Malaria

International Edition: DOI: 10.1002/anie.201601967
German Edition: DOI: 10.1002/ange.201601967

Artemisinin—A Gift from Traditional Chinese Medicine to the World (Nobel Lecture)**

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artemisinin · malaria · traditional Chinese medicine

1. Introduction

1.1. Malaria

Malaria has long been a devastating and life-threatening global epidemic disease in human history. Hippocrates, a Greek physician, described the disease as "marsh fevers", "agues", "tertian fevers", "quartan fevers", and "intermitten fevers" in his treatise *On Airs, Waters, and Places* in 400 B.C.[1] A detailed description of malaria symptoms can also be found in *Huangdi Neijing, Inner Casnon of the Yellow Emperor*, written between the Chun Qiu and Qin Dynasties, 770–207 B.C., one of the earliest reports of traditional Chinese medicine.[2]

Since malaria commonly originated and spread in the humid areas surrounding marshes and swamps, the disease was considered to be associated with the "bad air" hovering around the region, which is how the word "malaria"—a combination of medieval italian "mal" (bad) and "aria" (air)—was derived.[3]

It was not known that the disease was caused by parasites until a French scientist, Charles Louis Alphonse Laveran, discovered the single-celled Plasmodium parasite in blood smears from malaria patients in 1880.[4] In 1897, Ronald Ross, a British military doctor, found Plasmodium "eggs" (oocysts), in the guts of female mosquitos and later verified that Anopheles mosquitos were responsible for the transmission of malaria parasites between subjects.[5] These findings explained how the disease was transferred from malaria patients to the healthy population through a vector—female Antopheles mosquitos. Laveran and Ross both received the Nobel Prize in Physiology or Medicine in recognition of their exceptional contributions in understanding the origins of malaria.

There are over one hundred species of *Plasmodium*. Five of them infect humans, with *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium vivax*, and deadly *Plasmodium falciparum* causing malaria whereas *Plasmodium knowlesi* hardly poses any threat to humans. Camillo Golgi, an Italian scientist and Nobel Laurate, raised the idea of differentiating the *Plasmodium* species in 1886 when he demonstrated the correlation between the periodicity of the paroxysm (chill and fever pattern in the patient) with the 72-hour life cycle in the development of *Plasmodium malariae*. From the observation of 48-hour development cycles from the other patients, he came to a conclusion that there must be more than one species of malaria parasite responsible for these different patterns of cyclical infection.[6]

Human malaria symptoms are closely associated with the complex life cycle of malaria parasites. Malaria parasites present as sporozoite, merozoite, gametocyte, gamecyte, and oocyst through their life cycle, either in the vector (the definitive host) or in the infected subjects, for example, humans (the secondary host). Healthy individuals are infected by invasion of the thread-like sporozoites after being bitten by mosquitos. The sporozoites then, through the blood circulation, enter the liver cells, where each sporozoite develops into a schizont containing thousands of tiny rounded merozoites over a period of one or two weeks. The schizont releases the merozoites into the bloodstream when it matures and bursts. For some malaria species, for example, *Plasmodium vivax* and *Plasmodium ovale*, some sporozoites will develop into hypnozoites, which can reside in the liver for months or years before developing into schizonts. This causes relapses in infected people. The merozoites, once escaped from the liver cells into the blood stream, are taken up by the red blood cells where they asexually produce new infective merozoites until the red cells burst, which initiates another round of asexual multiplication. Some of the merozoites develop into gametocytes that, once taken by female *Anopheles* mosquitos through a blood meal, mature to form sperm-like male gametes or large, egg-like female gametes. Fertilization of gametes produces an oocyst filled with infectious sporozoites in the mosquitos' guts. The oocyst then bursts and releases sporozoites, which migrate to the mosquitos' salivary glands, ready for transfer to the next victims. Since all forms of *Plasmodium* parasites are hidden in either liver or red cells during most of their life cycles and well camouflaged from the immune system, it is more challenging to trigger defense through either natural immune response or vaccination.[7]

Treatment of malaria relies on chemotherapy by using medicines that act on various phases of the *Plasmodium* parasites in their life cycles. These medicines include quinoline compounds, sulfadoxine/pyrimethamine, mefloquine (Lariam), lumefantrine, doxycycline, artemisinin, and artemisinin-based combination therapies (ACTs). Most com-

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monly used ACTs consist of an artemisinin component plus other antimalarial drugs such as mefloquine (ASMQ), lumefantrine (Coartem), amodiaquine (ASAQ), piperaquine (Duo-Cotecxin), and pyronaridine (Pyramax).

Vector control, such as the use of insect repellants, insecticide-treated mosquito nets (INTs), indoor residual spraying, as well as eliminating stagnant water is still the main approach for malaria prevention, although some malaria vaccines are under development. Some preventative medicines, for example, chloroquine, doxycycline, mefloquine (Lariam), primaquine, and a combination of atovaquone and proguanil (Malarone) may be used, should prophylaxis be deemed necessary.[8]

To promote the early diagnosis and effective treatment of malaria, the WHO published a third edition of *Guidelines for the treatment of malaria* in April 2015. The guidance recommends that "All cases of suspected malaria be confirmed using parasite-based diagnostic testing (either microscopy or rapid diagnostic test) before administering treatment. Results of parasitological confirmation can be available in 30 minutes or less. Treatment, solely on the basis of symptoms should only be considered when a parasitological diagnosis is not possible."[9]

In addition, in order to address the increasing incidences of artemisinin-tolerant or -resistant malaria, the WHO issued a *Global Plan for Artemisinin Resistance Containment (GPARC)* and *Emergency Response to Artemisinin Resistance in the Greater Mekong Subregion* in which a systematic tier approach is recommended through a situational management of controlling, containing, and eliminating the occurrence and spreading of artemisinin-resistant malaria.[10,11]

"Expanding access to artemisinin-based combination therapies (ACTs) in malaria-endemic countries has been integral to the remarkable recent success in reducing the global malaria burden. No alternative antimalarial medicine is currently available that offers the same level of efficacy and tolerability as ACTs. The emergence of artemisinin resistance in the Greater Mekong subregion 1 (GMS) is therefore a matter of great concern. Resistance to other antimalarial medicine was also detected first in the GMS, eventually appearing elsewhere. In Africa, there is evidence that the spread of resistance coincided with increases in child mortality and morbidity."[9]

1.2. Traditional Chinese Medicine's Views on Malaria

Malaria was known as a disease by Chinese ancestors a long time ago. A Chinese character inscription (疟; malaria in Chinese) was found in the oracle ruins between 1401 and 1122 B.C. Comprehensive descriptions of malaria symptoms, epidemic, and relief of its unique periodic fevers and colds were given in the subsequent ancient medical literature such as *Zhou Li* (a classical book in ancient China, Zhou Dynasty, 1046—256 B.C.), *Inner Canon of Yellow Emperor* (between the Chun Qiu and Qin Dynasties, 770–206 B.C.), *Synopsis of Golden Chamber* (Han Dynasty, 206 B.C–220 A.D.), *On Causes and Symptoms of Chu* (Sui Dynasty, 581–618 A.D.), *Qian Jin Fang* and *Wai Tai Mi Yao* (Tang Dynasty, 618–907 A.D.), *Malaria on Sparse* (Ming Dynasty, 1368–1644 A.D.), and *Malignant Malaria Guide* (Qing Dynasty, 1644–1911 A.D.). Several ancient scripts from the central Asian countries, states of Assyria and India, also described some basic features of malaria.

In fact, the basic understanding of malaria was common and in agreement between traditional Western and Chinese medicines. Chinese ancestors believed that malaria was caused by the invasion of "exogenous evil" in the human body. The term "exogenous evil" was further explained as "Malaria gas", "Pathogen of malaria disease", and "Miasm, Miasma". This consensus remained in traditional Chinese medicine for more than two thousand years since first described in *Inner Canon of Yellow Emperor* (between the Chun Qiu and Qin Dynasties, 770–206 B.C.). Similarly, in the medieval period, the western medical practitioners believed that breathing in rotten gases from marshes and swamps was a cause of malaria.

Malaria was one of the epidemic diseases with the most comprehensive records in traditional Chinese medical scripts. For example, Pu Ji Fang's, *Prescription for Universal Relief* (Ming Dynasty, 1368–1644 A.D.)—one of the most comprehensive Chinese medicine prescription literatures—contained at least four chapters titled "Chu Nue Men" on malaria.

1.3. Qinghao

The term "Qinghao" is a general synonym in Chinese for the herbs of the *Artemisia* family.

Qinghao is one of the most common herbs which has been prescribed in traditional Chinese medical practice for over two thousand years. In Chinese medical terms, it offers the functions of cooling and detoxifying blood, eliminating osteopyrexia and fever, freeing from summer heat, preventing recurrence of malaria fevers, and removing jaundice.

In Sheng Nong's *Herbal Classic* (Qin and Han Dynasty, around 221 B.C. to 220 A.D.), the oldest herbal classic existing in China, Qinghao was listed in an inferior category (under the name of 草蒿) with a description of having an inherent nature of "bitterness and cold" and its main clinical application was relieving itches caused by scabies and scabs, treating a malignant sore, killing lice, retaining warmth of joints, and improving vision acuity.[12]

Although the herb Qinghao was documented in the traditional Chinese medical literature, however, few details were given on either the species or effective parts of the plant when clinical application was mentioned.

