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 is 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.
DOI: 10.7176/JHMN/60-01
Publication date: March 31st 2019

1. INTRODUCTION
Malaria is a mosquito borne infectious 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. falcifarum 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 indigenous 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 industrialized countries (Kassaye et al., 2006).

Phytochemicals differ from phytoneutrients 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, with 1,793,832 (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 protozoa 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.](image1)

![Figure 2. Trophozoite form of the malarial parasite within peripheral erythrocytes (Thomas, 2018).](image2)

![Figure 3. A mature schizont within an erythrocyte (Thomas, 2018).](image3)
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 - 41°C 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 diagnosis are 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 should 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 to quantify 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, primaquine, and mefloquine (ii) antifolate compounds (pyrimethamine, proguanil, dapsone, and sulfadoxine), (iii) artemisinin and derivatives (artemisinin, artesunate, artemether, arteether, dihydroartemisinin), and (iv) hydroxynaphthoquinone (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. malariaeaeor P. ovale, the drug of choice is chloroquine). In malaria-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 quinines 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 sulfadoxine 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.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of Resistance</th>
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<tbody>
<tr>
<td>Chloroquine</td>
<td>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 Pfmdr1 and Pfmdr2 may also contribute Warhurst, 2001).</td>
</tr>
<tr>
<td>Mefloquine, halofantrine, quinine</td>
<td>Mefloquine and halofantrine resistance may be related to amplification of Pfmdr1, increased expression of Pgh1, mutations in pfcr, 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).</td>
</tr>
<tr>
<td>Cycloguanil, chlorcycloguanil, pyrimethamine</td>
<td>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).</td>
</tr>
<tr>
<td>Sulfonamides and Sulfones (sulfadoxine, dapsone)</td>
<td>Point mutations in mitochondrially encoded cytochrome b gene (Srivastava, 1999).</td>
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<tr>
<td>Atovaquone</td>
<td></td>
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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 pharmacokinetic 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), homozygote hemoglobin C (90% reduction), hemoglobin E, and ovalocytosis carrier status have been reporte to 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 Edstein, 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](image)

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 antihelmint, 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, antischistosomal, wound management, venereal disease management, anticancer/tumor, antioxidant, hypoglycemic/anti-diabetic, 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 (Fissehaet al., 2014), malaria, ascarasis 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 falciparum*, *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<sub>50</sub> 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, vernoldie, vernoldin and hydroxyl vernoldin which exhibited antiplasmodial activity of IC<sub>50</sub> value lower than 4 µg/ml (Tona et al., 2004). Besides, Masaba (2000) discovered that acetone-water extract from *V.amygdalina* leaves showed lower IC<sub>50</sub> value (25.5 µg/ml) against *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, 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*.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Extract</th>
<th>Route</th>
<th>LD_{50} (mg/kg b.w.)</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>Aqueous (500-2000 mg/kg/day for 14 consecutive days)</td>
<td>Oral</td>
<td>-</td>
<td>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).</td>
</tr>
<tr>
<td></td>
<td>25% of <em>V. amygdalina</em> dry powder (or equivalent amount of ethanol extract or crude/purified saponins) for 2 weeks</td>
<td>Oral</td>
<td>-</td>
<td>Reduction of body and liver weights and increase of urinary and fecal output associated with stomach and small intestines enlargement (Igile et al., 1995a).</td>
</tr>
<tr>
<td></td>
<td>Aqueous (62.5 and 125 mg/kg)</td>
<td>Oral</td>
<td>-</td>
<td>Serum glutamate oxaloacetate transaminase (sGOT), serum glutamate pyruvate transaminase (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).</td>
</tr>
<tr>
<td></td>
<td>Aqueous (87.53 to 92.57g/kg)</td>
<td>Oral</td>
<td>-</td>
<td>No change in organ damage, blood count and liver enzyme profile (AST and ALT) (Amoleet et al., 2006).</td>
</tr>
<tr>
<td></td>
<td>Aqueous (50 and 100 mg/kg)</td>
<td>IP</td>
<td>-</td>
<td>No change of liver function diagnostic enzymes level (total bilirubin, conjugated bilirubin, unconjugated bilirubin, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) (Oyeyemi and Nwanjo, 2006).</td>
</tr>
<tr>
<td></td>
<td>Ethanol (up to 1000 mg/kg for 1 month)</td>
<td>Oral</td>
<td>-</td>
<td>No change in liver (ALT, AST, ALP, total and conjugated bilirubin) and kidney (creatinine) enzymes marker level (Eko et al., 2007).</td>
</tr>
<tr>
<td></td>
<td>Cold water (for 24 h)</td>
<td>IP</td>
<td>500 to 1265.22</td>
<td>Diarrhea and abortion (Awe et al., 1999) due to cathartic effect through weak contractile effect on smooth muscle.</td>
</tr>
<tr>
<td>Rat</td>
<td>Aqueous (250 and 500 mg/kg/day for 5 consecutive days)</td>
<td>Oral</td>
<td>-</td>
<td>Reduction of spermatozoa mobility and viability in dosage dependent mode. Hypoplasia of the seminiferous tubules was also observed in the treated rats. (Ogyeimi et al., 2008).</td>
</tr>
<tr>
<td></td>
<td>Aqueous extract of <em>V. amygdalina</em>, <em>Ocimum gratissimum</em> and <em>Gongronema latifolia</em> (ratio 1:1:1 at 16 g/kg b.w. (p.o) and 2.5 g/kg b.w. (i.p.)</td>
<td>Oral,IP</td>
<td>-</td>
<td>No significant change in general behaviour (Iwanya et al., 2010).</td>
</tr>
<tr>
<td></td>
<td>Methanol (50, 100 and 200 mg/kg)</td>
<td>Oral</td>
<td>-</td>
<td>Diarrhea and abortion (Awe et al., 1999) due to cathartic effect through weak contractile effect on smooth muscle.</td>
</tr>
<tr>
<td></td>
<td>Powder in standard food (25-75%)</td>
<td>Oral</td>
<td>-</td>
<td>Skin of the rat turned lighter without alteration of tissues architecture and cellular morphology. (Ibrahim et al., 2001).</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Aqueous</td>
<td>IP</td>
<td>1112</td>
<td>(Akah and Okafor, 2006)</td>
</tr>
<tr>
<td></td>
<td>Aqueous (0.3 mg/ml)</td>
<td>injection</td>
<td>-</td>
<td>Increase of uterus, intestine and jejunum contractilions which sustained for 30 minutes with elevated concentrations used (Caiment-Leblond, 1957; Kamatenesi-Mugisha et al., 2005).</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Aqueous (10 and 100 mg/kg)</td>
<td>Oral</td>
<td>-</td>
<td>Increase of uterus 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 <em>V. amygdalina</em> as an oxytocic plant in assistance of child birth traditionally. (Gantfon et al., 2008).</td>
</tr>
<tr>
<td>Mice</td>
<td>Lipophilic extract</td>
<td>In vitro</td>
<td>IC50 6.48 µg/ml</td>
<td>Induced mito depressive 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).</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Cold water extract</td>
<td>In vitro</td>
<td>1% after 8h of incubation</td>
<td></td>
</tr>
</tbody>
</table>

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

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