PLANTS AGAINST MALARIA
PART 2: *ARTEMISIA ANNUA* (QINGHAOSU OR THE SWEET WORMWOOD)

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In a previous article the history of Cinchona (the Peruvian bark) was described: Cinchona has been known in the West for almost 400 years.[1] In contrast, this second article deals with *Artemisia annua*, known to the Chinese herbal practitioners for at least 1,000 years but introduced into Western allopathic medicine only in the last 20.

The major difference between Cinchona and Artemisia is the speed with which the active principle of the latter, artemisinin, was isolated, synthesised and then modified to produce effective therapeutic entities, largely due to the efficiency of modern chemical techniques.

THE DIVINE PLOUGHMAN AND THE YELLOW EMPEROR

In China, in the mists of antiquity around 2800 BC, lived an individual – half-man, half-god – who became known as the Divine Ploughman (Sheng Nung).[2] Sheng Nung's works were later set down by an anonymous scribe in 101 BC. The demigod codified rules for the cultivation of crops such as rice and millet; and having tested many herbs himself, described those that were poisonous and those that might be useful in medicine. Amongst the herbs described by the Ploughman was Qinghaosu as an agent effective in the treatment of fever. It should be remembered that the Ancients considered fever as a specific disease and not simply a symptom.[1]

Similarly, the Yellow Emperor and his cabinet members, Gi Po and Lei Kim, developed techniques to diagnose and treat disease around 2700 BC, and later these were recorded in the Emperor's book.[2] In this period the Chinese also developed basic methods of chemistry including using fire to dry herbs for preservation and the use of wine (Jiu) to extract them. This led on to the techniques of distillation and condensation.

FOUL WEATHER DISEASE (NUE JI)

Malaria is common in south-west China where it is known as Nue Ji, foul weather disease. In this area of China it is both endemic and, in the rainy season, epidemic. As recently as 1995, in the rainy (or monsoon season), three million people were given prophylactic treatment, and yet 58,000 cases of malaria occurred. The mosquito still had its victims (Figure 1).

As the centuries passed in China, it was claimed that several different herbs were effective against Nue Ji. These included Qinghaosu (*Artemisia annua*), Chang Shan (*Dichroa febrifuga*), Ma Bian Cao (*Verbena officinalis*) and

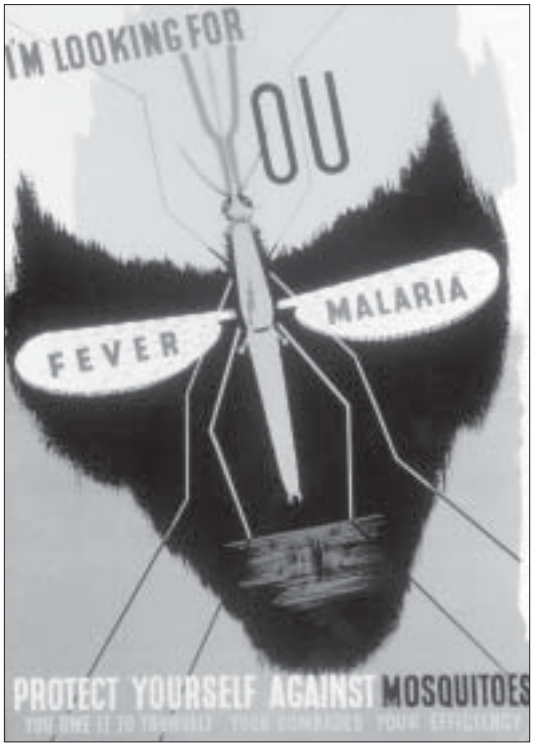


FIGURE 1

Beware the mosquito. World War 11 poster. The Wellcome Library, London.

Pai Chien Cao (*Desmodium pulchellium*).[2] The first to gain scientific validation is Qinghaosu.

