ISSN 0974 – 5211

Journal of Natural Products

Volume 7 (2014)

www.JournalofNaturalProducts.Com

Review

Enhancement of *Artemisinin* production: a Biotherapeutic Agent

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(Received 08 November 2013; Revised 08 Jan -12 May 2014; Accepted 17 May 2014)

ABSTRACT

Artemisinin is an important and highly effective biotherapeutic agent in the plant, *Artemisia annua*. Its efficacy against malaria and many other infectious diseases has increases the demand of this drug in international market. The major problem with this drug is its short supply, mainly because the low level of artemisinin content in plant. Conventional breeders, biotechnologist and other researchers are trying to enhance artemisinin production.

Keywords: Artemisinin; *A. annua;* Metabolic pathways; Genetic engineering; Synthetic biology.

Abbreviations: ACTs- Artemisinin combination therapies; DDT- dichlorodiphenyltrichloroethane; AD- After death; BC-Before Christ; HPLC- High performance liquid chromatography; GC-Gas chromatography; GCMS-Gas chromatography–mass spectrometry; AEC-Artemisinin Enterprises Conference; MMV- Medicines for Malaria Venture; iOWH- Institute for One World Health; E-MAL-Eradication malaria.

INTRODUCTION

Malaria is a deadly disease producing 200–400 million new infections and 1–3 million deaths each year (WHO, 2013). This disease is occurring mainly in African and Asian countries and is spreading through mosquito which carries most virulent parasite *Plasmodium falciparum.* Initially, first line therapies were used to cure malaria but resistant to first-line drug therapies become increased. However, artemisinin- based combination therapies (ACTs) shows about 100% effectiveness against these drug-resistant parasites. Unfortunately, because of their high cost and low productivity, ACTs are still beyond the reach of poor people (Rinaldi, 2004). Artemisinin is an important antimalarial drug found in Chinese medicinal plant *Artemisia annua* L.

Earlier two major strategies were used to fight against malaria before artemisinin and other recent drugs: (i) spraying of DDT, but due to unavoidable side effects on the reproduction of animals and its persistence in food chains it was unsuccessful. As a consequence the occurrence of malaria is now spreading. (ii) The ample use of quinine and chloroquine for treatment of malaria and as prophylaxis. The mission to fight malaria in such situation become more difficult especially sub tropical countries. Moreover, it is anticipated that due to global warming, vector born diseases are moving towards high altitude. Therefore, there is a continuous search for new remedies against malaria (Rathore, et al., 2005; Wright, 2010).

The rise of modern molecular biology techniques provides a cutting edges technology platform to develop new technologies especially in the area of life threatened disease like malaria, cancer and AIDS etc. but these technologies rely on research and development which are more costly. However, such technologies also provide solutions to fight deadly disease. With this vision Bill &

Melinda Gates Foundation grand funds to Amyris Biotechnologies, the Institute for One World Health, and the University of California, Berkeley to produce artemisinin (an promising antimalarial drug) by using synthetic biology, industrial fermentation, chemical synthesis and drug development. This unique partnership is using innovative technology to reduce the cost of ACTs, thereby making these life-saving therapies more accessible to people in the developing world (AE report, 2008).

ARTEMISININ IN *ARTEMISIA ANNUA*

History: Based on the traditional medicinal usage for thousands of years, a number of modern drugs have also been isolated from natural sources like plants, bacteria, fungus etc. One of such plants is *Artemisia annua* L (2n=18; family Asteraceae). *A. annua* (commonly known as wormwood) has also been mentioned in the Bible (Rev 8:10,11). In 340 AD, Ge Hong prescribed aerial part of *Artemisia* for treatment of fever in the 'Chinese hand book of prescriptions for emergency treatments' and in 1527, Li Shi Zhen, a Chinese herbalist / pharmacologist mentioned the use of *huang hua hao* (or yellow flower, later identified as *A. annua*) for treatment of children's fever and qinghao (*A. apiacea*) as a treatment for disease, now known as malaria (Klayman, 1993). In 1967, Chinese researchers had evaluated the effectiveness of several traditional herbal remedies against malaria and found hot water or ethanol is extractable of *A. annua* to have no antimalarial effect (Klayman, et al., 1984; Write, et al., 2010). In 1971, a low temperature extraction of *A. annua* with diethyl ether yielded a complex having antimalarial activity on both infected mice and monkeys. The main active principle, named artemisinin was responsible for this activity; is a sesquiterpene lactone having natural endoperoxide (Anon, 1979).

Artemisinin and its derivatives: Artemisinin was isolated for the first time in 1972 and its structure was established by combined spectral, chemical and X ray analysis (Anon 1979; Liu, et al., 1979; Zhongshan, et al., 1985; Lepan, et al., 1988). It's simple and quick analytical method for estimation was developed by Gupta et al. (1996) and several other researchers. This compound contains an endoperoxide moiety, lacks nitrogen-containing heterocyclic ring system which is a rare feature in natural products (Fig 1). The peroxide bridge of artemisinin, can undergo electrochemical reduction, Acton, et al. (1985) developed HPLC detection procedure that can be used for sensitive selection and rapid assay of artemisinin in crude plant material. Non-volatile sesquiterpenes can be recovered from plant by solvent extraction, some of which show high antimalarial activity. There are about 20 known sesquiterpenes including artemisitene and artemisinic acid (Akhila, et al., 1990). Artemisinin and its related sesquiterpenes in *A.annua* can be analyzed by means of thin layer chromatography on silica gel plate. Artemisinin and its bioprecursors were determined by Vendenberghi, et al., (1995) and Gupta, et al., (1998) through HPLC. Microwave assisted extraction of artemisinin was reported by Hao, et al., (2002). Relative distribution of artemisinin in different parts of the plant was studied by Charles, et al., (1990).

Artemisinin is poorly soluble in water. The number of chemical extraction was developed by several researchers to evolve the procedure and derivatisation. Artemether, Arteethar and artesunate are most common derivatives of Artemisinin. Artemether is formed by conversion of dihydroartemisinin into methyl ester in a two-step procedure and is stable at room temperature (Valecha and Tripathi, 1997). It was first synthesized by Li Yin in 1978 (Jansen and Zhimin, 1997). Mohmad, et al., (1999) determined artemether in plasma through gas chromatography- mass spectrometry (GCMS).

Artesunate is an ester derivative of artemisinin (Barradell and Filton, 1995; Valecha and Tripathi, 1997) and is more soluble in water.

Artelinate / artelinic acid is prepared by two-step batches of 10g artemisinin; first by using reduction of artemisinin which is converted into dihydroartemisinin and then dihydroartemisinin is converted into a mixture of α-and β- artelinic acid (Shrimali, et al., 1998).

