



Artemisinin—A Gift from Traditional Chinese Medicine to the World

Nobel Lecture, December 7, 2015

by Tu Youyou

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Dear respected Chairman, General Secretary, Esteemed Nobel Laureates, Ladies and Gentlemen,

It is my great honor to give this lecture today at Karolinska Institute. The title of my presentation is: Artemisinin—A Gift from Traditional Chinese Medicine to the World.

I would like to thank the Nobel Assembly and the Nobel Foundation for awarding me the 2015 Nobel Prize in Physiology or Medicine. This is not only an honor for myself, but also a recognition and motivation for all scientists in China. I would also like to express my sincere appreciation for the great hospitality of the Swedish people which I have received during my short stay over the last few days.

Thanks to Dr. William C. Campbell and Dr. Satoshi Omura for their excellent and inspiring presentations. The story I will tell today is about the diligence and dedication of Chinese scientists during the search for antimalarial drugs from traditional Chinese medicine forty years ago under considerably under-resourced research conditions.

INTRODUCTION

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 “intermittent 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 黄帝内经 (Huangdi Neijing, the *Inner Canon of the Yellow Emperor*, written around the time of the Chun Qiu and Qin Dynasties, 770–207 B.C.), the earliest traditional Chinese medical literature source [2].

Since malaria commonly originated and spread in humid areas surrounding marshes and swamps, the disease was considered associated with “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 the 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 mosquitoes and later verified that *Anopheles* mosquitoes were responsible for 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 *Anopheles* mosquitoes. Both Laveran and Ross received the Nobel Prize in Physiology or Medicine in recognition of their exceptional contribution in understanding the origins of malaria.

There are over a hundred species of *Plasmodium*. Five of them infect humans, among which *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium vivax*, and deadly *Plasmodium falciparum* cause malaria whereas *Plasmodium knowlesi* hardly poses any threat to humans. Camillo Golgi, an Italian scientist and Nobel Laureate, raised an idea for differentiation of the *Plasmodium* species in 1886 when he demonstrated the correlation between the periodicity of paroxysms (the chill and fever pattern in the patient) with the 72-hour life cycle of development of *Plasmodium malariae*. In observing 48-hour cycles of development from other patients, he came to the 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 sporozoites, merozoites, gametocytes, gametes and oocysts through their life cycle either in the vector (the definitive host) or in the infected subjects, e.g. humans (the secondary host). Healthy individuals are infected by an invasion of the thread-like sporozoites following a mosquito bite. The sporozoites then, through 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 they have escaped from the liver cells to 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* mosquitoes through 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 mosquitoes' guts. The oocyst then bursts and releases sporozoites, which migrate to mosquitoes' salivary glands, ready to attack their next victim. Since all forms of *Plasmodium* parasites are hidden in either the liver or red blood cells during most of their life cycles, they are well camouflaged from the immune system. This makes it more challenging to trigger a defense through either a natural immune response or vaccination [7].

Treatment of malaria relies on chemotherapy, using medicines that act on various phases of *Plasmodium* parasite life cycles. These medicines include quinoline compounds, sulfadoxine/pyrimethamine, mefloquine (Lariam), lumefantrine, doxycycline, artemisinin and artemisinin-based combination therapies (ACTs). The most commonly 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 use of insect repellants, insecticide-treated mosquitoes nets (INTs), indoor residual spraying as well as elimination of stagnant water etc. 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 early diagnosis and effective treatment of malaria, the World Health Organization (WHO) published the third edition of its *Guidelines for the treatment of malaria* in April 2015. The organization 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 its *Global Plan for Artemisinin Resistance Containment (GPARC)* and *Emergency Response to Artemisinin Resistance in the Greater Mekong Subregion* in which a systematical tier approach is recommended via situational management in controlling, containing and eliminating occurrence and spread 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 offering the same level of efficacy and tolerability as ACTs. The emergence of artemisinin resistance in the Greater Mekong subregion (GMS) is therefore a matter of great concern. Resistance to other antimalarial medicine was also detected first in GMS, eventually appearing elsewhere. In Africa there is evidence that the spread of resistance coincided with increases in child mortality and morbidity” [9].

Traditional Chinese medicine's views on malaria

Malaria was known as a disease by our Chinese ancestors long time ago. A Chinese character inscription 疟 (malaria in Chinese) was found in the oracle ruins from between 1401 and 1122 B.C. Comprehensive descriptions on malaria symptoms, epidemics and relief of its unique periodic fevers and chills were provided in subsequent ancient medical literature, such as 周礼 (*Zhou Li*, a classical book in ancient China, the Zhou Dynasty, 1046–256 B.C.), 黄帝内经 (*The Inner Canon of the Yellow Emperor*, from around the time of the Chun Qiu and Qin Dynasties, 770–207 B.C.), 金匱要略 (*The Synopsis of Prescriptions of the Golden Chamber*, the Han Dynasty, 206 B.C–220 A.D.), 诸病源候论 (*On Causes and Symptoms of Diseases*, the Sui Dynasty, 581–618 A.D.), 千金方 (*Qian Jin Fang*) and 外台密要 (*Wai Tai Mi Yao*) (the Tang Dynasty, 618–907 A.D.), 疟症论疏 (a book on malaria, the Ming Dynasty, 1368–1644 A.D.) and 瘴疟指南 (*Malignant Malaria Guide*, the Qing Dynasty, 1644–1911 A.D.). Several ancient texts from the central Asian countries, Assyria and India also described some basic features of malaria.

In fact, traditional Western and Chinese medicine agreed on their basic understanding of malaria. Our Chinese ancestors believed that malaria was caused by an invasion of 外邪 (exogenous evil) into the human body. The term “exogenous evil” was further explained as 疟气 (malaria gas), 疟邪 (pathogen of malaria disease), 瘴毒 and 瘴气 (miasm, miasma). This consensus remained in traditional Chinese medicine for more than two thousand years since it was first

described in 黄帝内经 (in the *Inner Canon of the Yellow Emperor*, from around the time of the Chun Qiu and Qin Dynasties, 770–207 B.C.). Similarly, in the medieval period, Western medical practitioners believed that inhaling rotten gases from marshes and swamps was the cause of malaria.

