CHAPTER I

INTRODUCTION

The use of plants as medicine goes back to early man. Neanderthal remains have been found to contain the remnants of medicinal herbs (Holt et al., 2002). The Nei Ching is one of the earliest health science anthologies ever produced and dates back to the thirteenth century BC (Nakanishi, 1999). Some of the first records on the use of natural products in medicine were written in cuneiform in Mesopotamia on clay tablets and date to approximately 2600 BC (Cragg et al., 2001; Nakanishi, 1999). Some 3000 years before, human kind was well aware of the medicinal properties of some plants growing around him (Sofowora, 1982). The use of plants to cure diseases and relieve physical suffering has started from the earliest times of mankind’s history (Hill, 1989). The sources of important pharmaceuticals are plants being used by indigenous people (Balic et al., 1996).

Even today, many of these agents continue to exist in one form or another as medicines for inflammation, influenza, coughing and parasitic infestation. Chinese herb guides document the use of herbaceous plant as far back in time as 2000 BC (Holt et al., 2002). In fact, the Chinese Material Medica has been repeatedly documented over centuries starting at about 1100 BC (Cragg et al., 2001). The best known of these documents is the Ebers Papyrus, which documents nearly 1000 different substances and formulations, most of which are plant based medicines (Nakanishi, 1999).
Asclepius (in 1500 BC) was a physician in ancient Greece who achieved fame in part because of his use of plants in medicine (Holt et al., 2002). A collection of Ayurvedic hymns in India from 1000 BC and earlier describes the uses of over 1000 different herbs. This work served as basis for Tibetan Medicine translated from Sanskrit during the eighth century (Cragg et al., 2001). Theophrastus, a philosopher and natural scientist in approximately 300 BC, wrote a History of Plants in which he describes the medicinal qualities of herbs and the ability to cultivate them. The Greek botanist Pedanious Dioscorides in approximately AD 100 produced a work entitled De Materia Medica, which today is still a very well known European document on the use of herbs in medicines. Galen (AD 130-200) practiced and taught pharmacy and medicine in Rome and published over two dozen books on his areas of interest. Monks in monasteries in the Middle Ages (fifth to the twelfth centuries) copied manuscripts about herbs and their uses (Cragg et al., 2001; Holt et al., 2002).

However, it should not go unrecognized that it was the Arabs who were responsible for maintaining the documentation of much of the Greek and Roman knowledge of herbs and natural products and expanding that information with their own knowledge of Chinese and Indian herbal medicine (Cragg et al., 2001). Li Shih – Chen produced a Chinese drug encyclopedia during the Ming Dynasty entitled “Pen–ts‘as kang mu” in AD 1596, which records 1898 herbal drugs and 8160 prescriptions (Nakanishi, 1999).

In 1785, Whitering published the use of Digitalis purpurea, and this eventually led to the isolation of digoxin, a cardiotonic agent (Cragg et al., 2002). In 1816, Serturner isolated the analgesic, morphine, from Papaver somniferum, and the isolation of the anti-malarial drug, quinine, from the bark of Cinchona pubescens was reported in 1820. Some other discovery highlights were the isolation of atropine from Atropa belladona in 1831, cocaine
from *Erythroxylum coca* in 1860, and ephedrine from *Ephedra sinica* in 1887 (Buss *et al*., 1995). The alkaloids vinblastine and vincristine from the Madagascar periwinkle *Catharanthus roseus* became available in the 1960’s and are now extensively used in the treatment of different cancer disease types (Neuss *et al*., 1990). Another promising anticancer drug is taxol, which was isolated from *Taxus brevifolia* in 1971. In the early 1970’s artemisinin, a potent and essentially non-toxic anti-malarial agent was isolated from *Artemisia annua*, a Chinese medicinal plant (Kalyman, 1993).

Historically, botanicals have been our most fruitful arena in the search for new medicine. Searching new drug from traditionally used medicinal plants can, therefore, be the shortest path to success. In search for new medicines, the average success rate for identifying useful medicines from plants is one in 125 (Mc Caleb, 1997). The success rate for new drugs from randomly synthesized chemicals is only one in 10,000 (Chadwick and Marsh, 1994).

Traditionally used vasoactive medicinal plants were studied and based on their result, it was suggested that ethno-directed collection is more efficient means of drug discovery than random plant screens. So looking for new medicinal compounds from natural sources, especially plants, makes a great deal of sense and leads to saving of both time and money (Slish *et al*., 1999).

**Nature an important source for developing novel leads for medicines:**

Despite great progress in medicinal chemistry, the discovery of a novel drug has become more and more difficult. The reasons are many, but include the fact that for major diseases good drugs are available. Moreover, developing a better drug that is active on the
same target which is less expensive becomes increasingly difficult. As a result, in 2003 only 21 novel drugs were brought to the market. Interestingly, of the 877 novel medicines that were developed in the period 1981-2002, 6% were natural products, 27% were derivatives of natural products, and 16% were synthetics developed on the model of a natural product, demonstrating that nature is an important source for developing novel leads for medicines (Newman et al., 2003).

Due to limited availability and/or affordability of pharmaceutical medicines in many tropical countries, the majority of the populations depend on traditional medical remedies (Zirihi et al., 2005). At least 80% of the world population is estimated to be still using such traditional medicines in primary health care, including 40,000 to 70,000 medicinal plants representing about 20% of all higher-plant species. In most cases, very little is known of these plants used in traditional medicines. A search in NAPRALERT, the most important scientific database on plants and their bioactive constituents, some 10 years ago suggested that only about 15% of all plant species had been studied to some extent for their phytochemistry and only about 5% for one or more biological activities (Verpoorte, 2000).

Although extensive research on medicinal plants is published every year, only a few plants have been comprehensively studied for pharmacological activity. Considering these facts, traditional medicinal plants obviously represent a great source of novel leads for drug development.
Use of Medicinal Plants in drug discovery an incentive for conservation:

Ecological economists and policy minded ecologists have offered economic rationales for the preservation of biological diversity, reasoning that if people benefit materially from intact eco systems, they will have an incentive to protect them (Simpson et al., 1996). Many aspects of nature - intrinsic, spiritual, and aesthetic - are of incalculable value and will always be undersupplied by market forces (Gowdy, 1997).

One of the promising approaches for enhancing natural value is to search among plants for biochemically active molecules that may be developed for treatment of disease. At least 7,000 compounds used in Western medicines are derived from plants and they continue to have great importance. Approximately 40% of the new drugs approved by the U.S. Food and Drug Administration from 1983 to 1994 were natural products and derivatives (Cragg et al., 1997) and more than half of the 150 top - selling drugs of 1993 were derived from or modeled after natural active ingredients (Grifo et al., 1997).

