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Antimalarial Drug – *Artemisia annua*

Namdeo A.G*. Mahadik K. R and Kadam S.S.

Bharati Vidyapeeth Deemed University, Poona College of Pharmacy,
Erandwane Educational Campus,Pune 411 038
**Corresponding author: ajay_namdeo@rediffmail.com*

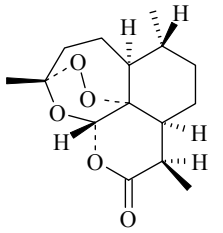
ABSTRACT - The objective of this review is to highlight the importance of anti-malarial compound artemisinin, a sesquiterpene from Chinese plant *Artemisia annua* L. It has been used for centuries as a folk remedy in China for the chills and fever caused by malaria. Artemisinin and its derivatives are effective against both chloroquine-resistant and sensitive strains of *P. falciparum*. Many derivatives of dihydroartemisinin (artemether, arteether, artesunic acid and artelinic acid) are either in use or being evaluated for use. Low yield of artemisinin in plant, increasing demand and extremely difficult synthesis in laboratory has motivated scientists worldwide to investigate an economical alternative.

KEY WORDS: Anti-malarial, artemisinin, *Artemisia annua, P. falciparum,* chloroquin-resistant.

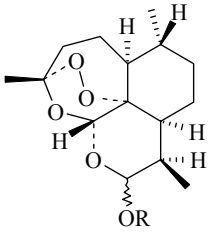
INTRODUCTION

Malaria is the world's most dangerous parasitic infection, causing more than a million deaths and 500 million cases annually (1). Malaria is the classical example of a disease that affects the productivity of individuals, families and the whole due to morbidity and mortality (2). The first antimalarial drug was quinine, isolated from the bark of Cinchona species (Rubiaceae) in 1820. In 1940, another antimalarial drug chloroquin was synthesized and until recently, this was the only used for the treatment of malaria. Unfortunately, after an early success, the malarial parasite *Plasmodium falciparum* became resistant to chloroquin. Treatment of chloroquin-resistant malaria was done with alternative drug or drug combinations, which were rather expensive and sometimes toxic. Furthermore, these combinations were not always based on pharmacokinetic principles due to inadequate knowledge of metabolism and mechanism of action of most antimalarial drugs. Artemisinin and its derivatives represent a new class of antimalarial that is effective against drug-resistant *P. falciparum* strains and therefore they are of utmost importance in the current antimalarial campaign (3, 4).

Artemisinin (Fig. 1) or Qinghaosu is a sesquiterpene lactone endoperoxide isolated from the Chinese herb *Artemisia annua* L. (Asteraceae) (Fig. 2.). The genus *Artemisia* includes ca 400 species. Many of these species have been screened for the presence of artemisinin but only *A. annua* and to a lower extent *A. apiacea* were found to produce artemisinin. Sweet or annual wormwood, *A. annua* is an annual herb native to Asia, most probably China. The plant has been used in traditional Chinese medicine as a remedy for chills and fevers for more than 2000 years. The plant now grows wild in many countries (5). Artemisinin was isolated in pure form in 1972, and its structure was determined in 1979 (6). Artemisinin has been reported to accumulate in leaves, small green stems, buds, flowers and seeds (7-9). Duke et. al. (10) showed that artemisinin is accumulated in the glandular trichomes of *A. annua*.



Artemisinin



Semi-synthetic derivatives of artemi

R = H	artemisinin
R = CH_3	artemether
R = CH_2CH_3	arteether
R = $COCH_2CH_2COONa$	artesunate

Figure 1. Structure of Artemisinin and its derivatives

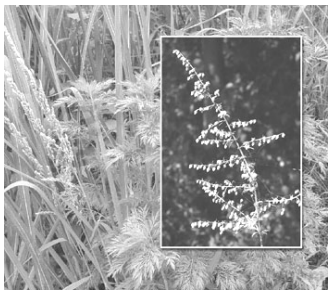


Figure 2. The Anti-malarial drug: Artemisia annua

Now artemisinin and its derivatives have been recognized as a new generation of powerful antimalarial drug for combating the most popular infectious disease malaria worldwide. Artemether and artesunnate were approved by the Chinese authority and collected in the "Essential Medicine List" by WHO. These derivatives have been successfully applied to remedy several million malaria-suffering patients since their advent. Meanwhile many research papers have been published to record rapid progress of artemisinin research from different disciplines of botany, chemistry, pharmacology, and clinical medicine etc.