According to the plant taxonomy, there are at least six species in the Artemisia family, which includes *Artemisia annua* L., *Artemisia apiacea* Hance, *Artemisia scoparia* Waldst. et kit., *Artemisia capillaries* Thunb., *Artemisia japonica* Thunb., and *Artemisia eriopoda* Bunge. Our studies confirmed that only *Artemisia annua* L. contains a meaningful quantity of artemisinin.[13]

Relief of malaria symptoms, that is, periodic fevers, using Qinghao was first recorded by Ge Hong in *A Handbook of Prescriptions for Emergencies* (East Jin Dynasty, around 317–420 A.D.). The application was subsequently mentioned in

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other literatures such as Sheng Ji Zonglu, *General Records of Holy Universal Relief* (Song Dynasty, 960–1279 A.D.), Danxi Xinfa, *Mastery of Medicine* (Yuan Dynasty, 1271–1368 A.D.), Pu Ji Fang, *Prescription for Universal Relief* (Ming Dynasty, 1368–1644 A.D.) in which Qinghao Soup, Qinghao Pills, and Qinghao Powders were described for relieving malaria symptoms. In addition to a summary of experience from earlier practitioners, Li Shi Zhen recorded his own practice in treating periodic "fevers and colds" in *Compendium of Materia Medica* (Ming Dynasty, 1368–1644). Malaria-related information could also be found in *Essentials of Materia Medica* (Qing Dynasty, 1644–1911 A.D.) and *Detailed Analysis of Epidemic Warm Diseases* (Qing Dynasty, 1644–1911 A.D.).

In addition to the documentation in the traditional Chinese medical literatures, some empirical formulas were also very popular in some regions, for example, a recipe from the Jiangsu province mentioned "Collecting Qinghao leaves on the day of 'The Dragon Boat Festival day ' and drying in the shade, mixing with an equal amount of cortex cinnamomi powders (taking weight unit to equal approximately 3.72 g) together with warm wine when having colds and with cold wine when having fevers, between 3 and 5 am in the morning on the day of the malaria episode, avoiding stimulating foods while taking medicines" for reducing malaria symptoms.

Without doubt, clinical practice inherited from traditional Chinese medical literatures for alleviating malaria symptoms by utilizing Qinghao provided some useful information in identifying a lead for the discovery of artemisinin.

2. Discovery of Artemisinin

2.1. Background

Malaria was effectively treated and controlled by chloroquine and quinolines for a long period of time until the development of drug-resistant malaria in the late 1960s following a catastrophic failure in a global attempt to eradicate malaria. The resurgence of malaria and rapidly increased mortality due to a loss of effective treatment presented a serious global challenge, in particular in the regions with a prevalence of malaria associated with the drug-resistant Plasmodium parasites, especially *Plasmodium falciparum*.

Southern East Asian was one of the most severe endemic areas in the late 1960s. As reported during the Vietnam War, the casualties to the US military force caused by medical disability due to the full seasonal prevalence of malaria was four to five times higher than the actual number of casualties from direct combat in 1964. Malaria infected nearly half of the total military individuals, that is, around 500 000 US soldiers in 1965. Fighting against malaria became one of the top medical priorities as well as challenges for the US army in Vietnam. A program coordinated through the Division of Experimental Therapeutics at the Walter Reed Army Institute of Research (WRAIR) in Washington, DC, was launched to search for new antimalarial drugs. The program involved numerous research institutes and a vast investment. Up to 1972, over 214 000 compounds were screened by the Walter Reed Army Institute of Research which, however, ended up with no breakthrough findings or discovery of novel antimalarial medicines.

Confidential antimalarial research was initiated by the Chinese military force in 1964. Research on novel antimalarial medicines became an important political assignment for medical researchers in the Chinese army.

A national agency for malaria control, abbreviated as the national 523 office (for confidential purposes, the project was named after the date of the 23rd May when the project was initiated. The agency was terminated in March 1980) was established in 1967 with a mission to organize and coordinate antimalarial drug research activities in seven provinces and cities across the country. Several thousands of compounds were screened between 1967 and 1969. However, no effective antimalarial drugs were identified.[15]

2.2. Initial Screening

In 1969, two directors and one member of the national project 523 office visited the Institutes of Chinese Materia Medica of the Academy of Traditional Chinese Medicine, seeking help in searching for novel antimalarial drugs from Chinese medicines. I was appointed by the leadership team at the Academy of Traditional Chinese Medicine to build and head the project 523 research group at the institute.

I started by collecting information on the relevant traditional Chinese medicines. Within three months, I gathered over 2000 herbal, animal, and mineral prescriptions for either internal or external uses through reviewing ancient traditional Chinese medical literatures, folk recipes, and interviewing experienced Chinese medical practitioners for potential prescriptions and herbal recipes. I then narrowed down the prescriptions from 2000 to 640 and summarized the recipes in a brochure *Antimalarial Collections of Recipes and Prescriptions*. I circulated copies of the brochure to other research groups outside the institute for reference through the national project 523 office in April 1969.

We started with experiments on dichroine using animal models. The study was soon given up due to its severe side effects. From May 1969, aqueous and ethanol extracts of over 100 herbs were prepared and tested in rodent malaria, with few promising results found up to June 1971. The paragraph in the summary of national malaria control research meeting shown in Figure 1 summarized the antimalarial drug research that "over one hundred clinical verification were conducted, some of them (herbal medicines) showing some clinical

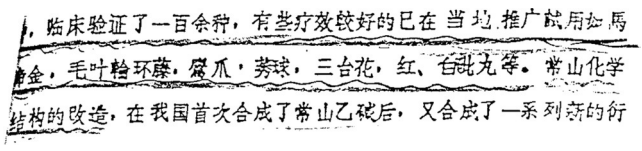

Figure 1. Copy of a paragraph from the summary of the National Malaria Control Research Meeting issued by the National Leading Group Office for Malaria Control on 1st June 1971.

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relevance have been further tested locally, which include herbal *dichodrae*, *ktze cycleanine*, *talon*, *ball atrazine*, *clerodendron serratum*, red and white arsenic pills etc."[16]

2.3. Extract Sample No. 191 and Focus on Qinghao Research

We started to focus on herb Qinghao in 1971, but received no promising results after multiple attempts. In September 1971, a modified procedure was designed to reduce the extraction temperature by immersing or distilling Qinghao using ethyl ether. The obtained extracts were then treated with an alkaline solution to retain the neutral portion by removing acidic impurities.

In the experiments carried out on 4th October 1971, sample No. 191, that is, the neutral portion of the Qinghao ethyl ether extract was found to be 100% effective on rodent malaria when administered orally at a dose of 1.0 g kg^{-1} for three consecutive days (Figure 2). The same results were observed when tested in malaria monkeys between December 1971 and January 1972. This breakthrough finding became a critical step in the discovery of artemisinin.

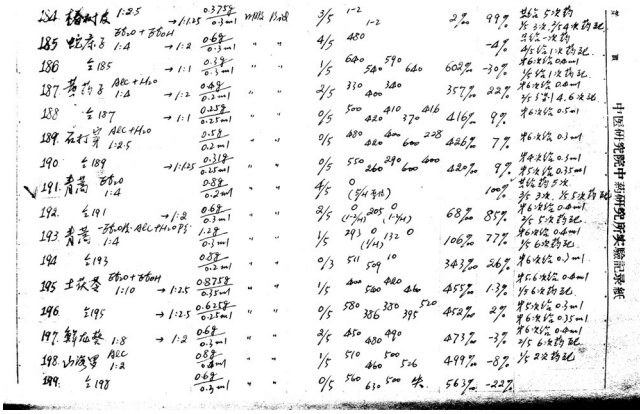

Figure 2. Copy of the original laboratory notebook record showing 100% inhibition of malaria parasites by the Qinghao neutral extract when tested on a rodent malaria model.

In the same studies, extracts from airpotato yam, pomegranate, rhizoma *smilacis glabrae*, and of Qinghao using other solvents were also tested, with negative or no comparable results.

I reported the findings in the nationwide project 523 meeting held in Nanjing on 8th March 1972 that "We have screened over one hundred types of single and combination herbal recipes since July 1971 and found that the Qinghao ether extract showed 95–100% inhibition of rodent malaria. We performed further purification to retain the effective neutral portion by removing the non-effective toxic acidic portion. We observed the same efficacy when testing the Qinghao ether extract and the neutral portion on the monkey malaria model in late December" (Figure 3).

This report attracted overwhelming interest and trigged nationwide collaboration in research on Qinghao and Qinghao extracts. We received multiple letters from other institutes requesting the sharing of information on our findings and experience,[17] which we responded to with thorough explanations (Figure 4).

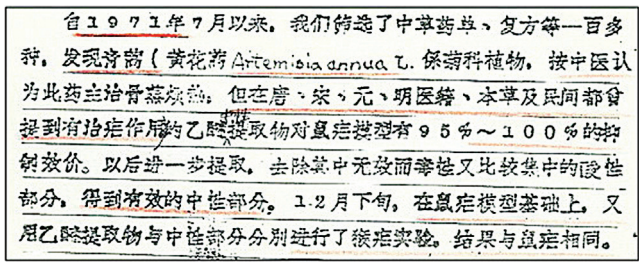

Figure 3. Copy of a paragraph of Tu Youyou's presentation in the "523" Project meeting held on 8th March 1972.

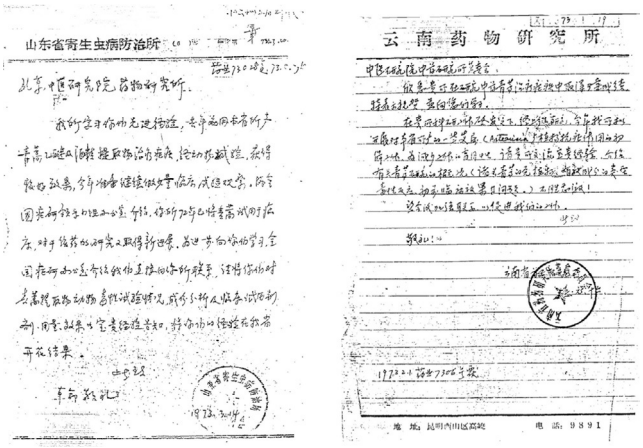

Figure 4. Copy of the letters from the Shangdong Institute of Parasitic Diseases (left) and Yunnan Provincial Institute of Materia Medica (right) requesting sharing the information on the findings made by the team at the Institute of Chinese Materia Medica, Academy of Traditional Chinese Medicine.

