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A disease is an abnormal situation affecting the body of an organism. It is often construed to be a medical provision associated with specific symptoms and signs (Dorland’s Medical Dictionary). It may be caused by external factors, for example, infectious disease, or it may be caused by internal dysfunctions, for example, autoimmune disease. In humans, "disease" is often used more broadly to refer to any condition that causes pain, dysfunction, distress, social problems, or death to the person afflicted, or similar problems for those in contact with the person. In this broader sense, it sometimes includes disabilities, injuries, disorders, syndromes, infections abnormal behavior, isolated symptoms, and typical variations of structure and function, while in other contexts and for other purposes these may be considered distinguishable categories. Diseases usually affect people not only physically, but also emotionally, as contracting and living with many diseases can alter one's perspective on life, and their personality.

Malaria:

The word Malaria arises from the Latin word “malus aria,” which means bad or evil air because it was originally thought that this disease was caused by foul air and particularly by vapors given off by swamps. It was also called swamp fever, and it is one of the ancient infection to man.

Discovery of Malarial Parasite:

The evolutionary history of Mammalian Plasmodia started with adaptation of Coccidia of the intestinal epithelium to some tissues of the internal organs and then to an invasion of free cells in the blood. The next step was possibilities of transmission of the parasites from one animal to another by bloodsucking arthropod vectors. The great
antiquity of the malaria infection is confirmed by the fact that well over 100 parasite species similar to those of man are found in a wide range of vertebrates from reptiles or birds to higher apes. None of the parasites, except for those found in some monkey, can be transmitted to man (Chwatt, 1985).

Prehistoric man was subjected to malaria. It was probable that the disease originated in Africa, which was believed to be the cradle of the human race. Fossil mosquitoes were found in geological strata 30 million years old and there was no doubt that they have spread the infection through the warmer regions of the globe long before the dawn of history. Malaria established itself in the new world was subjected to speculation, as no reliable historical or other data exist at these points.

The breakthrough in history of malaria was connected with first therapeutic advance, that at the beginning of the seventeenth century came in discovery of the value of “Peruvian bark” for treatment of fevers. The use of these remedy spread rapidly all over Europe, and it soon became obvious that only certain fevers were easily cured by this drug (Chwatt, 1985).

The most important event in the history of malaria took place towards the end of the nineteenth century, when the science of bacteriology and pathology discovered the cause of infectious diseases, observing the morbid changes in the organ and tissues and also perceived the role of insects in the transmission of some infections (Chwatt, 1985).

It was in 1880 that Laveran, first saw and described plasmodia in the red blood cells (RBC) of man. Soon after that Romanowsky in Russia developed a new method of staining the malaria parasites in blood film and this, together with the improvement of the microscope, made further studies of plasmodia very much easier (Chwatt, 1985).
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In 1897 Ronald Ross working in Secunderabad (India) found a developing form of malaria parasite in the body of mosquito that had previously fed on a patient with the plasmodia in his blood (Chwatt, 1985). The concept of malaria eradication was adopted by the World Health Assembly in 1955 and two years later the World Health Organization (WHO) launched the global campaign. Its results were excellent in Europe, North America, some parts of Asia, Union of Soviet Socialist Republics (USSR), Australia and less good in tropical countries (Chwatt, 1985).

Malaria is one of the major parasitic, and the most prevalent disease continues to be one of the greatest health problems facing the tropical and subtropical countries of the world. Malaria is amongst the most deadly communicable diseases in the world. Mortality and morbidity caused by malaria are a matter of great concern throughout the world. Even though casualty in children below the age of five years is very high, the disease affects all age groups. WHO estimated that out of 225 million clinical cases, the number of death case was estimated to 7,81,000 (WHO, 2010). WHO experts say that the number of the people worldwide infected with malaria is still increasing with the rate of about 5% annually, despite the extensive programs by the WHO (Dutta, 1995).

There are mainly four species of malarial parasites that infect human beings viz.,

1. Plasmodium falciparum
2. Plasmodium ovale
3. Plasmodium vivax
4. Plasmodium malariae

A fifth species, Plasmodium knowlesi, is typically found in nature, was found first in macaques (monkeys) and has been identified as a clinically significant pathogen in
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humans also (Figtree, et al., 2010). *P. falciparum* infects the Red Blood cells that adheres to and accumulates in the placenta in pregnant women. Pregnancy exacerbates malaria through a nonspecific hormone-dependent depression of the immune system. The protective antiplasmodial activity is suppressed at pregnancy, which has clinical consequences with important public health implications on pregnant women (Okwa, 2003). Malaria accounts for 6.5% of abortions, 15% of premature deliveries and 0.7% death in utero (WHO, 2010).

The major problem now a days in tropical and subtropical regions are resistance of *Plasmodium falciparum* to antimalarial drugs, especially to chloroquine (CQ). Since effective vaccines are not available, chemotherapy represents one of the few methods for the 1.6 billion humans at risk of infection (Peters, 1985).

In India chloroquine resistant malaria *P. falciparum* was first reported in Assam in 1973 and spreading to other parts of India either due to the drug pressure or due to migratory population. Moreover, the spread of *P. falciparum* malaria in many parts of the country has lead to complicated cerebral malaria in recent years. It has been reported that *P. falciparum* is becoming resistant to different anti-malarial drugs viz., quinine, sulfadoxine/pyrimethamine, mefloquine, etc. in different parts of India (Dhiman et al., 2001).

*P. falciparum* normally takes 7-14 days to show symptoms while *P. vivax* & *P. ovale* normally take 8-14 days (but in some cases parasite can survive for some months in the human host) and *P. malariae* 7-30 days. The two features that actually separate *P. falciparum* from the other human malaria are the ability to attack erythrocytes of all ages, causing high parasitaemia and enhanced growth and the capability to adhere to vascular
endothelium through sequestration (Okwa, 2003). Symptoms of malaria infection are not always dramatic, and can easily be dismissed as unimportant, symptoms may appear and disappears in phases and may come out and go at various time frames.

The clinical symptoms of malaria are primarily due to schizont rupture and destruction of erythrocytes. Malaria can have a gradual or a fulminant course with nonspecific symptoms. The presentation of malaria often resembles those of common viral infections; this may lead to a delay in diagnosis (Murphy and Oldfield, 1996). The majority of patients experience fever (>92% of cases), chills (79%), headaches (70%), and diaphoresis (64%) (Genton and D’Acremont, 2001). Other common symptoms include dizziness, malaise, myalgia, abdominal pain, nausea, vomiting, mild diarrhea, and dry cough. Physical signs include fever, tachycardia, jaundice, pallor, orthostatic hypotension, hepatomegaly, and splenomegaly. Clinical examination in nonimmune persons may be completely unremarkable, even without fever (Trampuz, et al., 2003).

Human Plasmodium sps. has two hosts, the asexual phase of malarial parasite occurs in man, which is considered the intermediate (secondary) host. The sexual phase of malaria parasite is takes place in the definitive (primitive) host i.e. female Anopheles mosquitoes.

Female Anopheles mosquitoes transmit malaria parasites. Only female Anopheles feed on the blood of humans and transmits disease. Not all anopheline are vectors of malaria. Out of the 58 anopheline species reported so far in India, nine had been recognizing as species complexes of which these are considered to play an important role in malaria transmission Major malaria vectors in India are: Anopheles stephensi, Anopheles dirus, Anopheles minimus, Anopheles sundaicus, Anopheles fluviatilis,
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**Biology of *Plasmodium* Parasites:**

The *Plasmodium* genus of protozoan parasites has a life cycle, which is split between a vertebrate host and an insect vector. With the exception of *P. malariae* (which may affect the higher primates) these species are exclusively parasites of man. The vector is always a mosquito, and is always an Anopheline mosquito. There are approximately 3,500 species of mosquitoes grouped into 41 genera. Human malaria is transmitted only by females of the genus *Anopheles*. Of the approximately 430 *Anopheles* species, only 30-40 transmit malaria (i.e., are "vectors") in nature. The great majority of cases of malaria occur in sub-Saharan Africa and are transmitted primarily by *A. gambiae s.s.*, *A. funestus*, and *A. arabiensis*. Outside of Africa a variety of *Anophelines* serve as malaria vectors including, but not limited to, *A. albimanus, A. darlingi, A. maculatus, A. stephensi, A. barbirostis, A. punctulatus,* and *A. sinensis* (Kiszewski et al., 2004). The basic life cycle of the parasite is shown below:

**Life cycle of *Plasmodium***:

The life cycle of all *Plasmodium* species is very complicated. Infection in human begins with the bite of an infected feminine Anopheline mosquito. Sporozoites released from the salivary glands of the mosquito enter the bloodstream throughout feeding; quickly invade liver cells (hepatocytes). Sporozoites are cleared from the circulation within 30 minutes. In case of *P. falciparum* the liver-stage parasites differentiate and endure asexual multiplication leading to ten to thousands of merozoites, that burst, from the hepatocytes within next successive 14 days. Individual merozoites invade red blood
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cells (erythrocytes) and bear an extra-ordinary spherical shape after, multiplication 
manufacturing 12-16 merozoites inside a schizont. The length of this erythrocytic stage 
of the parasite life cycle depends on the parasite species: 48 hours for \textit{P. falciparum}, \textit{P. vivax}, and \textit{P. ovale} whereas, 72 hours for \textit{P. malariae}. The clinical manifestations of 
malaria, fever and chills, are related to the synchronous rupture of the infected 
erythrocyte. The released merozoites persist maintain to invade alternative erythrocytes. 
Not all of the merozoites turn into schizonts, some differentiate into sexual forms, male 
and female gametocytes. A female Anopheline mosquito takes up these gametocytes 
throughout a blood meal among the mosquito midgut, the male gametocyte undergoes a 
speedy nuclear division, manufacturing 8 flagellated microgametes, that fertilize the 
female macrogamete. The ensuing ookinete traverses the mosquito gut wall and encysts 
on the exterior of the gut wall as a oocyst. Soon the oocyst ruptures, releasing many 
sporozoites into the mosquito body cavity where they eventually migrate to the salivary 
gland of the mosquito (Fig. 1).

Asexual phase in man:
The sporozoites from the mosquito salivary gland are injected into the human 
because the mosquito injects anticoagulant saliva to confirm an excellent flowing meal 
(blood). These sporozoites flow into the blood for a limited period of time and then settle 
within the liver where they enter the parenchymal cells and multiply; this stage is called 
as pre-erythrocytic schizogony. Within the parenchymal cells, the sporozoites develop to 
crypto-schizont and in every schizonts, thousands of merozoites are created and when it 
ruptures the merozoites are released. The free merozoites released from the liver cells 
enters RBCs in which they again pass through a series of stages, like ring, trophozoite,
schizonts etc. called erythrocytic schizogony and then again, the schizont ruptures and produce merozoites, which either infect new erythrocytes or form micro/ macro gametocytes, which have no further activity within the human host. Two kinds of malaria, *P. vivax* and *P. ovale*, can relapse, and some parasites can remain dormant in the liver from several months to four years after a person is infected. Once the parasites leave the liver and re-enter the bloodstream, they invade and multiply in the red blood cells, periodically bursting the cells. Their further development leads to the formation of gametocytes (the parasite’s sexual stages), which are picked up and transmitted to others when another mosquito feeds on blood from the infected person. Whereas in *P. falciparum* malaria during RBC invasion, the merozoites induces a pit to form within the RBC surface, and this closes over the parasite to form a microscopic bubble the parasitophorous vacuole (PV), within the RBC, where the parasite stays through successive stages- ring, trophozoite and schizont until its merozoite offspring mature and leave. The membrane lining the parasitophorous vacuole membrane (PVM) receives new parasite protein and lipids, which enlarges because of the parasite which grows inside. By the time the schizont buds of its cluster merozoites, most of the RBC haemoglobin has been consumed by the parasite. The merozoite cluster is currently enclosed during a bag composed of two membranes, an inner PVM and an outer RBC membrane normally, these then rupture unleash the merozoite, for an additional round of RBC invasion (Bannister, 2001).

Major structural alterations of the host erythrocyte are electron-dense protrusions, or 'knobs', on the erythrocyte membrane of *P. falciparum* infected cells. The knobs are induced by the parasite where several parasite proteins are associated with the knobs.
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(Deitsch and Wellems, 1996). Two proteins which might participate in knob formation or affect the host erythrocyte submembranous cytoskeleton and indirectly induce knob formation are the knob-associated histidine rich protein (KAHRP) and erythrocyte membrane protein-2 (PfEMP2), also called Mature parasite-infected erythrocyte surface antigen (MESA). Neither KAHRP nor PfEMP2 are exposed on the outer surface of the erythrocyte, but are localized to the cytoplasmic face of the host membrane. Their exact roles in knob formation are not known, but may involve reorganizing the sub membrane cytoskeleton.

The knobs are believed to play a role in the sequestration of infected erythrocytes since they are points of contact between the infected erythrocyte and vascular endothelial cells and parasite species which express knobs exhibit the highest levels of sequestration. In addition, disruption of the knob-associated histidine rich protein (KAHRP) results in loss of knobs and the ability to cytoadhere under flow conditions (Crabb et al., 1997). A polymorphic protein, called PfEMP1, has also been localized to the knobs and is exposed on the host erythrocyte surface. The translocation of PfEMP1 to the erythrocyte surface depends in part on another erythrocyte membrane associated protein called PfEMP3 (Waterkeyn et al., 2000). PfEMP1 probably functions as a ligand which binds to receptors on host endothelial cells. Other proposed cytoadherence ligands include a modified band-3, called pfalhesin (Sherman et al., 1995), sequestrin, rifins and clag (Craig and Scherf, 2001).
Figure 1: Showing the life cycle of the Plasmodium.

(Courtesy - http://armymedical.tpub.com/md0913/md09130208.htm)
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PfEMP1 is a member of the var gene family (Smith et al., 2001). The 40-50 var genes exhibit a high degree of variability, but have a similar overall structure. PfEMP1 has a large extracellular N-terminal domain, a transmembrane region and a C-terminal intracellular domain. The C-terminal region is conserved between members of the var family and is believed to anchor PfEMP1 to the erythrocyte submembranous cytoskeleton. In particular, this acidic C-terminal domain may interact with the basic KAHRP of the knob (Waller et al., 1999) as well as spectrin and actin (Oh et al., 2000).

The extracellular domain is characterized by 1-5 copies of Duffy-binding like (DBL) domains. These DBL domains are similar to the receptor-binding region of the ligands involved in merozoite invasion. The DBL domains exhibit a conserved spacing of cysteine and hydrophobic residues, but otherwise show little homology. Phylogenetic analysis indicates that there are five distinct classes of DBL domains (Smith et al., 2001).

Sexual phase in mosquito:

A female Anopheline mosquito bites an infected man harboring male and female gametocytes, along with the blood the parasites also enters to the gut where all other stages are digested except the gametocytes, and exflagellation occurs to form 5-6 microgametes. Macrogametes fuses with the microgametes, and the fertilization occur. After fertilization the zygote, a motionless granular cell is formed and after 18 to 24 hrs it becomes elongated and motile. The resulting ookinite penetrate the wall of the midgut, where develops into an oocyst. Inside the oocyst, thousands of sporozoites are produced and when this oocyst ruptures, the sporozoites migrate to the salivary gland and when this mosquito happens to bite another human being, the transmission occurs (Fig. 1).
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Distribution and impact:

Malaria varies greatly around the world in the level of intensity, in the vectors that transmit it and in the species causing the disease. In order for the global strategy to be relevant for countries around the world, it must be applicable to different settings. Malaria is currently endemic in 109 countries and in territories of tropical and subtropical zones, spanning all continents of the world except Antarctica and Australia (Fig. 2). There was an estimated 216 million malaria cases in 2010 (WHO, 2010). The lack of acquired immunity makes infants and young children highly vulnerable to malaria. In areas of intense malaria transmission, most cases of severe malarial anemia and deaths occur in infants and young children. Pregnant women are also at high risk of malaria. Each year approximately 50 million women living in malaria endemic countries throughout the world become pregnant (Snow et al., 1999). In stable transmission areas, the major effect is malaria-related anemia in the mother and presence of parasites in the placenta resulting in low-birth weight which contributes substantially to child deaths. In unstable transmission settings, pregnant women have little or no immunity to malaria and their risk of developing severe disease as a result of malaria infection is two to three times higher than that of non-pregnant women living in the same area (Luxemburger et al., 1997). Consequently, malaria during pregnancy contributes to maternal deaths in both stable and unstable transmission areas. Therefore, pregnant women require special attention and targeted policies (http://rbm.who.int/gmap/1-3.html).
Figure 2: Showing the world distribution of malaria since mid 19th century.
(Courtesy: http://www.rbm.who.int/endemiccountries.html)

