CHAPTER-1

INTRODUCTION

1.1 General Introduction of Malaria

In 21st century, Malaria continues to be one of the major diseases in Africa, Asia and Latin America. Approximately 40% of world population is at risk of Malaria parasite infection (Simooya, 2005). Each year, more than one million people around the globe die off malaria and more than two billion people in over 100 countries and regions are threatened by the disease (WHO Report, 2008). The WHO Roll Back Malaria department has reported that recent estimates of global malaria burden have shown increasing levels of malaria morbidity and mortality. It reflects the deterioration of human health due to malaria in many developing countries; especially those in Africa, the morbidity and mortality from malaria are still very high. Africa is the most affected continent: about 90% of all malaria deaths occur in the areas south of the Sahara, and the great majority of these include children under the age of 5 years. The World Malaria Report 2012 summarizes data received from 104 malaria-endemic countries and territories for 2011 and it was reported that 99 of these countries had on-going malaria transmission. According to the latest World Health Organization (WHO) estimates, there were about 219 million cases of malaria in 2010 and an estimated 660,000 deaths (WHO, 2012).

Malaria is not just a disease commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development. Malaria has had serious impact on the social and economic development of mankind. The disease has
been associated with major negative economic effects on regions where it is widespread. Global fund disbursements for malaria control rose sharply during past 8 years and were around US$ 1.66 billion in 2011 and US$ 1.84 billion in 2012. National government funding for malaria programme has also been increasing in recent years, and stood at an estimated US$ 625 million in 2011 (WHO, 2012 Report).

As mentioned above, malaria is the global health issue and similarly, in India, it is a major public health problem and one which contributes noticeably to the overall malaria burden in Southeast Asia. The National Vector Borne Disease Control Program of India reported ~1.6 million cases and ~1100 malaria deaths in 2009. In 2012, there were an estimated 207 million cases of malaria (uncertainty interval: 135 – 287 million), which caused approximately 627 000 malaria deaths (uncertainty interval 473 000 – 789 000) (WHO, Report 2013). Several researcher argue that this is a serious underestimation and actual number of malaria cases per year is more than above figures, (between 9 to 50 times greater), with an approximate 13-fold underestimation of malaria-related mortality (Das et al., 2012).

1.2 Economic Loss due to Malaria

The loss in terms of labour days due to malaria was estimated to 1328.75 million man-days per year. The total expenditure incurred on morbidity due to malaria is Rs. 7.18 per capita per annum. The yearly economic loss due to malaria in India is approximately Rs. 76,660 million (Sharma et al., 1996). India had an estimated 10.6 million malaria cases in 2006 that account for approximately 60% of cases in the WHO South-East Asia Region. With over 100 million slides examined every year, all reported cases are
confirmed and about half are due to *Plasmodium falciparum*. However, the percentage of cases detected through active versus passive surveillance is not known. The State’s most affected are Uttar Pradesh, Bihar, Karnataka, Orissa, Rajasthan, Madhya Pradesh and Pondicherry. Approximately 30% of these cases and 15% of *P. falciparum* cases were in India. Mosquito control covering about 80 million households and protecting 40% of the population at risk. The programme delivered 8.5 million insecticide treated nets (ITNs), more than 3 million first-line treatments and 800,000 courses of Artemisinin based Combination Therapy (ACT) during 2006 and 2007. Funding to cope up the malaria has been increased to more than US$ 140 million in 2007, provided by government, the Global Fund and the World Bank (WHO, Report 2008). In India, malaria problem possess major challenges as a chloroquine-resistance and recently increasing cases of proportion of *P. falciparum* cases. The parasite resistance to insecticide and antimalarial drugs is growing and the alarming reports of emergence of multi-drug resistance poses a real threat to the impact of most of the malaria control programme.

