Establishment of artemisinin combination therapy as first line treatment for combating malaria: *Artemisia annua* cultivation in India needed for providing sustainable supply chain of artemisinin

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The *Plasmodium falciparum* malaria is responsible for about 500 million clinical attacks and one million deaths each year, mostly in Africa and southeast Asia. On account of the parasite having become resistant to the conventional antimalarials, artemisinin-based combination therapy (ACT) is the only reliable treatment option. The Roll Back Malaria (RBM) and Millennium Development Goals (MDG) campaigns of WHO and UNDP, respectively, have made little headway, mainly because of paucity of artemisinin in world market. Here we show that the cultivation and processing of *Artemisia annua*, presently the only resource of artemisinins, in India, in partnerships, can help build a sound supply chain of artemisinin.

**Keywords:** *Artemisia annua*, artemisinin combination therapy, malarial drug resistance, *Plasmodium falciparum* malaria, supply chain of artemisinin.

MALARIA incidence is on the rise and the clinical disease caused by *Plasmodium falciparum* is widely distributed and entrenched in areas worldwide where climates are suitable for transmission. It was estimated, by the use of epidemiological, demographic and geographical parameters, that there were 515 million clinical attacks of *P. falciparum* malaria in the year 2002. About 70% of the clinical events occurred in Africa, 23% in South East Asia, 2.9% in western Pacific region, 2.3% in the East Mediterranean, 0.7% in America and 0.1% in Europe. The present level of annual global incidence of malaria continues to be about 500 million, 350 million in the African region and 125 million in the South East Asian region, withstanding the global malaria eradication programmes, such as the Roll Back Malaria (RBM) initiative which aims to halve the burden of malaria by 2010 and Millennium Development Goal (MDG) that targets to halt the rising incidence of malaria by 2015.

The long-term fight against malaria involves multiple fronts, including vector mosquito control, use of insecticide treated bednets to limit transmission, prompt and accurate diagnosis of malaria afflicted and effective antimalarial therapy for patients. Deaths due to malaria are occurring in increasing numbers because of frequent failure of the conventional treatments using drugs such as chloroquine and sulphadoxine-pyrimethamine, against which *P. falciparum* populations have developed a high degree of resistance. Combination therapies (CTs) with formulations containing an artemisinin compound (ACTs) have emerged as a more reliable treatment option. The underlying reason for the dependability of artemisinin is the absence of any recorded cases of artemisinin-resistant malaria. World Health Organization (WHO) has recognized the use of ACTs in the treatment of malaria, as a long term measure to control spread of the disease under its RBM programme. For realization of the objectives of RBM and MDC programmes, the world needs ACT treatments for up to 500 million cases of *P. falciparum* malaria that occur annually. The gross insufficiency in supply of ACTs at the present time is related to short supply of artemisinin and its derivatives.

The amorphane sesquiterpene artemisinin is naturally formed in the shoot of *Artemisia annua* plant (Figure 1). No other organism is known to synthesize artemisinin. Variously prepared leaves of *A. annua* were used as traditional medicine for fever in China for many centuries. A traditional preparation of *A. annua* for the treatment of malaria is now undergoing trials, on the way to development of a standardized remedy. Artemether, arteether, arti-
sunate, artinulate and dihydroartemisinin are some of the antimalarials semisynthesized from artemisinin, which are relatively more potent than artemisinin and are alternatively being used as monotherapies and as component drugs in ACTs, against multi-drug-resistant *P. falciparum* malaria. Artemisinin and its derivatives are also serving as therapies against schistosomiasis disease caused by the protozoan species *Schistosoma japonicum*, *S. mansoni*, and *S. haematobium*, which cause 1.5 million disabilities each year. Artemisinins also possess lethal activity(ies) against cancer lines, fungi and bacteria. \( \alpha \)-arteether is known to block the function of quinolone-resistant DNA-gyrase in *Escherichia coli*, *Mycobacterium smegmatis* and *M. tuberculosis*. Artemisinin has been shown to be a cancer chemo-prevention agent as well as an immune-suppressan in mammals, and an inhibitor of chloroplast development/photosynthesis and plant growth in higher plants. Apparently, artemisinins possess several kinds of biological activities and the underlying molecular mechanisms are being investigated. The disruption of haemoglobin catabolism has been implicated in the parasiticidal action of artemisinin in malaria; the iron catalysis of the endoperoxide bridge of artemisinin generates carbon-centred free radicals, which alkylate heme and parasite proteins, essential for the survival of *Plasmodium*. Artemisinin may prove to be active against other protozoan infectious diseases such as leishmaniasis, where the parasite depends on the digestion of haemoglobin to meet its nutritional needs. The absence of artemisinin resistance in malarial parasites may be related to multiple biological activities inherent in the sesquiterpene lactone endoperoxide artemisinin structure (Figure 2), beginning to be revealed by the study of inhibitory actions of artemisinin and its precursors and products on a variety of prokaryotes and eukaryotes.

