3 *Artemisia annua* as a Traditional Herbal Antimalarial

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3.1 INTRODUCTION

The species *Artemisia annua* L (Asteraceae) is native to China. Its ancient Chinese name, Qing Hao, literally means "green herb." The genus *Artemisia* comprises over 400 species, many of which have an aromatic, bitter taste. There are two theories as to the origin of its name. Ferreira et al. (1997) say that it is named after the Greek goddess Artemis, meaning literally "she who heals sickness," who was in fact goddess of the hunt, of forests, and was thought to be responsible for sudden death in women (Guirand, 1959). Apparently plants of this genus, probably *Artemisia absinthium*, were used to control pain in childbirth and to induce abortions. Bruce-Chwatt (1982) says that *Artemisia* was named after Queen Artemisia of Caria (Turkey), who lived in the fourth century B.C. She was so aggrieved on the death of her husband and brother, King Mausolus of Halicarnassus, that she mixed his ashes with whatever she drank to make it taste bitter.

A. annua is so named because it is almost the only member of the genus with an annual cycle. It is a shrub, often growing over 2 m high (Ferreira et al., 1997; see Figure 3.1 and Figure 3.2). Its leaves and flowers contain artemisinin, first isolated in China in 1971; this is the constituent with the greatest antimalarial activity (see Table 3.3). Artemisinin has been found in only two other species, *Artemisia apiacea* and *Artemisia lancea* (Tan et al., 1998).

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FIGURE 3.1 *A. annua.* (Copyright © Merlin Willcox.)

FIGURE 3.2 *A. annua* hybrid growing in Brazil. (Copyright © Pedro Melillo de Magalhães .)

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Artemisinin is poorly soluble in oil or water, so is usually administered orally, although it can be given rectally (Ashton et al., 1998) and, when suspended in oil, intramuscularly (Titulaer et al., 1990). Synthetic derivatives that are water soluble (artesunate) and oil soluble (artemether) have been developed to enable intravenous and intramuscular administration, respectively (Van Agtmael et al., 1999a). It is now universally accepted that this family of compounds is among the most powerful antimalarial drugs ever discovered. The pharmacological and clinical evidence is well documented (Wright and Warhurst, 2002; Wilairatana and Looareesuwan, 2002).

Artemisinin cannot be synthesized cost effectively, so it is still extracted from *A. annua* aerial parts. Therefore, the science of commercial cultivation of *A. annua*, to maximize artemisinin yields, is already well developed (Laughlin et al., 2002). However, the end product is often unaffordable for the poor, especially as it is recommended to only be used in combination with other drugs (Nosten, 2002). The lowest cost of a course of Co-artem (artemether-lumefantrine), provided by the manufacturers to some countries in Africa, is $2.40 (François Nosten, personal communication), and a course of artemether suppositories for a 30-kg child would cost about $2 (F.H. Jansen, personal communication). This is above the commonly quoted affordable limit of $1 (but even that is more than many people can afford). Furthermore, its value makes it an attractive target for forgery. A survey in 2000–01 in Southeast Asia found that 38% of shop-bought oral artesunate samples were fake and contained no artesunate (Newton et al., 2001). A more recent survey has found that some fake artesunate packs are now indistinguishable from the genuine ones (Newton et al., 2003).

Therefore, some organizations have already set up programs for small-scale cultivation and local utilization of the herbal formulation of *A. annua*. For example, the NGO Anamed (see below) and Medicos Descalzos (barefoot doctors) in Guatemala promote its use.

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However, the science of small-scale cultivation and local production is far from perfected, and has not been studied to the same extent as that of large-scale cultivation and use of its pharmaceutical end product. This chapter sets out to address some of these practical considerations as far as is possible within the current state of knowledge, and to highlight key research priorities for the immediate future. Its aims are to review the literature on the classical ethnomedical use of *A. annua*, literature on the pharmacokinetics and pharmacodynamics of Herba *A. annua* and its constituents, and experience in its horticulture and cultivation. Common concerns about the use of herbal preparations of *A. annua* will be discussed.