Drawing on the accumulated medicinal knowledge of traditional societies and compensating the providers of knowledge for their information should allow traditional societies that have conserved biodiversity to benefit from their knowledge of biological resources, thereby increasing the incentive for continuing local conservation.

Use of Natural Products as anti malarial agents:

Natural products are important sources of biologically active compounds and have potential for development of novel anti - malarial drugs (Wright et al., 1990). For mammals,
including man, natural products are generally safer. Interest in plants as new anti-malarial has been stimulated by the isolation of artemisinin, a highly active compound against drug-resistant *P. falciparum* from *Artimisia annua* (Klayman, 1985).

The first anti-malarial developed was quinine, prepared from *Cinchona* bark (Guerra, 1977). The use of plants for the treatment of malaria extends over at least three continents, including several countries in Africa, America and Asia (Phillipson et al., 1987). It is worth mentioning that Indian medicinal plants are considered as a vast source of several pharmacological principles and compounds that are commonly used as home remedies against multiple ailments (Maity et al., 2009). However, scientific data on such medicinal plants are less. New strategies in malaria control can be discovered if there is recognition and validation of traditional medical practices and search for plant-derived drugs. Since many modern drugs such as quinine and artemisinin originate from plants, it is essential that other medicinal plants which have folklore reputation for anti-malarial properties are investigated in order to establish their safety and efficacy and to determine their potential as sources of new anti-malarial drugs (Gessler et al., 1994).

**Malaria in the past and present:**

Malaria is probably one of the oldest diseases known to mankind that had profound impact on our history. Malaria has always been a major plague of mankind and still is. Malaria has been around since ancient times and references to this disease occur in historical documents from China as early as about 2700 BC and from Mesopotamia about 2000 BC. Egyptian papyri from 1570 BC and Hindu texts as old as the sixth century BC talk about a disease that is certainly malaria. In ancient Greek, Hippocrates was the first to describe the
details of the disease and relate them to the time of the year and to the marshy places where people lived. In the 18th century the people related their typical fever to poisonous vapours of swamps and therefore it was widely named as malaria from the Italian “Mala aria” (Mala “bad” and aria “air”). For centuries it prevented any economic development in vast regions of the earth (http://www.malariasite.com/).

In 1880 the army physician A. Laveran (1845-1922) first described malaria parasites in human blood (Haas, 1999). Methylene blue eosin stain was accidentally discovered by D. L. Romanowsky in 1891 (Mohan, 2005). It could stain these parasites in blood smears which facilitated the search for other malaria parasites in birds, reptiles and mammals. In 1883 the American physician, Albert King, had accumulated the mass of evidence that was to become known as the mosquito – malaria doctrine. In 1884 Sir Patrick Manson suggested that transmission from person to person was by mosquitoes. He had earlier proved that the parasite of filariasis was taken up by female mosquitoes during their blood meal, and that the parasites continued their development in the abdomen of the mosquito. He considered that this could also be the case for the malaria parasite and his idea was supported by Sir Ronald Ross. Ross who was working in India found the pigmented cysts on the stomach wall of the Anopheles mosquito in 1897. A year later Ross worked out the complete life cycle of bird malaria and showed that the bite of an infected mosquito was the route of infection.

The life cycle of a parasite in humans remained incompletely understood and exoerythrocytic development of the parasite remained a mystery for a long time. In 1895, Mac Cullum observed developmental stages of *Plasmodium relictum* in the liver and spleen of infected birds. The question of the exoerythrocytic development of the human malaria parasite however remained unresolved until 1947 when H. Shortt and C. Garnham showed
that a phase of division in the liver preceded the development of parasites in the blood (Cox, 2010). Table -1 shows the time line for origin of malaria (http://www.malariasite.com/).

**Table - 1**

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<th>Time Line for Origin of Malaria</th>
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<tr>
<td>Half a billion years ago</td>
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<td>150 million to 200 million years ago</td>
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<td>130 million years ago</td>
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<td>130 million years ago</td>
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<td>100 million years ago</td>
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<tr>
<td>~5 million years ago</td>
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<tr>
<td>2-3 million years ago</td>
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<td>4000 – 10000 years ago</td>
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Today, Malaria is still a major public health problem, especially in tropical and subtropical regions. Malaria imposes great socio-economic burden on humanity and with six other diseases (diarrhea, HIV/AIDS, tuberculosis, measles, hepatitis-B and pneumonia), accounts for 85% of global infectious disease burden (Murray et al., 1996; Murray et al., 1997).
More than 120 *Plasmodium* species have been identified of which *P. falciparum*, *P. vivax*, *P. knowlesi*, *P. ovale* and *P. malariae* are able to infect human hosts. 2.2 billion people are exposed to the threat of *P. falciparum* malaria which is the most lethal form of this disease. With 70% and 25%, most clinical events attributed to *P. falciparum* are found in Africa and in South East Asia (Snow *et al.*, 2005).

There were 216 million cases of malaria in 2010; 81% of these were in the WHO African Region. An estimated 3.3 billion people were at risk of malaria in 2010 (WHO, 2011). An estimated 655000 persons died of malaria in 2010. 86% of the victims were children under 5 years of age, and 91% of malaria deaths occurred in the WHO African Region (WHO, 2011).

Malaria is also strongly associated with poverty. Taking into consideration factors such as tropical location, colonial history, and geographical isolation, it has been shown that countries with intensive malaria had income levels of only 33% of that of countries without malaria, whether or not the countries were in Africa. The impact of malaria on the economic growth of countries is large. The loss in growth of countries with endemic malaria is estimated to be as high as 1.3% per year and the annual loss in productivity in Africa due to malaria is calculated to be up to 12 billion US $ (Samba, 2001).

**Malaria in India:**

In the south-eastern Asian region of WHO, of ~1.4 billion people living in 11 countries (land area, 8,466,600 km, i.e., 6% of global area), 1.2 billion are exposed to the risk of malaria, most of whom live in India (Kondrachine, 1992). However, Southeast Asia
contributed to only 2.5 million cases to the global burden of malaria. Of this, India alone contributed 76% of the total cases. In 2009, 2.4 million parasitologically confirmed malaria cases and 3,320 deaths were reported in the southeast Asian region, and most of the cases in the region are due to *Plasmodium falciparum*. Taking into account clinical episodes, it has now been estimated with the help of epidemiologic models and geographical and demographic data that *Plasmodium falciparum* estimates outside Africa, especially in southeast Asia, are 200% higher than reported by the World Health Organisation (ie., 118.94 million , global estimates of 515 million cases) (Snow *et al.*, 2005).