2.4. First Clinical Trial on Qinghao Extract and Nationwide Collaboration in Subsequent Development

We subsequently carried out a clinical trial between August and October 1972 in the Hainan province (21 cases) and simultaneously in the Beijing 302 Hospital (9 cases). This was the first time the neutral Qinghao ethyl ether extract was tested in humans. In the trial carried out in the Hainan province, a total of 21 local and migrate malaria patients—9 infected by *Plasmodium falciparum* and 11 infected by *Plasmodium vivax*—were treated in three dose groups and all of them recovered from the fevers with full clearance of the malaria parasites. All 9 patients were successfully treated in the Beijing 302 Hospital. The results from the first clinical trial in Hainan and Beijing were reported in the nationwide project 523 meeting held in Beijing in November 1972. The national leading group office for malaria control issued a communication on malaria control research on 5th November 1972 to record the clinical findings (Figure 5).

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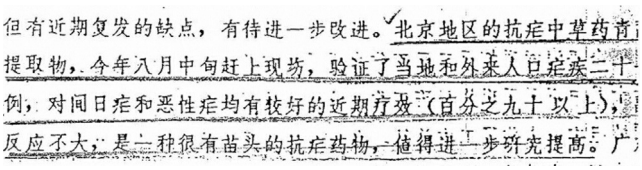
但有近期复发的缺点，有待进一步改进。北京地区的抗疟中草药青
提取物，今年八月中旬赶上现场，验证了当地和外来人口疟疾二十
例，对间日疟和恶性疟均有较好的近期疗效（百分之九十以上），
反应不大；是一种很有苗头的抗疟药物，值得进一步研究提高。广

Figure 5. Copy of a paragraph of a communication from the Malaria Control Research issued by the National Leading Group Office for Malaria Control on 5th November 1972.

"In the expedited clinical trial on the 21 cases of local and migrated malaria patients in August, the Qinghao extract from the Beijing (research) district showed relatively good efficacy (over 90%) against *Plasmodium vivax* and *Plasmodium falciparum*. This is a promising antimalarial drug with value for further improvement."[18]

Proving the efficacy of the neutral Qinghao ethyl ether extract in the experiments on rodent and monkey malaria models in October 1971 and the subsequent clinical trial between August and October 1972 steered the nationwide antimalarial research towards Qinghao.

Figure 6 summarizes the antimalarial research program carried out by the team at the Institute of Chinese Materia Medica, Academy of Traditional Chinese Medicine, in which the programs highlighted in blue were accomplished by the team at the Institute of Chinese Materia Medica, while the programs highlighted with blue and white were completed through the joint efforts of the teams at the Institute of Chinese Materia Medica and other institutes. The other research teams across the nation collaboratively completed those unhighlighted programs.

The team at the Institute of Chinese Materia Medica independently completed screening of Qinghao herbal extracts and herb Qinghao (*Artemisia annua* L. more specifically); obtained and proved the efficacy of the neutral Qinghao ethyl ether extract (Sample No. 191) in animal models in October 1971; completed the first clinical trial and proved the clinical efficacy of neutral Qinghao ethyl ether extract between August and October 1972; isolated and discovered artemisinin in November 1972; completed the first clinical trial on artimisinin between September and October 1973; discovered dihydroartemisinin; completed development activities, application, and received new drug approval for artemisinin in 1986 as well as dihydroartemisinin new drug approval in 1992. We collaborated with other institutes nationwide on the extended clinical trials between 1973 and 1978, determination of the stereostructure of artemisinin, research on dihydroartemisinin derivatives, search for Qinghao sources, optimization of manufacturing techniques, and research on new indications of dihydroartemisinin after 2003. Other institutes across the country synthesized and developed a number of artemisinin derivatives, that is, artemeter, artesunate, and arteether into new drugs.

2.5. Purification of Artemisinin and Chemistry Studies

We started further isolation and purification of the neutral Qinghao ethyl ether extract in parallel with the clinical trial and verification. In August 1972, we observed a good separation of the purified neutral extract by thin-layer chromatography on silica gel. In November 1972, an effective antimalarial compound was isolated from the neutral Qinghao ethyl ether extract by the team at the Institute of Chinese Materia Medica. The compound was later named artemisinin, or Qinghaosu in Chinese.

We started to determine the chemical structure of artemisinin in December 1972 through elemental analysis, spectrophotometry, mass spectroscopy, polarimetric analysis, and other techniques. These experiments confirmed that the compound had a completely new sesquiterpene structure with a formula of $C_{15}H_{22}O_5$, a molecular weight of 282, and contained no nitrogen.

The compound was further purified with different recrystallization processes and tested at the department of analytical chemistry of the Institute of Materia Medica, China Academy of Medical Sciences. Based on the elemental analysis and results from other studies, the colleagues at the Institute of Material Medica verified that the compound contained no nitrogen and had a potential formula of $C_{15}H_{22}O_5$ on 27th April 1973 (Figure 7). We started collabora-

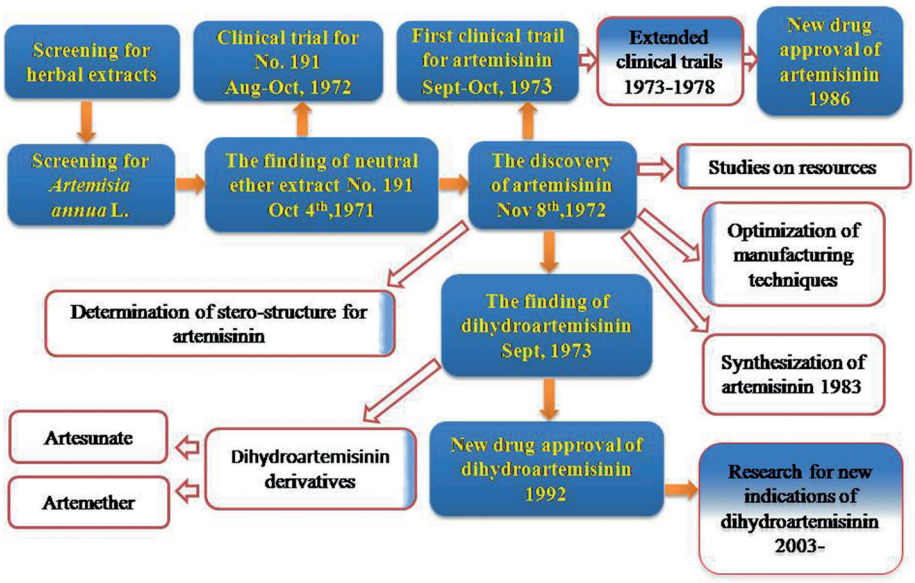

Figure 6. Summary of the work completed by the research team in the Institute of Chinese Materia Medica, Academy of Tradition Chinese Medicine (yellow text on blue background). The work completed by the collaboration between the Institute of Chinese Materia Medica and other institutes (black text on blue/white background), and the work completed by the collaboration between other research teams across the nation (black text in red outlined boxes).

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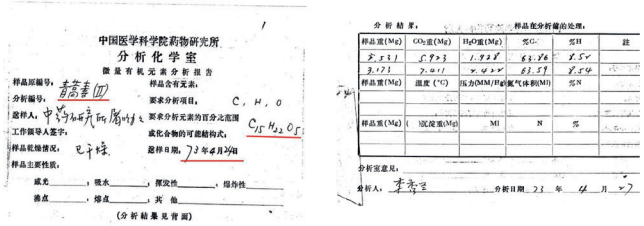

Figure 7. The elements analysis report by the Collaborative Institution, Institute of Matria Medica, Chinese Academy of Medical Sciences, on 27th April 1973.

tion with the Shanghai Institute of Organic Chemistry and the Institute of Biophysics of Chinese Academy of Sciences on the analysis of the chemical structure of artemisinin in 1974. The stereostructure was finally determined by X-ray crystallography, which verified that artemisinin was a new sesquiterpene lactone containing a peroxy group (Figures 8 and 9). This was the first application reported in China of determining the absolute molecular configuration by utilizing the scattering effects of oxygen atoms by the X-ray diffraction technique.[19,20] Table 1 presents some of the physical and chemical results during determination of the artemisnin chemical and stereostructure. The stereostructure of artemisinin was published in 1977 and cited in *Chemical Abstracts*.[20,21]

Table 1: Determination of the chemical and stereostructure of artemisinin.

Test	Result
appearance	white, needle-shaped, crystalline
melting point	156–157 °C
optical rotation	$[\alpha]^{17}_{D}$: +66.3°
mass spectrum	m/z 282.1472 $[M]^+$
elemental analysis	C: 63.72%, H: 7.86%
UV absorption	–
solubility	readily soluble in chloroform, acetone, ethyl acetate, benzene soluble in ethanol, ethyl ether slightly soluble in cold petroleum ether insoluble in water
IR (KBr)	1745 cm^{-1}, 831 cm^{-1}, 881 cm^{-1}, 1115 cm^{-1}
^{1}H NMR	(CCl_4, 100 MHz, hexamethyl disiloxane) δ: 0.93 (doublet, J = *6 Hz), 1.06 (doublet, J = 6 Hz), 1.36 (singlet), 3.08–3.44 (multiplet)
^{13}C NMR	(chloroform, 22.63 MHz) δ: 12, 19, 23 (quartet), 25, 25.1, 37, 35.5 (triplet), 32.5, 33, 45, 50, 93.5 (doublet), 79.5, 105, 172 (singlet)
X-ray crystallography	crystallographic parameters: D_2^4-P212121 lattice constant: a = 24.098 Å, b = 9.468 Å, c = 6.399 Å, measured density: d_o = 1.30 g cm^{-3} calculated density: ρ_{calcd} = 1.294 g cm^{-3} number of molecules in an asymmetric unit: 4

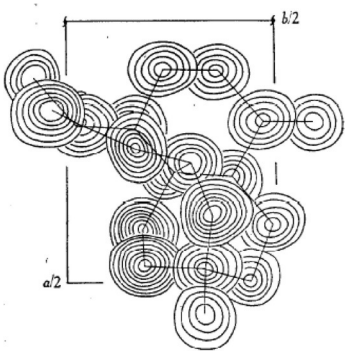

Figure 8. Three-dimensional electron density of the artemisinin crystal.[20]

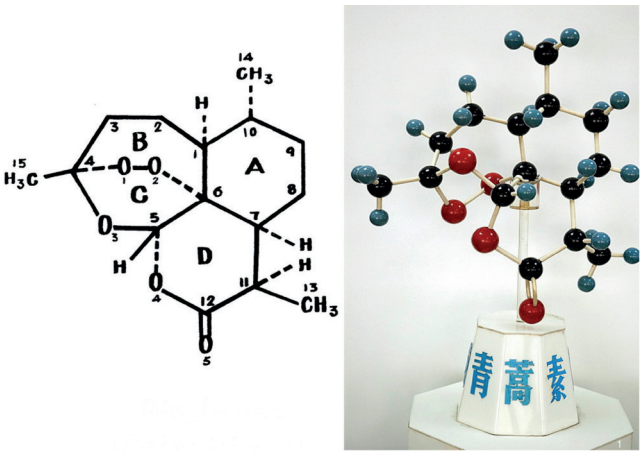

Figure 9. Chemical and stereostructures of artemisinin.