National scenario:

Malaria has been a problem in India for centuries. Details of this disease can be found even in the ancient Indian medical literature. In the 1930s there was no aspect of life in the country that was not affected by malaria. To combat this menace, the government of India launched the national malaria control program in April 1953, which was proved highly successful and within 5 years the incidence dropped to 2 million. Encouraged by this, the program was changed to a more ambitious national malaria eradication program, but since then the program had suffered from the repeated setback due to technical, operational and administrative reasons and thus cases are rising again. Malaria has now staged a dramatic comeback in India after its eradication in India and after its suppression in the early and mid sixties. Early setbacks in malaria eradication
coincided with dichlorodiphenyltrichloroethane (DDT) shortages. In the late 1960s malaria cases in urban areas started to multiply, upsurge of malaria was widespread. The biggest burden of malaria in India is borne by the most backward, poor and remote parts of the country whereas, from last few years Gujarat has shown a tremendous increase in positive malarial cases with much high death rates, concerning government to introduce various protective steps in various endemic regions of state to protect the people from infectious disease like malaria. The data till April 2012 of National Vector Borne Disease Control Programme (NVBDCP) shows the risk in Gujarat is significantly increasing and the condition is going to become worst, if proper steps on time to control the malaria would not be taken soon (Table 1 and 2).

The implementation of an urban malaria scheme in 1971-72 and modified plan of operation in 1977 improved the malaria situation. The impact was only on \textit{P. vivax} malaria. \textit{P. falciparum} showed a steady upward trend during 1970s and thereafter. Malaria at one time a rural disease, diversified under the pressure of developments in various ecotype. These ecotypes have identified as forest malaria, rural malaria, urban malaria, border malaria, industrial malaria, and migration malaria. Further, malaria in 1990s has returned with new features not witnessed during the pre-eradication days. These are the resistant to insecticides; pronounced exophilic vector behavior; extensive vector breeding grounds created by water resource development projects; urbanization and industrialization, resistance in \textit{P. falciparum} to chloroquine and other anti-malarial drugs; toxic effects of chemical used for vectors. Malaria control has become an enterprise, and its management requires decentralization and approaches based on local transmission multi-sectoral action and community participation (Sharma, 1996).
Malaria control operations:

The discovery of the insecticide DDT in 1942, by Paul Muller, the Nobel Prize Laureate in physiology and medicine, and its use in Italy in 1944, made the idea of global eradication of malaria seems possible. Subsequently, widespread systematic control measures such as spraying with DDT, coating marshes with paraffin (to kill Anopheles mosquito larvae), draining stagnant water and the widespread use of nets and cheap, effective drug such as chloroquine were implemented with impressive results. Despite the initial success, there was a complete failure to eradicate malaria in many countries due to a number of factors. Although the technical difficulties such as mosquito and parasite resistance to insecticides and antimalarials respectively have played a part, the main failure to reduce the disease is probably due to social, technical, economical and political factors preventing efficient application of control measures.

Drug resistance:

Resistance to antimalarial medicine is proving to be a difficult drawback in malaria management in most elements of the globe. Since early 1960s the sensitivity of the parasites to chloroquine, the most effective and most generally used drug for treating malaria, has been on the decline. Newer antimalarials were discovered in an endeavor to tackle this drawback, however these drugs are either expensive or have undesirable facet effects. Moreover, after a variable length of time, the parasites, particularly the falciparum sps., have started showing resistance to newer medicines also. The resistance to antimalarial drug, especially chloroquine, for P. falciparum is one of the principle factors contributing to the world wide increase in the morbidity and mortality due to malaria. Different approaches have been developed to monitor the extend of antimalarial
drug resistance and to determine the biological mechanisms by which the parasite has evaded the action of drug (Menard et al., 2006).

Artesunate is part of the artemisinin group of drugs that treat malaria. It is a semi-synthetic derivative of artemisinin that is water-soluble. Artemisinin and its derivatives have become essential components of antimalarial treatment. These plant-derived peroxides are unique among antimalarial drugs in killing the young intraerythrocytic malaria parasites, thereby preventing their development to more pathological mature stages. This results in rapid clinical and parasitological responses to treatment and life-saving benefit in severe malaria. Artemisinin combination treatments (ACTs) are now first-line drugs for uncomplicated *falciparum* malaria, but access to ACTs is still limited in most malaria-endemic countries. The first well-documented evidence of artemisinin resistance in patients was only recently reported from studies conducted along the Cambodia-Thailand border (WHO, 2011).

Drug resistance is probably the greatest challenge to the most malaria-control programs. Molecular marker for parasite resistance such as *Plasmodium falciparum* chloroquine resistance transporter (*pfcrt*), *Plasmodium falciparum* Multidrug-Resistant -1 (*pfmdr-1*) and dihydrofolate reductase (*dhfr*) have the potential to be used in an integrated fashion to provide timely information that is useful to policy makers (Menard et al., 2006).

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

The plasma membrane of the cell was the structure which ultimately determines the interaction between the cell and its environment. It must act as a permeability barrier allowing the inward passage of required molecules, but not be so unselective that the
contents of the cell were released indiscriminately. It must have the considerable strength to remain intact throughout the life span of the cell. Another essential property is "sidedness" i.e., the inner and outer surfaces of the membrane must function differently, 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 fall into 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 region exposed on both sides of the membrane, integral protein is generally insoluble in water, had substantial regions where most of the exposed amino acid units are hydrophobic.

Peripheral protein, are those proteins which were not inserted into the bilayer but reside 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, generally have an excess 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:

Several proteins of the red cell membrane have been well characterized recently and, of these, two are of particular interest. The first separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as the component 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 invades red blood cell, 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.

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 a suitable red cell, considerable cohesive forces are generated and there is visible morphological deformation of the host cell. This radiates 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 an ability of the blood stage parasite trophozoites 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 adhere to capillary walls of various organs leading to sequestration of the late stages from peripheral circulation.

Cytoadherence enables *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 invade erythrocytes by a multistep process that is mediated by specific molecular interaction 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 require interaction with Duffy blood group
antigen for invasion and invade both Duffy-positive and Duffy-negative erythrocytes. Sialic acid residues of glycophorin A, have been identified as invasion receptors for \textit{P. falciparum} (Pasvol \textit{et al.}, 1982).

**Pharmacology of current malaria chemotherapy:**

Chemotherapy is the primary means of treating protozoan infections. Successful chemotherapy depends on a large part on the ability to exploit metabolic differences between the pathogen and the host. A problem confronting chemotherapy is the ability of the pathogen to mutate and become drug resistance.

Antimalarial drugs may be divided into the following categories:

1) Causal prophylactic agents (acting against primary tissue stages)
2) Anti-relapse drugs (acting against latent tissue stages)
3) Blood schizontocides
4) Gametocytocides
5) Sporontocides

The majority of these drugs are blood schizonticidal agents that are effective against the erythrocytic stage of the plasmodia life cycle. Few drugs like Primaquine affect the gametocytes.

**Mechanism of action of anti-malarial drugs:**

**Quinolines and related drugs:**

\begin{itemize}
  \item Quinina
  \item Chloroquine
  \item Amodiaquine
\end{itemize}
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Quinoline antimalarials and related aryl alcohols owe their origins to quinine—an active ingredient of Cinchona bark. Quinine has liabilities associated with toxicity (such as tinnitus) and, because it requires thrice daily administration over 7 days, this can result in poor compliance. The dependence on raw material for its extraction and the opportunities presented by its structural elucidation led to the development of the fully synthetic 4-aminoquinoline antimalarials—notably chloroquine and later amodiaquine, which are inexpensive and administered over 3 days (Tilley et al., 2001).

Chloroquine has been the medicine of alternative in most part of malaria endemic areas for a few time until, resistance was developed by the notorious *falciparum* sps. It is a terribly potent schizonticidal drug against erythrocytic stage of all four *Plasmodium* sps. It has no impact on sporozoites, hypnozoites or gametocytes. It is a weak base, and concentrate itself in the parasites lysosomes, possibly by a parasite-specific drug-concentrating mechanism. The by-product of the parasitic digestion of the haemoglobin, haem (ferriprotoporphorin IX) is very toxic to the parasite. Plasmodial haem polymerase converts haem to harmless haemozoin (a red pigment associated with malaria). Chloroquine inhibits this enzyme and so a built up of haem kills the parasite by membranolytic action. Chloroquine also causes fragmentation of the parasite's Ribonucleic acid (RNA) and intercalates in its Deoxyribonucleic acid (DNA) (Ward, 1988).
Until the event of chloroquine resistance, quinine was the primary line of drug within the treatment of malaria. It absolutely taking its original place, since resistance has developed against chloroquine. Quinine may be a potent blood schizonticidal drug against the four plasmodia species. It is an anti-erythrocytic stages drug and has no impact on the exoerythrocytic section or the gametocytic phase (Rimchala et al., 1996).