3.2 ETHNOPHARMACOLOGY

3.2.1 *A. annua* as a Traditional Antimalarial

The earliest record of the medicinal use of the shrub *A. annua* (Qing Hao) dates back to 168 B.C. in the "Fifty-two prescriptions" discovered in one of the Han Dynasty tombs in Mawangdui; this advocated the use of *A. annua* for the treatment of hemorrhoids. It is likely that the plant had been used for some time before this, as it appears in the *Shen Nong Ben Cao Jing*, the foundation text of Chinese herbal medicine. This was first written in 200 A.D., but represents the cumulative knowledge of herbal medicine transmitted orally over many centuries (Shou-zhong, 1997). This text claims that among other properties, Qing Hao "relieves lodged heat in the joints," which could be interpreted as treating rheumatoid arthritis, or possibly a number of febrile conditions.

Zhang Ji (150–219 A.D.) in his classic text *On Cold Damage* (*Shang Han Lun*) recommends a decoction of *A. annua* to treat fevers with sweating and jaundice (Mitchell et al., 1999). He recommends that fruits of *Gardenia jasminoides* and roots of rhubarb (*Rheum palmatum*) should be added to the decoction. The *Handbook of Prescriptions for Emergency Treatment* (*Zhouhou Beiji Fang*), written in 340 A.D., also recommends the use of Qing Hao for fevers. The mode of preparation was a cold aqueous extraction: a handful of the aerial parts of the plant should be soaked in two Sheng (approximately 1 to 2 l) of water, and the juice should all be drunk (Tu, 1999). Different preparations have subsequently been recommended in different texts: a decoction of 30 g

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TABLE 3.1
Ingredients with Which *A. annua* Is Combined for Different Types of Fever

Type of Fever	Added Ingredient	Part
No sweating, but dizziness and stifling sensation in chest	*Dolichos lablab* L (Leguminosae)	Seed
	Talcum	Powder
Night sweats and anemia	*Lycium barbarum* L (Solanaceae)	Stem
	Cynanchum atratum Bge (Asclepiadaceae)	Root
Heat smoldering in *yin* regions of body	*Rehmannia glutinosa* (Scrophulariaceae)	Root
	Amyda sinensis	Tortoise shell
Damp summer heat with nausea, stifling sensation in chest, and intense fever	*Scutellaria baicalensis* Georgi (Labiatae)	Root
	Pinellia ternata (Thunb) Breit (Araceae)	Rhizome

Source: Bensky, D. and Gamble, A., (1993), *Chinese Herbal Medicine Materia Medica*, Eastland Press, Inc., Seattle, WA.

of *A. annua* daily, pills, powdered dried leaves in a dose of 3 g per day for 5 days, and fresh plant juice at a dose of 3 g daily (QACRG, 1979; Foster and Chongxi, 1992). Li Shizhen (1596) described the use of Qing Hao for malarial fevers in his *Bencao Gangmu*.

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Mixtures with other medicines have also been used; for example, in 1798 Wenbing Tiaobian (quoted in Laughlin et al., 2002) used *A. annua* combined with *Rehmannia glutinosa* (Scrophulariaceae), *Anemarrhena asphedeloides* (Liliaceae), *Paeonia suffruticosa* (Ranunculaceae), and *Carapax Amydae sinensis* (tortoise shell). There exist several other combinations, according to the type of fever (see Table 3.1). The leaves of *A. annua* are also burned in China as a fumigant insecticide to kill mosquitoes (Foster and Chongxi, 1992).

3.2.2 Anamed Recommendations

Anamed (Action for Nature and Medicine) is an NGO promoting the use of traditional medicines (www.anamed.org). It distributes seeds of a recently developed artemisinin-rich genotype of *A. annua* (Delabays, 1997; De Magalhães, 1996) for cultivation and preparation as an herbal antimalarial. Two hundred forty partner organizations in developing countries are participating in this program, and their feedback has helped the organization to refine its recommendations, which are summarized below.