Malaria was one of the epidemic diseases with the most comprehensive records in traditional Chinese medical literature. For example, 普济方 (Pu Ji Fang, *Prescription for Universal Relief*, the Ming Dynasty, 1368–1644 A.D.), one of the most comprehensive Chinese medicine prescription texts, contained at least four chapters entitled 诸疟门 (Chu Nue Men) on malaria.

The herb Qinghao

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

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

In 神农本草经 (*Sheng Nong's Herbal Classic*, the Qin and Han Dynasty, around 221 B.C. to 220 A.D.), the oldest herbal classic 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 in relieving itches caused by scabies and scabs, treating malignant sores, killing lice, retaining warmth in joints, and improving visual acuity [12].

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

According to plant taxonomy, there are at least six species in the *Artemisia* family: *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 meaningful quantities of artemisinin [13].

Relief of malaria symptoms, *i.e.* periodic fevers using Qinghao was first recorded by 葛洪 (Ge Hong) in 肘后备急方 (*A Handbook of Prescriptions for Emergencies*, the East Jin Dynasty, around 317–420 A.D.). The application was subsequently mentioned in other literature such as 圣济总录 (Sheng Ji Zonglu, *General Records of Holy Universal Relief*, the Song Dynasty, 960–1279 A.D.), 丹溪心法 (Danxi Xinfa, *Danxi, Mastery of Medicine*, the Yuan Dynasty, 1271–1368

A.D.), 普济方 (Pu Ji Fang, *Prescription for Universal Relief*, the Ming Dynasty, 1368–1644 A.D.) in which Qinghao soup, Qinghao pills for malaria relief, and Qinghao powders were described for relieving malaria symptoms. In addition to a summary of experience from earlier practitioners, 李时珍 (Li Shi Zhen) recorded his own practice in treating periodic “fevers and colds” in 本草纲目 (*Compendium of Materia Medica*, the Ming Dynasty, 1368–1644). Malaria-related information could also be found in 本草备要 (*Essentials of Materia Medica*, the Qing Dynasty, 1644–1911 A.D.) and 温病条辨 (*Detailed Analysis of Epidemic Warm Diseases*, the Qing Dynasty, 1644–1911 A.D.).

In addition to the documentation in the traditional Chinese medical literature, some empirical formulas were also very popular in some regions, for example, a recipe from Jiangsu province mentioned collecting Qinghao leaves on the day of 端午 (The Dragon Boat Festival) and drying them in the shade, mixing with equal amount of cortex cinnamomi powders, taking 一钱 (a weight unit, equal to approximately 3.72 grams) together with warm wine when having colds and with cold wine when having fevers in 五更 (time traditionally used in China, 3 to 5 a.m.) on the day of a malaria episode, avoiding stimulating foods while taking medicines “to reduce malaria symptoms.”

No doubt, clinical practice in alleviating malaria symptoms utilizing Qinghao—inherited from traditional Chinese medical literature—provided some useful information leading to the discovery of artemisinin.

DISCOVERY OF ARTEMISININ

Background

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

South East Asia was one of the most severe endemic areas in the late 1960s. As reported, during the Vietnam War, casualties in the US military force caused by medical disability due to the full seasonal prevalence of malaria reached four to five times higher than casualties from actual direct combat in 1964. Malaria infected nearly half of total military individuals or around five hundred thousand US soldiers in 1965. Fighting malaria became one of the top medical priorities

and 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 discoveries of novel antimalarial medicines.

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

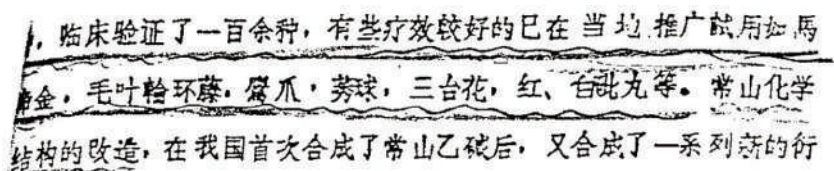
A national office for malaria control, known as the 523 Office (for purposes of confidentiality, the project was named for May 23, the date when it was initiated; the Office was terminated in March 1980) was established in 1967 with a mission of organizing and coordinating antimalarial drug research activities in seven provinces and cities across the country. Several thousand compounds were screened between 1967 and 1969. However, no effective antimalarial drugs were identified [15].

Initial screening

In 1969, two directors and one member from the National Project 523 Office visited the Institute 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 two thousand herbal, animal and mineral prescriptions for either internal or external uses by reviewing ancient traditional Chinese medical literatures and folk recipes, and interviewing experienced Chinese medical practitioners for potential prescriptions and herbal recipes. I then narrowed down the prescriptions from two thousand to 640 and summarized the recipes in a brochure entitled 抗疟单秘验方集 (“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 abandoned due to its severe side effects. From May 1969, aqueous and ethanol extracts of over hundred herbs were prepared and tested in rodent



，临床验证了一百余种，有些疗效较好的已在当地推广试用如马
 金，毛叶轮环藤，腐爪，葵球，三台花，红、白砒丸等。常山化学
 结构的改造，在我国首次合成了常山乙醚后，又合成了一系列新的衍

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 June 1, 1971.

malaria with few promising results found up to June 1971. The paragraph in the summary of national malaria control research meeting shown on Figure 1 updated the antimalarial drug research, saying that “over a hundred clinical verifications were conducted; some of the (herbal medicines) showing some clinical relevance has been further tested locally, including herbal *dichodrae*, *ktze cycleanine*, *talon*, *ball atrazine*, *clerodendron serratum*, red and white arsenic pills etc” [16].