As reported recently, 406 million Indians were at risk of stable *Plasmodium falciparum* transmission in 2007 with an uncertainty point estimate of 101.5 million clinical cases (95% i.e., 31.0 – 187.0 million cases) (Hay *et al.*, 2010). Experts estimated that as many as 40% of India’s malaria cases are caused by *P. falciparum* (Kumar, 1999).

Figure 1 shows the trend of malaria cases and deaths 2001-2010 (Source: National Vector Borne Disease Control Programme; http://www.nvbdcp.gov.in). Figure shows that the malaria cases have consistently declined from 2.08 to 1.49 million during 2001-2010. Similarly, *Plasmodium falciparum* (Pf) cases have declined from 1.0 to 0.77 million during the same period. Less than 2000 deaths were reported during all the years within this period with a peak in 2006, when an epidemic was reported in the NE States. The intensity of the problem of Malaria in our country and the epidemics that effuse due to its spread provides the main impetus that guides this research towards developing a safer and more effective natural drug against the malarial menace.
Malaria prevalence according to age and sex in India:

There is very limited information on age and sex specific seasonal prevalence of malaria in different paradigms in the country. In the available studies, age and sex classifications used are arbitrary (Das et al., 1997; Dev et al., 1995; Prakash et al., 1997; Dutta et al., 1999; Shukla et al., 1995; Dhiman et al., 2001; Srivastava et al., 1995).

The burden is generally higher in men than women in all age group. Children in the States of Assam (Das et al., 1997; Dev et al., 1995; Prakash et al., 1997) Arunachal Pradesh, (Dutta et al., 1999) and Rajasthan (Shukla et al., 1995) had a higher incidence of malaria than adults, whereas in the Indo-Gangetic plains, the situation was reversed (Dhiman et al., 2001; Srivastava et al., 1995).

Rapid rise of malaria cases in 2012:

A 60% rise in malaria cases was recorded in Ahmedabad in 2012 suggesting the rapid increase in the spread of this disease. Actual figures may be higher as this only pertains to
cases reported from 57 Urban Health Centres and Government General Hospitals. Till the first week of November, the city recorded 15,343 malaria cases as compared to 9,646 cases in the same period last year.

<table>
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<th>Malaria</th>
<th>Falciparum malaria</th>
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<td>2011</td>
<td>2012</td>
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<tr>
<td>9,646</td>
<td>15,343</td>
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<td>2011</td>
<td>2012</td>
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<td>3,376</td>
<td>2,242</td>
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What is evident from the data was that between July and August, which was the beginning of the monsoon, there was a drastic spike with 2,575 malaria cases. Similarly, 2,242 falciparum cases were recorded in 2012, while there were 3,376 cases in the same period in 2011. These statistics have been quoted from the Times of India (The Times of India, Ahmedabad, Friday, November 9, 2012).

Malaria evidently has a devastating socio-economic impact on affected countries. The drastic economic loss due to endemic malarial cases result in serious implications and hence medical research directed towards the targeted control of the disease is now imperative.

**The malaria parasite (*Plasmodium*) and its life cycle:**

Since this study has been aimed at targeting the plasmodium parasite directly in its cultured growth, it is imperative to elucidate here the main phases of its life cycle, in order to emphasize the possible site and mode of action of our test material.
Malaria parasites are protozoa of the genus *Plasmodium*. Genealogically they are organized in the phylum of Apicomplexa, suborder of Haemosporidae and family of Plasmodiidae. The malaria parasites are transmitted by the female *Anopheles* mosquito. They possess a very complex life cycle. The natural ecology of the malaria parasites involves the *Plasmodium* infecting successfully two different hosts, humans and the female *Anopheles* mosquito. The parasite must therefore adapt to very different environmental and host specific conditions such as cellular metabolism, temperature, etc. all *Plasmodium* species have a common ancestor which evolved as early as 130 million years ago with a two host life cycle in Dipterans and vertebrates suggesting a strong co-evolution of the parasite and its host (Carter et al., 2002).

The complex life cycle of *Plasmodium* includes three stages of two distinct proliferation forms with massive intracellular asexual multiplication, the sporogony (mosquito midgut) and schizogony (liver and blood). During a blood meal, a malaria infected female *Anopheles* mosquito inoculates the parasite in its sporozoite form into the skin of the human host. The sporozoites migrate in the skin of the victim, eventually enter the blood stream and travel to the liver. The sporozoites enter the liver sinusoids either through the hepatic arteriole or the portal venule. Kupffer cells are the resident macrophages of the liver and are strategically positioned on the sinusoidal lumen of the liver. Sporozoites actively invade these cells, safely transverse them before finally invading hepatocytes (Frevert, 2004).

During invasion the parasitophorous vacuole membrane (PVM) forms around the sporozoite. The liver stage undergoes nuclear replication and matures to hepatic schizonts followed by the release of tens of thousands of merozoites into the blood stream (Amino et
The liver stage infection in *P. falciparum* takes a period of seven days (Van Buskirk, 2009).

Intensive studies on the development of the parasite in hepatocytes the case of the rodent malaria, *Plasmodium berghei* merozoites are released directly into the blood stream. The parasites induce the death and detachment of their host hepatocytes, followed by the budding of parasite–filled vesicles (merosomes) into the sinusoid lumen of the liver (Amino et al., 2006).

In the case of *P. vivax* and *P. ovale*, some of the sporozoites entering hepatocytes (the propositions vary, depending on the strain) do not develop into exo-erythrocytic schizonts directly, but instead form hypnozoites. These small parasite forms (4-5µm in diameter) can remain dormant in the liver for years. At a given point in time, although the triggering signal is still unknown, the hypnozoites develop into exoerythrocytic schizonts, producing thousands of merozoites, thereby causing relapses of the disease. Merozoites enter into the blood stream, invade Erythrocytes and become enclosed in a parasitophorous vacuole, thus starting the asexual blood stage of the life cycle, the erythrocytic schizogony. The first stage of the intraerythrocytic development is the ring stage, which is followed by the metabolically very active trophozoite stage. *Plasmodium* development is finally completed with the fully developed schizont stage which holds 16-32 daughter merozoites. The length of the intraerythrocytic cycle differ between different *Plasmodium* species and strains, typically 48 hours (*P. falciparum, vivax* and *ovale*) or 72 hours (*P. malariae*), which explains the periodicity of the fever paroxysms experienced by the patient. Upon primary rupture of the parasitophorous vacuole followed by rupture of the erythrocyte membrane, the merozoites are released into the blood stream where they invade new erythrocytes (Wickham et al., 2003).
A few merozoites instead of developing into schizont mature to sexually differentiated gametocytes. When a female Anopheles mosquito ingests blood from an infected person, the infected as well as the uninfected erythrocytes are digested, but it is the gametocytes which undergo further development. The gametocytes differentiate into male microgametes and the female macrogametes within the midgut lumen of the mosquito. The male microgametes fertilize the female macrogamete and the zygote develops into an ookinet, which penetrates the midgut epithelium and forms an oocyst on the basal membrane. After rupture of the mature oocyst more than 1,000 sporozoites are released and some invade the mosquito’s salivary glands via the haemolymph to be injected into a new human host when the female mosquito feeds again (Han et al., 2000). Figure 2 shows the life cycle of the malaria parasite (www.dpd.cdc.gov).