Hirt (2001) recommends an infusion of 5 g of dried leaves on which 1 l of boiling water is poured and left to cool for 15 minutes (for a 60-kg adult; 2.5 g of leaves for a 30-kg child and 1.25 g for a 15-kg child). This method extracts 55% of the artemisinin into the water, and 35 to 40% remains in the leaves. Only 5% is lost, in contrast to a decoction (when the plant is boiled in water for several minutes), where 50% of the artemisinin is lost, because it is not heat stable. An infusion in full fat milk can increase the proportion of artemisinin extracted to 80% (De Magalhães et al., 2003). Anamed advises that as artemisinin reacts with iron, the tea should be prepared in pots made of other materials.

Anamed recommends the dose of 250 ml of the infusion, taken every 6 hours for 7 days; this dose is based on that in the Chinese pharmacopoeia, which recommends a dose of 4.5 to 9 g daily. An alternative is to swallow 1 g of dried leaves three times a day, but here Anamed has only made a few positive observations. Anamed is also observing patients treated with an enema using double the dose of leaves and half the amount of water; there have been some positive results, but further research is awaited (Hirt, personal communication).

3.2.3 *A. annua* and Artemisinin

Artemisinin is a sesquiterpene lactone, containing an unusual endoperoxide group, which is believed to be responsible for the antimalarial activity of *A. annua*. Unlike quinine-related drugs and antifolate drugs, artemisinin and its derivatives are gametocytocidal and reduce the transmission potential of falciparum malaria (Price et al., 1996). The mechanism of action is explained in detail in Chapter 15.

Artemisinin was isolated from cold-ether (rather than hot-water) extracts of the plant (Yu and Zhong, 2002). Aqueous decoctions of *A. annua* were found to be ineffective against *Plasmodium berghei* in mice, and the serum of rabbits treated with this decoction was ineffective against *Plasmodium falciparum in vitro*, but ether extracts were active (Lwin et al., 1991). This is not entirely surprising because decoctions contain less artemisinin than infusions (see above). Furthermore, preparations containing whole *Artemisia* leaves are likely to have a higher artemisinin content than filtered decoctions or infusions, if equivalent doses are used, because much of the artemisinin will remain in the leaves rather than be dissolved in the water. Second, the mouse model is not always an accurate predictor of effectiveness in humans. Absorption and metabolism of artemisinin and other active ingredients may differ, and *P. berghei* may have different properties and sensitivities from *P. falciparum*.

The dose of artemisinin in 5 g of dried leaves (with a relatively high content of about 1.4% artemisinin) would be no more than 70 mg. One liter of infusion made from 5 g of leaves contains 12 mg of artemisinin (Mueller et al., 2000). The dose recommended by the World Health Organization (WHO) is 20 mg/kg as a divided loading dose on the first day, followed by 10 mg/kg once daily for 6 days (Phillips-Howard, 2002). For a 60-kg adult, this works out as 1200 mg on the first day, followed by 600 mg daily.

The large discrepancy between the artemisinin content of the infusion and the recommended doses of artemisinin has led to two important concerns:

1. Herbal preparations of *A. annua* could not possibly be effective.
2. Subtherapeutic concentrations of artemisinin will favor the evolution of resistant parasites.

A third concern is whether herbal preparations are safe and well tolerated. Each of these points will be addressed in detail below.

3.3 CLINICAL EFFICACY

Artemisinin and its derivatives are the most potent and rapidly acting antimalarial drugs: the parasite biomass is reduced by 10,000-fold per asexual life cycle, compared to 100- to 1000-fold for other antimalarials. They also decrease gametocyte carriage by 90%, thus reducing transmission of malaria (White et al., 1999). A meta-analysis of trials, with data on 1919 patients, has shown that artemether is at least as effective as quinine and is associated with fewer serious adverse effects (Artemether-Quinine Meta-analysis study group, 2001).

