Perspectives of the *Artemisia annua* Dry Leaf Therapy (ALT) for Malaria and of its Re-Purposement as An Affordable Cure for Artemisinin-Treatable Illnesses

RICHA GOEL[1], RAJ KUMARI[2], VIJENDER SINGH[3], RICHA PANDEY[4], RENU KUMARI[5], SUCHI SRIVASTAVA[6] and SUSHIL KUMAR[5,7,*]

[1]*Department of Pharmacognosy, KIET School of Pharmacy, Ghaziabad 201 206, India*
[2]*Department of Pharmacognosy, ITS College of Pharmacy, Ghaziabad 201 206, India*
[3]*School of Pharmacy, Sharda University, Greater Noida, Ghaziabad 201 306, India*
[4]*Division of Natural Product Chemistry, CSIR-Indian Institute of Chemical Technology (IICT), Uppal Road, Tarnaka, Hyderabad 500 001, India*
[5]*National Institute of Plant Genome Research (NIPGR), Aruna Asaf Ali Marg, New Delhi 110 067, India*
[6]*Division of Plant Microbe Interactions, CSIR-National Botanical Research Institute (NBRI), Lucknow 226 001, India*
[7]*SKA Institution for Research, Education and Development, 4/11 Sarv Priya Vihar, New Delhi 110 016, India*

Malarial diseases continue to risk the lives of more than 3 billion people in 97 countries in the world, causing sickness in several million people and death to half a million patients. The preponderate malaria causing apicomplexan protozoan parasite species *Plasmodium falciparum* and *Plasmodium vivax* have become genetically resistant to most of the approved antimalarial drugs, including the artemisinin-based combination therapies (ACTs). At this time, there is a vigorous need to make enough efforts to meet the challenge of combating multi-drug resistant malaria by (a) speeding up the trials in progress on relatively more effective, new and mechanistically different antimalarial pharmaceuticals, (b) production of effective vaccines against *falciparum* and *vivax* malaria, (c) devising of new ways to use the presently available anti-malarials such as by using three-drugs ACTs and by using the different two-drug and three-drug ACTs sequentially, and (d) induction of *Artemisia annua dry leaf therapy* (ALT) of recent origin, but of ancient precedent, as an effective treatment for acute and complicated malaria. Here, a perspective type review is presented of the: pre-ALT antimalarial drugs, methodology of their usage and consequences of resistance development; safety, efficacy, affordability, quality maintenance and resilience to resistance development aspects of ALT; and possibilities of ALT re-purposement for treating many infectious-metabolic disorder related- and cancerous-diseases. In conclusion, an urgent need is emphasized for pilot studies and clinical trials on ALT to attest its deployment as anti-malarial and cure for diseases beyond malaria.

Keywords: Antimalarial Drug-Resistance; Antimalarial Pharmaceuticals; Auto-Immune Diseases; Cancers; Infectious Diseases; Non-Artemisinic Secondary Metabolites.

Introduction

Malaria, which results from the transmission of malarial parasite infection to humans by the bites of infected mosquitoes, is the deadliest infectious disease of tropical and sub-tropical climates. Malaria Eradication Scientific Alliance (MESA) has recently updated the research agenda for malaria elimination and eradication (malERA.2017). In recent years (such

*Author for Correspondence: E-mail: sushil2000_01@yahoo.co.in

as 2015, 2016), 3.5 billion people in 97 countries were at risk of getting infected with malarial parasite(s) (Fig. 1). Actually, in each of these years, several hundred million humans got malarial infections and about half a million patients, preponderantly young children, elderly, and pregnant women, succumbed to the disease. In 2016, about 90% of malaria in Southeast-cum-South Asia region was contributed by India (World 2016 Malaria report). In approximately last ten years, since the introduction of artemisinin combination therapy (ACT) as the treatment of malaria and regulation of parasite transmission, at least ten countries have become largely malaria free. During this period, due to success in control of the disease causing parasite by chemotherapeutic treatments, such as ACT, prophylaxis, and control of mosquito attacks by use of pyrethroid insecticide impregnated bednets and indoor insect repellants (Landier *et al.*, 2016; Dondrop *et al.* 2017; Sluydts *et al.*, 2017), the loss of life from malaria has been halved. For the last 72 years, from the time chloroquine was introduced as a substitute/ alternative of quinine in malaria treatment, the disease has been contained by use of five classes

of individual pharmaceuticals (aminoquinolones, aminoalcohols, antifolates, hydronapthoquinone and endoperoxides) and their combinations. However, malaraial parasites have developed genetic resistance against most (perhaps all) of the effective anti-malarials and their combinations. Besides, the resistant parasites have become geographically widespread. The vector mosquitoes have also developed resistance to insecticides used to impregnate bednets. The new affordable antimalarial chemical compounds and vaccines undergoing tests and trials are thought to be atleast a decade away (Dondrop, 2017; White, 2017; Kazmin *et al.*, 2017; Lopaticki *et al.* 2017; Sissoka *et al.*, 2017; Nasamu *et al.*, 2017; Bisland *et al.* 2018; Cowell *et al.* 2018; Kisalu *et al.* 2018). All these factors have posed a grave challenge for the control of malarial disease worldwide in coming years. Discussion is in progress on ways to increase the life span of currently available pharmaceuticals by employing them in alternate combinations, to resist the resistance power in the parasite and combat parasite transmission. At this time, when new effective and affordable malarial treatments are being eagerly
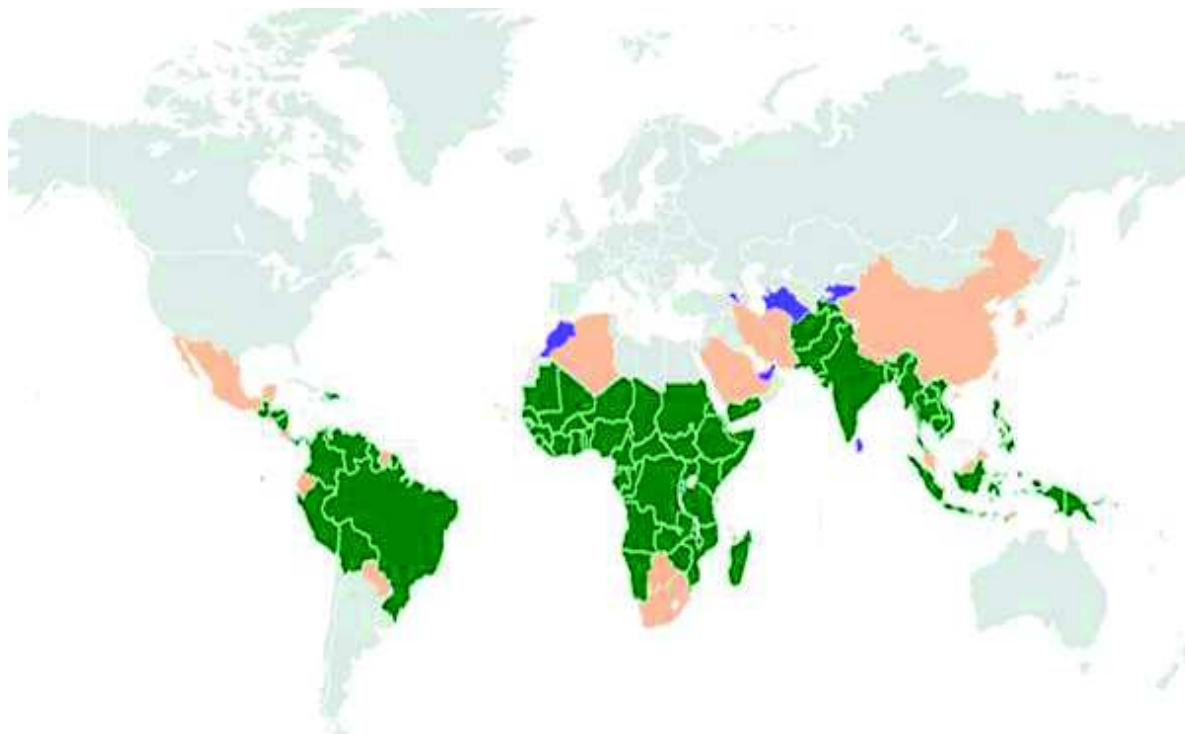

Fig. 1: World map of malaria endemic areas: the green coloured areas = transmission rate of malaria is high; peach coloured areas: relatively lower malaria transmission rate; indigo colored areas = recently became malaria free; slate coloured areas = have been malaria free for long time (reproduced with requested permission: Rabinovich R N *et al.*, Plos Med 14, e1002456)

awaited, a botanical treatment that appears to clear (artemisinin) resistant malaria has been recently described. Daddy *et al.* (2017) have reported success in curing 18 cases of severe malaria by administering to the patients tablets made of dry leaves of *Artemisia annua* (the natural rich source of the pharmaceutical artemisinin) plants. This treatment called *Artemisia annua dry leaf therapy* (ALT) was found to have cured malaria caused by parasites resistant to the currently used antimalarials, including artemisinin derivatives (Fig. 2). Perspectives of this novel, highly promising and innovative development are discussed in this review.
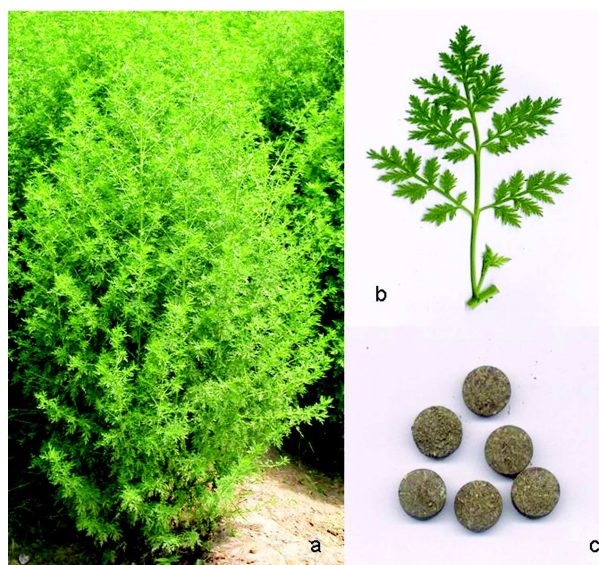

Fig. 2: ALT tablets made from dry *Artemisia annua* cv Sanjeevani leaves. a = A plant of *A. annua* cv Sanjeevani at pre-flowering development stage; b = *A. annua* freshly harvested leaf; and c = Tablets made by compressing the dried *A. annua* leaves

To delineate the importance of origin of ALT as a safe, efficacious and affordable antimalarial treatment, with multi-repurposing possibilities, this review (perspective) deals with several relevant subject matter areas. The first section defines characteristics of different species of malaria causing parasites, their vectors and endemicity alongwith several features of disease development. This is followed by chemical, biological and antimalarial properties of currently used (approved) antimalarials. Next are described the choices and methodologies of administration of antimalarials to adult, child, pregnant and lactating women patients of acute and complicated

malarias. Nature of mutations that render parasites species resistant to individual and multiple antimalarial drugs and circumstances that allowed soft and hard sweeps of certain artemisinin resistant mutation(s) is discussed next. This is followed by a section on discussions taking place to devise strategies to combat drug resistant malaria, especially artemisinin resistance. The next section covers aspects about the origin, empirical basis and evidence on clinical efficacy of ALT. Following it are sections that discuss the quality maintenance and cost aspects of ALT. The final section brings into focus the possibilities of repurposing ALT as treatment for a variety of autoimmune, metabolic and cancerous diseases.

Kinds of Malaria and Symptoms

There are about 200 different unicellular eukaryotic apicomplexan obligate narrow host-range parasite species of the genus *Plasmodium*, transmitted by dipteran insect species, whose infection can cause various kinds of malarial diseases in a wide range of vertebrates. The five major species of *Plasmodium* that cause malaria in humans are *falciparum* (Pf), *knowlesi* (Pk), *malariae* (Pm), *ovale* (Po) and *vivax* (Pv). The important properties of these human malarial parasites are comparatively summarized (and the references concerned with this section are also given) in the Table 1. Among the human malarial parasites, Pk is known to be a zoonotic species whose infection in several species of macacque monkeys produces malaria-like symptoms. Recently, another zoonotic species- *Plasmodium simium* (Ps)- has been found to cause malaria in humans in the Atlantic Forest Area of Brazil. The natural hosts for Ps are monkeys of the genera *Aloulta, Brachyteles, Cebus* and *Sapajus* (Brasil *et al.*, 2017).

The insect hosts of *Plasmodium* species are anopheline mosquitoes. Out of about 515 known species of *Anopheles*, approximately 70 are vectors of human malaria (Sinka *et al.*, 2012). Each of Pf, Pk, Pm, Po, Pv and Ps is transmitted to humans by several to many *Anopheles* species, in the geographical areas of their occurrence. The genomes of human malarial *Plasmodium* species and of the major *Anopheles* vector species have been sequenced (Neafsy *et al.*, 2015; Rufledge *et al.*, 2017). The vector for Ps has been identified as *Anopheles kertezsia cruzii* (Brasil *et al.*, 2017). Phylogenetically,

Table 1: Properties of malaria caused in humans by infection of different species of the alveolate parasite Plasmodium (Phylum: Apicomplexan; Family: Plasmodiidae)

S.No.	Characters		*Plasmodium falciparum (Pf)*	*Plasmodium vivax (Pv)*	*Plasmodium ovale*		*Plasmodium malariae (Pm)*	*Plasmodium knowlesi (Pk)*
					curtisi (Poc)	wallikeri (Pow)		
1	(A) Features of (n=14) genome	Size (Mb)	23.3	29.1	33.5	33.5	33.6	24.4
2		Estimated gene number	5355	6671	7165	6340	6559	5284
3		G+C content (%)	19	40	29	29	24	39
4		Pre-erythrocytic growth in hepatocytes (hepatic schizogony) (number of days = d)	5-7	6-9	8-9		14-16	6-9
5		Whether relapse causing hypnozoites are formed in liver ?	No	Yes	Yes		No	No
6		Incubation period (d)	8-15	10-21	12-20		18-60	10-12
7		Fever cycle (erythrocytic schizogony) (number of hours = h)	Tertian (48)	Tertian (48)	Tertian (48)		Quartan (72)	Quotidian (24)
8		Nature of red blood cells affected	All types of erythrocytes	Reticulocytes	Reticulocytes		Mature erythrocytes	All kinds of erythrocytes
9	(B) Features of life cycle in human host	Size of parasitaemia (number of parasites per μL of blood (x10^3)	20-500	20-50	9-10		5-10	0.5-10
10		Whether cytoadherence of parasite causes microvascular dysfunction ?	Yes	Rarely (if at all)		Rarely (if at all)	Rarely (if at all)	Yes
11		Whether severe malaria developes ?	Yes	Yes	No		No	Yes
12		Whether recrudescence occurs ?	Yes (when treatment fails)	Yes (when treatment fails)	Rare		Yes (sometimes after 30 to 50 y from the primary attack)	Yes
13		Time of appearance of gametocytes (d after the start of parasitaemia)	8-14	0	0		0	Not known
14	(C) Features of life cycle in mosquito host	Transmission causing *Anopheles* mosquito vector species	Many species (>70), most prominent are: *gambiae, culicifacies* and *stephensi*	Many species (>71), most prominent are: *aquasalis, culicifacies, stephensi, darlingi* and *dirus*	Several species (~10), most prominent are: *funestus, gambiae, stephensi, freeborni, dirus, farauti* and *atroparvus*		Many species (>30), most prominent are: *culicifacies, aconitus, arabiensis, atroparvus* and *freeborni*	Several species including: *craceus, hackeri, latens* and *bala-bacensis*

Table 1: Contd ....

16	(D) Major geographical areas of prevalence	World-wide tropical and subtropical areas (especially in Africa, Asia and Mediterranean)	World-wide subtropical areas (especially in Asia, Latin America and Africa)	Tropical regions of Africa and Asia and in Pacific islands, sympatrically (subspecies)	World-wide tropical and subtropical areas (including Pacific islands)	Southeast Asia and South Asia
17	(E) Remarks	*Pf* is the preponderant cause of malaria. The falciparum malaria is the deadliest and if not treated timely the acute (or un-complicated) malaria turns into cerebral (or complicated) malaria	*Pv* can cause severe disease and death due to splenome-galy. The Duffy blood group deficient in Africa when infected are often symptomless	In some cases relapse can occur as late as 4-5 years from initial inoculation	It is less life-threatning than *vivax* and *falciparum* malarias. However, it can cause chronic lifetime infection	This parasite is zoonotic, also causes malaria in the monkeys *Macaca fascicularis, M. nemestrina* and *Presbytis melalophos*. The disease in humans is mild, but can be lethal (mortalityH~2%). The Duffy blood group people in West Africa are often insensitive to this parasite. Transmission occurs from humans to monkey and *vice versa*. Human to human transmission is rare (perhaps via the vector *A. dirus*)

References: Antinori *et al*. (2017); Barber *et al*. (2017); Epstein *et al*. (2017); Rutledge *et al*. (2017); White (2017); York (2017); Anvikar *et al*. (2016); Camargo-Ayala *et al*. (2016); Doctor *et al*. (2016); Molina-Cruz *et al*. (2016); Sutherland (2016); Josling and Leianas (2015); Sharma *et al*. (2015); St. Laurent *et al*. (2015); Tainchum (2015); WHO (2015c); Naing *et al*. (2014); Visser *et al*. (2014); White *et al*. (2014); Hii and Rueda (2013); Singh and Daneshwar (2013); Bousema and Drakeley (2011); Pain *et al*. (2008); Collins and Jaffrey (2007 and 2005); Carlton *et al*. (2008); Kakkilaya 2003; Gardner *et al*. (2002); Silvestrini *et al*. (2000); Hansbook *et al*. (1996); Garnham (1966); www.antimicrobe.org/new/bo5.asp; www.cdc.gov/malaria/about/biology/parasites.htm; emedicine.medscape.com/article/221134; www.parasitesinhumans.org/plasmodium-falciparum-malaria.htm.

distance-wise the parasite species are related to each other as follows: Pf → Po → Pm → Pk, Pv and Ps (Hall, 2012; Brasil *et al.*, 2017). In terms of the frequencies of malaria infections caused by them in humans, parasites fall in the following order: Pf > Pv > Po, Pm > Pk > Ps. The malarias caused by Pf, Pv and Pk can be fatal if not treated. The Po and Pm caused malarias are less severe and generally not lethal. Pv, Ps and Po caused infections can remain dormant in the liver for up to many months. Pm infection can remain latent for years. The Duffy blood group deficient (*ackr1* = atypical chemokine receptor 1) humans (who are largely the inhabitants of West Africa) are resistant to infection by Pv and Pk because the parasites are unable to invade their *Fy a⁻b⁻* erythrocytes (de Carvalho and de Carvalho, 2011).

The Pf, Pv, Po, Pm, Pk and Ps malarias have differential distribution. Pv is the most widespread malaria; it is the major malaria causing parasite in subtropical areas of Asia, Americas and Africa. Nearly half of the malarial cases that occur outside of Africa are related to Pv infection. More dangerous than Pv malaria, Pf malaria is predominant in Africa, but also occurs in tropical regions of Asia and in Middle East. Pf malaria is responsible for 90% of malarial deaths in Africa. The distribution of Pm malaria is similar to that of Pf malaria except that it is much less frequent. Both Po and Pm are the cause of malaria in Pacific islands. There are two subspecies of Po called *P. curtisi* and *P. wallekeri*, both are cause of malaria in Africa and Asia, sympatrically. Together, Po and Pm account for about 10 million cases of new malaria each year. Malaria caused by Pk occurs largely in southeast- and south-Asia. The Ps malaria is limited to Brazil. In areas where frequency of occurrence of malarial infections is high, mixed infections of more than one Plasmodium species have been observed (Mayxay *et al.*, 2004). Recently, a rare case of malaria caused by infection of Pf, Pv, Po and Pm has been reported from forest area in Central India (Krishna *et al.*, 2017) which has high incidence of mixed infection.

Initial symptoms of malaria are often as non-specific as one or more of the following type of sickness: fever, chills, sweating, fast heart rate, sore throat, cough, pneumonia, headache, muscular pain, joint pain, fatigue, difficulty in swallowing, hyper-

salivation, jaundice, nausea, weakness, vomiting, constipation and enlargement of spleen. Laboratory diagnosis is essential to confirm malaria. The most reliable diagnosis is the detection of parasite-infected red blood cells through microscopic examination of thick and thin blood films. The rapid diagnostic tests (RDTs), based on detection of parasite antigens, can be used, but should not substitute for the needed microscopic tests (Kakkilaya, 2003). Once diagnosed, a confirmed malaria patient should immediately begin receiving the WHO prescribed treatment at the earliest.

The findings of microscopic test/s are helpful in classifying malaria as uncomplicated or severe. In cases of the non-complicated malaria, the parasitemia (% of parasitized red blood cells) is lower than 2%. If parasitemia is 10%, the malarial patient is facing the severe form of disease. The symptoms of the severe malaria include high fever and one or more of the following conditions: renal impairment (dark urine and limited output) acidosis, hypoglycemia, spontaneous bleeding, breathing difficulties, severe anemia, prostration or coma. Young children and pregnant women are not only more vulnerable to malarial infection but also prone to developing severe malaria. Consequences of severe malaria in pregnant woman include miscarriage, stillbirth, premature birth and birth defects in neonates. Generally, all kinds of malaria cause bone loss due to chronic bone inflammation and adversely affect functioning of skeletal and heart muscles due to poor supply of nutrients and oxygen (Marrelli and Brotto, 2016; Lee *et al.*, 2017). There occurs macrovascular dysfunction in Pf and Pk malaria due to adherence of infected cells to walls of blood vessels (White, 2017). The above kinds of deficits imposed by malaria, span of morbidity, possibility of death can all be checked by anti-malarial drug treatment which also aims to clear malarial parasites from the body of malarial patient such that malaria does not relapses and transmission to mosquitoes is blocked. Antimalarial drugs are also used as chemoprophylaxis, in mass drug administration campaigns to limit the spread of malaria in endemic areas, and to travelers visiting the malaria endemic areas.

Currently Used Antimalarial Drugs

The antimalarial drugs include; quinine, mefloquine,

halofantrine and lumefantrine (aryl aminoalcohols); chloroquine, amodiaquine and piperaquine (4-aminoquinolones); primaquine (8-aminoquinoline); pyronaridine (mannich base); atovaquone (napthaquinone); proguanil, pyrimethamine and sulfadoxine (antifolates); tetracycline, doxycycline and clindamycin (antibiotics); and artesunate, dihydro-artemisinin and artemether (artemisinin derived endoperoxides). The Table 2 illustrates chemical structures, purpose and regimen of administration to malaria patients, biological effects on *Plasmodium* parasites and prescription properties. There are large inter-class and intra-class differences in the properties of the drugs. *In vivo* half-life of artemisinins is short (0.5 to few hours) as compared to that of lumefantrine, pyronaridine, pyrimethamine, sulfadoxine, piperaquine and chloroquine (3 to 60 days). Quinine and artemether are highly insoluble in water and are usable for parenteral application. Artemisinins are very fast acting drugs. Quinine, chloroquine, piperaquine and artemisinins are able to block transmission of parasites to mosquitoes. Primaquin too blocks transmission but also prevents Pv and Po malaria relapses. Quinine, mefloquine, lumefantrine, atovaquone and artemisinins do not allow multiplication of parasite in mosquitoes. Unlike proguanil, pyrimethamine, sulfadoxine and atovaquone target singular but different parasite functions, whereas artemisinin derivatives, chloroquine and quinine exemplify antimalarials which target multiple functions in the parasites.

To overcome deficiencies of individual chemotherapeutics and to slow down resistance development, anti-malarials are now used in combinations. The following combinatorial regimen have been recommended by WHO to cure various kinds of malaria (Table 2): chloroquine + primaquine (against Po and Pv malaria); quinine + tetracycline or clindamycin (against severe malaria); ACTs = artemether + lumefantrine or mefloquine, dihydroartemisinin + piperaquine, artesunate + pyronaridine or sulfadoxine + pyrimethamine or artesunate + amadiquine (against uncomplicated malarias, especially those caused by Pf). Primaquine or alternatively tafenoquine is given additionally to stop relapses and transmission; both are contraindicated for G6PD deficient patient (Table 3: Elmes *et al.* 2008; Graves *et al.*, 2018). The tertian malaria caused by Ps is curable by chloroquine + primaquine treatment (Brasil *et al.*, 2017). The combinations used for

chemoprophylaxis in endemic areas are atavoquone + proguanil and proguanil + chloromycetin. For chemoprophylaxis mefloquin and doxycycline are also used preferably singly.

Life Cycle Stages at which Plasmodia are Killed by the Anti-Malarial Drugs

The Fig. 3 gives a diagramme of the life cycle stages of malarial parasites in human host and mosquito vector. Table 1 has quantitative data about parasite life cycle stages in all the five kinds of malaria. Life cycle stages at which the anti-malarials kill the parasites are identified in Table 2. The references from which information is described in this section, and summarized in Fig. 1, Table 1 and Table 2, was collated are given in the figures and tables.

The human host is inoculated with a small number (>10) of sporozoites when an infected female mosquito bites to obtain a blood meal. Sporozoites reach liver through blood and lymphatic system and invade hepatocytes. There is no antimalarial drug that blocks this process. The parasites in liver cells develop into schizonts, wherein the parasite undergoes many divisions to produce thousands of merozoites. The liver tissue schizonts rupture and release the merozoites into the blood stream. There are no clinical symptoms of malarial illness all through this stage. Merozoites developing in their liver schizonts are killed by several antimalarial drugs: primaquine, atavoquone, proguanil, pyrimethamine, tetracycline, doxycycline and artemisinin derivatives (artemisinins). The liver stage is completed in about 5-9 days from inoculation, except that in Pm malaria, it takes up to 16 days. Some Pv and Po infected hepatocytes produce hypnozoites. These dormant parasites can get activated and cause relapsed malaria, any time up to 5 years. Hypnozoites are eliminated from the liver by the drug primaquine.