2.6. Artemisinin Structure–Efficacy Correlation and Artemisinin Derivatives

In order to determine the functional groups in the artemisinin molecule, we chemically modified the peroxy and carboxy groups of the molecule. We produced deoxyartemisinin through reduction of the peroxy group to an epoxy group by subjecting the artemisinin to palladium and calcium carbonate in methanol under room temperature and pressure, and then treated it with an acetone/*n*-hexane mixture.

We also produced dihydroartemisinin by reducing the carboxy group to the hydroxy group using sodium borohydride. Dihydroartemisinin was further reduced to dihydrodeoxyartemisinin by reduction in the methanolic palladium and calcium carbonate solution. Some new compounds were obtained by derivatizing at the hydroxy group of dihydroartemisinin.

Figure 10 shows the effective doses and observation of the clearance of malaria parasites when the structure-modified compounds were administered. The results showed that the dose was reduced from 50–100 mg kg^{-1} day^{-1} for artemisinin to 12.5 mg kg^{-1} day^{-1} and 6 mg kg^{-1} day^{-1} for dihydroartemisinin and the acetate of dihydroartemisinin, respectively. The dose was similar for deoxyartemisinin and artemisinin. However, deoxyartemisinin was unable to clear malaria parasites. This study verified that the peroxy group in the artemisnin molecule was critical for its antimalarial function,

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Compound	Graph of chemical structures	Dose mg/kg/day × 3	Clearance of parasites
Artemisinin		50-100	Yes
Dihydroartemisinin		12.5	Yes
Acetate of dihydroartemisinin		6	Yes
Deoxyartemisinin		100	No

Figure 10. Structure–activity relationship of compounds derived from artemisinin.

while reducing the carboxy group to a hydroxy group improved the efficacy as well as allowing derivatization of artemisinin to form new compounds. This led to the development of dihydroartemisinin and other compounds such as artemether, artesunate, and arteether into new antimalarial drugs (Figure 11). Up to now, no clinical application has been reported with other artemisinin derivatives except for the four presented here.

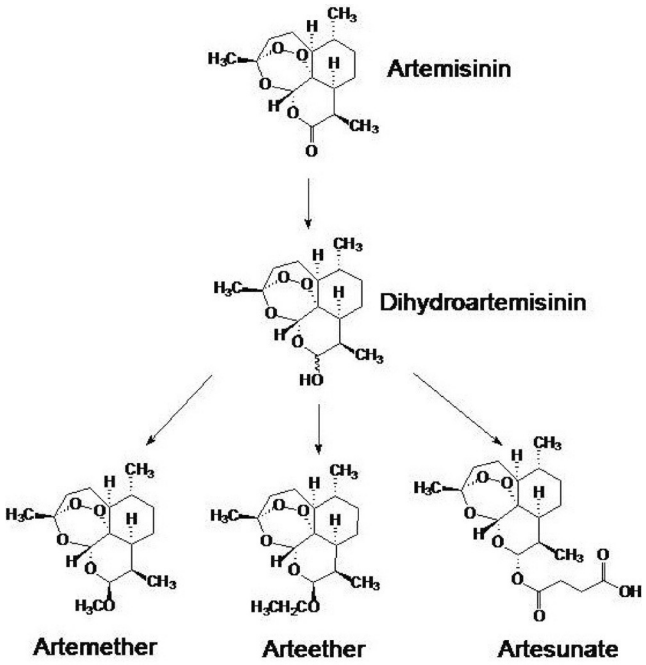

Figure 11. Artemisinin and artemisinin derivatives.

2.7. New Antimalaria Medicines: Artemisinin and Dihydroartemisinin

The team at the Institute of Chinese Materia Medica carried out a series of development activities on the chemistry, pharmacology, pharmacokinetics, stability, and clinical trials on artemisinin and dihydroartemisinin according to regulatory requirements. The China Ministry of Health granted the Institute of Chinese Materia Medica a New Drug Certificate for artemisinin (Figure 12, left) in 1986 and dihydroartemisinin (Figure 12, right) in 1992. Dihydroartemisinin is ten times more potent than artemisinin clinically, again demonstrating the "high efficacy, rapid action, and low toxicity" of the drugs in the artemisnin category.

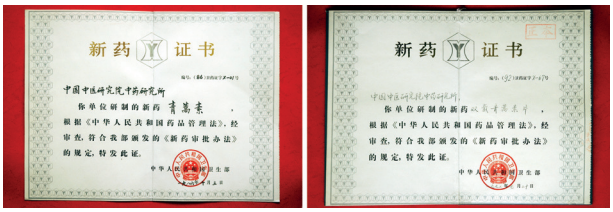

Figure 12. Artemisinin New Drug Certificate granted in 1986 (left) and the dihydroartemisinin New Drug Certificate granted in 1992 (right).

2.8. Worldwide Attention to Artemisinin

The World Health Organization (WHO), the World Bank, and United Nations Development Program (UNDP) held the 4th joint Malaria Chemotherapy Science Working Group meeting in Beijing in 1981 (Figure 13). A series of presentations on artemisinin and its clinical application including my report "Studies on the Chemistry of Qinghaosu" received positive and enthusiastic responses. In the 1980s, several thousand malaria patients were successfully treated with artemisinin and its derivatives in China.

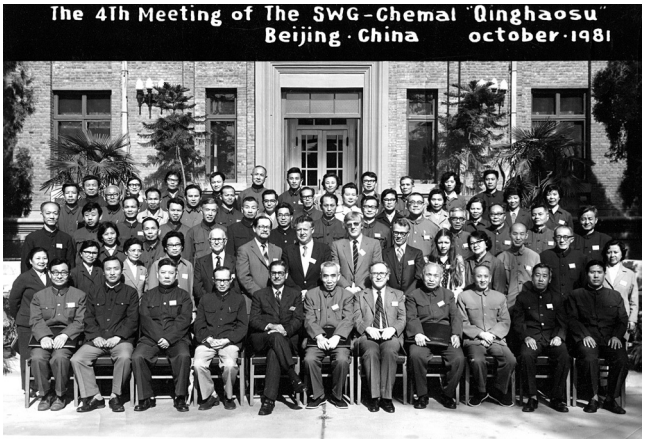

Figure 13. Delegates at the 4th Meeting of The SWG-Chemal "Qinghaosu" held by the World Health Organization (WHO), the World Bank, and United Nations Development Program (UNDP) in Beijing in 1981.

3. Discovery of Artemisinin Was not an Easy Win

After this brief review, you may think that this is no more than an ordinary drug-discovery process. However, it was not a simple and easy journey in the discovery of artemisinin from

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Qinghao, a Chinese herb medicine with over 2000 years of clinical application, especially in the 1970s when research was significantly under-resourced in China.

3.1. Commitment to the Clearly Defined Goal Assures Success in Discovery

The Institute of Chinese Materia Medica of Academy of Traditional Chinese Medicine joined the antimalarial drug research project 523 in 1969. I was appointed the head to build the project 523 research group in the institute by the academy's leadership team, and was responsible for searching for novel antimalarial drugs from Chinese medicines. It was a confidential military program with a high priority. As a young scientist in her early career life, I felt overwhelmed by the trust and responsibility received for such a challenging and critically important task. I had no choice but fully devote myself to accomplishing my duties (Figure 14).

Figure 14. Scene from the TV program "To Develop and Provide the Best Drugs for the People around the World".

3.2. Knowledge Is Prologue to Discovery

Figure 15 shows a photo taken soon after I joined the Institute of Chinese Materia Medica. Professor Lou Zhicen (left), a famous pharmacognosist, was mentoring on how to differentiate herbs. I graduated from Beijing Medical College in 1955 after four years of training on modern pharmaceutical sciences and later attended a training course on the theories and practices of traditional Chinese medicine which was designed for professionals with a modern (western) medicine training background between 1959 and 1962. "Fortune favors the prepared mind" and "What's past is prologue". My prologue of integrated training in modern and Chinese medicines prepared me for the challenges when the opportunities in searching for antimalarial Chinese medicines became available.

Figure 15. Professor Lou Zhicen (left), a famous pharmacognosist, mentoring on how to differentiate herbs.

3.3. Information Collating and Accurate Deciphering Are the Foundations for Success in Research

After accepting the tasks, I collected over 2000 herbal, animal, and mineral prescriptions for either internal or external uses by reviewing ancient traditional Chinese medical literatures and folk recipes, and interviewing well-known experienced Chinese medical doctors, who provided me with prescriptions and herbal recipes. I summarized 640 prescriptions in a brochure *Antimalarial Collections of Recipes and Prescriptions* (Figure 16). It was the information collection and deciphering that laid a sound foundation for the discovery of artemisinin. This also differentiates the approaches taken by Chinese medicine and general phytochemistry when searching for novel drugs.

