situations, and these 2should be taken into account when developing RDT policy (WHO, 2010).

The most practical of rapid malaria tests are the rapid antigen detection tests (RDTs), which detect parasite proteins in finger-prick blood samples. RDTs can identify only *P. falciparum* and *P. vivax*. Important shortcomings of RDTs include their inability toquantify parasitemia and suboptimal test performance with low-level parasitemia. Furthermore, some RDTs are unreliable as tests of cure because antigenemia may persist for prolonged periods even after treatment. But their simplicity may make them attractive and useful alternatives to blood smears, particularly in laboratories where expertise in reading blood films is lacking or in centers where malaria is infrequently encountered. Based on clinical studies involving both travelers to and residents of endemic areas, the overall sensitivity and specificity of RDTs for the detection of falciparum malaria are over 90%. However, sensitivity falls dramatically with low level parasitemia, and at present RDTs cannot be used alone to exclude malaria (Kathryn *et al*., 2004).

2.7.3. Polymerase chain reaction (PCR)

Techniques to detect parasite deoxyribonucleic acid (DNA), based on the polymerase chain reaction, are highly sensitive and very useful for detecting mixed infections, in particular at low parasite densities. They are also useful for studies on drug resistance and other specialized epidemiological investigations, but they are not generally available for large-scale field use in malaria endemic areas (WHO, 2010).However, most PCR assays do not have sufficiently rapid turnaround times to be clinically useful; therefore, PCR remains largely an investigational tool (Kathryn *et al*., 2004).

2.8. Treatment of malaria

Management of malaria includes general measures to be taken to save life of the person and prevention of recrudescence using drugs and other supportive measures (Kathryn *et al*., 2004). Treatment of malaria depends on the infecting plasmodia species, the geographic area of acquisition (which affects the likelihood of drug resistance) and severity of infection (Kathryn *et al*., 2004; Sweetman, 2005). Falciparum malaria in nonimmune person is a medical emergency and requires rapid initiation of therapy (even in cases where the species cannot be immediately identified, the patient should be assumed to have drug resistant falciparum malaria until proven (Kathryn *et al*., 2004).

Once diagnosed and confirmed, malaria is treated with one or more drugs that have been licensed to be used as antimalarial agents. The currently available antimalarial drugs are grouped into four classes according to their chemical structure and biological activity: (i) quinoline based antimalarials which include quinine and its derivatives such as chloroquine, amodiaquine, premaquine, and mefloquine (ii) antifolate compounds (pyrimethamine , proguanil, dapsone, and sulfadoxine), (iii) artemisinin and derivatives (artemisinin, artesunate, artemether, arteether, dihydroartemisinin), and (iv) hydroxynapthoquinone (atovaquone) (Na-Bangchangb and Karbwang, 2009).

The first-line treatment of uncomplicated *P. falciparum* malaria is artemether lumefantrine administered 2 times a day for 3 days. For infants less than five kg of body weight and pregnant women, oral quinine 8mg/kg administered 3 times a day for 7 days is the first line treatment (for the treatment of malaria caused by *P.vivax*, *P.malariae*or *P.ovale*, the drug of choice is chloroquine). Inmalaria-free areas and where compliance can be insured, in order to eliminate hypnozoite forms (relapsing stages) of *P.vivax* from the liver and to bring about radical cure, primaquine may be administered daily for 14 days starting after chloroquine treatment is completed. However, in malarious areas where there is a high risk of re-infection, and where the main purpose of treatment is to bring about clinical cure rather than radical cure, administration of primaquine is therefore, not recommended (Gezahegn , 2004).Second-line treatment is oral quinine if condition of the patient permits. Otherwise intravenous (IV) or intramuscular (IM) administration of quinine should be given (Gezahegn, 2004). In treatment of severe malaria, supportive therapy in reducing hyperpyrexia, controlling convulsions, maintaining fluid balance and correcting hypoglycaemia should be done (Sweetman, 2005).

Along with these measures for management of other complications in severe malaria, loading dose of intramuscular or slow intravenous quinine should be used. Maintenance dose should be followed twelve hours after the start of the loading dose until the patient can take oral medication. Artemether-lumefantrine or oral quinine could be administered if intramuscular or slow intravenous quinineis not available (Gezahegn, 2004). Radical treatment and curative treatment comprise main aspects of treatment. Various pharmacological options available for this purpose are chloroquine, mefloquine, quinine, primaquine, pyremethamine, artemisinin derivatives like artesunate, artemether, arteether and amino alcohols like lumefantrine and halofantrine along with tetracycline, doxycyclines and sulfadoxime etc (Bahekar and Kale , 2013).

2.9. Antimalarial resistance

Parasite resistance to antimalarial medicines is a major threat to achieving malaria control and eventual elimination. Antimalarial resistance in *P. Falciparum* parasites results in an enormous public health and economic burden (Stainesl and Krishna, 2012). Antimalarial resistance spreads when parasites are exposed to the selective window of drug concentrations that are sufficient to kill sensitive but not resistant parasites. Drugs with longer terminal elimination half-lives have the advantage of providing a longer post-treatment prophylactic effect, which appears to be important for their action in intermittent preventive therapy (IPT) in high-risk groups such as pregnant women, infants and young children. However, these longacting antimalarials have the disadvantage of residual concentrations inhibiting sensitive parasites far longer than resistant parasites, thus fuelling the spread of resistance. The window of selection is prolonged with an increase in resistance or in the terminal elimination half-life (unless these terminal concentration are too low even to kill sensitive parasites) (Stainesl and Krishna, 2012). Antimalarial resistance spreads because gametocyte carriage and infectivity to mosquitoes is consistently higher in patients infected with drug-resistant compared with drug-sensitive parasites. An increase in gametocyte numbers has been identified as the first indication that an antimalarial is beginning to fail and emphasizes the need for the treatment policy implemented to include drugs that will kill the sexual stages. Combining antimalarials with differing modes of action is expected to reduce the probability of a resistant (mutant) parasite surviving treatment. Despite their mismatched elimination half-lives, ACTs are preferred to other combination therapies given their potential to reduce malaria transmission – due to their rapid clearance of asexual parasites together with their partial gametocidal activity (Stainesl and Krishna, 2012). Some of the selected antimalarial drugs and its mechanisms of resistance described as depicted in table 1.