The artemisinin antimalarials are safely tolerated, have short half-lives and act rapidly in patients, against the young asexual and early sexual forms of *P. falciparum* parasite life cycle. Thus they combine properties of potency, anti-infectivity and protection from resistance development. Loose or fixed-dose combinations of artesunate (AS), artemether (AM) or dihydroartemisinin on the one hand and amodiaquine (AQ), chloroquine (CQ), halofantrine (HF), lumefantrine (LF), mefloquine (MQ), piperaquine (PQ), and quinine (QN) on the other hand are the preferred treatments for uncomplicated and severe malaria.

**Figure 1.** Seeds (left) and leaf (middle) of *Artemisia annua* cv Jeevanaraksha (family Asteraceae) plant and artemisinin (right) present richly in the leaves of this cultivar.

**Figure 2.** Diagrammatic representation of various kinds of inhibitory actions of artemisinins on the intra-erythrocytic asexual and sexual developmental stages of malarial parasite. a. *Plasmodium falciparum* parasite shown as a sporozoite which is transmitted to man by the bite of an infected mosquito. Sporozoites penetrate the liver cells, multiply asexually, split liver cells and infect red blood cells (erythrocytes). Parasite cells carry apicoplast, a relict plastid (or chloroplast), whose metabolism is essential for the parasite. b. Apicoplast. The apicoplast present in the parasite in all stages of its development is a target of artemisinin. Artemisinin will inhibit the anabolic functions of apicoplast, including those concerned with biosynthesis of heme, isopentenyl diphasphate and fatty acids, essential for the parasite, by way of its inhibition of apicoplast genome. c. Schizont in an erythrocyte. Merozoites liberated by liver cells enter blood corpuscles (erythrocytes) and become trophozoites and schizonts. The parasite multiplies asexually in erythrocytes. Temperature of malaria patient rises when the parasite schizonts growing in erythrocytes burst, releasing merozoites. Merozoites infect new erythrocytes for further asexual multiplication of parasite. Plasmodia also produce gametes in erythrocytes by a sexual cycle of growth. When mosquito sucks blood from a malarial patient, gametes get transferred to it. Fertilization occurs in the gut of the mosquito to serve as initiator of another cycle of malarial infection. Artemisinin has inhibitory action on sexual and asexual intra-erythrocyte stages of plasmodia. It stops parasitaemia as well as transmission. The endoperoxide bridge of artemisinin generates carbon-centred, short-lived radicals through its iron-mediated catalysis. These radicals incapacitate a variety of macromolecules, whose activities are essential for plasmodium growth and development. The heme-artemisinin adduct itself is highly toxic. The inactivation of sarco-endoplasmic reticulum Ca\(^{2+}\) ATPase by artemisinin-generated free radicals, further incapacitates asexually and sexually multiplying plasmodia.
(PPQ), pyronaridine (PRN), sulphadoxine/pyrimethamine (S/P) or trimethoprim (TMP) on the other hand, have been tried or used/are being used in South East Asia, Latin America and/or Africa.