Mefloquine interferes with transportation of haemoglobin artifact and alternative substances from the host cell to the parasites food vacuole (Hellgren et al., 1997; Weidekamm et al., 1998). Resistance has been reported, however if combined with chloroquine or quinine, that is overcome (Tilley et al., 2001).

The aryl alcohol lumefantrine is similar to mefloquine and halofantrine, and no neurotoxicity was seen during animal toxicology studies in preclinical development (Skeleton-Stroud and Mull, 1998). It is unclear whether a 2-day (4-dose) regimen or a 3-day (6-dose) regimen is the most appropriate treatment in Africa (WHO, 2001). The need for higher dosing to ensure efficacy is supported by evidence of the cross-resistance of lumefantrine with mefloquine in both Thailand (Noedl et al., 2001) and Cameroon (Basco et al., 1998), and the dependence on food for optimal adsorption of lumefantrine from the gut (Ezzet et al., 2000).

Primaquine, the lone representative of 8-aminoquinolines among licensed antimalarials, has been in use for over 50 years. It remains the only drug licensed for the prevention of malaria relapse (i.e., killing liver stages of the parasite). Primaquine also kills asexual blood stages and sterilizes the sexual-stage gametocytes. Thus, primaquine can prevent infection or relapse, cure disease, and prevent transmission of the infection. This broad range of activities represents the promise held in this family of compounds. A
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new 8-aminoquinoline, tafenoquine (WR 238605), is now in clinical trials and may revolutionize the prevention of malaria in travelers. Moreover, tafenoquine may provide an urgently needed weapon to combat epidemic malaria with a single regimen of therapy. The factor that most jeopardizes such utility is another trait shared by many 8-aminoquinolines, hemolytic toxicity in people lacking glucose-6-phosphate dehydrogenase (G6PD). G6PD deficiency is one of the most common genetic abnormalities in human beings, and it is especially common where malaria is or has been endemic (Brueckner et al., 2001). Primaquine was preferred to eliminate *P. falciparum* at the early stage of infection, when parasite develops in the liver, thus preventing the clinical disease. Primaquine is interfering with the mitochondrial function of *Plasmodium* (Robert et al., 2001).

**Folate Antagonists:**

![Pyrimethamine](image1)

![Atovaquone](image2)

![Proguanil](image3)

The antifolate class of antimalarial drugs is not derived from plants and owes its origins to compounds generated through a knowledge of cell biology and synthetic medicinal chemistry. Fully reduced folate cofactors are essential for the key one-carbon transfer reactions needed for nucleotide biosynthesis and amino-acid metabolism (Sherman, 1998). At present, the most significant antifolate used to treat malaria is undoubtedly the combination of the 2,4-diaminopyrimidine pyrimethamine, an inhibitor of dihydrofolate reductase (DHFR), and sulphadoxine, a sulphonamide that interferes
with the action of dihydropteroate synthase (DHPS), another enzyme in the folate pathway (Gregson and Plowe, 2005). The two components of the sulphadoxine/pyrimethamine combination act as synergists with each other, enhancing their activity and reducing the propensity for resistance development. The long half-life of the components may also account for the fact that sulphadoxine/pyrimethamine is extremely useful for intermittent treatment in pregnancy (Wolfe et al., 2001). The hydroxynaphthoquinone atovaquone interferes with mitochondrial electron transport. Rapid resistance develops against atovaquone (Looareesuwan, et al., 1999), owing to a point mutation in cytochrome c reductase (Vaidya, 2001). The addition of proguanil to atovaquone results in a synergistic activity that prevents the rapid development of resistance. At present atovaquone/proguanil is used primarily as a prophylactic agent, and its price is too high for it to achieve widespread use in developing countries. One reason for this cost is the complexity of the synthetic route to atovaquone (Ridley, 2002).

**Artemisinins:**

Several semisynthetic derivatives of artemisinin - the active ingredient of the Chinese herb ‘qinghao’ (*Artemisia annua*), which was used traditionally for treating fevers. These derivatives include artemether, arteether and artesunate, which are all metabolized to dihydroartemisinin - the main active agent in the body. These drugs are
fast acting and act against gametocytes, the sexual stages of the parasite that infect mosquitoes. The short half-lives of both the parent semisynthetic derivatives and the dihydroartemisinin metabolite necessitate treatment over 5-7 days when these compounds are used alone. They are therefore being used increasingly in combination with longer half-life drugs to reduce treatment time and increase individual compliance (WHO, 2001). It is anticipated that rapid clearance of the parasites by artemisinin derivatives (White, 1997), will reduce the chances of resistance development to the partner drugs (White, 1999).

**Antibiotics:**

Common antibiotics acting against bacterial protein synthesis such as tetracycline, doxycycline and clindamycin inhibit parasite growth and are being used increasingly in combination with other antimalarial treatments to augment their activity (WHO, 2001). In parts of southeast Asia, quinine plus tetracycline and quinine plus doxycycline are commonly used combinations, but their use in Africa is limited because both antibiotics are contra-indicated in children under 8 years of age (WHO, 2001). Clindamycin is recommended in combination with other antimalarials in some situations. These antibiotics are all thought to inhibit parasite growth through the inhibition of 'prokaryote-like' protein biosynthesis in the apicoplast - an organelle that is unique to apicomplexan parasites such as *Plasmodium* (Fichera and Roos, 1997; Clough and Wilson, 2001).

**Drugs used in prophylaxis:**

Malaria prophylaxis means preventive treatment of malaria. Drugs used for prophylaxis are said to be affecting in blocking the link between the exoerythrocytic and also the erythrocytic stage, therefore forestall the event of clinical attacks. Travelers take
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it one week before entering endemic zones and carry on taking one month after leaving. None of the chemoprophylaxis is a 100% yet and some may have slight side effects that hinder compliance (WHO, 2010).

Drug Resistance in Malaria:

Resistance of *Plasmodium falciparum* to chloroquine, a 4-aminoquinoline, was first observed almost 50 years ago. Today, chloroquine resistance occurs almost everywhere that *P. falciparum* occurs. Strains of *falciparum* parasites have developed resistance to most of the commonly used antimalarials, including sulfadoxine-pyrimethamine (Fansidar) and mefloquine. Resistance commonly develops within 10-15 years after an antimalarial is introduced (Table No. 3) (Wongsrichanalai *et al.*, 2002).

Unique aspects of antimalarial drug discovery:

Cost effective:

After the discovery of the most potential plant products as antimalarials quinine and artemisinin, the discovery towards the plant originated compounds or their analogs either synthetic or semi synthetic compounds had amplified. After the resistance to most of the antimalarial drugs the only hope was from the plants because the existence drug therapy to completely eliminate or cure the disease like malaria is costly for the poor people as the risk for the disease is much high in them because of poor economic and living conditions. CQ is a cost effective antimalarial drug with a relatively good safety profile (Greenwood *et al.*, 2005). However, CQ is no longer used alone due to the emergence and spread of *P. falciparum* CQ-resistant strains and, more recently, of *P. vivax* (Tjitra *et al.*, 2008). Artemisinin is a desirable substitute to the widely used chloroquine-based antimalarial drugs. The Plasmodium parasite that causes malaria has
become resistant to these traditional drugs. While faster acting and more effective, artemisinin is expensive and supplies are often limited. Artemisinin is currently extracted from plants. Unfortunately, the extraction makes large-scale production too costly for countries where the drug is needed most. The methods also employ volatile organic solvents that levy a heavy environmental toll.