Table 1. Selected antimalarial drugs and its mechanisms of resistance.

Drug	Mechanism of Resistance
Chloroquine	Decreased intraparasite accumulation of chloroquine. Probably predominantly mediated by mutations in PfCRT, a transmembrane protein in the parasite phagolysosome, resulting in increased efflux of chloroquine from the lysosome (Warhurst, 2001)). Mutations in multidrug resistance (MDR) P-glycoprotein pumps (Pgh) encoded by Pfmdr1and Pfmdr2may also contribute Warhurst, 2001).
Mefloquine, halofantrine, quinine	Mefloquine and halofantrine resistance may be related to amplification of Pfmdr1, increased expression of Pgh1, mutations in pfcrt, and increased efflux of drug (Milhous and Kyle.,1998). The details of quinine resistance have not been established, although quinine and mefloquine resistance often correlate (White, 1998).
Cycloguanil, chlorcycloguanil, pyrimethamine	Resistance mediated through point mutations in dihydrofolate Reductase (dhfr) and dihydrofolate Synthase (dhps) genes, although more efficient use of available folate may also contribute (White, 1998).
Sulfonamides and Sulfones (sulfadoxine, dapsone)	
Atovaquone	Point mutations in mitochondrially encoded cytochrome b gene (Srivastava, 1999).

2.9.1. Determinants of drug resistance and treatment failure

Clinically relevant resistance to antimalarial drugs emerges primarily through increasesin the prevalence of resistance-conferring gene mutations in an environment of selective drug pressure. Parasites survive the presence of drugs through mutation. Mutations occur at random because of replication errors. Many of those mutations are lethal and the parasite will die. Occasionally, a mutation will confer on a parasite a survival advantage when a given drug is present. This parasite, provided it is not cleared by the host's immune system, will proliferate and its progeny carry that mutation. Additional survival advantage could be gained by further mutations. This process generates populations of parasites with different abilities to survive a drug. Over time, the parasite populations with the greatest survival advantage will predominate (Rosenthal, 2001).

The range of naturally occurring drug susceptibilities within a parasite population is a function of the size of the circulating population of parasites (the "biomass" or "parasite burden"); a larger parasite biomass increases the chances for parasite mutation, broadening the distribution of parasite susceptibilities within that population. If a mutation does not compromise other biologic functions or confers a net survival advantage in the presence of a given drug, the possibility exists, provided the parasite carrying that mutation escapes the host's immune defenses, that the parasites with the mutation will be selected for and transmitted (Rosenthal, 2001).

2.9.2. Factors involved in the generation of drug resistance

2.9.2.1. Pharmaco-biological factors

Pharmacological factors that influence the rate of development of resistance include the drug's pharmacokinetic and pharmacodynamic characteristics, as well as the drug's intrinsic propensity to generate resistance. Drugs with a long residence time in the organism and a slow rate of reduction of the parasite "biomass" are more vulnerable to resistance. Equally vulnerable are drugs against which resistance develops through single-point mutations in the target molecule (also referred to as single nucleotide polymorphism (SNP), such as the antifolates and atovaquone. By contrast,the quinolines (chloroquine [CQ], quinine) have enjoyed a longer therapeutic life-span because resistance to these drugs is apparently multigenic-the greater the number of genes involved, the slower resistance will evolve(Rosenthal, 2001).

2.9.2.2. Epidemiological factors

One determinant of resistance development is the self-fertilization that occursbetween male and female gametocytes when they are picked up by a mosquito during ablood meal. Such reassortment determines the mutations that are carried by the ensuinggeneration of parasites that are transmitted to the next individual(s). In this process, theintensity of transmission plays a critical, yet undetermined, role, which is principallyrelated to the number of parasite clones carried by each individual (Rosenthal, 2001).

The intensity of transmission may influence resistance indirectly, via mechanisms such as immunity and drug use. Intense, continuous transmission favors the early acquisition of immunity, at the cost of increased mortality. This should, in theory, confine use of antimalarial drugs-thus limiting the ensuing selection pressure on parasites-to the younger age groups (primarily, children under 5 yr). In practice, this is not always true, and antimalarials are often used by all age groups to treat fever. Additionally,high rates of transmission increase the probability that

parasites will be exposed to subtherapeutic drug levels, especially drugs with long half-lives. People living in areas where transmission occurs at lower rates have fewer malaria attacks but, because the level of acquired immunity is far less, remain at greater risk of severe malarial illness for their entire lives(Rosenthal, 2001).

2.9.2.3. Operational and behavioral factors

The way that drugs are used by both health care providers and patients plays an important role in determining drugs' useful life-spans. Of particular concern are the manner in which drug policies are formulated and implemented and the extent to which official policy can influence practice: specifically, whether drugs are available only with a physician's prescription or whether they are readily available on the open market; whether antimalarials are prescribed only to patients with a proven malaria infection or whether they are prescribed on the basis of a clinical suspicion alone; the extent to which providers (whether formal or informal) and users of antimalarial drugs adhere to official recommendations; and whether the cost or complexity of the recommended regimen might encourage incomplete dosing(Rosenthal, 2001).

The way that people use antimalarial drugs greatly affects the degree of selective drug pressure; many behaviors result in exposure of parasites to inadequate or sub therapeutic drug levels, which, in turn, facilitate development of resistance. One example is the interplay between people's perceptions of illness and the likelihood of completing a full treatment course. Because people from many cultures perceive illness in terms of symptoms rather than causes, community perceptions of illness and their beliefs and practices related to treating those symptoms can differ greatly from Western biomedical definitions of malaria-related illness. Once the symptoms are gone, the illness is perceived to be gone as well. Patients' treating themselves with drugs that produce rapid relief of symptoms, such as artemisinin compounds apparently do, may be more likely to stop before the complete regimen is completed, thereby exposing parasites to a sub therapeutic dose (Rosenthal, 2001).