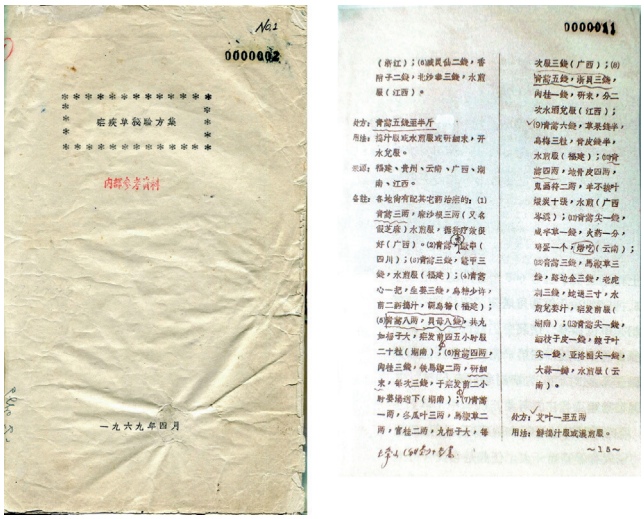

Figure 16. Antimalarial collections of recipes and prescriptions.

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3.4. Thorough Literature Reviewing Inspires an Idea Leading to Success

I reviewed the traditional Chinese literatures again when our research stalled following numerous failures. In reading Ge Hong's *A Handbook of Prescriptions for Emergencies* (East Jin Dynasty, around 317–420 A.D.; Figure 17), I further

Figure 17. Ge Hong's *A Handbook of Prescriptions for Emergencies* (East Jin Dynasty, around 317–420 A.D.).

digested the sentence "A Handful of Qinghao Immersed in Two Liters of Water, Wring out the Juice and Drink It All" when Qinghao was mentioned for alleviating malaria symptoms, whereas most of the herbs were typically boiled in water and made into a decoction before being taken by the patients.

This unique way of using Qinghao suddenly prompted an idea that the heating might need to be avoided during extraction in order to preserve the herbal activities, I subsequently redesigned the experiments by extracting the leaves and stems of Qinghao separately at a low temperature using water, ethanol, and ethyl ether.[22]

The earliest mention of Qinghao's application as a herbal medicine was found on the silk manuscripts entitled *Prescriptions for Fifty-two Kinds of Disease* unearthed from the third Han Tomb at Mawangduei. Its medical application was also recorded in Sheng Nong's Herbal Classic, *Bu Yi Lei Gong Bao Zhi* and *Compendium of Materia Medica* (Figure 18).

Although the herb Qinghao was widely documented in the traditional Chinese medical literatures, few details were given on either the species or effective parts of the plant when clinical application was mentioned.

According to plant taxonomy, there are at least six species in the Artemisia family, which includes *Artemisia annua* L., *Artemisia apiacea* Hance, *Artemisia scoparia* Waldst. et kit., *Artemisia capillaries* Thunb., *Artemisia japonica* Thunb., and *Artemisia eriopoda* Bunge. However, no clear classification was given for Qinghao (as a general name of the Artemisia family) regardless of a lot of mentioning of the name Qinghao in those literatures, neither were the effective parts of the

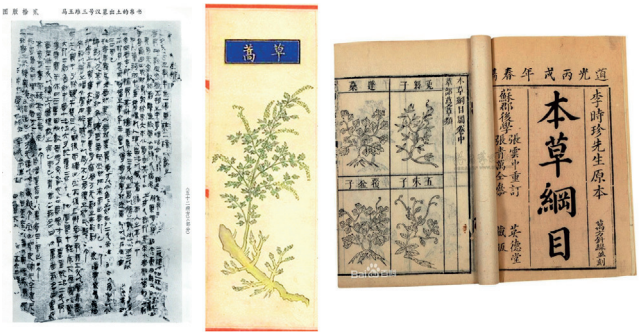

Figure 18. *Prescriptions for Fifty-Two Kinds of Disease*, unearthed from the third Han tomb at Mawangduei (left), *Bu Yi Lei Gong Bao Zhi* (middle), and *Compendium of Materia Medica* (right).

plant. All species in the Qinghao (Artemisia) family were used. By the time when research on artemisinin was carried out, two of the Qinghao (Artemisia) species were listed in the *Chinese Pharmacopoeia* and four others were also being prescribed.

Our studies confirmed that only *Artemisia annua* L. contains meaningful quantity of artemisinin. We subsequently carried out a thorough study on the herb Qinghao.

Figures 19 and 20 shows illustrations of plants and the epidermis structures of leaves of different species in the Artemisia family.[23] Figure 21 shows the thin-layer chromatographic spectra of extracts from *Artemisia annua* L., *Artemisia scoparia* Waldst. et kit., *Artemisia eriopoda* Bunge, *Artemisia capillaris* Thunb., *Artemisia japonica* Thunb., *and Artemisia apiacea* Hance.[23]

Samples no. 2 (*Artemisia annua* L. from Hainan Province) and no. 3 *(Artemisia annua* L. from Beijing) have the peaks

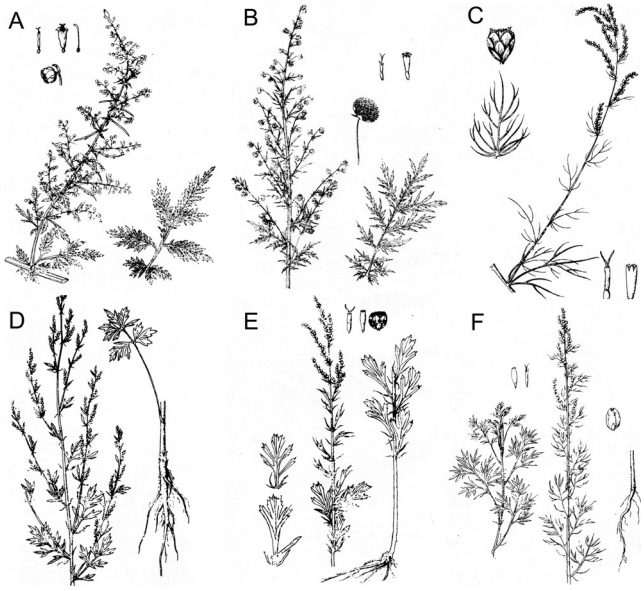

Figure 19. Illustration of six species in the Artemisia family. A) *Artemisia annua* L., B) *Artemisia apiacea* Hance C) *Artemisia capillaris* Thunb., D) *Artemisia eriopoda* Bunge, E) *Artemisia japonica* Thunb., F) *Artemisia scoparia* Waldst. et kit.

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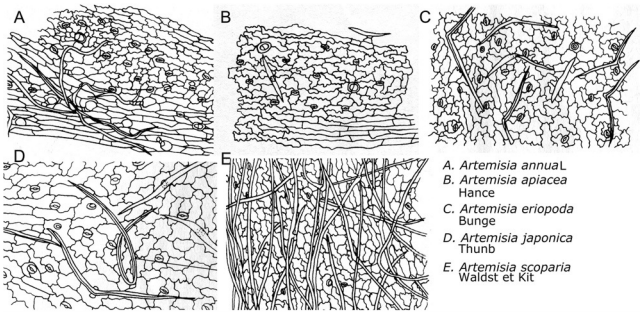

Figure 20. Illustrative epidermis structures of leaves of different species in the *Artemisia* family (*Artemisia capillaries* Thunb. has an epidermis structure similar to *Artemisia scoparia* Waldst. et kit.).

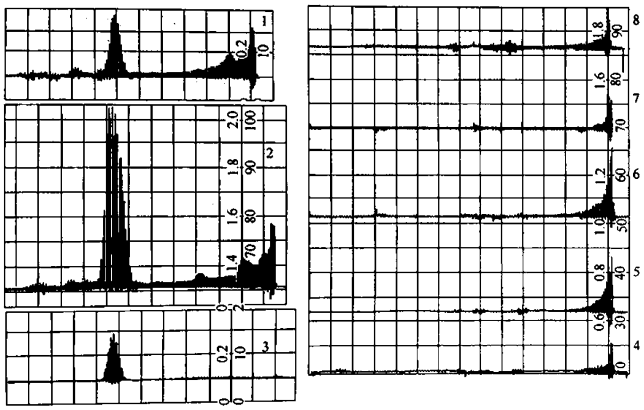

Figure 21. Thin-layer chromatographic spectra of the extracts (Kieselgel 60 F254, mobile phase: petroleum ether/ethyl acetate = 85/15) from 1) artemisinin reference standard, 2) *Artemisia annua* L. from Hainan Province, 3) *Artemisia annua* L. from Beijing, 4) *Artemisia scoparia* Waldst. et kit., 5) *Artemisia eriopoda* Bunge, 6) *Artemisia capillaris* Thunb., 7) *Artemisia japonica* Thunb., 8) *Artemisia apiacea* Hance.

eluting at the same retention time as the artemisinin reference standard (sample no. 1), while no. 4 *(Artemisia scoparia* Waldst. et kit.), no. 5 *(Artemisia eriopoda* Bunge*)*, no. 6 *(Artemisia capillaris* Thunb.), no. 7 *(Artemisia japonica* Thunb.), and no. 8 *(Artemisia apiacea* Hance) do not have any peaks or do not contain artemisinin. The peak from sample no. 2 (*Artemisia annua* L. from Hainan Province) was much higher than that from sample no. 3 *(Artemisia annua* L. from Beijing), suggesting that *Artemisia annua* L. growing in the Hainan province contained more artemisinin compared to the *Artemisia annua* L. collected from Beijing.

In addition to the confusion in finding the right plant, variables such as the part and origin of the plant, its harvest season, low artemisinin content in the plant, extraction, and purification process added extra difficult in the discovery of artemisinin. Success in identifying the effectiveness of the neutral Qinghao ethyl ether extract is not a simple and easy task. Without doubt, traditional Chinese medicine provides a rich resource. Nevertheless, it requires careful consideration to explore and improve.