However, the currently available funding for malaria prevention and control is far below the actual demands to reach global malaria targets. An estimated US$ 5.1 billion is needed every year between 2011 and 2020 to achieve universal access to malaria interventions. In 2011, only US$ 2.3 billion was available, less than half of what is needed.
1.3 Malarial Parasite

Malaria is a preventable and treatable mosquito-borne disease and is caused by *Plasmodium* parasites. It is a very serious and even fatal infectious disease and parasites are transmitted mainly to people through the bites of infected *Anopheles* mosquitoes, called "malaria vectors", which bite mainly between dusk and dawn. The parasites multiply within red blood cells, causing symptoms that include symptoms of anemia (light headedness, shortness of breath, tachycardia etc.), as well as other general symptoms such as fever, chills, nausea, flu-like illness, and in severe cases, coma and death. There are four parasite species that cause malaria in humans:

- *Plasmodium falciparum*
- *Plasmodium vivax*
- *Plasmodium malariae*
- *Plasmodium ovale.*

*P. falciparum* and *P. vivax* are the most common parasite with high rate of mortality and mortality. *P. falciparum* is the most deadly and account for the most serious version of the disease and death due to its high levels of death rate and spread of antimalarial drug-resistance (Mendis et al., 2001). It is estimated that 213.5 million clinical cases of malaria is due to *P. falciparum* in Africa (Loset and Kaur, 2009). *P. falciparum* infects as many as 400 million people a year (WHO, 2005a) and whose main victims are children under 5 years of age in Africa and Asia. In recent years, some human cases of malaria have also occurred with *Plasmodium knowlesi* – a species that causes
malaria among monkeys and occurs in certain forested areas of South-East Asia (Report, 2012; WHO, 2013 fact sheet).

1.4 Traditional Methods for Prevention and Treatment of Malaria

Today, malaria can be prevented, diagnosed and treated with a blend of available tools. The primary tools used for prevention are long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS) in which insecticides are sprayed on the walls of homes and outskirt of home, and intermittent preventive treatment and care for pregnant women (IPTp) to prevent malaria infection because due low immunity leads to high transmission settings. Other vector control measures (eg., larviciding and environmental management) are used when appropriate based on scientific evidence. Medicines and diagnostics are used for malaria case management. Malaria can be confirmed by parasitological diagnosis with either microscopy or a rapid diagnostic test (RDT) (WHO-Malaria, report 2012).

Malaria is an infectious disease which has affected human beings since the dawn of recorded history. By the middle of the last century (1960), however, many scientists felt that malaria was on the run away to vanquish. Two factors were primarily responsible for this perceived reduction in the severity of the malarial threat. Firstly, the Anopheles mosquito, which transmits the disease to humans, could at last be controlled by widespread application of the insecticide DDT. Secondly, the Plasmodium parasite, which causes malaria, could also be effectively controlled by the use of synthetic analogues of quinine (a natural product, obtained from the bark of the Cinchona tree), such as chloroquine, which had been developed earlier to World War II. By the 1960's,
however, malaria was back with a vengeance. To treat malaria, quinine and quinoline-based drugs such as chloroquine, mefloquine, and primaquine were widely used till early sixties (Woerdenbag et al., 1990).

The problem of increasing global burden of malaria is very high due to the development of resistance. The mosquitoes were developing resistance to DDT, which was soon to be banned in any case because of environmental concerns (Brown, 2010). This parasite has developed resistance to the most first line and second line drugs available in the market. The \textit{P. falciparum} parasite, which is responsible for cerebral malaria, an often fatal complication, was also developing resistance to chloroquine (Cowman and Foote, 1990; White, 1992). Thailand and South America were the first regions to be affected, but resistance to chloroquine spread to many other parts of the world and it is particularly serious in South East Asia. It was against this background of increasing resistance, and of the on-going wars in neighboring Cambodia and Vietnam, that the Chinese government began a major initiative to discover new anti-malarials from plants used in Traditional Chinese Medicine. In spite of lots of research carried out to treat malaria, still we could not be able develop an antimalarial vaccine. The most effective way to avoid infection is to protect body in the form of suitable clothing, mosquito nets, repellent and insecticides.