Specific mention of this herb continues with its description in the *Recipes for 52 Kinds of Diseases* found in the Han dynasty tomb at Mawangdu (*c.* 168 BC) and in Ge Hong's *Prescriptions for Emergency Treatments* (Zhou Hou Bei Ii Fang, *c.* AD 340). The culmination of all this work was the publication by Xue-Meng (AD 1719–1805) of his monumental ten volume treatise on Materia Medica called the *Ben Cao Kong Mi.* In this work Xue-Meng states categorically that 'Huang Hua Guo cures hot or cold fevers.'[2]

Amongst several other plants Huang Hua Guo contains Qinghaosu. In passing, one should mention that the plant had also been known to the Koreans as Gae-Tang-Sook and used as a febrifuge and stomachic. This is an interesting example of parallel development.

All these references over several thousand years, while intriguing, leave us with the same pressing questions which also bogged down progress with Cinchona for centuries,

namely: which plant is it?; what is the active compound?; and is this compound specifically plasmodicidal?

MODERN CHINESE DEVELOPMENT

Traditional Chinese herbal remedies are often mixtures based upon the principles of Yin and Yang: that is, the so-called 'active' agent is balanced by other agents that will counteract the adverse effects of the former; for example, a so-called 'hot' drug will be balanced by a 'cold' drug, or a 'wet' one by a 'drying up' compound.

In 1967, the Government of the People's Republic of China embarked upon a long-term systematic examination of all the indigenous plants contained in their many traditional herbal remedies. The magnitude of this task is apparent and it continues to the present day. Between 3,000 and 5,000 herbs have to be evaluated and the occurrence of multiple combinations has been a besetting problem.

The early studies in Qinghaosu established that the preparation was very active against malaria and that the active herb in the preparation was almost certainly *Artemisia annua*.[3,4] This plant is known in the West as the sweet or annual wormwood (Figure 2). Initial attempts to isolate the active principle or principles from the plant failed, but in 1971 success was achieved by extraction into diethyl ether at low temperature.

The extract cured mice infected with *Plasmodium berghei* and, in 1972, further work culminated in the isolation of a crystalline compound called by the Chinese Qinghaosu

FIGURE 2
Artemisia annua.
The sweet wormwood or Qinghaosu.

(or artemisinine after the generic name of the plant).[5] As is shown in Figure 3 the compound is a terpene, not an alkaloid or an amine. Chemical abstracts have therefore suggested that the name of the compound should be shortened to artemisinin deleting the final 'e' and this is now the commonly used name.

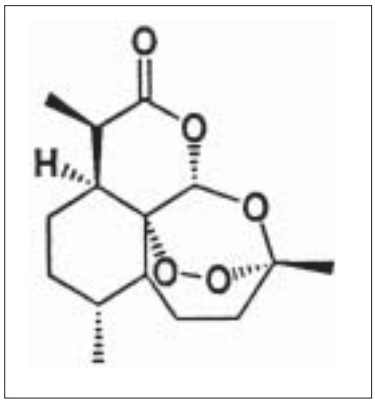


FIGURE 3
The chemical structure of Artemisinin (Qinghaosu).

PLANT GENUS ARTEMESIA

The genus Artemisia is a member of the family Compositae and comprises more than 300 species of annual, biennial and perennial herbs (and shrubs).[6] The plants have pinnate, divided, silky, hairy leaves and these are often grey to white in colour. The flowers are pendant and daisy-like but with no rays.

The genus was named after Artemis, the Greek goddess (Figure 4). She is chiefly associated with the wildlife of the earth, human fertility, reproduction and childbirth. The daughter of Zeus and Leto, she was the twin sister of Apollo. Often represented as many breasted, she appeared at Ephesus, in Asia Minor, as the Great Mother. She also had a strong association with the moon – as did many of the goddesses of fertility.[7]

The genus Artemisia is an important one and includes *A. absinthium* (the bitter wormwood), *A. dracunculus* (tarragon), *A. vulgaris* (the mugwort) and *A. ponticum* (the Roman wormwood).

