2. REVIEW OF LITERATURE

Malaria is probably one of the oldest diseases known to mankind. Ancient Chinese, Indian and Egyptian manuscripts have mentioned about this disease. Edwin Smith Surgical Papyrus (1600 B.C.) indicated about it while the Greek physician Hippocrates was the first to describe the manifestations of the disease (Boyd, 1949). In the 7th century A.D., the Italians named the disease malaria, which literally means 'bad air', due to misconception that foul-smelling vapours emanating from the swamps near Rome cause this sickness.

The first detailed clinical description of malaria and its treatment with the Cinchona bark was presented by the Genevan physician Morton (1696). Meckel (1847) was the first to observe black granules embedded in protoplasmic masses in the blood of a severely ill malarious patient. Later in 1880, Alphonse Laveran, discovered the causative agent of human malaria (Laveran, 1884). Gerhardt (1884) elucidated that infection could be passed by inoculation of blood from infected to healthy person. Marchiafava and Celli (1885) suggested the generic name Plasmodium and Mechnikov (1887) established that malaria parasite belongs to Protozoa. Ramanowsky (1890) introduced a stain, which could differentially stain blood cells, parasite cytoplasm and chromatin. This discovery greatly facilitated the detailed study of malaria parasite. Differential description of P. vivax and P. malariae was given by Grassi and Feletti (1892) and for P. falciparum by Welch (1897). The fourth human malaria parasite, P. ovale was described later by Stephens (1922). It was Manson (1894), who brought up the idea of
involvement of mosquito for extrinsic development of malaria parasite. This thought encouraged Sir Ronald Ross to investigate the fate of *Plasmodium* in various mosquito species in India. Ross (1898) succeeded in completely elucidating the sporogony of *Plasmodium reticulum* in *Culex pipens fatigans*. His pioneering work on establishing the main features of the parasitic life cycle earned him the Noble Prize in 1902, in the field of medicines.

Ultrastructure of malaria parasites, changes in the membrane of infected red blood cells and the mechanism of merozoite invasion into red cells have been studied in detail by Ladda et al. (1969) and Aikawa (1977). *In vitro* culture of *P. falciparum* was maintained by Trager and Jensen (1976). The first rodent malarial parasite (*P. berghei*) was discovered by Vinckei and Lips (1948) in the blood of a thicket rat, *Grammomys surdaster*. Other rodent parasite species were reported later i.e. *P. vinckei* (1952), *P. chabaudi* (1965) and *P. yoelii* (1974) (Carter and Diggs, 1977). The greatest challenge to tame parasite lies in its ability to quickly adapt and overcome eradication efforts. Malaria quickly rebounded after mass insecticide spraying campaigns during 1950s and 1960s. The rising problem of resistance to the classical antimalarial drugs and the problem of recrudescence to artemisinin have emerged as major challenges in field of chemotherapy.

**CHEMOTHERAPY**

The first recorded treatment of malaria dates back to 1600, when the bark of the *Cinchona* tree was first used by the native Peruvian Indians to treat the intermittent fevers associated with this illness. Chemotherapy of malaria includes quinine and its derivatives, antifolate drugs, artemisinin and its derivative drugs in combination with other antimalarial drugs.
QUINOLINE DERIVATIVES

Quinine has been used for 300 years ever since the extract of bark of Cinchona tree was first shown to have antimalarial activity. The bark was found to contain four potent anti-malarial alkaloids, which include quinine, quinidine, cinchonine and cinchonidine (Hodge, 1948; Karle and Bhattacharjee, 1999). Among which, quinine was found to be most potent against malaria. Quinine is the L-steroisomer of quinidine. Quinine acts mainly on the mature trophozoite stages of parasite and do not prevent sequestration of ring stages of P. falciparum. It also kills the sexual stages of P. vivax, P. ovale and P. malariae except mature gametocytes of P. falciparum. However, it is ineffective against the pre-erythrocytic stages of Plasmodium. Even then, it is the only drug, which has remained largely effective for the treatment of malaria to date (Zhang et al., 1999). The mechanism of its antimalarial action is not well understood, although it has been found to interact weakly with haem, thereby inhibiting haem polymerization in food vacuole of parasite (Robert et al., 2001). Quinine is a relatively safe drug, if dose recommendations are followed. Massive haemolysis with renal failure has been linked epidemiologically and historically to quinine but its etiology remains uncertain (Bruce-Chwatt, 1987). Over dosage of drug usually causes a complex of symptoms known as cinchonism, which is characterized in its mild form by tinnitus, impaired high tone hearing, headache, nausea, dizziness, dysphoria and sometimes disturbed vision (Taylor and White, 2004). Combination therapy of quinine with tetracycline is still effectively used for treatment of uncomplicated cases with 85% efficacy in Thailand (Pukrittayakaemee et al., 2000). Association of quinine and clindamycine significantly shortened the duration of treatment with respect to quinine used alone (Parola et al., 2001).

The requirement of this drug was very high but the production of Cinchona bark was not sufficient. This stimulated the search for
synthetic and newer drug development. Quinoline derivatives include 4-aminoquinoline drugs and 8-aminoquinoline (Fig. 2.1).

Fig. 2.1: Structures of the main quinoline-containing drugs.
4-Aminoquinoline drugs

4-aminoquinoline drugs include quinine, chloroquine, amodiaquine, mefloquine and Halofantrine like compounds. These compounds have been mainstay in malaria chemotherapy for 40 years. They are easily synthesized, cheap and well tolerated.

Chloroquine {7-chloro-4 (4-diethylaminol-methylbutylamino) quinoline} was introduced during World War II, as a synthetic 4-aminoquinoline compound (Loeb et al., 1946). It has been the most widely used antimalarial drug due to its low toxicity and cost (Penisko and Keystone, 1990; Trape, 2001). When taken orally, chloroquine is completely absorbed from gastrointestinal tract. It binds to plasma proteins, thus eliminated very slowly from body via kidney. The estimated elimination half life period of chloroquine is 1-2 months. In the liver, chloroquine is metabolized into monodesethylchloroquine. Chloroquine interrupts with the haem polymerization (Deen et al., 2008). It enters the red blood cell, inhabiting parasite cell and digestive vacuole by simple diffusion (Fig. 2.2).

![Fig. 2.2: Mechanism of action of chloroquine](image)

In digestive vacuole (pH 4.7), it becomes protonated (CQ2+) and binds to haem (FP) to form FP-Chloroquine complex. Action of
the toxic FP-chloroquine and FP results in cell lysis and ultimately leads to parasite cell autodigestion. CQ is safe in its use, when drug regimen is followed. In overdose, CQ is a dangerous drug, commonly associated with shock and cardiac arrhythmias (Riou et al., 1988). Of its symptomatic adverse effects, pruritus is worthy of note. This is more common in Africans than in Caucasians (Mnyika and Kihamia, 1991). CQ is eliminated slowly and accumulates in some tissues, notably the retina leading to eye disorders (keratopathy and retinopathy).

Major problem with chloroquine is growing resistance of *Plasmodium* to it. The resistance of chloroquine to *P. falciparum* first appeared in South-East Asia followed by South America (Spencer, 1985; Wernsdorfer, 1994). Since then chloroquine resistance has spread far beyond the first focus and now prevails in all parts of world. In India, chloroquine resistance was detected in 1973, in Karbi-Anglog district in Assam (Sehgal et al., 1973). Gradually, it has spread towards the west and south, covering almost entire country (Clyde, 1987). The chloroquine resistance is severe in North-East and South-Eastern regions in India, with high mortality and morbidity (Arora et al., 2008). Chloroquine resistance results from either decreased uptake or increased excretion of the drug by resistance parasite. However, exact mechanism responsible for this difference in chloroquine accumulation and chloroquine resistance has been unknown. The development of resistance to chloroquine has been found due to the mutations occurring in transporter-like genes, present on the surface of parasite food vacuole (Fidock et al., 2000; Sidhu et al., 2002). It is no longer recommended for *P. falciparum* malaria. However, its combination treatment with primaquine is the treatment of choice for *P. vivax* and *P. ovale*. The rising problem of resistance to chloroquine led to the development of three new antimalarial drugs 4-aminoquinoline (amodiaquine), a quinolinemethanol (mefloquine), and phenathrene methanol (halofantrine).
Amodiaquine is a Mannich base, 4-aminoquinoline, with a mode of action similar to that of chloroquine. A regimen of 10 mg of amodiaquine base per day for 3 days (total dose 30 mg/kg) is recommended for malaria treatment. Amodiaquine is more active than chloroquine against low-level chloroquine resistant parasites (Olliaro et al., 1996). Amodiaquine readily absorbed from the gastrointestinal tract. Several studies have shown the efficacy and good tolerability of amodiaquine (Massaga et al., 2003). It is rapidly converted in the liver to the active metabolite desethylamodiaquine, which contributes nearly all of its anti-malarial effect. There is insufficient data on the terminal plasma elimination half-life of desethylamodiaquine. However, in areas with high-level chloroquine resistance, amodiaquine is also ineffective (Lemnge et al., 2006; Mandi et al., 2008; Nsimba et al., 2008). The adverse effects of amodiaquine are thought to be similar to those of chloroquine. However, amodiaquine is more palatable than chloroquine, as it is associated with less cardiotoxicity and pruritis as compared to chloroquine. Large doses of amodiaquine have been reported to cause syncope, spasticity, convulsions and involuntary movements. Amodiaquine can induce toxic hepatitis and fatal agranulocytosis (Bell et al., 2008). The toxicity of amodiaquine seems to be related to the immunogenic properties of quinine imine produced by autooxidation of parent drug. Studies regarding its combination with atresunate have been proved effective against *P. falciparum* (Adjuik et al., 2002; Brasseur et al., 2007). The mechanism of resistance against amodiaquine has been found to be similar to chloroquine resistance.