Extract Sample No 191 and Focus on Qinghao Research

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

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

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

I reported our findings at the nationwide Project 523 meeting held in Nanjing on March 8, 1972, saying that “We have screened over a hundred types of single and combination herbal recipes since July 1971 and found that 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 we tested the

184 樟脑皮 1:2.5	→ 1:12.5	0.375g 0.3ml	WMS B.28	3/5	1-2	2%	99%	实验 5次药
185 蛇床子 1:4	→ 1:2	0.6g 0.3ml	"	4/5	480		-4%	实验 5次药
186 全 185	→ 1:1	0.3g 0.3ml	"	1/5	640 590 660	603%	-30%	实验 1次药
187 黄药子 AEC+H ₂ O 1:4	→ 1:2	0.6g 0.3ml	"	3/5	310 380 400	357%	22%	实验 1次药
188 全 187	→ 1:1	0.3g 0.3ml	"	0/5	500 420 410 416	416%	9%	实验 1次药
189 石打穿 AEC+H ₂ O 1:2.5	→ 1:1	0.5g 0.3ml	"	0/5	480 420 400 228	426%	7%	实验 1次药
190 全 189	→ 1:12.5	0.375g 0.3ml	"	0/5	550 260 290 400	420%	9%	实验 1次药
191 青蒿 1:4	→ 1:2	0.6g 0.3ml	"	4/5	0 (5/1 3/16)		100%	实验 5次药
192 全 191	→ 1:2	0.6g 0.3ml	"	3/5	0 (1/4) 205 0 (1/4)	68%	85%	实验 5次药
193 青蒿 AEC+H ₂ O 1:4	→ 1:2	0.6g 0.3ml	"	1/5	293 0 132 0 (1/4)	106%	77%	实验 5次药
194 全 193	→ 1:2	0.6g 0.3ml	"	0/3	511 509 10	343%	26%	实验 5次药
195 土茯苓 1:10	→ 1:2.5	0.875g 0.35ml	"	1/5	400 500 480 460	455%	13%	实验 5次药
196 全 195	→ 1:2.5	0.875g 0.35ml	"	0/5	580 386 330 395	452%	2%	实验 5次药
197 鲜瓜蒌 1:8	→ 1:2	0.6g 0.3ml	"	3/5	450 480 490	473%	-3%	实验 5次药
198 山慈姑 AEC 1:2	→ 1:2	0.6g 0.3ml	"	1/5	510 460 500 526	499%	-8%	实验 5次药
199 全 198	→ 1:2	0.6g 0.3ml	"	0/5	560 610 500 480	563%	-22%	实验 5次药

FIGURE 2. Copy of the original laboratory notebook record showing 100% inhibition of the malaria parasite by Qinghao neutral extract when testing on a rodent malaria model.

Qinghao ether extract and the neutral portion on the monkey malaria model in late December.” (Figure 3)

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

自1971年7月以来，我们筛选了中草药、复方等一百多种，发现青蒿（黄花蒿 *Artemisia annua* L. 菊科植物，按中医认为此药治骨蒸烦热，但在唐、宋、元、明医籍、本草及民间都曾提到有治疟作用的乙醇提取物对鼠疟模型有95%~100%的抑制效价。以后进一步提取，去除其中无效而毒性又比较集中的极性部分，得到有效的中性部分，12月下旬，在鼠疟模型基础上，又用乙醇提取物与中性部分分别进行了疟疾实验，结果与鼠疟相同。

FIGURE 3. Copy of a paragraph of Tu Youyou's presentation at the 523 Project meeting held on March 8, 1972.

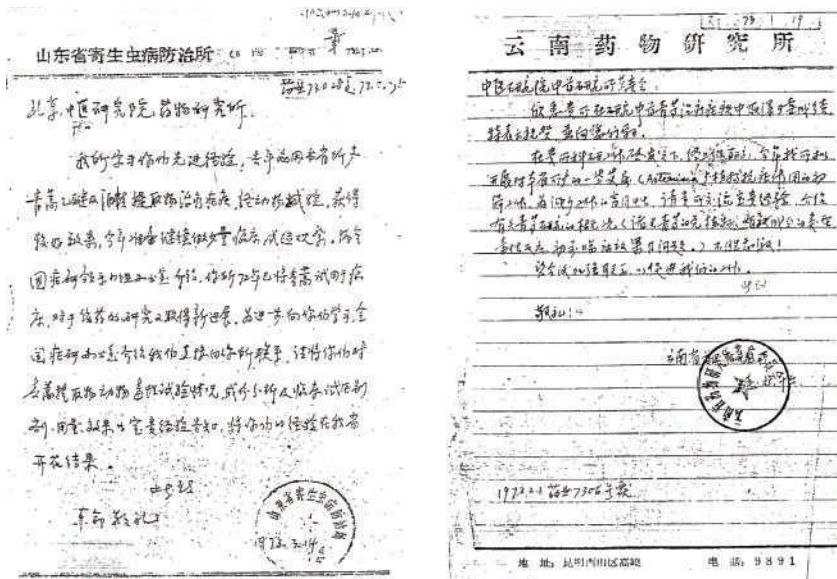


FIGURE 4. Copy of the letters from the Shandong Institute of Parasitic Diseases (left) and Yunnan Provincial Institute of Materia Medica (right) requesting sharing of information on the findings made by the team at the Institute of Chinese Materia Medica, Academy of Traditional Chinese Medicine.

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 Hainan province (twenty-one cases) and simultaneously at Beijing 302 hospital (nine cases). This was the first time the neutral Qinghao ethyl ether extract was tested in humans. In the trial carried out in Hainan province, a total of twenty-one local and migrant malaria patients, nine infected by *Plasmodium falciparum* and eleven infected by *Plasmodium vivax*, were treated in three dose groups and all of them recovered from the fevers with full clearance of malaria parasites. All nine patients were successfully treated at Beijing 302 hospital. The results from the first clinical trial in Hainan and Beijing 302 hospital were reported in the nationwide project 523 meeting held in Beijing in November 1972. The national office for malaria control issued a communication on malaria control research on November 5, 1972 to record the clinical findings (Figure 5).