3.5. Persistency in the Face of Challenges

Research conditions were relatively poor in China in the 1970s. In order to produce a sufficient quantity of the Qinghao extract for clinical trials, the team carried out extraction using several household water vats (Figure 22). Some team members' health deteriorated due to long-term exposure to large quantities of organic solvent and insufficient ventilation equipment. In order to launch a clinical trial sooner while not compromising patient safety, based on the limited safety data from the animal study, the team members and myself volunteered to take Qinghao extract ourselves to assure its safety. In 1973, unsatisfied results were observed in the clinical trial using artemisinin tablets, the team carried out a thorough investigation and verified poor disintegration of the tablets as the root cause, which allowed us to quickly resume the trial using capsules and confirmed artemisinin's clinical efficacy in time.

Figure 22. Under-resourced research conditions in 1970s China.

3.6. Collaborative Team Efforts Expedited Translation from Scientific Discovery to Effective Medicine

An antimalarial drug research symposium was held by the national project 523 office in Nanjing on 8th March 1972. In this meeting, on behalf of the Institute of Chinese Materia Medica, I reported the positive results of the Qinghao extract No. 191 observed in animal studies performed on rodent malaria and monkeys. The presentation received significant interest. On 17th November 1972, I reported the results of the successful treatment of 30 clinical cases at the national conference held in Beijing. This triggered a nationwide collaboration in research on Qinghao for malaria treatment.

Today, I would like to express my sincere appreciation again to my fellow project 523 colleagues at the Academy of Traditional Chinese Medicine for their devotion and exceptional contributions during the discovery and subsequent application of artemisinin. I would like to, once again, thank and congratulate the colleagues from Shangdong Provincial

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Institute of Chinese Medicine, Yunnan Provincial Institute of Materia Medica, the Institute of Biophysics of Chinese Academy of Sciences, Shanghai Institute of Organic Chemistry of Chinese Academy of Sciences, Guangzhou University of Chinese Medicine, Academy of Military Medical Sciences, and many other institutes for their invaluable contributions in their respective responsible areas during collaboration and their help and care of the malaria patients (Figure 23).

Figure 23. Invention certificate for progress in Antimalarial Research issued by the National Congress of Science and Technology, 1978.

I would also like to express my sincere respect to the national 523 office leadership team for their continuous efforts in organizing and coordinating the antimalarial research programs.

Without collective efforts, we would not be able to present artemisinin—our gift to the world in such a short period of time.

4. Malaria Control and Resistance or Tolerance to Artemisinin Drugs

4.1. Malaria Remains a Severe Challenge to Global Public Health

"The findings in this year's World Malaria Report demonstrate that the world is continuing to make impressive progress in reducing malaria cases and deaths", commented Dr. Margaret Chan, Director General of the World Health Organization in the recent World Malaria Report.[24]

The report indicated a positive sign in malaria control as a result of continuous intervention that "Since the year 2000, average malaria infection prevalence declined 46% in children aged 2–10, from 26% to 14% in 2013. The number of malaria infections at any one time dropped 26%, from 173 million to 128 million in 2103. Malaria mortality rates have decreased by 47% worldwide and by 54% in the WHO Africa Region.

By 2015, if the annual rate of decrease over the past 13 years is maintained, malaria mortality rates are projected to decrease by 55% globally and by 62% in the WHO Africa Region. Malaria mortality rates in children aged under 5 years are projected to decrease by 61% globally and 67% in the WHO Africa Region."

Nevertheless, statistically, there are approximately 3.3 billion people across 97 countries or regions still at a risk of malaria contraction and around 1.2 billion people live in the high risk regions where the infection rate is as high as or over 1/1000.[24]

According to the latest statistical estimation, approximately 198 million cases of malaria occurred globally in 2013, which caused 580000 deaths, with 90% from severely affected African countries and 78% being children below age five. Only 70% of malaria patients receive artemisinin combination therapies (ACTs) in Africa and as high as 56 million to 69 million child malaria patients do not have ACTs available to them.[24]

4.2. The Severe Warning of Parasites Resistant to Artemisinin

Plasmodium falciparum resistance to artemisinin has been detected in five countries of the Greater Mekong subregion: Cambodia, the Lao People's Democratic Republic, Myanmar, Thailand, and Vietnam. In many areas along the Cambodia–Thailand border, *Plasmodium falciparum* has become resistant to most available antimalarial medicines.

Tolerance of the *Plasmodium falciparum* to the mono artemisinin therapy has increased significantly. Although the artemisinin-based combination therapies are still highly efficacious, however, an increase in the rate of treatment failure with artesunate/mefloquine in Thailand and with dihydroartemisinin/piperaquine in Cambodia has been reported. There was evidence of genetic changes in the parasites, that is, mutations in the Kelch 13 (K13) propeller domain associated with reduced susceptibility and slow clearance.[25]

An even more serious concern or severe warning is that the resistance to artemisnin is not only detected in the Greater Mekong subregion but also appears in some African regions.[25]

4.3. Global Plan for Artemisinin-Resistant Containment

The WHO launched the Global Plan for Artemisinin Resistant Containment (GPARC) in January 2011, with a goal to maximize protection by artemisinin combination therapies as an effective treatment for *Plasmodium falciparum* malaria. Artemisinin resistance has been confirmed within the Greater Mekong subregion, and potential epidemic risk is under critical review. Over 100 experts involved in the program reached a unanimous agreement that the chance of containing and eradicating artemisinin-resistant malaria is very limited and there is an urgent need to constrain artemisinin resistance.

A proactive matrix approach by stopping the spread of resistant parasites, increasing monitoring and surveillance to evaluate the artemisinin resistance threat; improving access to diagnostics and rational treatment with artemisinin combination therapies, investing in artemsinin-resistance-related research, and motivating action and mobilizing resources is encouraged by the WHO for containing or eliminating

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artemisinin resistance where it already exists and preventing artemisinin resistance where it has not yet appeared.[8]

To protect the efficacy of artemisinin combination therapies, I strongly urge a global compliance to the WHO's Global Plan for Artemisinin Resistant Containment. This is our responsibility as scientists and medical doctors in the field.

5. Chinese Medicine, A Great Treasure

Before concluding, I would like to discuss briefly Chinese medicine. Chinese medicine and pharmacology are great treasure troves. We should explore them and raise them to a higher level (Figure 24). Artemisinin was explored from this

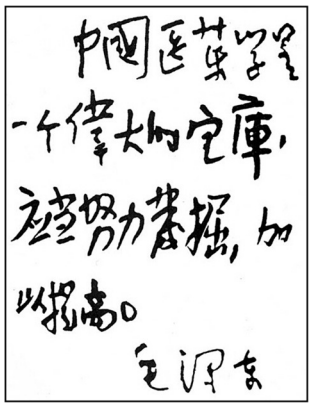

Figure 24. Handwriting of Mao Zedong "Chinese medicine and pharmacology are a great treasure trove. We should explore them and raise them to a higher level".

resource. From research experience gained from artemisinin discovery, we learnt strengths of both Chinese and Western medicines. There is great potential and future advances if these strengths can be fully integrated. We have a substantial amount of natural resource from which our fellow medical researchers can develop novel medicines.

Since Sheng Nong's *Tasting hundred herbs*, we have accumulated substantial experience in clinical practice, integrated and summarized medical application of most natural resources over the last thousands of years of Chinese medicine. Adopting, exploring, developing, and advancing these practices would allow us to discover more novel medicines beneficial to global healthcare.

To end my lecture, I would like to share with you a well-known poem, "On the stork tower", by Wang Zhihuan from the Tang Dynasty (688–742 AD):

"The sun along the mountain bows; The Yellow River seawards flows;
You will enjoy a grander sight; By climbing to a greater height."

Let's reach to a greater height to appreciate Chinese culture and find the beauty and treasure in the territory of traditional Chinese medicine!

Acknowledgements

I would like to acknowledge all colleagues in China and overseas for their contributions in the discovery, research, and clinical application of artemisinin. I am deeply grateful for all my family members for their continuous understanding and support. I sincerely appreciate your kind attention. Thank you all.

Biography

My Childhood

I was born on 30th December 1930 in Ningbo, a city on the east coast of China with a rich culture and over 7000 years of history. Although it was an age of turmoil in China when I was a child, I was lucky enough to have completed a good education from the primary to the middle schools.

My father worked in a bank while my mother looked after my four brothers and me, the only girl in our family. According to our recently discovered family tree, my ancestors lived in Ningbo for many generations. Our family's long history of highly valuing children's education and always considering this as a family's top priority allowed me to have good opportunities for attending the best schools in the region—the private Ningbo Chongde Primary School (1936–1941) and later the private Ningbo Maoxi Primary School (1941–1943), the private Ningbo Qizheng Middle School (1943–1945), and private Ningbo Yongjiang Girl School (1945–1946).

I unfortunately contracted tuberculosis at the age of sixteen and had to take a two year break with treatment at home before I resumed my study at the private Ningbo Xiaoshi High School (1948–1950) and Ningbo High School (1950–1951). This experience led me to make a decision to choose medical research for my advanced education and career—if I could learn and have the (medical) skills, I could not only keep myself healthy but also cure many other patients. After graduation from the high school, I sat the university entrance examination and fortunately I was accepted by the Department of Pharmacy and became a student in the Medical School of Peking University.