The merozoites released from liver invade red blood cells (RBCs). In the infected erythrocytes (RBCs), parasite forms schizonts in which it produces 16-32 merozoites per schizont. When these schizonts have ruptured and the released merozoites have invaded fresh erythrocytes, an erythrocytic-schizont cycle of parasite infection is completed. Only upon completion of 3-8 such cycles, the parasite density in blood reaches the pyrogenic level of $50/\mu L$. At this stage febrile paroxysm lasting 8-12 h appears. In this period the body temperature progressively rises and
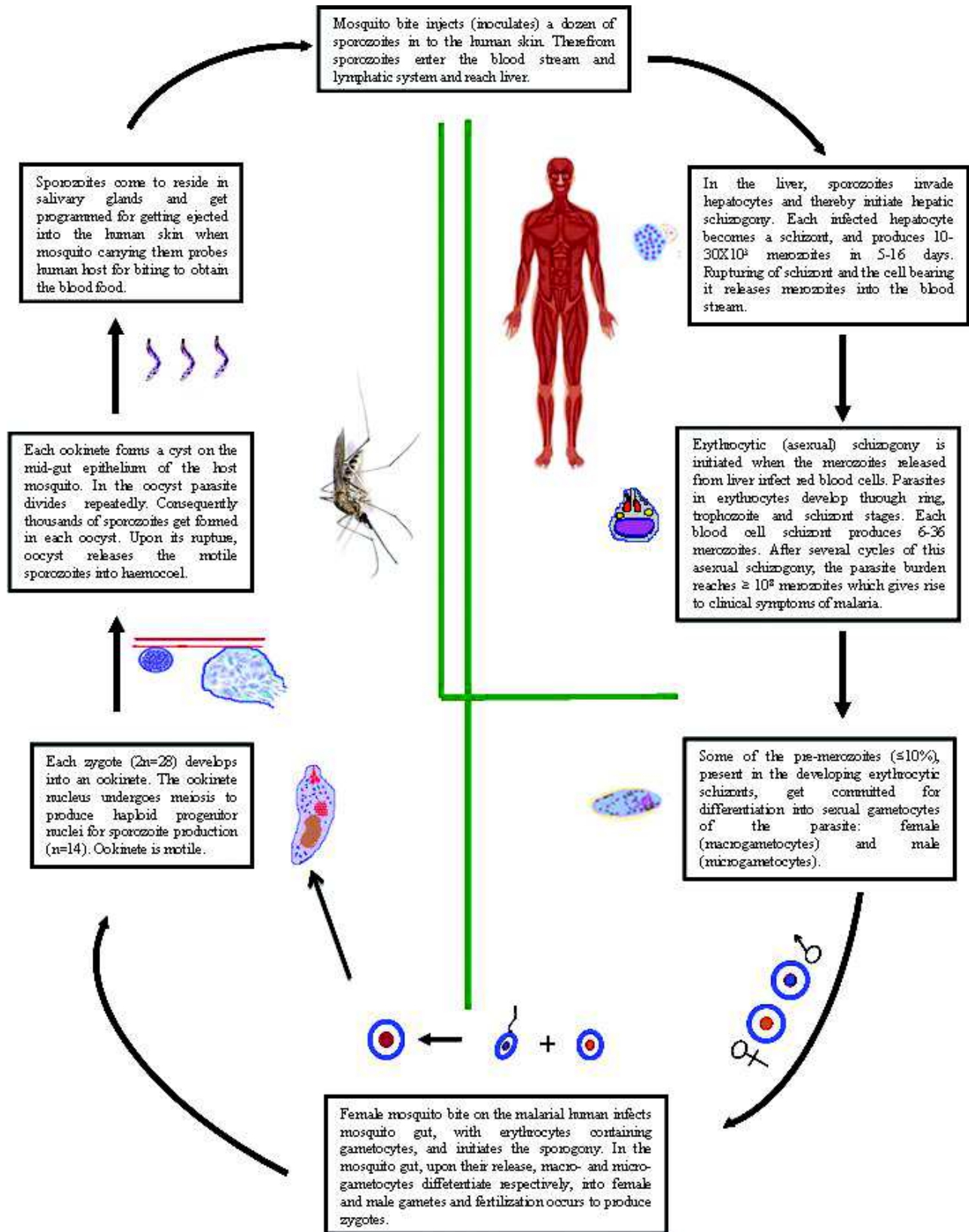
Mosquito bite injects (inoculates) a dozen of sporozoites in to the human skin. Therefrom sporozoites enter the blood stream and lymphatic system and reach liver.

Sporozoites come to reside in salivary glands and get programmed for getting ejected into the human skin when mosquito carrying them probes human host for biting to obtain the blood food.

In the liver, sporozoites invade hepatocytes and thereby initiate hepatic schizogony. Each infected hepatocyte becomes a schizont, and produces 10-30X10^3 merozoites in 5-16 days. Rupturing of schizont and the cell bearing it releases merozoites into the blood stream.

Each ookinete forms a cyst on the mid-gut epithelium of the host mosquito. In the oocyst parasite divides repeatedly. Consequently thousands of sporozoites get formed in each oocyst. Upon its rupture, oocyst releases the motile sporozoites into haemocoel.

Erythrocytic (asexual) schizogony is initiated when the merozoites released from liver infect red blood cells. Parasites in erythrocytes develop through ring, trophozoite and schizont stages. Each blood cell schizont produces 6-36 merozoites. After several cycles of this asexual schizogony, the parasite burden reaches ≥ 10^8 merozoites which gives rise to clinical symptoms of malaria.

Each zygote (2n=28) develops into an ookinete. The ookinete nucleus undergoes meiosis to produce haploid progenitor nuclei for sporozoite production (n=14). Ookinete is motile.

Some of the pre-merozoites (≤10%), present in the developing erythrocytic schizonts, get committed for differentiation into sexual gametocytes of the parasite: female (macrogametocytes) and male (microgametocytes).

Female mosquito bite on the malarial human infects mosquito gut, with erythrocytes containing gametocytes, and initiates the sporogony. In the mosquito gut, upon their release, macro- and micro-gametocytes differentiate respectively, into female and male gametes and fertilization occurs to produce zygotes.

Fig. 3: Life cycle of the malaria causing parasites of *Plasmodium* species (adapted after Kumar et al. 2015). Five species of the unicellular protozoan Plasmodium that cause malaria in humans are *falciparum, vivax, ovale, malariae* and *knowlesi*. Genome sequences of all of these species are now known (Table 1). Humans get the transmitted malarial parasites

when infected anopheline female mosquito bites them to obtain blood as its food. There are more than 500 species of Anopheles mosquito of which more than 60 serve as malaria vector. Genome sequences of the entire major malaria vector *Anopheles* species have been described (Neafsey *et al.*, 2015). Life cycle of malarial *Plasmodium* species is complex and consists of several stages in each of the vertebrate and mosquito hosts. There are two asexual stages (in liver and red blood cells) and a sexual stage (in red blood cells) of Plasmodium life cycle in humans. **(a) Hepatic schizogony:** In this asexual phase the parasites inoculated into human host by mosquito, in the form of sporozoites, multiply asexually in liver parenchyma cells to produce a huge population of merozoites. Each sporozoite produces a few thousands of merozoites in schizonts. Not shown in the figure is an alternative pathway taken by parasites of *P. vivax* and *P. ovale* at this stage, wherein parasites enter a dormant phase to become hypnozoites. These latent parasites are the cause of relapse malarias, after several to many months from the initiation of infection as they reenter hepatic schizogony. **(b) Erythrocytic schizogony:** The liver produced merozoites infect red blood cells, wherein they pass into schizont stage after completion of ring and trophozoite stages. Each erythrocytic schizont produces many merozoites via asexual cell proliferation. Thus parasite load on the host increases enormously which leads to the appearance of clinical symptoms of malaria. **(c) Gametocytogenesis:** The pre-merozoites present in fraction of red blood cell schizonts get committed to sexual differentiation. Two types of gametocytes are formed: macro (female)- and micro-(male). The gametocytes are precursors of gametes. Gametocytes get ingested by the female mosquitoes that bite infected humans to obtain their blood meal. There are four stages of parasite development in the mosquito host. **(d) Gametogenesis:** Gametocytes ingested from human host enter into gametogenesis in the lumen of mosquito gut. Microgametocyte exits erythrocyte and differentiates into a female gamete. The microgametocyte upon exit from erythrocyte undergoes divisions there to produce eight flagellated male gametes by a process that has been called exflagellation. Fertilization involves fusion between a male gamete and a female gamete (both haploid like merozoites and gametocytes) to form zygote(s). **(e) Ookineteization:** One or more than one zygote is formed, depending on the number of female gametocytes ingested by the mosquito. Each zygote turns into a motile ookinete, during this processes its diploid nucleus undergoes meiosis to produce haploid nuclei. This is the stage wherein genetic recombination occurs resulting in the production of new genotypes of the parasite. **(f) Oocyst development:** Ookinete moves to form a cyst on the midgut epithelium. In the oocyst so produced, the haploid parasites multiply repeatedly to form thousands of sporozoites. **(g) Programming of sporozoites:** Oocyst ruptures and sporozoites get released into haemcoel. Sporozoites migrate to the salivary glands where they are programmed to eject and enter human skin at the time when infected mosquito carrying them probes the human host for a bite to obtain a blood meal. *P. knowlesi* has several macacque monkeys as vertebrate hosts in addition to humans. It has been experimentally shown that the following types of malaria transmission can occur: mosquito → monkey or human; monkey → monkey; human → human. However, there is little evidence for the natural human to human transmission of *knowlesi* malaria. Whereas the natural transmission of all kinds of malaria in human occurs when infected mosquito bites, malaria of all kinds can also be transmitted by mother to newborn, blood transfusion, organ transplanting or usage of contaminated needles or syringes.
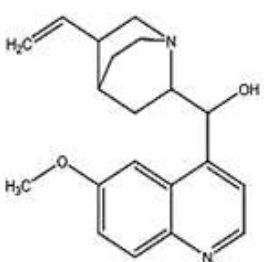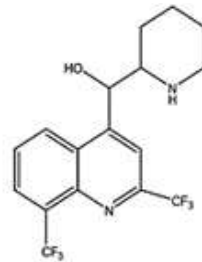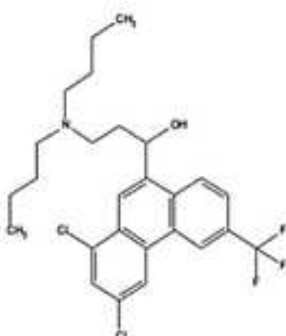
References

Barber *et al.*, 2017; White, 2017; Doerig *et al.*, 2015; Josling and Llianas, 2015; Guttery *et al.*, 2012; Janse *et al.*, 1986
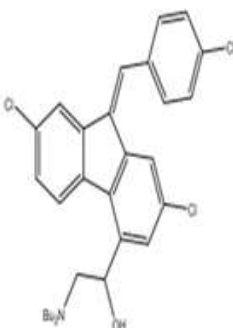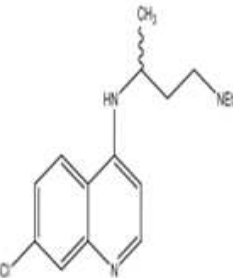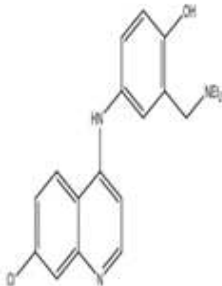
peaks, skin vasodilates, headache, myalgia and other symptoms are felt, and the period ends following sweating and recession of clinical symptoms. The period from inoculation of sporozoites to the first appearance of clinical symptoms is the incubation period of malaria, which varies according to the causal speacies. It is 8-15, 10-12, 10-21, 10-20 and 18-60 days respectively in Pf, Pk, Pv, Po and Pm malaria. The duration of erythrocytic infection to schizont rupture is 24 h in the Pk malaria, 48 h in Pf, Pv and Po malaria, and 72 h in Pm malaria. The pattern of appearance of fever and clinical symptoms is variable with mixed infection malarias. All of the 19 drugs listed in Table 2 are schizonticidal for erythrocytic scizonts and are therefore able to provide relief from malaria. The severe malaria results when erythrocytic multiplication cycles continue uninterrupledly.
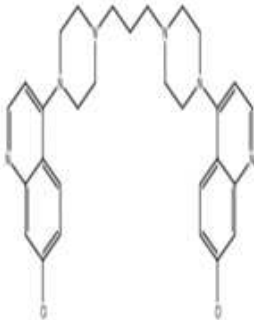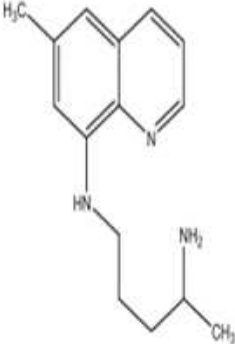
Individuals who have immunity, on account of previous malarial infections, can tolerate parasite densities such as $10^4/\mu L$. In immune patients, the parasite multiplication is slower and parasite clearance is faster, than in patients undergoing the first malaria infection. It is to be noted that a malaria attack does not make the individual fully immune to the subsequent attack of malarial diseases.
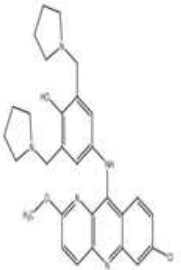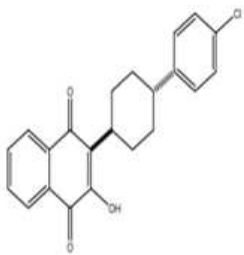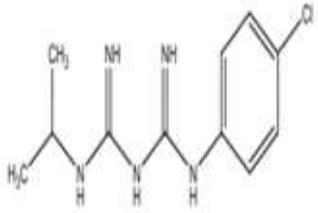
Some erythrocytic merozoites (>1%) grow and differentiate via five stages into male gametocytes (microgametocytes) and female gametocytes (macrogametocytes), the precursors, respectively, of male and female gametes. In all kinds of malaria, except Pf malaria, gametocytogenesis occurs in the peripheral blood. However, in Pf malaria, several young stages are completed in deep tissues, such as
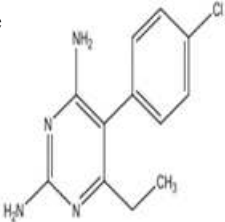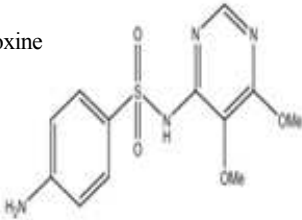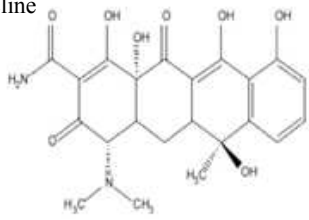
Table 2: Structure, activity and related features of different classes of antimalarials, that are presently in use against various developmental stages of *Plasmodium falciparum* (*Pf*), Plasmodium vivax (Pv), Plasmodium ovale (Po), Plasmodium malariae (Pm) and Plasmodium knowlesi (Pk) caused human malaria(s) and genetic markers of resistance detected against the antimalarials in P. falciparum and P. vivax
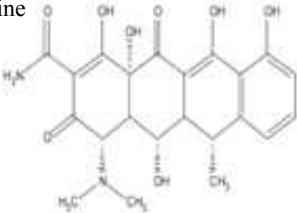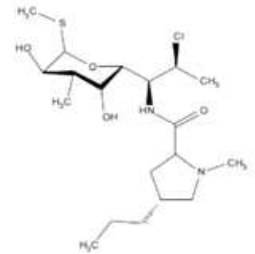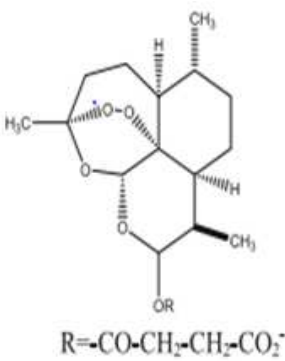
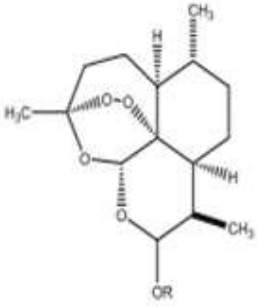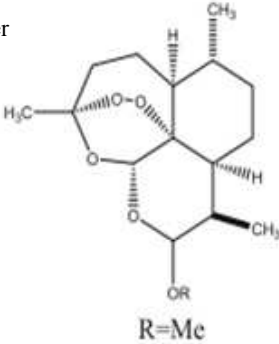| S.No.	Antimalarial compound (drug)				Prescription-cum-adminis-tration	Malarial parasite lifecycle stage(s) on which known to be active	Current knowledge about mecha-nism of action on parasite(s) and indication (purpose) of usage	Genetic markers, in *Plasmodium falciparum* (*Pf*) and *P. vivax* (*Pv*) arasites, for the resistance that has developed against the antimalarial	Remarks	Reference(s)
	Chemical class	Name	Structure	Biological half life (hours=h; days = d)						
I	II	III	IV	V	VI	VII	VIII	IX	X	XI
1	(A) Aryl amino-alcohols	Quinine		10-12 h	Parenteral or oral	Blood schiz-onticidal, gametocyto-cidal and sporontocidal	Inhibits heme detoxification; used to treat malaria unresponsive to other drugs and to control transmission	Single nucleotide polymorphisms (SNPs) in *Pfmdr 1a, Pfcrtb, Pfnhe1c, Pfmrp1d* and gene amplifi-cation in *Pfmdr1*	The drug is not to be given to the patients of tinnitus and optic neuritis	Cowman *et al.* (1994); Dondorp *et al.* (2010a); Achan *et al.* (2011); Lelievre *et al.* (2012); Gupta *et al.* (2014); Cui et al. (2015); Antony and Panja (2016); Lalloo *et al.* (2016); Dellicour *et al.* (2017)
2		Mefloquine		14-18 d	Oral 250mg tablets (Larium; is relatively expensive)	Blood schizont-icidal and sporon-tocidal against *Pf, Pv, Po* and *Pm* malaria	Inhibits heme detoxification and parasite's ribosomes; used in treatment of acute uncompli-cated malaria	SNPs and copy number increase in *Pfmdr1*; SNPs in *Pvmdr1*	It is not to be given to pregnant women and neuropsy-chiatric patients	Cowman *et al.* (1994); Sullivan *et al.* (1998); Price *et al.* (2004); Lelievre *et al.* (2012); Gargano *et al.* (2012); Gogtay and Ferner (2015); Grigg *et al.* (2016); Livezey *et al.* (2016)
3		Halofantrine		1-90 h	Oral 250mg tablets (Halfan; is relatively expensive), 1500mg in six doses over 18h period	Blood schizont-icidal in *Pf, Pv, Po* and *Pm* malaria	As above	As above	It is contra-indicated for cardiac disease patients	Croft *et al.* (2007); Friedman and Caflisch (2009); Oluyomi *et al.* 2009; Plucinski *et al.* (2015)

Richa Goel et al.

I	II	III IV	V	VI	VII	VIII	IX	X	XI
4		Lumefantrine	3-6 d	Oral (artemisinin-based combination therapy = ACT) tablets of 120mg lumefantrine and 20mg of artemether (Coartem); four tablets to be taken twice a day for 4 days	Blood schizonticidal and sporontocidal	Inhibits heme de-toxification; used together with artemether to treat acute malaria	SNPs and copy number increase in *Pfmdr1*	Safe medicine, including for children of 5 kg body weight	Ezzet *et al.* (2000); Sidhu *et al.* (2002&6 Naobya *et al.* (2010); Delves *et al.* (2012a,b); Slack *et al.* (2012); Agarwal *et al.* (2013); Hodel *et al.* (2013); Masanja *et al.* (2013); Laman *et al.* (2014); Plucinski *et al.* (2015); Soniler *et al.* (2017)
5	(B) 4-Amino-quinolines	Chloroquin	30-60 d	Oral tablets of 250 and 500 mg (Aralen; is the cheapest antimalarial drug); 2.5g is administered in 48h	Blood schizonticidal in *Pf, Pv, Po* & *Pm* malaria, and gametocytocidal in *Pv* malaria	Inhibits heme detoxification; used to treat acute uncomplicated malaria & along with Primaquine as first line cure for *Pv* malaria	SNPs in *Pfcrt, Pfmdr1,* and *Pfmrp1*; and *Pvmdr1,* and *Pvcrt1*	Contraindicated for the patients of hepatic disease	Sullivan *et al.* (1998); Woofton (2002); Rottman *et al.* (2010); Caldarelli *et al.* (2012); Krafts *et al.* (2012); Ecker *et al.* (2012); Chandra *et al.* (2013); Price *et al.* (2014); Galatas *et al.* (2017); Mwanza *et al.* (2017); Nyunt *et al.* (2017)
6		Amodiaquine	5h	Oral tablets of 200mg; 25-35mg/kg body weight over 3d period	Blood schizonticidal	Inhibits heme detoxification; treatment of acute uncomplicated malaria and alongwith sulfadoxine plus pyrimethamine for prophylaxis in travellers	SNPs and copy number increase in *Pfmdr1* and SNPs in *Pfcrt*	Contraindicated in patients of kidney and liver diseases and pregnant and breast feeding women	Kerb *et al.* (2009); Sa *et al.* (2009); Cairns *et al.* (2010); Bashir *et al.* (2010); Sutherland *et al.* (2011); Nair *et al.* (2012); Qiao *et al.* (2013); Maiga *et al.* (2016); York (2017)

I	II	III	IV	V	VI	VII	VIII	IX	X	XI
7		Piperaquine		30d	Oral 18mg/kg body weight of Piperaquine plus 4mg/kg body weight of dehydroartemisinin (ACT) for 3d	Blood schizonticidal; given in combination with dehydroartemisinin to treat acute malaria and for transmission control and prophylaxis	Inhibits heme detoxification; the ACT is safe and effective against acute malaria in adults and children	*Pf plasmepsin*-2, -3e gene amplification	Contraindicated in pregnancy	Davis *et al*. (2005); Moore *et al*. (2008); Dalessandro (2009); Friedman and Caflisch (2009); Naobya *et al*. (2010); Rijken *et al*. (2011); Delves *et al*. (2012a,b); Levelievre *et al*. (2012); Stack *et al*. (2012); Agarwal *et al*. (2013); Hodel *et al*. (2013); Masanja *et al*. (2013); Amato *et al*. (2016); Witkowski *et al*. (2017); Hoglund *et al*. (2017); Bassat and Menendez (2017); Thanh *et al*. (2017)
8	(C) 8-Amino-quinoline	Primaquine		7h	Oral 15mg and 26.3mg tablets	Eliminates hypnozoites, liver stage schizonts, and gametocytes and has very weak blood stage activity against *Pf* malaria	Impairs mitochondrial functions; to cure and prevent relapse of *Pv* and *Po* malaria and prevention of *Pv* malaria; stops transmission from humans to mosquitoes	Resistance is known but not the markers	Contraindicated for pregnant and breast feeding women and glucose 6 phosphate dehydrogenase (G6PD) deficient individuals	Rieckmann *et al*. (1968); Dutta *et al*. (1989); Hill *et al*. (2002); Baird and Hoffman (2004); Mazier *et al*. (2009); Wells *et al*. (2010); Dondorp *et al*. (2010); Dow *et al*. (2011); Kheng *et al*. (2011); Bediako *et al*. (2011); Graves *et al*.(2009&12); La Crue *et al*. (2013); Howes *et al*. (2013); Duffy and Avery (2013a,b); White (2013); White *et al*. (2014); Eziefula *et al*. (2014); Ashley *et al*. (2014); Rajgor *et al*. (2014); Devine *et al*. (2017); Milligans *et al*. (2017); Dicko *et al*. (2018); Graves *et al*. (2018); Tine *et al*. (2017)

I	II	III	IV	V	VI	VII	VIII	IX	X	XI
9	(D) Mannich base	Pyronaridine		<3-8d	Oral (ACT) tablets of 180 mg Pyronaridine tetraphosphate plus 60 mg of Artesunate (Pyramax); given one/d for 3d	Blood schizonticidal	Inhibits β-haemetin formation	SNPs in *Pfmrp1*	Not to be given to the patients of liver disease	Chang *et al.* (1992); Auparakkitanon *et al.* (2006); Croft *et al.* (2012); Peatey *et al.* (2012); Delves *et al.* (2012); Duffy and Avery (2013a,b); Gupta *et al.* (2014); Leang *et al.* (2016)
10	(E) Napthaquinone	Atovaquone		59h	Oral tablet of 1g Atovaquone and 400mg Proguanil (Malarone); one tablet/d for 3d, lower doses for children and 25% strength tablet for prevention	Liver stage and blood stage schizonticidal, and sporonticidal	Acts against mitochondrial bc1 complex and inhibits parasite's respiration process; given in combiation with Proguanil to treat acute malaria and for prophylaxis	SNPs in *Pfcytb*[b]	Not to be given to the patients of hypersensitive reactions and renal impairment	Rieckmann *et al.* (1968); Fowler *et al.* (1995); Srivastava and Vaidya (1999); Farnert *et al.* (2003); Gil *et al.* (2003); Camus *et al.* (2004); Osci-Akoto *et al.* (2009); La Crue *et al.* (2013); Cordel *et al.* (2013); Goodman *et al.* (2016); Saunders *et al.* (2016); Staines *et al.* (2017)
11	(F) Antifolates	Proguanil		24h	As above	Mild liver stage schizonticidal and blood stage schizonticidal for *Pf, Pv, Po* malaria	Inhibits dihydrofolate reductase (DHFR) synthesis or folate synthesis; the drug is administered together with Atovaquone or Chloroquine to treat acute malaria and for prophylaxis	SNPs in *Pfdhfrg* and *Pvdhfr*	As above	Fowler *et al.* (1995); Srivastava and Vaidya (1999); Boggild *et al.* (2007); Osci-Akoto *et al.* (2009); La Crue *et al.* (2013); Cordel *et al.* (2013); Cottrell *et al.* (2014); Lachish *et al.* (2016); Lalloo *et al.* (2016); Saunders *et al.* (2016); Staines *et al.* (2017)

I	II	III	IV	V	VI	VII	VIII	IX	X	XI
12		Pyrimethamine		<3->4 d	Oral tablet of 25mg Pyrimethamine and 500mg of Sulfado-xine (Fansidar; is a cost effective drug); adults to take 2-3 tablets/week	Mild liver stage schizonticidal, blood stage schizonticidal and sporonto-cidal	Inhibits dihy-drofolate reduc-tase (DHFR) synthesis and thereby folate synthesis; it is given along with sulfadoxine to cure acute malaria, even in pregnant women	As above	Not to be given to the patients of megaloblastic anemia and folate deficiency	Sibley *et al.* (2001); Gatton *et al.* (2004); Mugittu *et al.* (2005); Donderp *et al.* (2010); Tanaka and Williams (2011); Gujjar *et al.* (2011); Kiszewski *et al.* (2011); Huang *et al.* (2012); Calderon *et al.* (2012); Duffy and Avery (2013a,b); Gadalis (2013); Mishra (2014); Misra *et al.* (2014); Desai *et al.* (2016); Goel *et al.* (2016); Cisse *et al.* (2017); Maiga *et al.* (2017); Ayubu and Kidima (2017)
13		Sulfadoxine		<4->8 d	As above	Blood stage schizonticidal in Pf malaria but relatively ineffe-ctive in *Pv* malaria	Inhibits the dihydropteroate synthesis (DHPS) and thereby folate synthesis; indication as above	SNPs in *Pfdhpsh* and *Pvdhps*	As above and also not to be given in the patients of sulphur allergy	As above and Rieckmann *et al.* (1968); Pukrittayakamee *et al.* (2000); Ayubu and Kidima (2017); Guhman and Slutsker (2017)
14	(G) Anti-biotics	Tetracycline		8-11h	Oral 250mg tablets (Sumycin and other brands; is cost effective	Slow acting liver and blood stage schizonticidal	Protein syn-thesis inhibitor; given along with Quinine	Resistance is not known	Not to be given to children of <8y age	Ruiz-Sanchez *et al.* (1956), Dahl *et al.* (2006); Briolant *et al.* (2008); Tan *et al.* (2011); Gaillard *et al.* (2015 and 2016)