“In the expedited clinical trial on the twenty-one cases of local and migrant malaria patients in August, the Qinghao extract from the Beijing (research) district showed relatively good efficacy (over

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蒿提取物，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 from the communication on malaria control research issued by the National Leading Group Office for Malaria Control on November 5, 1972.

90%) against *Plasmodium vivax* and *Plasmodium falciparum*. This is a promising antimalarial drug with potential for further improvement” [18].

Proving the efficacy of 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 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

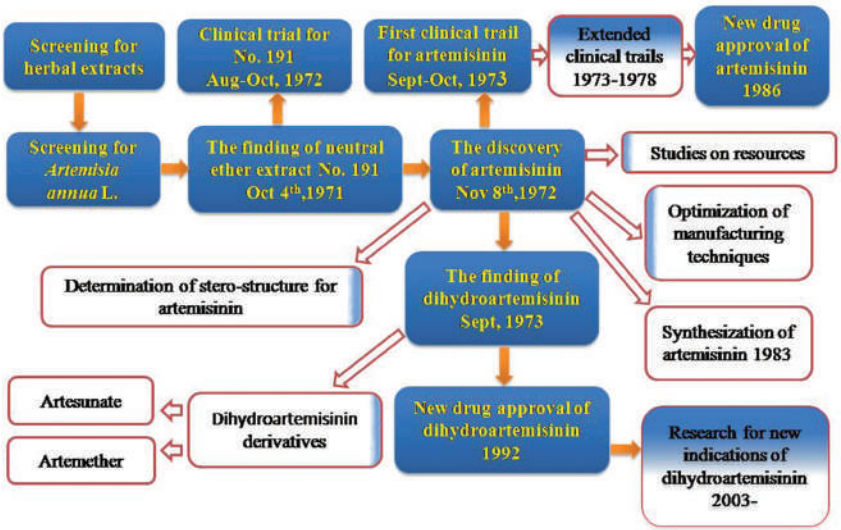


FIGURE 6. Summary of the work completed by the research team at the Institute of Chinese Materia Medica, Academy of Traditional Chinese Medicine (boxes with blue background). Work completed through collaboration between the Institute of Chinese Materia Medica and other institutes (Boxes transitioning from blue to white), and work completed through collaboration between other research teams across the nation (boxes in white).

Medicine in which the programs highlighted in blue were accomplished by the team at the Institute of Chinese Materia Medica while the programs highlighted in blue and white were completed through the joint efforts by the teams at the Institute of Chinese Materia Medica and other institutes. Other research teams across the nation collaboratively completed the non-highlighted programs.

The team at the Institute of Chinese Materia Medica independently completed screening on Qinghao herbal extracts, and herb Qinghao (*Artemisia annua* L. more specifically), proved the efficacy of 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 artemisinin between September and October 1973, discovered dihydroartemisinin, completed development activities, applied for and received artemisinin new drug approval in 1986 and dihydroartemisinin new drug approval in 1992. We collaborated with other institutes nationwide on extended clinical trials between 1973 and 1978, determination of the stereo structure of artemisinin, research on dihydroartemisinin derivatives, searches for Qinghao resources, optimization of manufacturing techniques, and research on new indications for dihydroartemisinin after 2003. Other institutes across the country synthesized and developed a number of artemisinin derivatives, *i.e.* artemeter, artesunate and arteether into new drugs.

Purification of artemisinin and chemistry studies

We started further isolation and purification of neutral Qinghao ethyl ether extract parallel with the clinical trial and verification. In August 1972, we observed a good separation of the purified neutral extract by silica gel thin-layer chromatography. 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 spectrum, polarimetric analysis and other techniques.

The compound was further purified with different re-crystallization 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, 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 April 27, 1973 (Figure 7). We started 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 stereo-structure 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 one of the first applications reported in China in determining an absolute molecular configuration utilizing the scattering effects of oxygen atoms by X-ray diffraction technique [19, 20]. Table 1 presents some of the physical and chemical test results for artemisinin chemical and stereo structure determination. The stereo structure of artemisinin was published in 1977 and cited by *Chemical Abstracts* [20, 21].

中国医学科学院药物研究所
分析化学室
微量有机元素分析报告

样品原编号: 青蒿素(IV)

分析编号: 73-4-1

送样人: 中国科学院上海有机化学研究所

工作领导人签字: 李秀三

样品干燥情况: 已干燥

样品主要性质:

样品含有元素:

要求分析项目: C, H, O

要求分析元素的百分比范围: $C_{15}H_{22}O_5$

或化合物的可能结构式:

送样日期: 73年4月24日

感光 _____, 吸水 _____, 挥发性 _____, 爆炸性 _____

沸点 _____, 熔点 _____, 其他 _____

(分析结果见背面)

分析结果:

样品在分析前的处理:

样品重(Mg)	CO ₂ 重(Mg)	H ₂ O重(Mg)	%C	%H	注
2.531	5.923	1.928	63.86	8.51	
3.173	7.411	2.422	63.59	8.54	
样品重(Mg)	温度(°C)	压力(MM/Hg)	氮气体积(Ml)	%N	
样品重(Mg)	()沉淀重(Mg)	Ml	N	%	

分析室意见:

分析人: 李秀三

分析日期: 73年4月27

FIGURE 7. The elements analysis report by the collaborative institution, the Institute of Matria Medica, Chinese Academy of Medical Sciences, on April 27, 1973.