![Fig. 2 showing the life cycle of P. falciparum.](image-url)
Cultivation of *P. falciparum* In vitro:

The early studies of Bass and Johns (1912) shown steps to the successful development of continuous cultures of *P. falciparum*. Malaria parasite cultures were constantly short-term, lasting only a few days with diminishing numbers, until the parasites died out. Not only was it exceptionally tiresome to continually begin new cultures, but investigators worked with parasites that were unusual in the sense that the overall population was dying. During that period, many reported advances were capable to lengthen the time of cultivation by only a few days but always with the inevitable terminal results. Most of the early investigators on *in vitro* cultivation used the bird malaria parasite *P. lophurae* or the simian parasite *P. knowlesi*, but the first successful cultivation by Trager and Jensen (1976) of malaria parasite was of *P. falciparum*, the most important of the human malaria species. Most researchers do not retain information how hard it was to work with malaria parasites, especially *in vitro*. Using fundamentally the same procedures, other species of malarial parasites now have been cultured, including *P. fragile*, *P. inui*, and *P. cynomolgi*, but these species present no real compensation over the use of *P. falciparum*. Methods for the cultivation of *P. vivax* have been reported but these cultures apparently require a incessant resource of human reticulocytes, which limits the suitability and presents a nearly impossible barrier to all but a few laboratories (Golenda et al., 1997). The evaluation on the impact of continuous cultures of *P. falciparum* underscores the remarkable contributions of this technique on malaria research (Trager and Jensen, 1997).
Serum replacements:

Freshly prepared human high-density lipoprotein fraction (concentration range of 0.25 to 0.50 mg/ml) was used to support growth of *P. falciparum*, with results comparable to those obtained using human serum (Grellier *et al.*, 1991). Other lipoprotein fractions, low- and very-low-density lipoproteins, formed little or no growth. Growth-promoting factor GF 21 (containing an ammonium sulfate fraction of adult bovine serum plus insulin, transferrin, and sodium selenite) was used with Daigo's T basal medium for serum-free growth of *P. falciparum* (Asahi and Kanazawa, 1994).

RPMI 1640 was supplemented with adenosine, unsaturated C18 fatty acids, and fatty acid-free bovine serum albumin for serum-free growth, but growth rates of parasites were lower than those in plasma-containing medium (Willet and Canfield, 1984). Pooling sera minimized variations in growth-promoting properties of serum samples obtained from different humans (Jenson, 1988) and rabbits (Sax and Rieckmann, 1980).

Commercial serum replacements:

Nutridoma-SR (4%), is used to support the growth of several strains of *P. falciparum* from different global locations, with a resulting parasitemia of about 10% within 3 to 4 days (Lingnau *et al.*, 1994). With a lower concentration of Nutridoma-SR (1%) combined with Albumax I (0.5%), a purified serum albumin preparation improved results were obtained.
Cultures were sustained for 30 to 50 days, with parasitemias of 10%, compared to parasitemias of >15% obtained with human serum. They found that cultures raised in higher concentrations of Nutridoma-SR (2 or 4%) were nonviable or gave lower levels of parasitemia (parasitemia being the level of infection of blood cells) (Flores et al., 1997). Albumax used for cultivation of P. falciparum, with parasitemias reaching as high as 85% after 7 days with continuously passaged plasmodia (Binh et al., 1997). Albumax II (0.5%) used for growth of P. falciparum, achieved parasitemia of about 6 and 12% for two different malaria strains. They found that it was necessary to add hypoxanthine to the growth medium in order to obtain these levels of parasitemia (Cranmer et al., 1997). Plasma, without prior heat treatment, has been used for large-scale growth of P. falciparum, clotting was avoided by use of plastic culture vessels or siliconized glassware (Read and Hyde, 1993).

**Membrane characteristics of malarial parasite and red blood cells:**

The plasma membrane of the cell is the formation which eventually decides the interaction between the cell and its environment. It must act as a permeability barrier permitting the entry of required molecules, but not be so indiscriminate that the contents of the cell were released at random. It must have the substantial strength to remain integral throughout the life span of the cell. Another vital property is “sidedness” i.e., the inner and outer surfaces of the membrane must function in a different way, if they do not, substances might be pumped in at one point and out at another. Also receptors for various chemical signals and markers that identify a cell to its neighbors were found on the outside—thus they would be useless inside (Wernsdorfer, and McGregor, 1988). Protein’s associated with membrane are of two classes: the integral protein and peripheral proteins. Integral proteins,
are those with portion of each molecule embedded in the lipid bilayer, so it had a part exposed on both sides of the membrane, integral protein is usually insoluble in water, had considerable regions where most of the exposed amino acid units are hydrophobic. Peripheral protein, are those proteins which were not embedded into the bilayer but are located on one or other of the surfaces, usually the cytoplasmic surface. Each of these proteins is bounded to an integral protein, and it like the protein of cell cytoplasm, usually have an surplus of hydrophilic amino acids at the surface of the molecule and an excess of hydrophobic ones inside. The proteins and glycoproteins were considered to be integrals since they were not solubilized without the aid of detergents or other methods of disruption (Wernsdorfer and McGregor, 1988).

**RBCs peripheral proteins:**

Red cell membranes have two main proteins, which are Spectrin and Actin, and they lie complexed together, on the internal surface of the membrane in loose ionic association with other cytoskeletal proteins (Wernsdorfer and McGregor, 1988).

**RBCs integral proteins:**

Numerous proteins of the red cell membrane have been well characterized lately and, of these, two are of particular interest. The first separated by sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) as the constituent with molecular
weight of 90,000-100,000 daltons and is known as the band 3 proteins. The second is glycophorin of which they are three types, A, B and C. Glycophorin A accounts for about 75% - 80% of the major glycoproteins in the human erythrocytes (Wernsdorfer and McGregor, 1988).