Since 20\textsuperscript{th} century, for the prevention and treatment of malaria various medicinal methods have been available, but increasing resistance to formally effective treatment has again made malaria therapy a major problem. Artemisinin, a new antimalarial drug is a Sesquiterpene lactone endoperoxide isolated from the Chinese herb \textit{Artemisia annua}. L
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(Asteraceae) and currently it is only hope to cure malaria. The herb *Artemisia annua* has been used for many centuries in Chinese traditional medicine as a treatment for fever and malaria. In 1971, Chinese chemists isolated from the leafy portions of plant the substance responsible for its reputed medicinal action (Klayman, 1985). In order to effectively curb the spread of malaria in the world, WHO has recommended the use of artemisinin-based combination therapies (ACT) for the treatment of this disease (WHO, 2004). Artemisinin is a sesquiterpene contain 1, 2, 4-trioxane ring structure and this ring structure is responsible for the antimalarial activity of this natural product.

1.5 Artemisinin Chemistry and Function

Artemisinin (Figure 1) is the most efficacious antimalarial drug in the world to date (Abdin *et al.*, 2003) and it is only produced in *A. annua* L. plants in very low amounts. Chinese scientists first isolated artemisinin from *A. annua* plants and the structure was later characterized by others as a sesquiterpene lactone with an endoperoxide linkage (Abdin *et al.*, 2003). This endoperoxide bridge rarely exists in natural products but is essential for the medical function of artemisinin (Woerdenbag *et al.*, 1990; Balint, 2001).

Artemisinin based drugs are the only antimalarials recommended by the World Health Organization (WHO) because of their safety and efficacy against all kind of malaria including cerebral malaria. Antitumor and antimicrobial functions have also been reported (Meshnick *et al.*, 1996; Singh and Lai, 2004; WHO, 2005a). The drug Artemisinin is one of the most promising next generation antimalarial because of its effectiveness against all strains of *Plasmodium*, now resistant to frontline drugs (Ferreira *et al.*, 2005).
Artemisinin and its derivatives are proven antimalarial compounds against *P. falciparum*. Artemether, arteether, artesunate, dihydroartemisinin are some of the antimalarial semi-synthesised from artemisinin. Combination therapies (CTs) with formulation containing an artemisinin compound (ACTs) have emerged as more reliable treatment option (Kumar and Srivastava, 2005). The WHO now recommends that *P. falciparum* malaria is always treated using a combination of two drugs that act at different biochemical sites within the parasite (WHO, 2006). If a parasite mutation producing resistance arises spontaneously during treatment, the parasite should then be killed by the partner drug, thereby reducing or delaying the development of resistance to the artemisinin derivatives, and increasing the useful lifetime of the individual drugs (White 1996; 1999; WHO, 2006). This policy emerged at the time when ACTs were primarily being considered, but other possibilities such as amodiaquine combined with sulfadoxine-pyrimethamine (non-ACTs) are also available. Artemisinin derivatives are therefore usually given with another longer-acting drug, with a different mode of action,
in a combination known as artemisinin-based combination therapy or ACT. These combinations can then be taken for shorter durations than artemisinin alone (White, 1999; WHO, 2006). The artemisinin derivatives also reduce the development of gametocytes (the sexual form of the malaria parasite that is capable of infecting mosquitoes) and consequently the carriage of gametocytes in the peripheral blood (Price 1996; Targett 2001). This reduction in infectivity has the potential to reduce the post-treatment transmission of malaria (particularly in areas of low or seasonal transmission), which may have significant public health benefits (WHO 2006). At present, the best solution for malaria problem is to use Artemisinin based Combination therapies (ACTs). It is a Combination of Artemisinin drug with several other antimalarials drugs for examples artesunate-amodiaquine (AS-AQ), Artesunate-sulphadoxine-pyrimethamine (ASSP), Artesunate-Chloroguanil-Dapsone, Artesunate-mefloquine (AS-MQ), dihydroartemisinin -piperaquine (DH-PP), artemether -lumefantrine (AM-LM) (Whengang et al., 2010). ACT, combination of artesunate-sulphadoxine pyrimethamine, has been therefore launched in high burden states for treatment of P. falciparum to allocate both rapid parasite clearance and reduce selection pressure (Jain, 2003).
1.6 Introduction of *Artemisia annua* L.