Arteether is prepared in two-steps using batches of 10 g artemisinin. The first step in the preparation of arteether involves reduction of artemisinin with sodium borohydride into dihydroartemisinin and subsequent esterification of dihydroartemisinin by Lewis acid catalysed reaction, affording an epimeric (80:20 mixture of β- and α- isomers) ether of dihydroartemisinin. Both the epimers are separated by column chromatography and crystallized to yield crystalline β arteether and oily α arteether (Jain, et al., 2000). The study of Dutta, et al., (1989) strongly advocated for the future clinical evaluation of arteether to control multiple drug resistance and high risk *Plasmodium falciparum* cases in the areas where chloroquine, mefloqunine and quinine resistant malaria occurs or increasing rapidly.Based on antimalarial activity, some old drugs are replaced by new drugs (Table 2).

ENHANCEMENT OF ARTEMISININ CONTENT BY DIFFERENT WAYS

Conventional breeding: The species *A. annua* is dominated in China and Vietnam, but it is also now grows in Tanzania, South Africa, India and Madagascar etc. The active pharmaceutical ingredient (API) or 'raw artemisinin' is extracted from the leaves, just before flowering, and from planting to extraction plant takes more than 9 months. Soil, climate, altitude and grower's knowledge can affect the content of artemisinin substantially. Problems related to scale-up production in large extent have been solved by selection of plants for high artemisinin content and adaptation to the environment in which they are to be grown.

Conventional researchers approach has been used to increase artemisinin content in plant but rate of success is very low (Delabays, et al., 1993). Multiple cross, top cross and poly cross design are generally used for genetic improvement in this crop. Indian Researchers have developed very challenging varieties of this plant e.g. Jewanraksha, Suraksh, and CIM Arogya (Paul, et al., 2014) using poly cross breeding. They have achieved 0.1% to 1.2% artemisinin and 0.8% artemisinin per hector. Due to high open pollinated behavior, recurrent selection using gene pool exploitation showed very successful strategy to improve this crop genetically (Kumar, et al., 1999; Khanuja, et al., 2008; Paul, et al., 2010; Graham, et al., 2010). High yielding varieties have enough potential to increase artemisinin in supply chain (Fig 2).

Generally in nature, synthesis of secondary metabolites is biased towards the physiological and environmental conditions (different types of stress) of plants (Paul and Shakhya, 2013). Physiological stress such as water deficit can trigger secondary metabolite synthesis which also reduces the time and cost of drying of crop and also increase crop profit margin (Jaleel, et al., 2007; Marchese, et al., 2010). Simultaneously, supply of micro and macro nutrient, vermi compost, nitrogen and phosphate also trigger artemisinin pathway specific genes (Ozguven, et al., 2008; Keshavarzi, et al., 2012). Reports suggested that addition of microbes such as *Azotobacter, Azospirillum, Pseudomonas* etc can also play an important role in artemisinin synthesis (Keshavarzi, et al., 2012). Plant growth regulators (Cytokinines, Auxins, GA3 etc.) and secondary messengers (Jasmonic acid Salisylic acid etc) also enhance aretemisinin content (Guo, et al., 2010). The accumulation of artemisinin content was also found in different species of *Artemisia* (Mannan, et al., 2010). Developmental stages were also affecting the artemisinin content. Optimum artemisinin content was observed at pre flowering stage and correlation coefficient between artemisinin content and density of capitate glands on the surface (0.987) was reported by Ferreira et al., (1992;1995). Exogenous applications of methyl jasmonate (MeJA) and 2-isopentenyladenine (2-iP) in artemisinin production and main genes of biosynthetic pathway was induced. Artemisinin content was increased in both the applications, but no correlation was found between gene expression and its content (Nugyen, et al., 2011)

BIOTECHNOLOGICAL TOOLS

Cell and tissue culture and genetic transformation: Cell/tissue culture is also a good approach for artemisinin enhancement but for large scale production, it may be quite cost effective. Hydroponics and *in vitro* plant culture are other interesting options (Jain, et al., 1996), which have not yet confirmed their potential but can play an important role to enhance artemisinin concentration (Martinaz and Staba,1988; Mathur and Kumar, 1996; Table 2). The *A. annua* shoots along with *Piriformospora indica* (Sharma and Agrawal, 2013), a mycorrhiza-like fungus and MS basal media with hormonal combinations showed significant enhancement in artemisinin content (Li, et al., 2012). The artemisinin content in plants regenerated from stem explants using 0.1 mg/l TDZ showed 0.36 μg/mg dry weight and two-fold higher than that of *in vitro* grown plants of same age of *A. annua*. This can be a potential system for a rapid propagation of shoots from stem explants and makes it possible to develop a clonal propagation of *A. annua* (Lualon, et al., 2008). Ri-mediated transformation of *A. annua* (Banerjee, et al., 1997) with a recombinant farnesyl diphosphate synthase gene for artemisinin production was reported by Chen, et al., (1999). The antisense squalene synthase (SQS) gene was transferred into *A. annua* via *Agrobacterium* mediated transformation, and artemisinin content in transgenic line was more in comparison to the control. The results demonstrated that inhibiting pathway competing for precursor of artemisinin by anti-sense technology is an effective means of increasing artemisinin content of *A. annua* plants. High efficiency of genetic transformation and regeneration of *Artemisia annua* L. via *Agrobacterium tumefaciens*-mediated procedure was reported by several workers (Ghosh, et al., 1997; Lui, et al., 2011). Supply of MeJA and 2-iP affect transcription of the main genes HMG-CoA reductase (HMGR), farnesyl pyrophosphate synthase (FDS), amorpha-4,11-diene synthase (ADS), cytochrome P450 monooxygenase (CYP71AV1), cytochrome P450 reductase (CPR), and artemisinic aldehyde

reductase (DBR2). In this study expression level of all the studied genes showed different level of expression at different time intervels (Nguyen, et al., 2011).

Expression of pathway specific genes in microorganism: Modern synthetic biology technique is a cost-effective solution for supply of artemisinin. The genetic engineering and recombinant DNA technology has potential to revolutionize the drug delivery systems. The production of artemisinin under the Artemisinin Project team, using synthetic biology to assemble the genes of biosynthetic pathway the plant A. *annua* into microbes. The Berkeley people and scientists at Amyris Biotechnologies are completing the synthetic biologic process to produce artemisinic acid, a precursor to artemisinin. Berkeley scientists are elucidating the gene of metabolic pathway of *A. annua* inserting this pathway into microorganisms and optimizing the resulting microbial strains for commercial production of precursor through fermentation (Tsuruta, et al., 2009). Over-expression of HMG-CoA reductase and amorpha-4,11-diene synthase genes in *A. annua* L. and its influence on artemisinin content (Alam and Abdin, 2011). *HMGR* and *FPS* genes were also use for enhancement of artemisinin (Wang, et al., 2011).