I	II	III	IV	V	VI	VII	VIII	IX	X	XI
15		Doxycycline		15-25h	Oral 250mg tablets; is highly cost effective	As above	Inhibitor of protein synthesis; given along with fast acting anti-malarial to cure acute malaria and as prophylaxis for travellers	As above	Not to be given to pregnant women	As above and Gaillard *et al.* (2017)
16		Clindamycin		3h	Oral 75mg to 900mg capsules; is cost effective	Blood schizonticidal	Inhibition of protein synthesis, given along with Quinine to patients unable to tolerate Tetracycline	As above	Not to be given to patients of intestinal disease	Burkhardt *et al.* (2007); Daulay *et al.* (2011); Obonya and Juma (2012); Al-Zaydani *et al.* (2016), Lalloo *et al.* (2016); Gaillard *et al.* (2017)
17	(H) Endope- roxides	Artesunate	R=-CO-CH₂-CH₂-CO₂⁻	0.5-1.4h	Oral as ACT@ 4mg/kg body wt of Artesunate with 10mg/kg body wt of Amodiaquine or 25mg/kg body wt of Mefloquine or 25mg/kg body wt of each of Sulfado-xine and Pyri-methamine; all for 3d; or Artesunate as suppository	Fast acting liver and blood stage schizonticidal, incomplete game-tocytocidal and sporontocidal; given along with combination ACT in acute malaria; parenterally and as suppository in cerebral malaria prescribed to cure acute malaria as ACT partner and in severe malaria	Fe (II) present in heme proteins clears the peroxide bond, the free radicals thus generated react with essential parasite proteins inactivating them, such as sarcoplasmic endoplasmic reti-culum calcium ATPase and heme metabolism;	SNPs in *Pf kelch-13*[i]; (the haptotype *C580Y* is undergoing hard selective sweep in Southeast Asia); amplification of *Pfmdr1*	Not to be given to the patients of respiratory disease, and pregnant and lactating women	Pukrittayakamee *et al.* (2000); Dondorp *et al.* (2009); Rottman *et al.* (2010); Charman *et al.* (2011); Zhang *et al.* (2012); Barber *et al.* (2013); Delves *et al.* (2013); Klonis *et al.* (2013); Ashley *et al.* (2014a); Ashley *et al.* (2014b); Ariey *et al.* (2014); Henriques *et al.* (2014); White (2014); Gopalkrishnan and Kumar (2015); Strainer *et al.* (2015); Cui *et al.* (2015); Tilley *et al.* (2016); wwwarn (2016); Antony and Panja (2016); Al-Zaydani *et al.* (2016); Kremsner *et al.* (2016); Hemming-Shroeden and Lo (2017); Imwong *et al.* (2017); Abraham (2017)

I	II	III	IV	V	VI	VII	VIII	IX	X	XI
18		Dihydro-artemisinin	R=H	0.5-0.75h	As shown at serial no. 7	Fastest acting Artemisinin derivative that does not allow trophozoite formation and is gametocytocidal and sporontocidal	Mode of action as above; treatment of acute malaria as an ACT component	As above	As above	Sinclair *et al.* (2009); Keating (2012); Tarning *et al.* (2012); Gargano *et al.* (2012); Zani *et al.* (2014a,b); Amartunga *et al.* (2016); Tillay *et al.* (2016); Kakaru *et al.* (2016); Anato *et al.* (2017); Bassat and Menandez (2017), Phu *et al.* (2017)
19		Artemether	R=Me	4-11h	As shown at serial no. 4; the drug is lipid soluble and is used orally, rectally and intramuscularly	Mode of action like Artesunate; As an ACT it is a frontline drug for acute malaria	As above	As above	As above	Mc Gready *et al.* (2008); Hatz *et al.* (2008); Achan *et al.* (2009); Sutherland *et al.* (2012); Agarwal *et al.* (2013); Hodel *et al.* (2013); Esu *et al.* (2014); Chandira *et al.* (2014); Sonden *et al.* (2017); Rutledge *et al.* (2017); Chikwu *et al.* (2017); Phuc *et al.* (2017)

a-i = Antimalarial resistant drug alleles: a, *mdr1* = Multi-drug resistance gene on chromosome 5; b, *crt* = Chloroquine resistance transporter gene on chromosome 7; c, *nhe1* = Sodium/hydrogen exchanger gene on chromosome 13; d, *mrp1* = Multi-drug resistance associated proteins gene on chromosome 1; e, *plasmepsin-2, -3* = Aspartyl (protease plasmepsin gene located on chromosome 4; f, *cyt b* = cytochrome B gene on mitochondrial genome; g, *dhfr* = Dihydrofolate reductase gene on chromosome 8; h, *dhps* = Dihydropteroate synthetase gene on chromosome 4; i, *kelch-13* = Kelch 13 propeller gene located on chromosome 13, (the gene product has three domains an apicomplex domain, a BIB/POZ domain and a ß-propeller Kelch domain)

bone marrow and only the final stage is accomplished in the blood stream. Generally, the gametocytes are short-lived, except that in Pf malaria, once formed they remain viable for months. The gametocytes are not responsible for any clinical symptoms. The following anti-malarials are able to block gametocyte formation: quinine and chloroquine in Pv and Pm malarias; and primaquine and artemisinins in all kinds of malarias. The artemisinins do not eliminate gametocytes completely.

The route of malaria spread in human is human → female mosquito → human. Mosquitoes transmit the gametocytes from infected humans as a part of their blood meal. In the mosquito gut, the gametocytes exit erythrocytes and undergo gametogenesis. A female gametocyte develops into a female gamete. A male gametocyte divides into 8 male gametes, each male gamete is flagellated. The gametes, gametocytes, merozoites and sporozoites are all haploid (n = 14). The haploid male and female gametes fuse to form a zygote (2n = 28) which differentiates into an ookinete. The ookinete migrates to the epithelial cell wall of the gut to form an oocyst. The oocyst undergoes meiosis and subsequently the haploid products in it multiply to produce thousands of sporozoites. Upon rupture of oocysts, the sporozoites migrate to salivary glands where they get programmed for inoculation into human host via carrier mosquitoe's bite. The sporogony cycle from gamete formation to sporozoite production in oocysts and their accumulation in salivary glands is blocked by several antimalarial drugs, including the following: quinine, mefloquine, lumefantrine, atavaquone, proguanil, primaquine, methylene blue and pyromethamine.

Treatments Based on Currently Used Antimalarial Drugs

The Table 3 presents a summarized account of first line treatments, for acute and severe malaria caused by different species of malaria parasites in adults, pregnant women and young children, recommended by WHO. The recommendations are a result of the scores of trials carried out, for the cure of different kind of malaria in endemic areas owing to their occurrence in Africa, Americas, Pacific islands, Southeast-and South-Asia, on adult men and women, pregnant and lactating women and young children. Some of the references on which the WHO recommendations are based are given at the bottom of the Table 3. The dosages of drugs for children are to be adjusted with the body weight. Some drugs are prescribed when malarial patients suffer from concurrent ailments or inherited metabolic deficiencies, the prescriptions for each of the antimalarial drugs are given in the Table 2. Most importantly, primaquine is not to be administered to pregnant and/or breast feeding women. Because severe malaria patients can suffer from blockage in blood flow, filling up of fluid in lung's air sacks, clotting in blood vessel, renal failure, and/or seizures etc., they must be treated under intensive care environment. Any concurrent bacterial infection in malaria patients should receive immediate attention, along with malaria treatment.

The options for the treatment of uncomplicated malaria in adult men and women are: artemether + lumefantrine; dihydro-artemisinin + piperaquine; atavaquone + proguanil; quinine + doxycycline. Along with a drug combination, a dose of primaquine ensures control of transmission. The treatment for Pv, Po or mixed malaria is one of the following: artemether + lumefantrine; dihydro-artemisinin + piperaquine; chloroquine. For Pm and Pk malaria, the drug recommended is chloroquine. The drug options for children against Pf malaria are: artemether + lumefantrine; dihydroartemisinin + piperaquine; atavaquone + proguanil; quinine + clindamycin. Chloroquine is the drug recommended for children against Pv, Po, Pm and Pk malaria. The pregnant women afflicted with any kind of malaria are recommended to use quinine + clindamycine or artemether + lumefantrine and those having non- Pf malaria are also recommended chloroquine. The treatment options for the patients with severe malaria in adult men and women caused by all kinds of parasites are: intravenous artesunate for one or more days until the patient can swallow tablets, but not more than 5 days, followed by a full course of artemether + lumefantrine, dihydro-artemisinin + piperaquine or quinine + tetracycline; or intravenous quinine for 2 days or until the patient can begin to swallow tablets, followed by a full course of quinine + doxycycline. In the severe malarial cases caused by Pv or Pk, the intravenous treatments are to be followed by full course of chloroquine. The pregnant women patients with severe Pf malaria are to be given intravenous artesunate or quinine treatments, like that for adult

Table 3: The prevalent antimalarial treatment regimens against un-complicated and complicated malaria(s) in adults, pregnant and breast feeding women and children

S.No.	Indicative malarial condition	Malaria caused by *Plasmodium falciparum*	*Plasmodium vivax, P. ovale* or of these mixed with *P. falciparum*	*P. vivax, P. ovale, P. malariae* or *P. knowlesi*
1	(A) Uncomplicated malaria: (i) in adults	Oral: Artemether + Lumefantrine (the drug(s) of choice)[a]; Dihydroartemisinin + Piperaquine (not to be administered to patients suffering from cardiac condition(s))[b]; Atovaquone+ Proguanil (known to produce pronounced gastrointestinal side effects)[c]; Quinine + Doxycycline[d]; and a single dose of 0.25mg/kg body weight of Primaquine on the first day or 15 mg/Kg of Methylene blue for three days	Oral: Artemether + Lumefantrine[a]; Dihydroartemisinin + Piperaquine[b] or Chloroquinem	Oral: Chloroquine[m]
2	(ii) in pregnant women	Oral: Quinine + Clindamycin (in: all trimesters)[e]; or Artemether + Lumefantrine (in all trimesters)	As in column 2; item 2; or Chloroquine[m]	As in column 3; item 2
3	(iii) in children (< 12 years)	Oral: Quinine + Clindamycin[e]; Atovaquone + Proguanil[f]; Artemether + Lumefantrine[g]; or Dihydroartemisinin + Piperaquine[h]	Chloroquinen	Chloroquinen
4	(B) Severe or complicated malaria: (i) in adults	Intravenous Artesunate for 24h or more (or until the patient can swallow tablets, but not more than 5 days)[i], followed by a full course of Artemether + Lumefantrine[a] or of Dihydroartemisinin + Piperaquineb or Intravenous Artesunate treatmenti followed by a full course of Quinine + Doxycycline[d]; or alternatively Intravenous Quinine[j] for 48 hours or until the patient is able to swallow tablets, followed by oral Quinine + Doxycycline[k]	As in column 2; item 4; or intravenous artesunate treatment followed by full course of Chloroquine	As in column 2; item 4
5	(ii) in pregnant women	Intravenous Artesunatei, followed by Artemether + Lumefantrine or Dihydroartemisinin + Piperaquine as in item 4 above; or Intravenous Quinine followed by a course of Quinine + Clindamycinl	Oral: Chloroquine (in all trimesters); Artemether + Lumefantrine (in all trimesters)	As in column 2; item 4
6	(iii) in children (< 12 years)	A rectal suppository dose of upto 100mg (10mg/kg body weight) Artesunate followed by intravenous Artesunate or Quinine and thereafter dihydroartemisinin + Piperaquine or Quinine + Clindamycin; as for adults with dosage adjusted as per body weight	As in column 2	As in column 2
7	Relapse (prevention): (i) in adults	Not applicable	Primaquineo (not to be administered to Glucose-6-phosphate dehydrogenase = G6PD deficient)[p]	As in column 3
8	(ii) in pregnant and breast feeding women	As above	Chloroquineq followed by Primaquine upon withdrawl of breast feeding	As in column 3

a = 4 tablets (such as of Coartem) followed by 4 tablets at 0, 8, 24, 36, 48 and 60 hours; b = 3 or 4 tablets (such as of Eurartesim) daily for 3 days; c = 4 tablets (such as of Malarone) daily for 3 days; d = 600mg Quinine sulphate every 8h for 5-7 days and 200mg Doxycycline daily; e = 600 mg Quinine sulphate every 8 h plus 450 mg Clindamycin every 8 hours for 7 days; f = 1 to 4 Malarone paediatric tablets (as per body weight from = 10 kg to = 40 kg); g = 1-4 tablets at 0, 8, 24, 36, 48 and 60 hours (as per body weight from = 15 kg to = 35 kg); h = ½ to 3 tablets followed by equal amount at 24 and 48 hours (as per body weight = 10 kg to = 60 kg); i = 2.4 mg/kg body weight injection of Artesunate at 0, 12 and 24 h and thereafter daily; j = starting dose of 20mg/kg body weight of Quinine hydrochloride in 5% dextrose over a 4h period, followed by 10mg/kg body weight of Quinine hydrochloride every 8h for upto 48h and later every 12h; k = 600mg Quinine sulphate three times a day for 5 to 7 days from the start of Quinine therapy, plus oral 200mg of Doxycycline each day for 7 days; l = Intravenous Quinine therapy to be followed by oral quinine, like in k, except in place of Doxycycline, Clindamycin (450mg) will be administered 3 times a day for a period of 7 days; m = 620mg at 0h, 310mg at 8h and 310mg on day2 and 3; n = 10 mg starting dose, then 5mg/kg at 8h and also on day 2 and 3; o = 15 to 30mg/day or 0.2-0.5 mg/kg body weight/day

for 14 days depending on body weight; p = The G6PD deficients may be adminstered 0.75mg/kg of Primaquine per week for 8 weeks; q = 500mg each week

References: Dicko *et al*. (2018) ; Abraham (2017); Antinori *et al*. (2017); Dellicour *et al*. (2017); Phuc *et al*. (2017); Plucinski *et al*. (2017); Thanh *et al*. (2017); Tine *et al*. (2017); Baird *et al*. (2016); Gutman *et al*. (2016); Herchline (2016); Kakaru *et al*. (2016); Kremsner *et al*. (2016); Lalloo *et al*. (2016); Pekyi *et al*. (2016); Sirima *et al*. (2016); Verlinden *et al*. (2016); White (2016); Kheng *et al*. (2015); Kumar *et al*. (2015); Shanks *et al*. (2015); Sharma *et al*. (2015); Eziefula *et al*. (2014); White *et al*. (2014); WHO (2014); Barber *et al*. (2013); Cordel *et al*. (2013); Delves *et al*. (2013); Gogtay *et al*. (2013); Singh and Daneshwar (2013); Eder *et al*. (2012); Keating (2012); Obonyo and Juma (2012); Achan *et al*. (2011); Daulay *et al*. (2011); Rijken *et al*. (2011); Kiszewski *et al*. (2011); Dondorp *et al*. (2010); RCOG (2010); Achan *et al*. (2009); Sinclair *et al*. (2009); Hatz *et al*. (2008); Mc Gready *et al*. (2008); Hill *et al*. (2006); Karunajeewa *et al*. (2006); Kumar and Srivastava (2005); Maitland *et al*. (2005); Baird and Hoffman (2004); Dondorp *et al*. (2005); Newton *et al*. (2013); van Hansbrock *et al*. (1996); White *et al*. (1983); WHO-HTM-GMP-2017.9-org.pdf; www.contrmicrb.org/new/bo.5.asp; www.cdc.gov/malaria/diagnosis_treatment/treatment.html; www.who.int/malaria/publications/atoz/9789241548528/en; www//apps.who.int/iris/bitstream/10665/181162/1/9789241509244_eng.pdf

men and women. Whereas artesunate treated pregnant women patients are to be given a full oral course of artemether + lumefantrine or dihydro artemisinin + piperaquine, those who received intravenous quinine will be given full oral course of quinine + clindamycin. The severely ill pregnant women, with any non-Pf malaria, will be given a full course of oral chloroquine or atremether plus lumefantrin, irrespective of the trimester of pregnancy. Young children with complicated malaria are to be first treated with artesunate given rectally, followed by the treatments (with dose adjustment according to the patients's body weight) recommended for severely ill adult patients.

Natural Selection of Genetic Resistance to Currently Used Antimalarial Drugs, in Malarial Parasites

Like other organisms, human malarial parasite species are endowed to evolve, by induction of new mutations, amplification of variation by genetic recombination and selection of variant genotypes, for improved adaptation to changing environmental conditions, which in their case include; lethal anti-malarial drugs used singly and in combinations. The genetic variants of parasites become resistant to a drug when the gene product(s) targeted by the drug have become modified or the drug does not reach the site of its action because the process of its transport is disabled or it is effluxed out from its site of action. Drug resistance is acquired via selection of mutations that result in over expression and/or increased activity of one or more of target gene function(s) or deactivation of one or more host protective gene function(s). Several life cycle characteristics of malarial parasites, such as these mentioned below, are favourable for selection of drug

resistance imparting mutations: (a) rapid rate of genome replication (Agarwal *et al.*, 2017) ; (b) high mutation rate per genome replication cycle (Hamilton *et al.*, 2017; William *et al.*, 2017); (c) large population size within infected human and mosquito hosts (Table 1; Churcher *et al.*, 2017); and (d) enormity of overall population size of parasites in humans and mosquito hosts in malaria endemic areas (Duffy *et al.*, 2017). Besides, selection of drug resistant mutations also depends on the drug pharmacokinetics, purity and dosage; irregular and sub-optimal intake of drug favours selection of genetic drug resistance in parasite (Anyanwu *et al.*, 2017). The combinatorial use of drugs with differing pharmacokinetics and mode of action, such as in ACTs, delay development of resistance against the drugs (Dondorp *et al.*, 2009). Drug resistance in malarial parasites is known to have arisen faster for those drugs that targeted only one gene function in them. For example, resistance against proguanil, atavaquone, pyrimethamine and sulfadoxine was selected and became observable within one or two years of their large scale usage (Paloque *et al.*, 2016). Contrariwise, resistance against artemisinins, which have multiple targets in parasites, was observable 26 years after their large scale therapeutic adoption (Paloque *et al.*, 2016).

The malarial parasite cells have in them a nuclear genome (n =14), and copies of mitochondrial and apicoplast organelle genomes (Gardner *et al.*, 2002; Vaidya and Mather, 2009; Rai *et al.*, 2017). The comparison of genome sequences of the sensitive and resistant isolates (clones) of parasites have allowed identification and mapping of molecular markers (point mutation, deletion and insertion polymorphisms) associated with anti-malarial

resistance. The molecular markers for resistance to different drugs have been found to be located on several different nuclear chromosomes and resistance to atovaquone to be located on the mitochondrial genome (Sharma, 2005; Antony and Parija, 2016; Menard and Dondorp, 2017). In the Table 2, the column VIII gives information about the target(s) of the 19 drugs; and the column IX lists molecular markers found associated with resistance selected in parasites for 15 drugs. The chromosomes in which drug resistant mutations are located are identified in the legend of Table 2.

Resistance of parasites towards tetracycline, doxycycline and clindamycin has not been observed and the identity of gene polymorphism(s) responsible for resistance against primaquine is yet to be established. It will be seen that mutations in the *CRT* and *MDR1* genes render the parasite variably resistant to several drugs. The *Pf crt* polymorphism affects response of the parasite to quinine, amodiaquine, piperaquine and lumefantrine in addition to giving resistance to chloroquine. *Pf mdr1* poses resistance to chloroquine, quinine, mefloquine, halofantrine, lumefantrine and artemisinin. There are examples of different mutant alleles in the same gene inducing differential responses in the parasite; mutations in the amino terminal and carboxy terminal of *Pf MDR1* are of this type (Cui *et al.*, 2015; Antony and Parija, 2016; Menard and Dondorp, 2017). Mutational polymorphism in the *Pf kelch* 13 gene has been responsible for resistance to artemisinin (Ashley *et al.*, 2014; Miotto *et al.*, 2015; Takala-Harrison *et al.*, 2015; Amato *et al.*, 2016; Li *et al.*, 2017); 20 SNPs are known to have arisen independently in different locations – an example of multiple origin of soft sweep (adaptation to ART; Pennings and Hermisson, 2006). Among the *Pf k 13* mutations, the *C580Y* allele not only imparted ART resistance to *Plasmodium falciparum*, but made the parasite fitter (Imwong *et al.*, 2017). It got selected in the background of *Pf crt, Pf dhps, Pfdhfr* and *Pfmrd1* mutations and its hard sweep has spread the allele widely in southeast Asia (WHO-multidrug-resistant-lineages-; Hemming-Schroedar and Lo, 2017; Imwong *et al.*, 2017; Thanh *et al.*, 2017). A symptom of ART resistance due to a *C580Y* type mutation is recrudescence or reappearance of Pf infection upon depletion or removal of artemisinin.

Many ring stage intra-erythrocytic *C580Y* parasites enter a quiescent state, because upregulation of the UPR (unfolded protein response) signaling pathway in them allows repair of proteins that underwent alkylation or oxidation, by free radicals released by disintegration of the peroxide bridge in ART when it comes in contact with iron from haemoglobin. The quiescent parasites are supported by minimal energy metabolism taking place in the parasite's apicoplast and mitochondria. Quiescent parasites breakout as soon as ART is depleted (Mbengue *et al.*, 2015; Paloque *et al.*, 2016). Recently (Rossi *et al.*, 2017), emergence of *P. falciparum* strains with *pfmdr1*, *pfplasmepsin2* and *pfkelch13 580Y* triple mutations has been recorded in Cambodia.

There is emergent danger of this (*C580Y* type) and other multi-drug resistant strains getting entry into India and Africa. There is also evidence for *de novo* ACT resistance development in African areas of high Pf malaria endemicity (Lu *et al.*, 2017; Daddy *et al.*, 2017). Another observation of great concern is that artemisinin-resistant Pf clinical isolates, including perhaps the ACT resistant ones, can infect diverse mosquito species that transmit malaria in Southeast Asia and Africa (St. Laurent *et al.*, 2015). WHO has developed a global technical strategy to control and eliminate malaria worldwide by 2030 (WHO:GST_Malaria_Eng.pdf). Some leading workers in this field have proposed additional strategies for malarial treatment and vector control and there has been emergence of a new treatment- the *A. annua* dry leaf therapy (ALT) for treatment of drug resistant malaria, which are discussed below.

Strategies Proposed to Treat and Control Multidrug-Resistant Malaria

The studies summarized in table 2 shows that resistance has developed against antimalarial drug in current use singly or in two drug combinations. It is visualized that in the absence of new drugs and vaccines (Kazmin *et al.*, 2017; Zhang *et al.*, 2018) in the near future, there is an urgent need to use the existing drugs in better ways and new combinations. The two treatments advised for chloroquine resistant Pv malaria are: (a) dihydroartemisinin + piperaquine with a dose of primaquine (White, 2016), and (b) administration of verapmil, the calcium channel blocker which serves as a chemo sensitizer, along

with chloroquine to improve drug efficiency (Verlinden *et al.*, 2016). The possible treatments advised for ACT resistant Pf malaria are: (a) A new ACT combination of artesunate + pyronaridine to be introduced as treatment (White, 2016). (b) ACTs such as dihydroartemisinin + piperaquine and artesunate + mefloquine be used rotationally (Dondorp *et al.*, 2017; Menard and Dondorp, 2017). (c) The period of use of prevalent ACTs to be extended from 3 days to up to 7 days (Dondorp *et al.*, 2017; Menard and Dondorp, 2017). (d) ACTs to be used as combinations of a artemisinin drug with two partner drugs, such as artemether + lumefantrine + amodiaquine, and dihydroartemisinin + piperaquine + mefloquine (Shanks *et al.*, 2015; Verlinden *et al.*, 2016; Dondorp *et al.*, 2017; Menard and Dondorp, 2017). (e) The double and triple drug ACTs to be used sequentially (Verlinden *et al.*, 2016). (f) The combination of fosmidomicin and piperaquine to serve as a sure cure (Mombo-Ngoma *et al.* 2017). Another important suggestion is administration of a dose of the drug ivermectin in endemic areas along with the ACT or singly periodically on a mass scale (Dondorp *et al.*, 2017; Menard and Dondorp, 2017). Ivermectin taken by mosquitoes along with the blood meal of ivermectin administered humans will have killing effect on them, thereby drastically controlling the malarial transmission (Chaccour *et al.*, 2013; Kumar *et al.*, 2015).

An entirely new strategy to treat multi-drug (ACT) resistant malaria has been developed, wherein tablets made of dried leaves of the *A. annua* plant (natural resource of artemisinin drugs) are used (Daddy *et al.*, 2017). The origin and essential features of this highly affordable malaria therapy are discussed below.

Artemisia Annua Dry Leaf Antimalarial Therapy (ALT)

The ALT has been earlier called as the whole Plant based Artemisinin Combination Therapy (pACT; Weathers *et al.*, 2014a). pACT was called a combination therapy because of the involvement of artemisinin and other metabolites present in the leaves of *A. annua* in the antimalarial therapeutic effect of *Artemisia annua* dry leaves (Weathers *et al.*, 2011, 2013, 2014b a and b; Elfawal *et al.* 2012; Onimus *et al.*, 2013; Weathers and Towler, 2014; Desrosiers and Weathers, 2016; Daddy *et al.*, 2017). ALT is unlike

the conventional ACTs (mentioned in Tables 2 and 3), in which the artemisinin component, extracted from *A. annua* (Kumar *et al.*, 2000; Misra *et al.*, 2014) or artemisinin synthesizing transgenic tobacco or *Physcomitrella patens* whole plant (Malhotra *et al.*, 2016; Ikram *et al.*, 2017), or semi-synthesized from *Artemisia annua* produced natural precursor(s) (Paddon *et al.*, 2013; Paddon and Keasling, 2014; Ikram and Simonsen, 2017; Kung *et al.* 2018) is present in its derived pharmaceutical forms such as artesunate, artemether and dihydroartemisinin. ALT is a non-pharmaceutical antimalarial treatment that depends on artemisinin and many other metabolites naturally biosynthesized and present in the leaves of *Artemisia annua* plant, but for many of which the mode(s) of antimalarial action remains to be revealed. To get WHO recommendation, ALT has to go through extensive, essential, fundamental and clinical research which needs to demonstrate that ALT is safe, efficacious and would not promote development of resistance to artemisinin in malarial parasites (WHO, 2012).

ALT uses standardized tablets as the anti-malarial drug prepared by compressing the dried pulverized leaves, harvested from cultivated plants of specific variety(ies) of *Artemisia annua*, which contain >1% artemisinin (Weathers and Towler, 2014; Daddy *et al.*, 2017). The origin of ALT, as a dependable medicine against multi-drug-resistant malaria, is based on information from historical texts and a number of experimental findings. Some of the important empirical basis for ALT is annotated below:

(a) There is recorded evidence that the Chinese people have been using *A. annua* material as a remedy for fever and chills, such as those associated with malaria. One of the effective material consumed as traditional medicine was the consumption of juicy extract of water soaked *A. annua* leafy stems. The chinese traditional medicine literature does not report any case of resistance development against *A. annua* treatment used (Bensky and Barolet, 1990; Dhingra *et al.*, 1999; Hongwen and Shouming, 2002; Hsu, 2006).

(b) The *A. annua* plant material has been used by human populations in various parts of the world, where the species existed naturally for various

purposes, including for medicinal uses and as an item of food for livestock and humans, without notice of any harmful effects (Bansky and Barolet, 1990; Brisibe *et al.*, 2009; Yimer and Sahu, 2016) and therefore the species has been granted the GRAS (Generally Recognized As Safe) rating. Accordingly, *A. annua* leaves in amounts d" 30 g dry weight / day can be safely consumed (Duke, 2001; Wall and Watson, 2017).