The bitter wormwood (*A. absinthium*) was used to eliminate intestinal worms and as an insect repellent. Most importantly, it was also employed for many years as a flavouring agent for the drink absinthe. However, this plant contains the neurotoxin thujone and the poison can produce addiction and convulsions. As a result of these untoward effects absinthe was banned in most European countries at the end of the nineteenth century.[8] Tarragon (*A. dracunculus*) is widely used both as a flavouring in cooking and also to add taste to vinegar. Mugwort (*A. vulgaris*) is a venerable herb with a long history. A good description was given by Dioscorides in

FIGURE 4
Artémis de Versailles, 'Diane chasseresse' accompagnée d'une biche. Museé du Louvre, Paris. © Photo RMN – Hervé Lewandowski.

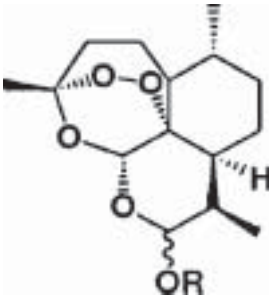
the fourth century who claimed that it gave succour to women in labour. This effect of mugwort on all women's ailments also featured in the thirteenth century Welsh herbal of the Physicians of Myddfai. From time immemorial this Artemisia was the herb burnt by the Chinese in the practice of moxibustion.

When artemisinin was isolated from *A. annua*, other species in the same genus were evaluated for their content of the terpene by means of their activity against *Plasmodium falciparum*. Most of the species studied were inactive compared with *A. annua,* but *A. aspiacea* and *A. capillares* had some antimalarial activity.

CHEMISTRY OF THE ACTIVE PRINCIPLE(S)

The yield of artemisinin from *A. annua* varies from 0·01 to 0·5% by weight. The Chinese authorities reported that the best yields came from plants growing in the Sichuan province. They established that the compound had an empirical formula $C_{15}H_{22}O_5$ and the structure, when established, proved to be that of a sesquiterpene with a characteristic lactone ring (Figure 3). The structure of the compound was confirmed in 1983 by a total synthesis of the terpene from isopulegol.[9]

When artemisinin is subjected to chemical reduction, the compound dihydroartemisinin is produced and this proved to be twice as effective as the parent compound when tested against *Plasmodium* species. However, there was a problem with the dihydro compound: its relative insolubility. To overcome this problem three sets of derivatives were produced: ethers, esters and carbonates (Figure 5). As a result of this programme of partial synthesis several compounds were brought forward for clinical trial, including arteether and artesunate.



R = CH_3: Artemether

R = C_2H_5: Arteether

R = $COCH_2CH_2CO_2Na$: Artesunate (Na)

FIGURE 5
The chemical structure of the derivatives of artemisinin.

THE USE OF ARTEMISININ AND ITS DERIVATIVES IN THE TREATMENT OF CLINICAL MALARIA

The first definitive trials of artemisinin were carried out in 1979 by the Chinese Coordinating Research Group. They treated 2,099 patients with malaria with the terpene (ratio *P. falciparum*:*P. vivax* is 3:1). They reported an astonishing result: clinical cure in all patients! In addition they treated 143 cases of chloroquine-resistant falciparum malaria and 141 cases of cerebral malaria with good results.[3] Since this early ground-breaking trial with its dramatic outcome, many other trials have been carried out in China and south-east Asia confirming this first Chinese study. The most striking results have been seen in the treatment of the dangerous condition cerebral malaria where 'cure' rates of up to 90% have been achieved by artemisinin, artemether and sodium artesunate.[10]

It should be emphasised that the artemisinin derivatives do not cure malaria as recrudescence is common. To eradicate the parasite a supplementary drug is necessary, indeed mandatory, for example sulphadoxine or mefloquine (see below). When malaria occurs in isolated rural areas standard therapy may prove difficult. Arteether can be given intramuscularly and artesunate suppositories are effective in patients who are too ill to swallow (or are in a coma as a result of cerebral malaria).[11]