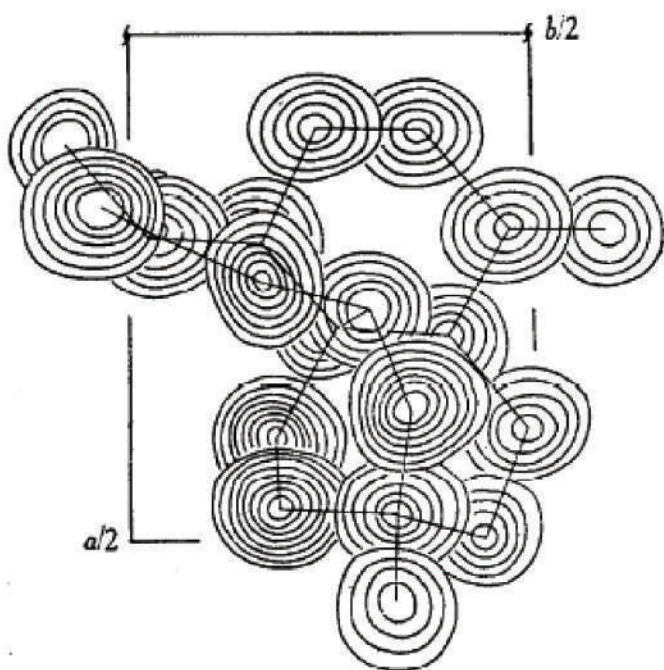


FIGURE 8. Three-dimensional electron density of the artemisinin crystal [20].

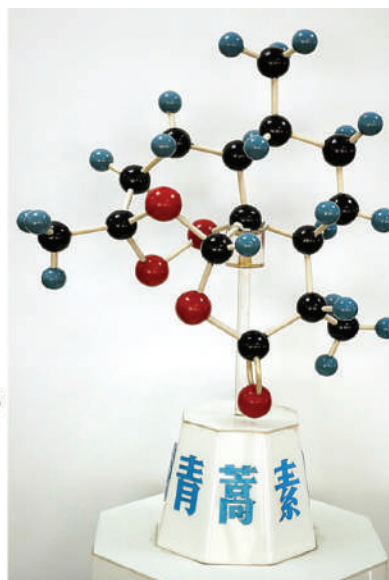
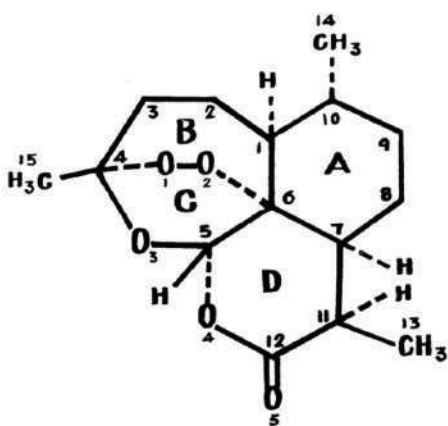


FIGURE 9. Chemical and stereo structures of artemisinin.

TABLE 1. Determination of chemical and stereo structure of artemisinin

Test	Result
Appearance	White, needle shape crystals
Melting point	156–157 °C
Optical rotation	$[\alpha]_D^{17}$: +66.3 °
High resolution 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)	1745cm ⁻¹ , 831cm ⁻¹ , 881cm ⁻¹ , 1115cm ⁻¹
¹ H-NMR	(CCl ₄ , 100M Hz, Hexamethyl disiloxane) δ 0.93 (doublet, J=6Hz), 1.06 (doublet, J=6Hz), 1.36 (singlet), 3.08–3.44 (multiplet)
¹³ C-NMR	(Chloroform, 22.63M Hz) 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 —P ₂₁₂₁₂₁ 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: d _c = 1.294 g/cm ³ Number of molecules in an asymmetric unit: 4

Artemisinin structure—efficacy correlation and artemisinin derivatives

In order to determine the functional groups in the artemisinin molecule, we chemically modified the peroxy and carboxyl groups of the molecule.

We produced deoxyartemisinin through reduction of the peroxy group to an epoxy group by subjecting the artemisinin in the palladium and calcium carbonate methanol solution under room temperature and pressure, and then treating it using an acetone and n-hexane mixture.

We also produced dihydroartemisinin by reducing the carboxyl group to a hydroxyl group using sodium borohydride. Dihydroartemisinin was further reduced to dihydro-deoxyartemisinin by reacting in a palladium and calcium carbonate methanol solution.

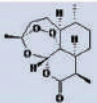
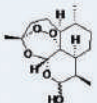
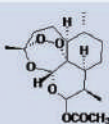
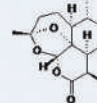
Compound	Graph of chemical structures	Dose mg/kg/day × 3	Clearance of parasites
Artemisinin		50-100	Yes
Dihydroartemisinin		12.5	Yes
Acetate of dihydroartemisinin		6	Yes
Deoxyartemisinin		100	No

FIGURE 10. Structure—Activity relationship of compounds derived from artemisinin.

Some new compounds were obtained by derivatizing through the hydroxyl group of dihydroartemisinin.

Figure 10 shows the effective doses and observation in clearance of malaria parasites when the structure-modified compounds were administered. The results showed that the dose was reduced from 50–100 mg/kg/day for artemisinin to 12.5 mg/kg/day and 6 mg/kg/day for dihydroartemisinin and acetate of dihydroartemisinin. The dose was similar between deoxyartemisinin and artemisinin. However, deoxyartemisinin was unable to clear malaria parasites. This study verified that the peroxy group in the artemisinin molecule was critical for its antimalarial function while reducing the carboxyl group to hydroxyl group improved the efficacy as well as allowed 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.