Interaction of merozoite and red blood cells:

When merozoite attack red blood cells, it undergoes a complex sequence of events that begins with its adherence to the host cell. Miller (1977) has described this process, which takes only about 30 seconds which consists of four phases:

1. The attachment of the merozoite.
2. The deformation of the erythrocyte membrane
3. The entry of the merozoite into the erythrocyte by way of an invagination of the erythrocyte membrane.
4. Finally the resealing of the erythrocyte membrane (Wernsdorfer and McGregor, 1988).

The process of attachment and invasion appear to be separating events as indicated by the fact that cytochalasin B treated merozoites will attach to but not invade susceptible cells (Miller et al., 1979). It was reported that merozoites can attach to red cells initially by any part of their cell surface but an invasion only occurs if the merozoite is orientated so that the apical complex of the merozoite is in contact with the red cell surface (Miller, 1977). Following attachment of the apical region of the merozoite to an appropriate red cell, considerable cohesive forces are generated and there is visible morphological deformation of the host cell. This radiate from the point of attachment. Although generally this is followed by the rapid expansion of the erythrocyte membrane to form parasitophorous vacuole
enclosing the merozoite, the merozoite can still get detached at this stage and invade another cell (Wernsdorfer and McGregor, 1988).

**Characteristics of *P. falciparum* for the cytoadherence and erythrocyte invasion:**

Cytoadherence refers to a capability of the blood stage parasite trophozoits and schizonts to adhere to the vascular endothelium in the human host and bind to uninfected erythrocytes to form rosettes. *P. falciparum* infected trophozoite and schizont stick to capillary walls of various organs leading to sequestration of the late stages from peripheral circulation. Cytoadherence allows *P. falciparum* to avoid passage through the spleen where infected erythrocytes are destroyed. Cytoadherence of *P. falciparum*-infected erythrocytes in brain capillaries have been implicated in cerebral malaria (Pillai and Usha Devi, 2001).

Malaria parasites are obligate intracellular parasites that attack erythrocytes by a multistep process that is mediated by specific molecular communication between the erythrocyte receptors and the parasite ligands. For example, the human malaria parasite *P. vivax* is absolutely dependent mainly on the Duffy blood group antigen for the invasion of human erythrocytes. Duffy –negative erythrocytes lack the Duffy blood group antigen and are completely resistant to an invasion by *P. vivax* (Okoyeh et al., 1999). *P.falciparum*, on the other hand, does not need interaction with Duffy blood group antigen for invasion and invade both Duffy-positive and Duffy-negative erythrocytes. Salic acid residues of the glycophorin A, have been identified as invasion receptors for *P. falciparum* (Pasvol et al., 1982).
Antimalarial drug resistance:

The cases of malaria identified as caused by *P. falciparum* are on the rise because this parasite has developed resistance towards most of the commonly used anti-malarial drugs (Wellems *et al.*, 2001; Warhurst, 2002; Kshirsagar, 2006; Hyde, 2007; Bethell *et al.*, 2011; Shah *et al.*, 2011).

The anti-malarial Chloroquine (CQ) is a wonder drug for its efficacy, minimal side effects and affordability. However, the spread of Chloroquine resistance has been one of the main reasons for resurgence of *falciparum* malaria, especially in the African continent. In the past, CQ had been an effective drug in India against malaria (Mishra *et al.*, 1996; Sharma, 1999; Sharma, 2007). However, the CQ resistance (CQR) in *P. falciparum* malaria is rampant in India, it was first reported from Assam in 1973 (Mishra *et al.*, 1996). Thereafter, the number of CQR falciparum malaria cases increased exponentially in Assam and elsewhere in the country (Sharma *et al.*, 1996; Sharma, 2007) Replacement drugs such as sulphadoxine – pyrimethamine (SP) could not sustain for long due to the development of rapid resistance in the parasite. Other drugs such as amodeaquine, mefloquine, atovaquone, etc. could not fit the bill due to a variety of reasons, including development of cross resistance, side effects and cost considerations. Quinine, despite its toxicity and reported resistance development, continues to be a final option. In this current scenario, with around 300 million malaria cases and a million deaths per year, the advent of the use of aretminisnin (ART) derivatives has provided a new direction for malaria containment (Govindarajan *et al.*, 2012). However, this has lead to a new problem of increased parasite resistance, which in turn triggers the need for research towards developing effective alternatives to the existing drugs.
**Artesunate (a derivative of artemisinin) resistance:**

Artesunate – based combination therapy (ACT) to treat malaria has been implemented in most of the countries (Eastman, 1998). In India, artesunate is combined with SP (ASP) and is given to malaria patients in most parts of the country where CQR levels are very high. Artesunate resistance has been reported from some South East Asian countries (Djimde et al., 2001; Mok et al., 2011; Bethell et al., 2011). However, considering the higher frequency of SPR – associated mutations among Indian isolates, it may not take a very long time for the emergence of drug – resistant parasite against ASP (Sharma, 2012). Such a situation has heralded the need for alternative antiplasmodial therapy (Bagavan et al., 2011). The key weakness of major available anti malarials are shown in the Table 2 and Table 3 (Well et al., 2010).
Table 2: Table showing Key Weaknesses of the Major Available Antimalarials (Excluding ACTs)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Key Weaknesses</th>
<th>Indications</th>
</tr>
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</table>
| Quinine               | • Does not kill the pre-erythrocytic stages of malaria parasites or the mature gametocytes of *P. falciparum*. Difficult to administer in severe malaria | • Uncomplicated *P. falciparum*, *P. vivax*, or unidentified malaria.  
  • Safe in pregnancy |
| Chloroquine           | • Widespread drug resistance has made it almost useless for the treatment of *P. falciparum* in most of the world.  
  • It does not produce radical cure of *P. vivax* and *P. ovale*  
  • Low safety margin and very dangerous if overdosed | • Chloroquine-sensitive *P. falciparum* or unidentified malaria  
  • Uncomplicated *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*  
  • Used for prophylaxis |
| Amodiaquine           | • There is some cross resistance with chloroquine  
  • GI side effects | • Uncomplicated *P. falciparum* malaria |
| Mefloquine (Lariam™)  | • Associated with adverse neuro-logical side effects such as mood disturbance and dizziness, and GI side effects  
  • High cost | • Uncomplicated *P. falciparum*, *P. vivax*, or unidentified malaria  
  • Used for prophylaxis -- pro-posed for IPTp |
| Primaquine            | • Hemolytic side effects in G6PD-deficient patients  
  • Must be taken daily for 14 days to be effective | • Uncomplicated *P. vivax* and *P. ovale*, anti-gametocyte activity in other species |
| Sulfadoxine-pyrimethamine | • Resistant strains in East Africa  
  • Rare severe adverse effects: Stephen Johnson Syndrome | • Used for IPTp and proposed for use in IPTi  
  • Can be used in pregnancy  
  • Used for prophylaxis |
| Intravenous artesunate | • No GMP formulation available. Need to compare intravenous and intramuscular delivery (and potentially intra-rectal) | • Severe malaria |