(c) In a study, batches of healthy mice were orally fed on one hand, with an amount of artemisinin in its pure form, and on the other, were fed an equal amount of artemisinin in the form of *A. annua* dried leaves. The blood stream of mice fed with dry leaves contained > 40 times more artemisinin, as compared to mice fed with pure artemisinin. Mice were required to be fed with > 45 fold more of pure artemisinin (as a component of the normal mouse food) than artemisinin in dry *A. annua* leaves so that artemisinin could be detected in mouse blood stream (Weathers *et al.*, 2011).

(d) In another study, it was observed that oral administration of the *Artemisia annua* leaves to the *Plasmodium chabaudi* - infected mice killed the parasite without causing toxicity to mice (ICIPE 2005; Elfawal *et al.*, 2012; Onimus *et al.*, 2013). It was further found that parasitaemia in the infected mice was reduced at least five fold more by a single dose of *A. annua* leaves as compared to an equivalent dose of pure artemisinin, and the effect of dry leaves lasted longer than that of pure artemisinin (Elfawal *et al.*, 2012). The experiments at c and d above suggested that the presence of metabolites other than artemisinin in the dry leaves of *A. annua* improved both the bioavailability of artemisinin in the blood stream and therapeutic efficacy of artemisinin in the infected red blood cells. These possibilities were evidenced by correlating the phytochemistry of *A. annua* leaves with the response of healthy and parasite infected mice to feeding of pure artemisinin versus *A. annua* leaves, as above and below in e and f. Recently, using CaCo-2 model of intestinal transport, the digestates of *A. annua* dried leaves were found to improve the artemisinin transport by 37 % (Desrosiers

and Weathers 2018).

(e) The leaves of *A. annua* plants are known to contain a number of classes of secondary metabolites including; artemisinic compounds other than artemisinin (Bhakuni *et al.*, 2001; Brown, 2010; Mesa *et al.*, 2015; Towler and Weathers, 2015; Weathers *et al.*, 2017). Many of these possess varying levels of anti-plasmodial activity, albeit much weaker than in artemisinin. The non-artemisinin, antimalarial compounds affect the survival of parasites via mechanisms that are independent of that for artemisinin, or which determine the availability or activity of artemisinin at its site(s) of action. Some of the metabolites of *Artemisia annua* characterized for possession of their own kind of anti-*Plasmodium* activity, according to their chemical class, are as follows: artemisinic compounds = arteannuin B, artemisinic acid, dihydroartemisinic acid (Fig. 4; Allen *et al.*, 1997; Suberu *et al.*, 2013; Weathers *et al.*, 2014); coumarin = scopoletin (Fig. 5; Ezcokonkwo and Obidoa, 2001; Ferreira *et al.*, 2010; Malik *et al.*, 2011); flavonoids = artematin, casticin, circilineol, chrysoplenetin, chrysophenol-D, eupatorin, kaempferol, luteolin, myrcetin, quercetin (Fig. 6; Elford *et al.*, 1987; Liu *et al.*, 1992; Chiung-Sheue *et al.*, 1992; Lehane and Saliba, 2008; Ferreira *et al.*, 2010); phenolic acids = chlorogenic and rosmarinic acids (Fig. 5; de Magaehaes *et al.*, 2012; Suberu *et al.*, 2013; Towler and Weathers, 2014; Daddy *et al.*, 2017); saponins (Fig. 5; Francis *et al.*, 2002); sulfated polysaccharides (Xiao *et al.*, 1996; Clark *et al.*, 1997; Francis *et al.*, 2002; Andrews *et al.*, 2005); terpenes = artemisia alcohol, artemisia ketone, borneol, camphene, camphor, caryophyllene, 1, 8-cineole, germacrene D, limonene, myrcene, nerolidol, α-pinene, phytol , sabinene, spathulenol, α-terpineol (Fig. 7; Goulart *et al.*, 2004; van Zyl *et al.*, 2006; Grace *et al.*, 2012; Suberu *et al.*, 2013; Bilia *et al.*, 2014; Weathers *et al.*, 2014c; Towler and Weathers, 2015; Daddy *et al.*, 2017). The flavonoids and phenolic acids in general inhibit the cytochrome enzymes, present in liver and intestine, that metabolize artemisinin to deoxyartemisinin, thereby increasing the bioavailability of artemisinin in the blood stream
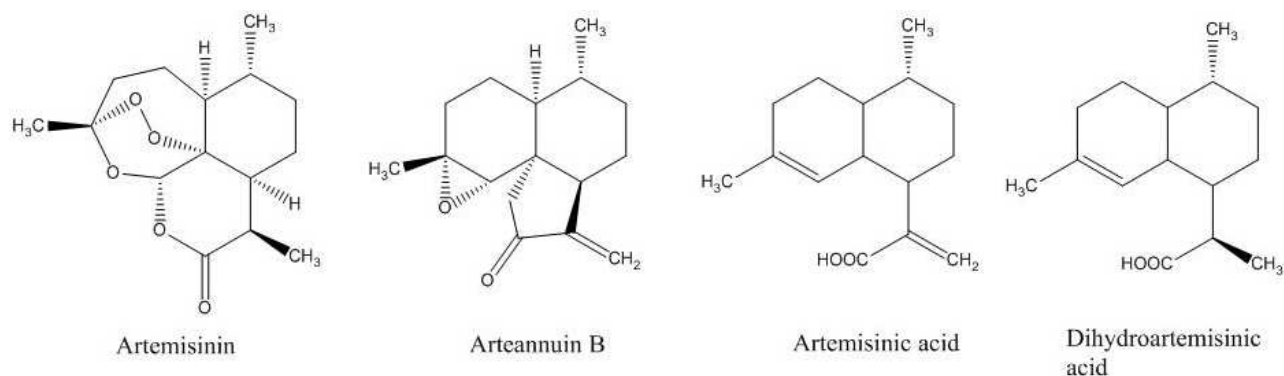
Artemisinin   Arteannuin B   Artemisinic acid   Dihydroartemisinic acid

Fig. 4: Molecular structures of important artemisinic compounds present in the leaves of *Artemisia annua*

Scopoletin

Chlorogenic acid

3-*O*-Methylquercetin

Rosmarinic acid
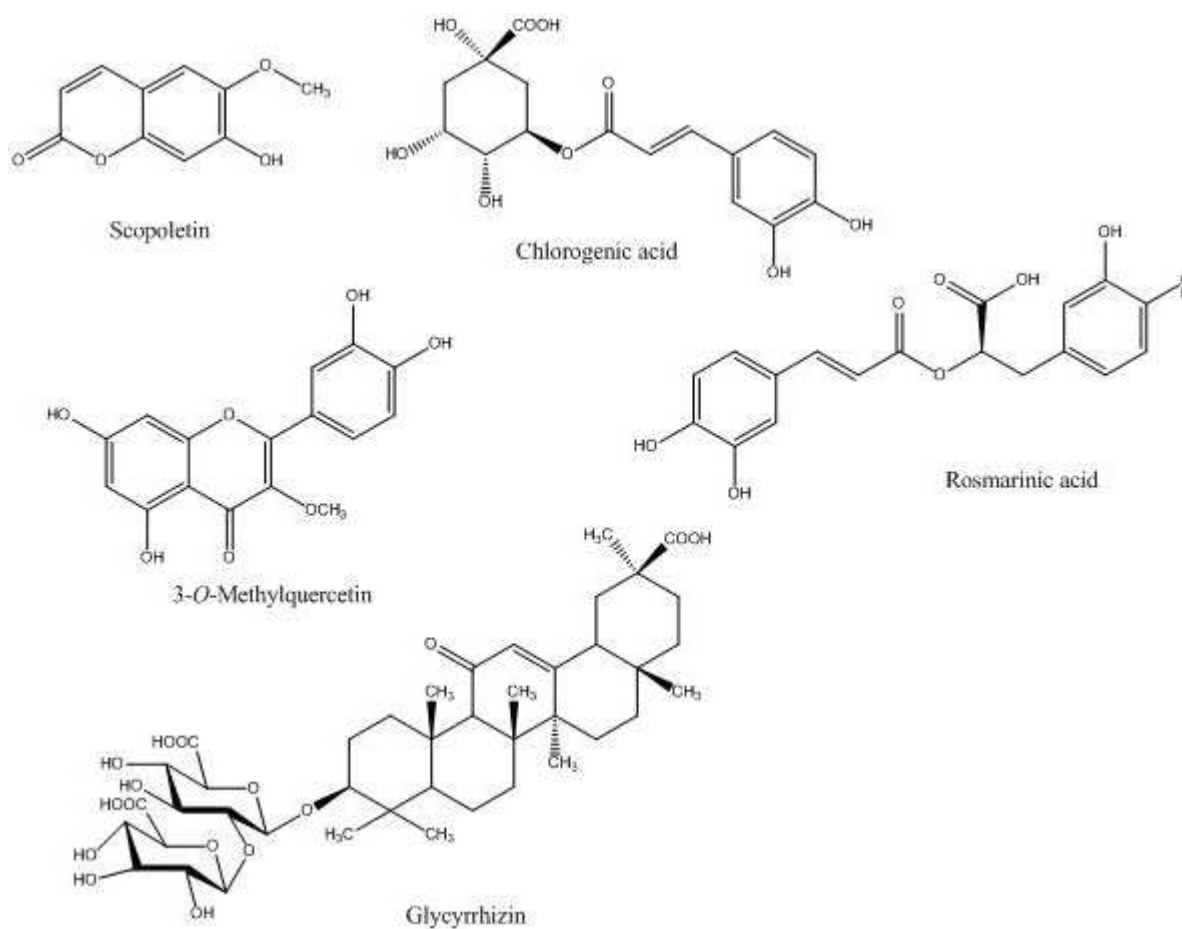
Glycyrrhizin

Fig. 5: Molecular structures of some of the secondary metabolites, representing various classes, present in the leaves of *Artemisia annua*: scopoletin (coumarin), chlorogenic and rosmaric acids (phenolic acids), glycyrrhizin (saponin) and quercetin-3-methylether (methylated quercetin)

(Elford *et al.*, 1987; Liu *et al.*, 1999; Svensson *et al.*, 1999; Svensson and Ashton, 1999; Sanella *et al.*, 2007; Lehane and Saliba, 2008; Sergent *et al.*, 2009; Choi *et al.*, 2011; de Magalhaes *et al.*, 2012; Suberu *et al.*, 2013; Towler and Weathers, 2015). The methoxylated flavonoids increase the activity of artemisinin against the intra-erythrocytic plasmodia, by blocking the conversion of heme bound to artemisinin to hemozoin, such that there is an enhancement in the release of free radicals from the clearance of the peroxide bridge and eventually in the
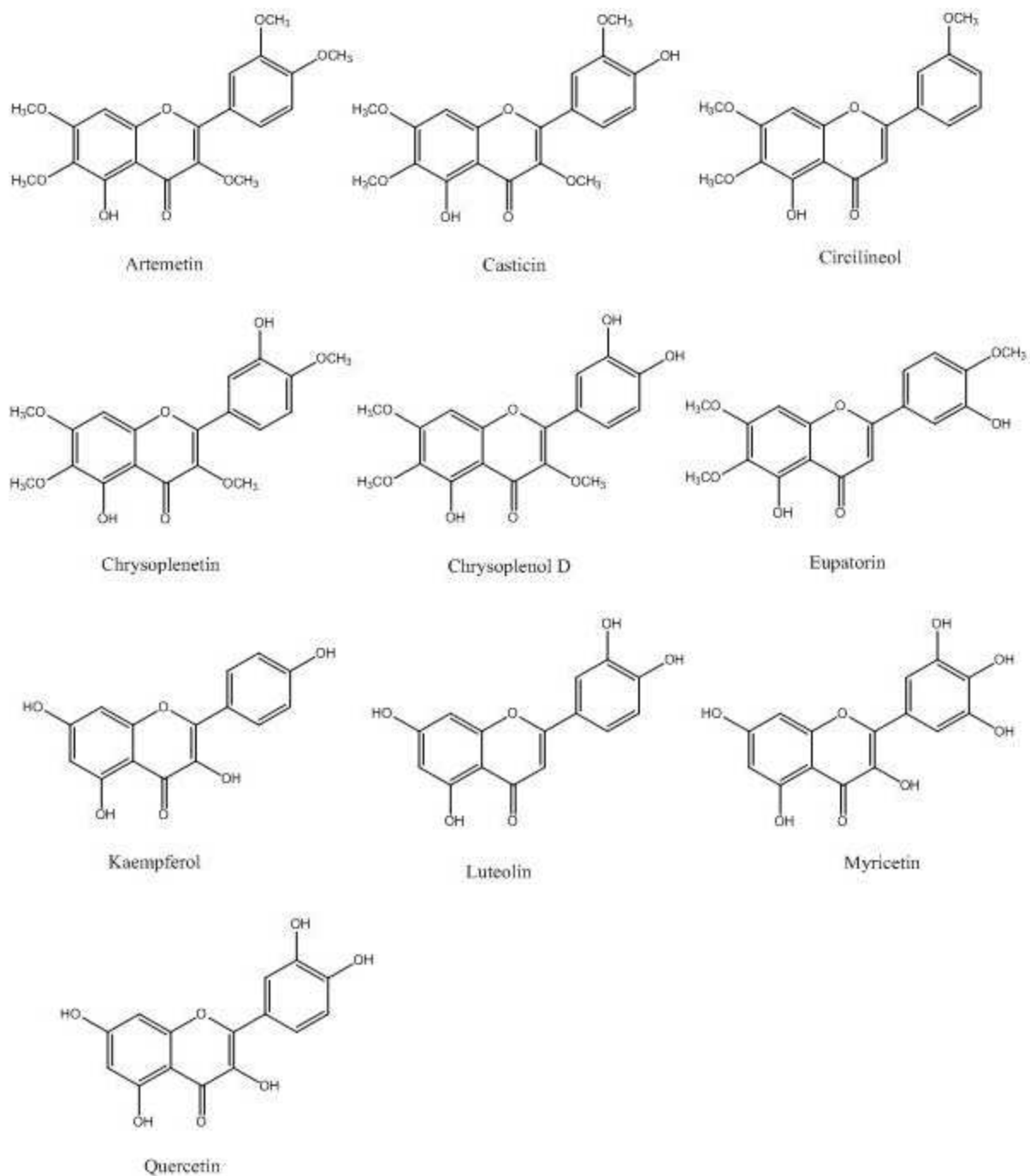
Fig. 6: Molecular structures of some of the important flavonoids present in the leaves of *Artemisia annua*

killing of parasites (Rodriguez *et al.*, 1972; Elford, 1987; Bilia *et al.*, 2002 and 2008). The finding, that cytochrome enzymes, which metabolize artemisinin to deoxyartemisinin (Whirl-Carrillo *et al.*, 2012) in the liver of the infected animals, are suppressed by flavonoids and phenolic acids was indicated in an animal-

experiment by Weathers et al. (2014b). Separate batches of mice infected with *P. chabaudi* were fed with pure artemisinin and with artemisinin in *A. annua* dry leaves @ 100mg/ kg body weight and monitored for 2h for the presence of artemisinin and deoxyartemisinin in the blood stream. In mice fed with pure artemisinin, the
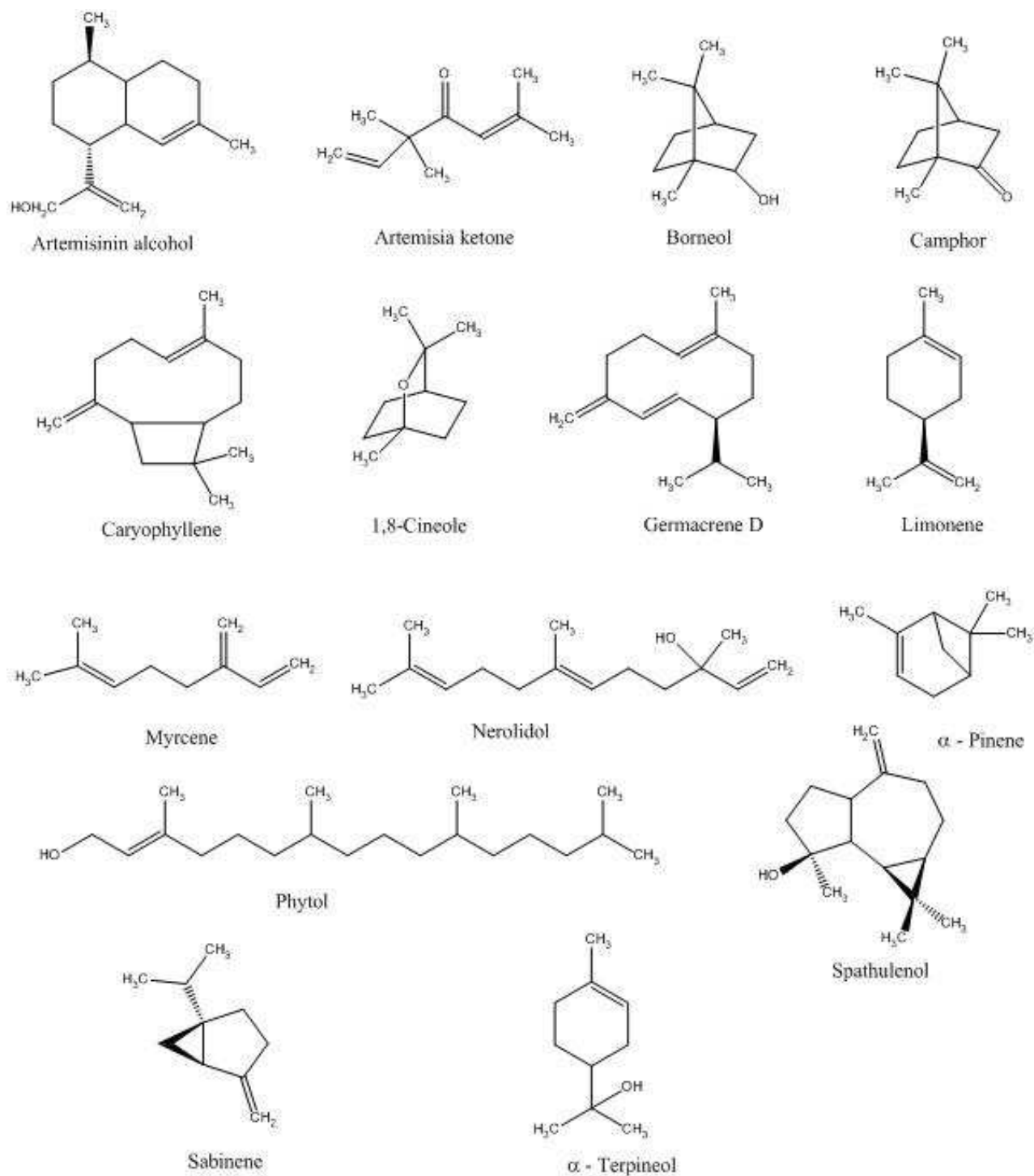
Fig. 7: Molecular structures of some of the terpenes present in the leaves of *Artemisia annua*

drug was detectable in the blood only after 60 min, whereas, artemisinin content in the blood constantly increased over the 120 min period in mice fed with artemisinin in *A. annua* dry leaves. In the leaf-fed mice, the content of deoxyartemisinin in the blood stream was much lesser than that in pure artemisinin-fed mice.

(f)   ALT was shown to be effective against artemisinin resistant malarial infections and its

treatment resilient to resistance development in the animal model systems (Elfawal *et al.*, 2015). Administration of a single oral dose of *A. annua* dry leaves (24 mg artemisinin/kg body weight) to rodents infected with artemisinin resistant *P. yoelli* cured their parasitaemia, whereas, an equivalent dose of pure artemisinin proved to be ineffective on corresponding animals. It was further shown that the stable resistance to *A. annua* dry leaf treatment, in *P. chabaudi* infected mice occurred 2.7 times slower than acquirement of resistance to pure artemisinin. Achievement of resistance to dry *A. annua* leaf treatment in *P. chabaudi* infected mice was found to be 1.6 times lower than that for the treatment with artesunate + mefloquine (ACT).

(g)   The clinical use of ALT treatment on human patients with severe Pf malaria in the Democratic Republic of Congo proved the efficacy of ALT (Daddy *et al.*, 2017). For ALT treatment, tablets of 500 mg weight, each containing 5.5 mg artemisinin, were prepared by compressing powdered dry leaves of Anamed-A3 variety of *A. annua*. The patients administered with the ALT treatment were 6 males and 12 females, of 14 months to 60 years of age, whose malaria did not get cured, neither with artemether + lumefantrine, nor with intravenous artesunate treatment. The malaria patients had entered the severe phase which included such symptoms as loss of consciousness, convulsions, frustration, shock, respiratory distress, pulmonary edema, bleeding, gastric distress and jaundice. Among the patients, the adults were administered one tablet twice daily for 5 days, children of 5-15 kg body weight and 15-30 kg body weight, were respectively given quarter and half tablet twice daily for 5 days, and those in coma or too young to swallow tablets, the tablet-dose was crushed, mixed with water and delivered via nasogastric tube. All the patients got cured of their malarial disease and there were no adverse side effects. In ALT treatment on another set of patients, rectal administration of dried pulverized leaves of *Artemisia annua* was found effective in curing Pf malaria (Abolaji *et al.*, 2016). More extensive studies are needed that will cover 28 days of follow up after treatment with ALT.

From the evidence described above, about the roles of diverse phytochemicals present in the leaves of *A. annua* in augmenting the inhibitory/lethal effects of artemisinin in ALT on infections of *Plasmodium* species on animal model systems and about clinical efficacy and safety of ALT on human malaria patients, it is possible to conclude that ALT is an inexpensive but safe and effective option for treating acute and severe malaria. Since multiple secondary metabolites with independent lethal mode of action on malarial parasite are involved in the efficacy of ALT, it is possible to further conclude that it will take considerable time period before any resistance evolves against ALT treatment in malarial parasites or via it against artemisinin. It has been advised that safety of ART treatment in pregnant women be evaluated and that nausea resulting from oral intake of dry leaf tablets may be controlled by encapsulation or use of anthelmentics or sweet substances (Yarnell, 2014). Should there be recrudescence, the ALT treatment may be repeated or alternatively a triple ACT treatment be given.

ALT: Establishment of the Compositional Consistency of Tablets

Like for pharmaceuticals, stringent control over the quality of *A. annua* dry leaf tablets, during their manufacturing process, is essential for ALT's inclusion in the first line antimalarial therapeutics. To achieve this objective in practice, all individual steps of the process must be standardized. To obtain leaves of high artemisinin content, only the identified genotypes of *A. annua* should be grown under consistent and specified cultivation conditions (cultivation_of_ Artemisia_in_Africa_and_Asia.pdf; Ferreira *et al.*, 1997; Kumar and Srivastava, 2005). To retain the secondary metabolites in high concentrations, the harvested shoots of field grown plants must be dried under clean and ambient conditions (Ferreira *et al.*, 1992; Laughlin, 2002) to retain the secondary metabolites present in them in high concentrations. From the dry shoots, leaves are to be mechanical separated from stem on clean surface, the dry leaves produced from different fields should be homogenized, sieved, pulverized using a blade cutter or equivalent instrument, characterized and converted into tablets of standard weight, size and content of artemisinin and a few flavonoids and terpenes, under hygienic conditions (Weathers and Towler, 2014; Weathers *et*

al., 2014a).

The *A. annua* crops can be cultivated in temperate and sub-tropical agroenvironments, such as those available in the countries of central and southern Europe, Central Asia, Southeast Asia, South Asia, East Africa, South America and in Australia. Several genetically improved and bred varieties of wide adaptability, whose leaves upon drying contain 0.7 to 1.2 % artemisinin, are readily available, including Anamed (A3), Jeevanraksha, Arogya and Sanjeevani (de Magalhaes *et al.*, 1997; Kumar *et al.*, 1999; Delabays *et al.*, 1993 and 2001; Ferreira *et al.*, 2005; Kumar and Srivastava, 2005; Simonnet *et al.*, 2008; Paul *et al.*, 2010; Gupta *et al.*, 2016). Besides, several to many seed industry bred varieties of *A. annua* are also available.

A. annua is a short day-flowering, open-pollinated annual shrubby species that completes its life cycle in upto one year time. The sowing and harvesting times of *A. annua* crops to obtain high quality produce of leaves have been prescribed according to the agro-climates of country-wise geographical locations of cultivation and variety(ies) (Ferreira *et al.*, 1992 and 1994; Laughlin, 1994 and 2002; Ram *et al.*, 1997; Gupta *et al.*, 2002; Kumar *et al.*, 2002 and 2004; de Magalhaes *et al.*, 2004; Brisibe *et al.*, 2009; Luo *et al.*, 2009; Goel *et al.*, 2011; Jiang *et al.*, 2013; Pop *et al.*, 2017). The nursery grown plants of 1 month or more age are transplanted in fields @ 20-70 thousand plants/ ha, depending on the plant architecture and average field duration of plant population of the variety used. Nursery plants are raised by spreading the seeds on a wet soil surface, in a farmyard manure fertilized field. The amount of seeds required for planting 1 ha of crop is 3-5 g. Fields of sandy to sandy loam soil type are used and fertilized with manure and fertilizers @ N:P:K::60:40:40 kg/ha. The transplanted *A. annua* crop, to produce dry leaves for ALT, is harvested before flowering occurs on plants. The plant shoots are dried at temperatures <40ºC, in field, under shade or in specially designed temperature controlled chambers. The desirable moisture content in the dried leaves is 10-12%. Dry leaves are stored and transported in the form of large blocks by compressing leaves in moulds.

Artemisia annua has been in commercial cultivation by farmers in India for more than 15 years, under the public-institution assisted farmer-company partnerships (Fig. 8; Kumar *et al.*, 2015). In recent years, such farmer-company partnerships have covered 2500 h/y, largely in the North-West India and in this region preponderantly in the Indo-Gangetic plains area. According to the agro-climate of the Indo-Gangetic plains, the most suitable time for the sowing of nursery is 15 December to 15 January. Seedlings are transplanted into the fields vacated by potato crops between 20 February and 1 March. This summer crop of *A. annua* is harvested between May 28 and June 5 (several weeks before the onset of monsoon rains) and shoots are dried under shaded conditions. Alternatively or additionally, the plants growing in nursery are transplanted in fields vacated by wheat crop from 15 May onwards, and the resulting crop is harvested between 21 September and 1 October (after the withdrawal of monsoon rains and with the

Fig. 8: *Artemisia annua* **cv Sanjeevani crops in farmer's fields in the village Shivpura of district Unnao in the state Uttar Pradesh of the Indo-Gangetic plains area of India [Pictures shared by Sanjay Kumar of CIMAP, Lucknow; Kumar *et al.*, 2015]**

onset of inflorescence development, but before flowering occurs). The autumn crop is dried in temperature controlled chambers. The yield of dry leaves, respectively, from the summer and autumn harvested crop is 2.5 and 3.5 T/ha, with 0.8 to 1.2% artemisinin content, depending on the variety used; highest levels of ART (1-1.4%) are present in the leaves harvested from crops of Sanjeevani variety (Sanjay Kumar, Ramesh Srivastava and Anil Gupta, personal communication).