Mefloquine was developed in 1970 at Walter Reed Army Institute of Research, U.S., as a synthetic analogue of quinine. It is a potent long acting blood schizontocide, which is active against *P. falciparum* resistant strains to chloroquine and sulfadoxine-pyrimethamine combinations (Maguire et al., 2006). A standard dose of 25 mg/kg has been found to provide better protection against
P. falciparum. Mefloquine has long elimination half life period varying between 10-40 days in adults depending upon lipid contents (Slutsker et al., 1990; Karbwang et al., 1991). It is metabolized in the liver to carboxymefloquine, an inactive antimalarial metabolite, which eliminates slowly and has similar toxic profile to parent compound. Mefloquine exerts severe and permanent adverse side-effects, so its use is contraindicated. It is known to cause severe depression, anxiety, paranoia, aggression, nightmares, insomnia, seizures, birth defects, peripheral motor-sensory neuropathy (Jha et al., 2006). Mefloquine should be used with caution in patients with heart block, patients taking beta blockers, patients with history of epilepsy and psychiatric disease. It should be avoided in first trimester of pregnancy. Mefloquine resistance was first observed in late 1980 near the Thai-Cambodian borders (Shanks, 1995; Wongsrichanalai et al., 2001). Resistance in P. falciparum to Mefloquine in India was detected in Surat district in Gujarat state (Sharma, 1996).

Halofantrine contains a substituted phenanthrene. It is related to the antimalarial drugs, quinine and lumefantrine. It is only recommended in the areas with multidrug resistant (MDR) P. falciparum and P. vivax infections. A standard dose of 8 mg of halofantrine base per kg in three doses at 6 hrs interval is generally recommended for malaria treatment. Its systemic absorption is unpredictable but increase six fold in presence of fatty foods. Halofantrine is metabolized in the liver into biological active desbutyl metabolite, which has been found to exert its antimalarial effect. The elimination half life period of halofantrine is 4-5 days. Halofantrine has no role presently in the malaria control programmes, because of its high cost and cross resistance to mefloquine. Halofantrine can cause abdominal pain, diarrhoea, vomiting, rash, headache, itching and elevated liver enzymes concentration. It should not be given to patients with cardiac conduction defects (Bryson and Goa, 1992; Luzzi and Peto, 1993; White, 1996). The most dangerous side effect due to halofantrine is cardiac arrhythmias (Leite et al., 2007). Even at
standard doses, it causes significant QT prolongation (Nicholas and White, 2007).

More recently another amino alcohol benflumetol and azacrine type Mannich base, pyronaridine have been introduced into the field. Pyronaridine was first synthesized in China, in 1970. It is structurally related to chloroquine, being a substituted 1-aza-acridine and also a substituted 1, 5-napthyridine. It has been found effective against chloroquine-sensitive as well as chloroquine-resistant parasites. Its high activity has been validated in clinical trials in China, Thailand and Cameroon. However, it has more gastrointestinal side-effects than chloroquine. Extensive experience has been gained in the use of pyronaridine for malaria in Hunan and Yunan Provinces, where it has been found to be safe and effective. Efforts are being made for its use in combination therapy with artesunate. Although these drugs are not quinoline based, however their mode of action is similar to that of other quinoline drugs.

Quinoline derivative drugs like chloroquine, exert their antimalarial effect by binding to toxic haem, thereby prevent the detoxification of haem. Haem is released during proteolysis of hemoglobin and detoxified by crystallization in the parasites acidic food vacuole. Other haem detoxification pathways, that had been considered include – peroxidative degradation and glutathione dependent degradation. The haem chloroquine complex strongly promotes the peroxidative cleavage of phospholipid membrane. This mode of action is thought to be similar for other quinolines.

Unfortunately, resistance to each of these drugs has now been reported in many areas of the world. The genetic basis of resistance has been revealed by careful and extensive recombination mapping of the progeny of a genetic cross between chloroquine resistant and chloroquine sensitive phenotypes. Analysis of these data revealed a genetic linkage between loci on chromosomes 5, 7 and 13 are associated with chloroquine resistance. The relevant genes on
chromosomes 5 and 7 might be pfmdr1 and pfcr but the gene on chromosome 13 has yet to be identified. The recombination study has resulted in the identification of a key gene, named pfcr with 36 kb region residing on chromosome 7, encodes a 49 kDa protein with 10 predicted transmembrane domains exhibits mutations that shows complete linkage to the chloroquine resistance phenotype in laboratory adapted strains of P. falciparum (Fidock et al., 2000). Several mutations in the pfcr gene have shown correlations with the chloroquine resistance phenotype. A substitution of a threonine (T) for a lysine (K) at residue 76 (K76T) has been found to show a perfect and consistent correlation with chloroquine resistance (Cooper et al., 2005). At least three other changes are always seen in combination with K76T mutation, of which transmembrane domain 1, 4 and 9 are thought to play particular important roles in determining the transport characteristics of CRT with respect to different drugs (Cooper et al., 2007). cDNA version of pfcr resistance parasite transfected into sensitive parasites, bestow the resistance phenotype and confirm the central role of codon (Bray et al., 2005).

The membrane of the food vacuole is expected to carry a number of different transporters. A MDR-like transporter, designated pfmdr1, has been identified on the food vacuole membrane. Pfmdr1 resides on chromosome 5, encodes 162 kDa protein with 12 predicted transmembrane domains and two ATP binding folds, thought to import solutes into food vacuole, including drugs mefloquine, halofantrine and artemisinin (Rohrbach et al., 2006). Amplification of pfmdr1 to copy numbers up to five, appears to be a primary mechanism, whereby parasite becomes resistant to other important quinoline or methanol-based drugs, particularly in Asia (Sidhu et al., 2006; Uhlemann et al., 2007), although amino acid polymorphisms in Pgh1 can also have significant effect for exerting chloroquine resistance (Sidhu et al., 2005).
8-Aminoquinoline Drugs

The early synthetic work in Germany produced the 8-aminoquinolines, primaquine and pamaquine. 8-aminoquinoline drugs act on plasmodial mitochondria through generation of toxic metabolites or by causing oxidative stress or both of these mechanisms leading to the parasite death (Baker and McChesney, 1988; Ni et al., 1992). Primaquine is tissue stage schizontocidal drug having a unique broad spectrum, killing liver stages and asexual blood stages of *P. vivax* (Pukrittayakaemee et al., 1994). In epidemic regions, primaquine is widely used as gametocytocide and recommended for prophylaxis (Baird and Hoffman, 2004). A standard regimen of 30 mg dose has been found to provide complete protection against *P. falciparum* and *P. vivax*. Primaquine is absorbed well after oral administration, but owing to its short half-life, it is required to be administered daily. Its use is contraindicated in fetus and the person with G6-PD deficiency (Baird et al., 2003).

Primaquine has been found to cause some side effects, which include nausea, vomiting, abdominal pain, cramps and granulocytopenia. To resolve its toxicity problem and enhance its efficacy, the basic side chain of pamaquine base was attached to a number of heterocyclic ring systems, which led to the development of acridine derivative, quinacrine (also known as atebrine or mepacrine) (Coatney, 1963). Further scientific efforts have led to the development of compounds WR 225448 and tafenoquine (WR 238605) in 1975 and 1979, respectively. Another 8-aminoquinoline drug, which has been in preclinical trails, is CDRI80/53 (bulaquine). This derivative differs from primaquine only by 2, 4-dihydrofuran group present in the basic side chain anchored onto quinoline nucleus in 8 position. It has been found to be more active than primaquine in rodent and simian models in preclinical trials (Peters et al., 1993a,b,c; De Alencar et al., 1997). Tafenoquine (Fig. 2.1) is primaquine analogue with larger half-life of 14 days. It has larger
therapeutic index than primaquine thus used for the prevention of relapses of *P. vivax* (Walsh *et al.*, 1999) and chemoprophylaxis of *P. falciparum* (Lell *et al.*, 2000).

Study of natural products has led to the isolation of a newer morphine compound namely, tazopsine from *Strychnopsis thouarsii*. Tazopsine has been found to exert inhibitory effect on liver stage of parasite. Further research work on tazopsine compound, which has led to the formation of N-cyclopentenyl tazopsine. This compound has shown significant parasite inhibitory profile, thus it may serve as an alternate for primaquine (Caraz *et al.*, 2006).

**ANTIFOLATE DRUGS**

Other important drugs against malaria includes antifolate drugs (dihydrofolate reductase inhibitors, sulfonamids and sulfones), which are effective both as prophylactic and therapeutic agents. These drugs interfere differentially with folate metabolism of parasite pathway. Antifolate agents used in the treatment of malaria infection are subdivided into two classes: inhibitors of dihydropteroate synthase (DHPS), known as class I antifolates and inhibitors of dihydrofolate reductase (DHFR), the class II antifolates (Fig. 2.3).