GI: Gastrointestinal; GMP: Good manufacturing practice; G6PD: Glucose-6-phosphate dehydrogenase; IPTp: Intermittent preventative treatment in pregnancy; IPTi: Intermittent preventative treatment in infants; WHO: World Health Organization.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Key Weaknesses</th>
<th>Indications</th>
</tr>
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</table>
| Artemether-lume-fantrine (Coartem®/Coart emé Dispersible) | • Administration: twice per day for 3 days  
• Low bioavailability, potential dependence on fatty foods  
• Half-life a little shorter than the other ACTs  
• Some reports of resistant strains | • Uncomplicated P. falciparum or unidentified malaria |
| Amodiaquine-artesunate (Coarsucam®) | • Resistance to amodiaquine can compromise efficacy  
• Common GI side effects  
• Contraindicated for prophylaxis because of a risk of hepatotoxicity and agranulocytosis with chronic use  
• No approval by stringent regulatory authority (but prequalified by the WHO) | • Uncomplicated P. falciparum malaria |
| Artesunate-mefloquine (ASMQ) | • Psychiatric and gastrointestinal adverse events  
• No approval by stringent regulatory authority  
• No prequalification by WHO  
• No plans for pediatric formulation  
• High cost of goods ($2.50 per treatment) due to cost of mefloquine | • Uncomplicated P. falciparum malaria and chloroquine-resistant blood stage P. vivax malaria |
| Dihydroartemisinin-piperaquine (Eurartesim™) | • On the WHO treatment guidelines, but no product approved by WHO prequalification.  
• Pediatric formulation still in development – for 2012 launch | • Uncomplicated P. falciparum malaria |
| Pyronaridine-artesunate (Pyramax®) | • Safety data limited to the current clinical trial data  
• Pediatric formulation (sachet) still in development – for launch in 2012 | • Uncomplicated P. falciparum malaria and blood stage P. vivax malaria |
| Naphthoquine-artesiminin (Arco) | • No ICH clinical study, or large phase III study  
• Little information on the safety  
• Not submitted to a stringent regulatory authority or included in the WHO treatment guidelines | • Uncomplicated P. falciparum malaria |
| Arterolane maleate-piperazine phosphate (Arterolane) | • Concerns about efficacy, based on relatively poor activity as monotherapy in Phase IIa study  
• No studies carried out in children, or juvenile toxicology data so far  
• In clinical Phase III -- Indian launch expected in early 2011  
• Not submitted to a stringent regulatory authority or included in WHO treatment guidelines | • Uncomplicated P. falciparum malaria |
The highly adaptive character of the malarial parasite accentuates the difficulty of obtaining an anti-malarial vaccine along traditional lines (Kamaraj et al., 2012). The problem of drug resistance and the difficulty of creating an efficient vaccine with less or low toxicity, are the main principles that underline the urgent need for new antimalarial drug, with least side effects. The ideal chemotherapeutic compounds should be easy to administer, store and of low cost. One possible source for such affordable treatment lies in the use of traditional herbal remedies (Kamaraj et al., 2011). Since many modern drugs such as quinine and artemisinin originated from plants, it is essential that other medicinal plants which have folklore reputation for anti-malarial properties are investigated in order to establish their safety and efficacy and to determine their potential as sources of new anti malarial drugs (Gessler et al., 1994).

Asase et al., (2005) have suggested that the continuous search for novel and more effective antimalarial compounds especially from medicinal plant extracts is of utmost importance in combating malaria infection. Similar observations have been made by other researchers as well (Ramazani et al., 2010; Ravikumar et al., 2012), since these are natural products with minimal side effects. Hence the present study was focused on the screening of various extracts of some traditionally used medicinal plants. The plants selected in this study, after pilot observations include three selected plants viz., Tylophora indica, Plumeria rubra and Xanthium strumarium (Plate I; Figs. 1, 2 and 3). The characteristics of these plants have been elucidated here to emphasize their significance in pharmacognosy and phytochemistry and the relevance of selecting these plants.
Plate I

Fig. 1: Tylophora indica

Fig. 2: Plumaria rubra

Fig. 3: Xanthium strumarium
Tylophora indica

Kingdom: Plantae
Order: Gentianales
Family: Apocynaceae
Subfamily: Asclepiadoideae
Genus: Tylophora
Species: Tylophora indica

Morphology

Tylophora is a small, slender, perennial, twinning or climbing herb. Leaves are elliptic to ovale (3.8 – 6.0 cm, 6.0-10.5 cm), petioles are up to 12 mm long. Flowers are minute (1-1.5 cm across) and corolla is greenish yellow or greenish purple in color. Fruit is a follicle (Shah et al., 1976; Kiritikar et al., 2001).

Habit and Habitat:

Tylophora indica is found in planes, hilly slopes and forests. It forms dense patches in the forests in moist, humid conditions in open hill slopes and narrow valley. The plant grows in the area with lesser rainfall (Sabitha et al., 2012). Tylophora grows in wide range of well drained soil and prefers scanty localities (Nadkarni et al., 1976).
Distribution:

It is a twinning perennial plant distributed throughout Southern and Eastern parts of India in plains, forests and hilly places. It is indigenous to India and inhabits up to an elevation of 1260 m in the sub-Himalayan tract. It also grows in plains and hilly places of India up to an altitude of 1000 m in Bengal, Assam, Odisha and Southern India (Gupta et al., 2010). About 60 species found in tropical, sub-tropical Asia, Africa and Australia and about 35 species are reported from China (Sabitha et al., 2012). Some species found in India are *Tylophora indica*, *Tylophora rotundifolia*, *Tylophora fasciculate*, *Tylophora apiculata*, *Tylophora anomala*, *Tylophora sylvatica*, *Tylophora hetero-phyla*, etc.