The fixed dose combinations available in the form of blister packs are: AS–MQ, Artelkin™ and AM–LF, Coartem® (artemether + lumefantrine or benflumetol) and such fixed dose combinations under development are: AS–AQ, AS–MQ and AS–S/P. Novartis and WHO have entered into an agreement which entails the former to make available Coartem drug to the public sector of the developing countries at a preferential price. In the context of ACTs having been identified as the first line of malarial therapy it is important to remember that widespread or locational loci-specific resistance of P. falciparum malaria is recorded against the non-artemisinin drugs that go into ACTs, such as AQ, CQ, HF, MQ, PPQ, TMP and S/P. and cross-resistance to LF may also be present on account of similarity of its structure and action to quinine, MQ and HF. It is clear that artemisinin component of ACTs will provide the reliability to ACTs as the first line of antimalarial treatment.

The structure of artemisinin molecule which harbours a peroxide bond in 1,2,4-trioxane heterocycle is highly complex and the total chemical synthesis of artemisinin is uneconomical. Recently, a synthetic molecule 1,2,4-trioxolane-7 or OZ277 or RBX-11160, which has in it a pharmacoporic peroxide bond and possesses antimalarial activity has entered the phase-2 and phase-3 trials. It is believed that the molecule will have low production cost, will be easier to formulate for three-day oral treatment regimen, and has good potency and pharmacokinetic properties, although it is structurally much different from artemisinin. The results of clinical trial with OZ277 will be known in about five years from now. The structural similarities and differences between artemisinin and OZ277 mean that they may share some biological activities and also possess differential biological activities. Attempts to produce artemisinic acid, an intermediate in the biosynthetic pathway of artemisinin, in genetically modified Escherichia coli by transfer of mevalonate pathway genes from yeast and/or A. annua and those governing steps subsequent to the compound amorphapha-4,11-diene from A. annua are in progress. The synthesis of amorphadiene in E. coli has been achieved. There is deficiency in knowledge about the intermediates and enzymes that catalyse the pathway of artemisinin biosynthesis beyond amorphadiene, to artemisinic acid. It is hoped that E. coli strains capable of synthesizing recombinant artemisinic acid will be available in about five years time. It is proposed to derive artemisinin semisynthetically from artemisinic acid produced in genetically engineered E. coli. The molecule OZ277 or semisynthetic artemisinin is unlikely to be available in the near future. Thus, the RBM must rely on artemisinin produced from A. annua medicinal crop plant.

In view of five years having gone by since the RBM initiative, comprehensive efforts are required immediately to reach ACTs to those who need it worldwide, at affordable costs. Significantly the progress of RBM programme is limited by the high cost of ACTs. While antimalarial drugs such as QC and S/P cost US$ 0.1 to 0.2 per treatment course, the cost of ACT treatment course is US$ 1.20 to 3.50; the cost of AS–AM treatment course now is US$ 2.40. Malaria control programmes may make good progress if the cost of ACT course is brought down to US$ 1.0. This means that the cost of production of artemisinin must be cut severalfold by making A. annua cultivation and artemisinin extraction processes more cost-effective.

A number of biotechnology projects worldwide are aimed at biopharming/producing by farming a variety of valuable molecules: drugs, vaccines, antibodies, hormones, vitamins, proteins, enzymes, etc. In such programmes, cultivable plants will be serving as hosts to genes of concerned pathway(s) transferred from other organisms. It will be ironic if the A. annua plant, whose genome has all the essential genes for artemisinin metabolism, cannot be exploited to produce artemisinin at low costs in desired quantities. This challenging problem is a test case for protagonists of biotechnology and biopharming. Conventional breeding and genetic engineering approaches should allow construction of A. annua genotypes rich in artemisinin. Besides, improvements in the cultivation, post-harvest processing, artemisinin extraction and artemisinin storage technologies should permit stable supply of artemisinin at low prices. Indeed, there is need to generate new knowledge and exploit the progress in R&D already made in these directions.