The classification of plant is as below

Kingdom : *Plantae*

Subkingdom : *Tracheobionta*

Superdivision : *Spermatophyta*

Division : *Magnoliophyta*

Class : *Magnoliopsida*

Subclass : *Asteridae*

Order : *Asterales*

Family : *Asteraceae*

Genus : *Artemisia*

Species : *annua* L.

*A. annua* is also known as sweet wormwood in the United States, and Qing Hao in China. As a Chinese annual herb, the pharmaceutical value of *A. annua* has been recognized since 168 B.C. and it has been used to treat fevers, emorrhoids, and malaria in China for centuries (Abdin et al., 2003). *A. annua* is an aromatic annual herb that occurs mostly in China. The genus *Artemisia* includes 400 species. The numerous amounts of secondary metabolites are occurred in *Artemisia*, it serves as a source of highly aromatic volatile oils, mainly artemisia ketone, artemisia alcohol 1.8-cineole camphor; germacrene
D, beta-caryophyllene camphene hydrate, alpha-pinene, and myrcene. Non-volatile sesquiterpenes can be recovered from the plant by solvent extraction, some of which show high antimalarial activity. It was reported that more than 20 known sesquiterpenes including artemisinin (arteannuin A), arteannuin B, artemisitene, and artemisinin acid (Ferreira and Janick, 2009). Artemisinin is present in *A. annua* in 0.01-0.8% of dry weight (Namdeo, 2006).

1.7 Geographical Distribution

*Artemisinin annua* occurs in the temperate, cool temperate and subtropical zones (mainly in Asia) of the world. Its origin is China and grows mainly in the middle, eastern and southern parts of Europe and in the northern, middle and eastern parts of Asia. However, it also grows in the Mediterranean region and countries in North Africa, as well as in south and south-west Asia (Rong and Yourum, 1991). China, Vietnam, Tanzania, Kenya are the few countries where *Artemisia annua* cultivated at bulk level. In India, it is cultivated at small scale in Kashmir Valley especially Leh and Laddakh area. *A. annua* for industrial use is mainly collected from the wild.

1.8 Morphology of *A. annua*

*A. annua* is a short day plant (Ferreira and Janick, 1995). The mature plant with a single stem can reach about 2m in height. Aromatic leaves are about 2.5-5.0 cm long, deeply dissected and alternately branched around the stem. Two weeks after receiving an inductive stimulus, vegetative shoots develop into inflorescent shoots (Ferreira and Janick, 1995). The 2-3mm yellow nodding capitula are in loose panicles composed of
many greenish or yellowish central florets which are bisexual and with little nectar and pistillate marginal florets. The central flowers which can be fertile or sterile are perfect and the anemophilous pollen is trifoliate and smooth, and may or may not have spines (Ferreira and Janick, 1996). Both non-glandular filamentous, 5-celled T-shaped, and biseriate 10-celled glandular trichomes have been found on the surfaces of leaves, stems, and flowers. At least 40 volatile compounds and a lot of nonvolatile compounds have been extracted from A. annua and identified (Ferreira and Janick, 1995; 1996).