Medicinal Properties:

The medicinal properties of *Tylophora* sps. are many. Its effects against jaundice (Kritikar et al., 1991), inflammation (Chopra et al., 1986), tumor (Huang et al., 2004), asthmatic (Huntley et al., 2000), histaminic (Gopalkrishnan et al., 1980), hypotensive, analgesic, convulsant and rheumatic activities (Gopalkrishnan et al., 1979) have been reported. The leaves are used as diaphoretic, emetic and expectorant (Parrotta et al., 2001). Also the anticancer activity of tylophorin has been reviewed (Gopalkrishnan et al., 1980). It has also shown immunomodulatory, antioxidant (Jagetia et al., 2004) and smooth muscle relaxant (Gopalkrishnan et al., 1979) activities. Thus taking into consideration its immense medicinal potential and the fact that it traditionally used in certain rural areas of our state, this species of the genus *Tylophora* has been selected for the present investigation.
**Plumeria rubra (L)**

Kingdom : Plantae

Subkingdom : Tracheobionta

Superdivision : Spermatophyta

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Asteridae

Order : Gentianales

Family : Apocynaceae

Genus : Plumeria

Species : *Plumeria rubra*

**Morphology**

It grows up to a height of 25 feet with 35 spread (Gopi *et al.*, 2011). Plant growth rate is slow. About eight species of *Plumeria rubra* occur in India. The ascending branches have ascending leaves which are simple alternate, spiral, petiole undissected, elliptic or ovate shape, base tapering (narrow alternuate) or oblique, margins entire or undulate, apex acuminate or acute or obtuse. Pink or red color
flowers, spreading cymes fruits elongated (Bobbarala et al., 2000). Flowering period is August to October.

**Habit and Habitat:**

*Plumeria* is a deciduous tree with thick, widely distributed in common rather moist garden, in lawns and in open plantation is unusual in appearance. Plant loses leaves for a short time during the winter. The plants are widely cultivated in the tropical and subtropical regions throughout the world (Sulaiman et al., 2008).

**Distribution:**

*Plumeria* is indigenous to tropical America and is found from Southern Mexico to Northern South America and also most abundant in India (Burkill, 1935). However, due to its ease of propagation through cuttings, many species and hybrids of *Plumeria* are now widely cultivated and distributed in the warmer regions of the world (Omata et al., 1991).

**Medicinal Properties:**

The decoction of the bark and roots of *P. rubra* is traditionally used to treat asthma, ease constipation, promote menstruation and reduce fever. The latex is used to soothe irritation (Wiart, 2002). The flowers are aromatic and widely used in
pectoral syrups. The flower decoctions of *P. rubra* was reported to use in Mexico for control of diabetes mellitus. The leaves of *P. rubra* are used in ulcers, leprosy, inflammations and rubefacient (Bobbarala *et al.*, 2000). The fruit is reported to be eaten in West Indies. In India, however, it has been used as an abortifacient (Zaheer *et al.*, 2010). Flavone glycoside isolated from *Plumeria rubra* shows antioxidant and hypolipidemic activity (Devprakash *et al.*, 2012).

Flavone glycosides on treatment to the hyperglycemic animals had shown no significant alteration in the blood glucose and serum total cholesterol, while a significant reduction in the level of serum triglycerides was observed when compared with the alloxan injected hyperglycemic control animals (Hazeena Begum *et al.*, 2010). Methanolic extract of *P. rubra* (leaf and flower) were able to show antimicrobial action against different bacteria. Result shows methanolic extract of leaf and flower of *P. rubra* inhibits the growth of the 14 indicator bacteria with the zone inhibition between 12-28 mm. The extract *P. rubra* flowers found more active than the leaf parts against Bacillus Cercus with zone of inhibition of 28 (Egwaikhide *et al.*, 2009). *Plumaria rubra* containing fulvoplumierin act as inhibitors of human immunodeficiency virus type 1 (HIV) reverse transcriptase (Tan, 1991).

**Chemical composition:**

The flower of *Plumaria rubra* consist 1-diethoxyethane, benzaldehyde, geraniol, citral, methylbenzoate, nerolidol, naphathalene, linalool, benzylbenzoate and methylsalicilate.
The bark of plumeria rubra consists scopoletin, β-Sitosterol, Plumieride, fulvoplumerin. The root contains plumericine, β-dihydropumericin isoplumericin, B-dihydropumericin acid, Fulvoplumerin and plumieride. Rubrinol: an antibacterial triterpenoid, together with teraxasteryl acetate, lupeol, stignasterol, and oleanolic acid.

**Xanthium strumarium L.**

Scientific classification:
- **Kingdom**: Plantae
- **Subkingdom**: Trachiobionta
- **Division**: Magnoliophyta
- **Class**: Magnoliopsida
- **Subclass**: Asteridae
- **Order**: Asterales
- **Family**: Asteraceae
- **Genus**: Xanthium
- **Species**: *Xanthium strumarium*

**Morphology:**

Leaves of *X. strumarium* are broadly alternate, are triangular-ovate or suborbicular, light and bright green in colour in an alternate pattern with irregular lobes and relatively inconspicuous teeth, 5-15 cm long, often three-lobed, with prominent veins, long petiole, scabrous on both sides (Kamboj, *et al.*, 2010) stems are round or slightly ribbed, often speckled with purple and have short white hairs scattered across the surface; flower heads are in terminal
and axillary racemoses, and are white or green; numerous male uppermost, female ovoid, covered with hooked bristles. Fruits are obovoid, enclosed in the hardened involucre, with two hooked beaks and hooked bristles. Flowering time in India is August-September. This weed is easily dispersed through animals as the fruits have hooked bristles and two strong hooked beaks. It flowers from July to October, and the seeds ripen from August to October. The flowers are monoecious and are pollinated by insects. The plant is self-fertile. The fruits are harvested when ripe and dried for use (Agharkar, 1991).

**Habits and Habitat:**

*X. strumarium* is a cocklebur or burweed commonly found as a weed in roadsides, rice fields, hedges throughout the tropical parts of India (Oudhia, 2001). *X. strumarium* is an annual herb (Kamboj et al., 2010).