Need is felt internationally for new genotypes of *A. annua* and for methodologies of plant population propagation, such that the individual plants under cultivation have the same genotype or largely similar genotypes. Since *A. annua* is an open pollinated crop, individual plants in populations of its registered varieties Anamed (A3), Jeevanraksha, Sanjeevani and others demonstrate phenotypic differences arising from segregation of alleles of thousands of genes which are present in heterozygous condition. A genomic study has confirmed presence of heterozygosity at a large number of protein coding genes, amongst 63226 genes identified in *A. annua* (Shen *et al.* 2018). The quality of dry leaf tablets from any available variety is the result of an average phenotype of its cultivated populations. In the future it is desirable to have ALT tablets from plants of a single genotype. There are several possibilities to pursue this aim. One of these is to develop elite inbred lines through selfing in existing varieties for 6 or more generations. The seeds of chosen inbred line will be always produced in isolation. Second, F1 hybrids of two selected inbred lines, selected for heterosis, may be chosen for cultivation. Again, F1 seeds will be produced from co-cultivation in isolation of the parental inbred lines whose own seeds will be produced in isolation. Special genotypes, an important one being photo-period independent early flowering, could be developed in the background of chosen singular genotype(s). When suitable genotype(s) have become available for mono-genotype-culture, an alternative method to produce planting material on a mass scale could be the deployment of micro-propagation procedures (Mathur and Kumar, 1996; Paul *et al.*, 2012; Gopinath *et al.*, 2014; Fei and Weathers, 2015, 2016). Any one selected plant from Jeevanraksha, Sanjeevani or Anamed (A3) could become a clonal variety with the use of micropropagation for genotype multiplication.

Cost-effectiveness of ALT Treatment

The ALT treatment in comparison to ACT treatment is highly cost effective. In the Indo-Gangetic plains area, the cost of cultivation, harvesting and processing of harvested shoots to obtain dry leaves of *A. annua* var Jeevanraksha, Arogya or Sanjeevani (all genetically related), and profit for farmers, under the farmer-private company partnership scheme, for two hectares of crop yield of 50 tons of dry leaves is ~ Rs. 2,00,000 (or ~US$ 3,500). The cost of producing 10 million tablets of 500 mg dry leaves each can therefore be speculated as ~ Rs 5,00,000 (or ~ US$ 8500). Considering the expenditure of all kinds on supply chain of ALT tablets, the cost of a "10 tablets treatment" of an adult is estimated as less than Rs 1 (or less than US Cents 17). The ALT treatment in India will be at least 60 to 150 fold less costly than an ACT treatment. It is possible to conclude that large scale adoption of ALT treatment as advised above can tremendously advance the aim of WHO and 97 malaria endemic countries, including India, to significantly reduce or eliminate the burden of malaria by 2030 (www.rollbackmalaria.org/about-rbm/aim-2016-2030; nvbdcp.gov.in>Doc>National-framework-for-malaria-elimination-in-India-2016-2030). ALT capsules have the added advantage of use as suppositories.

Possibilities of Using ALT Beyond Malaria

A variety of disease conditions in humans and livestock are known to respond curatively to artemisinic-, terpenoid-, and flavonoid-compounds present in *A. annua* leaves. There is thus a strong possibility that ALT tablets may prove to be of therapeutic value against many diseases beyond malaria. From a search of the enormous body of publications on the subject of artemisinin (including derivatives)- and *A. annua*-therapeutics, the tables 4 and 5 provide a short list of disease conditions which are controlled by treatment with artemisinin and/or its derivatives (artemisinins), *A. annua's* dry leaf powder and/or leaf extracts (termed here as artemisannua). The Tables 4 and 5 also summarize some representative robust evidence which demonstrates that many virus-, bacteria-, fungi, protozoa-, and helminths-caused infectious diseases on the one hand and autoimmune-, and digestive systems/metabolic-disorders, and cancers on the other hand are attenuated/prevented by the treatment with artemisinins and artemisannua.

Table 4: Summarized evidence which shows that Artemisia annua leaf powder and/or leaf extract, artemisinin and/or artemisinin derivatives are inhibitory and putatively curative against a variety of infectious diseases in humans and livestock animals

S.No.	Disease(s)	Causal infectious organism/ agent	Host	Nature of study	Therapeutic test material(s)/ compound(s) used	Observation(s)	Reference(s)
(A) Virus caused:							
1	Hepatitis B	Hepatitis B virus (HBV)	Human derived Hep.G2/2.2.15 cell line	*In vitro*	Artemisinin and artesunate	Both the drugs strongly inhibited viral replication without causing cytotoxicity	Romero *et al.* (2005)
2	Hepatitis C	Hepatitis C virus	Human derived HuH-2 cell line (HCV)	*In vitro*	Artemisinin	Viral replication was inhibited; this effect was potentiated by iron donated by hemin, without cytotoxicity	Paeshuyse *et al.* (2006)
3	Cytome-galovirus	Human cytome-galovirus	Human fibroblast cells	*In vitro*	Artesunate	Viral replication was inhibited, very strongly in the presence	Kaptein *et al.* (2006)
			Human	*In vivo*	As above	Viral load was reduced of iron, without toxicity	Shapiro *et al.* (2008)
4	Herpes virus	Human herpes virus 6A (HHC-6a)	Cultured human cells	*In vitro*	As above	Viral early and late gene expression and replication was inhibited and thereby viral multiplication was arrested	Milbrandt *et al.* (2009)
(B) Bacterial caused:							
5	Tubercu-losis	*Mycobac-terium tuberculo-sis* H37Rv	MGI 960 system, and Ogawa slant medium assay	*In vitro*	As above	A single dose strongly inhibited bacterial growth measured 21 days after treatment	Choi (2017)
6		As above	Sprague Dawley rats	*In vivo*	As above	3.5mg/kg dose a day for 4 weeks cured the rats of infection without causing toxicity	As above
(C) Fungus caused:							
7	Aspergi-llosis	*Aspergillus fumigatus*	Fungus	*In vitro*	Artemisinin	The drug killed the fungus by targetting fungal oxidative phosphorylation and cell wall and ergosterol synthesis pathways	Gautam *et al.* (2011)
(D) Protozoan caused:							
8	a) Acantha-moebiasis	*Acantha-moeba castellani* (free living amoeba)	Cultured trophozoites	*In vitro*	Artemether	Amoebae were killed	Deng *et al.* (2015)
9		Acantha-moeba castellani 309 and Ac32	As above	*In vitro*	Artemisia annua leaves- water, alcohol or chloroform extract	Amoebae were killed	Derda *et al.* (2016)
10		As above	BALB/C mice (Mus musculus)	*In vivo*	As above	As compared to untreated infected mice, the treated infected mice survived for 2 to 4 fold longer time period	As above

11	b) Cocci-diosis	*Eimeria tenella*	Domesticated chicken (*Gallus domesticus*)	*In vivo*	*Artemisia annua* dry leaves	Addition of dried leaves @ 20g/kg to feed was coccidostatic as well as growth promoter	Brisbe *et al.* (2008); Bosselman and Gylling (2013)
12		As above	As above	*In vivo*	As above	Addition of dried leaves @ 1.5% eliminated coccidiosis	Dragan *et al.* (2014)
13	c) Leishma-niasis	*Leishmania donovani* (Visceral infection)	Infected human macrophages	*In vitro*	Artemisinin	Parasite load was reduced; the anti-leishmanial activity was via apoptosis of parasites	Sen *et al.* (2007 & 2010)
14		As above	BALB/C mice	*In vivo*	Leaves and seeds of Artemisia annua or their hexane extract	Parasite load decreased on account of apoptosis of promastigotes	Mutiso *et al.* (2011); Islamuddin *et al.* (2012)
15		As above	As above	*In vivo*	Essential oil of leaves of *Artemisia annua*	Intra-peritonial administration of the essential oil reduced the parasite burden in spleen and liver by 90% without toxicity to test animals	Islamuddin *et al.* (2014)
16		As above	As above	*In vivo*	Artemisinin	Administration of the drug loaded nanoparticles reduced the parasite burden and spleen- and hepato-megaly	Want et al. (2015)
17		*Leishmania major* (cutaneous infection)	As above	*In vivo*	As above	Lesion size was reduced via induction of apoptosis in promastigotes	Ghaffarifar *et al.* (2015)
18		As above	As above	*In vivo*	Artemisinin ointment	Ulcers were healed	As above
19		*Leishmania panamensis* (cutaneous infection)	Infected human U-937 macrophage cell line	*In vitro*	*Artemisia annua* leaf powder	Amastigotes were inhibited without toxicity to macrophages and genotoxicity to lymphocytes	Mesa *et al.* (2017)
20		As above	Hamster (*Mesocricatus auratus*)	*In vivo*	As above	Intracellular amastigotes present in the ulcers were killed and 5 out of 6 treated hamsters were cured after 30 days treatment with 500mg/kg/day	As above
21		As above	Humans	*In vivo*	As above	Two patients were cured after 45 days of treatment with 30g of leaf powder (666mg/day)	As above
22	d) Toxipla-smosis	*Toxoplasma gondii* (Obligate apicomplexan parasite)	Human forskin fibroblast (HFF) cells	*In vitro*	Artesunate	Infected cells killed much like intra-erythrocytic malarial parasites	Gomes *et al.* (2012)
23		As above Kunming *Mus musculus*	CDI, OFI,	*In vivo*	Artesunate, dihydroarte-misinin and their combi-nation	All three treatments reduced the infection and improved survival time period of the diseased animals	Sarciron *et al.* (2000)
24	e) Trypa-nosomiasis	*Trypano-soma brucei* (African sleeping sickness)	*Mus musculus*	*In vivo*	Artemether	5 days of treatment eliminated the parasite	Akande and Fagbemi (2011); Yimar and Sahu 2016

25		As above	Rattus rattus	*In vivo*	As above	Growth of parasite was arrested and life span was extended	Oluyomi *et al.* (2009)
26		As above	Cultured trypomastigotes	*In vitro*	Artemisinin	Parasite growth was arrested and the mechanism of parasitic killing was like that of intra-erythrocytic malarial parasites	Mishina *et al.* (2007)
27		*Trypano-soma cruzi* (Chagas disease)	Cultured epimastigotes	*In vitro*	As above	As above	As above
28		As above	Cultured in Vero cells (kidney epithelial cells of African green monkey)	*In vitro*	Artesunate	All intracellular stages of parasite were inhibited	Akande and Fagbemi (2011); Olivera *et al.* (2015)
(E) Metazoan caused:							
29	a) Schisto-somiasis	*Schistosoma mansoni* (Helminth flatworm; common in Africa and South America)	Humans	*In vivo*	Artemether	The drug prevented as well as reduced the parasite's infection	Utzinger *et al.* (2000); Kaiser and Utzinger (2007)
30		As above	BALB/C mice	*In vivo*	Dihydroar-temisinin	A single dose of 300mg/kg was found on 21st day post infection to have reduced much of parasite burden	Li *et al.* (2012)
31		*Schistosoma japonicum* (common in Southeast Asia)	As above	*In vivo*	Artemether, artesunate and dihydro-artemisinin	All were effective; 200, 300 and 400mg/kg drug administered on 20,21 and 22 day post infection strongly reduced the parasite load	As above

References to review type papers: Efferth (2008); Ho *et al.* (2014); Efferth *et al.* 2016 ; Muthamiselvan *et al.* (2016); Yimer and Sahu (2016); Mesa *et al.* (2017)

Table 4 summarizes results of some studies on the effects of artemisinins and artemisannua on viral-, bacterial-, fungal-, protozoan- and helminth-infections, in cell lines *in vitro* and/or on model animals or humans *in vivo*. The drugs artemisinin and artesunate have been found to inhibit replication/multiplication of hepatitis causing hepatitis B (HBV) and C (HCV) viruses and sore inducing herpes virus and its close relative cytomegalovirus in cultured human cells. The *in vitro* growth of *Mycobacterium tuberculosis* (the bacterium which causes tuberculosis in humans), as well as the tubercular bacterial growth in infected mice, has been found to be arrested by artesunate. Addition of artemisinin to the culture of *Aspergillus fumigatus* (which causes aspergillosis in human) has been observed to stop the growth of fungus.

Artemether and extracts of *A. annua* leaves have proved lethal to *in vitro* growing *Acanthamoeba castellani* (a cause of amoebiasis in humans). Treatment of mice infected with *Acanthamoeba* with water-, alcohol- or chloroform- extract of *Artemisia annua* leaves was observed to have increased the life span of diseased animals. Feeding of *A. annua* leaves to the broiler chickens infected with *Eimeria tenella* parasites saved the infected animals from development of coccidiosis disease. Growth of both visceral and cutaneous leishmaniasis causing *Leishmania* parasites, in human macrophage cultures, was found to be attenuated by the treatment of artemisinin. Analogously, the leishmania infections in model animals were also observed to have been arrested by treatment with artemisinin or *A. annua*

Table 5: Curative effect of *Artemisia annua* leaf extracts, artemisinin or its derivatives on the diseases of human /model animal immune and digestive systems and cancers of various organs

S.No.	Type of disease(s)	Organism(s)/ system(s) used for testing	Disease condition: origin/method by which induced	Therapeutic agent tested for its efficacy	Observation(s)	Reference (s)
A. Autoimmune						
1.	Rheumatoid arthritis	Rat	Collagen	Artesunate	The inflammatory symptoms were attenuated by intra-peritoneal treatment with the drug (3-5 mg/kg/d)	Li *et al*. 2013
2.	Crohn's disease (Inflammatory bowel)	Mouse	Variously	As above	The symptoms of disease were ameliorated by administration of the drug @ 150 mg/kg/d	Yang *et al*. 2012
3.	Allergenic asthma	BALB/C mouse	Ovalalbumin	As above	The treatment suppressed both the inflammation and oxidative damage associated with severe asthma	Cheng *et al*. 2011
4.	Lupus nephritis	B6D2F1 and DBA/2 mice	Pristane	Artemisinin	The drug relived the symptoms	Wu *et al*. 2010 of the diseases
5.	Uveitis	Long-Evans rat	Lipopolys-accharide	Artesunate	The drug suppressed the uveitis	Wang *et al*. 2011 induction
B. Digestive system						
6.	Obesity	C57BL/6 mouse	Nutrition rich diet	Boiled water extract of *Artemisia annua* dry leaves	Oral administration of the extract reduced the weight of animals without affecting their appetite	Baek *et al*. 2015
7.	As above	Sprague Dawley rats	As above	*Artemisia annua* leaves	Body weight, adipose tissue mass, adipocyte cell size, total cholesterol level were decreased	Song *et al*. 2017
8.	Fatty liver	CB7BL/6J mouse	High fat diet	Dehydrated water extract of *Artemisia annua* leaves	Twelve weeks treatment prevented hepatic fibrosis, obesity and inflammation of liver by reducing the accumulation of lipids	Kim *et al*. 2011
9.	Diabetes	Albino rat	Alloxan	*Artemisia annua* leaf extract	One month of treatment prevented hyperglycemia and ameliorated diabetes related metabolite abnormalities	Helal *et al*. 2014
10.	As above	Zebrafish, mouse, rat, human pancreatic islet	Type I diabetes impairment	Artemether	The glucagon producing pancreatic α cell got transformed into insulin producing β cells via activation of GABA receptors by loss of Arx function and thereby insulin production got restored	Li *et al*. 2017
E. Cancers						
11.	Squamous cell carcinoma of oral cavity	Human cells	Gingival epithelial cancerous (1HGK) cells	Dihydroarte-misinin	The drug was apoptotically cytotoxic to cancerous cells	Yamachika *et al*. 2004

12.	Pancreatic cancer	Human cells	PANC-1, Bx Pc-8 and CFPAC-1 pancreatic cancer cell lines and HL-7702 normal hepatic cell line	Artesunate	The drug caused oncosis-like cell death multi-fold more on cancer cells than on normal cells	Du *et al.* 2010
13.	As above	Human cancer cell xenograft on mouse	Panc-1tumor xenograft in mouse	As above	Artesunate caused dose dependent tumor regression	As above
14.	Hepatocellular cancer	Human	Hep G-2 and BWTG-3 cells	As above	Artesunate reduced the cancer cell viability in a dose dependent manner	Vandewynckel *et al.* 2012
15.	As above	Mouse	Diethyl nitro-samine induced tumor in liver	As above	Tumor burden was reduced without hepatotoxicity	As above
16.	Gastric cancer	Human cells	SGC-7901, BGC-823 and AG5 gastric cancer and GES-1 normal cell lives	As above	Cancer cells were killed by oncosis like process, but there was no significant effect on non-cancerous cells	Zhou et al. 2013
17.	As above	Human tumor xenograft on mouse	Gastric tumors were xenografted on nude mice	As above	The treatment regressed the tumors without detriment to animals	As above
18.	Colorectal cancer	Human	Patients	As above	Administration of 200 mg drug daily for 14 days cured 8 out of 9 patients	Magalhaes *et al.* 2012 ; Krishna *et al.* 2014
19.	Gall bladder cancer	As above	GBC-SD and NOZ gall bladder cancer cell lines	Artemisinin	The treatment stopped cell prolife-ration and caused cell killing by apoptosis	Jia *et al.* 2016
20.	As above	As above	The xenograft of above cancers on BALB/C mice	As above	The xenograft growth was inhibited by drug treatment	As above
21.	Renal cancer	As above	Renal cell carcinoma cell lines: Caki-1, 786-O and SN12C-GFP-SR Lu 2	Artesunate	Cancer cells were killed by oncosis via ROS generation and ATP depletion	Jeong *et al.* 2015
22.	As above	Human cancer xenograft on mice	Xenograft of 786-o-Luc cells planted subcutaneously	As above	Intra-peritoneal administration of the drug repressed the tumor (growth, metastasis and angiogenesis were all inhibited)	As above
23.	Cervical cancer	Human	HPV-39 inhibi-ted cervical cancer cells	Artemisinin	The cancerous cells stopped proli-ferating and were killed apoptotically by the effect of the drug	Mondal and Chatterji 2015

leaf powder. Artesunate was observed to inhibit the *Toxiplasma gondii* infection of cultured human cells and of mice *in vivo*. Trypanosomiasis (human African sleeping sickness) like disease caused by *Trypanosoma brucci* infection in experimental mice and rats was found to have been cured by the artemether treatment. Artemisinin and artesunate treatments given individually inhibited the growth of *T. brucci* and *T. cruzi* (the cause of chagas disease in humans) in cultured human cells. Infection in humans and in experimental mice of *Schistosoma mansoni* as well as *S. japonicum* (both the species are cause of schistosomiasis disease) was observed to get inhibited by the treatment with each of these drugs- artemether, dihydroartemisinin, and artesunate.

A summary of the results of some representative studies on the effects of artemisinins and artemisannua on rheumatoid arthritis, Crohn's disease, asthma, lupus and uveitis (autoimmune disorders), obesity and diabetes (metabolic disorders) and eight kinds of cancers is listed in the table 5. In different studies, artesunate was found to cure/ suppress and relieve symptoms of collagen induced rheumatoid arthritis, Crohn's disease, ovalabumin induced asthma, and lipopolysaccharide induced uveitis, all in model animals. Obesity and fatty liver diseases caused by consumption of high fat/ nutrition diet in experimental animals were found to be cured by treatment with *A. annua* leaf extracts. The *A. annua* leaf extracts also cured alloxan induced diabetes in rats. It was found that artemether treatment, to type1 diabetic zebrafish, mice and rats and human pancreatic islets, transformed the pancreatic á cells into β cells such that insulin synthesis started relieving the type 1 diabetes symptoms. Cells of human cell lines of pancreatic-, hepatocellular-, gastric-, colorectal- and renal- cancer stopped proliferating and got killed by an oncosis- like process upon treatment with artesunate. Also the xenographs of pancreatic-, hepatocellular-, gastric- and renal- cancers in animal models were found to regress upon treatment with artesunate. The artemisinin treatment produced analogous results on *in vitro* and *in vivo* gall bladder cancer and on *in vitro* cervical cancer. The experimental findings, that artemisannua controlled obesity and diabetes in model animals strongly suggest ALT as a treatment for these diseases in humans.

Clearly, the above discussion suggests that the mechanisms of biological actions of artemisinins and artemisannua are such that these agents serve as broad spectrum therapeutics so as to cure a variety of human diseases. These observations raise the possibility that perhaps ALT can substitute for artemisinins and artemisannua and ALT could be a therapy for multiple diseases beyond malaria. In view of the above, the need for pilot studies and clinical trials on quality controlled ALT tablets for studying the response of their administration to patients of each of the different non-malarial, as well as malarial diseases, that respond to artemisinins and artemisannua, can not be overemphasized.

Concluding Remarks

In the approximately last ten years, the incidence of malaria disease was reduced by 20% and mortality among malaria patients by 30%. This was mainly achieved by use of two-drug ACTs and chloroquine in the treatment, respectively, of *falciparum* and *vivax* malaria and by use of primaquine treatment to block the transmission of parasites from humans to mosquitoes (Table 3). However, the *falciparum* and *vivax* malarial parasites have developed genetic resistance against a large majority of the approved antimalarial pharmaceuticals in some of their populations in malaria endemic areas, thereby making the drugs ineffective (Table 2). There has been independent development of artemisinin resistance in Southeast Asia and Africa; consequently ACT treatments too have become ineffective in parts of these geographical areas. To meet the challenge of multi-drug resistant falciparum malarial strains, treatment with three-drug ACTs has been advised. This year, a new treatment (ALT) has been added to cure the acute and complicated malaria caused by ACT-resistant falciparum parasites. The ALT treatment comprises of capsules filled with or tablets made from *A. annua* dry leaf powder, derived from cultivated plants of specific variety(ies) bred for e" 1% artemisinin content and a combination of other therapeutically active metabolites naturally present. A regimen of two 500 mg leaf powder tablets a day for 5 days was found to cure adults suffering from ACT resistant complicated falciparum malaria that was unresponsive to ACT or iv artesunate (most likely artemisinin resistant). The ALT treatment's malaria curing property has been related to antimalarial

activities of artemisinin, several other artemisinic compounds, many terpenes and flavonoids and other types of molecules present in the dry *A. annua* leaves. ALT is safe and seems resilient against artemisinin drug resistance development. The cost of an ALT-treatment was estimated to be about 100 fold lower than that of an ACT-treatment. Extensive putative use of ALT has gained importance since a recent policy statement of WHO (September 10, 2017) emphasizes on the importance of affordability for everyone of safe, efficacious and quality medical products (http://odishatv.in/health/afforability-medicine-who-wants-bold-steps-237875/). The ALT, besides being an efficacious antimalarial treatment has properties which raise possibilities of its multi-repurposement as a treatment against all those diseases which respond curatively to artemisinin, its derivatives and *A. annua* leaf powder or its extracts. This list includes diseases as diverse as hepatitis, tuberculosis, leishmaniasis, toxoplasmosis, trypanosomiasis, schistosomiasis, asthma, rheumatoid arthritis, diabetes, and cancers of various body organs. There is now an urgent need for (a) further evaluation of artemisinin efficacy against several of the listed diseases *in vivo* models, and (b) pilot studies and clinical trials to attest ALT treatment for varied malaria and diseases beyond malaria for which artemisinin efficacy has been experimentally established, for the benefit of billions of patients of above listed diseases.