There are less data on the efficacy of the whole herb. The larger clinical trials of different *A. annua* preparations are summarized in Table 3.2. Chang and But (1986) report on a trial of the dilute alcohol extract of the herb, given as a total dose of 72 g of crude extract over 3 days in divided doses. The cure rate was said to be 100% in 485 cases of *Plasmodium vivax* and 105 cases of *P. falciparum*. However, the definition of cure was not clear; it would be impossible to achieve a cure rate of 100% with a 3-day course of pure artemisinin.

Hirt and Lindsey (2000) reported a 93% parasite clearance rate in 254 patients who had taken a 7-day course of *A. annua* infusion in the Democratic Republic of Congo, mainly for falciparum

TABLE 3.2
Clinical Trials of *A. annua* Preparations for Patients with Malaria

Efficacy	Recrudescence Rate	N[a]	Species	Preparation	Total Dose (g)	Days	Reference
100%	?	485	Vivax	Crude alcohol	72	3	Chang and But, 1986
100%	?	105	Falciparum	Crude alcohol	72	3	Chang and But, 1986
100%	8%	50	Vivax	Oil-based capsules	129	6	Yao-De et al., 1992
100%	?	5	Falciparum	Aqueous infusion	20	5	Mueller et al., 2000
93%	13%	254	Falciparum + others	Aqueous infusion	35	7	Hirt and Lindsey, 2000
92%	?	48	Falciparum	Aqueous decoction	20	4	Mueller et al., 2000

[a] *N* = number of subjects.

TABLE 3.3
Clinical Trial of *A. annua* in Gelatine Capsules

Drug	N[a]	Days of Treatment	Total Dose (g)	FCT[b] (hours)	PCT[c] (hours)	Recrudescence (%)
COEA	53	3	73.6	18.3	33.7	12/36 (33)
COEA	50	6	128.8	18.4	32.9	4/50 (8)
QHET	41	3	80.0	21.8	33.0	13/41 (32)
Chloroquine	20	3	1.2	23.7	49.9	0/20 (0)

[a] *N* = number of subjects.
[b] FCT = fever clearance time.
[c] PCT = parasite clearance time.

Source: Yao-De, W. et al., (1992), *Chung Kuo Chi Sheng Chung Hsueh Yu Chi Sheng Chung Ping Tsa Chih*, 10, 290–294.

malaria, although some had infections with other *Plasmodium* species. In a subset of 31 patients followed long-term, the recrudescence rate was 13% after 1 month (Hirt, 2001).

AU: Please introduce COEA and QHET.

Yao-De et al. (1992) report on a clinical trial of two types of gelatine capsules containing the herb *A. annua*, COEA and QHET (Table 3.3). One of these, called COEA, contained oil to enhance absorption of the artemisinin. All the patients were infected with *P. vivax*; 53 were treated with COEA for 3 days, and 50 were treated for 6 days. The dosage was 36.8 g on the first day, followed by 18.4 g on subsequent days, to give the total doses recorded in Table 3.3. Parasite clearance times and fever clearance times were significantly faster for all the herbal preparations than for chloroquine. Patients were followed up for 30 days, with a blood film examined for parasitemia every 10 days. About one in three patients who had taken the capsules for 3 days only experienced a recrudescence, but this was reduced to only 8% in those who had taken the capsules for 6 days.

Mueller et al. (2000) evaluated two preparations of *A. annua* for patients with falciparum malaria. Although numbers were small, they found that both the infusion (1 l of boiling water added to 5 g of dried leaves, and mixture left to cool for 15 minutes before filtering) and the

decoction (5 g of dried leaves placed in 1 l of water, boiled for five minutes, and then filtered) were effective. In each case, the dose was 250 ml four times a day, although the infusion group was treated for 5 days and the decoction group for 4 days. The results were good in both groups (see Table 3.2) — this is surprising, given that the decoction contains less artemisinin and was ineffective in mice (see above). One reason may have been that the patients had some immunity to malaria. Unfortunately, there was no follow-up after day 7, so there are no data on recrudescence. A further trial by the same group showed that recrudescence rates are high (Lutz Heide, personal communication). Parasite clearance occurred by day 7 in 75% of patients treated with *A. annua* (cf. in 35% treated with CQ), but by day 28 only 55% were still free of parasites (cf. 90% for quinine, 35% for CQ).