2.9.3. Clinical consequences of drug resistance and treatment failure

2.9.3.1. Reduced treatment efficacy

The most obvious consequence of antimalarial drug resistance is a failure of the drug to produce a rapid and complete cure. This consequence can be manifested in a variety of ways (Rosenthal, 2001).

2.9.3.2. Delayed initial therapeutic response

This is considered by many to be the first sign of resistance. The rate of parasite and fever reduction is influenced not only by parasite susceptibility but also by host factors and the drug's pharamacokinetic and pharmacodynamic characteristics (Rosenthal, 2001).

2.9.3.3. Parasitologic recrudescence and return of clinical symptoms

In some settings, recrudescent infections tend to be clinically silent. Nonetheless, the return of clinical symptoms associated with recrudescent parasitemia is probably the most common clinical consequence of failed treatment (Rosenthal, 2001).

2.9.4. Public health consequences of drug resistance

2.9.4.1. Increases in malaria transmission

At the simplest level, poor therapeutic efficacy fails to remove parasites from infected individuals, thereby maintaining a larger population of parasitemic individuals in a given area and maintaining a larger biomass of parasites contributing to malaria transmission. Drug resistance is also more directly associated with a potential for increased transmission by enhancing gametocyte carriage. This can be a result of longer parasite clearance times, which are associated with increase gametocyte carriage, or increased frequency of recrudescent infections, which are twice as likely to carry gametocytes compared with primary infections (Rosenthal, 2001).

2.9.4.2. Frequency of severe illness

On an individual basis, the primary concern with failed malaria treatment is progression to severe, potentially life-threatening or fatal illness. Crude estimates of the number of clinical attacks of malaria among African children range from 1 to 5 per year; of these, an estimated 2% of these attacks are severe. Malaria morbidity and mortality has nonetheless increased because of the number of severe malaria cases that develop because of ineffective first-line treatment (Rosenthal, 2001).

2.9.4.3. Mortality rates

Estimates of the impact of effective treatment on the mortality rate of malaria range from a 50-fold decrease in probability of mortality among uncomplicated malaria cases (from about 5% to 0.1%) to a 5-fold decrease in probability of mortality among patients with severe illness (from nearly 100% to 15–20%) (58). As treatment fails, the overall case-fatality rate undoubtedly rises (Rosenthal, 2001).

2.10. Immunity against malaria

During its complex, multi-stage life cycle, the malaria parasite not only expresses a great variety of proteins at different stages, but these proteins also keep changing often. As a result, a natural infection with malaria parasites leads to only a partial and short lived immunity that is unable to protect the individual against a new infection (Doolan *et al*., 2009).

2.10.1. Natural or innate immunity

Natural or innate immunity to malaria is an inherent refractoriness of the host that prevents the establishment of the infection or an immediate inhibitory response against the introduction of the parasite. The innate immunity is naturally present in the host and is not dependent on any previous infection. Alterations in the structure of hemoglobin or in certain enzymes have been found to confer protection against either the infection or its severe manifestations and these traits are often found in areas of high malaria transmission. Duffy negativity in red cells protects against *P. vivax* infection. Certain thalassemias (50% reduction in infection), homozygte hemoglobin C (90% reduction), hemoglobin E, and ovalocytosis carrier status have been reportedto confer protection against *P. falciparum* or *P. vivax*. Glucose 6 phosphate dehydrogenase deficiency (50% protection) and sickle cell hemoglobin (90% protection) confer protection against severe malaria and related mortality (Doolan *et al*., 2009).

2.10.2. Acquired or adaptive immunity

Acquired or adaptive immunity against malaria develops after infection and its protective efficacy varies depending on the characteristics of the host, place of stay, number of infections suffered etc. It has been graded as anti-disease immunity (that protects against clinical disease), anti-parasite immunity (protects against high parasitemia), and sterilizing immunity (protects against new infections by maintaining a low-grade, asymptomatic parasitemia; also called premunition), with a considerable overlap between these. Following infection with malaria parasites, a nonimmune individual commonly develops an acute clinical illness with very low levels of parasitemia and the infection may progress to severe disease and death. After a couple of more infections, anti-disease immunity develops and causes suppression of clinical symptoms even in the presence of heavy parasitemia and also reduces the risk of severe disease. Frequent and multiple infection slowly lead to the development of anti-parasite immunity that results in very low or undetectable parasitemia. Sterilizing immunity, though never fully achieved, results in a high degree of immune responsiveness, low levels of parasitemia, and an asymptomatic carrier status. Premunition suggests an immunity mediated directly by the presence of the parasites themselves and not as much the result of previous infections (Doolan *et al*., 2009).

The acquisition of immunity against malaria is, therefore, very slow and not very effective and remains species specific and strain specific. People living in unstable endemic areas tend to acquire only partial immunity (Doolan *et al*., 2009). The acquired anti malaria immunity does not last long. In the absence of re-infection for about 6 months or 1 year, as may happen when the person leaves the malarious area, the acquired immunity turns ineffective and the individual becomes vulnerable to the full impact of a malarial infection once again (Doolan *et al*., 2009).

2.11. Prevention of malaria

The current approach to manage the disease includes vector control to disrupt transmission from mosquito to human, prevention of infection and treatment after infection (Tripathi *et al*., 2005). Prevention of the disease includes vector control to disrupt transmission from mosquito to human which can be achieved using insecticide treated nets and genetically engineered mosquitoes (Tripathi *et al*., 2005;). But nowadays high insecticide resistance resulting from insensitive acetyl cholinesterase (AchE) has emerged in mosquitoes (Weill *et al.,* 2004).

2.11.1. Avoiding the bite

The best way to avoid getting bitten by a mosquito is to avoid mosquitoes entirely. Mosquitoes are most active at dusk and dawn, so avoiding outdoor activity at those times limits exposure to the insect. Wearing protective clothing, particularly light-colored clothing that radiates infrared light much less than darker clothes, helps to avoid a mosquito's bite (Marcus, 2009).