![Fig. 2.3: Structure of antifolate drugs](image-url)
The combination of DHFR and DHPS inhibitors are synergistic, hence they are used in combination therapy in the treatment of malaria. Inhibitor of DHFR includes the proguanil and pyrimethamine drugs. Proguanil was the first reported antifolate antimalarial to be discovered as a result of an intensive British research programme during Second World War. Despite lower schizontocidal activity against quinine (Covell et al., 1949), early reports on the use of proguanil (Paludrine) for malaria treatment were very exciting (Maegraith et al., 1945; Jones et al., 1948; Seaton and Lourie, 1949), as it was found to be safe in its use. Thereafter, it developed resistance in Southeast Asia, which soon spread widely and casted shadow on further development of this drug. Proguanil is metabolized to chlorcycloguanil (Triazine form), which inhibits DHFR of parasite. Combination therapy of proguanil with chloroquine has been found effective in malaria treatment (Peters, 1971; Kain et al., 2001; Petersen, 2004). Proguanil has recently been combined with atovaquone, an inhibitor of electron-transport to the cytochrome bc1 complex (coenzyme Q). This combination is known as Malarone. It was found to be synergistic and thus being used as prophylactic agent against malaria (Kain, 2003). Potency of proguanil led to the discovery of its various analogues, which includes chlorproguanil, cloxicguanil (BRL50216) and BRL 6231 (WR 99210). These drugs are being used in combination with dapsone for prophylaxis and treatment of malaria (Shanks et al., 1992; Mutabingwa et al., 2001). Recently, proguanil/dapson combination has been combined with artesunate (Krudsoods et al., 2005), however, chlorproguanil/dapson combination provides more efficacy over proguanil/dapson and cycloguanil/dapson combination (Winstanley et al., 1995; Watkins et al., 1997).

Pyrimethamine has been the most widely used antimalarial antifolate agent for the malaria treatment. It belongs to 2, 4-diaminopyrimidine derivative family. The interest in the antimalarial activity of this family of compounds was sparked in 1940s, when they
were synthesized and tested as analogues of folic acid in the
treatment of tumors (Hitchings et al., 1950). Falco et al. (1951)
observed that the structure of these compounds and proguanil were
similar and hypothesized that 2, 4 diaminopyrimidine could have
antimalarial activity. Thus, the screening of 2, 4 diaminopyrimidine
compounds led to the development of pyrimethamine. Pyrimethamine
is recommended in combination with various sulfa drugs, which
include sulfadoxine, sulphene (known as Fansider and Metakelfin
respectively) and sulfadiazine, sulfametopyrazine, sulfametoxine,
sulfaphenazole and sulfisoxazole (McGregor et al., 1963; Powell et
al., 1967).

Sulphadoxine/pyrimethamine (SP) combination acts by
reciprocal potentiation of its two components, achieved by a
sequential blockade of two enzymes involved in the biosynthesis of
folinic acid within the parasites. Sulphadoxine, like other
sulphonamides, is a structural analog of p-aminobenzoic acid
(PABA), which competitively inhibits dihydrofolic acid synthesis by
inhibiting dihydropteroate synthetase, which is necessary for the
conversion of PABA to folic acid. Pyrimethamine is a folic acid
antagonist having mechanism of action similar to that of trimethoprim.
Pyrimethamine inhibits the reduction of dihydrofolic acid to
tetrahydrofolic acid (folinic acid) by inhibiting dihydrofolate reductase.
Pyrimethamine interferes with the synthesis of tetrahydrofolic acid in
malarial parasites at a point immediately succeeding to
sulphonamides, where it acts. The combination of sulphadoxine and
pyrimethamine results in a synergistic action against susceptible
parasites. It has been also found effective against certain strains of
Plasmodium falciparum that are resistant to chloroquine. SP attacks
the different development stages of the parasite, thereby reducing
the chances of recrudescence. The protective effect of a single dose
lasts for approximately four weeks.
The drug is contraindicated in patients with severe renal problems, infants and nursing women. Fatalities associated with the administration of sulphadoxine and pyrimethamine includes, Stevens-Johnson syndrome and toxic epidermal necrolysis. Sulpha drugs block the synthesis of de novo folate synthesis, which led to the development of DHPS inhibitor for the malaria treatment. This family includes dapsone, which is the most potent DHPS inhibitor of malaria ever described. Dapsone was synthesized at the beginning of 20th century, in 1908, as a result of a search for molecules to produce azo dyes (Fromm and Whittmann, 1908). This compound was not tested as an antimicrobial until the late 1930s, when Buttle et al. (1937) and Fourneau et al. (1937) assessed its chemotherapeutic effects against bacterial cells. This sulphone based agent was found to suppress the growth of various pathological agents including mucobacteria and malaria parasite. It has been used in monotherapy in past for the treatment of malaria. However, because of high toxicity, the development of this drug was abandoned. Dapson was combined with pyrimethamine as Maloprim and with chlorproguanil (Lapdap) for the treatment of malaria (Winstanley et al., 1997). In vitro and in vivo analysis have demonstrated that chlorcycloguanil/dapsone combination is more potent than that of pyrimethamine and sulfadoxine combination (Nzila-Mounda et al., 1998; Mutabingwa et al., 2001; Bukirwa et al., 2004). In addition, an artemisinin based combination of chlorproguanil/dapsone with artesunate is being developed (Wootten et al., 2005).

The antifolate resistance to DHFR drugs (pyrimethamine, cycloguanil and chloroguanil) has arisen due to point mutations in dhfr-ts genes (Zolg et al., 1989; Foote et al., 1990; Plowe et al., 1998). The first step in pyrimethamine resistance arises with a single mutation, causing serine to asparagine change at position 108 of dhfr, which is followed by ancillary mutation of asparagine to isoleucine at codon 51 and cysteine to arginine at 59 codon. More
than 1000-fold increase in pyrimethamine resistance occurs, when a fourth mutation, Ile to Leu at codon 164 is added (Hyde, 2002).

**ANTIBIOTICS**

Some antibiotics that act on prokaryotic organisms such as tetracycline, doxycycline and azithromycin have also shown antimalarial activity (Kevin *et al.*, 2007). The antibiotics are thought to inhibit parasite growth through the inhibition of ‘prokaryote like’ protein biosynthesis in the apicoplast – that is unique to apicomplexan parasites (Fichera and Ross, 1997; Clough *et al.*, 1999).

Tetracycline is a broad-spectrum antimicrobial drug that has potent but slow action against the asexual blood stages of *Plasmodium* species. It is also active against the primary intrahepatic stages of *P. falciparum*. Tetracycline can be used in combination with quinine in the treatment of *falciparum* malaria to decrease the risk of recrudescence. The combination of quinine plus tetracycline given over 5-7 days is still highly effective in multidrug resistance areas (Looareesuwan *et al.*, 1994). The half life period of tetracycline has been recorded to 8 hrs. Tetracycline has been found to induce gastrointestinal effects, which include epigastric distress, abdominal discomfort, nausea, vomiting and diarrhea. These effects can be reduced, if this drug is taken with a meal (Rang and Dale, 1987).

Doxycycline is related to oxytetracycline and has an identical spectrum of activity. It differs from tetracyclines in terms of good absorptive power and long plasma half life. Doxycycline combination with tetracyclines can be used for therapy in quinine susceptible areas. Doxycycline offers a considerable operational advantage over tetracycline, due to higher efficacy and low cost. 200 mg concentration of doxycycline given once a day for 5 days in combination with mefloquine or artesunate has been found effective to treat multiresistant uncomplicated *falciparum* malaria in Thailand.
Doxycycline is completely absorbed from the gastrointestinal tract. Its peak plasma concentration reached around 2 hr after oral administration. It binds with plasma proteins (80-90%) and has a biological half-life of 15-25 h. Doxycycline (Vibramycin) is an alternative to mefloquine or malarone in areas with high levels of chloroquine resistance.

Azithromycin is the most potent antimalarial macrolide antibiotic. It has been found to act synergistically with quinine against *Plasmodium falciparum*. It is an efficient blood schizontocide but has a relatively slow action (Olliaro and Trigg, 1995). A large chemoprophylaxis trial in Kenya using 250 mg/day concentration of azithromycin has shown protective efficacy of 80% in *P. falciparum* infections (Anderson et al., 1995). In a similar trial in Indonesia, the protective efficacy was 100% for *P. vivax* but was not found enough for *P. falciparum* (Taylor et al., 1999). In further efforts, combination therapy of azithromycin with quinine for three days has been found to be safe, well tolerated and effective in curing drug-resistant *P. falciparum* malaria (Miller et al., 2006).

**ATOVAQUONE**

Atovaquone is a naphthoquinone belonging to a new family of antimalarial compounds (Fig. 2.4).