The traditional sources of artemisinin have been the wild and cultivated populations of A. annua in certain identified regions of China and Vietnam. To meet the new artemisinin supply challenge, A. annua crop cultivation requires to be extended to geographical areas where high yields of artemisinin can be obtained by use of cultivars developed for adaptation to such regions. The often reported yield of artemisinin from one hectare (ha) of land in China, Vietnam and Africa is 15 kg. Technologies standardized in India allow harvests as big as 75 kg/ha. Their deployment should help bring down the cost of production of artemisinin severalfold.

Considerable progress has been made in the agricultural and chemical technologies of artemisinin production in India. Four artemisinin-rich cultivars have been developed, namely Jeevanraksha, Jeevanraksha-2, Aroyga and Jwahart; in whole young leaves of these varieties, the respective artemisinin content is 0.8, 1.0–1.2 and 1.2%. These are poly-crop products of serial selections in the progeny of a cross between accessions with low concentrations of artemisinin. Plants of these varieties complete their life cycle in about one year in the Indo-Gangetic plains. Upon transplantation of 8-week-old seedlings in field in February, second generation seeds are ready for harvest in November. A multi-harvest rattoon cultivation technology has been standardized in which upper herbage is harvested four times to give an yield of 75 kg artemisinin.
inin/ha in contrast to a maximum yield$^{62,63}$ of artemisinin at 25 kg/ha reported from Brazil. New processes for the extraction of artemisinin and semisynthesis of artemether, arteether, artesunate and dihydroartemisinin have also been patented$^{58,64-67}$. It is widely realized that combined usage of the most cost-effective agricultural$^{56,57,63}$, extraction (Figure 3)$^{58,64,67-69}$ and semisynthesis$^{65,66,70,71}$ technologies developed in various laboratories worldwide can bring down the cost of artemisinin component of ACT treatment course to less than US$ 1.0.

Indian experience shows that farmers readily adopt industrial crops such as those of essential oil-yielding plants, if they are sure of selling their produce and making a profit$^{72}$. Farmers of the Indo-Gangetic plains who plant menthol mint after winter potato/wheat/Bengal gram crop in March/April, and harvest it before planting rice in July/August, have doubled their profits and India has become the main international supplier of mint oils$^{72,73}$. Farmers will grow A. annua if their produce will be picked up by industries/processors at pre-fixed price, yielding a profit of say US$ 100 to 250 per month of crop occupation period. A. annua cultivation schedules have also been developed for small farmers, who like to take food crops in their fields, particularly to save a part of the produce for their own use. Such farmers, for example, can follow the following rotations: potato–Artemisia–rice or potato–mint–Artemisia and produce A. annua herbage in each hectare of land planted, which is equivalent of about 40 kg artemisinin$^{74}$. All types of A. annua-farmers will have as bonus about half a tonne of woody stem and root biomass per kg of artemisinin extracted from their produce$^{75}$. There is need to crop A. annua in about 20,000 ha in different seasons and locations, to meet the initial requirements of setting up an international supply chain of artemisinin for the RBM programme.

India should join the global effort in RBM initiative by producing artemisinin in large amounts and thereby make possible the successful implementation of WHO goals. Indian public and private institutions should develop partnerships with their counterparts in other countries who will actively participate in the RBM endeavour. Our analysis of the world scenario further shows that in terms of the continued prevalence of malaria, need and supply of artemisinin for ACT formulations, developments taking place towards availability of artemisinin from genetically modified microorganisms and of synthetic structural analogoue, robustness of artemisinins as safe and stable antimalariais, exploration of artemisinins as a possible cure for certain
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cancers and protozoal, fungal and bacterial diseases, Indian S&T institutions should pursue with renewed vigour R&D in *A. annua* and its products. Indeed *A. annua* is a model plant system for biopharming and this plant can be a resource of its own, as well as heterologous products.

Briefly, the RBM programme requires 500 tonnes of artemisinin for use in ACT formulations. Deficiency in the supply chain of artemisinin can be remediated by cultivation of *A. annua* in India. By use of *A. annua*-related agronomic and processing-technologies developed in India, the cost of treatment course of ACTs is expected to come down to the desired and affordable level of US$ 1.0. *A. annua* plant can serve as a model for biopharming purposes.

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