My University Life

My choice of leaning pharmacy was driven by my interests, curiosity, and a desire to seek new medicines for patients. In 1941, an Institute of Chinese Materia Medica was founded at Peking University. The institute later developed into the Department of Pharmacy in the Medical School in 1943. In 1952, the second year of my university training, the Medical School was separated from Peking University and became an independent Beijing Medical College. By this time, significant efforts and investment were made in building the university's infrastructure and curriculum. Most pharmacy courses such as pharmacognosy, medicinal chemistry, and phytochemistry were designed and taught by the

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returnees, such as professors Lin Qishou and Lou Zhicen, who received educations and advanced degrees in western countries. Although pharmacognostical study or "crude drug" was my major, my training was not limited to the field and I had great chances to attend all basic training in the pharmaceutical sciences. In the pharmacognosy course, Professor Lou Zhicen conveyed knowledge on the origin of the medicinal plants and trained us how to classify, distinguish, and identify those plants based on their botanical description. In the phytochemistry course, Professor Lin Qishou gave a comprehensive introduction and hands-on training on how to extract active ingredients from the plants, how to select proper extraction solvents, how to carry out chemistry studies and determine the structures of the chemicals isolated from the plants. These courses provided scientific insight into the herbs and plants and more, importantly, explained how those herbal medicines function in a view different from the traditional Chinese medicine.

My First Job and a Whole Life's Commitment

In December 2015, we celebrated the 60th anniversary of the China Academy of Chinese Medical Sciences (CACMS). This was also the 60th anniversary of my career. After graduation from the university in 1955, I was assigned to work at the Institute of Chinese Materia Medica of the newly established Academy of Traditional Chinese Medicine under the China Ministry of Health. The academy has been growing and expanding rapidly over the last 60 years, along with changing its name from the Academy of Traditional Chinese Medicine to China Academy of Traditional Chinese Medicine and now China Academy of Chinese Medical Sciences. However, its mission of focusing on professional training, research, and continuous exploring and development of Chinese medicines for human healthcare through utilization of evolving sciences and technologies has never changed. It is the academy's mission and establishment that have provided me with good opportunities to utilize my knowledge, skills, and experience while exposed to new areas of research.

My first research project was on *Lobelia chinensis*, a herb commonly prescribed in traditional Chinese medicine for the treatment of Schistosomiasis, a disease caused by Schistosoma parasitic flat worms. In fact, my first publication was on the pharmacognostical study of *Lobelia chinensis* co-authored with my mentor Professor Lou Zhicen in 1958. I completed another study on the pharmacognostical evaluation of *Radix Stellariae* before I went for full-time training on Chinese medical theory and practice organized by the Ministry of Health for professionals with a western (modern) medical background between 1959 and 1962. This training further added an in-depth knowledge on traditional Chinese medicine to my western (modern) medical background.

Over the last 60 years, I have held different responsibilities at the academy, from the head of the Chemistry Department (1973–1990) to the head of the Artemisinin Research Center of China Academy of Chinese Medical Sciences (since 1997) and various academic assignments, from associate professor (1979–1985), professor (since 1985), and now a chief professor of the China Academy of Chinese Medical Sciences.

Western and Traditional Chinese Medicine—A Unique Combination

China was lacking in medical resources in the early 1950s. There were only around 20000 physicians and several 10000 traditional Chinese medical practitioners in the country. To fully utilize the limited resource and explore Chinese medicines, the Chinese leadership launched programs in an effort to promote the ideas of enhancing the healthcare service through the "combination of western and traditional Chinese medicines".

The medical school graduates or young doctors were encouraged to learn traditional Chinese medicines, while the experienced traditional Chinese medical practitioners enriched their knowledge by attending training on western medicine. This unique combination was proven not only of benefit for the patients but also enabled further exploration and development of Chinese medicine and its application through modern scientific approaches.

The Ministry of Health of China organized a number of full-time training courses in the late 1950s in which scientists with a western medical background were given opportunities for systemic training on traditional Chinese medicine. In this two and half year training, I learnt traditional Chinese medical theory and gained experience in clinical practice. Another training I had was on the processing of Chinese Materia Medica. This processing skill is a unique and exclusive pharmaceutical technology and has been widely used for the preparation of Chinese materia medica. The traditional way of processing was developed and summarized from thousands of years of experience in traditional Chinese medical practices with a belief that the processing could alter the properties and functions of remedies, increase medical potency, and reduce toxicity and side effects. In fact, differences in chemical compositions have been detected between herbs treated with different processes. Knowledge on the processing in combination with the scientific explanation benefited my work enormously.

Assignment of Antimalarial Drug Research Task

Malaria is a life-threatening epidemic disease. It was, however, effectively treated and controlled by chloroquine and quinolines for a long period of time until the development of drug-resistant malaria plasmodium parasites, namely p*lasmodium falciparum* in the late 1960s following a catastrophic failure in a global attempt to eradicate malaria. The resurgence of malaria and rapidly increased mortality posed a significant global challenge, especially in the southern East Asian countries. In the 1960s, the Division of Experimental Therapeutics at the Walter Reed Army Institute of Research (WRAIR) in Washington, DC, launched programs to search for novel therapies to support its military presence in South-East Asia. The US military force involved in the Vietnam War

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suffered massive casualties due to disabilities caused by malaria infection. Up until 1972, over 214000 compounds, were screened with no positive outcomes.

In China, the military institutes started confidential antimalarial research in 1964. In 1967, the Chinese leadership set up a national leading group office for malaria control (abbreviated as the national 523 office) to coordinate nationwide research. Several thousands of compounds were screened between 1967 and 1969, but no useful medicines were found.

In 1969, two directors and another member from the national 523 office visited the Academy of Traditional Chinese Medicine and Institute of Chinese Materia Medica, seeking help in searching for novel remedies from Chinese medicine.

This was in the middle of the great culture revolution in China. Almost every institute was impacted and all research projects stalled. A lot of experienced experts were sidelined. After thoughtful consideration, the academy's leadership team appointed me to head and build a project 523 research group at the Institute of Chinese Materia Medica. My task was to search for antimalarial drugs from traditional Chinese medicine.

As a young scientist, I was so overwhelmed and motivated by the trust and responsibility. I also felt a huge pressure from the high visibility, priority, challenges as well as the tight schedule of the task. The other challenge was the impact on my family life. By the time I accepted the task, my elder daughter was four years old and my younger daughter was only one. My husband had to be away from home to attend the training campus. So that I could focus on the research, I left my younger daughter with my parents in Ningbo and sent my elder daughter to a full-time nursery, where she had to live with her teacher's family while I was away from home for the project. This continued for several years. My younger daughter couldn't recognize me when I visited my parents three years later and my elder daughter hid behind her teacher when I picked her up on returning to Beijing after a clinical investigation.

Traditional Chinese Medicine and its Relevance to Malaria

Our long journey in the search for antimalarial drugs began by the collection of relevant information and recipes from traditional Chinese medicine.

Malaria was one of the epidemic diseases with the most comprehensive records in the traditional Chinese medical literatures such as *Zhou Li*, a classical book in ancient China published in the Zhou Dynasty (1046–256 B.C.). Other literatures includes *Inner Canon of Yellow Emperor* published between the Chun Qiu and Qin Dynasties (770–206 B.C.), *Synopsis of Golden Chamber* published in the Han Dynasty (206 B.C to 220 A.D.), *General Treatise on the Cause and Symptoms of Diseases* published in the Sui Dynasty (581–618 A.D.), *Qian Jin Fang* and *Wai Tai Mi Yao* published in the Tang Dynasty (618–907 A.D.), *Malaria on Sparse* published in the Ming Dynasty (1368–1644 A.D.), *Malignant Malaria Guide* published in the Qing Dynasty (1644–1911 A.D.), *Prescription for Universal Relief* published in the Ming Dynasty (1368–1644 A.D.).

After thorough reviewing of those traditional Chinese medical literatures, folk recipes, and interviewing experienced Chinese medical practitioners, I collected over 2000 herbal, animal, and mineral prescriptions within three months after the project initiation. From the 2000 recipes, I summarized 640 prescriptions in a brochure *Antimalarial Collections of Recipes and Prescriptions*. I circulated copies of the brochure to other research groups outside the institute for reference through the national project 523 office in April 1969.

A Handful of Qinghao Immersed in Two Liters of Water, Wring out the Juice, and Drink It All

We started our experiments on dichroine using animal models. The study was soon stopped due to its severe side effects. From May 1969, extracts of over 100 herbs were prepared and tested in rodent malaria, with few promising results found up to June 1971.

After multiple attempts and failures, I refocused on reviewing the traditional Chinese medical literatures. One of the herbs, Qinghao (Chinese name of herbs in the Artemisia family), showed some effects in inhibiting malaria parasites during initial screening, but the result was inconsistent and not reproducible. I repeatedly read relevant paragraphs in those literatures where the use of Qinghao was recorded to relieve malaria symptoms.

In Ge Hong's *A Handbook of Prescriptions for Emergencies*, I noticed one sentence "A Handful of Qinghao Immersed in Two Liters of Water, Wring out the Juice and Drink It All" when Qinghao was mentioned for alleviating malaria fevers. Most herbs were typically boiled in water and made into a decoction before taken by the patients.

This unique way of using Qinghao prompted me to think that the heating during extraction might have destroyed the active components and the high temperature might need to be avoided in order to preserve the herb's activities. Ge Hong's handbook also mentioned "wring out the juice". This reminded me that the leaves of Qinghao might be one of the main components prescribed. I redesigned the experiments so that the stems and leaves of Qinghao were extracted separately at a reduced temperature using water, ethanol, and ethyl ether.

Sample No. 191, a Symbolic Breakthrough in Artemisinin Discovery

We produced extracts from different herbs, including Qinghao, using the modified process and subsequently tested those ethyl ether, ethanol, and aqueous extracts on rodent malaria. On 4th October 1971, we observed that sample no. 191, the Qinghao ethyl ether extract, showed 100% effectivity in inhibiting malaria parasites in rodent malaria. In the subsequent experiments, we separated the extracts into a neutral portion and a toxic acidic portion. The neutral

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portion showed the same effect when tested in malaria monkeys between December 1971 and January 1972.

On 8th March 1972, I reported the findings at the national project 523 meeting held in Nanjing. This encouraging news received overwhelming interest from the antimalarial drug research teams across the country.

"Sheng Nong Tasted Hundreds of Herbs"—Why Can't We?