**Analogs of existing antimalarials and other diseases:**

Another approach to antimalarial chemotherapy is to improve upon existing antimalarials by chemical modifications of these compounds. This approach does not require knowledge of the mechanism of action or the biological target of the parent compound. Indeed, this approach was responsible for the development of many existing antimalarials. For example, chloroquine, primaquine and mefloquine were discovered through chemical strategies to improve upon quinine (Stocks *et al.*, 2002). More recently, 4-aminoquinolines that are closely related to chloroquine appear to offer the antimalarial potency of the parent drug, even against chloroquine-resistant parasites (Kaschula *et al.*, 2002). An 8- aminoquinoline, tafenoquine, offers improved activity against hepatic-stage parasites over that of the parent compound, primaquine (Walsh *et al.*, 1999), and is effective for antimalarial chemoprophylaxis (Lell *et al.*, 2003). Since halofantrine use is limited by toxicity, the analog lumefantrine was developed and is now a component of the new combination co-artemether. New folate antagonists and new endoperoxides related to artemisinin are under investigation (Posner *et al.*, 2003).

**Least drug resistance:**

Plants produce a vast and diverse assortment of organic compounds, the great majority of which do not appear to participate directly in growth and development. These
substances, traditionally referred to as secondary metabolites, often are differentially
distributed among limited taxonomic groups within the plant kingdom. Their functions,
many of which remain unknown, are being elucidated with increasing frequency. The
current chemotherapeutic drugs for malaria are almost resistant so the only alternative to
fight against the disease tends to be the drugs from the plant source, as had always proven
the best in the combat towards the increasing emergence in malaria. The mixture of
compounds (crude drug) or the isolated compound can be used against the malaria to
overcome the disease burden for some time as in meantime, the combinations of the
artificial drug or semi synthetic drugs and be brought to treat malaria in the market.

Less toxic:

Plant products are always used by the people to treat various diseases. Ancient
people came to know about the toxic effects of the plants by side effects in the animals
grazing those plants or by the people who had came in physical contact or had taken that
plant to treat any disease. The plants used to treat fever and other clinically similar
symptoms analogous to that of malaria had very rarely shown any kind of toxic effects
and if shown is so less that it can be neglected. The plants have mixture of phytochemical
components, some of which may have toxic effects, if taken as pure form but in the crude
form it may not have so worsen effects to the body hence may use to treat diseases.

New targets:

Although it’s not possible to discuss all of the many scientific opportunities that
are presenting themselves for malaria drug discovery, several other areas deserve to be
highlighted, because malaria parasites are microaerophilic homolactate fermenters and rely
on glycolysis for ATP production, lactate dehydrogenase is being explored as a drug
target (Dunn, et al., 1996), because the malarial parasite requires enormous amounts of glucose uptake from the host to support its growth and the identification of a glucose transporter by functional transfection of the gene into Xenopus oocytes had shown significance (Woodrow et al., 1999). Identification of other transporter molecules including an anion-selective channel that is responsible for transporting solutes and nutrients (Desai et al., 2000, Kirk 2001) may prove new target to tackle this problem. Proteases involved in erythrocyte invasion also offer potential (Blackman, 2000), as do some highly active compounds that may interfere with phospholipid biosynthesis (Vial, et al., 1999). Cell signalling pathways, particularly through protein kinases, offer huge potential for drug discovery (Kappes et al., 1999), and there is a wealth of medicinal chemistry experience available in this area from industry. Until protein kinases can be linked to specific functions, however, it will be difficult to assign a relevance to particular genes for drug discovery. Some studies suggest that combination of inhibitors acting on pathways that converge to produce a final product could be more effective than a single inhibitor and would reduce the rate of evolution of drug resistance (Macreadie et al., 2000).

In vitro cultivation of malaria parasite:

The short-term cultures have been used to study certain aspects of metabolism of the parasite and the mode of action of anti malarial drug (McCormick et al., 1971). One result indicating the possibility of continuous cultivation was reported by Anderson (1953) with P. gallinaceum. He used the medium consisting of chicken erythrocytes extract prepared in chicken serum and found that after continuous culture for some days, with a dilution with fresh blood cells every 2nd day, the parasitaemia was the same as it
Introduction

had been initially, showing that extensive multiplication had occurred. Other methods proved to be more cumbersome without giving better result. Trager (1971) reasoned that at least for species such as *P. coatneyi* and *P. falciparum* which spends 2/3rd of each 48 hours cycle sequestered in deep organs and attached to capillary walls, it might be desirable to have a stationary settled layer of red cells with a slow flow of medium over them. This led to the development of the so-called flow vial, which gave a second cycle of reinvasion but no overall increase in parasitaemia. Experiments, with a number of tissue culture media revealed the great superiority of Roswell Park Memorial Institute medium-1640 (RPMI-1640) supplemented with 25mM N-2 hydroxyethyl peprazine N-2 ethane sulphonic acid buffer (HEPES) (Trager and Jensen, 1976). RPMI-1640 is a medium originally prepared (Moore *et al.*, 1967) for the culture of human leucocytes. When this medium with 25 mM HEPES and 15% rhesus monkey serum was used with *P. coatneyi* in flow vials, the parasite continued to cycle for over a week and persisted for two weeks with decreased numbers (Trager and Jensen, 1980).

**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, produced 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 better results were obtained. Cultures were maintained 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 parasitemias 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).
Plants selected for the study:

In the developing countries, where malaria is a serious issue people depend on traditional medicines as they are comparatively inexpensive. As scientific data on the efficacy of these natural products are scarce, it is important that the medicinal plants are screened and investigated to establish their efficacy as potential sources of new antimalarial drugs. Based on the therapeutics uses for other ailments we have selected the following plants in the present study to investigate their efficacy to use as potential antimalarials.

*Calotropis procera* (Ait.) R. Br.L.N.

Kingdom: Plantae
(Unranked): Angiosperms
(Unranked): Eudicots
(Unranked): Asterids
Order: Gentianales
Family: Asclepiadaceae
Genus: *Calotropis*
Species: *C. procera*

English Name: Swallow-Wort, Sodom Apple, Dead Sea Apple
Common name: Aak, Madar, Aakha, Bhoot baba.
Tamil name: Vellai Erukku.
Introduction

Gujarati name: Akado, Aakado, Akda, Myhara, Retoakah.
Marathi name: Lalrui, Akanda, Lalakara, Rui.

Certotropis procera (Asclepiadaceae) is a xerophylos species which is found to be erect, tall, large, much branched and perennial shrub or small tree that grows to a height of 5.4 m., with milky latex throughout. Bark is soft and corky. Branches are stout and terete. Leaves are sub-sessile, opposite, decussate, broadly ovate-oblong, elliptic or obovate, acute, thick, glaucous and green, covered with fine cottony pubescent hair on young but glabrous later and cordate leaf base. Flowers are umbellate-cymes and tomentose on young. Calyx are glabrous, ovate and acute. Corolla are glabrous, lobes erect, ovate, acute, with 5-6 coronal scales, latterly compressed and equally of exceeding the staminal column. Follicles are sub-globose or ellipsoid or ovoid. Seeds are broadly ovate, acute, flattened, minutely tomentose, brown in color.

It is found in most parts of the world in dry, sandy and alkaline soils and warm climate. In India it is found from Punjab and Rajasthan to Assam and Kanyakumari, upto an altitude of 1050 meters. It grows abundantly in Rajasthan and Gujarat. It is found in waste lands and grows as a weed in agricultural lands. It grows well on rubbish heaps, waste and fallow lands, roadsides and sand dunes.