Acknowledgements

References

Abraham G (2017) Malaria in pregnancy: is artemisinin based treatment safe *Evidence based Med* **15** 63-65

Abolaji G T, Olooto F M and Williams F E (2016) Development of value added tea bags and capsules of Artemisia Annua Anamed (A3) whole plant for malaria treatment *Agrosearch* **16** 69-73

Achan J, Talisuna A O, Erhart A, Yeka A, Tibenderana J K, *et al.* (2011) Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria *Malar J* **10** 144

Achan J, Tibenderana J K, Kyabayinze D, Wabwire Mangen F, Kamya M R, *et al.* (2009) Effectiveness of quinine versus artemether-lumefantrine for treating uncomplicated falciparum malaria in Ugandan children: randomised trial *BMJ* **339** b2763-b2763

Agarwal A, McMorrow M, Onyango P, Otieno K, Odero C, *et al.* (2013) A randomized trial of artemether-lumefantrine and dihydroartemisinin-piperaquine in the treatment of uncomplicated malaria among children in western Kenya *Malar J* **12** 254

Agarwal M, Bhowmick K, Shah K, Krishnamachari A and Dhar S K (2017) Identification and characterization of ARS-like sequences as putative origin(s) of replication in human malaria parasite *Plasmodium falciparum FEBS J* **284** 2674-2695

Akande F and Fagbemi B (2011) *In vivo* and *in vitro* effects of artemisinin group of drugs on trypanosomosis in mice *J Nat Sci Eng Tech* **10** 73-80

Al-Zaydani I A, Al-Hakami A, Kumar A, Abdalla S A, Otaif M, *et al.* (2016) Severe unresolving *Plasmodium falciparum* malaria following artemisinin combination therapy: Emergence of drug resistance in Saudi Arabia *Indian J Med Microbiol* **34** 553-557

Allen P C, Lydon J and Danforth H D (1997) Effects of components of *Artemisia annua* on coccidia infections in chickens *Poult Sci* **76** 1156-1163

Amaratunga C, Lim P, Suon S, Sreng S, Mao S, *et al.* (2016) Dihydroartemisinin-piperaquine resistance in *Plasmodium falciparum* malaria in Cambodia: A multisite prospective cohort study *Lancet Infect Dis* **16** 357-365

Amato R, Lim P, Miotto O, Amaratunga C, Dek D, *et al.* (2017) Genetic markers associated with dihydroartemisinin-piperaquine failure in *Plasmodium falciparum* malaria in Cambodia: a genotype-phenotype association study *Lancet Infect Dis* **17** 164-173

Amato R, Miotto O, Woodrow C J, Almagro-Garcia J, Sinha I, *et al.* (2016) Genomic epidemiology of artemisinin resistant malaria *Elife* **5** e08714

Andrews K T, Klatt N, Adams Y, Mischnick P and Schwartz-Albiez R (2005) Inhibition of chondroitin-4-sulfate-specific adhesion of *Plasmodium falciparum*-infected erythrocytes by sulfated polysaccharides *Infect Immun* **73** 4288-4294

Antinori S, Corona A, Castelli A, Rech R, Borghi B, *et al.* (2017) Severe *Plasmodium falciparum* malaria in the intensive care unit: A 6-year experience in Milano, Italy *Travel Med*

Infect Dis **17** 43-49

Antony H A and Parija S C (2016) Antimalarial drug resistance: An overview *Trop Parasitol* **6** 30-41

Anvikar A R, Shah N, Dhariwal A C, Sonal G S, Pradhan M M, *et al*. (2016) Epidemiology of *Plasmodium vivax* malaria in India *Am J Trop Med Hyg* **95** 108-120

Anyanwu P E, Fulton J, Evans E and Paget T (2017) Exploring the role of socioeconomic factors in the development and spread of anti-malarial drug resistance: a qualitative study *Malar J* **16** 203

Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois A-C, *et al*. (2014) A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria *Nature* **505** 50-55

Ashley E A, Recht J and White N J (2014b) Primaquine: the risks and the benefits *Malar J* **13** 418

Ashley E A, Dhorda M, Fairhurst R M, Amaratunga C, Lim P, *et al*. (2014a) Spread of artemisinin resistance in *Plasmodium falciparum* malaria *N Engl J Med* **371** 411-423

Auparakkitanon S, Chapoomram S, Kuaha K, Chirachariyavej T and Wilairat P (2006) Targeting of hematin by the antimalarial pyronaridine *Antimicrob Agents Chemother* **50** 2197-2200

Ayubu M B and Kidima W B (2017) Monitoring compliance and acceptability of intermittent preventive treatment of malaria using sulfadoxine pyrimethamine after ten years of implementation in Tanzania *Malar Res Treat* **2017** 1-5

Baek H K, Shim H, Lim H, Shim M, Kim C K, *et al*. (2015) Anti-adipogenic effect of *Artemisia annua* in diet-induced-obesity mice model *J Vet Sci* **16** 389-396

Baird J K and Hoffman S L (2004) Primaquine therapy for malaria *Clin Infect Dis* **39** 1336-1345

Baird J K, Valecha N, Duparc S, White N J and Price R N (2016) Diagnosis and treatment of *Plasmodium vivax* malaria *Am J Trop Med Hyg* **95** 35-51

Barber B E, William T, Grigg M J, Menon J, Auburn S, *et al*. (2013) A prospective comparative study of Knowlesi, falciparum, and vivax malaria in sabah, Malaysia: High proportion with severe disease from plasmodium knowlesi and plasmodium vivax but No mortality with early Referral and artesunate therapy *Clin Infect Dis* **56** 383-397

Barber B E *et al*. (2017) World malaria report: time to acknowledge *Plasmodium knowlesi* malaria *Malar J* **16** 135

Bassat Q and Menéndez C (2017) Dihydroartemisinin-piperaquine: If it works for control, can we use it for elimination? *The Lancet Inf Dis* **17** 121-122

Bediako A *et al*. (2011) Pharmacokinetics of amodiaquine after a single over dose in Cohanian children with uncomplicated

malaria *J Pharma Sci & Res* **3** 1420-1426

Bensky D and Barolet R (1990) Chinese Herbal Medicines: Formulas and Strategies. Eastland Press, Seatle, USA

Beshir K, Sutherland C J, Merinopoulos I, Durrani N, Leslie T, *et al*. (2010) Amodiaquine resistance in *Plasmodium falciparum* malaria in Afghanistan is associated with the pfcrt SVMNT allele at codons 72 to 76 *Antimicrob Agents Chemother* **54** 3714-3716

Bethell D, Se Y, Lon C, Socheat D, Saunders D, *et al*. (2010) Dose-dependent risk of neutropenia after 7-day courses of artesunate monotherapy in Cambodian patients with acute *Plasmodium falciparum* malaria *Clin Infect Dis* **51** e105-114

Bhakuni R, Jain D, Sharma R and Kumar S (2001) Secondary metabolites of *Artemisia annua* and their biological activity *Curr Sci* **80** 35-48

Bilia A R, Lazari D, Messori L, Taglioli V, Temperini C and Vincieri F F (2002) Simple and rapid physico-chemical methods to examine action of antimalarial drugs with hemin: Its application to *Artemisia annua* constituents *Life Sci* **70** 769-778

Bilia A R, Sannella A R, Vincieri F F, Messori L, Casini A, Gabbiani C, Severini C and Majori G (2008) Antiplasmodial effects of a few selected natural flavonoids and their modulation of Artemisinin activity *Nat Prod Commun* **3** 1999-2002

Bilia A R, Santomauro F, Sacco C, Bergonzi M C and Donato R (2014) Essential oil of artemisia annua L.: An extraordinary component with numerous antimicrobial properties *Evidence-Based Complement Altern Med* 2014

Bisland E, van Vliet L, Williams K, Feltham J, Carrasco M P, Fotoran W L, *et al*. (2018) Plasmodium dihydrofolate reductase is a second enzyme target for the antimalarial action of triclosan *Sci Rep* **8** Article number 1038

Boggild A K, Parise M E, Lewis L S and Kain K C (2007) Atovaquone-proguanil: Report from the CDC expert meeting on malaria chemoprophylaxis *Am J Trop Med Hyg* **76** 208-223

Bosselmann A S and Gylling M (2013) Economic potential of a Danish production of *Artemisia annua* based feed additives for broilers. Frieleriksbey: Department of Food and Resource Economics, University of Copenhegan. (IFRO report; No. 224) IFRO_Report_224 pdf

Bousema T and Drakeley C (2011) Epidemiology and infectivity of *Plasmodium falciparum* and *Plasmodium vivax* gametocytes in relation to malaria control and elimination *Clin Microbiol Rev* **24** 377-410

Brasil P, Zalis M G, de Pina-Costa A, Siqueira A M, Júnior C B, Silva S, Areas A L L, Pelajo-Machado M, *et al*. (2017)

Outbreak of human malaria caused by *Plasmodium simium* in the Atlantic Forest in Rio de Janeiro: A molecular epidemiological investigation *Lancet Glob Health* **5** e1038-e1046

Briolant S, Fusaï T, Rogier C and Pradines B (2008) Tetracycline antibiotics in malaria *Open Trop Med J* **1** 31-46

Brisibe E A, Umoren U E, Brisibe F, Magalhaes P M, Ferreira J P S *et al*. (2009) Nutritional characterization and antioxidant capacity of different tissues of *Artemisia annua Food Chemist* **115** 1240-1246

Brisibe E A, Umoren U E, Owai P U and Brisibe F (2008) Dietary inclusion of dried *Artemisia annua* leaves for management of coccidiosis and growth enhancement in chickens *African Journal of Biotechnology* **7** 4083-4092

Brown G D (2010) The biosynthesis of artemisinin (Qinghaosu) and the phytochemistry of *Artemisia annua* L. (Qinghao) *Molecules* **15** 7603-98

Brown H W (1967) Malaria parasites and other haemosporidia *JAMA* **201** 336

Burkhardt D, Wiesner J, Stoesser N, Ramharter M, Uhlemann A C, *et al*. (2007) Delayed parasite elimination in human infections treated with clindamycin parallels "delayed death" of *Plasmodium falciparum* in vitro *Int J Parasitol* **37** 777–785

Cairns M, Cisse B, Sokhna C, Cames C, Simondon K, *et al*. (2010) Amodiaquine dosage and tolerability for intermittent preventive treatment to prevent malaria in children *Antimicrob Agents Chemother* **54** 1265-1274

Caldarelli SA, Hamel M, Duckert J F, Ouattara M, Calas M, *et al*. (2012) Disulfide prodrugs of albitiazolium (T3/SAR97276): Synthesis and biological activities *J Med Chem* **55** 4619-4628

Calderón F, Vidal-Mas J, Burrows J, De La Rosa J C, Jiménez-Díaz M B, *et al*. (2012) A divergent SAR study allows optimization of a potent 5-HT2c inhibitor to a promising antimalarial scaffold *ACS Med Chem Lett* **3** 373-377

Camargo-Ayala P A, Cubides J R, Niño C H, Camargo M, Rodríguez-Celis C A, *et al*. (2016) High *Plasmodium malariae* prevalence in an endemic area of the colombian amazon region *PLoS One* **11** e0159968

Camus D, Djossou F, Schilthuis H J, Hogh B, Dutoit E, *et al*. (2004) Atovaquone-proguanil versus chloroquine-proguanil for malaria prophylaxis in nonimmune pediatric travelers: results of an international, randomized, open-label study *Clin Infect Dis* **38** 1716-1723

Carlton J M, Adams J H, Silva J C, Bidwell S L, Lorenzi H, *et al*. (2008) Comparative genomics of the neglected human malaria parasite *Plasmodium vivax Nature* **455** 757–763

CDC Malaria treatment (United States) (2014) www.cdc.gov/malaria/diagnosis_treatment/treatment.html.

Chaccour C J, Kobylinski K C, Bassat Q, Bousema T, Drakeley C, *et al*. (2013) Ivermectin to reduce malaria transmission: a research agenda for a promising new tool for elimination *Malar J* **12** 153

Chandira R M, Palanisamy P, Jayakar B and Pasupathi A (2014) Formulation and evaluation of fast dissolving tablets of Artemether and Lumefantrine *J Pharm Res* **8** 1467-1474

Chandra R S, Orazem J, Ubben D, Duparc S, Robbins J and Vandenbroucke P (2013 Creative solutions to extraordinary challenges in clinical trials: methodology of a phase III trial of azithromycin and chloroquine fixed-dose combination in pregnant women in Africa *Malar J* **12** 122

Chang C, Lin-Hua T and Jantanavivat C (1992) Studies on a new antimalarial compound: Pyronaridine *Trans R Soc Trop Med Hyg* **86** 7-10

Charman S A, Arbe-Barnes S, Bathurst I C, Brun R, Campbell M, *et al*. (2011) Synthetic ozonide drug candidate OZ439 offers new hope for a single-dose cure of uncomplicated malaria *Proc Natl Acad Sci U S A* **108** 4400-4405

Cheng C, Ho W E, Goh F Y, Guan S P, Kong L R, *et al*. (2011) Anti-malarial drug artesunate attenuates experimental allergic asthma via inhibition of the phosphoinositide 3-kinase/Akt pathway *PLoS One* **6** e20932

Choi J S, Piao Y J and Kang K W (2011) Effects of quercetin on the bioavailability of doxorubicin in rats: Role of CYP3A4 and P-gp inhibition by quercetin *Arch Pharm Res* **34** 607-613

Choi W (2017) Novel pharmacological activity of artesunate and artemisinin: Their potential as anti-tubercular agents *J Clin Med* **6** 30

Chukwu L C, *et al*. (2017) Pattern of parasitological response and symptom clearance in patients treated with artemether-lumefantrine combination in the treatment of uncomplicated malaria in Elele, a malaria endemic area in Rivers State Nigeria *The FASEB J* **31** 1002

Churcher T S, Sinden R E, Edwards N J, Poulton I D, *et al*. (2017) Probability of transmission of malaria from mosquito to human is regulated by mosquito parasite density in naïve and vaccinated hosts *PLoS Pathog* **13** e1006108

Cisse M, Awandare G A, Soulama A, Tinto H, Hayette M-P and Guiguemdé R T (2017) Recent uptake of intermittent preventive treatment during pregnancy with sulfadoxine–pyrimethamine is associated with increased prevalence of Pfdhfr mutations in Bobo-Dioulasso, Burkina Faso *Malar J* **16** 38

Clark D L, Su S and Davidson E A (1997) Saccharide anions as

inhibitors of the malaria parasite *Glycoconj J* **14** 473-479

Collins W E and Jeffery G M (2005) *Plasmodium ovale*: Parasite and disease *Clin Microb Rev* **20** 579-592

Cordel H, Cailhol J, Matheron S, Bloch M, Godineau N, *et al.* (2013) Atovaquone-proguanil in the treatment of imported uncomplicated *Plasmodium falciparum* malaria: a prospective observational study of 553 cases *Malar J* **12** 399

Cottrell G, Musset L, Hubert V, Le Bras J and Clain J (2014) Emergence of resistance to atovaquone-proguanil in malaria parasites: Insights from computational modeling and clinical case reports *Antimicrob Agents Chemother* **58** 4504-4514

Cowman A F, Galatis D and Thompson J K (1994) Selection for mefloquine resistance in *Plasmodium falciparum* is linked to amplification of the pfmdr1 gene and cross-resistance to halofantrine and quinine *Proc Natl Acad Sci U S A* **91** 1143-1147

Cowell A N, Istvan E S, Lukens A K, Gomez-Lorenzo M G, Vanaerschot M, Sakata-Kato, *et al.* (2018) Mapping the malarial parasite druggable genome by using in vitro evolution and chemogenomics *Science* **359** 197-199

Croft A M (2007) Developing safe antimalaria drugs: Key lessons from mefloquine and halofantrine *Int J Risk Saf Med* **19** 153-161

Croft S L, Duparc S, Arbe-Barnes S J, Craft J, Shin C-S, *et al.* (2012) Review of pyronaridine anti-malarial properties and product characteristics *Malar J* **11** 270

Cui L, Mharakurwa S, Ndiaye D, Rathod P K and Rosenthal P J (2015) Antimalarial drug resistance: Literature review and activities and findings of the ICEMR network *Am J Trop Med Hyg* **93** (Suppl 3) 57-68

Daddy N B, Kalisya L M, Bagire P G, Watt R L, Towler M J and Weathers P J (2017) *Artemisia annua* dried leaf tablets treated malaria resistant to ACT and i.v. artesunate: Case reports *Phytomedicine* **32** 37-40

Dahl E L, Shock J L, Shenai B R, Gut J, DeRisi J L and Rosenthal P J (2006) Tetracyclines specifically target the apicoplast of the malaria parasite *Plasmodium falciparum Antimicrob Agents Chemother* **50** 3124-3131

Daulay D A P, Trisnawati Y, Lubis S, Pasaribu S, *et al.* (2011) Comparison of quinine-doxycycline and quinine-clindamycin for falciparum malaria in children *Paediatrica Indonesia* **51** 187-191

Davis T M E, Hung T Y, Sim I K, Karunajeewa H A and Ilett K F (2005) Piperaquine: A resurgent antimalarial drug *Drugs* **65** 75-87

de Carvalho G B and de Carvalho G B (2011) Duffy Blood

Group System and the malaria adaptation process in humans *Rev Bras Hematol Hemoter* **33** 55-64

De Magalhães P M, Pereira B, Sartoratto A, De Oliveira J and Debrunner N (1999) New hybrid lines of the antimalarial species *Artemisia annua* L. *Acta Hortic* **502** 377-381

de Magalhaes P M, Pereira B and Sartoratta A (2004) Yields of antimalarial *Artemisia annua* L. species. Acta Hort 629, I5H5 *Proc XXVI IHC_Future of Medicinal and Aromatic Plants* Eds LE 421-424

Delabays N, Collet G and Benakis A (1993) Selection and breeding for high artemisinin (quinghaso) yielding strains of *Artemisia annua Acta Hort* **330** 203-207

Delabays N, Simonnet X and Gaudin M (2001) The genetics of artemisinin content in Artemisia annua L. and the breeding of high yielding cultivars *Curr Med Chem* **8** 1795-1801

Dellicour S, Sevene E, McGready R, Tinto H, Mosha D, *et al.* (2017) First-trimester artemisinin derivatives and quinine treatments and the risk of adverse pregnancy outcomes in Africa and Asia: A meta-analysis of observational studies *PLoS Med* **14** e1002290

Delves M, Plouffe D, Scheurer C, Meister S, Wittlin S, *et al.* (2012b) The activities of current antimalarial drugs on the life cycle stages of plasmodium: A comparative study with human and rodent parasites *PLoS Med* **9** e1001169

Delves M J, Ramakrishnan C, Blagborough A M, Leroy D, Wells T N C and Sinden R E (2012a) A high-throughput assay for the identification of malarial transmission-blocking drugs and vaccines *Int J Parasitol* **42** 999-1006

Delves M J, Ruecker A, Straschil U, Lelièvre J, Marques S, *et al.* (2013) Male and female *Plasmodium falciparum* mature gametocytes show different responses to antimalarial drugs *Antimicrob Agents Chemother* **57** 3268-3274

Deng Y, Ran W, Man S, Li X, Gao H, *et al.* (2015) Artemether exhibits amoebicidal activity against *Acanthamoeba castellanii* through inhibition of the serine biosynthesis pathway *Antimicrob Agents Chemother* **59** 4680-4688

Derda M, Hadaœ E, Cholewiñski M, Skrzypczak £, Grzondziel A and Wojtkowiak-Giera A (2016) *Artemisia annua* L. as a plant with potential use in the treatment of acanthamoebiasis *Parasitol Res* **115** 1635-1639

Desai M, Gutman J, Taylor S M, Wiegand R E, Khairallah C, *et al.* (2016) Impact of sulfadoxine-pyrimethamine resistance on effectiveness of intermittent preventive therapy for Malaria in pregnancy at clearing infections and preventing low birth weight *Clin Infect Dis* **62** 323-333

Desrosiers M R and Weathers P J (2016) Effect of leaf digestion and artemisinin solubility for use in oral consumption of dried *Artemisia annua* leaves to treat malaria *J*

Ethnopharmacol **190** 313-318

Desrosiers M R and Weathers P J (2018) Artenisinin permeability via CaCo-2 cells increases after simulated digestion of *Artemisia annua* leaves *J Ethnopharmacol* **210** 254-259

Devine A, Parmiter M, Chu CS, Bancone G, Nosten F, *et al.* (2017) Using G6PD tests to enable the safe treatment of *Plasmodium vivax* infections with primaquine on the Thailand-Myanmar border: A cost-effectiveness analysis *PLoS Negl Trop Dis* **11** e0005602

Dhingra V, Rao K V and Narasu M L (1999) Artemisinin: Present status and perspectives *Biochemical Education* **27** 105-109

Dicko A, Roh M E, Diawara H, Mahamar A, Soumare H M, *et al.* (2018) Efficacy and safety of primaquine and methylene blue for prevention of *Plasmodium falciparum* transmission in Mali: a phase 2, simple-blind, randomized controlled trial *The Lancet Infect Disease* doi.org/10.1016/S1473-3099(18)30044-6

Doctor S M, Liu Y, Anderson O G, Whitesell A N, Mwandagalirwa M K, *et al.* (2016) Low prevalence of *Plasmodium malariae* and *Plasmodium ovale* mono-infections among children in the Democratic Republic of the Congo: a population-based, cross-sectional study *Malar J* **15** 350

Doerig C, Rayner J C, Scherf A and Tobin A B (2015) Post-translational protein modifications in malaria parasites *Nat Rev Microbiol* **13** 160-172

Dondorp A M (2017) New genetic marker for piperaquine resistance in *Plasmodium falciparum Lancet Infect Dis* **17** 119-121

Dondorp A M, Fanello C I, Hendriksen I C, Gomes E, Seni A, *et al.* (2010) Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): An open-label, randomised trial *Lancet* **376** 1647-1657

Dondorp A M, Nosten F, Yi P, Das D, Phyo A P, *et al.* (2009) Artemisinin resistance in *Plasmodium falciparum* malaria *N Engl J Med* **361** 455-467

Dondorp A M, Smithuis F M, Woodrow C and Seidlein L von (2017) How to contain artemisinin- and multidrug-resistant falciparum malaria *Trends Parasitol* **33** 353-363

Dow G S, Gettayacamin M M, Hansukjariya P P, imerbsin R R, Komcharoen S S, *et al.* (2011) Radical curative efficacy of tafenoquine combination regimens in Plasmodium cynomolgi-infected Rhesus monkeys (*Macaca mulatta*) *Malar J* **10** 212

Drãgan L, Györke A, Ferreira J F, Pop I A, Dunca I, *et al.* (2014) Effects of *Artemisia annua* and *Foeniculum vulgare* on

chickens highly infected with *Eimeria tenella* (Phylum Apicomplexa) *Acta Vet Scand* **56** 22

Du J-H, Zhang H-D, Ma Z-J and Ji K-M (2010) Artesunate induces oncosis-like cell death in vitro and has antitumor activity against pancreatic cancer xenografts in vivo *Cancer Chemother Pharmacol* **65** 895-902

Duffy C W, Ba H, Assefa S, Ahouidi A D, Deh Y B, *et al.* (2017) Population genetic structure and adaptation of malaria parasites on the edge of endemic distribution *Mol Ecol* **26** 2880-2894

Duffy S and Avery V M (2012) Development and optimization of a novel 384-well anti-malarial imaging assay validated for high-throughput screening *Am J Trop Med Hyg* **86** 84-92

Duffy S and Avery V M (2013) Identification of inhibitors of *Plasmodium falciparum* gametocyte development *Malar J* **12** 408

Duke J A (2001) Handbook of phytochemical constituents of CoRAs herbs and other economic plants *CRC Press*

Dutta G P, Puri S K, Bhaduri A P and Seth M (1989) Radical curative activity of a new 8-aminoquinoline derivative (CDRI 80/53) against *Plasmodium cynomolgi* B in monkeys *Am J Trop Med Hyg* **41** 635-637

D¼Alessandro U (2009) Progress in the development of piperaquine combinations for the treatment of malaria *Curr Opin Infect Dis* **22** 588-592

Ecker A, Lehane A M, Clain J and Fidock D A (2012) PfCRT and its role in antimalarial drug resistance *Trends Parasitol* **28** 504-514

Eder M, Farne H, Cargill T, Abbara A and Davidson R N (2012) Intravenous artesunate versus intravenous quinine in the treatment of severe falciparum malaria: a retrospective evaluation from a UK centre *Pathog Glob Health* **106** 181-187

Efferth T (2007) Willmar Schwabe Award 2006: Antiplasmodial and antitumor activity of artemisinin - From bench to bedside *Planta Med* **73** 209-309

Efferth T (2017) From ancient herb to modern drug: *Artemisia annua* and artemisinin for cancer therapy *Semin Cancer Biol* **46** 65-83

Efferth T (2017) Cancer combination therapies with artemisinin-type drugs *Biochemical Pharmacology* **139** 56-70

Efferth T, Herrmann F, Tahrani A and Wink M (2011) Cytotoxic activity of secondary metabolites derived from *Artemisia annua* L. towards cancer cells in comparison to its designated active constituent artemisinin *Phytomedicine* **18** 959-969

Efferth T, Romero M R, Osman A G, ElSohly M, Wink M, *et al.* (2016) Expanding the therapeutic spectrum of artemisinin activity against infectious diseases beyond malaria and novel pharmacological developments *World J Tradition Clin Med* **2** 1-23

Efferth T, Romero M R, Wolf D G, Stamminger T, Marin J J G and Marschall M (2008) The antiviral activities of artemisinin and artesunate *Clin Infect Dis* **47** 804-811

Elfawal M A, Towler M J, Reich N G, Golenbock D, Weathers P J and Rich S M (2012) Dried whole plant *Artemisia annua* as an antimalarial therapy *PLoS One* **7** e52746

Elfawal M A, Towler M J, Reich N G, Weathers P J and Rich S M (2015) Dried whole-plant *Artemisia annua* slows evolution of malaria drug resistance and overcomes resistance to artemisinin *Proc Natl Acad Sci USA* **112** 821-826

Elford B C, Roberts M F, Phillipson J D and Wilson R J M (1987) Potentiation of the antimalarial activity of qinghaosu by methoxylated flavones *Trans R Soc Trop Med Hyg* **81** 434436

Elmes N J, Nasveld P E, Kitchener S J, Kocisko D A and Edstein M D (2008) The efficacy and tolerability of three different regimens of tafenoquines versus primaquine for post-exposure prophylaxis of *Plasmodium vivax* malaria in south west Pacific *Trans Royal Soc Trop Med Hyg* **102** 1095-1101

Epstein J E, Paolino K M, Richie T L, Sedegah M, Singer A, *et al.* (2017) Protection against *Plasmodium falciparum* malaria by PfSPZ Vaccine *JCI Insight* **2** e89154

Esu E, Effa E E, Opie O N, Uwaoma A and Meremikwu M M (2014) Artemether for severe malaria *Cochrane database Syst Rev* **9** CD010678

Eziefula A C, Bousema T, Yeung S, Kamya M, Owaraganise A, *et al.* (2014) Single dose primaquine for clearance of *Plasmodium falciparum* gametocytes in children with uncomplicated malaria in Uganda: A randomised, controlled, double-blind, dose-ranging trial *Lancet Infect Dis* **14** 130-139

Ezzet F, Van Vugt M, Nosten F, Looareesuwan S and White N J (2000) Pharmacokinetics and pharmacodynamics of lumefantrine (benflumetol) in acute falciparum malaria *Antimicrob Agents Chemother* **44** 697-704

Färnert A, Lindberg J, Gil P, Swedberg G, Berqvist Y, *et al.* (2003) Evidence of *Plasmodium falciparum* malaria resistant to antovaquone and proguanil hdrochloride: case reports *Bmj* **326** 628-629

Fei L and Weathers P J (2015) From leaf explants to rooted plantlets in a mist reactor *In Vitro Cell Dev Biol Plant* **51** 669-681

Fei L and Weathers P J (2016) From leaf explants to hanging rooted plantlets in a mist reactor *Plant Cell Tiss Org Cult* **124** 265-274

Ferreira J F S and Janick J (1995) Production and detection of artemisinin from *Artemisia annua Int Symp Med Aromat Plants* **209** 41-49

Ferreira J F S, Laughlin J C, Delabays N and de Magalhães P M (2005) Cultivation and genetics of *Artemisia annua* L. for increased production of the antimalarial artemisinin *Plant Genet Resour Charact Util* **3** 206-229

Ferreira J F S, Luthria D L, Sasaki T and Heyerick A (2010) Flavonoids from *Artemisia annua* L. As antioxidants and their potential synergism with artemisinin against malaria and cancer *Molecules* **15** 3135-3170

Ferreira J F S, Simon J E and Janick J (2010) Artemisia *annua*/ : Botany, Horticulture, Pharmacology. In *Horticultural Reviews*, pp. 319-71

Fowler R E, Sinden R E and Pudney M (1995) Inhibitory activity of the anti-malarial atovaquone (566C80) against ookinetes, oocysts, and sporozoites of *Plasmodium berghei J Parasitol* **81** 452-458

Francis G, Kerem Z, Makkar H P S and Becker K (2002) The biological action of saponins in animal systems: a review *Br J Nutr* **88** 587-605

Friedman R and Caflisch A (2009) Discovery of plasmepsin inhibitors by fragment-based docking and consensus scoring *Chem Med Chem* **4** 1317-1326

Gadalla N B, Abdallah T M, Atwal S, Sutherland C J and Adam I (2013) Selection of pfdhfr/pfdhps alleles and declining artesunate/sulphadoxine-pyrimethamine efficacy against *Plasmodium falciparum* eight years after deployment in eastern Sudan *Malar J* **12** 255

Gaillard T, Briolant S, Madamet M and Pradines B (2017) The end of a dogma: the safety of doxycycline use in young children for malaria treatment *Malar J* **16** 148

Gaillard T, Madamet M and Pradines B (2015) Tetracyclines in malaria *Malar J* **14** 445

Gaillard T, Madamet M, Tsombeng F F, Dormoi J and Pradines B (2016) Antibiotics in malaria therapy: which antibiotics except tetracyclines and macrolides may be used against malaria? *Malar J* **15** 556

Galatas B, Nhamussua L, Candrinho B, Mabote L, Cisteró P, *et al.* (2017) *In-vivo* efficacy of chloroquine to clear asymptomatic infections in mozambican adults: a randomized, placebo-controlled trial with implications for elimination strategies *Sci Rep* **7** 1356

Gardner M J, Hall N, Fung E, White O, Berriman M, et al. (2002)

Genome sequence of the human malaria parasite *Plasmodium falciparum Nature* **419** 498-511

Gargano N, Ubben D, Tommasini S, Bacchieri A, Corsi M, *et al*. (2012) Therapeutic efficacy and safety of dihydroartemisinin-piperaquine versus artesunate-mefloquine in uncomplicated *Plasmodium falciparum* malaria in India *Malar J* **11** 233