Annual plant, aromatic, green, glabrous or with scattered, small, approximate hairs. Stem erect, ribbed, brownish or violet-brown, naturally grows to 30–100 cm high (cultivated plants may reach 200 cm high). Leaves alveolate-punctate glandular; lower leaves petiolate, 3–5 cm long and 2–4 cm wide, ovate, thrice pinnately cut, their lobules oblong-lanceolate, short-acuminate, entire or with 1–2 teeth, 1–2 mm long and 0.5 mm wide; middle and cauline leaves twice pinnately cut; upper leaves sessile, smaller and less compound; uppermost leaves bracteal, simple with fewer lateral lobes. Capitula globose, 2.0–2.5 mm in diameter, in long pyramidal paniculate inflorescence. Involucrre glabrous, outer involucral bracts linear-oblong, green; inner oval or almost round, with wide scarious border, lustrous. Receptacle convex, glabrous. Peripheral florets pistillate, 10–20, filiform, punctate-glandular; their stigma lobes narrowly linear, obtuse, exserted from corolla tube; disk florets bisexual, 10–30, their corollas cupshaped-tubular, glabrous; anthers narrowly linear, apical appendages of anthers long, acute, basal appendages very short, subacute; style shorter than stamens, stigma lobes linear, straight, weakly divergent, apically ciliate. Achenes 0.6–0.8 mm long, oblong-ovate, flattened, with small round areola at apex, scariously bordered (Shishkin and Bobrov, 1995).
Genus *Artemisia* belongs to the botanical family of *Asteraceae* which contains about 23000 species. Most studies have concentrated on such species as *A. absinthium* L., *A. pontica* L., *A. judaica* L., *A. vulgaris* L., *A. dubia* Wall. ex Bess., *A. scoparia* Waldst and *Kit.*, and *A. annua* L. (Adekenov et al., 1982; Liu et al., 2003; 2004; Sujatha et al., 2007; Grech-Baran et al., 2010). Members of the *Asteraceae* family produce a large number of various secondary metabolites that show biological activity. Among them, sesquiterpene lactones and flavonoids are the most interesting ones from the pharmacological point of view. These substances are known for their reported medical efficacy e.g. strong anti-inflammatory, antimalarial, antioxidant, antitumor activity, as well as for the fact that they increase immunity and decrease the risk of atherosclerosis, arthritis and gastrointestinal disorders (Liu et al., 2004; Stojanowska, 2010; Kazemi et al., 2011).

The biological production of artemisinin for malarial therapy was largely discussed by Zhao et al. (2007). In 2012, Crespo-Ortiz and Wei, reported that artemisinin and its analogs, which are naturally occurring antimalarials, show a potent anticancer activity. In primary cancer cultures and cell lines, their antitumor actions consists of an inhibiting cancer proliferation.

### 1.9 Uses of Artemisinin

It is well known antimalarial drug, several reports stated that these drug could be used for the treatment of cancer and killing of parasite responsible for *Leishmania donawali*. Cancer and Malaria, AIDS, TB are among the top of major health and developmental challenges of the world. In spite of lots of research on cancer biology and drug development, still remain major problem for human health. In addition the vast
majority of people affected by such major diseases do not have financial resources to cover the cost of treatment. For these reasons one of the major goals of the scientific community has been the discovery of new effective and accessible drugs particularly from traditional and medicinal plants sources. Natural plant compounds provide a potential source of such chemotherapeutic agents that act on various types of cancers. Taxol, an anticancerous drug which is of plant origin, gained widespread attention due to its potency. There are other few drugs used to cure cancer such as Vinblastin and Vincristine obtained from Catharanthus roseaus plant. The above said secondary metabolites have very high economical value due to its bioactivity and low scale production.

Cancer is one of the most common devastating disease affecting millions of people per year. Cancer has been estimated as the second leading cause of death in humans. So there has been an intense search on various biological sources to develop a novel anti-cancer drug to combat this disease. Plants have proved to be an important natural source of anti-cancer therapy for several years. About 30 plant derived compounds have been isolated so far and are currently under clinical trials. These anti-cancer compounds have been found to be clinically active against various types of cancer cells. Further research in this area may lead to better treatment of cancer.