To complete the artemisinin biosynthetic pathway, amorphadiene undergoes three oxidation steps to form artemisinic acid. The discovery of *CYP71AV1* gene (Teoh, et al., 2006) is an important step which produces cytochrome P450s. In the entire process, yeast was used as screening platform, the single gene, *CYP71AV1*, which catalyzes all three oxidation steps, is required for conversion of amorphadiene into artemisinic acid. Through these steps artemisinic acid producing strain of yeast was formed. The endogenous farnesyl pyrophosphate (FPP) pathway, expressing *A. annua* genes, amorphadiene synthase, and *CYP71AV1* and its redox partner (CPR) was incorporated in yeast strain. Optimization of CYP71AV1 gene expression in microbial strain is still in the process to produce amorphadiene. In *A. annua* plant, artemisinic acid is subsequently oxidized to yield artemisinin (Arsenault, et al., 2008; Liu, et al., 2011). However, a singlet oxygen generating enzyme capable of peroxidizing artemisinic acid has not been identified. And it is still postulated that transformation of artemisinic acid to artemisinin is performed in a light-driven reaction. Hence, these steps are difficult to complete through microbial fermentation process. The required chemical conversion has been considered as further technique for this step. With this vision up to artemisinic acid was produced from microorganism and then converted chemically to artemisinin (Ro, et al., 2006) and than can be converted further into derivatives such as arteether, artemether and artesunate (Fig 3).

FUTURE POSSIBILITIES OF ENHANCEMENT/ PRODUCTION OF ARTEMISININ

In India, CIMAP (Central Institute of Medicinal and Aromatic Plants- CSIR) has developed number of varieties and transferred many technologies to many companies. Based on the Artemisinin Enterprise conference 2008 (four partners are actively involved in this discussions). University of York has described the project delivery in the form of increased artemisinin content up to 5% by 2015. The other partners of AE are iOWH and Barkley scientist their goal in this aspect is to ferment the genetically engineered yeast strain to produce arrtemisinic acid. This will be used as raw material for chemical synthesis of artemisinin. And the fourth partner MMV has described their part by doing clinical trial and launches the product by 2015. For this purposes currently many technologies has been used for artemisinin production (AE report, 2008; Fig 4).

CONCLUSION

Continuous research is going with the aim to enhance artemisinin production but artemisinin is still beyond the reach of poor peoples and *A. annua* is only source of artemisinin production. Due to its poor water soluble nature, derivatives of artemisinin in water soluble form have been developed which can be used as drug. In the entire process of drug development efforts involving phytochemistry coupled with semisynthetic approach to produce bioactive derivatives of artemisinin namely arteether (Jain, et al., 2000), artemether (Valecha and Tripathi, 1997), artesunate (Barradell and Filton, 1997), artelinate (Shrimali, et al., 1998) and dihydroartemisinin (Jain, et al., 2001), have opened up new avenues for a strategic battery of antimalarial phyto-molecules. Recently resistance to artemisinin and its derivatives has been reported from Cambodia (Noedl, et al., 2008; François, et al., 2009). Nevertheless artemisinins have still been crucial to recent successes in reducing the malaria

burden, and artemisinin- based combination therapies are essential to all plans for malaria elimination.

Acknowledgements: The authors are grateful to Director of G. B. Pant Institute of Himalayan and Environment Development (An autonomous institute of the Ministry of Environment and Forestry, MoEF, Govt. of India) Almora for Computer and library facility.

REFERENCES

Acton, N., Klayman, D.L., Rollman I.J., (1985): Reductive electrochemical HPLC assay for artemisinin 'quinghaosu'. *Planta Med*., 51: 445-446.

Akhila, A., Rani, K., Thakur, R.S., (1990): Biosynthesis of artemisinic acid in *Artemisia annua*. *Phytochemistry*, 29: 2129-2132.

Alam, P., Abdin, M.Z., (2011): Over-expression of HMG-CoA reductase and amorpha-4,11-diene synthase genes in *Artemisia annua* L. and its influence on artemisinin content. *Plant Cell Rep.*, 30(10):1919-28.

Anon., (1979): Quinghaosu antimalarial coordinating research group. Antimalarial studies on Quinghaosu. *Chin. Med. J.*, 92: 811-816.

Arsenault, P.R., Wobbe, K.K., Weathers, P.J., (2008): Recent advances in artemisinin production through heterologous expression. *Curr. Med. Chem*., 15(27): 2886.

Artemisinin Enterprises Conference. 2008 Report. www.york.ac.uk/org/cnap/.../pdfs/AE conference -report-web.pdf

Banerjee, S., Zehra, M., Gupta, M. M., Kumar. S., (1997): Agrobacterium rhizogenes-mediated transformation of *Artemisia annua*: production of transgenic plants. *Planta Med*., 63:467–469

Bangchangk, N.A., Karbwang, J., Thomas, C. G., (1994): Pharmacokinetics of artemether after oral administration to healthy Thaimales and patients with acute, uncomplicated falciparum malaria. *Ba. J. Clin. Pharmacol.*, 37: 249-253.

Barradell, L.B., Fitton, A., (1995): Artesunate. A review of its pharmacology and therapeutic efficacy in the treatment of malarial. *Drugs*, 50 (4): 714-741.

Batty, K.T., Davis, T.M.E., Thu, L.T.A., (1996): Selective high performance liquid chromatography determination of artesunate and α- and β-dihydroartemisinin in patients with falciparum malaria. *J. Chromatogr. B. Biomed. Appl.*, 677: 345-350.

Benakis, A., Paris, M., Plessas, C., (1993): Pharmacokinetics of sodium artesunate after iii and iv administration (abstract). *Am. J. Trop. Med. Hyg*., 293 Suppl: 293.

Brown, G.D., (1994): Secondary Metabolism in Tissue Culture of *Artemisia annua*. *J. Nat. Prod.*, 57 (7): 975–977

Cai, G., Li G.,Ye H., Li G., (1995): Hairy root culture of Artemisia annua L. by Ri plasmid transformation and biosynthesis of artemisinin. *Chin. J. Biotechnol*., 11: 4 227-235.