![Fig. 2.4: Structure of Atovaquone](image)

Hooker (1936) was the first to recognize the presence of naphthaquinone in the plant extracts and synthesized lapachol (a 2-
alkynaphthoquinone). It was originally developed as an antimalarial compound but was registered for the treatment of infections caused by *Pneumocystis carinii* and *Toxoplasma gondii* associated with AIDS. Atovaquone acts against ubiquinol-cytochromeC oxidoreductase (complex III), which is required for a number of parasite biochemical processes (Vaidya, 2001). The half-life of atovaquone is approximately 60 hrs. Atovaquone monotherapy has shown recrudescence phenomenon in patients with *P. falciparum* infection (Looareesuwan *et al.*, 1996). Thus combination therapy was generally recommended for it. The combination therapy of atovaquone with the antifolate proguanil has been proved very effective. This combination is now marketed as Malarone. It is highly efficacious against *P. falciparum*, including strains, that are resistant to chloroquine and mefloquine, with cure rates of 94-100% (Radloff *et al.*, 1996; Anabwani *et al.*, 1999; Looareesuwan *et al.*, 1999). Atovaquone-proguanil offers an alternative for chemoprophylaxis in those persons travelling to chloroquine-resistant *P. falciparum* areas and who cannot take mefloquine or doxycycline. Atovaquone is absorbed slowly from the gastrointestinal tract and subjected to wide individual variability. Absorption is greatly increased, if the drug is taken with a fatty meal. Adverse effects include abdominal pain, nausea, vomiting, diarrhoea, headache, anorexia and coughing.

**ARTEMISININ AND ITS DERIVATIVES**

Ancient Chinese herbal remedy *Artemisia annua* (sweet wormwood) led to development of artemisinin and its derivatives. It was used by Chinese herbal medicine practitioners for last 2000 years. Chinese scientists screened a series of traditional remedies for drug activities in 1967 and found that extracts of qinghao had potent antimalarial activity. In 1972, the active ingredient was purified and first named qinghaosu (essence of qinghao) and then later renamed artemisinin (Fig. 2.5).
Fig. 2.5: Structure of artemisinin and its derivatives

Artemisinin is a sesquiterpene lactone with peroxide bridge linkage. It has low solubility in water and oil, thus it can be administered orally, rectally and intramuscularly (Barradell and Fitton, 1995). Unfortunately, oral administration is not possible in patients with severe malaria due to extreme vomiting. To resolve this problem, several semi-synthetic artemisinin derivatives have been developed. These derivatives include the water soluble artesunate and the oil soluble artemether and arteether (Golenser et al., 2006).

Related compounds that are found in A. annua, are arteannuin B and artemisinic acid. Artemisinic acid is the precursor for artemisinin in the biotransformation pathway (Mueller et al., 2000; Kim and Sasaki, 2004; Lapkin et al., 2006). The peroxide bridge is the active group for antimalarial activity and therefore, it does not need the complex ring structure (Meshnick et al., 1996). This makes it easier to synthesize simplified analogs such as trioxanes. Fenozan 50F and WR279137 are newly developed semisynthetic compounds, which have been proved as an effective antimalarial agents against gametocyte and sporozoite stages of P. falciparum (Peters et al., 1993a,b,c; Posner et al., 1994; Fleck et al., 1997).

All artemisinin compounds induce a very rapid reduction of parasitaemia starting almost immediately after administration (Balint, 2001). Various studies have suggested that the drug is most effective against trophozoites (Geary et al., 1989), late rings and early trophozoites (Ter Kuile et al., 1993; Skinner et al., 1996).
Comparison of drug activity against eukaryotic cell lines, related to activity against malaria parasite was performed in vitro to predict drug specificity against *P. falciparum*. The effects of artemisinin and its derivatives were examined on chloroquine sensitive and resistant strains. The IC$_{50}$ of parasite strains differs, when treated with chloroquine (16.5 nM against sensitive strains/232.6 nM against resistant strains). However, there was not much difference between the IC$_{50}$ values of artemisinin (14.5 nM) against chloroquine sensitive and resistant strains (17.0 nM).

Many studies regarding resolving the mechanism of action of artemisinin has suggested the involvement of peroxide bridge (Avery *et al.*, 2003). The complex ring structure of artemisinin has not been found to play any role in exerting antimalarial effect, however, it is necessary for the stability of molecule. Despite relying on artemisinin and its derivative drugs, the exact mechanism of action is still unresolved. Artemisinin has been found specifically to inhibit Sarco/Endoplasmic Reticulum Ca$^{2+}$-ATPase (SERCA) (Eckstein-Ludwig *et al.*, 2003; Liu *et al.*, 2006). It is responsible for the maintenance of calcium ion concentrations, which is important for the generation of calcium-mediated signaling and the correct folding and post-translational processing of proteins. In *Plasmodium falciparum*, PfATP6 acts as SERCA-type Ca$^{2+}$-ATPase. Artemisinin and its derivative compounds have been found to inhibit specific PfATP6 activity. Modeling of PfATP6 demonstrated that artemisinin bind to the protein by hydrophobic interactions, while leaving the peroxide bonds exposed. This allows cleavage of the peroxide bridge by iron to generate carbon-centered radicals, leading to enzyme inactivation and parasite death. It has been demonstrated that mutations in SERCA results in the rising problem of resistance of artemisinin and its derivatives.

The other possible mechanism of artemisinin action is the production of reactive species (Van Agtmael *et al.*, 1999; Ittarat *et al.*, ...
During the blood stage of parasite, more than 70% haemoglobin within infected erythrocytes is digested. Haem is released, which is toxic for parasite. Therefore, it is neutralized by polymerization into haemozoin. It was found that haem or Fe$^{2+}$ catalyzes the opening of peroxide bridge in artemisinin and leads to the formation of free radicals. The initially formed oxygen radicals rearrange to primary and secondary carbon centered radicals, intermediates in the formation of known metabolites. These intermediates are finally involved in alkylation of protein. Both the proposed mechanisms of action depend on the activation of peroxide group leading to production of reactive species.

The action of artemisinin and its derivatives is different from that of the other antimalarial drugs, although both artemisinins and quinolines interact with haem. Artemisinins have a very fast action and parasites clearance time is much shorter as compared to other antimalarials. Artemisinin and its derivatives are metabolized to dihydro-artemisinin (DHA), which has been found to exert its antimalarial effect same as that of parent drug (Burk et al., 2005). There is a significant difference in the half-lives of different artemisinin derivatives. Artesunate is metabolized almost immediately, while artemether and arteether are metabolized more slowly and DHA has a half-life of 1 hr.

Treatment with artemisinin causes reduction of parasite below detectable limit, without eliminating all parasites. These parasites further grow leading to a recrudescence of malaria symptoms. In order to completely eliminate the parasites and prevent the emergence of resistant *P. falciparum*, combination with other long acting drugs is necessary.

There is currently no evidence for clinically relevant artemisinin resistance. However, wide use of artemisinin and its derivatives may lead to the development of resistance. Artemisinin-resistant strains of *P. falciparum* (Inselburg, 1985) and *P. yoelii* (Peters and Robinson,
A COMPARATIVE STUDY OF SOME ANTIMALARIALS ON CLEARANCE OF BLOOD STAGE *Plasmodium berghei* INFECTION

1999) have been obtained in the laboratory. Various clinical isolates and lab strains of *P. falciparum* have been found to vary in sensitivities to artemisinin *in vitro* (Le Bras, 1998), but this does not seem to be clinically significant. Two *P. falciparum*-infected cases were found to fail supervised artesunate therapy, but their parasites appear to be fully sensitive to artemisinin *in vitro* (Luxemburger *et al.*, 1998). Some artemisinin failures have also been reported from India (Gogtay *et al.*, 2000) and Sierra Leone (Sahr *et al.*, 2001) but the recrudescent parasites were not tested for sensitivity to artemisinin *in vitro*. In general, recrudescence after monotherapy with artemisinin derivatives, is thought to be due to pharmacological factors such as host metabolism and there is no convincing evidence that the failure of artemisinin derivatives in humans is due to parasite resistance. Adverse effects are rare in patients treated with artemisinin derivatives. In a prospective study of over 3,500 patients in Thailand, there was no evidence for serious adverse effects (Price *et al.*, 1999). Artemisinin derivatives also appear to be safe for pregnant women (McGready *et al.*, 1998; Alkadi, 2007).

**ARTEMISININ COMBINATION THERAPY (ACT)**

World Health Organization has endorsed ACT as first-line treatment for the *Plasmodium falciparum* infections (WHO, 2006). ACTs combine the rapid schizontocidal activity of an artemisinin derivative (artesunate, artemether or dihydroartemisinin) with a longer-half-life partner drug. Combination chemotherapy tends to delay the onset of resistance. The important combination with artemisinin and its derivatives includes:-

**Artesunate and Chloroquine**

This combination has been thoroughly tested in randomised controlled trials and has demonstrated that it is well tolerated with few side effects. However, in one study there was less than 85% cure
in areas, where chloroquine resistance was known. It is not approved for use in combination therapy and is unadvised in areas of high *P. falciparum* resistance.

**Artesunate and Amodiaquine**

This combination has also been tested and proved to be more efficacious and similarly well tolerated to the chloroquine combination. The cure rate was greater than 90%, potentially providing a viable alternative, where levels of chloroquine resistance are high (Ibrahium *et al.*, 2007). The main disadvantage is a suggested link with neutropenia. Dosage is recommended as 4 mg/kg of artesunate and 10 mg/kg of amodiaquine per day for 3 days.

**Artesunate and Mefloquine**

This has been used as an efficacious first-line treatment regimen in Peruvian patients (Gutman *et al.*, 2009). Mefloquine is known to cause vomiting in children and induces some neuropsychiatric and cardiotoxic effects. These adverse reactions seem to be reduced, when the drug is combined with artesunate (Nosten, 1998). This is not considered a viable option to be introduced in Africa due to the long half-life of mefloquine, which potentially could exert a high selection pressure on parasites (Rogers *et al.*, 2009). The standard dose required is 4 mg/kg per day of artesunate plus 25 mg/kg of mefloquine as a split dose of 15 mg/kg on day 2 and 10 mg/kg on day 3 has been proven effective in Peruvian patients.