Chemical Constituents:

The plants contain the cardenolide, Proceragenin, while the root bark contains benzoylinesolone and benzoylisolinelone. The leaves and stalk contain calotropin, and calotropagenin while the flower contains calotropenyl acetate, and multiflavenol and the latex contains uzarigenin, and terpenol ester (Yoganarasimhan, 2011). Chemical investigation of this plant has shown the presence of triterpenoids, calotropursenyl acetate
and calopfriedelenyl, a norditerpenyl ester, calotropternyl ester oleanene triterpenes like calotropoleanly ester, procerleanol A and B (Ansari and Ali 2001) and cardiac glycosides calotropogenin, calotropin, uscharin, calotoxin and calactin (Ahmed et al., 2005) The plant also has been investigated for the presence of cardenolides (Seiber et al., 1982) and anthocyanins (Ahmed et al., 2006). Phytochemical investigation of root of Calotropis procera Linn yields two new phytoconstituents, procerursenyl acetate and Proceranol, together with the known compounds N-dotriacont-6-ene, glycercyl mono-oleoyl-2-phosphate, methyl myrisate, methyl behenate and glycercyl-1, 2-dicapriate-3-phosphate. The structures of the new compounds have been identified as urs-18 alpha-II-12, 20 (30)-diene-3 beta-yl acetate and n-triacontan-10 beta-ol on the basis of spectral data analysis and chemical reactions. The root bark has also been found to possess α-amyrin (Saber et al., 1969) β-amyrin (Saxena and Saxena, 1979) lupeol, β-sitosterol (Saber et al., 1969) and flavanols like quercetin-3-rutinoside (Lal et al., 1985). In the leaves, mudarine is the principal active constituent as well as a bitter yellow acid, resin and 3 toxic glycosides calotropin, uscharin and calotoxin. The latex contains a powerful bacteriolytic enzyme, a very toxic glycoside calactin (the concentration of which is increased following insect or grasshopper attack as a defense mechanism), calotropin D I, calotropin D II, calotropin F I, calotropin F II and a non toxic proteolytic enzyme calotropin (2% - 3%). This calotropin is more proteolytic than papain, and bromelain, coagulates milk, digests meat, gelatin and casein. The whole plant contains a- and b- amyrin, teraxasterol, gigantin, giganteol, isogiganteol, b-sitosterol and a wax (Vadlapudi et al., 2012).
Medicinal Uses:

The crude extract of *C. procera* and its protein fraction were found to possess high fibrinolytic and anticoagulant activity in rabbit and human plasma (Hussain *et al.*, 2011). The alcoholic extract of leaves and roots were found to have anticancer activity against human epidermal carcinoma of the nasopharynx in tissue culture (Dhar *et al.*, 1968). The aqueous and alcoholic extract has slight depression followed by stimulation of the rate and force of myoeydial contraction of isolated frog’s heart. It also induce increase in blood pressure in dog, marked contractions in rabbit duodenum, rat's ileum and uterine horn of virgin rat. Aqueous extract has mild diuretic effect on rat. Latex has anti-inflammatory properties. Petroleum ether extract of flowers showed abortifacient activity (Basu and Chaudhuri, 1991). Its latex is used in leprosy, eczema, inflammation, cutaneous infections, syphilis, malarial and low hectic fevers, and as abortifacient (Kumar and Basu, 1994). Different parts as well as latex of *Calotropis procera* have been reported to have emetic, purgative and anthelmintic effects in traditional medicine (Jain *et al.*, 1996). The effect of crude fractions of *C. procera*’s flowers, buds and root were tested against a chloroquine sensitive strain, MRC 20 and a chloroquine resistant strain, MRC 76 of *Plasmodium falciparum* (Sharma and Sharma, 1999). Leaves: in rheumatism, as an anti-inflammatory and antimicrobial and Roots: as hepatoprotective agents, against colds and coughs, syphilis and elephantiasis, as an anti-inflammatory, analgesic, antimalarial and antimicrobial. Flowers: as cytostatic, abortifacient and antimalarial, in asthma and piles and villagers in Bikaner district ingest almost all plant parts in various dietary combinations for malarial fevers and pyrexias (Sharma and Sharma, 2000). Aqueous extract of the flowers has been found to exhibit analgesic, antipyretic and anti-
inflammatory activity. The alcoholic extract from different parts has been found to possess antimicrobial and spermicical activity (Kamath and Rana, 2002). The plant contains several useful enzymes. A protease was purified to homogeneity from the latex of medicinal plant *C. procera*. The enzyme hydrolyses denatured natural substances like casein, azoalbumin, and azocasein with high specific activity (Dubey and Jagannadham, 2003).

**Citrullus colocynthis (L.) Schrad.**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
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<tbody>
<tr>
<td>(unranked)</td>
<td>Angiosperms</td>
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<tr>
<td>(unranked)</td>
<td>Eudicots</td>
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<td>(unranked)</td>
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<td>Cucurbitaceae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Citrullus</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>C. colocynthis</em></td>
</tr>
<tr>
<td>Common name</td>
<td>Bitter apple, Bitter cucumber, Egusi, or vine of Sodom (Gavakshi, Indarvaruni)</td>
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<tr>
<td>Hindi name</td>
<td>Makkal, Badi indrayan</td>
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<tr>
<td>Malayalam</td>
<td>Valiya Kattuvellari, Valiya pekkummatti</td>
</tr>
<tr>
<td>Gujarati name</td>
<td>Indravana, Indrak</td>
</tr>
</tbody>
</table>

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Citrullus colocynthis, is a viny plant native to the Mediterranean Basin and Asia, especially Turkey (especially in regions such as İzmir), Nubia, and Trieste. Colocynth is widely distributed throughout India. It grows in a state of nature in the arid tracts of north-west, central and south India, and is met with in the Punjab, Sind and on the Coromandal coast. The roots and the whole fruit without the seeds are commonly used in India whereas only the pulp is official in the British Pharmacopoeia. The Indian varieties of Colocynth differ a little from the imported varieties and are nearly globular in shape and usually of the size of an orange or smaller with a surface marbled with green and yellow white patches. When fresh, the pulp is spongy and juicy but when dry the fruit becomes yellowish white and contains a scanty yellowish pulp embedded inside the fruit. All parts of the plant are very bitter, and contain traces of an alkaloids and the bitter principle colocynthin (Chopra and Chopra, 2006).

Chemical constituents:

There is practically no difference in the chemical composition between the Indian and European varieties; both owe their physiological activity to the alkaloid and the bitter principle colocynthin. The alkaloid is only present is very minute quantity. The average yield of the better principle is not less than 2 percent on the weight of dry pulp which compares favorably with the standard in the British Pharmacopoeia. The substance referred to as colocynthin (citruline) and believed to be a glycoside, is now known to be a mixture of alkaloids and crystalline alcohol, citrullol. The pulp also contains α-elaterin, hentriacontane, a phytosterol and a mixture of fatty acids (Chopra and Chopra, 2006). The plant is good source of vitamins including ascorbic acid, riboflavin and thiamine.
Introduction

The plant has high amount of ascorbic acid making it a highly effective antioxidant (Asyaz et al., 2010).

Medicinal Uses:

*Colocynth* is a very old remedy in the hindu medicine. The fruit has been described as cathartic and useful in biliousness, constipation, fever and intestinal parasites. The mohammedan physicians use this drug extensively in their practice as a drastic purgative, in ascites and jaundice and in various uterine conditions, especially in amenorrhea. There is also mention of the drug in Greek and Roman medicine. Even in moderate doses it is seldom prescribed except as adjuvant to other cathartics. *Coloncynth* is used in medicine as a drastic purgative, and in the form of solid extract enters into, any of the purgative pills of modern pharmacy. Although a fair amount of Indian *Colocynthia* is used in the country, large quantities of the fruit as well as preparations made from it are annually imported from Europe, Arabia and Syria. In Spain and Cyprus, *Colocynthia* apples are actually cultivated for purpose of export. In fact imported *Colocynthia* fruits and solid extracts are more in evidence on the market than the preparations made from the drug of Indian origin (Chopra and Chopra, 2006). Immature fruits and seeds are demonstrated the most efficient of all and were used in Tunisia, as in the rest of the Mediterranean region, the parts of plants most often used for medicinal purposes are fruits and/or seeds (Marzouk et al., 2009). A fruit of *Citrullus colocynthis* is traditionally used for the treatment of diabetes, microbial diseases, ulcer, inflammation, jaundice and urinary diseases in Asian and African countries (Nmila et al., 2000). Oil obtained from seeds is applied on head for hair loss. Fresh juice of the leaves is externally applied daily for a month, which is claimed to be effective in baldness. Fresh juice of *Colocynthia* is mixed
with equal quantity of sodium chloride. It is warmed over fire till it dries up, this powder is given 1 gram twice a day after meal as a stomachic and appetizer given in constipation. The half piece (approx. 100 gm) of ripened fruit is given twice a day to cattle for treating indigestion, colic pain, cough and intestinal worms (Qurshi et al., 2010).