Charles, D.J., Simon, J.E., Wood, K.V., Heinstein, P., (1990): Germplasm variation in artemisinin content of *Artemisia annua* using an alterantive method of artemisinin analysis from crude plant extracts. *J. Nat. Prod.*, 53: 157-160.

Chen, D.H., Liu, C.J., Ye, H.C., Li, G.F., Liu, B.Y., Meng, Y.L., Chen, X.Y., (1999): Ri-mediated transformation of Artemisia annua with a recombinant farnesyl diphosphate synthase gene for artemisinin production. *Plant Cell Tissue Organ. Cult*., 57: 157-162.

Chen, D., Ye, H., Li, G., (2000): Expression of a chimeric farnesyl diphosphate synthase gene in Artemisia annua L. transgenic plants via Agrobacterium tumefaciens-mediated transformation. *Plant Sci*., 155:179–185

Delabays, N., Benakis, A., Collet, G., (1993): Selection and breeding for high atemisinin (qinghaosu) yielding strains of *Artemisia annua. Acta Hort.*, 330: 203-206.

Duc, D.D., de Verise, P.J., Khan N.X. et al., (1994): The pharmacokinetic of a single dose of artemisinin in healthy Vietnamese Subjects. *Am. J. Trop. Med. Hyg*., 51: 785-790.

Dutta, G.P., Bajpai, R., Vishwakarma, R.A., (1989): Antimalarial efficacy of Arteether against multiple drug resistant strains of *Plasmodium Yoelii* Nigeriensis. *Pharmacological Research*, 21(4): 415-419.

Ferreira, J.F.S., Janick. J., (1995a): Floral morphology of *Artemisia annua* with special reference of trichomes. *Int. J. Plant Sci.*, 156: 807-815.

Ferreira, J.F.S., Charles, D., Simon, J.E., Janick, J., (1992): Effect of drying methods on recovery and yield of artemisinin from *Artemisia annua* L. *Hort. Science* 27: 650 (Abstr 565).

François, N., Yi, P., Das, D., Phyo, A.P., Joel, T., Kmlafa,W. Hanpithakpong, Lee, S.J., Silamut, P.R.K., Imwong, M., Chotivanich, K., Lim, P., Herdman, T., Yeung, S.S.A.S., Nicholas, P.S., Day, P.J., Lindegardh, N., Socheat, D., White, N.J., (2009): Artemisinin Resistance in *Plasmodium falciparum* Malaria, *N. Engl, J. Med.*, 361: 455-467.

Fulzele, D.P., Sipahimalani, A.T., Heble, M.R., (1991): Tissue cultures of *Artemisia annua*: organogenesis and artemisinin production. *Phytother. Res.*, 5:149-153.

Fulzele, D.P, Heble, M.R, Rao, P.S., (1995): Production of terpenoid from Artemisia annua L. plantlet cultures in bioreactor. *J. Biotechnol.*, 40: 139-143.

Ganesan, C.M., Paulsamy, S., (2011): Standardized protocol for the *in vitro* culture of *Artemisia annua* L. A medicinal plant at high altitudes of Nilgiris, the Western Ghats. *J. Res. Bio.*, 3: 173-178

Ghosh, B., Mukherjee, S., Jha, S., (1997): Genetic transformation of *Artemisia annua* by *Agrobacterium tumifaciencs* and artemisinin synthesis in transformed cultures. *Plant Science*, 122:193-199.

Graham, I.A., Besser, K., Blumer, S., Branigan, C.A., Czechowski, T., Elias, L., Guterman, I., Harvey, D., Isaac, P.G., Khan, A.M., Larson, T.R., Li, Y., Pawson, T., Penfield, T., Rae, A.M., Rathbone, D.A., Reid, S., Ross, J., Smallwood, M.F., Segura, V., Townsend, T., Vyas, D., Winzer, T., Bowles, D., (2010): The Genetic Map of *Artemisia annua* L. Identifies Loci Affecting Yield of the Antimalarial Drug Artemisinin. *Science*, (327) 5963: 328-331.

Gulati, A., Bharel, S., Jain, S.K., Abdin, M.Z., Srivastawa, P.S., (1996): In vitro micropropagation and flowering in *Artemisia annua*. *J. Plant Biochem. Biotechnol*., 5: 31-35.

Gui, L. Q., Peggins, J.O., Fleckenstein, L.L., Masonic, K., Heiffer, M.H., Brewer, T.G., (1998): The pharmacokinetics and bioavailability of dihydroartemisinin, Arteether, Artmether, Artesunic acid and Artelinic acid in rats. *J. Pharm. Pharmacol.*, 50:173-182.

Gupta, M.M., Jain, D.C., Verma, R.K., Gupta, A.P., Bhartariya, K.S., (1998): Simultaeneous determination of artemisinin, artennuien B and artemisinic acid in *Artemisia annua* by High performance Liquid Chromatography with the combination of ODS and CN columns. *J. Medi. Arom. Plant Sci.*, 19: 960-972.

Hao, Jin-Yu., Wei, H., Shun-de, H., Bo-Yong, X., Xiu, D., (2002): Microwave assisted extraction of artemisinin from *Artemisia annua* L. *Separation and Purification Techonology, 28*: 191-196.

Heng, K.W., Chan, D.J.C., Chan, L.K., (2013): Production of cell biomass and artemisinin via batch cell culture of *Artemisia annua*. *Adva. Envir.Bio.*, 7(12): 3737-3742

Jain, D.C., Bhakuni, R.S., Gupta, M.M., Sharma, R.P., Kahol, A.P., Dutta, G.P., Kumar, S., (2000): Domestication of *Artemisia annua* plant and development of new antimalarial drug Arteether in India. *J. Sci. Indu. Res.*, 59: 1-11.

Jain, D.C., Bhakuni, R.S., Sharma, R.P., Kumar, S., Dutta, G.P., (2001): Formulation of dihydroartemisinin for the control of wide spectrum of malaria. United States Patent No 6,214,864

Jain, D.C., Mathur, A.K., Gupta, M.M., Singh, A.K., Verma, R.K., Gupta, A.P., Kumar, S., (1996): Isolation of high artemisinin yeilding clones of *Artemisia annua*. *Phytochemistry*, 43: 993-1001.

Jaleel, A., Manivannan, C.P., Sankar, B., Kishore, K.A., Gopi, R., Panneerselvam, R., (2007): Pseudomonas fluoresense enhances biomass yield and ajmalicine production *in Catharanthus roseus* under water deficit stress. Colloids and Surface B: *Biointerfaces*., 60: 7- 11.