**Artemether and Lumefantrine**

This combination has been extensively tested in 16 clinical trials, proving effective in children under the age of 5 years and has been shown to be better tolerated than artesunate plus mefloquine combinations (Faucher *et al.*, 2009). There are no serious side effects documented but the drug is not recommended in pregnant or lactating women due to limited safety testing in these groups. This is
A COMPARATIVE STUDY OF SOME ANTIMALARIALS ON CLEARANCE OF BLOOD STAGE PLASMODIUM BERGHEI INFECTION

the most viable option for widespread use and is available in fixed-dose formulas, thus increasing compliance and adherence.

Artesunate and Sulfadoxine/Pyrimethamine

This is a well tolerated combination but the overall level of efficacy still depends on the level of resistance to sulfadoxine and pyrimethamine thus limiting its usage (Priotto et al., 2003). It is recommended in doses of 4 mg/kg of artesunate per day for 3 days and a single dose of 25 mg/kg of SP.

Piperaquine-dihydroartemisinin-trimethoprim (Artecom)

Piperaquine-dihydroartemisinin-trimethoprim (Artecom) and Artecom combined with Primaquine has been studied in resistant areas of China and Vietnam. The drug has been shown to be highly efficacious (greater than 90%) even to strains resistant to primaquine (Davis et al., 2005). Prior to introduction more information is required on safety and tolerability in pregnant women and children and toxicology data.

Pyronaridine and artesunate

This combination tested both in vitro as well as in vivo have shown efficacy of combination therapy of pyronaridine with artesunate (Vivas et al., 2008). It has been found to demonstrate a clinical response rate of 100% in one trial in Hainan (an area with high levels of P. falciparum resistance to Pyronaridine).

Chlorproguanil-Dapsone and artesunate (Lapdap plus)

Lapdap plus is the most tested drug currently under development and could be introduced in African countries imminently (Bukirwa et al., 2004). It is not recommended as a monotherapy due to concerns of resistance developing, thus threatening the future use of related compounds.

The use of an artemisinin derivative as one of the combination partners has become the standard recommendation for treatment of
uncomplicated malaria, owing to its potent anti-malarial capacity. ACT treatment quickly reduces body parasite number, which not only relieve symptoms rapidly, but also reduce the chance of emergence of clones resistant against the partner drug (White, 1996).

PLANT PRODUCTS

The rising problem of resistance to the present antimalarial drugs, stresses the need to adopt newer strategies to cope up with this devastating disease. Natural product derived compounds offer an appealing and effective approach to chemotherapy. This approach has identified the most important drugs currently available to treat severe malaria, which includes quinine and derivatives of artemisinin. In the case of artemisinin, relatively simple chemical modification of natural product parent compound have led to a series of highly potent antimalarials, that will probably play a critical role in the treatment of severe malaria over next decade. Further more extensive research is required for the evaluation of traditional medicinal plants, which have been in use since ever. With the exception of quinine and artemisinin, few detailed pharmacological investigations have been carried out on plant derived antimalarial compounds. Such studies are important not only because they contribute to our knowledge of traditional antimalarial plants, which hopefully will lead to the more effective use of these species in malaria treatment but they may also contribute to the development of new antimalarial agents. Plant species contains a wide diversity of chemical types and it is likely that some of these will have novel mode of action against malaria parasites, so that the elucidation of their mechanism of action could lead to the identification of new biochemical targets against which new compounds may be developed.

Bearing this in mind, the research of new antimalarial drugs among plants used in the traditional medicine appears to be particularly important. The study of medicinal plants has been
considered as a worthwhile approach for the discovery of new drugs. The Research Initiative on Traditional Antimalarial Methods (RITAM) supports the research on herbal antimalarials (TDR, 2000). The purpose of RITAM is to facilitate exchange and collaboration among those studying and using plants in controlling malaria with an interest in developing a coordinated strategy for a more effective, evidence-based use of traditional antimalarial methods (Willcox and Bodeker, 2000). Large number of compounds belonging to different molecular classes have been isolated and identified till now but only a limited number of active compounds have shown their efficacy against cerebral malaria.

*In vitro* culture of *P. falciparum* have been developed in human red blood cells (Trager and Jensen, 1976). This technique is useful to assess *in vitro* antimalarial activity of crude extracts prior to the isolation of active principles. *In vitro* tests are more rapid and less expensive as compared to *in vivo* tests, however, they are not more predictable as compared to *in vivo* results (Fereira-da-Cruz *et al.*, 2000). In contrast to *in vitro*, *in vivo* results are more predictable and authentic. They give a measure of toxicity at the same time. Detailed evaluation of antimalarial drugs is done either in the *Aotus* monkey (*Aotus trivirgatus*) using *P. falciparum* infection or in the Rhesus monkey (*Macaca mulata*) with *P. cynomolgi* infection (Dhawan and Srimal, 1997). Phillipson and Wright (1991) reported the chronological progress of biological testing for antimalarial activity. Around the 1950s, the screening of crude plant extract was based on avian malarias utilizing *in vivo* tests against *Plasmodium gallinaceum* in chicks and against *P. cathelemrium* and *P. lophurae* in ducklings.

In India, *Azadirachta indica* (neem) is widely used in the treatment of various diseases, including malaria. Sharma and Sharma (1998) reviewed the plants showing antiplasmodial activity from crude extracts to isolated compounds and listed the compounds in the three major groups: alkaloids, terpines and quassinoids with
some aromatic and miscellaneous compounds. They reported *in vitro* and *in vivo* activity of 231 crude plant extracts, showing antiplasmodial activity against different species of *Plasmodia*. With the problems of increasing levels of drug resistance and difficulties in poor areas of being able to afford and access effective antimalarial drugs, traditional medicines could be an important and sustainable source of treatment. The identification of new active compounds with effective mode of action from traditional medicinal plants may prove fruitful for the effective treatment of malaria in future.

There are four basic approaches for the selection of plants that may contain new biological agents: the random selection, the taxonomic, the phytochemical and the ethnopharmacological approach. With the random approach, all the available species are collected, without looking at prior knowledge and experience. In the taxonomic approach, plants of a given genus or family are deemed to be of interest and are sought from diverse locations and evaluated. In the ethnopharmacological approach, credence is given to information on the traditional use of the plant and according to this information, the plant are collected and evaluated. For all approaches, the material collected is evaluated through a range of bioassays. Selected active extracts then undergo bioassays-directed fractionation procedures in order to isolate the active principle(s). In the phytochemical approach, particular compound types, for example, indole alkaloids are regarded as being of biological interest, and plants likely to have related compounds are collected and evaluated (Cordell *et al.*, 1991).

Several papers dealing with the research of new antimalarial drugs from plants can be found in the literature and the ethnopharmacological approach is prevalent in this kind of research. Many reports regarding studies on plant extract’s antimalarial activity of traditional plants used by the population of endemic countries are available (Milliken, 1997; Milliken and Albert, 1997; Kamei *et al.*, 1991).
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2000; Marshall et al., 2000; Deharo et al., 2001; Simonsen et al., 2001; Horgen et al., 2001). Nevertheless, the discovery of active compounds resulting from these studies rarely occurs. Often, the pharmacological screening of herbal extracts implies many problems (Grabley et al., 1999). Their chemical complexity makes high throughput screening difficult and the results less significant. Side compounds such as lipids and tannins may exert unspecific, false-positive effects in the pharmacological assays. Therefore, preliminary chemical analysis and the separation of the compounds is a very important step.

NATURAL COMPOUNDS WITH ANTIMALARIAL ACTIVITY

Investigation of a range of plants from various countries, which are used as traditional medicine for the treatment of malaria has led to the discovery of large number of compounds with significant antimalarial activity. Table 2.1 lists some of these compounds which belong to different secondary metabolic classes and traditional plant from which they have been isolated.

Table 2.1: Example of classes of compounds with antimalarial activity isolated from traditional medicines

<table>
<thead>
<tr>
<th>Class of Compound</th>
<th>Compound</th>
<th>Plant</th>
<th>Part of Plant</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinone</td>
<td>1-hydroxybenzoic acid</td>
<td>Psychotria complementans</td>
<td>Stem &amp; roots</td>
<td>Panama</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>Lupeol</td>
<td>Vernonia brasiliana</td>
<td>Leaves</td>
<td>Brazil</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Ancistroheynine A</td>
<td>Ancistocladus heyneanus</td>
<td>roots</td>
<td>India</td>
</tr>
<tr>
<td>Sesquiterpenoids</td>
<td>16.17 Dihydrobrachycalyxolide</td>
<td>Vernonia brachycalyx</td>
<td>Leaves</td>
<td>Kenya</td>
</tr>
<tr>
<td>Quassinoids</td>
<td>Bruceolide</td>
<td>Brucea javanica</td>
<td>Fruits</td>
<td>China</td>
</tr>
<tr>
<td>Limonoids</td>
<td>Fissinolide</td>
<td>Khaya senegalensis</td>
<td>Bark</td>
<td>Sudan</td>
</tr>
<tr>
<td>Lignans</td>
<td>(+)-Nyasol</td>
<td>Asparagus africanus</td>
<td>Roots</td>
<td>Kenya</td>
</tr>
<tr>
<td>Coumarines</td>
<td>O-Methylexostemin</td>
<td>Exostema mexicanum</td>
<td>Stem &amp; Bark</td>
<td>Latin America</td>
</tr>
</tbody>
</table>
Quinones

Chemically, quinones are compounds with 1,4-diketocyclohexa-2,5-dienoid moiety. The structure of many naturally occurring quinones is based on the benzoquinone, naphthoquinone or anthraquinone ring system. A number of natural and synthetic naphthoquinones have been found to possess antiplasmodial activity (Carvalho et al., 1988). Among naphthoquinones isolated from higher plants, plumbagin isolated from Droseraceae possess the strongest antimalarial activity against *P. falciparum* with an IC$_{50}$ of 0.27 μM (Likhitwitayawuid et al., 1998). Further testing of semi synthetic compound revealed that the presence of quinone structure was essential for its antiplasmodial activity. Some traditionally used species of Bignoniaceae, in the Colombian Amazon regions, has been found to contain furano-naphthoquinones, which exhibit significant IC$_{50}$ of 0.677 and 0.167 μM against *P. falciparum* and *P. berghei* respectively (Perez et al., 1997). However, *in vivo* studies using the *P. berghei* mouse model are required to evaluate their potential toxicity in a mammalian host.