Gatton M L, Martin L B and Cheng Q (2004) Evolution of resistance to sulfadoxine-pyrimethamine in *Plasmodium falciparum Antimicrob Agents Chemother* **48** 2116–2123

Gautam P, Upadhyay S K, Hassan W, Madan T, Srideshmukh R *et al*. (2011) Transcriptic and proteomic profile of *Aspergillus fumigatus* on exposure to artemisinin *Mycopathologia* **172** 331-346

Ghaffarifar F, Esavand Heydari F, Dalimi A, Hassan Z M, Delavari M and Mikaeiloo H (2015) Evaluation of apoptotic and antileishmanial activities of artemisinin on promastigotes and BALB/C mice infected with *Leishmania major Iran J Parasitol* **10** 258-267

Gil J P, Nogueira F, Strömberg-Nörklit J, Lindberg J, Carrolo M, Casimiro C, Lopes D, Arez A P, Cravo P V and Rosário V E (2003) Detection of atovaquone and malarone resistance conferring mutations in *Plasmodium falciparum* cytochrome b gene (Cyt b) *Mol Cell Probes* **17** 85-89

Global Malaria Progamme (2015) Control and elimination of *Plamodium vivax* Malaria - A Technical Brief

Goel D et al. (2016) Artesunate-, sulfadoxine-, and pyrimethamine-induced cardiotoxicity *BLDE Univ J Health Sci* Case Report **1** 54-56

Goel R, Goel D, Chaudhary S, Sharma V and Kumar S (2011) Cropping of the artemisinin (antimalarial drug) yielding *Artemisia annua* cultivars, over a ten year period in the agroclimate of north-west India,has not led to the species becoming a weed *National Academy Science Letters* **34** 307-315

Gomes T C, Andrade Júnior H F de, Lescano S A Z and Amato-Neto V (2012) In vitro action of antiparasitic drugs, especially artesunate, against *Toxoplasma gondii Rev Soc Bras Med Trop* **45** 485-490

Goodman C D, Siregar J E, Mollard V, Vega-Rodriguez J, Syafruddin D, *et al*. (2016) Parasites resistant to the antimalarial atovaquone fail to transmit by mosquitoes *Science* **352** 349-353

Gopalakrishnan A M and Kumar N (2015) Antimalarial action of artesunate involves DNA damage mediated by reactive oxygen species *Antimicrob Agents Chemother* **59** 317-325

Gopinath B et al. (2014) In vitro propagation of an important medicinal plant *Artemisia annua* L. from axillary bud explants *Adv Appl Sci Res* **5** 254-258

Goulart H R, Kimura E A, Peres V J, Couto A S, Duarte F A A and Katzin A M (2004) Terpenes arrest parasite development and inhibit biosynthesis of isoprenoids in *Plasmodium falciparum Antimicrob Agents Chemother* **48** 2502-2509

Grace M H, Lategan C, Graziose R, Smith P J, Raskin I and Lila M A (2012) Antiplasmodial activity of the ethnobotanical plant *Cassia fistula Nat Prod Commun* **7** 1263-1266

Graves P M, Choi L, Gelband H and Garner P (2018) Primaquine or other 8-amino quinolines for reducing *Plasmodium falciparum* transmission *Cochrane Darabase of Syst Rev* doi.10.1002/14651858.CD008152.pub5

Graves P M, Gelband H and Ribeiro I (2009) Primaquine for reducing transmission of *Plasmodium falciparum* malaria *Lancet Infect Dis* **16** 674-684

Grigg M J, William T, Menon J, Barber B E, Wilkes C S, *et al*. (2016) Efficacy of artesunate-mefloquine for chloroquine-resistant *Plasmodium vivax* malaria in Malaysia: An open-label, randomized, controlled trial *Clin Infect Dis* **62** 1403-1411

Gujjar R, E l Mazouni F, White K L, White J, Creason S, *et al*. (2011) Lead optimization of aryl and aralkyl amine-based triazolopyrimidine inhibitors of *Plasmodium falciparum* dihydroorotate dehydrogenase with antimalarial activity in mice *J Med Chem* **54** 3935–3949

Gupta A, Gupta M M, Srivastava A, Bansal R P, Lal R K, *et al*. (2016) CIM-Sanjeevani: A high artemisinin yielding population of Artemisia (*Artemisia annua*) *J Med Arom Plant Sci* **38** 78-83

Gupta A P, Chin W H, Zhu L, Mok S, Luah Y, *et al*. (2013) Dynamic epigenetic regulation of gene expression during the life cycle of malaria parasite *Plasmodium falciparum PLoS Pathog* **9** e1003170

Gupta B, Xu S, Wang Z, Sun L, Miao J, *et al*. (2014) *Plasmodium falciparum* multidrug resistance protein 1 (*pfmrp1*) gene and its association with *in vitro* drug susceptibility of parasite isolates from northeast Mynmar *J Antimicrob Chemother* **89** 2110-2117

Gupta S K, Singh P, Bajpai P, Ram G, Singh D, *et al*. (2002) Morphogenetic variation for artemisinin and volatile oil in *Artemisia annua Ind Crops Prod* **16** 217-224

Gutman J, Kovacs S, Dorsey G, Stergachis A and ter Kuile F O (2017) Safety, tolerability, and efficacy of repeated doses of dihydroartemisinin-piperaquine for prevention and treatment of malaria: a systematic review and meta-analysis *Lancet Infect Dis* **17** 184-193

Gutman J and Slutsker L (2017) Intermittent preventive treatment with sulfadoxine-pyrimethamine: More than just an Antimalarial? *Am J Trop Med Hyg* **11** 9-10

Guttery D S, Holder A A and Tewari R (2012) Sexual development in plasmodium: Lessons from functional analyses *PLoS Pathog* **8** e1002404

Hall N (2012) Genomic insights into the other malaria *Nat Genet* **44** 962-963

Hamilton W L, Claessens A, Otto T D, Kekre M, Fairhurst R M, *et al*. (2017) Extreme mutation bias and high AT content in *Plasmodium falciparum Nucleic Acids Res* **45** 1889-1901

Harrison T R (1970) Harrison's principles of internal medicine 6ᵗʰ ed, McGraw-Hill, NY

Hatz C, Soto J, Nothdurft H D, Zoller T, Weitzel T, *et al*. (2008) Treatment of acute uncomplicated falciparum malaria with artemether-lumefantrine in nonimmune populations: a safety, efficacy, and pharmacokinetic study *Am J Trop Med Hyg* **78** 241-247

Helal E G E, *et al*. (2014) Anti-diabetic effect of *Artemisia annua* (Kaysom) is alloxan induced diabetic rats *Egypt J Hosp Med* **57** 422-430

Henriques G, Hallett R L, Beshir K B, Gadalla N B, Johnson R E, *et al*. (2014) Directional selection at the pfmdr1, pfcrt, pfubp1, and pfap2mu loci of *Plasmodium falciparum* in Kenyan children treated with ACT *J Infect Dis* **210** 2001-2008

Herchline T E (2016) Malaria treatment and management emedicine.medscope.com/article/221134-treatment-overview#a1

Hii J and Rueda L M (2013) Malaria vectors in the Greater Mekong Subregion: overview of malaria vectors and remaining challenges *Southeast Asian J Trop Med Public Health* **44** Suppl 1 73-165

Hill D R, Baird J K, Parise M E, Lewis L S, Ryan E T and Magill A J (2006) Primaquine: Report From Cdc Expert Meeting on Malaria Chemoprophylaxis I *Am J Trop Med Hyg* **75** 402-415

Ho W E, Cheng C, Peh H Y, Xu F, Tannenbaum S R, *et al*. (2012) Anti-malarial drug artesunate ameliorates oxidative lung damage in experimental allergic asthma *Free Radic Biol Med* **53** 498–507

Ho W E, Peh H Y, Chan T K and Wong W S F (2014) Artemisinins: Pharmacological actions beyond anti-malarial *Pharmacol Ther* **142** 126-139

Hodel E, Guidi M, Zanolari B, Mercier T, Duong S, *et al*. (2013) Population pharmacokinetics of mefloquine, piperaquine and artemether-lumefantrine in Cambodian and Tanzanian

malaria patients *Malar J* **12** 235

Hoglund R M, Adam I, Hanpithakpong W, Ashton M, Lindegardh N, *et al*. (2012) A population pharmacokinetic model of piperaquine in pregnant and non-pregnant women with uncomplicated *Plasmodium falciparum* malaria in Sudan *Malar J* **11** 398

Hongwen Y and Shouming Z (2002) Chapter 9 In : C. W. Wright, Artemisia, New York, N.Y Taylor and Francis p 148-158

Howes R E, Battle K E, Satyagraha A W, Baird J K and Hay S I (2013) G6PD Deficiency. Global Distribution, Genetic Variants and Primaquine Therapy *Adv Parasitol* **81** 135-201

Hsu E (2006) The history of qing hao in the Chinese materia medica *Trans R Soc Trop Med Hyg* **100** 505-508

Huang H, Lu W, Li X, Cong X, Ma H, *et al*. (2012) Design and synthesis of small molecular dual inhibitor of falcipain-2 and dihydrofolate reductase as antimalarial agent *Bioorganic Med Chem Lett* **22** 958-962

Ikram N K B K and Simonsen H T (2017) A review of biotechnological Artemisinin production in plants *Front Plant Sci* **8** 1966

ICIPE (2005) Whole leaf *Artemisia annua* -based antimalarial drug: report on proof-of-concept studies http://bot.ly//lvCHQkH.2005

Ikram N K B K, Kashkovli A B, Peramuna A P, vander Krol A R, Bouwmeester H, *et al*. (2017) Stable production of the antimalarial drug artemisinin in the moss *Physcomitrella patens Front Bioeng Biotechnol* **5** 47

Imwong M, Suwannasin K, Kunasol C, Sutawong K, Mayxay M, *et al*. (2017) The spread of artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong subregion: a molecular epidemiology observational study *Lancet Infect Dis* **17** 491-497

Islamuddin M, Chouhan G, Want M Y, Tyagi M, Abdin M Z, *et al*. (2014) Leishmanicidal activities of *Artemisia annua* leaf essential oil against Visceral Leishmaniasis *Front Microbiol* **5** 626

Islamuddin M, Farooque A, Dwarakanath B S, Sahal D and Afrin F (2012) Extracts of *Artemisia annua* leaves and seeds mediate programmed cell death in *Leishmania donovani J Med Microbiol* **61**(PART12) 1709-1718

Janse CJ, Ponnudurai T, Lensen A H, Meuwissen J H, Ramesar J, *et al*. (1988) DNA synthesis in gametocytes of *Plasmodium falciparum Parasitology* **96** 1-7

Jeong D E, Song H J, Lim S, Lee S J, Lim J E, *et al*. (2015) Repurposing the anti-malarial drug artesunate as a novel therapeutic agent for metastatic renal cell carcinoma due to

its attenuation of tumor growth, metastasis, and angiogenesis *Oncotarget* **6** 33046-33064

Jia J, Qin Y, Zhang L, Guo C, Wang Y, *et al*. (2016) Artemisinin inhibits gallbladder cancer cell lines through triggering cell cycle arrest and apoptosis *Mol Med Rep* **13** 4461-4468

Jiang X, Zhang H, Wang M, Wang L and Zhang L (2013) Comparison analysis of different parts and geographical origins from southwestern China on artemisinin content of *Artemisia annua Curr Trends Technol Sci* **2** 293

Josling G A and Llinás M (2015) Sexual development in Plasmodium parasites: knowing when it's time to commit *Nat Rev Microbiol* **13** 573-587

Kakkilaya B S (2003) Rapid diagnosis of malaria *Lab Med* **8** 602-608

Kakuru A, Jagannathan P, Muhindo M K, Natureeba P, Awori P, *et al*. (2016) Dihydroartemisinin-Piperaquine for the prevention of malaria in pregnancy *N Engl J Med* **374** 928-939

Kaptein S J F, Efferth T, Leis M, Rechter S, Auerochs S, *et al*. (2006) The anti-malaria drug artesunate inhibits replication of cytomegalovirus in vitro and in vivo *Antiviral Res* **69** 60-69

Karunajeewa H A, Reeder J, Lorry K, Dabod E, Hamzah J, Page-Sharp M, *et al*. (2006) Artesunate suppositories versus intramuscular artemether for treatment of severe malaria in children in Papua New Guinea *Antimicrob Agents Chemother* **50** 968-974

Kazmin D, Nakaya H I, lee E K, Johnson M J, vander Most R A *et al*. (2017) Systems analysis of protective immune responses to RTS,S malaria vaccination in humans *Proc Natl Acad Sci USA* **114** 2425-2430

Keating G M (2012) Dihydroartemisinin/piperaquine: A review of its use in the treatment of uncomplicated *Plasmodium falciparum* malaria *Drugs* **72** 937-961

Keiser J and Utzinger J (2007) Artemisinins and synthetic trioxolanes in the treatment of helminth infections *Curr Opin Infect Dis* **20** 605-612

Kerb R, Fux R, Mörike K, Kremsner P G, Gil J P, *et al*. (2009) Pharmacogenetics of antimalarial drugs: effect on metabolism and transport *Lancet Infect Dis* **9** 760-774

Kheng S, Muth S, Taylor W R J, Tops N, Kosal K, *et al*. (2015) Tolerability and safety of weekly primaquine against relapse of *Plasmodium vivax* in Cambodians with glucose-6-phosphate dehydrogenase deficiency *BMC Med* **13** 203

Kim K E, Ko K, Heo R W, Yi C O, Shin H J, *et al*. (2016) *Artemisia annua* leaf extract attenuates hepatic steatosis and inflammation in high-fat diet-fed mice *J Med Food* **19** 290-299

Kisalu N K, Idris A H, Weidle C, Flores-Garcia Y, *et al*. (2018) A human monoclonal antibody prevents malaria infection by targetting a new site of vulnerability in the parasite *Nature Med* doi:10.1038/nm.4512

Kiszewski A E (2011) Blocking *Plasmodium falciparum* malaria transmission with drugs: The gametocytocidal and sporontocidal properties of current and prospective antimalarials *Pharmaceuticals (Basel)* **4** 44-68

Klonis N, Creek D J and Tilley L (2013) Iron and heme metabolism in *Plasmodium falciparum* and the mechanism of action of artemisinins *Curr Opin Microbiol* **16** 722-727

Krafts K, Hempelmann E and Skorsha-Stania A (2012) From methylene blue to chloroquine: a brief review of the development of an antimalarial therapy *Parasitol Res* **111** 1-6

Kremsner P G, Adegnika A A, Hounkpatin A B, Zinsou J F, Taylor T E, *et al*. (2016) Intramuscular artesunate for severe malaria in African children: a multicenter randomized controlled trial *PLoS Med* **13** e1001938

Krishna S, Bhandari S, Bharti P K, Basak S and Singh N (2017) A rare case of quadruple malaria infection from the highly malaria-endemic area of Bastar, Chhattisgarh, India *PLoS Negl Trop Dis* **11** e0005558

Krishna S, Ganapathu S, Ster I C, Saeed M E, Cowan M *et al*. (2014) A randomized, double-blind placebo-controlled pilot study of oral artesunate therapy for colorectal cancer *Ebio Med* **2** 82-90

Kumar S, Banerjee S, Dwivedi S, Gupta M M, Verma R K *et al*. (1999) Registration of Jeevanraksha and Suraksha varieties of the antimalarial medicinal plant *Artemisia annua J Med Arom Plant Sci* **21** 47-48

Kumar S, Gupta S K, Gupta M M, Verma R K, Jain D C *et al*. (2002) Method for maximization of artemisinin production by the plant *Artemisia anuua* US Patent no. 6,393,763B1

Kumar S, Gupta S K, Singh P, Bajpai P, Gupta M M *et al*. (2004) High yields of artemisinin by multi-harvest of *Artemisia annua* crops *Indust Crops Prod* **19** 77-90

Kumar S, Kumari R and Pandey R (2015) New insight-guided approaches to detect, cure, prevent and eliminate malaria *Protoplasma* **251** 717-753

Kumar S, Ram S, Verma D K, Dangesh A and Tomar V K S (2015) Public-private partnership towards rural development: a study of *Artemisia annua* in Uttar Pradesh *Curr Sci* **109** 1237-1239

Kumar S and Srivastava S (2005) Establishment of artemisinin combination therapy as first line treatment for combating

malaria: *Artemisia annua* cultivation in India needed for providing sustainable supply chain of artemisinin *Curr Sci* **89** 1097-1102

Kung S H, Lund S, Murarka A, McPhee D and Paddon C J (2018) Approaches and recent developments for the commercial production of semi-synthetic artemisinin *Front Plant Sci* doi.org/10.3389/fpls.2018.00087

La Crue A N, Saenz F E, Cross R M, Udenze K O, Monastyrskyi A, Stein S, Mutka T S, Manetsch R and Kyle D E (2013) 4(1H)-quinolones with liver stage activity against *Plasmodium berghei Antimicrob Agents Chemother* **57** 417-424

Lachish T, Bar-Meir M, Eisenberg N and Schwartz E (2016) Effectiveness of twice a week prophylaxis with atovaquone-proguanil (Malarone??) in long-term travellers to West Africa *J Travel Med* **23** pii: taw064

Lalloo D G, Shingadia D, Bell D J, Beeching N J, Whitty C J M and Chiodini P L (2016) UK malaria treatment guidelines 2016 *J Infect* **72** 635-649

Laman M, Moore B R, Benjamin J M, Yadi G, Bona C, *et al.* (2014) Artemisinin-Naphthoquine versus Artemether-Lumefantrine for uncomplicated malaria in Papua New Guinean children: An open-label randomized trial *PLoS Med* **11** e1001773

Landier J, Parker D M, Thu A M, Carrara V I, Lwin K M, *et al.* (2016) The role of early detection and treatment in malaria elimination *Malar J* **15** 363

Laughlin J C (1994) Agricultural production of artemisinin—A review *Trans R Soc Trop Med Hyg* **88** 21-22

Laughlin J C (2002) Post-harvest drying treatment effects on amtimalarial constituents of *Artemiasia annua* L. *Acta Horticulturae* **576** 315-320

Leang R, Canavati S E, Khim N, Vestergaard L S, Borghini Fuhrer I, *et al.* (2016) Efficacy and safety of pyronaridine-artesunate for treatment of uncomplicated *Plasmodium falciparum* malaria in western Cambodia *Antimicrob Agents Chemother* **60** 3884-3890

Lee M S J, Maruyama K, Fujita Y, Konishi A, Lelliott P M *et al.* (2017) Plasmodium products persist in the bone marrow and promote chronic bone loss *Sci Immunol* **2** eaam8093

Lehane A M and Saliba K J (2008) Common dietary flavonoids inhibit the growth of the intraerythrocytic malaria parasite *BMC Res Notes* **1** 26

Lelivre J, Almela M J, Lozano S, Miguel C, Franco V, *et al.* (2012) Activity of clinically relevant antimalarial drugs on *Plasmodium falciparum* mature gametocytes in an atp bioluminescence "transmission blocking" assay *PLoS One* **7** e35019

Li H, Wang W, Qu G, Li Y, Tao Y, *et al.* (2012) Effect of the in vivo activity of dihydroartemisinin against Schistosoma mansoni infection in mice *Parasitol Res* **110** 1727-1732

Li H J, Wang W, Li Y Z, Qu G L, Xing Y T, *et al.* (2011) Effects of artemether, artesunate and dihydroartemisinin administered orally at multiple doses or combination in treatment of mice infected with *Schistosoma japonicum Parasitol Res* **109** 515-519

Li J, Casteels T, Frogne T, Ingvorsen C, Honoré C, *et al.* (2017) Artemisinins target GABA A receptor signaling and impair á cell identity *Cell* **168** 86-100.e15

Li Y, Wang S, Wang Y, Zhou C, Chen G, *et al.* (2013) Inhibitory effect of the antimalarial agent artesunate on collagen-induced arthritis in rats through nuclear factor kappa B and mitogen-activated protein kinase signaling pathway *Transl Res* **161** 89-98

Liu K C S C, Yang S L, Roberts M F, Elford B C and Phillipson J D (1992) Antimalarial activity of *Artemisia annua* flavonoids from whole plants and cell cultures *Plant Cell Rep* **11** 637-640

Liu K C S C, Yang S L, Roberts M F, Elford B C and Phillipson J D (1999) The contribution of flavonoids to the antimalarial activity of *Artemisia annua Planta Medica* **55** 654-655

Livezey J, Oliver T and Cantilena L (2016) Prolonged neuropsychiatric symptoms in a military service member exposed to mefloquine *Drug Saf - Case Reports* **3** 7

Loo C S N, Lam N S K, Yu D, Su X zhuan and Lu F (2017) Artemisinin and its derivatives in treating protozoan infections beyond malaria *Pharmacol Res* **117** 192-217

Lu F, Culleton R, Zhang M, Ramaprasad A and Von Seidlein L (2017) Emergence of Indigenous Artemisinin-Resistant *Plasmodium falciparum* in Africa *N Engl J Med* **376** 991-993

Luo A, Cai Z, Jing Y and Shen J (2009) Artemisinin content determination and AFLP analysis of Artemisia grown in Chongqing *Mol Plant Breed* **7** 1144-1148

Malaria parasites. www.cdc.gov/malaria/about/biology/parasites.htm

Maitlaud K, Nadel S, Pollard A J, Williams T N, Newton C R and Levin M (2006) Management of severe malaria in children: proposed guidelines for the United Kingdom *BMJ* **331** 337-343

Magalhaes P, Dupont I, Hendrick X A, Joly A, Raas T *et al.* (2012) Anti-inflammatory effect and modulation of cytochrome P450 activities by *Artemisia annua* tea infusions in human intestinal caco-2 cells *Food Chem* **134** 864-871

Maiga H, Lasry I, Diarra M, Sagara J, Bamadio A, *et al*. (2016) Seasonal malaria chemoprevention with sulphadoxine-pyrimethamine and amodiaquine selects DHER-DHPS quintuple genotype in Mali *PLoS One* **11** e0162718

Malhotra K, Subramaniyan M, Rawat K, Kalamuddin M, Qureshi M I, *et al*. (2016) Compartmentalized metabolic engineering for artemisinin biosynthesis and effective malaria treatment by oral delivery of plant cells *Mol Plant* **9** 1464-1477

Malik A, Kushnoor A, Saini V, Singhal S, Kumar S and Yadav Y C (2011) *In vitro* antioxidant properties of scopoletin *J Chem Pharm Res* **3** 659-665

Marrelli M T and Brotto M (2016) The effect of malaria and anti-malarial drugs on skeletal and cardiac muscles *Malar J* **15** 524

Masanja I M, Selemani M, Khatib R A, Amuri B, Kuepfer I, *et al*. (2013) Correct dosing of artemether-lumefantrine for management of uncomplicated malaria in rural Tanzania: do facility and patient characteristics matter? *Malar J* **12** 446

Mathur A K and Kumar S (1996) Micropropagation of *Artemisia annua* via the inflorescence *J Herbs Spices and Med Plants* **4** 61-71

Mayxay M, Pukrittayakamee S, Newton P N and White N J (2004) Mixed-species malaria infections in humans *Trends Parasitol* **20** 233-240

Mazier D, Rénia L and Snounou G (2009) A pre-emptive strike against malaria's stealthy hepatic forms *Nat Rev Drug Discov* **8** 854-864

Mbengue A, Bhattacharjee S, Pandharkar T, Liu H, Estiu G, *et al*. (2015) A molecular mechanism of artemisinin resistance in *Plasmodium falciparum* malaria *Nature* **520** 683-687

McGready R, Tan S O, Ashley E A, Pimanpanarak M, Viladpainguen J, *et al*. (2008) A randomised controlled trial of artemether-lumefantrine versus artesunate for uncomplicated *Plasmodium falciparum* treatment in pregnancy *PLoS Med* **5** 1699-1715

Medicine for malaria value. www.mmv.org/research.development/artesunate-rd-portfolio

Menard D and Dondorp A (2017) Antimalarial drug resistance: a threat to malaria elimination *Cold Spring Harb Perspect Med* **7** 1-24

Mesa L E, Lutgen P, Velez I D, Segura A M and Robledo S M (2015) *Artemisia annua* L., potential source of molecules with pharmacological activity in human diseases *Am J Phytomed Clinic Therapeutic* **3** 436-450

Mesa L E, Vasquez D, Lutgen P, Vélez I D, Restrepo A M, *et al*. (2017) In vitro and in vivo antileishmanial activity of *Artemisia annua* L. leaf powder and its potential usefulness in the treatment of uncomplicated cutaneous leishmaniasis in humans *Rev Soc Bras Med Trop* **50** 52-60

Milbroudt J, Auerochs S, Korn K and Marschall M (2009) Senstivity of human herpes virus 6 and other human herpes viruses to the broad-spectrum antiinfective drug artesunate *Clin Virol* **46** 24-28

Milligan R, Daher A and Graves P M (2017) Primaquine at alternative dosing schedules for preventing relapse in people with *Plasmodium vivax* malaria *Cochrane Database Syst Rev* **2017**

Miotto O, Amato R, Ashley E A, MacInnis B, Almagro-Garcia J, *et al*. (2015) Genetic architecture of artemisinin-resistant *Plasmodium falciparum Nat Genet* **47** 226-234

Mishina Y V, Krishna S, Haynes R K and Meade J C (2007) Artemisinins inhibit *Trypanosoma cruzi* and *Trypanosoma brucei* rhodesiense in vitro growth *Antimicrob Agents Chemother* **51** 1852-1854

Mishra N (2014) Declining efficacy of artesunate plus sulfadoxine-pyrimethamine in northeastern India *Malaria J* **13** 284

Misra H, Mehta D, Mehta B K and Jain D C (2014) Extraction of artemisinin , an active antimalarial phytopharmaceutical from dried leaves of *Artemisia annua* L., using microwaves and a validated HPTLC-visible method for its quantitative determination *Hindawi* **2014** 1-11

Molina-Cruz A, Zilversmit M M, Neafsey D E, Hartl D L and Barillas-Mury C (2016) Mosquito vectors and the globalization of *Plasmodium falciparum* malaria *Annu Rev Genet* **50** 447-465

Mombo-Ngoma G, Remppis J, Sievers M, Manego R J, Endamne L, Kabuwende L, *et al*. (2017) Efficacy and safety of fosmidomycin-piperaquine therapy for uncomplicated falciparum malaria- a single arm, age-de-escalation proof of concept studies in Gibbon *Clin Infect Dis* doi:10.1093/cid/cia1122

Mondal A and Chatterji U (2015) Artemisinin represses telomerase subunits and induces apoptosis in HPV-39 infected human cervical cancer cells *J Cell Biochem* **116** 1968-1981

Mordmüller B, Surat G, Lagler H, Chakravarty S, Ishizuka A S, *et al*. (2017) Sterile protection against human malaria by chemoattenuated PfSPZ vaccine *Nature* **542** 445-449

Mugittu K, Abdulla S, Falk N, Masanja H, Felger I, *et al*. (2005) Efficacy of sulfadoxine-pyrimethamine in Tanzania after two years as first-line drug for uncomplicated malaria: assessment protocol and implication for treatment policy strategies *Malar J* **4** 55

Muthamilselvan T, Kuo T F, Wu Y C and Yang W C (2016)

Herbal remedies for coccidiosis control: A review of plants, compounds, and anticoccidial actions *Evid Based Complement Alternat Med* **2016** 2657981