Jansen, F.H., Zhimin, Y., (1997): Who discovered Artemisinin: In the meeting with Youyang Government at Wulingshan Pharma Factory, Chongqing. Fhj@dafra.be.

Jing, F., Zhang, L., Li, M., Tang, Y., Wang, Y., Wang, Y., Wang, Q., Pan, Q., Wang, G., Tang, K., (2009): Abscisic acid (ABA) treatment increases artemisinin content in *Artemisia annua* by enhancing the expression of genes in artemisinin biosynthetic pathway. Biologia, 64(2): 319-323.

Jones, A.M.P., Saxena, P.K., (2013): Inhibition of Phenylpropanoid Biosynthesis in *Artemisia annua* L.: A Novel Approach to Reduce Oxidative Browning in Plant Tissue Culture. *PLoS ONE*, 8(10): e76802. doi:10.1371/journal.pone.0076802

Kager, P.A., Schultz, M.J., Zijlstra, E.E. *et al*., (1994): Arteether administration in human: preliminary studies of pharmacokinitic safety and tolerance. *Trans. R. Soc. Trop. Med.Hyg*., 88: S1/31/S1/32.

Keshavarzi, M.H.B., Mousavi-Nik, S.M., Abdin, M.Z., (2012): The effect of biological and chemical fertilizers on chlorophyll and artemisinin content in *Artemisia annua L.* The 1th International and The 4th National Congress on Recycling of Organic Waste in Agriculture 26 – 27 April, Isfahan, Iran

Khanuja, S. P. S., Paul, S., Shasany, A.K., Gupta, A.K., Darokar, M.P., Gupta, M.M., Verma, R.K., et al., (2008): High artemisinin yielding plant genotype 'CIM-AROGYA'. US Patent 7,375,260.

Klayman, D.L., Lin, A.J., Acton, N., Scovill, J.P., Hoch, J.M., Milhous, W.K., Theorides, A.D., (1984): Isolation of artemisinin (quinghaosu) from *Artemisia annua* growing in the United States. *J. Nat. Prod.*, 47: 715-717.

Klayman, D.L., (1993): *Artemisia annua*: From weed to respectable ant malarial plant. P. 242-255. In: Kinghorn, A.D., Baladrin, M.F. (eds), *Human medicinal agents from plants.* Am. Chem. Soc. Symp. Ser. Washington, DC.

Kumar, S., Banerjee, S., Dwivedi, S., Gupta, M.M., Verma, R.K., Jain, D.C., Khanuja, S.P.S., Mathur, A.K., Bagchi, G.D., Zehra, M., Mehta, V.K., Naqvi, A.A., Paul, S., Govind, R., Ram, M., Saikia, D., Sangwan, R.S., Santha Kumar, T.R., Shasany, A.K., Darokar, M.P., Singh, A.K., Singh, A., (1999): Registration of 'Jeevanraksha' and 'Suraksha' varieties of the antimalarial medicinal plant *Artemisia annua*. *J. Medi. Arom. Plant Sci.*, 21: 47-48.

Lepan, I., Golic, L., Jupeij, M., (1988): Crystal and Molecular structure of Quinghaosu: a redetermination. *Acta Pharm. Jugosl*., 38: 71-77.

Li, J., Zhao, G-Z., Varma, A., Qin, S., Xiong, Z. et al., (2012): An Endophytic *Pseudonocardia* Species Induces the Production of Artemisinin in *Artemisia annua*. *PLoS ONE*, 7(12): e51410. doi:10.1371/journal.pone.0051410.

Liu, B., Wang, H., Du, Z., Li, G. Ye, H., (2011): Metabolic engineering of artemisinin biosynthesis in *Artemisia annua* L. *Plant Cell Rep.*, 30:689–694.

Lui, C.Z., *et al*., (1997): Production of artemisinin by hairy root cultures of *Artemisia annua* L. *Biotechnology letter*, 19: 927-929.

Liu, J.M., Ni, M.Y., Fan, J.F., (1979): Structure and reaction of arteannuin. *Acta Chem Sci*., 37:129-143.

Liu, C. Z., Hao**,** Chen, G., Yu, C., Wang., Fun, O., (2003): Comparision of various bioreactors on growth and artemisinin biosynthesis of *Artemisia annua* L. Shoot Culture. *Process Biochemistry*, 39: 45-49.

Liu, C. Z., Chen, G., Yu C., Wang, Fan, O., (2002): Effect of light irradiation on hairy root growth and artemisinin biosynthesis of *A. annua* L. *Process* Biochemistry, 38: 581-585.

Liu, C.Z., Yu C., Chen, G., Fan, O., He-chun, Y., Goa, F. L., (1998): Production of artemisinin by shoot culture of *Artemisia annua* L. in a modified inner loop mist bioreactor. *Plant Science*, 135: 211-217

Lualon, W., De-Eknamkulb, W., Tanakac, H., Shoyamad, Y., Putaluna, W., (2008): Artemisinin production by Shoot Regeneration of *Artemisia annua* L. Using Thidiazuron. Z. *Naturforsc.* 63c: 96D100.

Mannan, A., Ahmed, I., Arshad, W., Asim, M.F., Qureshi, R.A., Hussain, I., Mirza, B., (2010): Survey of artemisinin production by diverse *Artemisia* species in northern Pakistan. *Malaria Journal*, 9:310 doi:10.1186/1475-2875-9-310

Marchese, J.A., Ferreira, J.F.S., Rehder, V.L.G., Rodrigues, O., (2010): Water deficit effect on the accumulation of biomass and artemisinin in annual wormwood (*Artemisia annua* L., Asteraceae) *Braz. J. Plant Physiol*., 22(1): 1-9.

Martinez, B.C., Staba, J., (1988): The production of artemisinin in *Artemisia annua* L. tissue culture. *Adv.Cell Cult.*, 6: 69-87.

Mathur, A.K., Kumar, S., (1996): Micro-propagation of *Artemisia annua* via the inflorescence. *J. Herb Spices and Medi. Plants*, 4(1): 61-71.

Mohamed, S.S., Khalid, S.A., Ward, S.A., Wan, T.S.M., Tang, H.P.O., Zheng, M., Haynes, R.R., Edwards, G., (1999): Simultaneous determination of artemether and its major metabolite dihydroartemisinin in plasma by gas chromatography- mass spectrometry- selected ion monitoring. *J. Chrom.*, 251-260.

Nair, M.S., Acton, N., Klayman, D.L., Kendrick, K., Basile, D.V., Mante, S., (1986): Production of artemisinin in tissue cultures of *Artemisia annua*. J. Nat. Prod., 49(3):504-7.