Alkaloids

Alkaloids are one of the major classes of compounds possessing antimalarial activity. The most important and oldest drug quinine belongs to this class of compounds. Alkaloids are the physically active nitrogenous bases derived from many biogenetic precursors. A large number of naturally occurring alkaloids, belonging to different groups, have been reported to possess antimalarial activity against different malarial models.

Rasoaanaivo et al. (1998) isolated a series of benzyl-tetrahydroisoquinolines alkaloids (hervelines, reticuline and laudanosine) from *Hernandia voyronii* (Hernandiaceae), plant traditionally used in Madagascar as coadjuvant to chloroquine in the treatment of malaria. Regarding the biological effects of these
compounds, the authors pointed out a moderate in vitro antiplasmodial activity but they have shown an interesting chloroquine-potentiating action. According to the authors, the benzyltetrahydroisoquinoline moiety and the methylation of the hydroxy group at C-12' might be responsible for the bioactivity of the herveline series. These results account for the traditional use of the crude extract of *H. voyronii* against malaria. Similarly, a bisbenzylisoquinoline alkaloid dchatrinc, isolated from the wood of *Beilschmiedia madang* (Lauraceae), has been found to possess significant IC$_{50}$ (0.017 μM) comparable to quinine against *P. falciparum* (Kitagawa *et al.*, 1993).

Naphthylisoquinoline alkaloids have been isolated from the liana families (Ancistrocladaceae and Dioncophyllaceae) of traditional medicinal plants of African and Asian countries for the treatment of malaria. These compounds have a considerable in vitro antiplasmodial potential against erythrocytic forms of both *P. falciparum* and *P. berghei* (Francois *et al.*, 1995). In vivo experiments pointed out that the percentage of parasite suppression with the naphthylisoquinoline alkaloids was generally higher than those obtained with the reference antimalarial drugs (Francois *et al.*, 1997). Dioncophylline C, in particular, has been considered as a promising candidate for further development in the preclinical and clinical phase due to its low toxicity, efficacy and bioavailability in the bloodstream.

*Sparattanthelium amazonum*, a traditional medicinal plant has been found to contain Aporphine alkaloid, (−)-roemrefidine. This compound has been found to possess an interesting in vivo antiplasmodial activity with ED$_{50}$ (5.98 mg/kg/day) in *P. berghei* infected mice (Munoz *et al.*, 1999). This compound acts with a different mechanism of action having no effect on the erythrocytic reinvasion. Further study carried out to determine the toxicity of the compound does not show cytotoxicity against different cancer cell lines (KB, HEp-2, and HeLa).
The azaaporphinoids hadranthine A, sampangine and 3-methoxy-sampangine were found to be the active antimalarial constituents of *Duguetia hadrantha* (Annonaceae) (Muhammad *et al.*, 2001). Because these alkaloids were more active against chloroquine-resistant *P. falciparum* clone W2 (IC$_{50}$ 6.8 to 120 ng/ml), chloroquine IC$_{50}$ (140 ng/ml), without cytotoxicity toward different cell lines, they warrant further investigation as potential antimalarial lead compounds.

Cryptolepine is an indolquinoline alkaloid extracted from roots (Kirby *et al.*, 1995) and leaves (Paulo *et al.*, 2000) of *Cryptolepis sanguinolenta* (Asclepiadaceae). This plant is widely used as traditional source of treatment for malaria in Ghana. *In vitro* activity of cryptolepine was found to be approximately twice as strong (IC$_{50}$ 0.134 µM) as compared with chloroquine (IC$_{50}$ 0.230 µM), but it was proven to be inactive in *P. berghei* infected mouse model. However, Cimanga *et al.* (1997) have shown a significant chemosuppressive effect of cryptolepine against *P. yoelii* and the same activity of its hydrochloride also against *P. berghei* infected mouse model. Boye and Oku-Ampofo (1983) have reported a successful clinical trial with an aqueous decoction of *C. sanguinolenta* root. It was generally considered that cryptolepine intercalate with DNA exerted cytotoxic properties to parasite however, Wright *et al.* (2001) have suggested some different mechanism of its action, which further needs to be evaluated.

The indole alkaloids (icajine, isoretuline, and strychnobrasiline) have been isolated from *Strychnos* spp. did not exhibit significant antiplasmodial activity *in vitro* (Frederich *et al.*, 2001). However, these compounds have been found to reverse chloroquine resistance against two different chloroquine-resistant strains of *P. falciparum* at a concentration between 2.5 and 25 µg/ml. Icajine has been found to present synergistic effect with mefloquine, when tested in the mefloquine resistant strain F32 of *P. falciparum*. No clear
structure/activity relationships could be established for these three compounds, although active alkaloids do not have any substitution on their indole moiety. Icajine affects both types of resistance at concentration nontoxic for human cells (HCT-116 human cancer cell line). Thus it could be used to comprehend the chloroquine- and mefloquine resistance of *P. falciparum*.

Voacamine, a bisindole alkaloid has been found to exhibit significant *in vitro* antiplasmodial activity against both chloroquine sensitive D6 (IC$_{50}$ 238 ng/ml) and chloroquine resistant W2 (IC$_{50}$ 290 ng/ml) strains (Federici *et al.*, 2000). Voacamine was firstly isolated from *Voacanga* species (Janot and Goutarel, 1955) and then from other Apocynaceae species such as *Peschiera fuchsiaefolia*. For this compound a potential effect on the nuclear division of the parasite, possibly on the DNA protein synthesis has been hypothesized (Ramanitrahasimbola *et al.*, 2001), however, exact mode of its action, further needs to be elucidated.

**Quassinoids**

The quassinoids are heavily oxygenated lactones with majority of C$_{20}$ basic skeleton, named as picrasane. However, C$_{18}$, C$_{19}$ and C$_{25}$ quassinoids are also known. They have varying number of different oxygen containing groups. Quassinoids have been found to possess wide spectrum of biological properties with antineoplastic and antimalarial activities. The first study about the antimalarial activity of Simaroubaceae’s extracts date back to Spencer *et al.* (1947). Following these observations, different authors assert that a number of quassinoids (modified triterpenes), brucein A, brucein B, brucein D and brusatol, have been found to exert *in vitro* antimalarial activity against *P. falciparum* (Fandeur *et al.*, 1985; Pavanand *et al.*, 1986; Anderson *et al.*, 1991). These compounds showed a significant antimalarial activity, but having general low cytotoxicity. Structure activity relationship studies of quassinoids have suggested that an α, β-unsaturated chetone in ring-A and an oxymethylene bridge in ring-
C are generally considered necessary for antimalarial activity (Okano et al., 1990). The synthetic compound 3,15-di-O-acetylbruceolide, a derivative from bruceoside has been found to show significant antiplasmodial activity (Kim et al., 2000). This compound has been found to possess a potent in vitro antimalarial activity against *P. falciparum*, which was equivalent to that of chloroquine (Murakami et al., 1998). The results obtained from Kim et al. (2000) showed that concentration of 3,15-di-O-acetylbruceolide (0.46 ± 0.06 mg/kg per day) exhibited 50% suppression of *P. berghei* infected mice, while the ED$_{50}$ values of chloroquine and artemisinin were 0.2 and 5.6 mg/kg per day. The authors have hypothesized several phenomena, involving in the morphological changes in the parasites along with the protein inhibition of parasite, when treated with 3,15-di-O-acetylbruceolide. The importance of protein synthesis during transformation of trophozoites to schizonts has been reported previously (Kirby et al., 1989).

Cedronin belongs to the few quassinoids with C 19 skeleton. It exhibited IC$_{50}$ value similar for chloroquine resistant and sensitive strains, suggesting that quassinoids may act upon malarial parasite with different mode of action from that of chloroquine. The compound also has been further found to exhibited low toxicity with effective in vivo activity against *P. vinkei petri* with ED$_{50}$ value of 1.8 mg/kg/day (Moretti et al., 1994).