2.11.2. Eliminating breeding grounds

Other means of avoiding mosquitoes involve limiting their opportunities to breed and find you when you are vulnerable. Mosquitoes can also breed in swimming pools, birdbaths, fountains, animal watering troughs, roof gutters, and even in carelessly discarded cans and beverage containers. Denying mosquito's access to such objects, by keeping them dry whenever possible, or removing litter where water can accumulate, limits the opportunities of mosquitoes to reproduce. In cases where water must stand, as in swimming pools, chemicals such as chlorine can be added to the water to kill mosquito larvae. In some cases, mosquito fish (*Gambusia affinis*) can be introduced into water (Marcus, 2009).

2.11.3. Using protective barriers

Protective barriers are anything that physically blocks a mosquito's access to its prey. By reducing the number of mosquitoes that enter a house, window screens do reduce the risk of malaria to the people inside it. Another protective barrier that can work quite effectively is bed netting. Bed netting can be even more effective when impregnated with insect repellents or, better still, insecticide. In Ethiopia, in particular, the Carter Center has been providing people with bed netting impregnated with pyrethrum, a natural insecticide extracted from chrysanthemums (Marcus, 2009).

2.11.4. Using repellents

Certain areas such as deep woods or swamps have mosquito populations that may be so dense and/or hungry that

the mosquitos may be active at all hours, including in bright sunlight. Moreover, there are times when people have to be out at dusk or dawn. In such cases, chemical mosquito's repellents can be used to prevent bites. The repellent makes the person wearing it seem too unappealing to bite (Marcus, 2009).

2.11.5. Using chemoprophylaxis

Antimalarial drugs which act at different stages of malaria parasite can be used for prophylaxis or treatment of malaria. They are classified as blood schizonticides, tissue schizonticides and gametocides (Michel *et al*., 2002).Mass chemoprophylaxis cannot be done for all people in malaria endemic areas. However, it can be done for those at high risk of malaria like children and pregnant women, particularly primigravidae, though costly (*Kathryn et al*., 2004 and Sweetman, 2005). Special risk group exposed to malaria such as long term travelers, children, pregnant women, aircrew, migrants to visit malarious areas need prophylaxis (Shanks and Edestein, 2005).

Malaria related morbidity and mortality can be reduced in children less than 5 years of age by either intermittent or continuous chemoprophylaxis. Chemoprophylaxis during pregnancy increases infant birth weight and survival, although this effect is largely limited to primi gravidae. Travelers to malaria endemic areas are also recommended to take drugs to prevent malaria (Kathryn *et al*., 2004). Chemoprophylaxis should be started 2 weeks before departure and continued for four weeks after return from the malarious area. For non-immune travelers visiting malarious areas for a period of 2- 3 months, weekly mefloquine administered at 5 mg/kg is the recommended drug for chemoprophylaxis (Michel *et al*, 2002; Fairhurst and Wellems, 2006).

3. Vernonia amygdalina

Medicinal plants are various plants thought by some to have medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science or approved by regulatory agencies such as the United States Food and Drug Administration or European Food Safety Authority to have medicinal effects. A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs (Audu *et al*., 2012).

3.1. Brief description

V. amygdalina Del, commonly called bitter leaf in English and 'Girawa' in Amharic, is a perennial shrub of 2-5m in height that grows throughout tropical Africa. It has a rough bark with dense black straits, and elliptic leaves that are about 6 mm in length. The leaves are green and have a characteristic odor and bitter taste (Ijeh and Ejike, 2011).

Figure 5. Picture of *V. amygdalina* leaf

3.1.1. Scientific classification of Vernonia amygdalina

Kingdom: Plantae
Division: Angiosperms
Order: Asterales
Family: Asteraceae
Genus: Vernonia
Species: *V. amygdalina*
Botanical Name: *Vernonia amygdalina* (Audu *et al*., 2012).

3.1.2. Habitat and distribution

Vernonia amygdalina (Del.) commonly called bitter leaf is the most widely cultivated species of the genus Vernonia which has about 1,000 species of shrubs (Munaya, 2013).It is grown in many countries, in savannah zones and cultivated. Although most popularly used for food, it has also, been traditionally used for its medicinal

properties. True to its name, bitter leaf is bitter to taste but surprisingly delicious in meals (Abosi and Raseroka, 2003). It is found in wide range of bush land, wood land and forest habitat 1200-3000 mean above sea level (masl) in Bale, Wollo, Gondar, Gojam, Wellega, Shewa, Illubabor, Kefa, Hararge and Gamo Gofa floristic regions (Mesfin, 2004).

3.1.3. Medicinal uses

V. amygdalina Del. is probably the most used medicinal plant in the genus Vernonia (Erasto *et al*., 2006). The observation that an apparently sick wild chimpanzee chewed *V. amygdalina* Del. and seemed to return to normal activity after a while. Traditional medicine, practitioners use the plant as an antihelminth, anti-malarial, and as a laxative. Others use it as a digestive tonic, appetizer, febrifuge and for the topical treatment of wounds (Ijeh and Ejike,2011).

V. amygdalina Del. has antibacterial, antiplasmodial/antimalarial, amoebicidal, antifungal, antileishmanial, antischistosomial, wound management, venereal disease management, anticancer/tumor, antioxidant, hypoglycemic/antidiabetic, hepatoprotection, nephroprotection, serum lipid modulation, gastric secretion, analgesic, antifertility and insecticidal properties (Ijeh and Ejike,2011). Its Leaf decoction is used to treat fever, malaria, diarrhea, dysentery, hepatitis and cough as a laxative and as fertility inducer. They are also used as medicine for scabies, headache, and stomach ache. traditional root extract are also use as treatment against malaria and gastrointestinal disorders. It is also useful as a control agent against disease in plant. The ash from burnt branches is used to control seed-borne fungi (Aspergillus, Fusarium and Penicillium spp) (Odeh and Usman, 2014).

In Ethiopia *Vernonia amygdalina* is used for the treatment of internal worms, stomach ache, malaria and 'mich' in Amaro Woreda (Fissehae*t al*., 2014), malaria, ascarisis and around in Hawassa city, southern Ethiopia (Reta,2013), flariasis and ascariasis (Tolosa, 2007). Elsewhere in Ethiopia used against menstruation pain, as purgative and vermifuge, in wound dressing and against urinary inflammations and against malaria, evil eye and diarrhoea (Debela, 2001).