Nguyen, K.T., Arsenault, P.R., Weathers, P.J., (2011): Trichomes + roots + ROS = artemisinin: regulating artemisinin biosynthesis in *Artemisia annua* L. *In Vitro Cell Dev Biol Plant*, 47(3): 329–338.

Noedl, H., Se, Y., Schaecher, K., Smith, B.L., Socheat, D., Fukuda, M.M., (2008): Evidence of Artemisinin-Resistant Malaria in Western Cambodia. *N. Engl. J. Med*., 359(24): 2619-2620

Ozguven, M., Sever, B., Orhan, I., Sekeroglu, N., Kirpic, M. Kartal, M., Pesin, I., Kaya, Z., (2008): Effects of varying nitrogen doses on yield, yield components and artemisinin content of *Artemisia annua L. Ind. Crops Prod*., 27: 60– 64.

Paniego, N.B., Giulietti, A.M., (1994): *Artemisia annua* L: dedifferentiated and differentiated cultures. *Plant Cell Tiss. Organ Cult.*, 36: 163-168.

Paul, S., Khanuja, S.P.S., Shasany, A.K., Gupta, M.M., Darokar, M.P., Saikia, D., Gupta, A.K., (2010). Enhancement of artemisinin content through four cycles of recurrent selection with relation to heritability, correlation and molecular marker in *Artemisia annua* L. *Planta Med.*, 76: 1468-1472.

Paul, S., Khanuja, S.P.S., Gupta, M.M., (2014): Breeding strategy for genetic improvement up to four generations in relation to artemisinin with canopy and other secondary metabolites in *Artemisia annua* L. *Ind. Crop Prod*.,56:67-73

Paul, S., Shakya, K., (2013): Arsenic, Chromium and NaCl induced artemisinin biosynthesis in *Artemisia annua* L.: A valuable antimalarial plant. *Ecotoxicology and Environment safety*, 98:59-65

Rinaldi, A., (2004): Fighting malaria at the crossroads: the tools to battle the disease exist, but the lack of political will in developed nations jeopardizes their success. *Embo Rep*., 5: 847-851.

Rathore, D., McCutchan, T.F., Sullivan, M., Kumar, S., (2005): Antimalarial drugs: current status and new developments. *Expert Opin Investig Drugs*., 14:871–883.

Ro, D.K., Paradise, E.M., Ouellet, M., Fisher, K.J., Newman, K.L., Ndungu, J.M., Ho, K.A., Eachus, R.A., Ham, T.S., Kirby, J., Chang, M.C.Y., Withers, S.T., Shiba, Y., Sarpong, R., Keasling, J.D., (2006): Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature*, 440:940–943.

Sharma, G., Agrawal, V., (2013): Marked enhancement in the artemisinin content and biomass productivity in *Artemisia annua* L. shoots co-cultivated with *Piriformospora indica*. *World J Microbiol Biotechnol.*, 29(6):1133-8

Shrimali, M., Bhattacharya, A.K., Jain, D.C., Bhakuni, R.S., Sharma, R.P., (1998): Sodium artelinate: A potential antimalarial. *Indian Journal of Chemistry*, 37B: 1161-1163.

Teoh, K.H., Polichuk, D.R., Reed, D.W., Nowak, G., Covello, P.S., (2006): *Artemisia annua* L. (asteraceae) trichome specific cDNAs reveal CYP71AV1, a cytochrome P450 with a key role in the biosynthesis of the antimalarial sesquiterpene lactone artemisinin. *FEBS Lett*., 580:1411–1416.

Titulaer, H.A.C., Zuidema, J., Kager, P.A., Wetsteyn, J.C.F.M., Lugt, C.B., Merkus, F.W.H.M., (1990): The pharmacokinetics of artemisinin after oral, intramuscular and rectal administration to volunteers. *J. Pharm. Pharmacol*., 42:810–813.

Tsuruta, H., Paddon, C.J., Eng, D., Lenihan, J.R., Horning, T., Anthony, L.C., Regentin, R., Keasling, J.D., Renninger, N.S., Newman, J.D., (2009): High-level production of amorpha-4,11-diene, a precursor of the antimalarial agent artemisinin, in *Escherichia coli*. *PLoS ONE*, 4: e4489.

Valecha, N., Tripathi, K.D., (1997): Artemisinin: Current Status in Malaria. *India Journal of Pharmacology*, 29: 71-75.

Vandenberghe, D.R., Vergauwe, A.N., VanMontagu, M., Vanden, E., (1995): Simultaeneous determination of artemisinin and its bioprecures *Artemisia annua. J. Nat. Pro.*, 58(5): 798-803.

Wang, Y., Jing, F., Yu, S., Chen, Y., Wang, T., Liu, P., Wang, G., Sun, X., Tang, K., (2011): Co-overexpression of the *HMGR* and *FPS* genes enhances artemisinin content in *Artemisia annua* L. *J. Med. Plants Res.*, 5(15): 3396-3403.

Weathers, P.J., Bunk, G., McCoy, M.C., (2005): The effect of phytohormones on growth and artemisinin production in *Artemisia annua* L. hairy roots. *In vitro Cell Dev. Biol*.- Plant, 41: 47-53.

Weathers, P.J., DeJesus-Gonzalez, L., Kim, Y.J., Souret, F.F., Towler, M.J., (2004): Alteration of biomass and artemisinin production in *Artemisia annua* hairy roots by media sterilization method and sugars. *Plant Cell Rep*., 23: 414-418.

Weathers, P.J., Hemmavanh, D.D., Walcerz, D.B., Cheetham, R.D., Smith, T.C., (1997): Interactive effects of nitrate and phosphate salts, sucrose, and inoculum's culture age on growth and sesquiterpene production in *Artemisia annua* L. hairy root cultures in vitro. *Cell Dev. Biol.-Plant*, 33:306-312.

WHO, (2013): World Health Organization report, http://www.who.int/malaria/publications/world_malaria_report_2013/report/en/

Wright, C.W., Linley, P.A., Brun, R., Wittlin, S., Hsu, E., (2010): Ancient Chinese Methods Are Remarkably Effective for the Preparation of Artemisinin-Rich Extracts of Qing Hao with Potent Antimalarial Activity. *Molecules*, 15: 804-812.

Woerdenbag, H.J., Lüers, J.F.J., van Uden, W., Pras, N., Malingré, T.M., Alfermann, A.W., (1993): Production of the new antimalarial drug artemisinin in shoot cultures of *Artemisia annua* L. *Plant Cell, Tissue and Organ Culture*, 32(2): 247-257.