Qinghao has been used as a traditional medicine for at least 2000 years in China (11). The earliest written record in silk so far discovered is the "Recipes for 52 kinds of diseases", which was unearthed from the Mawangdui Tomb of the West Han Dynasty (168 BC) in Changsha, Hunan Province (12). The first record of qinghao for the treatment of malaria (fever) was described in "The Handbook of Prescriptions for emergency treatments" written by Ge Hong (AD 281-340) Since then a series of Chinese medicine books including the most famous book "Compendium of Medical Herbs" (Bencao Gangmu) by Li Shizen in 1596, have described the application of qinghao for fever remedy. In the phytotaxonomy qinghao is *Artemisia annua* L. Composites, so Qinghaosu is also named as artemisinin or seldom as arteannuin.

The structure determination of qinghaosu was performed by a joint research group consisted of the researchers from the institute of Chinese Materia Medica and Shanghai Institute of Organic Chemistry during the mid 1970's (13,14). It was easy to propose that this compound seemed to be a sesquiterpene from ^{1}H NMR, ^{13}C NMR, HRMS, and elemental analysis, hence the molecular weight was 282 and the molecular formula was $C_{15}H_{22}O_5$. Following the discovery of artemisinin from qinghao in the early 1970's, *A. annua* has become one of the most extensively investigated thereafter. Qinghaosu is identified in all *A. annua* plant from different geographical origin while its contents are varied drastically with its growing area and stages of plant development. Qinghaosu is present in the leaves and flowers of *A. annua* in ~ 0.01-1.1 % dry weight, with the highest content just before flowering. Apart from *A. annua* qinghaosu was detected in only other species, i.e. *A. apiacea* (15). Different *A. annua* materials including the leaves, stems/ flowers, roots and seeds as wells endophytes inside *A. annua* have been employed for phytochemical investigations. More than 150 natural products have been reported belonging to different chemical structure types.

From indigenous *Artemisia annua* L., continuous phytochemical studies by Chinese researchers in the early 1980s led to the excavation of another 10 sesquiterpenes including deoxy-artemisinin (16), artemisinin D (17), artemisinin F (18), artemisinin E (19), artemisinin A (16, 17), epoxyarteannuinic acid (19), artemisinic acid (20, 21), artemisinic acid methyl ester (22) artemisinol (22) and arteannuin B (16). From biogenetic viewpoint, artemisinic acid or its11, 13-dihydroanalogue, dihydro-artemisinic acid which was isolated later from *A. annua* is late precursors in the biogenesis of qinghaosu. By the year 1991, 16 closely related sesquiterpenes had been isolated from aerial part of *A. annua* and briefly summarized by Zaman and Sharma. (23). A bisnor-sesquiterpene, norannuic acid was reported in 1993 (24) and three more sesquiterpenes were isolated and reported in 1994 by Misra et al. (25). Sy et. al. (26) isolated seven new sesquiterpenes in 1998. Two amorphane sesquiterpenes, deoxyarteannuin B and dihydro-deoxyarteannuin B were introduced to sesquiterpene family isolated from aerial pars of *A. annua* in 2001. Recently, the first phytochemical investigation of natural products from the seeds on *A. annua* was conducted by Brown et. al. led to discovery of fourteen new sesquiterpenes (27). In addition, two sesquiterpene plant hormones, abscisic acid and its methyl ester were found in an Indian growing *A. annua* (28).

Apart from sesquiterpenes from *A. annua*, essential oils are another active research interest as it could be potentially used in perfumery, cosmetics and aromatherapy. Depending on its geographical origin, the oil yield in *A. annua* ranges between 0.02-0.49% on fresh weight basis and 0.04-1.9% on dry weight basis (29). Major components in the oil were reported to be artemisia ketone, isoartemisia ketone, 1, 8-cineole and

camphor. Up to date, 46 flavonods have been isolated from *A. annua* (30-33). Other chemical compounds in *A. annua* includes carbohydrates, traces of glycosides, resins etc.