3.1.4. Antimalarial properties of *V. amygdalina Del.*

Malaria is said to be responsible for approximately one million infant deaths every year in sub-Saharan Africa (Abosi and Raseroka, 2003). What is worrisome is that the parasite is becoming resistant to a number of the current drugs for malaria treatment available in the market. Abosi and Raseroka (2003) reported that the ethanolic extract of the leaves and root-bark of *Vernonia amygdalina* suppressed parasitemia (induced by inoculation with *Plasmodium berghei*) in mice by 67% and 54%, respectively in four days. The aqueous extract of the leaves of the plant has also been shown to reduce the load of *P. berghei* in mice by 73% when given intraperitoneally for 4 days (Njan *et al*., 2008). It is thought that the flavonoids, saponins and alkaloids (Sayed *et al*., 1987) and sesquiterpene and steroidal constituents (Phillipson *et al*., 1993) are responsible for the antiplasmodial properties of VA (Ijeh and Ejike, 2011).

The aqueous extract of *Vernonia amygdalina* leaves exhibit antimalarial activity on *Plasmodium falcifarum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae* even though some of these strains are resistant to conventional antimalarial drugs, they were susceptible to this plant especially at higher concentration (Odeh and Usman , 2014). So, in traditional practices ,*Vernonia amygdalina* (bitter leaf) is used in the management of parasitic infection most importantly, Malaria fever which is the most rampant of the parasitic infection(Odeh and Usman , 2014).

The ethanol, petroleum ether, dichloromethane, ethyl acetate, acetone-water and isoamyl alcohol extracts of *V. amygdalina*, showed antimalarial activity against *Plasmodium falciparum* (Dd2) *in vitro* (Masaba, 2000; Tona *et al*., 2004). The root extract of *V. amygdalina* displayed mild activity against chloroquine-sensitive *P. falciparum* with IC_{50} of 19 µg/ml but no activity against the chloroquine-resistant strain.

This antimalarial effect of *V. amygdalina* is contributed by its active compounds, or more specifically sesquiterpene lactones such as vernolepin, vernolin, vernolide, vernodalin and hydroxyl vernodalin which exhibited antiplasmodial activity of IC_{50} value lower than4 µg/ml (Tona *et al*., 2004). Besides, Masaba (2000)discovered that acetone-water extract from *V.amygdalina* leaves showed lower IC_{50} value (25.5 µg/mlagainst *P. falciparum* than water extract (76.7 µg/ml) after 48h.

Furthermore, Iwalokun (2008) showed that this aqueous leaves extract (62.5, 125 mg/kg) was able to work synergistically with chloroquine (5 and 30 mg/kg) against both chloroquine-sensitive and resistant *P.berghei* to shorten the parasite clearance time, prolong the recrudescent times and improve curing rate. The study has also suggested that administration of *V.amygdalina* ethanol extract 1 h prior to chloroquine intake can avoid the reduction in chloroquine bioavailability (Igboasoiyia *et al*., 2008).

3.1.5. Phytochemical constituents of V. amygdalina Del.

A wide array of phytochemicals (including anti-nutritional factors) has been shown to be present in *V. amygdalina*. A summary of the phytochemicals present in *V. amygdalina*. Stigmastane-type saponins such as vernoniosides A1, A2, A3 (Jisaka *et al*., 1992), A4, B2, B3 (Jisaka *et al*., 1993a), C, D and E (Ohigashi, 1994) have been shown to be present in the leaves. The A-series saponins have been shown to be responsible for the bitter taste of *V. amygdalina*. Other steroidal saponins have been identified in the plant. Sesquiterpene lactones are another class of

phytochemicals found abundantly in the leaves of *V. amygdalina*. Some of the identified Sesquiterpene lactones are vernolide, vernodalol,vernolepin, vernodalin and hydroxyvernolide flavonoids luteolin, luteolin 7-O-β-glucoroniside and luteolin 7-O-β-glucoside are found in the leaves of *V. amygdalina*. Other researchers have confirmed the presence of flavonoids in the plant. Other phytochemicals present in the leaves of *V. amygdalina* are terpenes, coumarins, phenolic acids, lignans, xanthones and anthraquinones (Ijeh and Ejike,2011).

3.1.6. Toxicity and Safety of V. amygdalina

Toxicology studies had been carried out via both *in vitro* and *in vivo* systems on various subjects to confirm the toxicity of *V. amygdalina*. As indicated in Table 2, *V. amygdalina* only induced mild toxic effect when administrated at very high concentration. More importantly, safe consumption dosage needs to be identified for women at different stages or vitality of pregnancy, to avoid abortion since it may induce uterine contraction (Yeap *et al*., 2010).

Table 2. *In vitro* and *in vivo* toxicity of *V. amygdalina*.