Zhao, K.C., Song, Z.Y., (1993): Pharmacokinetics of dihydroqinghaosu in human volunteers and comparison with qinghaosu. *Acta Pharmaceutical Sinica*, 28: 342-346.

Zhao, S., (1987): High performance liquid chromatographic determination of artemisinin (qinghaosu) in human plasma and saliva. *Analyst.*, 12: 661-664.

Zhongshan, W., Naksnima, T.T., Kopecky, K.R., Molina, J., (1985): Qinghaosu: H and C^{13} nuclear magnetic resonance spectral assynments and Luminiscence. *Can. J. chem*., 63: 3070-3074.

Zhou, Z.M., Huang, Y.X., Xie, G.H., Sun, Y., Wang, Y., Fu, L., Jian, H., Guo. Z., Li, G., (1988): HPLC with polarograph detection of artemisinin and its derivatives and application the method to the pharmacokineticc study of artemether. *J. Liq. Chromatogr*., 11: 1117-1137.

Table- 1: Pharmacokinetics of artemisinin and its derivatives.

S. N.	Drug type	Dose	Assay	Cmax (µg/L)	Tmax (h)	$T^{1/2}\beta$ (h)	Reference(s)
1	**Artemisinin**						
	Oral administratiom						
	12 (HM)	500mg	HPLC-ED	391	1.80	2.59	Duc, et al., (1994);
	11(MP)	500mg	HPLC-ED	364	2.90	2.70	
	10(HM)	400mg	HPLC-UV	260	1	1.90	Titulaer, et al., (1990);
		400mg IM	HPLC-UV	209	3.40	3.83	
	1(HM)	10mg/kg	HPLC-UV	1100	=4		Zhao, et al., (1987);
	19(MP)	500mg day 1	HPLC-UV	588	2.4	2.3	Hassan, et al., (1996);
	11(MP)	500mg day 6		116	3.1	2.2	
	Rectal administration		RIA				Zhao, et al., (1993);
	5 (HM)	15mg/kg	HPLC-ED	40	4.6	4.8	Zhou, et al., (1998)
	9(HM)	10mg/kg	HPLC-ED	170	11.3	4.1	
	6(MP)	10mg/kg		180	9.6	4.0	
2	**Dihydroartemisinin**						
	3(HM)	1.1mg/kg PO	RIA	130	1.33	1.63	Zhao, et al., (1993);
	3(HM)	2.2mg/kg PO	RIA	710	1.33	1.57	
	5(HM)	8mg/kg PR	RIA	100	4.70	4.82	Bangchangk, et al.,
	6(HM)	200mg artemether	HPLC-UV	379	6	10.6	(1994);
	6(MP)	200mg artemether	HPLC-UV	593	7.40	12.5	Benakis, et al., (1993);
	6(Severe MP)	2mg/kg artesunate IM	HPLC-ED	390		1.59	Batty, et al., (1996)
	6(Severe MP)	2mg/kg artesunate IV	HPLC-ED	2020		1.59	
	6(MP)	120mgartesunate IV	HPLC-UV	/200		0.57	
3	**Artesunate**						
	6 (Severe MP)	2mg/kg IM	HPLC-ED	510		0.49	Benakis, et al., (1993);
	6 (Severe MP)	2mg/kg IV	HPLC-ED	2640+1		0.49	Batty, et al., (1996)
	6 (MP)	120mg IV	HPLC-UV	800		3.5 MIN	
4	**Artemether**						
	6(HM)	200mg PO	HPLC-UV	118	3	3.1	Bangchang, et al.,
	6(MP)	200mg PO		231	3	4.2	(1994);
	6(HM)	6mg/kg	HPLC-ED	145	5.2	7.7	Zhou, et al., (1988)
	6 (HM)	10mg/kg	HPLC-ED	224	6.3	11.1	
5	**Arteether**						
	1(HM)	3.6mg/kg IM	HPLC-ED			23	Kager, et al.,(1994)
	1(HM)	3.6mg/kg and1.6mg/kg IM at 24,48,72,96hrs	HPLC-ED			69.30	
6	**Artelinic acid**						
		10mg/kg IV	HPLC-EC	12706+		1.35	Gui, et al., (1998)
		10mg/kg IM	HPLC-EC	1010	11.3	2.13	
				8032+1			
				011			

- Abbreviation:- Cmax = peak plasma drug concentration, HM= healthy male volunteers, HPLC(ED, UV)= high performance liquid chromatography (Electrochemical detection, ultraviolet detection), IV intra-venous, MP= patient with *falciparum* malaria, PO= oral, PR= rectal, RIA= radioimunoassay, $T^{1/2}\beta$ = elimination half life, T max = time to Cmax.

Table- 2: Enhancement of artemisinin content using different tissue culture methods.

S. N.	Work done	References
1	Regeneration of plants from *Agrobactrium rhizogenes* transformed hairy roots.	Banerjee, et al., (1997)
2	Relationship of artemisinin content of tissue cultured, greenhouse, grown and field grown plants of *Artemisia annua*	Ferreira, et al., (1997)
3	Tissue culture of *Artemisia annua*: Organognesis and artemisinin production.	Fulzele, et al., (1991)
4	Production of terpenoids from *Artemisia annua* L. plantlet cultures in bioreactors.	Fulzele, et al., (1995)
5	Effect of light irradiation on hairy root growth and artemisinin biosynthesis of *A. annua* L.	Liu, et al., (2002)
6	Comparison of various bioreactors on growth and artemisinin biosynthesis of *Artemisia annua* L. Shoot Culture	Liu, et al., (2003)
7	Production of artemisinin by shoot culture of *Artemisia annua* L. in a modified inner loop mist bioreactor.	Liu, et al., (1998)
8	Production of artemisinin by hairy root cultures of *Artemisia annua* L	Lui, et al., (1997)
9	Micropropagation of *Artemisia annua* via the inflorencence.	Mathur and Kumar, (1996)
10	*Artemisia annua* L: dedifferentiated and differentiated cultures.	Paniego and Giulietti, (1994)
11	Production of the new antimalarial drug artemisinin in shoot cultures of *A. annua* L.	Woerdenbag, et al., (1993)
12	Secondary Metabolism in Tissue Culture of *Artemisia annua*	Brown, (1994)
13	Production of artemisinin in tissue cultures of Artemisia annua.	Nair, et al., (1986)
14	Standardized protocol for the *in vitro* culture of *Artemisia annua* L. – A medicinal plant at high altitudes of Nilgiris, the Western Ghats.	Ganesan and Paulsamy, (2011)
15	Hairy root culture of Artemisiaannua L. by Ri plasmid transformation and biosynthesis of artemisinin	Cai, et al., (1995)
16	Ri-mediated transformation of Artemisia annua with a recombinant farnesyl diphosphate synthase gene for artemisinin production	Chen, et al ., (1999)
17	Expression of a chimeric farnesyl diphosphate synthase gene in Artemisia annua L. transgenic plants via Agrobacterium tumefaciens-mediated transformation	Chen, et al., (2000)
18	In vitro micropropagation and flowering in Artemisia annua.	Gulati, et al., (1996)
19	Abscisic acid (ABA) treatment increases artemisinin content in Artemisia annua by enhancing the expression of genes in artemisinin biosynthetic pathway.	Jing, et al., (2009)
20	The effect of phytohormones on growth and artemisinin production in Artemisia annua L. hairy roots.	Weathers, et al., (2005)
21	Alteration of biomass and artemisinin production in Artemisia annua hairy roots by media sterilization method and sugars	Weather, et al., (2004)
22	Interactive effects of nitrate and phosphate salts, sucrose, and inoculum's culture age on growth and sesquiterpene production in *Artemisia annua* L. hairy root cultures in vitro.	Weather, et al., (1997)
23	Inhibition of Phenylpropanoid Biosynthesis in *Artemisia annua* L.: A Novel Approach to Reduce Oxidative Browning in Plant Tissue Culture.	Jones and Saxena, (2013
24	Production of cell biomass and artemisinin via batch cell culture of *Artemisia annua*	Heng, et al., (2013)