From March 1972, the team started to produce large quantities of Qinghao extract in preparation for a clinical study. Most pharmaceutical workshops were shut down during the great culture revolution. Without manufacturing support, we had to extract herbs ourselves using household vats. The team worked very long hours every day, including weekends. Due to a lack of proper equipment and ventilation, and long-term exposure to the organic solvents, some of my team members included myself started to show unhealthy symptoms. This, however, did not stop our efforts.

Some conflicting information was seen in the animal toxicological studies. It was already in the middle of the summer and very limited time was available to us before the malaria epidemic season ended. We would delay the study for at least a year if we continued our debating on the toxicity. To expedite the safety evaluation, I requested to take the extracts voluntarily. The leaders at the institute approved my request. In July 1972, two other team members and myself took the extracts under close monitoring in the hospital. No side effects were observed in the one-week test window. Following the trial, another five members volunteered in the dose-escalation study. This safety evaluation won us precious time and allowed us to start and complete the clinical trial in time.

Traditional Chinese medicine started with a story of Sheng Nong "tasted a hundred herbs". Sheng Nong was an ancient Chinese medical practitioner. To understand the efficacy and toxicity of the herbs, he tasted over a hundred herbs himself and recorded all the details, which left us with a lot of precious information. Although Qinghao was prescribed as a herbal medicine for thousands of years, the dose of the actives in those prescriptions was much lower than that in the Qinghao extract we tested. Our desire to get the clinical trial completed and have medicines for our patients as soon as possible was a real driving for such an action.

Success in the First Clinical Trial

The first clinical trial on the Qinghao extract was carried out in the Hainan province between August and October 1972. We treated a total of 21 local and migrant malaria patients, 9 infected by *Plasmodium falciparum*, 11 infected by *Plasmodium vivax*, and one with mixed malaria infections.

The patients were divided into three groups with different dose regimens. We closely monitored the patients' body temperature and change in the numbers of parasites in the blood specimen. The trial was successful and all the patients recovered from the fevers, and no malaria parasites were detected after treatment. Nine malaria patients were also successfully treated with the Qinghao extract in Beijing No. 302 hospital.

The results from the first clinical trial in Hainan and Beijing No. 302 hospital were reported in the national project 523 meeting held in Beijing in November 1972. Success in the first clinical trial and previous evidence observed in the rodent malaria and monkey studies steered the nationwide antimalarial drug research towards Qinghao.

Artemisinin and Dihydroartemisinin

We started the isolation and purification of the neutral Qinghao ethyl ether extract in parallel with the clinical trial in 1972. Between April and June of 1972, a few crystals were isolated from the extract. The team finally isolated several crystals by silica-gel column chromatography in November 1972, one of which proved to be effective against malaria. The compound was later named artemisinin, or Qinghaosu in Chinese.

We carried out a clinical trial on artemisinin between August and October 1973 using artemisinin tablets which, however, did not yield the desired results. We examined the tablets returned from the clinical center and found that the tablets were too hard to disintegrate. We resumed the study using artemisinin capsules at the end of September 1973. Since it was already toward the end of the epidemic season, we only treated three patients, and all of them recovered after administration of artemisinin capsules.

Dihydroartemisinin was found in September 1973 in an experiment where I tried to derivatize artemisinin for structure–activity relationship evaluation. The carboxy group related peak disappeared and was replaced by the hydroxy group related peak in the IR spectrum after a reduction reaction using sodium borohydride. The experiment result was verified in a repeat experiment carried out by other team members. In a subsequent test in rodent malaria, we noticed a significantly reduced dose was needed to achieve the same efficacy as artemisinin when dihydroartemisinin was administered.

We completed a series of development activities on the chemistry, pharmacology, pharmacokinetics, stability, and clinical trials on artemisinin and dihydroartemisinin according to regulatory requirements. The China Ministry of Health granted a New Drug Certificate to the Institute of Chinese Materia Medica for artemisinin in 1986 and dihydroartemisinin in 1992. Dihydroartemisinin is ten times more potent than artemisinin clinically, again demonstrating the "high efficacy, rapid action, and low toxicity" of the drugs in the artemisnin category.

"Bench to Bedside"—Collaboration Expedited Translation from a Discovery to a Medicine

We started to determine the chemical structure of artemisinin in December 1972. The first thing we verified was that the compound did not contain nitrogen. This gave us a hint that the compound we had found could be a new

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chemical, different from quinolines. The team later confirmed that the compound was a new sesquiterpene lactone containing a peroxy group with a formula of $C_{15}H_{22}O_5$ and a molecular weight of 282.

In the 1970s, instruments and technical capabilities were very limited at each individual institute. The team at the Institute of Chinese Materia Medica collaborated with the Institute of Materia Medica, China Academy of Medical Sciences who confirmed the formula of the artemisinin molecule. We started a collaboration with the Shanghai Institute of Organic Chemistry and the Institute of Biophysics of Chinese Academy of Sciences on artemisinin chemical structure analysis in 1974. The stereostructure was finally determined using X-ray crystallography by the Institute of Biophysics. This was one of the first applications reported in China on determining the absolute molecular configuration by utilizing the scattering effects of oxygen atoms by the X-ray diffraction technique.

Undoubtedly, collaboration and collective efforts expedited the translation from the discovery to the new medicine. Colleagues from the Academy of Traditional Chinese Medicine, Shangdong Provincial Institute of Chinese Medicine, Yunnan Provincial Institute of Materia Medica, the Institute of Biophysics of Chinese Academy of Sciences, Shanghai Institute of Organic Chemistry of Chinese Academy of Sciences, Guangzhou University of Chinese Medicine, Academy of Military Medical Sciences, and many other institutes made significant contributions in their respective responsible areas during the development. The leadership team from the national 523 office played an important role in ensuring logistic support and coordinating the nationwide collaboration.

Qingaho and Artemisia annua L.

Herb Qinghao was frequently mentioned in the traditional Chinese medical literatures for various clinical applications besides alleviating malaria symptoms. These applications include relieving itches caused by scabies and scabs, treating malignant sores, killing lice, retaining warmth in joints, and improving vision acuity. However, little explanation was given on either the species or effective parts of the plant in the traditional Chinese medical literatures.

According to plant taxonomy, there are at least six species in the Artemisia family, which includes Artemisia annua L., *Artemisia apiacea* Hance, *Artemisia scoparia* Waldst. et kit., *Artemisia capillaries* Thunb., *Artemisia japonica* Thunb., and *Artemisia eriopoda* Bunge. Traditional Chinese medical literatures only mentioned Qinghao (general name of Artemisia in Chinese). By the time research on artemisinin was carried out, two Qinghao (Artemisia) species were listed in the *Chinese Pharmacopoeia* and four others were also being prescribed.

We carried out a thorough investigation and confirmed that only *Artemisia annua* L. contains artemisinin. In addition to identification of the right species, we also verified the best regions for growing Qinghao, the best collection season, and the officinal part of the plant.

Our Discovery Saves Patient Lives while Scientific Communities Recognize our Contributions

I feel that nothing can be more rewarding than the fact that artemisinin, since its discovery, has saved many malaria patients' lives. Over the last decades, more than 200 million malaria patients have received artemisinin or artemisinin combination therapies.

The scientific communities never forget a significant contribution to healthcare. I appreciate the numerous awards granted by the government and organizations in China. This includes the Award for Progress in Antimalarial Research Achieved by the Project 523 Scientific Team by the China National Science Conference in 1978, National Scientific Discovery Award for Antimalarial Drug-Qinghaosu by the China Ministry of Science and Technology in 1979, Invention Award (as the first inventor) by the China National Congress for Science and Technology Awards in 1982, Award of Young and Middle-Aged Experts with Outstanding Contribution in China State Council in 1984, Highest Honorary Award by the China Academy of Traditional Chinese Medicine in 1992, The Top Ten National Achievements for Progress in Science and Technology by the China State Scientific and Technological Commission in 1992, First-Rate Award of National Achievements in Science and Technology by the National Award Committee for Advances in Science and Technology in 1992, National Model Worker by the China State Council in 1995, Award for Outstanding Achievement in Traditional Chinese Medicine by the Guangzhou Zhongjing Award Foundation for Traditional Chinese Medicine in 1995, Outstanding Scientific Achievement Award by the Hong Kong Qiu Shi Science and Technologies Foundation in 1996, Top Ten Healthcare Achievements in New China by the China Ministry of Health in 1997, Woman Inventor of the New Century by the China National Bureau of Intellectual Property in 2002, Golden Medal of the 14th National Invention Exhibition by the China National Bureau of Intellectual Property in 2003, Award for Development of Chinese Materia Medica by the Cyrus Chung Ying Tang Foundation in 2009, China GlaxoSmithKline Award for Outstanding Achievements in Life Science in 2011.

I sincerely appreciate the Prince Mahidol Award Foundation (Thailand) for presenting me the 2003 Prince Mahidol Award, the Albert and Mary Lasker Foundation (USA) for presenting me the 2011 Lasker-DeBakey Clinical Medical Research Award, and the Warren Alpert Foundation and Harvard Medical School (USA) for awarding me the 2015 Warren Alpert Foundation Prize (co-recipient). I am, once again, sincerely grateful to the Nobel Foundation (Sweden) for awarding me the 2015 Nobel Prize in Physiology or Medicine as a co-recipient.

Research Efforts Continue

The discovery of artemisinin inspires us in research approaches through the integration of diversified disciplines. Exploring the treasure of traditional Chinese medicine provides us with a unique path leading to success, whereas

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utilizing modern scientific techniques and approaches are no doubt an effective and efficient way to realize and expedite the discovery.

Efforts continue in research on artemisinin to understand its action mechanisms and prevent or delay the development of artemisinin-tolerant or -resistant malaria. Expanding clinical applications of artimisinin is also of interest to public health. We know what it can do, we need to know why and how it does it, what else it can do, and how it can do better.

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 10210–10226
Angew. Chem. **2016**, *128*, 10366–10382

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Received: February 25, 2016
Published online: August 4, 2016

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