AU: Please introduce CQ.

None of the above studies adhere to an ideal design (see Chapter 21). Nevertheless, they all suggest that herbal preparations of *A. annua* may be effective against malaria in humans. Yet even in the Chinese studies, using relatively large amounts of *A. annua*, the dose of artemisinin would be less than half the WHO-recommended dose (assuming artemisinin content of 1%, the 129-g total dose would contain 1290 mg of artemisinin). There is an evident discrepancy between the reported clinical efficacy of traditional formulations of *A. annua* and their low artemisinin content. Several possible mechanisms will be discussed below.

1. *Efficacy of lower doses of artemisinin*
   Ashton et al. (1998) have suggested that oral doses of 500 mg of artemisinin daily are unnecessarily high. Rectal doses of 500 mg are equally effective, although their relative bioavailability was only 30% (equivalent to an oral dose of 160 mg). There was a high degree of variability in the artemisinin plasma concentrations after both oral and rectal administration. Interestingly, clinical end points such as parasite and fever clearance times do not correlate with drug exposure.
2. *Improved bioavailability of artemisinin*
   Pure artemisinin has poor oral bioavailability, about 32% (Titulaer et al., 1990). This is not affected by food intake (Dien et al., 1997). Artemisinin is poorly soluble in water and oil, but dissolves in organic solvents. Infusions of *A. annua* contain greater amounts of artemisinin than an infusion made by pouring hot water onto pure artemisinin, implying that other plant constituents, for example, flavonoids, are helping to dissolve artemisinin by acting as detergents (Mueller et al., 2000). Saponins from other plants used in traditional mixtures may further amplify this effect.
3. *Inhibition of artemisinin catabolism*
   The bioavailability of artemisinin is reduced by a factor of 6.9 by the fifth day of repeated treatment (Sidhu et al., 1998). This may be due to autoinduction of liver enzymes (Svensson et al., 1998), although the half-life of artemisinin remains unchanged. The plasma concentration of the active metabolite dihydroartemisinin increases with repeated treatment, so this may compensate for the loss in artemisinin concentration (Van Agtmael et al., 1999b). *In vitro*, the enzymes mediating the catabolism of artemisinin are cytochrome P450 2B6 and 3A4 (Svensson and Ashton, 1999). There is a large variation of the level of these enzymes in human subjects, which may explain the interindividual variation in artemisinin kinetics. It is conceivable that other components of the plant (or of other plants used in traditional mixtures) may inhibit these enzymes. No studies have yet been performed on the pharmacokinetics of artemisinin from traditional preparations of the whole herb.
4. *Immunostimulation*
   Artemisinin stimulates the phagocytic activity of macrophages in the mouse abdominal cavity *in vivo* (Ye et al., 1982). Furthermore, after treatment with artemisinin, mouse macrophages were better at phagocytosing *P. berghei*-infected mouse erythrocytes

in vitro. The activity of macrophage acid phosphatase was also enhanced. This property of artemisinin may boost the human immune response to malaria.

5. *Other constituents with antimalarial activity*
   Other *Artemisia* species have antimalarial activity without containing artemisinin (Valecha et al., 1994). *A. absinthium* and *Artemisia abrotanum* were used to treat malaria in Europe, but their activity is attributable to other constitutents (Cubukcu et al., 1990; Deans and Kennedy, 2002). *Artemisia afra* extracts are effective against *P. falciparum in vitro*, and this activity is attributable to a complex mixture of flavonoids and sesquiterpene lactones, rather than to a single compound (Kraft et al., 2003). Some of these phytochemicals may also contribute to the activity of *A. annua.*