Plants have played an important role as a source of effective anti-cancer agents, and it is significant that over 60% of currently used anti-cancer agents are derived in one way or another from natural sources, including plants, marine organisms and microorganisms (Newman and Cragg, 2012). The search for anti-cancer agents from plant sources started in earnest in the 1950s with the discovery and development of the vinca alkaloids, vinblastine and vincristine, and the isolation of the cytotoxic podophyllotoxins.
Plant secondary metabolites have seemed to be an outstanding reservoir of new medical compounds. Many anti-cancer compounds have been obtained from various plant sources like Catharanthus roseus, Podophyllum species, Taxus brevifolia, Camptotheca acuminata, Betula alba, Cephalotaxus species, Erythroxylum perrveille, Curcuma longa, Ipomoea batatas, Centaurea schischkinii and many others. Researchers are still attempting to explore the bioavailability of anti-cancerous compounds in unexplored plant species (Nirmala et al., 2011). The herbal extract of Artemisia is also used for medicinal purpose as well as in agriculture as a natural herbicide which kill disease-carrying larvae and plant destroying molds and fungi on crops.

1.10 Supply and Demand Regime of Artemisinin

Artemisinin and its derivatives have attracted more and more attention and in 2001, WHO recommended that Artemisinin-based Combination Therapies (ACTs) should be adopted to treat malaria (Mandelbaum-Schmid, 2005) due to little or no cross-resistance with other antimalarial drugs, rapid reduction of the parasite, and efficacious activity against P. falciparum stains (Meshnick et al., 1996). The global demand for ACT has grown sharply since its recommendation by the World Health Organization in 2001. However, a combination of financing and programmatic uncertainties, limited suppliers of finished products, information opacity across the different tiers in the supply chain, and widespread fluctuations in raw material prices have together contributed to a market fraught with demand and supply uncertainties and price volatility. Various short-term solutions have been deployed to alleviate supply shortages caused by these challenges; however, new mechanisms are required to build resilience into the supply chain.
Currently, artemisinin is derived from a raw substance extracted from the plant *A. annua* L. which is the only one source. The major raw material needed for production of active pharmaceutical ingredient (API) comes from filed grown crops in few countries like China, Vietnam, and Tanzania etc. The cultivation *A. annua* requires a minimum of 6 months, and extraction and isolation, processing and packaging of the final product require at least 5-6 months depending on the product formulation. For field cultivation of plant and artemisinin production, several limiting factor play role such as it need to follow agronomical conditions design by the WHO for crop production and environmental variations, seasonal distribution acts as main key for production. For the germination of seeds of *Artemisia*, it needs low temperature so it renders cultivation in many parts of world. Shenzao provenance of China and Vietnam are the main producers of this plant. There are few trails going on cultivation of *Artemisia* in Leh and Ladakh part of India. At the present time 80% of the *Artemisia*/artemisinin is produced in China, 15% in Vietnam and the remaining in Kenya, Tanzania, Uganda, Madagascar and a small amount in India. Trials of *Artemisia* are being grown in Zimbabwe, South Africa and Nigeria.

The report estimates that between 17,000-27,000 hectares of *A. annua* would be required to satisfy global demand for Artemisinin Combination Therapies (ACTs), which could be grown by farmers in suitable areas of the developing world. However, if the sharp increase in demand for the pharmaceutical products is not predicted in proper time to permit for increased agricultural production, there could be temporary shortages in supply. Reliable forecasting of global ACT requirements is thus essential. ACTs are
expected to reach over 300M treatments in 2014, the highest since the start of the ACT scale-up in 2004.

In 2011, the average price of artemisinin was around US$550/kg. The global market for the production and extraction of Artemisia/artemisinin was between $82.5 million and $93.5 million. Amyris, Sanofi-aventis and IPCA, Guilin Pharma, Strides and Ajanta are the few companies for the manufacturing and commercialization of artemisinin-based drugs, with a goal of market availability by 2014 end. The companies assert that the new technology will diversify sources, increase supplies of high-quality artemisinin and lower the cost of ACTs. The few private companies have started trial for the microbial production of synthetic artemisinin, but still not achieved at commercial scale. It has been reported that, due to non-availability of raw materials the cost of ACTs is high and not affordable to poor people. There is urgent need of large searching of alternatives technologies for artemisinin production to fulfill the global demand and also to search new antimalarial drug.