Animal	Extract	Route	LD_{50} (mg/kgb.w.)	Remark
Mice	Aqueous (500-2000mg/kg/day for 14consecutive days)	Oral	-	No signs of toxicity or adverse toxicological effects at all doses except for decrease of red blood cell count and dose dependent increase of serum bilirubin (Njan *et al*., 2008).
	25% of *V. amygdalina* dry powder (or equivalent amount of ethanol extract or crude/purified saponins) for 2 weeks	Oral	-	Reduction of body and liver weights and increase of urinary and fecal output associated with stomach and small intestines enlargement (Igile *et al*., 1995a).
	Aqueous (62.5 and 125 mg/kg)	Oral		Serum glutamate oxaloacetate transaminase (sGOT), serum glutamate pyruvate tansaminase (sGPT) and lactate dehydrogenase (LDH) level rose around 6-33%. Increase of serum enzyme markers level was more severe when it was consumed with antimalarial drug chloroquine (Iwalokun, 2008).
	Aqueous (87.53 to 92.57g/kg)	Oral	-	No change in organ damage, blood count and liver enzyme profile (AST and ALT) (Amole*et al*., 2006).
	Aqueous (50 and 100 mg/kg)	IP	-	No change of liver function diagnostic enzymes level (total bilirubin, conjugated bilirubin, unconjugated bilirubin,alanine aminotransferase, aspartate aminotransferase and alkaline phoosphatase) (Ojiako and Nwanjo, 2006).
	Ethanol (up to 1000 mg/kg for 1 month)	Oral	-	No change in liver (ALT, AST, ALP, total and conjugated bilirubin) and kidney (creatinine) enzymes marker level (Ekpo *et al*., 2007).
	Cold water (for 24 h)	IP	500 to 1265.22	(Nwanjo, 2005; Ojiako and Nwanjo, 2006).
Rat	Aqueous (250 and 500 mg/kg/day for 5 consecutive days)	Oral	-	Reduction of spermatozoa mobility and viability in dosage dependent mode. Hypoplasia of the seminiferous tubules was also observed in the treated rats. (Oyeyemi *et al*.,2008).
	Aqueous extract of *V.amygdalina*, *Ocimum gratissimum* and *Gongronema latifolia* (ratio 1:1:1 at 16 g/kg b.w. (p.o) and 2.5 g/kg b.w. (i.p.)	Oral,IP	-	No significant change in general behaviour (Iroanya *et al*., 2010).
	Methanol (50, 100 and 200 mg/kg)	Oral		Diarrhea and abortion (Awe *et al*., 1999) due to cathartic effect through weak contractile effect on smooth muscle.
	Powder in standard food (25-75%)	Oral		Skin of the rat turned lighter without alteration of tissues architecture and cellular morphology. (Ibrahim et al., 2001).
Rabbit	Aqueous	IP	1112	(Akah and Okafor, 2006)
	Aqueous (0.3 mg/ml)	injection		Increase of uterine, intestine and jejunum contractionwhich sustained for 30 minutes with elevatedconcentrations used (Caiment-Leblond, 1957;Kamatenesi-Mugisha *et al*., 2005).
Guinea pig	Aqueous (10 and 100 mg/kg)	oral		Increase of uterine and mammary gland contraction amplitudes and thus increase milk production and help in infant's delivery (Ijeh et al., 2008). This supports the traditional use of *V. amygdalina* as an oxytocic plant in assistance of child birth traditionally. (Ganfon *et al.,* 2008)
Murine macrophages J774	Lipophilic extract	*In vitro*	IC50 6.48 µg/ml	Induced mitodepressive effect. Higher concentration of the extract or extensive incubation caused sticky effect on chromosomes during cell division and protein denaturation lead to nuclear disintegration and cell death. Presence of sesquiterpene lactones were suggested as the major contributors to this effect (Ene-Obong and Amadi, 1987).
Allium cepa root tip	Cold water extract	*In vitro*	1% after 8h of incubation	

Generally, *V. amgydalina* is safe to consume and is good for health unless it is consumed in very large

quantities and the potential danger of taking this plant is much lower than that of other common vegetables (Ojiako and Nwanjo, 2006). *V. amygdalina* may cause adverse effect over the male reproductive system without controlled regimen (Yeap *et al*., 2010).

4. CONCLUSION

Malaria is one of the life-threatening diseases caused by *Plasmodium* protozoa transmitted by an infective female *Anopheles* mosquito vector. Treatment is influenced by the species causing the infection, including *Plasmodium falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi. Hence, it should be prevented through* vector control using insecticide treated nets and genetically engineered mosquitoes. *Vernonia amygdalina* can be found in most of Ethiopian regions and used for the treatment of different alignments including malaria in rural communities. Therefore, the antimalarial activities the plant should be further investigated to be used for the treatment of malaria.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

5. REFERENCES

Abosi AO., Raseroka BH., 2003. *In vivo* antimalarial activity of *Vernonia amygdalina*. *Br. J Biomed. Sci*., **60** (20): 89-91.

Akah PA., Okafor CL., 2006. Blood sugar lowering effect of *Vernonia amygdalina* del, in an experimental rabbit model. *Phytother. Res*., **6**: 171-173.

Akinjogunla O J., Ekoi OH., Odeyemi AT., 2011. Phytochemical screening and *in-vitro* antibacterial assessment of aqueous leaf extracts of *Vernonia amygdalina* (asteraceae) *Andocimum gratissimum* (Lamiaceae) on mxifloxacin resistant *Escherichia coli* isolated from clinical and environmental samples. *Nature and Science*,**9**(7).

Akinjogunla OJ., Adegoke AA., Udokang IP.,Adebayo-Tayo BC.,2009. Antimicrobial potential of *Nymphaea lotus* (Nymphaeaceae) against wound pathogens. *J Med Plants Res.,***3**(3):138-141.

Amole OO., Izegbu MC., Onakoya JAA., Dada MO., 2006. Toxicity studies of the aqueous extract of *Vernonia amygdalina*. *Biomed. Res*., **17**: 39-40.

Audu SA., Taiwo AE., Ojuolape AR., Sani AS., Bukola AR., Mohammed I., 2012.A study review of documented phytochemistry of *Vernonia amygdalina* (Family Asteraceae) as the basis for pharmacologic activity of plant extract. *J Nat. Scie.Res*., **2**(7): 2224-3186.

Awe SO., Makinde JM., Olajide OA., 1999. Cathartic effect of the leaf extract of *Vernonia amygdalina*. *Fitoterapia*, **70**: 161–165.

Bahekar S., Kale R., 2013. Herbal Plants Used For the Treatment of Malaria- A Literature *Review.J Pharmacogn and Phytochem*.,**1**(6):141-142.

Biamonte MA., Wanner J., Roch KGL., 2013. Recent advances in malaria drug discovery. *Bioorg. Med. Chem. Lett*. **23**: 2829–2843.

Brian M., Greenwood DA., Fidock, Dennis EK., Stefan HI. Kappe, Pedro L. Alonso, Frank H., Collins., Patrick Ed., 2008.Malaria: progress, perils, and prospects for eradication. *J. Clin. Invest*. **118**:1266–1276.

Bryan GY., Samantha A., Tineke S., Kamala DP., Sornchai L., 2000.Synergism of multiple adhesion molecules in mediating cytoadherance of *Plasmodium falciparum* infected erythrocytes to microvascular endothelial cells under flow. *Blood*. **96**:2292-8.

CDC, 2015. Malaria: The history of malaria. Georgia, USA.