   A. annua contains many different classes of compounds: at least 28 monoterpenes, 30 sesquiterpenes, 12 triterpenoids and steroids, 36 flavonoids, 7 coumarins, and 4 aromatic and 9 aliphatic compounds (Bhakuni et al., 2002). Artemisinin is not the only antimalarial compound in *A. annua.* The callus of the plant has some antimalarial activity even though it contains no artemisinin (François et al., 1993). Furthermore, the water-soluble fraction of *A. annua*, after extraction of artemisinin, has an antipyretic effect (Chang and But, 1986).
6. *Synergistic activity of other constituents of* A. annua
   Artemisinin is only 1 of 29 sesquiterpenes in *A. annua*. Some of these are in much greater concentrations than artemisinin in wild strains of the plant: arteannuin B (two to four times) and artemisinic acid (seven to eight times). Both of these have antibacterial and antifungal properties (Dhingra et al., 2000). Arteannuin B used alone was found to be ineffective and toxic in rat malaria, but it potentiated the effect of artemisinin (Chang and But, 1986). However, in hybrid plants with a high artemisinin content, the concentration of artemisinic acid is much lower (Magalhães, personal communication).

   In addition, *A. annua* produces at least 36 flavonoids. Many of these have antimalarial activity *in vitro*, although the inhibitory concentration 50% (IC_{50}) is much higher than that of artemisinin (Table 3.4). Five of these, artemetin, casticin, chrysoplenetin, chrysosplenol-D, and cirsilineol, have been shown selectively to potentiate the *in vitro* activity of artemisinin against *P. falciparum* (Liu et al., 1992). Casticin, at a concentration of 5 µmol/l, induced a three- to fivefold reduction in the IC_{50} for artemisinin (Elford et al., 1987). Chrysosplenol-D has the strongest potentiating effect, and this is also the most abundant flavone in plant material (Liu et al., 1992). Interestingly, the flavones do not potentiate the antimalarial activity of chloroquine (Elford et al., 1987). Although they have no effect on hemin themselves, they do catalyze the reaction of artemisinin with hemin (Bilia et al., 2002) and may also help to solubilize artemisinin (see above).

TABLE 3.4
In Vitro* Antimalarial Activity of Constituents of *A. annua

Constituent	IC_{50} (µ*M*)
Artemisinin	0.03
Artemetin	26
Casticin	24
Chrysoplenetin	23
Chrysosplenol-D	32
Cirsilineol	36
Eupatorin	65

Source: Liu, K.C.-S. et al., (1992), *Plant Cell Rep*., 11, 637–640.

The effect of all the flavones in combination with artemisinin has not been investigated. Other flavones, and indeed other components of *A. annua*, may have a similar effect; they have not all been tested because it is difficult to purify them. The antimalarial properties of the traditional preparation of *A. annua* most probably reside in the combination of many constituents, not just artemisinin.

3.4 EVOLUTION OF RESISTANCE

One of the principle concerns regarding the use of *A. annua* for malaria is that the ingestion of low doses of artemisinin may accelerate the development of resistance to this drug. There is little evidence to support or refute this claim, and further research is needed.

It could be argued that some degree of resistance to artemisinin has already evolved in China. The IC_{50} of artemisinin varies according to the strain of *P. falciparum* and can be as low as 6 n*M* (Wongsrichanalai et al., 1997). Chinese strains (IC_{50} = 630 n*M*) are much less sensitive to artemisinin than African strains (IC_{50} = 25 n*M*). This could be explained by the long-standing local use of *A. annua*, or of artemisinin itself over the last 30 years (Wernsdorfer, 1999). The parasites would find it easier to develop resistance to a single agent than to a battery of antimalarial compounds such as those contained in whole plant extracts.