Unfortunately, the level of the production of artemisinin in *A. annua* plants is relatively low, only about 0.01 to 0.8% (DW) (Abdin *et al.*, 2003). A minimum of six months is required for cultivating *A. annua* (WHO, 2004). Due to its unique and complex structure, it is not economically practical to chemically synthesize artemisinin. To meet the therapeutic demand, enhanced production of artemisinin is highly desirable. To achieve above mention goal, there is need to facilitate cultivation of plant and *in vitro* production of artemisinin is also required with reliable quality. Both of these ways will ensure a sustainable supply to meet market demand. *In vitro* cultures may potentially...
constitute useful and easily manipulated systems for producing valuable biologically active compound in plants that do not require labor-intensive methods (McCabe et al., 1997). To treat malaria, the treatment courses needed increased dramatically from 2 million treatment courses in 2003 to 30 million courses in 2004 and 70 million treatment courses for 2005. This has, thus, already led to a shortage of artemisinin for ACTs. At least 130 million treatment courses of ACTs in 2006 will be required (WHO, 2005a). Usually for dosage, 0.6g artesunate or, for the combination artemether/lumefantrine, 0.48g artemether is needed for one ACT adult treatment course (WHO, 2005b). Because artemisinin and artemether are semisynthesized from artemisinin, at least 330 tons artemisinin are needed for just treating malaria infected patients in 2005 and at least 12,000 hectares are required to produce 70 million adult ACT treatments (WHO, 2005b). Therefore, the world market for artemisinin based products is now growing rapidly and the demand for artemisinin is increasing.

1.11 Approaches for Improving Artemisinin Production: *In Vitro* Production

Although artemisinin production can be increased through larger scale, field cultivation of *A. annua*, the length of cultivation and manufacturing time, the need for a large amount of land and labor, and the expense of extraction are still the major problems. Hence, alternative approaches are being studied to enhance artemisinin production using *in vitro* methods. In view of the low artemisinin detected in plant, tissue culture system has been used with great interest as alternative method for production of this drug. Simon *et al.* (1990) reported concentration of artemisinin ranging from 0.03% - 0.05% on dry weight basis. Results from experiments with undifferentiated callus and
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cell suspension culture of *A. annua* are disappointing with respect to artemisinin production (Jha *et al*., 1988; Tawfik *et al*., 1989; Fulzele *et al*., 1991).

Martinez and Staba (1988) have reported that roots originating from leaf segments contained artemisinin, if they were grown on Murashige and Skoog (MS) or Gamborg’s B5 medium supplemented with IBA or NAA at 0.05-0.02 mg/L. Also they reported that artemisinin content increased when roots developed into plants with a properly developed root system. The hairy roots, results of genetic transformation by *A. rhizogenes* have attractive properties of secondary metabolite production. Hairy root cultures provide a promising alternative to the biotechnological exploitation of plant cell culture (Liu *et al*., 2006). Transformed roots of *A. annua* have shown their capacity to produce artemisinin in significant quantity (Jazari *et al*., 1995; Weathers *et al*., 1994; 2003; 2004). Weathers and group (1997) at Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester have also demonstrated the capacity of these roots to grow in variety of bioreactor and production of artemisinin. They have developed gas phase nutrient mist bioreactor and liquid phase bubble column bioreactor for scale up of artemisinin in hairy roots of *A. annua* (Kim *et al*., 2001; 2002).