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 and stability of artemisinin and dihydroartemisinin and performed clinical trials

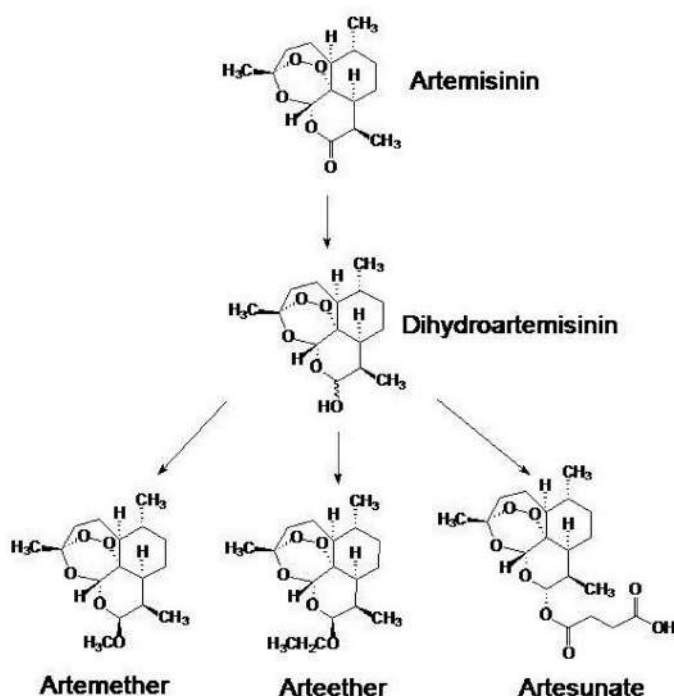


FIGURE 11. Artemisinin and artemisinin derivatives.

according to regulatory requirements. The China Ministry of Health granted an Artemisinin New Drug Certificate (Figure 12, left) to the Institute of Chinese Materia Medica in 1986 and a Dihydroartemisinin New Drug Certificate (Figure 12, right) in 1992, respectively. Dihydroartemisinin is ten times more potent than artemisinin clinically, again demonstrating the “high efficacy, rapid action and low toxicity” of the drugs in the artemisinin category.



FIGURE 12. The Artemisinin New Drug Certificate granted in 1986 (left) and the Dihydroartemisinin New Drug Certificate granted in 1992 (right).



FIGURE 13. Delegates to 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.

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.

DISCOVERY OF ARTEMISININ WAS NOT AN EASY WIN

After this brief review, you may comment that this is no more than an ordinary drug discovery process. However, it was not a simple and easy journey in the discovery of the artemisinin from Qinghao, a Chinese herbal medicine with over two thousand years of clinical application, especially in the 1970s when research was significantly under-resourced in China.

Commitment to a clearly defined goal assures success in discovery

The Institute of Chinese Materia Medica of the 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 at the institute by the



FIGURE 14. Screen shot from the TV program “To Develop and Provide the Best Drugs for People around the World.”

academy’s leadership team and was in charge of 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 to fully devote myself to accomplishing my duties (Figure 14).

Knowledge is prologue in 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 me 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 theories and practices of traditional Chinese medicine 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 both modern and Chinese medicine prepared me for the challenges when the opportunities to search for antimalarial Chinese medicines became available.

Information collating and accurate deciphering are the foundation for success in research

After accepting the tasks, I collected over two thousand herbal, animal and mineral prescriptions for either internal or external use by reviewing ancient



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

traditional Chinese medical literature and folk recipes, interviewing well-known and experienced Chinese medical doctors who provided me prescriptions and herbal recipes. I summarized six hundred forty prescriptions in a brochure 抗疟单秘验方集 (“Antimalarial Collections of Recipes and Prescriptions”) (Figure 16). It was this 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 in searching for novel drugs.

Thorough literature reviewing inspires an idea leading to success

I reviewed the traditional Chinese literature again when our research stalled, following numerous failures. In reading 肘后备急方 written by 葛洪 (Ge Hong's *A Handbook of Prescriptions for Emergencies*, the East Jin Dynasty, around 317–420 A.D.) (Figure 17), I further pondered the sentence 青蒿一握，以水二升渍，绞取汁，尽服之 (A handful of Qinghao immersed in two liters of water, wring out the juice and drink it all) which recommended cold Qinghao for alleviating malaria symptoms. Most herbs were typically boiled in water and made into decoction before taken by the patients.

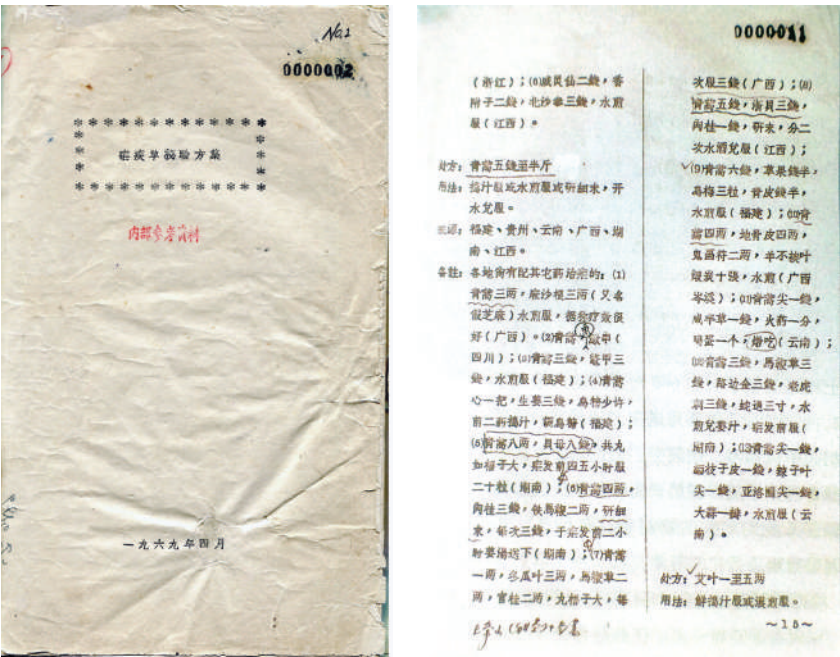


FIGURE 16. Antimalarial collections of recipes and prescriptions.