Debela H., 2001. Use and management of traditional plants by indigenous people of Boosat District Welenchiti Area. MSc thesis, Addis Ababa University.

Doolan D L., Dobaño C., Baird JK., 2009. Acquired Immunity to Malaria. *Clin. Microbiol Rev*. **22**(1):13–36.

Dunavan CP., 2005. Tackling malaria . *Scientific Amer*., **293**(6): 76-83.

Ekpo A., Eseyin OA., Ikpeme AO., Edoho EJ., 2007. Studies on some biochemical effects of *Vernonia amygdalina* in rats. *As. J. Biochem*.,**2**: 193-197.

Ene-Obong EE., Amadi OC., 1987. Contributions to the cytological effects of medicinal plants. I. The mitodepressive effects of water extracts of *Boerhaavia diffusa* and *Vernonia amygdalina* on *Allium cepa* root tip mitosis. *Cytologia*, : 469-474.

Erasto P., Grierson DS., Afolayan AJ., 2006. Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina*. *J. Ethnopharmacol*., **106**: 117-120.

Fairhurst RM., Wellems TE., 2006. Modulation of malaria virulence by determinants of *Plasmodium facliparum* erythrocyte membrane protein -1 display. *Curr. Opin in Haematol*., **13**(3): 124-30.

Fakhreldin M., Omer, J. Brian de Souza, Eleanor MR., 2003. Differential induction of TGF-{beta} Regulates proinflammatory cytokine production and determines the outcome of lethal and nonlethal *Plasmodium yoelii* Infections *J. Immunol.***17**1:5430-5436.

Fidock DA., Rosenthal PJ, Croft SL., Brun R., Nwaka S., 2004. Antimalarial drug discovery: efficacy models for compound screening. Nature reviews, drug discovery, **3**: 513-17.

FMOH., 2006. National five year strategic plan for malaria prevention and control in Ethiopia, 2006–2010, Ministry of Health, Addis Ababa, Ethiopia.

Ganfon H., Gbaguidi F., Frederich M., Moudachirou M., Quetin-Leclercq J., 2008. *In vitro* evaluation of antiplasmodial activity of plant samples used in traditional medicine in Benin. *Planta Med.,* PF6: 249.

Gezahegn T., 2004. Ministry of health, In: Malaria diagnosis and treatment, guidelines for health workers in Ethiopia, Addis Ababa.

Gitau EN., Newton CR., 2005. Review Article: blood – brain barrier in falciparum malaria. *Trop Mede and international Health*. **10**(3): 285-92.

Guerra CA., Sno RW., Hay SI., 2006. Defining the global spatial limits of malaria transmission in 2005. Advances in Parasitology, 62:157-179.

Henri CH., John N., Valéry C., Irene G., Georges EG., 2006. A unified hypothesis for the genesis of cerebral malaria: sequestration, inflammation and hemostasis leading to microcirculatory dysfunction. *Trends in Parasitol*. **22**(11):503-508.

Igile GO., Oleszek W., Burdan S., Jurzysta M., 1995a. Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. *J. Agric.Food Chem.,***43**: 2162-2166.

Ijeh II., Ejike CE. C.C., 2011. Current perspectives on the medicinal potentials of *Vernonia amygdalina* Del. *J Med Plants Res*. **5**(7), 1051-1061.

Ijeh II., Igwe KK., Ejike CECC., 2008. Effect of administration of aqueous extracts of *Vernonia amygdalina* .Del leaves to guinea pig dams on milk production and contraction of the mammary gland and uterus. African Journal of Traditional, Complementary and Alternative medicines, Abstracts of The World Congress on Medicinal and Aromatic Plants, Cape Town, pp. 439-440.

Iroanya O., Okpuzor J., Mbagwu H., 2010. Anti-nociceptive and antiphlogistic actions of a polyherbal decoction. *Int. J. Pharmacol*., **6**:31-36.

Iwalokun BA., 2008. Enhanced antimalarial effects of chloroquine by aqueous *Vernonia amygdalina* leaf extract in mice infected with chloroquine resistant and sensitive *Plasmodium berghei* strains. *Afr. Health Sci*., **8**: 25-35.

Jennifer L., Ally PL., Anang AS., Fred EC., Joseph L., 2005. searching for new antimalarial therapeutics amongst Known Drugs. *Chemical Biology and Drug Design*. **67**: 409.

Jisaka M., Ohigashi H., Takegawa K., Hirota M., Irie R., Huffman MA., Koshmizu K., 1993a. Steroid gluccosides from *Vernonia amygdalina*, a possible chimpanzee medicinal plant. *Phytochem*., **34**: 409-413

Jisaka M., Ohigashi H., Takagaki T., Nozaki H., Tada T.,Hiroto M., Irie R., Huffman MA., Nishida T., Kagi M., Koshimizu K., 1992. Bitter steroid glucosides, vernoniosides A1, A2, A3 and related B1from a possible medicinal plant - *Vernonia amygdalina* used by wild chimpanzees. *Tetrahedron*, **48**: 625-632.

Kantele A., Jokiranta TS., 2011. Review of cases with the emerging fifth human malaria parasite, *Plasmodium knowlesi*. *Clin Infect Dis*. 52:1356–1362.

Kassaye KD., Amberbir A., Getachew B., Mussema Y., 2006. A historical overview of traditional medicine practices and policy in Ethiopia. *Ethiop J Health Dev*. **20**(2):127-134.

Kathryn N., Kelvin C., Jay S., 2004. Malaria. *Canad. Med.l Ass. J*. **170**(11) :1693-1701.

Legoff G., Leong PT., Robert V., 2006. Molecular characterization of the malaria vector *Anopheles gambiae* s.s.in Madagascar. Medical and Veterinary Entomology, **20**: 259.

Malaria Operational Plan FY,2008. President's Malaria Initiative, Ethiopia.

Malaria Operational Plan FY, 2014. President's Malaria Initiative, Ethiopia, Pp10-13.

Marcus B., 2009. Deadly Diseases and Epidemics: Malaria, 2nd edi., by Infobase Publishing, New York, Pp.74-80.