Mutiso J M, Macharia J C, Barasa M, Taracha E, Bourdichon A J and Gicheru M M (2011) In vitro and in vivo antileishmanial efficacy of a combination therapy of diminazene and artesunate against *Leishmania donovani* in BALB/c mice *Rev Inst Med Trop Sao Paulo* **53** 129-132

Mwanza S, Nambozi M, Chileshe J, Joshi S, Malunga P, Kabuya J-B, Hachizovu S, *et al*. (2017) Chloroquine-sensitive *Plasmodium falciparum* in a high burden malaria area after over a decade of its withdrawl as first-line antimalarial medicine: Case of Nchelenge District *BMJ Global Health* **2** A17

Naing C, Whittaker M A, Nyunt Wai V and Mak J W (2014) Is *Plasmodium vivax* malaria a severe malaria?: A systematic review and meta-analysis *PLoS Negl Trop Dis* **8** e3071

Nair A, Abrahamsson B, Barends D M, Groot D W, Kopp S, *et al*. (2012) Biowaiver monographs for immediate-release solid oral dosage forms: Primaquine phosphate *J Pharm Sci* **101** 936-945

Nasamu A S, , Glushakova S, Russo I, Vaupel B, Oksman A, Kim A S, Fremont D H, Tolia N, *et al*. (2017) Plasmepsins IX and X are essential and druggable mediators of malaria parasite egress and invasion *Science* **38** 518-522

Newton P N, Angus B J, Chierakul W, Dondorp A, Ruangveerayuth R, *et al*. (2003) Randomized comparison of artesunate and quinine in the treatment of severe falciparum malaria *Clin Infect Dis* **37** 7-16

Nsobya S L, Kiggundu M, Nanyunja S, Joloba M, Greenhouse B and Rosenthal P J (2010) *In vitro* sensitivities of *Plasmodium falciparum* to different antimalarial drugs in Uganda *Antimicrob Agents Chemother* **54** 1200-1206

Nyunt M H, Han J-H, Wang B, Aye K M, Aye K H, *et al*. (2017) Clinical and molecular surveillance of drug resistant vivax malaria in Myanmar (2009-2016) *Malar J* **16** 117

Obonyo C O and Juma E A (2012) Clindamycin plus quinine for treating uncomplicated falciparum malaria: a systematic review and meta-analysis *Malar J* **11** 2

Oluyomi S A, Justine T E and Faoziyat S (2009) Depletion of parasitaemia by halofantrine hydrochloride and artemether in rats infected with African trypanosomes *Afr J Pharmacy and Pharmacol* **3** 432-438

Onimus M, Carteron S and Lutgen P (2013) The surprising efficiency of *Artemisia annua* powder capsules *Med Aromat Plants* **2** 125

Osei-Akoto A, Orton L C, Owusu-Oforis (2009) Atovaquone-proguanil for treating uncomplicated malaria *Cochrane Rev John Wiley and Sons*

Paddon C J and Keasling J D (2014) Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development *Nat Rev Microbiol* **12** 355-367

Paeshuyse J, Coelmont L, Vliegen I, hemel J Van, Vandenkerckhove J, *et al*. (2006) Hemin potentiates the anti-hepatitis C virus activity of the antimalarial drug artemisinin *Biochem Biophys Res Commun* **348** 139-144

Pain A, Böhme U, Berry A E, Mungall K, Finn R D, *et al*. (2008) The genome of the simian and human malaria parasite *Plasmodium knowlesi Nature* **455** 799-803

Paloque L, Ramadani A P, Mercereau-Puijalon O, Augereau J-M and Benoit-Vical F (2016) *Plasmodium falciparum*: multifaceted resistance to artemisinins *Malar J* **15** 149

Paul S, Khanuja S P S, Shasany A K, Gupta M M, Darokar M P, *et al*. (2010) Enhancement of artemisinin content through four cycles of recurrent selection with relation to heritability, correlation and molecular marker in *Artemisia annua* L. *Planta Med* **76** 1468-1472

Paul S, Gupta M M and Khanuja S P S (2012) Maintaining the artemisinin content through direct and indirect *in vitro* regeneration and their assessment of variation with the field from mother plants of *Artemisia annua* L. *Nat Prod Bioprospect* **2** 194-199

Peatey C L, Leroy D, Gardiner D L and Trenholme K R (2012) Anti-malarial drugs: how effective are they against *Plasmodium falciparum* gametocytes? *Malar J* **11** 34

Pekyi D, Ampromfi A A, Tinto H, Traoré-Coulibaly M, Tahita M C, *et al*. (2016) Four artemisinin-based treatments in African pregnant women with malaria *Malawi Med J* **28** 139-149

Pennings P S and Hermisson J (2006) Soft sweeps II - Molecular population genetics of adaptation from recurrent mutation or migration *Mol Biol Evol* **23** 1076-1084

Phuc B Q, Rasmussen C, Duong T, Dong L, Loi M, Ménard D, *et al*. (2017) Treatment failure of dihydroartemisinin/piperaquine for *Plasmodium falciparum* malaria, Vietnam *Emerg Infect Dis* **23** 715-717

Pinato D J and Stebbing J (2015) Artemisia: A divine dart against cancer? *The Lancet Oncology* **16** 759-760

Plucinski M M, Talundzic E, Morton L, Dimbu P R, Macaia AP, *et al*. (2015) Reply to "no robust evidence of lumefantrine resistance" *Antimicrob Agents Chemoether* **59** 5867-5868

Pop I A, Oroian I, Lobontiu I and Friss Z (2017) *Artemisia annua* L. culture technology in the climate of Transylvania *Procedia Eng* **181** 433-438

Price R N, von Seidlein L, Valecha N, Nosten F, Baird J K and White N J (2014) Global extent of chloroquine-resistant Plasmodium vivax: A systematic review and meta-analysis *Lancet Infect Dis* **14** 982-991

Rai P, Sharma D, Soni R, Khatoon N, Sharma B and Bhatt T K (2017) *Plasmodium falciparum* apicoplast and its transcriptional regulation through calcium signaling *L Microbiol* **55** 231-236

Rajgor D D, Gogtay N J, Kadam V S, Kocharekar M M, Parulekar M S, *et al.* (2014) Antirelapse efficacy of various primaquine regimens for *Plasmodium vivax Malar Res Treat* **2014** 347018

Ram M, Gupta M M, Dwivedi S and Kumar S (1997) Effect of plant density on the yields of artemisinin and essential oil in *Artemisia annua* cropped under low input cost management in North-Central India *Planta Med* **63** 372-374

RCOG=Royal College of Obstetricians and Gynaecologists (2010) The diagnosis and treatment of malaria in pregnancy Report no. : Green - top Guideline no. 54b

Rieckmann K H, McNamara J V, Frischer H, Stockert T A, Carson P E and Powell R D (1968) Gametocytocidal and sporontocidal effects of primaquine and of sulfadiazine with pyrimethamine in a chloroquine-resistant strain of *Plasmodium falciparum Bull World Health Organ* **38** 625-632

Rijken M J, McGready R, Phyo A P, Lindegardh N, Tarning J, Laochan N, Than H H, Mu O, Win A K, Singhasivanon P, White N and Nosten F (2011) Pharmacokinetics of dihydroartemisinin and piperaquine in pregnant and nonpregnant women with uncomplicated falciparum malaria *Antimicrob Agents Chemother* **55** 5500-5506

Rodríguez E, Carman N J, Vander Velde G, McReynolds J H, Mabry T J, *et al.* (1972) Methoxylated flavonoids from Artemisia *Phytochemistry* **11** 3509-3514

Romero M R, Efferth T, Serrano M A, Castaño B, MacIas R I R, *et al.* (2005) Effect of artemisinin/artesunate as inhibitors of hepatitis B virus production in an "in vitro" replicative system *Antiviral Res* **68** 75-83

Rossi G, De Smet M, Khim N, Kindermans J M and Menard D (2017) Emergence of *Plasmodium falciparum* triple mutant in Cambodia *Lancet Infect Dis* **17** 1233

Rottmann M, McNamara C, Yeung B K S, Lee M C S, Zou B, *et al.* (2010) Spiroindolones, a potent compound class for the treatment of malaria *Science* **329** 1175-1180

Ruiz Sanchez F, Ruiz-Sanchez A and Naranjo Grande I (1956) The treatment of malaria with tetracycline *Antibiotic Med* **3** 193-196

Rutledge G G, Böhme U, Sanders M, Reid A J, Cotton J A, *et al.* (2017) *Plasmodium malariae* and *P. ovale* genomes provide insights into malaria parasite evolution *Nature* **542** 101-104

Rutledge G G, Marr I, Huang G K L, Auburn S, Marfurt J, *et al.* (2017) Genomic characterization of recrudescent *Plasmodium malariae* after treatment with artemether/lumefantrine *Emerg Infect Dis* **23** 1300-1307

Sa J M, Twu O, Hayton K, Reyes S, Fay M P, *et al.* (2009) Geographic patterns of *Plasmodium falciparum* drug resistance distinguished by differential responses to amodiaquine and chloroquine *Proc Natl Acad Sci USA* **106** 18883-18889

Sannella A R, Messori L, Casini A, Vincieri F F, *et al.* (2007) Modulation of the *in vitro* antimalarial effects of artemisinin by selected flavonoids and by reducing agents *Planta Medica* **73** 865

Sarciron M E, Saccharin C, Petavy A F and Peyron F (2000) Effects of artesunate, dihydroartemisinin, and an artesunate-dihydroartemisinin combination against *Toxoplasma gondii Am J Trop Med Hyg* **62** 73-76

Saunders D L, Chaorattanakawee S, Gosi P, Lanteri C, Somethy S, *et al.* (2016) Atovaquone-proguanil remains a potential stopgap therapy for multidrug-resistant *Plasmodium falciparum* in areas along the Thai-cambodian border *Antimicrob Agents Chemother* **60** 1896-1898

Schreiber A, Härter G, Schubert A, Bunjes D, Mertens T and Michel D (2009) Antiviral treatment of cytomegalovirus infection and resistant strains *Expert Opin Pharmacother* **10** 191-209

Sen R, Bandyopadhyay S, Dutta A, Mandal G, Ganguly S, *et al.* (2007) Artemisinin triggers induction of cell-cycle arrest and apoptosis in Leishmania donovani promastigotes *J Med Microbiol* **56** 1213-1218

Sen R, Ganguly S, Saha P and Chatterjee M (2010) Efficacy of artemisinin in experimental visceral leishmaniasis *Int J Antimicrob Agents* **36** 43-49

Sergent T, Dupont I, Van Der Heiden E, Scippo M L, Pussemier L, *et al.* (2009) CYP1A1 and CYP3A4 modulation by dietary flavonoids in human intestinal Caco-2 cells *Toxicol Lett* **191** 216-222

Shanks G D, Edstein M D and Jacobus D (2015) Evolution from double to triple- antimalarial drug combinations *Trans Royal Soc Trop Med Hyg* **109** 182-188

Shen Q, Zhang L, Liao Z, Wang S, *et al.* (2018) The genome of *Artemisia annua* provides insight into the evolution of Asteraceae family and artemisinin biosynthesis *Mol Plant* doi.org/10.1016/j.molp.2018.03.015

Shapira M Y, Resnick I B, Chou S, Neumann A U, Lurain N S, *et al.* (2008) Artesunate as a potent antiviral agent in a patient with late drug resistant cytomegalovirus infection after hematopoietic stem cell transplantation *Clin Infect Dis* **46** 1455-1457

Sharma V P, Dev V and Phookan S (2015) Neglected *Plasmodium vivax* malaria in northeastern States of India *Indian J Med Res* **141** 546-555

Sharma Y D (2005) Genetic alteration in drug resistance markers of *Plasmodium falciparum Indian J Med Res* **121** 13-22

Sibley C H, Hyde J E, Sims P F, Plowe C V, Kublin J G, *et al.* (2001) Pyrimethamine-sulfadoxine resistance in *Plasmodium falciparum*: what next? *Trends Parasitol* **17** 582–588

Sidhu A B S, Uhlemann A, Valderramos S G, Valderramos J, Krishna S and Fidock D A (2006) Decreasing *pfmdr1* copy number in *Plasmodium falciparum* malaria heightens susceptibility to mefloquine, lumefantrine, halofantrine, quinine, and artemisinin *J Infect Dis* **194** 528-535

Sidhu A B S, Verdier-Pinard D and Fidock D A (2002) Chloroquine resistance in *Plasmodium falciparum* malaria parasites conferred by pfcrt mutations *Science* **298** 210-213

Silvestrini F, Alano P and Williams J L (2000) Commitment to the production of male and female gametocytes in the human malaria parasite *Plasmodium falciparum Parasitology* **121** S0031182099006691

Simonnet X, Quennoz M and Carlen C (2008) New *Artemisia annua* hybrids with high artemisinin content *Acta Hortic* **769** 371-373

Sinclair D, Zani B, Donegan S, Olliaro P and Garner P (2009) Artemisinin-based combination therapy for treating uncomplicated malaria *Cochrane database Syst Rev* CD007483

Singh B and Daneshvar C (2013) Human infections and detection of *Plasmodium knowlesi Clin Microbiol Rev* **26** 165-184

Sinka M E, Bangs M J, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, *et al.* (2012) A global map of dominant malaria vectors *Parasit Vectors* **5** 69

Sirima S B, Ogutu B, Lusingu J P A, Mtoro A, Mrango Z, *et al.* (2016) Comparison of artesunate-mefloquine and artemether-lumefantrine fixed-dose combinations for treatment of uncomplicated *Plasmodium falciparum* malaria in children younger than 5 years in sub Sahara Africa: a randomized, multicentre, phase 4 trial *Lancet Infect Dis* **16** 1123-1133

Sissoko M S, Healy S A, Katile A, Omaswa F, Zaidi I, *et al.* (2017) Safety and efficacy of PfSPZ vaccine against *Plasmodium falciparum* via direct venous inoculation in healthy malaria-exposed adults in Mali: A randomised, double-blind phase 1 trial *Lancet Infect Dis* **17** 498-509

Slack R D, Mott B T, Woodard L E, Triphati A, Sullivan D, *et al.* (2012) Malaria-infected mice are completely cured by one 6 mg/kg oral dose of a new monomeric trioxane sulfide combined with mefloquine *J Med Chem* **55** 291-296

Sluydts V, Durnez L, Heng S, Gryseels C, Canier L, *et al.* (2016) Efficacy of topical mosquito repellent (picaridin) plus long-lasting insecticidal nets versus long-lasting insecticidal nets alone for control of malaria: a cluster randomised controlled trial *Lancet Infect Dis* **16** 1169-1177

Sonden K, Wyss K, Jovel I, Vieira da Silva A, Pohanka A, *et al.* (2017) High rate of treatment failures in nonimmune travelers treated with artemether-lumefantrine for uncomplicated *Plasmodium falciparum* malaria in Sweden: retrospective comparative analysis of effectiveness and case series *Clin Infect Dis* **64** 199-206

Song Y, Lee S J, Jang S H, Kim T H, Kim H D, *et al.* (2017) Annual wormwood leaf inhibits the adipogenesis of 3T3-L1 and obesity in high-fat diet-induced obese rats *Nutrients* **9** pii: E554

Srivastava I K and Vaidya A B (1999) A mechanism for the synergistic antimalarial action of atovaquone and proguanil *Antimicrob Agents Chemother* **43** 1334-1339

St. Laurent B, Miller B, Burton T A, Amaratunga C, Men S, *et al.* (2015) Artemisinin-resistant *Plasmodium falciparum* clinical isolates can infect diverse mosquito vectors of Southeast Asia and Africa *Nat Commun* **6** 8614

Staines H M, Burrow R, Teo B H, Chis Ster I, Kremsner P G and Krishna S (2017) Clinical implications of Plasmodium resistance to atovaquone/proguanil: A systematic review and meta-analysis *J Antimicrob Chemother* doi: 10.1093/jac/dkx431

Straimer J, Gnadig N F, Witkowski B, Amaratunga C, Duru V, *et al.* (2015) K13-propeller mutations confer artemisinin resistance in *Plasmodium falciparum* clinical isolates *Science (80-.)* **347** 428-431

Suberu J O, Gorka A P, Jacobs L, Roepe P D, Sullivan N, *et al.* (2013) Anti-plasmodial polyvalent interactions in *Artemisia annua* L. aqueous extract - Possible synergistic and resistance mechanisms *PLoS One* **8**

Sullivan D J, Matile H, Ridley R G and Goldberg D E (1998) A common mechanism for blockade of heme polymerization by antimalarial quinolines *J Biol Chem* **273** 31103-31107

Sutherland C J (2016) Persistent Parasitism: the adaptive biology of malariae and ovale malaria *Trends Parasitol* **32** 808-819

Sutherland C J, Lansdell P, Sanders M, Muwanguzi J, van Schalkwyk D A, *et al.* (2017) Pfk13-independent treatment failure in four imported cases of *Plasmodium falciparum* malaria given artemether-lumefantrine in the UK *Antimicrob Agents Chemother* AAC.02382-16

Svensson U S and Ashton M (1999) Identification of the human cytochrome P450 enzymes involved in the in vitro metabolism of artemisinin *Br J Clin Pharmacol* **48** 528-535

Tainchum K, Kongmee M, Manguin S, Bangs M J and Chareonviriyaphap T (2015) Anopheles species diversity and distribution of the malaria vectors of Thailand *Trends Parasitol* **31** 109-119

Takala-Harrison S, Jacob C G, Arze C, Cummings M P, Silva J C, *et al.* (2015) Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia *J Infect Dis* **211** 670-679

Tan K R, Magill A J, Parise M E and Arguin P M (2011) Doxycycline for malaria chemoprophylaxis and treatment: Report from the CDC expert meeting on malaria chemoprophylaxis *Am J Trop Med Hyg* **84** 517-531

Tanaka T Q and Williamson K C (2011) A malaria gametocytocidal assay using oxidoreduction indicator, alamarBlue *Mol Biochem Parasitol* **177** 160-163

Thanh N V, Thuy-Nhien N, Tuyen N T K, Tong N T, Nha-Ca N T, *et al.* (2017) Rapid decline in the susceptibility of *Plasmodium falciparum* to dihydroartemisinin–piperaquine in the south of Vietnam *Malar J* **16** 27

Tilley L, Straimer J, Gnädig N F, Ralph S A and Fidock D A (2016) Artemisinin action and resistance in *Plasmodium falciparum Trends Parasitol* **32** 682-696

Tine R C, Sylla K, Faye B T, Poirot E, Fall F B, *et al.* (2017) safety and efficacy of adding a single low dose of primaquine to the treatment of adult patients with *Plasmodium falciparum* malaria in senegal, to reduce gametocyte carriage: A Randomized controlled trial *Clin Infect Dis* **65** 535-543

Towler M J and Weathers P J (2015) Variations in key artemisinic and other metabolites throughout plant development in *Artemisia annua* L. for potential therapeutic use *Ind Crops Prod* **67** 185-191

Utzinger J, N'Goran E K, N'Dri A, Lengeler C, Shuhua X and Tanner M (2000) Oral artemether for prevention of *Schistosoma mansoni* infection: Randomised controlled trial *Lancet* **355** 1320-1325

Vaidya A B and Mather M W (2009) Mitochondrial evolution and functions in malaria parasites *Annu Rev Microbiol* **63** 249-267

Van Hensbroek M B, Onyiorah E, Jaffar S, Schneider G, Palmer A, *et al.* (1996) A trial of artemether or quinine in children with cerebral malaria *N Engl J Med* **335** 69-75

Van Zyl R L, Seatlholo S T, Van Vuuren S F and Viljoen A M (2006) The biological activities of 20 nature identical essential oil constituents *J Essent Oil Res* **18** 129-133

Vandewynckel Y P, Laukens D, Geerts A, Vanhove C, Descamps B, *et al.* (2014) Therapeutic effects of artesunate in hepatocellular carcinoma: repurposing an ancient antimalarial agent *Eur J Gastroenterol Hepatol* **26** 861-870

Verlinden B K, Louw A and Birkholtz L M (2016) Resisting resistance: is there a solution for malaria? *Expert Opin Drug Discov* **11** 395-406

Visser B J, Wieten R W, Kroon D, Nagel I M, Bélard S, *et al.* (2014) Efficacy and safety of artemisinin combination therapy (ACT) for non-falciparum malaria: A systematic review *Malar J* **13** 463

Wall R and Watson J (2017) *Artemisia annua* L. GRAS research FDA_Artemisia_annua_GRAS_IQP

Wang X Q, Liu H L, Wang G B, Wu P F, Yan T, *et al.* (2011) Effect of artesunate on endotoxin-induced uveitis in rats *Investig Ophthalmol Vis Sci* **52** 916-919

Want M Y, Islammudin M, Chouhan G, Ozbak H A, Hemeg H A, *et al.* (2017) Nanoliposomal artemisinin for the treatment of murine visceral leishmaniasis *Int J Nanomedicine* **12** 2189-2204

Weathers P, Reed K, Hassanali A, Lutgen P and Engeu P O (2014c) Whole plant approaches to therapeutic use of *Artemisia annua* L. (Asteraceae) In: Aftab T, Ferreira J, Khan M., Naeem M. (eds) *Artemisia annua Pharmacology and Biotechnology* Springer, Berlin, Heidelberg

Weathers P J, Arsenault P R, Covello P S, McMickle A, Teoh K H and Reed D W (2011) Artemisinin production in *Artemisia annua*: Studies in planta and results of a novel delivery method for treating malaria and other neglected diseases *Phytochem Rev* **10** 173-183

Weathers P J, Cambra H M, Desrosiers M R, Rassias D and Towler M J (2017) Artemisinin the noble molecule: From plant to patient *Studies Nat Prod Chem* **52** 193-229

Weathers P J, Elfawal M A, Towler M J, Acquaah-Mensah G K and Rich S M (2014b) Pharmacokinetics of artemisinin delivered by oral consumption of *Artemisia annua* dried leaves in healthy vs. *Plasmodium chabaudi*-infected mice *J Ethnopharmacol* **153** 732-736

Weathers P J, Jordan N J, Lasin P and Towler M J (2014d) Simulated digestion of dried leaves of *Artemisia annua*

consumed as a treatment (pACT) for malaria *J Ethnopharmacol* **151** 858-863

Weathers P J and Towler M J (2014) Changes in key constituents of clonally propagated *Artemisia annua* L. during preparation of compressed leaf tablets for possible therapeutic use *Ind Crops Prod* **62** 173-178

Weathers P J, Towler M, Hassanali A, Lutgen P and Engeu P O (2014a) Dried-leaf *Artemisia annua*: A practical malaria therapeutic for developing countries? *World J Pharmacol* **3** 39-55

Whirl-Carrillo M, McDonagh E M, Hebert J M, Gong L, Sangkuhl K, *et al.* (2012) Pharmacogenomics knowledge for personalized medicine *Clin Pharmacol Ther* **92** 414-417

White N J (2005) Artesunate versus quinine for treatment of severe falciparum malaria: A randomised trial *Lancet* **366** 717-725

White N J (2013) Primaquine to prevent transmission of falciparum malaria *Lancet Infect Dis* **13** 175-181

White N J (2014) Malaria: A molecular marker of artemisinin resistance *Lancet* **383** 1439-1440

White N J (2016) Can new treatment developments combat resistance in malaria? *Expert Opin Pharmacother* 1303-1307

White N J (2017) Malaria parasite clearance *Malar J* **16** 88

White N J, Ashley E A, Recht J, Delves M J, Ruecker A, *et al.* (2014) Assessment of therapeutic responses to gametocytocidal drugs in *Plasmodium falciparum* malaria *Malar J* **13** 483

White N J, Looareesuwan S, Warrell D A, Warrell M J, Chanthavanich P, *et al.* (1983) Quinine loading dose in cerebral malaria *Am J Trop Med Hyg* **32** 1-5

WHO (2015a) Guidelines for treatment of malaria-3rd edition www.who.int/malaria/publications/atoz/978941548528/enl technical brief. www//apps.who.int/iris/bitshean/10665/181162/1/9789241509244_eng.pdf

WHO Artemisinin and artemisinin based combination therapy resistance. WHO-HTM-GMP-2017.9-eng.pdf

WHO: Severe malaria. Trop Med Int Health 2014; 19 (Supplement 1)

WHO (2012) Effectiveness of non-pharmaceutical forms of *Artemisia annua* L. against malaria. http:/www.who.int/malaria/position_statement_herbal_remedy_artemisia_annua l.pdf

Witkowski B, Duru V, Khim N, Ross L S, Saintpierre B, *et al.* (2017) A surrogate marker of piperaquine-resistant *Plasmodium falciparum* malaria: a phenotype–genotype

association study *Lancet Infect Dis* **17** 174-183

Wootton J C, Feng X, Ferdig M T, Cooper R A, Mu J, *et al.* (2002) Genetic diversity and chloroquine selective sweeps in *Plasmodium falciparum Nature* **418** 320-323

Wu X, Zhang W, Shi X, An P, Sun W and Wang Z (2010) Therapeutic effect of artemisinin on lupus nephritis mice and its mechanisms *Acta Biochim Biophys Sin (Shanghai)* **42** 916-923

WWARN Gametocyte Study Group (2016) Gametocyte carriage in uncomplicated *Plasmodium falciparum* malaria following treatment with artemisinin combination therapy: a systematic review and meta-analysis of individual patient data *BMC Med* **14** 79

Xiao L, Yang C, Patterson P S, Udhayakumar V and Lal A A (1996) Sulfated polyanions inhibit invasion of erythrocytes by plasmodial merozoites and cytoadherence of endothelial cells to parasitized erythrocytes *Infect Immun* **64** 1373-1378

Yamachika E, Habte T and Oda D (2004) Artemisinin: An alternative treatment for oral squamous cell carcinoma *Anticancer Res* **24** 2153-2160

Yarnell E (2014) *Artemisia annua* (Sweet Annie), other Artemisia species, artemisinin, artemisinin derivatives, and malaria *J Restor Med* **3** 69-84

Yimer S and Sahu O (2016) Traditional medicines for treatment of African diseases by *Artemisia annua Medic Sci Pub Health* **4** 22-32

York A (2017) Seasonal malaria chemoprevention in the Sahel *Lancet Infect Dis* **17** 588

Zani B, Gathu M, Donegan S, Olliaro P L and Sinclair D (2014a) Dihydroartemisinin-piperaquine for treating uncomplicated *Plasmodium falciparum* malaria *Cochrane database Syst Rev* CD010927

Zani B, Gathu M, Donegan S, Olliaro P L and Sinclair D (2014b) The Cochrane database of systematic reviews 1: CD010927 Dihydroartemisinin-piperaquine for treating uncomplicated *Plasmodium falciparum* malaria *Cochrane Database Syst Rev* CD010927

Zhang M, Wang C, Otto T D, Oberstaller J, *et al.* (2018) Uncovering the essential genes of the human malaria parasite *Plasmodium falciparum* by saturation mutagenesis *Science* **360** eaap7847

Zhou X, Sun W-J, Wang W-M, Chen K, Zheng J-H, *et al.* (2013) Artesunate inhibits the growth of gastric cancer cells through the mechanism of promoting oncosis both in vitro and in vivo *Anticancer Drugs* **24** 920-927.