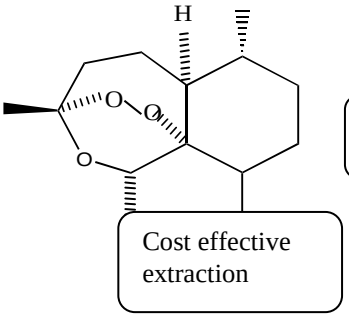

Figure- 1: Structure of artemisinin.

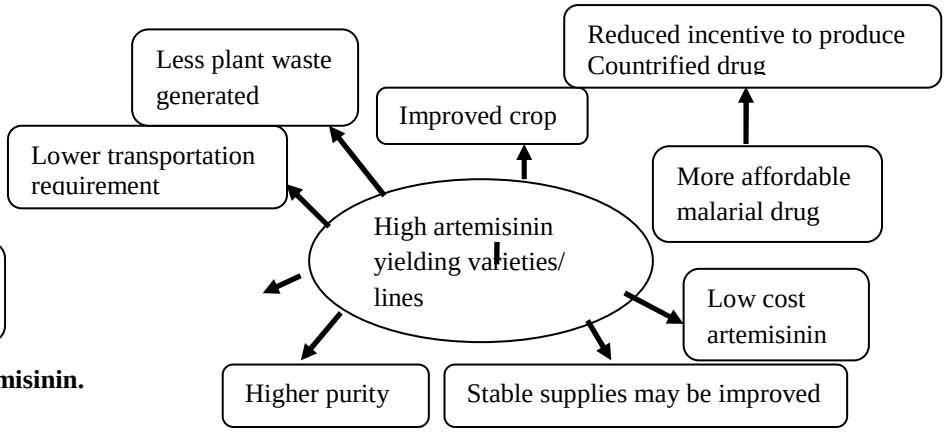

Figure- 2: Showed chain of production of artemisinin from high yielding varieties (AE report 2008).

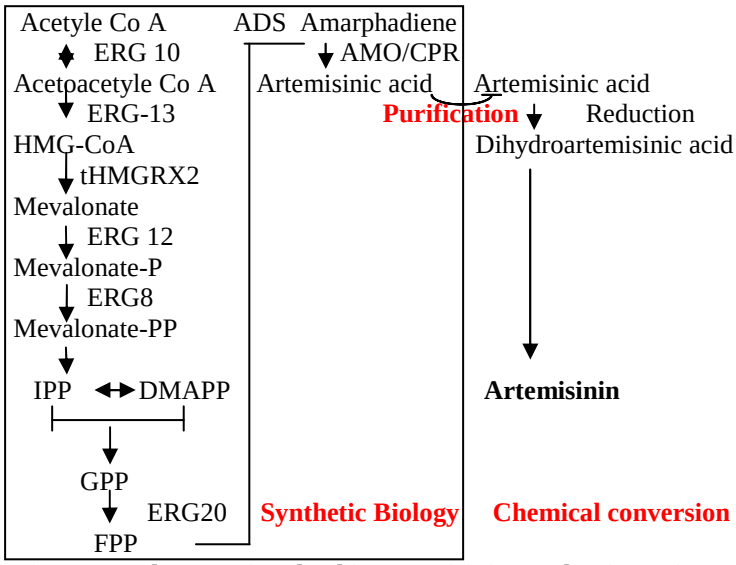

Figure- 3: The steps involved in artemisinin production using synthetic biology and chemical conversion (Ro et al., 2006).

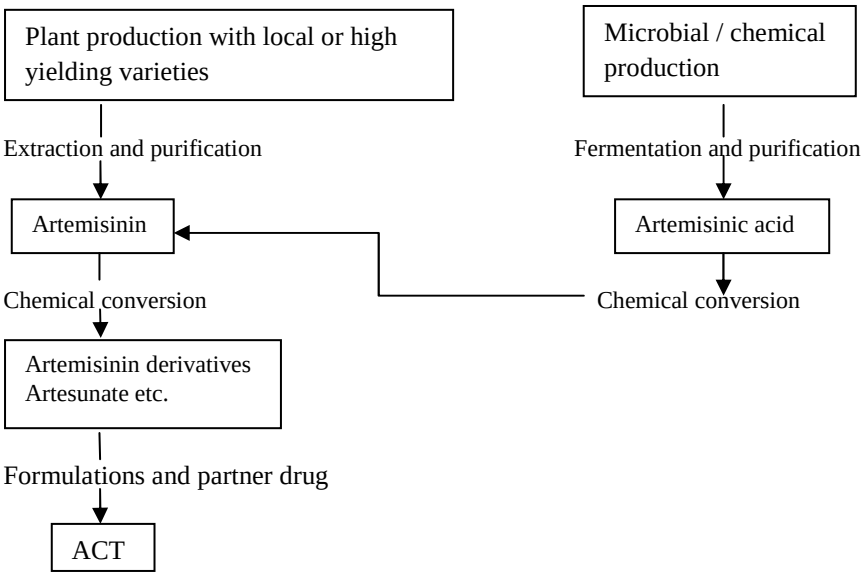

Figure- 4: Current technology used for artemisinin production (AE Report, 2008).