Transformed hairy roots of *A. annua* have been studied for improving artemisinin production. Compared to suspension cultures, hairy roots are more stable, grow faster, and may be easier to scale-up. Many different culture conditions including light, elicitors, and culture in bioreactors have been investigated (Towler *et al*., 2006; Weathers *et al*., 2006a, 2006b). However, artemisinin yields in hairy roots are not yet high enough to be economically attractive. Alternatively, shoot cultures of *A. annua* are also being studied.
Different culture conditions such as carbon sources, sugar concentration, $\text{NH}_4^+/\text{NO}_3^-$ ratio, phosphate concentration, phytohormones (Basile et al., 1993; Woerdenbag et al., 1993; Liu et al., 1998), addition of precursors, such as mevalonic acid, elicitors, or addition of metabolic inhibitors have been studied (Abdin et al., 2003). Shoots cultured in bioreactors have also been studied for scale up of products (Liu et al., 1998). Unfortunately, artemisinin production in shoot cultures, although greater than in hairy roots, is also still much less than in whole plants (Abdin et al., 2003). More recently, Martin et al. (2003) have introduced a portion of the artemisinin pathway into E. coli. If this effort succeeds, then E. coli may be used to produce high-yield terpenoid-based drugs including artemisinin in large-scale fermentations with expected costs of extraction also largely decreased. The relative low yield (0.01-0.8 %) of artemisinin in A. annua is a serious limitation to the commercialization of drug. Therefore, enhanced production of artemisinin either in cell or tissue culture or in the whole plant is highly desirable.

Artemisinin production in whole A. annua plant ranges from 0.01 to 0.8% (w/w) (Abdin et al., 2003). In whole plants, the artemisinin level in leaves and inflorescences are much higher than in stems, but in pollen or roots artemisinin is undetectable (Ferreira and Janick, 1996). Although some have reported that in a single plant, artemisinin production was higher in the upper leaves than the lower leaves (Simon et al., 1990; Duke et al., 1994). Others have found that artemisinin content was evenly distributed (Ferreira and Janick, 1996). At different development stages, artemisinin production in A. annua has been reported to be variable but, again, the reports on changes were inconsistent (Woerdenbag et al., 1990; Ferreira and Janick, 1996). Artemisinin is
apparently stored in the glandular trichomes of *A. annua* and the glands of old leaves normally rupture open and release their stored materials and, thus the artemisinin level in older leaves on whole plants is lower (Duke *et al.*, 1994; Ferreira and Janick, 1996).

In view of low concentration of artemisinin detected in the plants, biotechnological approaches such as root organ technology, bioreactor technology and downstream technology is essential for enhancement of production of artemisinin in *A. annua* plant. Biotechnological production of artemisinin is promising one. ACTs are much more expensive than other drugs because of relatively low yields of artemisinin in *A. annua*. Therefore, there is need of many efforts to enhance the production of artemisinin *in vivo* and *in vitro* by biotechnology. To fulfill the world demand of artemisinin, it is obvious that there is need for an additional source of artemisinin whose supply will be consistent, reliable and inexpensive. The chief motto of our study is to develop process to increase the production of antimalarial compound artemisinin *in vitro*. We have used biotechnological approaches such as micropropagation, root organ culture technology, bioreactor technology, which seems to be promising and economical for enhancement of artemisinin production in *A. annua* culture with production cycle taking less time compare to six month for field cultivation. At present, the only source of drug is extraction of field grown crops of *A. annua* which is subjected to certain limitation such as low temperature requirement, seasonal *and* somatic variation and geographical limitation, and high production cost. Here we can show that *in vitro* technology could be used a resource of artemisinin. Stable and consistence supply of artemisinin will reduce the cost of drug so that drug will available at affordable price to poor peoples.
The main emphasis of the present work was to study in vitro production of artemisinin that can be exploited as a large scale production. Root organ culture technology and solid phase extraction system was exploited for production of artemisinin. Root organ culture system is used for the production of secondary metabolite; it has several advantages such as their rapid growth, are quite easy to prepare and maintain, show a less variation and can be easily cloned to produce a large supply. The said work was carried out to screen the different probable biological activities of artemisinin. The artemisinin obtained from crude extract was used for screening of anti cancerous potential because the mode of action of artemisinin on malaria parasite shows that it could be used as an anticancer drug. This study is carried out on cancerous cell line. The isolated artemisinin was also tested for its biological activities like antibacterial and antioxidant activity.

1.12 Research Objectives

1. To Establish root organ culture of Artemisia annua.

2. Optimization of culture condition for maximum growth of roots in vitro

3. Development of solid phase extraction system for recovery of artemisinin