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

This unique way of using Qinghao suddenly gave me the idea that heating might need to be avoided during extraction, in order to preserve the herb's activity. 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 mentioning of Qinghao's application as an herbal medicine was found on the silk manuscripts entitled 五十二病方 (*Prescriptions for Fifty-two Kinds of Disease*) unearthed from the third Han Tomb at Mawangdui. Its medical application was also recorded in 神农本草经 (*Sheng Nong's Herbal Classic*), 补遗雷公炮制便览 (*Bu Yi Lei Gong Pao Zhi*) and 本草纲目 (*Compendium of Materia Medica*) (Figure 18) etc.

Although the herb Qinghao was widely 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 plant taxonomy, there are at least six species in the *Artemisia* family; *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 the Qinghao (the



FIGURE 18. *Prescriptions for Fifty-Two Kinds of Disease*, unearthed from the Third Han Tomb at Mawangdui (left), *Bu Yi Lei Gong Pao Zhi* (middle), and *Compendium of Materia Medica* (right).

general name of the *Artemisia* family) regardless of numerous mentions of the name Qinghao in the literature, nor did the texts specify the effective parts of the plant. All the species in Qinghao (*Artemisia*) family were used. By the time that research on artemisinin was carried out, two Qinghao (*Artemisia*) species were listed in the Chinese Pharmacopoeia and four others were also being prescribed.

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

Figures 19 and 20 show illustrative descriptions of plants and epidermis structures of leaves from different species in the *Artemisia* family [23]. Figure 21 shows the thin-layer chromatographic spectrums 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].

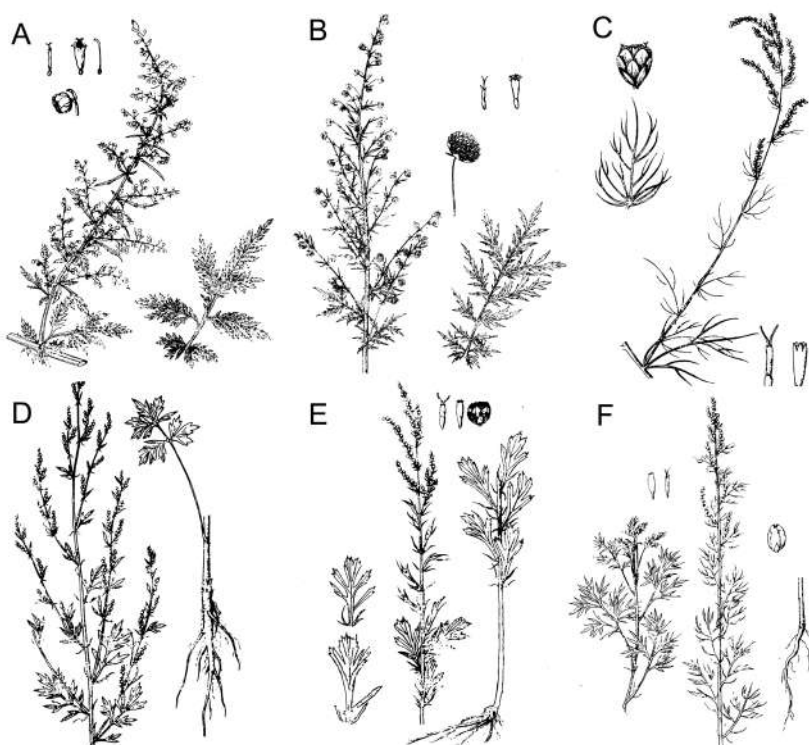


FIGURE 19. Illustrative description of six species in the artemisia family A. *Artemisia annua* L., B. *Artemisia apiacea* Hance C. *Artemisia capillaris* Thunb., D. *Artemisia eriopoda* Bunge, E. *Artemisia japonica* Thunb., F. *Artemisia scoparia* Waldst. et kit.

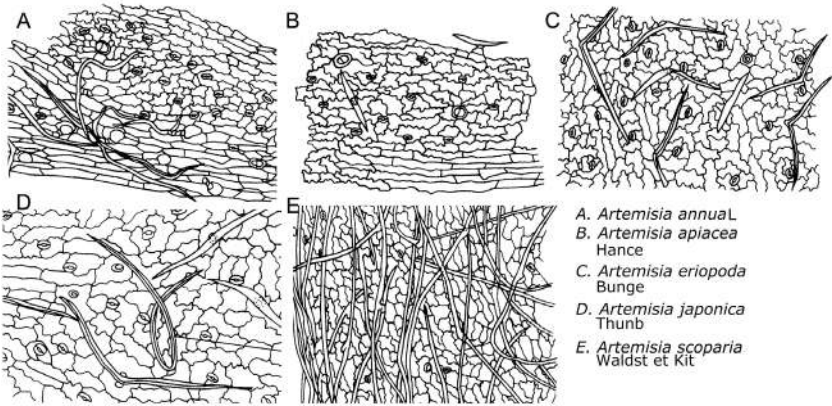


FIGURE 20. Illustrative epidermis structures of leaves from different species in the *Artemisia* family (*Artemisia capillaries* Thunb. has the epidermis structure similar to *Artemisia scoparia* Waldst. et Kit.).

Samples No 2 (*Artemisia annua* L. from Hainan province) and No 3 (*Artemisia annua* L. from Beijing) have peaks eluted 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)

