CHAPTER IV

SUMMARY AND CONCLUSION

Malaria is the most significant parasitic disease in the world, responsible for 500 million cases and 1 million deaths every year and the number of clinical attacks due to *Plasmodium falciparum* seems to be 50% higher. This situation has occurred due to progressive extent of resistance of this parasite to almost every marketed drug. The loss of effectiveness of the newer antimalarial drugs has also occurred at an alarming rate. Resistance to artemisinin derivatives and artemisinin combination therapy (ACT) was also reported. To evade resistance problem combination therapy associating long and short acting compounds with different modes of action was adopted. It offers resourceful but expensive treatments. Hence, there is a clear need to develop an effective, low-cost, least multi-drug resistance agent, which is also safe, less-toxic and more easily available.

Plants have been a vital part of life in many indigenous communities of our country and even in the State of Gujarat. Plants widely used as anti-malarials by traditional healers are considerably more active *in vitro* against *P. falciparum* than plants that are not widely used or not used at all, for the treatment of malaria. Also anti-malarial properties of *Cinchona* bark, known for more than 300 years and several semi synthetic derivatives of artemisinin - the active ingredient of the Chinese herb ‘Qinghao’ (*Artemisia annua*, which was used traditionally for treating fevers) being used progressively more over the past two decades, reveals that the antimalarial drugs used in the past have been derived from compounds from these medicinal plants or are structures modeled on lead compounds from plants. Although
there is an extensive use of traditional herbal remedies in the management of malaria, scientific understanding of these plants is mostly unknown.

The continuous cultivation of *P. falciparum*, achieved by Trager and Jensen in 1976 revolutionized the study on human malaria. Their methods have made it promising for research workers all over the world to study the clinically most important malaria parasite and hence formed the foundation of many of the current advances in malaria biochemistry, parasitology, immunology and chemotherapy. The availability of fresh human serum remains a chief restrictive factor in growing *P. falciparum* on a mass scale. In our laboratory due to the difficulty in attaining human serum, we have modified the *in vitro* maintenance of the *P. falciparum* isolates using the same plasma of the O+ve blood used for the isolation of fresh RBCs.

Three medicinal plants were selected to determine anti-malarial potency of the crude extracts of selected medicinal plants, ie. *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. The screening of plants for antiplasmodial activity was done on both the *Plasmodium falciparum* strains CQ sensitive 3D7 and CQ resistant RKL-9.

The effective concentration (EC₅₀) is the extract concentration that kills 50% of malaria parasites where activity is effective if EC₅₀ value ≤10 μg/ml. Our results revealed that many of the extracts of these plants exhibited EC₅₀ ≤10 μg/ml, for *Plasmodium*...
*Plasmodium falciparum* 3D7 strain and *Plasmodium falciparum* RKL 9 after *in-vitro* culture, which supported the fact that there are some potent phytochemical components present in these plants which manifest efficacy as antiplasmodial agents. The detailed information as presented in this thesis on the phytochemical and various biological properties of the extracts might provide evidence for the use of these plants in this context.

Certain markers of oxidative stress were evaluated in the present investigation to determine the buildup of free radicals and its possible implications. The decreased level of lipid peroxidation in the dosed plasmodium infected RBC could be attributed to the strong antioxidant molecules in the test extracts, in particular the methanolic and aqueous extracts of *Tylophora* and *Xanthium*. It is evident that the build-up of reactive oxygen species in the infected RBCs are effectively scavenged by the action of the protective enzymes like SOD, triggered on addition of the XM extract.

Haemoglobin and total protein concentration of the test samples were also carried out and the results were exhibiting promising antiplasmodial effects of the extracts. The preliminary decrease in the Hb% and raise in the total protein contents of the untreated samples could reverse the effects after the treatment.

The HPTLC separation and antioxidant activity of the secondary metabolites present was correlated with reference to the phytochemical studies conducted and the antioxidant compounds which may be responsible for the antiplasmodial activity were
highlighted with reference to the Rf values and the area of the separated compounds noticed in the HPTLC and standard Rf calculating methods.

Molecular docking through *in-silico* analysis and bioavailability studies of selected phytochemicals against *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), it was concluded that Tylophorine could be a potential inhibitor of Plasmodium development and life cycle of erythrocytic stages. This study also points out the importance of using novel molecules showing selective interaction towards *Plasmodium falciparum* Erythrocyte protein1 as useful strategies in malaria treatment.

In conclusion, the experimental work directed towards the primary objective of this study viz., to determine anti-malarial activities of the crude extracts of selected medicinal plants, did yield evidence of effective components which could have potent anti-plasmodial activity. The present study has met the objectives, to yield semi-purified extracts that could control the *Plasmodium* growth and multiplication, at the erythrocytic stages. However, future lines of research in this direction could add to the dimension of this study.