There is undoubtedly a high recrudescence rate when artemisinin is used alone, and if it is given in a short course; there are at least three factors that may contribute to this. First is the time-dependent reduction in bioavailability, although an escalating dose of artemisinin did not reduce the frequency of recrudescences (Gordi et al., 2002).* Second, low concentrations of dihydroartemisinin have a static rather than cidal effect on early trophozoites *in vitro*; these then remain in a metabolic resting state for up to 6 days followed by renewed growth. Third, the short half-life of artemisinin (approximately 2 hours) means that although parasitemia is reduced below detectable levels, the drug is not present in the plasma long enough to eliminate all the parasites. Parasite clearance would require effective drug levels during three to four life cycles (each one lasting 48 hours), which can be achieved by combination with a long-acting antimalarial drug (Van Agtmael et al., 1999a) or by administration of the drug every 4 to 6 hours for 7 days.

On the other hand, these pharmacokinetic properties will help to protect artemisinin against the evolution of resistance. The shorter the half-life, the less time parasites are exposed to subtherapeutic concentrations, and so the less the selective pressure for evolution of resistance. As artemisinin has a short half-life, parasites would be exposed to subtherapeutic doses for only a few hours. Drugs such as sulfadoxine-pyrimethamine have a much longer half-life, and parasites are exposed to subtherapeutic concentrations for 37 days after a single treatment (Watkins and Mosobo, 1993).

The traditional preparation of the herb may be effective in spite of its low artemisinin content (as discussed above) and thus may kill parasites before they can develop resistance. Studies in China have shown that the recrudescence rate can be reduced by combining *A. annua* with the roots of two other plants, *Astragalus membranaceus* (Leguminosae) and *Codonopsis pilosa* (Campanulaceae) (Chang and But, 1986). The mechanism for this has not yet been elucidated, but *A. membranaceus* is known to have immunostimulant properties (Foster and Chongxi, 1992). Modern drug combinations may unwittingly be mimicking the combinations of phytochemicals (and sometimes plant species) with synergistic activities contained in the traditional preparations of *A. annua*. Indeed, the pharmaceutical industry may find it beneficial to further investigate combinations of artemisinin with other compounds produced by *A. annua*, which may improve its antimalarial efficacy and reduce the risk of resistance.

* This may have been because the initial dose was low (100 mg) and was metabolized faster than the higher dose of 500 mg, which may have saturated first-pass metabolism.

TABLE 3.5
Therapeutic Index of *Artemisia* Extracts and Artemisinin in *P. berghei*-Infected Mice

Substance	LD_{50}[a] (mg/kg)	ED_{50}[b] (mg/kg)	Therapeutic Index (LD_{50}/ED_{50})
Crude plant	162,500	11,900	13.7
Ether extract (neutral fraction)	7425	2646	2.80
Dilute alcohol extract	4162	2526	1.64
Artemisinin	5105	139	36.80

[a] LD_{50} = dose lethal in 50% of mice.
[b] ED_{50} = dose effective in curing 50% of mice.

Source: Yao-De, W. et al., (1992), *Chung Kuo Chi Sheng Chung Hsueh Yu Chi Sheng Chung Ping Tsa Chih*, 10, 290–294; Chang, H.M. and But, P.P.H., (1986), *Pharmacology and Applications of Chinese Materia Media*, Vol. 1, World Scientific Publishing, Singapore.

3.5 SAFETY AND TOLERABILITY

The presence of multiple chemical constituents in herbal preparations of *A. annua* raises the question as to their safety. The lethal dose 50% (LD_{50}) and therapeutic index have been measured for the crude plant and are reassuringly high (see Table 3.5).

Artemisinin has a slightly negative chronotropic effect on the heart, causing mild hypotension. Toxicological studies on artemisinin are reviewed in detail later in this book (see Chapter 15), but clinically it seems to be very safe. The chief concerns over the safety of artemisinin originated from a study that showed that high-dose intramuscular arteether and artemether in dogs and rats caused a clinical neurological syndrome with gait disturbances and loss of some reflexes, and prominent brainstem lesions (Brewer et al., 1994). However, artemisinin has now been used in several million patients, with only one report of neurological side effects following artesunate treatment (WHO, 1998b; White et al., 1999; Wilairatana and Looareesuwan, 2002). In patients who died of severe malaria, neuropathological findings were similar in recipients of quinine and artemether, and there was no evidence for artemether-induced toxic effects (Hien et al., 2003).