**Distribution:**

The genus *Xanthium* is distributed in nearly all parts of the world (Lauras *et al.*, 2005). It is an annual herb found in tropical and temperate regions of Eurasia, North America and South America (Pullaiah, 2006). In Pakistan the noxious weed is found abundantly in plain to the height of 8000 feet throughout the country and in Kashmir (Naser, 1972). It is also found in fallow paddy fields and hotter parts of India, Sri Lanka and the Western Himalayas up to the height of 5000 feet (Nadkarni, 1976). The genus *Xanthium* is represented by its twenty-five species in the world (Han *et al.*, 2007). Out of this *X. strumarium, X. spinosum, X. macrocarpum* and *X. sibircum* are found in Pakistan and India (Sastri, 1976).
**Medicinal Properties:**

The species of *Xanthium* have been used as traditional herbal medicines for the treatments of nasal sinusitis, headache, urticaria, arthritis (Ma *et al.*, 1998; Han *et al.*, 2006), fever, scrofula, herpes and cancer (Sharma *et al.*, 2003; Mahmoud *et al.*, 2005). The whole plant *X. stumarium* is of great medicinal importance. Carboxyatrachloride possessed hypoglycemic activity (Horace *et al.*, 1983; Pullaiah, 2006) however, a sesquiterpene Xanthanol is an antimicrobial agent (Jawab *et al.*, 1988). Aerial parts also contain mixture of alkaloids, sesquiterpenoids and sesquiterpene lactones (Xanthinin, Xanthatin, Xanthumin). Xanthumin is CNS depressant and also shows antibacterial activity. The seeds are potential source of fatty oil comprises of saturated and unsaturated fatty acids (Sastri *et al.*, 1976). The seeds contain (-) Xanthienopyran which is a new inhibitor of superoxide anion generation by human neutrophils (Lee *et al.*, 2008). The fruits are rich in vitamin C and are effective in treating small pox. The fruits contain an anti-inflammatory agent, β.sitosterol. β. D glucoside (Sastri *et al.*, 1976). Caffeic acids found in fruits possess antihyper glycemic effect (Hsu *et al.*, 2000). The n-butanol fraction of fruits showed analgesic and anti-inflammatory activity (Han *et al.*, 2007). The fruits are used as tonic, diuretic, sedative and diaphoretic. The leaves are astringent, antisyphilitic and diuretic. The leaves contain an essential oil that shows potent antifungal activity (Sastri *et al.*, 1976). The sesquiterpene lactones found in leaves exhibit cytotoxic activity against human tumor cell lines. (Ahn *et al.*, 1995) (Kim *et al.*, 2003). The roots are better tonic useful in cancer, scrofula and herpes. (Sharma 2003). Besides, these, the extracts of this plant also exhibit antinociceptive (Lee *et al.*, 2008) antidiabetic, antitrypanosemal (Maitra *et al.*, 2006) antimitotic (Mahmoud *et al.*, 2005) anticancer (Mahmoud, 1998) and antitumor properties (Saxena *et al.*, 1995).
Chemical constituents:

The aerial parts of the plant contain sesquiterpene lactones, viz., xanthinin; its steroisomer, xanthumin, xanthatin (deacetylxanthinin) a toxic principle, a suplhated glycoside: xanthonumarin, atractyloside, carboxytractyloside; phytosterols, xanthanol, isoxanthanol, xanthinosin, 4-oxo-bedforndia acid, hydroquinone; xanthanolides (Minato, 1965; Willaman, 1970) caffeoylquinic acids; α and γ-tocopherol (Molina et al., 1991); thiazinedione (Qin et al., 2006) 4-oxo-1(5),2,11,(13)-xanthatriene-12,8-olide, known as “deacetyl xanthumin” an antifungal compound; linoleic acid. The main toxic compound isolated from the plant has been identified as carboxytractyloside, a kaurene glycoside previously called ‘xanthonumarium’ (Macleod et al., 1990). The fruits are rich in vitamin C. Thiazinediones isolated from the fruits (Han et al., 2006).

Although these selected medicinal plants are extensively used for varied medicinal purposes, its role in preventing or ameliorating malarial symptoms has not been scientifically investigated (Kamboj et al., 2010). Hence the present study has been focused towards obtaining the crude extract after serial extraction and validating its efficacy in in-vitro cultured Plasmodium. This could culminate in establishing its role and efficacy as a potent anti-plasmodial agent.

MOLECULAR DOCKING AND BIOAVAILABILITY STUDIES OF SELECTED PHYTOCHEMICALS AGAINST PLASMODIUM FALCIPARUM ERYTHROCYTE MEMBRANE PROTEIN 1 (PFEMP1)

The escalating resistance of malaria parasites, in particular Plasmodium falciparum, to antimalarial drugs is a key aspect in the persistence of this disease as a major worldwide
public health threat. Various potential biochemical targets have been proposed and are being pursued for the *de novo* design of novel anti-malarials (Olliaro *et al*., 1999).

*Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) is a clonally variant adhesion protein that mediates binding of infected erythrocytes (IE) to blood microvasculature and other host cells (Kraemer *et al*., 2006). Adherence of IEs to microvascular endothelium is a major virulence factor and, in conjunction with the related phenomenon of rosetting with uninfected erythrocytes, prevents parasitized erythrocyte circulation to the spleen, an organ where parasites may be destroyed (Miller *et al*., 2002).

In the present study, docking simulation was performed using Arguslab software (ArgusLab 4.0.1 2004) with PfEMP1 as the target and computationally selected phytochemical compounds were docked into the receptor’s binding site. Subsequently, the compounds were screened with ADMET (absorption, distribution, metabolism, excretion and toxicity) filtering protocol to assess their drug likeness.

**Objectives of the Study:**

The primary objective of this study was to investigate the antimalarial properties of the selected medicinal plants.

The specific objectives of the study were to –
Determine anti-malarial activities of the crude extracts of selected medicinal plants, i.e., *Tylophora indica*, *Plumaria rubra* and *Xanthium strumarium*. The selection of the plants was made on the basis of its easy availability, therapeutic value and degree of research work done so far.

Quantitative assessment of antimalarial drug susceptibility profile in fresh and culture adapted isolates of *Plasmodium falciparum*.

To carry out phytochemical analysis to identify and determine the active principles from the samples which exhibit potent antiplasmodial activity.

To determine the cytotoxicity of selected extracts and their effects on the red blood cells (RBC). Morphological and biochemical changes were also compared with the context.

To establish a scientific basis for the use of these plants.

To check the *in silico* analysis of the known phytocomponents of the selected plants against the *Plasmodium falciparum* Erythrocyte Membrane Protein 1 (PfEMP1). This novel approach was adopted to further validate the screening.

The present thesis comprises of:

**Chapter- I  Introduction:** wherein the topic of research has been introduced in the light of current reports, data and literature in the field.

**Chapter-II  Materials and Methods** which deals with the methodology employed.
Chapter-III  Results and Discussion. The results obtained have been explained in the context of the study.

Chapter-IV  Summary and Conclusion.

At the end, a bibliography is given in alphabetical and chronological order.

This investigation holds specific significance with regard to the rampant spread of the disease in our State, the growing resistance of the *Plasmodium* species and the negligible side effects endured using natural compounds from plants.