Masaba SC., 2000. The antimalarial activity of *Vernonia amygdalina* Del (Compositae). *Trans. Royal Society Trop.Med. Hyg.,* **94**: 694-695.

MDGs, 2014. The Millennium Development Goals Report, United nations, New York, p37.

Mesfin T., 2004. Flora of Ethiopia and Eritrea national herbarium v-4 part 2, p.75

Michel F., Jean MD., Luc A., Patric DM., Angenot., 2002. New trends in anti- malarial Agents. *Curr.Med. Chem*. **9**: 1435-1456.

Milhous WK., Kyle DE.,1998. Introduction to the modes of action of and mechanisms of resistance to antimalarials. In: Sherman IW, ed. Malaria: parasite biology, pathogenesis, and protection. Washington, DC: ASM Press, Pp.303–316.

Munaya C., 2013. Bitter leaf-based extracts cures hepatitis co-inferation and others. The Guardian Newspaper.

Njan AA., Adzu B., Agaba AG., Byarugaba D., Diaz-Llera S., Bangsberg DR., 2008. The analgesic and antiplasmodial activities and toxicology of *Vernonia amygdalina*. *J. Med. Food*, **11**: 574-581.

Odeh AP., Usman AA., 2014. Antimalarial activity and phytochemical analysis of aqueous leaf extract of *Vernonia amygdalina*, *J Zool. and Biosci Res*. **3**:28-31

Ojiako OA., Nwanjo HU., 2006. Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. *Afr. J. Biotechnol*., **5**: 1648-1651.

Oyeyemi MO., Oluwatoyin O., Ajala Leigh OO., Adesiji T., Fisayo., 2008. The spermiogram of male wistar rats treated with aqueous leaf extract of *Vernonia amygdalina*. *Folia Veterinaria*, **52**:98-101.

Phillipson JD., Wright CW, Kirby GC, Warhurst DC., 1993. Phytochemistry of some plants used in traditional medicine for the treatment of protozoal diseases. Abstracts, Int Symposium of the Phytochem Soc of Europe; University of Lausanne, Switzerland, p. L3.

Pravi CT., 2006. Medicinal plants: Traditional knowledge. I.K. International Pvt. Ltd. New Delhi, Pp. 216.

Qureshi R., Waheed A., Arshad M., Umbreen T., 2009. Medico-ethnobotanical inventory of Tehsil Chakwal, Pakistan. *Pak J. Bot*., **41**(2): 529-538.

RBMP, 2015. Evidence for Advocacy: Key statistics on the fight against malaria. Geneva, Switzerland.

Rogerson SJ., Grau GE., Hunt NHH., 2004. The 'microcircualtion' in severe malaria. *Microcirculation*. **11** (7): 599-76.

Rosenthal PJ., 2001. Antimalarial Chemotherapy: Mechanisms of action, resistance, and new directions in drug discovery, Humana Press Inc., New Jersey,Pp.66- 76.

Sayed MD., Zaki AY., El-Marzabai MM., Doss SL., 1987. *Med. plants Phytochem*., **21**: 944.

Sweetman SC., 2005. Matrindale: The Complete Drug Reference, 34th Edi., Pharmaceutical Press, London.

Shanks GD., Edstein MD., 2005. Modern malaria chemoprophylaxis. *Drugs*. **65**(15): 2091-110.

Singh S., 2011. Current scenario of control of malaria. *Trop Parasitol.***1**:52-3.

Snow RW., 2015. Global malaria eradication and the importance of P. falciparum epidemiology in Africa, BMC Med.3:23.

Snow RW., Guerra CA., Noor AM., Myint HY., Hay SI., 2005. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature*, ***434***:214-217.

Srivastava IK., Morrisey JM., Darrouzet E., Valkova-Valchanova M., Ohnishi T., Daldal F.,1999. Resistance mutations reveal the atovaquone binding domain of cytochrome b in malaria parasites. *Mol Microbiol*., **33**:704–711.

Stainesl H M., Krishna S., 2012. Treatment and Prevention of Malaria: Antimalarial Drug Chemistry, Action and Use, Springer Basel AG, Pp.1-6

The Carter Center's (TCC) Malaria Control Program (MCP), 2013.Summary proceedings 4th annual malaria control program review Ethiopia and Nigeria Held on March 8, 2013 at The Carter Center, Atlanta, Georgia.

Thomas E Herchline, 2018. Malaria. Wright State University, Boonshoft School of Medicine, Ohio.

Tona L., Cimanga RK., Mesia K., Musuamba CT., Bruyne TD., Apers S., Hernans N., Miert SV., Pieters L., Totte J., Vlietinck AJ., 2004. *In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. J. *Ethnopharmacol*., **93**: 27-32.

Tripathi RP., Mishra RC., Dwevedi N., Tewari N., Verma SS., 2005.Current status of malaria control. *Curr Med Chem*. **12**(22): 2643-59.

Vizioli J., Adam R., Bulet P., Charlet M, Lowenberger C, Blass C, Muller HM, Dimopoulos G., Hoffmann J., Kafatos FC., 2000. Cloning and analysis of a cecropin gene from the malaria vector mosquito, Anopheles gambiae. *Insect Molecular Biology.* **9** : 75.

Warhurst DC., 2001. A molecular marker for chloroquine-resistant falciparum malaria. *N Engl J Med.***344**:299–301.

Weill M., Malcom C., Chandre F., Mogensen K., Berthomiev A., Marquine M., Raymond M., 2004. The unique mutation in ace- 1 giving high insecticide resistance is easily detectable in mosquito vectors. *Insect molecular Biol*. **13**(1): 1-7.

White NJ., 1998. Drug resistance in malaria. *Br Med Bull*.**54**:703–715

WHO, 2010.Guidelines for the treatment of malaria, 2nd edi., Geneva, Pp.117-120

WHO, 2014.World malaria report, Geneva, Switzerland

Yeap SK., Ho WY., Beh BK., Liang WS., Ky H., Hadi A., Yousr N., Alitheen N B., 2010. *Vernonia amygdalina*, and ethnoveterinary and ethnomedical used green vegetable with multiple bioactivities. *J Medic. Plants Res.,***4**(25):2787-2812.