**Terpenoids**

The discovery of endoperoxide sesquiterpene lactone compound from *Artemisia annua* stimulates the research for other antimalarial compounds from traditional medicinal plants (Trigg, 1989), which comes out in the discovery of new sesquiterpenes lactones of the germacraneolide type from Asteraceae family. The isolated compounds, neurolenin A and B from traditionally used antimalarial plant *Neurolaena lobata* from Central America were found to exhibit significant antiplasmodial activity with an IC$_{50}$ of 0.92
μM, when compared with artemisinin. Two more interesting sesquiterpenoids compounds of the guaiane type, nardoperoxide and isonardoperoxide, were isolated from roots of *Nardostachys chinensis* (Valerianaceae) (Takaya et al., 1998) and found to exhibit significant antimalarial activity against *P. falciparum* (EC\(_{50}\) 1.5 and 0.6 μM), which was comparable with that of quinine (EC\(_{50}\) 0.11 μM). Studies against FM3A and KB cells showed selective cytotoxicity and antimalarial activity of compounds, which was comparable to those of quinine.

Isolated compound, dilactone 16, 17-dihydrobrachycalyxolide from traditionally used Kenyan plant *Vernonia brachycalyx* has been found to exhibit strong antiplasmodial activity (IC\(_{50}\) 5.9 to 32 μM), on different *P. falciparum* strains. However, it strongly suppresses the body's immune mechanism, if administered to humans (Oketch-Rabah et al., 1998). Further studies are required to synthesize, new derivatives without any toxicity.

The Meliaceae family, closely related to the Simaroubaceae, has bitter principles known as limnoids, which are polyoxygenated triterpenoids. They are biosynthetically connected to the quassinoids. Some plant species belonging to this family are widely used as antimalarials or antipyretics in traditional medicine (Obih et al., 1985; Weenen et al., 1990). A series of limnoids such as gedunin, nimbinin, nimbolide, dihydrogedunin and azadirachtin, have been known to possess antimalarial activities (Bray et al., 1990). Studies concerning the investigations on the structure-activity relationships of some gedunin derivatives are being made in this direction (Mackinnon et al., 1997; Bickii et al., 2000). It appeared that the α,β-unsaturated ketone moiety in ring A (that was hypothesized to be involved in the reaction with the parasite nucleic acid) and the 7-acetate function in ring B are important for antimalarial activity. Bickii et al. (2000) have also reported that the combination of chloroquine and the limnoid gedunin showed an additive effect.
Flavonoids and Xanthones

The antimalarial activity of these classes of compounds has also been found very significant. The ethanolic bark extract of *Garcinia dulcis* has been found to contain five xanthones among which garciniaxanthone has been found to exhibit significant antimalarial activity with an IC\textsubscript{50} value of 0.96\mu g/ml against *P. falciparum*. Flavonoids isolated from *Artemisia annua* were not found active against *P. falciparum* but demonstrated selective potentiating effect on antiplasmodial activity of artemisinin (Liu et al., 1992). Thus, more compounds needs to be explored from these classes of secondary metabolites.

Miscellaneous compounds

Various compounds with different chemical structures have been found to possess antimalarial property. The most active constituents isolated from the tubers of *Cyperous rotundus* (Cyperacae), the root bark of *Zanthoxylum gilletii* (Rutaceae) and the root bark of *Margaritaria discoidea* (Euphorbiaceae) were \textalpha-cypcronc, N-isobutyldeca-2,4-dienamide and securinine respectively. All these compounds have been found to exhibit significant antiplasmodial activity due to the presence of \textalpha, \textbeta unsaturated carbonyl moiety. This moiety was suspected to undergo chemical reaction with nucleophilic sites in the parasite DNA molecule, thereby inhibiting the growth of parasite (Achenbach et al., 1992).

Recently developed antimalarial, Ayush-64 is a combination of four plants namely *Alstonia scholoris, Picrorhiza kurroa royle, Swertia chirata* and *Caesalpinia crista linn* (Kazim et al., 1991) is a potent example of herbal therapy. Further combination of different plants and their potent compounds may lead to the formation of new antimalarial drugs.

*Xanthium strumarium, Ajuga bracteosa* and *Berberis aristata* are known for their antimalarial properties in traditional medicine.
These plants has been in field practice, however, scientific evidence regarding their use is very limited. Thus it becomes imperative to evaluate these medicinal plants for their *in vitro* and *in vivo* antiplasmodial activity.

**Ajuga bracteosa**

**Family**: Labiateae  
**Habitat**: The sub Himalayan tract  
**Ayurvedic Name**: Neelkanthi  
**Folk**: Ratapaati (Kumaon) Khurbanti (Punjab)  
**Action**: The aromatic leaves are regarded as stimulant, diuretic, tonic and apparent. It is said to be a better astringent given in the treatment of fever and also used as febrifuge. As an aromatic tonic, it is useful in Ague. It is also used to kill lice, regarded as depurative. Leaves are used in the treatment of malaria, as a substitute for *Cinchona* (Kirtikar and Basu, 1975). The herb is also used in gout, rheumatism, palsy and amenorrhoea. Ethyl alcohol extract of leaves has been found to have anticancerous activity. The juice of leaves is used as blood purifier. The powder of leaves is used for burns and boils. An alkaloid fraction showed stimulant action on perfused frog heart.

*A. bracteosa* Wall ex Benth. Lamiaceae is a perennial herb growing wild from Kashmir to Nepal in Western Himalaya at an altitude of 1300 M. Leaves of this plant are used as stimulant, diuretic and in the treatment of various diseases like rheumatism, goat, palsy and amenorrhoea (Kirtikar and Basu, 1918). The phytochemistry of this genus has been subjected to extensive investigation and is a source of saturated and unsaturated long chain compounds (Bhakuni *et al.*, 1987) and ecdysteroids (Beauchamp *et al.*, 1996). Clerodane diterpene class of compounds, found in this plant, has been reported
to have insect antifedant activity (Camps and Coll, 1993). A new clerodane diterpene bracteonin A (Verma et al., 2002) and a new phthalic acid ester (Singh et al., 2006) has been reported from this plant with various medicinal properties.

The phytochemistry of *Ajuga* has been subjected to extensive investigation and is a source of saturated and unsaturated long chain compounds (Bhakuni et al., 1987) and ecdysteroids (Beauchamp et al., 1996). Diterpenoids and triterpenoids like secondary metabolites with various medicinal properties have also been detected in the plant. Clerodane diterpene class of compounds present in this plant has been reported to have insect antifedent activity (Camps and Coll, 1993). The phytochemical investigation and isolation of various compound from *Ajuga remota* has led to the identification of various compounds like Ajugarin I, II and III along with ajugasterane C, ajugalactone, cyasterone, B ecdysane and ergosterol 5, 8 peroxide. Ergosterol 5, 8 peroxide isolated from *A. remota* has been found to exert significant IC50 (8.2 ± 1.1 μM) value against chloroquine sensitive (FCA20/GHA) strain of *P. falciparum* (Kuria et al., 2001). Furthermore, triterpenoid ergosterol 5, 8 peroxide acts as an antimicrobacterial compound (Cantrell et al., 1999). This compound has been found to inhibit the growth of protozoan parasite of Trypanosomatidae family such as *Trypanosoma cruzi* and various *Leishmania* species by interfering with the integrity of cell membrane (Linares et al., 2006). The active involvement of this compound in the plant extract to inhibit plasmodial growth cannot be denied. However, the active mode of action is yet to be elucidated.

*Xanthium strumarium*

**Family:** Asteraceae

**Habitat:** Tropical India

**Ayurvedic Name:** Shankheshwara, Arishta.
Folk: Bana-okraa

Action: Plant is used for leucoderma, ulcers, abscesses and malignant diseases. Roots antitumour, leaves and shoot applied externally on venereal sores, herpes and scrofula. Leaves contain sesquiterpene lactones xanthin, xanthiumin, xanthanol and iso xanthanol. Leaves contain isohexacosane, chlorobutanol, stearyl alcohol, beta sitosterol and palmitic acid. A highly toxic compound, carboxyatractyloside has been isolated from plant (Burrows and Tyrl, 1989). Beta-sitosterol glucoside is anti-inflammatory. Xanthiumin is a central nervous system depressant. Alcoholic solution of xanthiumin shows strong antibacterial activity against gram negative bacteria and fungi. A cytotoxic compound, xanthatin (a seco 4,5 guaianolide) has been detected in resin. Roots have been found to contain n-heptacosanol, stigmasterol, 3,4 dihydro oxycinnamic acid, beta-sitosterol, D-glucoside and campesterol compounds.

The phytochemistry of this genus has been subjected to extensive investigations. The aerial parts of *X. strumarium* contain Xanthanolide 11α, 13-dihydroxanthatin. Two sesquiterpene lactones (Xanthiatin and Xanthinosin) have been isolated from the bur of Xanthium. These compounds show moderate to high *in vitro* cytotoxic activity with an IC50 of 0.1 to 6.2 µg/ml in human cancer cell lines W1 Dr ATCC (colon), MDA-MB-231, ATCC (breast) and NC1-417 (lungs) (Ramirez-Erosa et al., 2007). Similarly 8-epixanthin and 8-epixanthin epoxide (*Xanthanolide sesquiterpene* lactone) have been isolated from leaves of *X. strumarium*, which are reported to inhibit cultured human tumor cell A549 (non-small cell lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF498 (central nervous system) and HCT-15 (colon). It also inhibits the farnesylation process of human lamin-B by farnesyl transferase (FTase). *In vitro* IC50 value was calculated as 64 and 58 µg/ml for 8-epixanthin and i-epixanthin epoxide respectively (Kim et al., 2003).
Methanolic extract of *Xanthium semen* has been found to be a potent inhibitor of nitric oxide (NO), prostaglandin (PGE2) and tumor necrosis factor-alpha (TNF-α) by blocking NF-Kappa B activations, which is responsible for its anti-inflammatory and antinociceptive activities (Han *et al.*, 2007). The leaves of plant have been found to exert *in vitro* and *in vivo* antitrypanosomal effect (Talakal *et al.*, 1995). The leaves of plants are used in the treatment of long standing cases of malaria (Chopra *et al.*, 1986).