ARTEMISININ IN COMBINATION THERAPY

Early on in the studies with artemisinin a significant

problem emerged. Recrudescence of malaria occurred in up to ten per cent of the patients studied. However, studies in Thailand showed that this 'failure' rate could be reduced to two to five per cent if the terpene was combined with mefloquine. Moreover, the emergence of resistance to mefloquine was delayed.[12]

In addition, the ability of this combination to kill gametocytes, delayed or prevented transmission of the malarial infection. Other combinations are under investigation to increase the cure rate in malaria and to prevent the development of resistance in the parasite. They include artemether/lumefantrin and artemether/atovaquone/proguanil.[12]

CEREBRAL MALARIA

Several randomised studies have compared the efficacy of artemether and quinine in cerebral malaria. Most of these trials demonstrated an equivalent cure rate for the new and the old compound.

Two worrying features emerged from these trials. In those patients who received artemisinin, both fever and coma appeared to be more prolonged than in those receiving quinine.

In dogs, various toxic effects had been described including haemolysis of red cells, vacuolation of cerebral neurones and prolongation of the cardiac QT interval on the electrocardiogram (ECG). In humans, the experience with the drug has, in general, been reassuring. No haemolysis of the red cells has been observed nor any definite neurological damage.[13] A residual concern has been prolongation of the QT interval which was observed in 25% of patients in one particular trial compared with 45% of the quinine-treated patients (in the same study). It has therefore been recommended that quinine and artemisinin should not be used as combined therapy. Artemisinin (and its derivatives) seem to be tolerated better than quinine when given by the intramuscular route.[13]

HOW DOES ARTEMISININ KILL THE MALARIAL PARASITE?

The manner in which this compound kills off the malarial parasite is a complex matter and several different mechanisms are at work.[14] The main actions seem to be:

1. disruption of haemoglobin catabolism in the plasmodial parasite;
2. damage to the haem detoxification system of the parasite;
3. generation of free radicals from the sesquiterpene lactone which attack the membranes of the parasite; and
4. alkylation of intracellular proteins in the parasite either by free radicals or by the haem-artemisinin complex.

The ability of the endoperoxide to generate free radicals is not a mechanism shared with the quinolone antimalarials and this might explain why artemisinin has proved superior to chloroquine. It has also stimulated the search for other plant derived (or semi-synthetic) terpenes that could attack the plasmodia in a similar way (see below). It is now very clear that artemisinin is the most exciting compound to be developed against malaria since the Second World War and that it is, on the whole, very safe.

A NEW ANGLE FOR THE PENTAGON

Since the 1941–5 war in the Pacific, the US General Staff have been painfully aware of the human cost of fighting in this theatre of action. The casualties would include not only combat deaths and disablement but those due to malaria, rickettsial diseases and arborviruses. The US's treaty with Taiwan would mean war with China if the former were to be attacked.

In the early 1980s, the Pentagon realised that the Chinese had Qinghaosu and would therefore have a definite military advantage in any land war in Asia. This was particularly important as chloroquine-resistance in plasmodia had become widespread. The US General Staff were thrown into a state of agitation and they asked the Walter Reed Institute, also located in Washington, if Qinghaosu could be identified and, if so, did it exist at any site in America. The answer in fact lay right on their own doorstep! Amongst a number of areas investigated was the Potomac River as it flows through Maryland into Washington and then down to the sea. *Artemisia annua* was located in several sites along the lower part of the river and down into the Potomac basin. Extraction of the herb was carried out and artemisinin recovered at a concentration of 0·06% w/w.[15] The Pentagon's panic was over!

It later turned out that *A. annua* was very well known to US botanists having been introduced from the near East and Asia in the nineteenth century. The early settlers had used it as a preservative and flavouring and called it Sweet Annie! The scramble for qinghaosu by the US military reminds us strongly of the rush for cinchona by the French, British and Dutch in the eighteenth and nineteenth centuries.[1]

OTHER POSSIBLE USES OF ARTEMESIA DERIVATIVES

Schistosomiasis and Clonorchiasis

In terms of its public health and socioeconomic significance, the blood fluke disease schistosomiasis ranks second only to malaria. Praziquantel has been established as the drug of choice for some years, as it is both effective and safe. Nevertheless, reinfection rates are high and there are worries about the emergence of resistance to this compound.