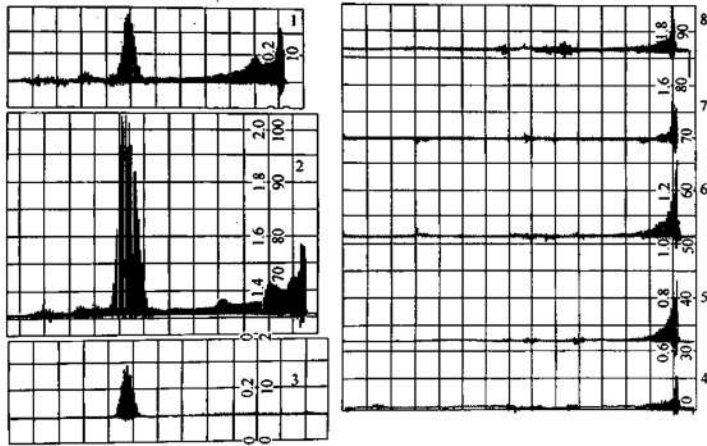


FIGURE 21. Thin-layer chromatographic spectrums of the extracts (Kieselgel 60 F254, mobile phase: Petroleum ether/ethyl acetate = 85/15) from 1. Artemisinin reference standard 2. *Artemisia annua* L. from Hainan province, 3. *Artemisia annua* L. from Beijing, 4. *Artemisia scoparia* Waldst. et kit.,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

do not have any peaks or do not contain artemisinin. The peak from the sample No. 2 (*Artemisia annua* L. from Hainan province) was much higher than that from the sample No. 3 (*Artemisia annua* L. from Beijing) suggesting that *Artemisia annua* L. growing in 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 etc. added extra difficulties in the discovery of artemisinin. Success in identifying effectiveness of neutral Qinghao ethyl ether extract was not a simple and easy win.

No doubt, traditional Chinese medicine provides a rich resource. Nevertheless, it requires our thoughtful consideration to explore and improve.

Persistence in the face of challenges

Research conditions were relatively poor in China in the 1970s. In order to produce sufficient quantities of Qinghao extract for clinical trials, our 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



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

solvents and insufficient ventilation equipment. In order to launch clinical trials 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, unsatisfactory 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.

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 March 8, 1972. At this meeting, on behalf of the Institute of Chinese Materia Medica, I reported the positive readouts of Qinghao extract No. 191 observed in animal studies performed on rodent malaria and monkeys. The presentation evoked significant interest. On November 17, 1972, I reported the results of the successful treatment of thirty clinical cases at the national conference held in Beijing. This triggered nationwide collaboration in research on Qinghao for malaria treatment.



FIGURE 23. Invention Certificate for Progress in Anti-malarial Research, issued by the National Congress of Science and Technology, 1978.

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

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 have been able to present artemisinin—our gift to the world—in such a short period of time.

MALARIA CONTROL AND RESISTANCE OR TOLERANCE TO ARTEMISININ DRUGS

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,” Dr. Margaret Chan, Director-General of World Health Organization, commented in the recent *World Malaria Report* [24].

The report indicated positive progress in malaria control as a result of continuous intervention: “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 2013. 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, approximately 3.3 billion people across 97 countries or regions are still at risk of contracting malaria and around 1.2 billion people live in high-risk regions where the annual infection rate is at or above one per 1000 [24].

According to the latest statistical estimate, approximately 198 million cases of malaria occurred globally in 2013, causing 584,000 deaths, with 90% of these in 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 millions to 69 millions of child malaria patients do not have ACTs available for them [24].

A severe warning about 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 mono artemisinin therapy has increased significantly. Although artemisinin-based combination therapies are still highly efficacious, increases in the rates of treatment failure with artesunate–mefloquine in Thailand and with dihydroartemisinin–piperaquine in Cambodia have been reported. There was evidence of genetic changes in the parasites, *i.e.* mutations in the Kelch 13 (K13) propeller domain associated with their reduced susceptibility and slow clearance [25].

It is an even more serious concern—or a severe warning—that resistance to artemisinin is not only detected in the Greater Mekong sub-region but has also appeared in some African regions [25].

Global plan for artemisinin-resistant containment

WHO launched the Global Plan for Artemisinin Resistant Containment (GPARC) in January 2011 with a goal to maximize protection to artemisinin combination therapies as an effective treatment for *Plasmodium falciparum* malaria. Artemisinin resistance has been confirmed within the Greater Mekong sub-region, and potential epidemic risk is undergoing a critical review. Over a hundred experts involved in the program reached 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 artemisinin resistance-related research, and motivating action and mobilizing resources is encouraged by WHO to contain or

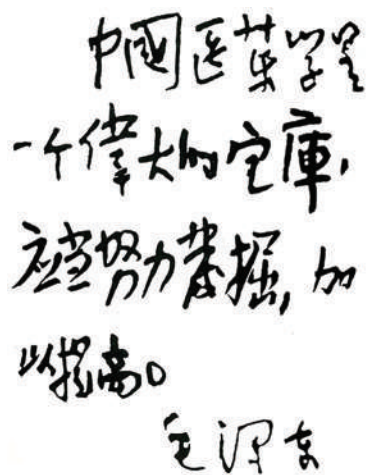
eliminate artemisinin resistance where it already exists and prevent artemisinin resistance where it has not yet appeared [8].

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

CHINESE MEDICINE, A GREAT TREASURE

Before concluding, I would like to briefly discuss Chinese medicine. "Chinese medicine and pharmacology are a great treasure-house. We should explore them and raise them to a higher level." (Figure 24). Artemisinin was explored using this resource. From our research experience in discovering artemisinin, we learned the strengths of both Chinese and Western medicine. There is great potential for future advances if these strengths can be fully integrated. We have a substantial amount of natural resources from which our fellow medical researchers can develop novel medicines.

Since "Tasting a hundred herbs by Sheng Nong," we have accumulated substantial experience in clinical practice, integrated and summarized the medical application of most nature resources over the past several thousand years through Chinese medicine. Adopting, exploring, developing and advancing these practices would allow us to discover more novel medicines beneficial to global healthcare.



中國醫學藥學
一個偉大的寶庫，
應該努力發掘，加
以提高。
毛澤東

FIGURE 24. Handwriting of Mao Zedong: "Chinese medicine and pharmacology are a great treasure-house. We should explore them and raise them to a higher level."

To end my lecture, I would like to share with you a well-known poem, “On the stork tower,” written during the Tang Dynasty by Wang Zhihuan (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 us 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 thank all my colleagues in China and overseas for their contributions to the discovery, research and clinical applications of artemisinin!

I am deeply grateful to all my family members for their continuous understanding and support!

I sincerely appreciate your kind attention!

Thank you all!

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