**Ephedra foliata** var. a Stapf.

| Kingdom: | Plantae |
| Division: | Gnetophyta |
| Class: | Gnetopsida |
| Order: | Ephedrales |
| Family: | Ephedraceae |
| Genus: | Ephedra |
| Species: | *E. foliata*. |
| Common names: | Soma, Ephedra, Asmania |
| Gujarati names: | Lana, Andho-khimp, Suo-Phagaro |

The medical use of *Ephedra* dates back to at least 2700 B.C., first found records was in Chinese medicines. *Ephedra foliata* is a species of *Ephedra* that is native to the Middle East and central Asia, from Egypt to India. Distributed in north west and eastern Africa and widespread across the Arabian peninsula, extending east to India (Freitag and Maier-Stolte 2003). The plant is commonly dioecious, rarely monoecious often climbing on the shrubs. It may reach as high as 2 m if supported by high shrubs. Leaves vary in size and may reach up to 2 cm long in early stages of growth, they also could be absent.
Ephedras are dioecious, with male and female cones occurring on separate plants. The cones are borne singly or in pairs or whorls at the branch nodes. *Ephedra* plants do not flower every year. The distribution of male and female *Ephedra* plants is not random; individuals on dry slopes are overrepresented by males, whereas those growing on run-on surfaces are 4 times as likely to be females as males (Freeman *et al*., 1976).

**Chemical constituents:**

Almost all commercial applications of *Ephedra*, extracts derive from the Ephedrine alkaloids that have been found in the stems in many Eurasian species. The herb contains multiple (1-2%) organic alkaloids. Its most important constituents (about 40-90%) are l-ephedrine, d-pseudoepidrine, flavanes, and volatile oil. Other ingredients are ephedroxane, 1-N-methylephedrine, d-nor-ephedrine, l-n-mthylephedrine, d-N-pseudoehidrine, d-nor-pseudoephidrine, d-n- dimethyipseudephdrine, beta-ephidrine, and 1-alpha-d-terpinol (Ling, 1995; Zhu, 1998).

**Medicinal uses:**

Powdered *Ephedra* stems are used in traditional herbal medicines as a hypertensive aid to treat asthma, nose and lung congestion, hay fever and several other ailments (Dowd *et al*., 1998). The plant has astringent taste and is strong in pine odor. Ephedrine is administered in cases of shinitis, asthma, hay fever and emphysema. Ephedrine is given orally for treating epilepsy, nocturnal enuresis, myasthenia gravis and urticaria accompanying angioneurotic edema, relieve congestion and swelling ephedrine salts are prescribed as nasal spray. This is also employed subcutaneously to prevent hypotension during anesthesia. Pseudoephedrine is also taken orally which is very effective as nasal decongestant. Moreover, various preparations from *Ephedra* are used to
treat fever, colds, coughs and headache etc. Although, the primary use of Ephedra and its products was for weight loss, energy enhancement and respiratory diseases management (Wang et al., 2006). Stimulates cardiovascular muscle, raises blood pressure, increases heartbeat, augments arrhythmia, dilates cardiovascular, cerebrovascular, and muscular vascular systems and increases blood flow. Ephidrine and pseudoephedrine increase the tone of skeleton muscles, and are useful in treating myasthenia gravis and fatigue. Diaphoretic and antifebrile, antiallergic (Ephedra water or alcohol extract inhibits the release of histamines), cough, bronchitis, allergic rhinitis, sinusitis, nasal congestion (Dong et al., 1998).

**Lantana camara Linn.**

Kingdom: Plantae
Subkingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Asteridae
Order: Lamiales
Family: Verbenaceae
Genus: *Lantana* L.
Species: *L. camara*
Introduction

Common names: Raimuniya, Indradhanu (Indian), Lantana Weed, Wild Sage, Yellow Sage.

Gujarati: Gandhati

Tamil name: Unni chedi.

*Lantana camara* is a significant weed of which there are some 650 varieties in over 60 countries or island groups. *L. camara* is a low erect or sub scandent, vigorous medium-sized aromatic shrub which grows to 1.2 - 2.4 meters (or even more) with quadrangular stems having stout recurred prickles and a strong odor of black currents; it grows; its root system is very strong, and it gives out a new flush of shoots even after repeated cuttings. The posture may be sub erect, scrambling, or occasionally clambering (ascending into shrubs or low trees, clinging to points of contact by means of prickles, branches, and leaves). The leaves are generally oval or broadly lance-shaped, 2 to 12 cm in length, and 2 to 6 cm broad, acute or subacute, crenate-serrate, rugose above, scabrid on both sides and a yellow-green to green color. Flower small, usually orange, sometimes varying from white to red in various shades and having a yellow throat, in axillary heads, almost throughout the year; Fruit form in small clusters similar in appearance to a blackberry, greenish-blue black, blackish, drupaceous, shining, with two nutlets, almost throughout the year. Found in diverse habitats and on a variety of soil types agricultural areas, coastland, natural forests, planted forests, range/grasslands, riparian zones, ruderal/disturbed, scrub/shrub lands, urban areas, wetlands. Disturbed areas such as beside roads, railway tracks and canals are also favorable for the species (Thaman, 1974; Winder and Harley, 1983, and Day et al., 2003).
IntrodudX&yv

Chemical constituents:

Several tri-terpenoids, napthaquinones, flavonoids, alkaloids, and glycosides isolated from this plant are known to exert diverse biological activities including cytotoxic and anticancer properties (Roy and Barua 1985; Sharma et al., 1987; Sharma and Sharma 1989; Liu 1995; Forestieri et al., 1996; Verma et al., 1997; Mishra et al., 1997). The cytotoxic activity may be due to the presence of toxic lanthanoids and alkaloids from this plant (Ghisalberti, 2000). Thirty nine and 46 constituents were identified in the leaf and flower oils, respectively. Sabinene (19.6–21.5%), 1,8-cineole (12.6–14.8%), β-caryophyllene (12.7–13.4%) and α-humulene (5.8–6.3%) were the major components of both oils. Two rare sesquiterpenoids humulene epoxide III and 8-hydroxybicyelogermaerene were also isolated from the oils (Prasad et al., 2011).

Medicinal uses:

*L. camara* has several uses, mainly as a herbal medicine and in some areas as firewood and mulch. The chemical constituents of *Lantana* extracts from the leaves had exhibited antimicrobial, fungicidal, insecticidal and nematicidal activity. Verbascoside, which possesses antimicrobial, immunosuppressive and antitumor activities, has also been isolated (Day et al., 2003). Lantanoside, linaroside and camarinic acid have been isolated and are being investigated as potential nematocides (Day et al., 2003). *Lantana* oil is sometimes used for the treatment of skin itches, as an antiseptic for wounds and externally for leprosy and scabies (Ghisalberti, 2000). Plant extracts are used in folk medicine for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atoxy of abdominal viscera (Day et al., 2003). Study of

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methanolic extract of *Lantana camara* against neostigmine as promotility agent showed an anticholinergic effect due to *L. camara* constituents. Results suggest a utility in secretory and functional diarrhea and other GI disorders. Further study showed significant inhibition of castor-oil induced diarrhea in mice (Sagar *et al.*, 2005). A recent literature survey showed that *Lantana camara* can clear diabetes. Oral administration of methanol extract of *Lantana camara* leaves in alloxan induced diabetic rats showed significant dose dependent reduction of blood glucose concentration (Ganesh, *et al.*, 2010).

*Rauvolfia tetraphylla* Linn.

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Gentianales
Family: Apocynaceae
Subfamily: Rauvolfioideae
Genus: *Rauvolfia*
Species: *R. tetraphylla.*
Common names: Devil-pepper, four-leaf devil-pepper, Snakerooot, Chandrika, Sarppaganti.
Hindi names: Chandrabhaga, Chota chand.
Malayalam names: Pampumkolli, Kattamalpori.
Rauvolfia tetraphylla L. is an endangered woody shrub belongs to the family Apocynaceae. The shrub have smooth bark and slender twigs. The bark of the young twigs is green, it turns light gray with age. The smooth leaves are about 2 inches wide and 6 inches long, and taper to sharp points at both ends. They have many lateral veins and are dark green and shiny above, light green and dull beneath. The leaves are born in short stems, and usually occur in whorls of 4, sometimes 3, or opposite. The small white flowers are borne in clusters in the leaf axils near the tips of the slender, slightly drooping branches. The pear-shaped, bluntly grooved fruits, containing 2 seeds are about 1/8 inches long, green when young and become reddish brown at maturity. All parts of the plants contain a milky sap, which is irritating to the skin. The bark and leaves are distinctly bitter to the taste. Trees grown in partial shade appear darker green than those growing in full sun. The trees, which blossom during the wet season, are propagated by seeds. This tree is wooded areas, in thickets on hillsides, and along stream beds on all to the virgin islands. It is also found in the moist area districts in the open fields (Oakes and Butcher, 1962).