**Berberis aristata**

**Family:** Berberidaceae  
**Habitat:** North western Himalayas, Nilgiris, Kullu and Kumaon  
**Ayurvedic Name:** Daaruharidraa, Daaru  
**English name:** Indian Barberry  
**Action:** Rasaut, Rasasranjana, bitter cholagogue, antidiarrhoeal, stomachic, laxative, diaphoretic, antipyretic, antiseptic used externally in ophthalmia, conjunctivitis, ulcer, sores and swollen gums. Root barks anti-inflammatory, hypoglycaemic hypotensive, antiamoebic, anticoagulant, antibacterial. Bark used in liver complaints, diarrhea, dysentery, cholera, gastric disorders, enlargement of spleen and for regulating metabolism. Berries are antiscorbutic laxative. Berberine hydrochloride and sulphate help in diagnosis of latent malaria by releasing the parasite into the blood stream. Alkaloid berberine possesses antibacterial and anti-inflammatory activities. It is used as an intestinal antiseptic and bitter stomachic. It also exhibits antineoplastic properties. Its synthetic derivative dihydroberberine is used in brain tumors.

The phytochemical investigation of *Berberine* has led to the isolation of a benzylisoquinoline alkaloid. This compound has been found to possess antimicrobial activities against a wide variety of
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microorganisms including Gram-positive and Gram-negative bacteria, fungi and protozoa (Amin et al., 1969; Birdsall and Kelly, 1997; Park et al., 1999; Lauk et al., 2007). Berberine has been found to possess activities against Methicillin-resistant *Staph. aureus* (Yu et al., 2005). However, berberine is being reported to be readily extruded by multidrug resistance pumps (MDRs), present in numerous bacterial cells and thereby bacteria escaping death (Stermitz et al., 2000). The crude extract from *Berberis* sp. was found to have better antimicrobial activity in comparison to alkaloid fraction and native berberine (Lauk et al., 2007). Apart from antimicrobial activities, berberine has been reported to possess anti-inflammatory, analgesic and antipyretic potential (Kupeli et al., 2002; Yesilada and Kupeli, 2007). Recent studies have also shown the antifilarial potential of the *Berberis aristata* root extract against *Setaria cerri* microfilariae (Rizvi et al., 2008).

**HOMEOPATHY**

Homeopathy is a gentle, painless, holistic system of healing, developed during the 1790s by Samuel Hahnemann, a German physician. Experimenting on himself with the anti-malarial drug quinine, Hahnemann noticed that large doses of the medicine actually caused malaria-like symptoms, while smaller doses cured the symptoms. From this, he advanced his concept of *Similia similibus curentur*, or "let like be cured with like." Hahnemann then developed an extensive system of medicine based on this concept. He named it homeopathy, from the Greek words *homoios* (the same) and *pathos* (suffering).

Homeopathic remedies are almost always made from natural materials — plant, animal or mineral substances — that have been treated to form mother tinctures or non-soluble powders. Liquid extracts are then potentized or increased in power, by a series of dilutions and successions or shakings. It is thought that succession is
necessary to transfer the energy of the natural substance to the solution. In addition, the potency of the remedy is regarded as increasing with each dilution. After the tincture has been diluted to the prescribed potency, the resulting solution is added to a bottle of sucrose/lactose tablets, which are stored in a cool, dark place. If the remedy is not soluble in water, it is ground to a fine powder and triturated with powdered lactose to achieve the desired potency.

Proponents of homeopathy over the years have included Louisa May Alcott, Charles Dickens, Benjamin Disraeli, Johann Wolfgang Goethe, Nathaniel Hawthorne, William James, Henry Wadsworth Longfellow, Pope Pius X, John D. Rockefeller, Harriet Beecher Stowe, William Thackeray, Daniel Webster and W. B. Yeats. England's Royal Family has employed homeopathic practitioners since the 1830s.

Homeopathic prescription differs in general from allopathic medicine in its tailoring of remedies to the patient's overall personality type and totality of symptoms, rather than to the disease. Whereas, a conventional physician would prescribe the same medication or treatment regimen to all patients with the common cold, for example, a homeopathic practitioner would ask detailed questions about each patient's symptoms and the modalities or factors that make them better or worse. As a result, the homeopath might prescribe six different remedies for six different patients with the same illness. In acute prescribing homeopathy, consultations are briefer as compared to constitutional homeopathic prescribing. A typical patient might spend just 10-15 min with the practitioner, compared to more than an hour for constitutional prescribing.

Homeopathy came to India in as early as in 1810, when some German physicians and missionaries came to Bengal. Dr. John Martin Honigberger was the first person, who is recognized to have brought homeopathy to India. In 1861, a virulent malaria fever wasragging over lower Bengal and it was at this juncture that the great philanthropist Babu Rajendra Lal Dutta, truly laid the foundation of
homeopathy in India and started its practice with astounding results. 'Rajendra Lal Dutta' is called the father of Indian Homeopathy. The homeopathy medicines come from three reigns that exist in the nature: animal, mineral and vegetal e.g. from the animal reign we have the snake poison (*Lachesis trigonogaster*), from the mineral reign we have the copper and mercury and from vegetal we have the extracts from plants.

*Cinchona* is a genus of about 40 species of the family Rubiaceae native to tropical shrubs or small growing to 5-15 m tall with evergreen foliage. The trees of this genus are the source of a variety of alkaloids, the most important is China, which is the best known of the traditional medicinal remedies obtained from *Cinchona officinalis*. This is general protoplasmic poison. It is rapidly absorbed from gastrointestinal tract, 60 to 70% is oxidized in the body and the remainder is rapidly excreted in the urine. It destroys the parasite in the red blood cells rapidly by binding strongly to blood proteins and form complexes, which are toxic to the malarial parasite. It is employed to persons with chloroquine-resistant strains of malaria. The name of the genus is due to the Linnaeus who named the trees in 1742.

*Cinchona* bark is found in Nilgiris and Khasi Hills, Assam, Sikkim in India. The bark of quinine has been found to contain various alkaloids. Also called China, it acts on blood and nervous system, inhibits enzyme action, thereby retarding tissue metabolism. It is best remedy in chronic liver troubles, anemia and intermittent fever as reported in homeopathy treatment (Kent, 1974). Mother tincture is a drug prepared from original plant source using alcohol as a solvent by process of immersion, maceration and percolation. Drug power of tincture means strength of drug e.g. D.P.-1/6 means 1 part is medicinal substance and 5 parts are solvents. Hahnemann classified drugs from vegetable kingdom for preparation of mother tincture into four classes depending on their juicy contents. *Cinchona*
belong to Class 4; which contains dried vegetables and animal substances. Class 1, Class 2, Class 3, includes drugs prepared from very juicy, medium juicy and less juicy plants respectively. Recent clinical trials in Europe have suggested a positive effect of homeopathic medicines on such conditions as allergic rhinitis (Reilly et al., 1986) and influenza (Ferley et al., 1989). Davis et al. (1975) reported that Cinchona interrupts schizogony in P. berghei.

The homeopathic mode of treatment often encourages use of drugs at such ultra-low doses and high dilutions that even the physical existence of a single molecule of the original drug substance becomes theoretically impossible. But homeopathy has sustained for over two hundred years despite periodical challenges thrown by scientists and non-believers regarding its scientific use. There has been a spurt of research activities on homeopathy in recent years, at clinical, physical, chemical, biological and medical levels with acceptable scientific norms and approach. While clinical effects of some homeopathic drugs could be convincingly shown, one of the greatest objections to this science lies in its inability to explain the mechanism of action of the microdoses based on scientific experimentations and proofs. Though many aspects of the mechanism of action still remain unclear, serious efforts have now been made to understand the molecular mechanism(s) of biological responses to the potentized form of homeopathic drugs. Khuda-Buksh (2003) has reviewed some of clinical trials carried on the molecular mechanism of action of the potentized homeopathic drugs.

Fenner (1925) and Farrington (1989) have listed 45 homeopathic remedies for malaria among which Arsenicum, Bryonia, Cinchona, Ipecacunha, Eucalyptus globus, Natrium muriaticum, Vertrum album, Pulsatilla, Rhus toxicondendron, Nux vomica, sulphur are most frequently used for the treatment of malaria. Eupatorium perfoliatum and Arsenicum album has been found experimentally effective with a level of 60% parasitaemia inhibition, when tested in
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P. berghei infected mice (Lira-Salazar et al., 2006). China is known to destroy the parasite by binding strongly to the blood proteins of parasite (Davis et al., 1975). Similarly Eucalyptus is used for the clearance of parasite from the spleen. It is best known for the treatment of relapsing fever. These two homeopathic drugs have been checked out for their efficacy against P. berghei infected mice in order to validate their use in malaria treatment.