In animal models of schistosomiasis, artemisinin had

shown activity against the trematodes *Schistosoma mansoni* and *Schistosoma japonicum*. This work has now been extended to humans and positive clinical trials have been reported from China in *S. japonicum* and from the Ivory Coast in *S. mansoni* infection.[16] Several possibilities emerge from these studies. First, artemisinin compounds could be used as an additional tool to control schistosomiasis in areas where malaria is not endemic, for example, Brazil, Venezuela and Egypt. Second, it could be used as a short-term prophylaxis for travellers; canal cleaners in the Sudan; and in emergency situations such as flooding.

Artemisinin has also been shown to be effective against *Clonorchis* (*Opisthorchis*) *sinensis,* the liver fluke of southern China, which is an important cause of cholangiohepatitis.[16] This area of China is also affected by endemic malaria so that caution will be needed in the application of artemisinin compounds in order not to select out resistant plasmodia.

PLANT HUNTING AGAINST MALARIA: PAST, PRESENT AND FUTURE

The histories of Artemisia and Cinchona encapsulate the chequered background of the use of plant products against malaria. First, the ancient folklore of the Chinese or Amerindian, followed by the chemical identification of the active principles and then their application in a more controlled way than with the crude plant extracts. In the particular case of quinine worries about supplies of the drug in World Wars I and II led to the development of synthetic antimalarials such as the quinolones and mefloquine.

The striking difference between the stories of Cinchona and Artemisia is the length of time that was required to identify and secure the active therapeutic compound: in the case of quinine almost 200 years (1630 to 1820) but in the case of artemisinin only ten years (1970 to 1980). This marked contrast reflects the ability and efficiency of modern chemical techniques to isolate, identify and synthesise an active principle and, of supreme importance, to supply sufficient quantities for authoritative clinical trials. Another substantial advantage to the modern investigator is the availability of *in vitro* culture methods for plasmodia; together with the *in vivo* model of *Plasmodium berghei* infection in mice. Resistance to artemisinin will surely develop and appropriate steps, such as the use of combination therapy must be taken now to limit or delay this emerging problem.

What are the future prospects in this area? In relation to artemisinin, the active moiety has been simplified to the 1, 2, 4-trioxane structure and some compounds with this basic group have been synthesised and are being screened for antimalarial activity. A group in Liverpool has synthesised new diamine containing analogues of artemisinin. These have the advantage of increased oral bioavailability, superior activity against chloroquine resistant parasites and also are not metabolised by P450 enzymes in the liver. Potentially therefore they may have better *in vivo* activity in human malaria.[18]

Could there be other plants that might have significant killing capacity against *Plasmodium*? The answer is almost certainly 'yes'. Promising leads include *Artabotrys uncinatus* (China: arteflene), *Azadirachta indica* (Asia: nimbolide) and *Brucea javanica* (China: terpenoids).[17] Others will no doubt follow. While the malarial vaccine is eagerly awaited, the search for natural and synthetic antimalarial compounds must be pursued actively. We should obviously proceed along as broad a front of attack as we can sustain as nature always fights back!

Finally, what lessons that we can learn from the stories of Cinchona and Artemisia? The first, perhaps, is that there is often an element of truth in the old folk myths concerning medicinal plants. The second, is that the full rigour of modern chemical and biological techniques should be applied to candidate compounds culminating in the gold standard of the double-blind randomised controlled clinical trial. Only in this way can we move from superstition and myth to scientific certainty.

As we wander round the apothecary's herb garden and contemplate the sweet wormwood, a nondescript grey Artemisia, we should reflect that within this unprepossessing plant lies chemical magic!

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