**Chemical constituents:**

Plant contains the alkaloid rauvolscine, 0.1% in root bark, 0.2% in stem bark and 0.2% in the leaves (Chopra et al., 1992). A new sarpagine-type alkaloid, N (α)-Demethylaccedine, has been isolated from the plant (Ghani, 2003). Ten indole alkaloids-ajmaline, yohimbine, -yohimbine, isoreserpine, corynanthine, deserpidine, reserpiline, isoreserpiline, aricine, and a new alkaloid, named lankanescine, have been isolated and identified from Rauvolfia canescens (Arambewela and Madawela, 2001).
Medicinal uses:

Hindus used this plant for centuries as a febrifuge and as an antidote to the bites of poisonous reptiles like snakes. It was also used to treat dysentery and other painful affections of the intestinal canal. The plant is medicinally important in the treatment of cardiovascular diseases, hypertension and various psychiatric diseases (Faisal and Anis, 2002). Plant pacifies vitiated kapha, vata, epilepsy, insomnia, wounds, fever, colic and urinary retention, in controlled dose. Overdose cause giddiness and is toxic. Some believed it caused uterine contraction and promoted the expulsion of the fetus. It was also mentioned as a stomachic that cures fever. In the western parts of India the root was used as a remedy for painful affections of the bowels. In the last decade its medicinal value has been accepted by the allopathic system. The secondary metabolites in Rauvolfia are found to be employed for centuries for the relief of various central nervous system disorders, both psychic and motor, including anxiety states, excitement, maniacal behavior associated with psychosis, schizophrenia, insanity insomnia and epilepsy. The leaf extract of the herbaceous plant, is used for treatment of cholera, eye disease and fever. It is also used as antihypertensive, as well as in intestinal disorders, diarrhea and dysentery (Anonymous, 1969). Plant was found to be Antimicrobial (Shariff et al., 2006).

*Sida acuta* Burm f. and *S. rhombifolia Linn.*

Kingdom: Plantae (spermatophyta)

Division: Phanerogams

Subdivision: Angiospermae
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Class: Dicotyledonae
Subclass: Polypatales
Series: Thalamiflorae
Order: Malvales
Family: Malvaceae
Genus: Sida

Sida acuta Burm. f.

Vernacular names: Bala, Rajabala, Brihannagahala, Phanijivaka, Pronijivaka, Vatyalikaor Vatyaluka, Vatya.

Sida rhombifolia Linn.

Vernacular names: Bala, Atibala, Mahabala, Pitabala, Pitapuspi, Sahadevi or Sahadeva, Prasadini, Kuruntotti.

The family comprises 197 genera and about 2865 species spread over tropical and subtropical regions of the world; some 22 genera and 110 species are found in India. The genus comprises of about 200 species in the world of which 12 are occurring in India. Most of the Sida species found in India are known by general name “Bala” and
referred under Bala Chatustaya in Ayurvedic System of Medicine. They have curative effect on gout. Plants are not velvety or hairy, carpels 3-10, carpellary awns shorter than calyx segment where calyx is not retrose scabrid. Carpels generally five, In *Sida acuta* leaves are ovate, cordate, rounded or truncate at base. In *Sida rhombifolia* the twigs are slender, green, and semi woody. The alternate leaves are variable in both shape and size. They have a 3 to 8 mm petiole with broadly ovate to lanceolate (often rhomboidal) blades 2 to 6 cm long and serrate at the margins, especially from the middle to the tip (Liogier, 1994).

**Chemical Constituents:**

The study of the fixed oil from the seeds of *Sida acuta* for its physico-chemical characteristics and fatty acid composition, reported that yield of oil was 12% and it consisted of oleic, lenoleic, palmitic, steric, myristic, palmitoleic acids, β-amyrin, β-sitosterol and an unknown waxy non-steroidal substance (Rao *et al*., 1973). From the roots of *Sida acuta* α-amyrin, starch, ecdysteron and the alkaloids ephedrine and cryptolepine were isolated (Rao *et al*., 1984). Chemical investigations of *arivalmookappachialai* (*Sida acuta*) an ingredient in siddha formulations, chemicals analysis of whole plant led to the isolation of α-amyrin, β-sitosterol and its glucoosides h-octacosnol, dimethylteraphthalate and four alkaloids (Saraswathy *et al*., 1998).

Gunatiaka *et al*., (1980) investigated several species of *Sida* viz. *S. acuta*, and *S. rhombifolia*, and reported that the important alkaloids present in these species are cryptolepine, ephedrine and vasicine.

Raja and Sudharkar (2000) listed the chemical compounds present in *Sida rhombifolia* consisting of isoquinoline alkaloids, indole alkaloids, flavonoids, lignans and
steroids. Venkatesh et al., (1999) carried out the study of various organic extracts of *Sida rhombifolia* leaves. Phytochemical screening of the extracts indicated the presence of alkaloid, steroid and / or triterpenoids and their glycosides, tannins, flavonoids and their glycosides, carbohydrates and absence of cardiac glycosides. Jadhav et al., (2007) investigated that methanolic extract of the whole plant of *Sida rhombifolia* L. and reported new ecdysteroid glycosides along with ecdysone and 20-hydroxyecdysone.

**Medicinal uses:**

‘Bala’ is a reputed drug in ayurveda for the treatment of rheumatism and it forms a chief ingredient of about 75 ayurvedic formulations (Remashree et al., 2008). The use of *Sida acuta* which is an important medicinal weed found in Gujarat commonly called as Bala, which is reported for its use as astringent, cooling, tonic, stomatic and febrifuge (Tripathi et al., 1996). Methanolic extract of *Sida acuta* found for the alteration of normal estrous cycle in rats and revented pregnancy (Kholkute et al., 1978). The powdered aerial parts showed significant edema suppressant activity similar to indomethacin. The total aqueous extract of the whole plant showed significant activity against carbon tetrachloride, paracetamol and rifampicin, induced hepatotoxicities in experimental albino rats (Rao and Mishra, 1998).

Flavonoids from *Sidaguri* (*Sida rhombifolia* L.) could inhibit the activity of Xanthine oxidase enzyme up to 55.29%, (Iswantini et al., 2009). The supraspinal antinociceptive activity of *S. rhombifolia* extracts produced a dose dependent antinociceptive effect in different potency without inducing a decrease of the functional motor coordination in the mice (Konate et al., 2012). Rao and Mishra (1997) carried out anti-inflammatory and hepatoprotective activities of *Sida rhombifolia*. The effects of
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different parts of *Sida rhombifolia* on chemical and drug induced hepatotoxicity and carrageenan induced paw oedema in rats were studied. Hepatotoxicity was induced in rats by treatment with carbon tetrachloride, paracetamol and rifampicin. The powdered roots, aerial parts and their aqueous extracts showed significant hepatoprotective activity. The methanolic extracts of the aerial part showed significant oedema suppression in rats. These result shows that the plant may exhibit stimulatory effects on hepatic regeneration or free radical scavenging effect etc. The oedema suppression may be due to the inhibitory activity effects on the release of histamine like substances. *Sida rhombifolia* extract demonstrate a marked antibacterial and antifungal activity. Fractions of the crude methanol extracts revealed that the bactericidal and fungicidal constituents of the plant were mainly distributed in their ethyl acetate and aqueous fractions (Muanza *et al.*, 1994).

*Vitex negundo* L.

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<tr>
<th>Kingdom:</th>
<th>Plantae</th>
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<td>(Unranked):</td>
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<tr>
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<td><em>V. negundo</em></td>
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<tr>
<td>Common names:</td>
<td>Five-leaved chaste tree, Sindhuvara, Nirgundi.</td>
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properties (Dharmasiri et al., 2003). Lignans, one class of natural compounds present in *V. negundo*, showed anti-cholinesterase activity in *in-vitro* (Azar and Abdul, 2004). However, no studies were conducted to explore the effect of *V. negundo* extract against memory impairment in *in-vivo*. *V. negundo* extract has potential therapeutic effects on improving the anti-amnesic activity in rats through inhibiting lipid peroxidation, augmenting endogenous antioxidant enzymes and decreasing acetylcholinesterase (AChE) activity in brain (Kanwal et al., 2010). In Ayurveda, Unani and Siddha for the management of pain, headache, inflammation, leucoderma, enlargement of the spleen, rheumatoid arthritis, gonorrhea, bronchitis, fever, cold and cough, the extract of these plant is used as juice, decoction and also as vapor (Panday and Chunekar, 1998; Sabnis, 2006; Anisuzzaman et al., 2007).