PHARMACOLOGY

Antimalarial drugs derived from natural *Artemisia annua* L. have many advantages: quick reduction of fevers, fast clearing parasites in blood (90% of malaria patients recovered within 48 hrs) and no significant side effects. Experimental and clinical studies reveal that artemisinin, Artemether and artesunnate are not only the potent antimalarial drugs but also the useful agents for other disease, especially as antiparasitic agent. In 1970s, Artemether and artesunnate were confirmed to be more active than artemisinin in animal models (34-36). Some components of *A. annua* Such as qinghaosu, artemisinin B, artemisinic acid, artemisitene, flavonoids and other terpenoids, showed antitumor activities at varying concentrations against L-1210, P-388, A-549, HT-29, MCF-7 and KB *in vitro* (37-39). It was found that dihydroartemisinin can selectively kill cancer cells in presence of holotransferrin, which can increase intracellular iron concentrations, and normal breast cells (HTB 125) and lymphocytes had non-significant changes. It seems the mechanisms of anticancer action and of antimalarial activity are similar (40-42). Lin, PY et al. (43) suggested that qinghaosu, artesunnate and artemether had both immunosuppressive and immunostimulating activities.

The outstanding antimalarial activity, the unique structure and relatively low yield (0.01-0.8%) of artemisinin in *A. annua* are serious limitations for commercialization of the drug has attracted great attention in the research area of chemical synthesis.

Various attempts for the chemical synthesis of artemisinin have been reported. Schmid and Hofheinz (44) converted (-) – isopulegol to artemisinin in 13 synthesis steps with an overall yield of 5%. Xing-Xiang Xu et. al. (45) reported a scheme for artemisinin synthesis, where the starting material was (+) – citronellal. Avery et. al. (46) used 10 step synthetic route with R-(+)-pulegone as the starting compound, which involves the ozonolysis of a vinylsilane. These syntheses are very complex and not feasible for large-scale production of artemisinin.

Studies have been conducted in various laboratories to elucidate the biochemical pathway of artemisinin and its regulation with an aim to improve artemisinin content of the plants. Akhila et. al. (47, 48) proposed a biosynthetic pathway for artemisinin. The following biosynthetic sequence is suggested: farensyl pyrophosphate (FPP) → germacrane skeleton → dihydrocostunolide → cadinanolide → arteannuin B → artemisinin. But their studies did not indicate artemisinic acid as a precursor of artemisinin. Sangwan et. al. (49) achieved *in vitro* and *in vivo* transformation of artemisinic acid to arteannuin B and artemisinin. Kim and Kim (50) reported the transformation of dihydroartemisinic acid into artemisinin by cell-free extracts from *A. annua*.

In view of low concentrations of artemisinin detected in the plants, tissue culture systems have been used with great interest as alternative methods for the production of this drug. However, the results so far are not very encouraging. The first trial of using tissue culture for artemisinin production came from He et. al. (51). The yield was 0.008% in shoots from callus of *A. annua* plants of Chinese origin. Simon et. al. (52) reported concentration ranging from 0.03% to 0.05% on dry weight (DW) basis. Results from experiments with undifferentiated callus and cell suspension cultures of *A. annua* are disappointing with respect to artemisinin production (53-56). Most workers (50-57) did not detect artemisinin in roots. Attempts were also made to improve the artemisinin production by omittence or addition of medium components like plant growth regulators (58), casein hydrolysate (59), by inclusion of precursors of artemisinin biosynthetic pathway and by addition of sterol synthesis inhibitors (60). Several attempts have been made for selection and breeding of high yielding strains of *A. annua* (61-63). Attempts to transform the *A. annua* plants using *Agrobacterium rhizogenes* (64, 65) resulted in transformed hairy roots.

As a response to increasing levels of antimalarial resistance, WHO recommends that all countries experiencing resistance to conventional mono therapies should use combinations use combination therapies preferably those containing artemisinin derivatives (ACTs- Artemisinin based Combination Therapies) for *falciparum* malaria. WHO currently recommends the following therapeutic options:

- artemether
- artesunate + maodiaquine
- artesunate + sulphdoxine/ pyremethamine
- artesunate + mefloquine
- amodiquine + sulphaodxine-pyrimethamine

Malaria is a highly treatable disease, and very effective treatment s available in the form of Artemisinin based Combination Therapies (ACTs). WHO call on all “Roll Back Malaria (RBM) “ partners to unite in a global

coalition to enable countries to accelerate access to ACTs and make these lifesaving medicines affordable to the people in need (4).

CONCLUSION

Presently, the only commercial source of the drug is extraction from field-grown leaves and flowering tops of *A. annua*, which are subjected to seasonal and somatic variations. Although the complete organic synthesis has been established, it is not yet economically feasible because of its complexity and low yield of active compounds. For these reasons, it is, essential to look for non-conventional, alternate strategies, which are economically viable for the commercial production of artemisinin.

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