Artemisinin is generally considered to be safe in the second and third trimesters of pregnancy (WHO, 1998b). However, early studies showed that relatively low doses of artemisinin (13 to 25 mg/kg, or 1/200 to 1/400 of the LD_{50}) cause fetal resorption in rodents; therefore, use in the first trimester is not recommended (WHO, 1994). However, in a series of 16 patients treated with artesunate in the first trimester of pregnancy, the rate of abortion (20%) was similar to that of the general population (McGready et al., 1998).

A literature search has not revealed any animal toxicity studies, but the herb extract has been evaluated in China and was deemed to be of low toxicity. Five hundred ninety patients were treated with the herb extract, and of these, 3.4% developed gastrointestinal symptoms such as nausea, vomiting, abdominal pain, and diarrhea. No adverse effects were observed in patients with cardiac, renal, or hepatic dysfunction, or in pregnant women (Chang and But, 1986). Interestingly, the pharmacokinetics of artemisinin are not affected in patients with cirrhosis of the liver (De Vries et al., 1997), but artemisinin does induce certain liver enzymes, and thus interacts with some other drugs such as omeprazole (Svensson et al., 1998). Observational studies in Africa found that 25% of malaria patients being treated with *A. annua* infusion had nausea, although none vomited. Other mild adverse events during treatment included dizziness, tinnitus, pruritus, and abdominal pain (Hirt, 2001).

A. annua can be regarded as an established traditional medicine, as it has been widely used and is included in the pharmacopoeia of the People's Republic of China (Mueller et al., 2000).

Nevertheless, any future clinical trials of *A. annua* preparations should carefully monitor subjective side effects as well as end-organ function (using a protocol such as that described in Chapter 18 of this volume).

3.6 CULTIVATION

A. annua is native not only to China but also to Japan, Korea, Vietnam, Myanmar, northern India, and southern Siberia through to eastern Europe (WHO, 1998a). It has been introduced to many other countries, in Europe, North America, and the Tropics (Laughlin et al., 2002). Seed varieties have been adapted by breeding for lower latitudes, and cultivation has been successfully achieved in many tropical countries, for example, in the Congo (Mueller et al., 2000), India (Mukherjee, 1991), and Brazil (Milliken, 1997; Carvalho et al., 1997; De Magalhães et al., 1997).

The tiny seeds succeed best when sown on top of well-aerated soil, as they germinate in the light (Hirt and Lindsey, 2000); in areas with a heavy soil, the plants can first be developed in a greenhouse. In order to maximize the yield of artemisinin, the critical factor is day length, because the plant usually grows in the long summer days at high latitudes and flowers when the day length shortens. The concentration of artemisinin peaks around the time of flowering, although in some cases this may be just before flowering, and in other cases during full flowering (Ferreira et al., 1995a; Laughlin et al., 2002). The artemisinin concentration peaks at a slightly different time in different areas, and identifying this will help to maximize the yield of artemisinin in harvested plants (Chang and But, 1986). In the Tropics, where days are shorter than in northern summers, flowering occurs earlier, reducing the biomass achieved. However, yields can be maximized at higher altitudes and with late-flowering varieties (Laughlin et al., 2002), or by artificially lengthening hours of daylight to over 13.5 hours (Ferreira et al., 1995a).

In wild-type plants, the greatest concentration of artemisinin is found in the inflorescence, although it occurs in all other aerial parts of the plant, except the seed (Ferreira et al., 1997). In artemisinin-rich plants, the greatest concentration of artemisinin occurs at the beginning of the flowering season (De Magalhães, personal communication). It used to be thought that sun and oven drying reduced the artemisinin content and that it was best to air-dry leaves in the shade (Laughlin et al., 2002). However, Simonnet et al. (2001) found that sun-drying plants in the field increased the artemisinin content (perhaps by promoting conversion of some precursors to artemisinin), but that if drying continued for more than a week, leaves were lost, decreasing the overall yield. The optimum would therefore seem to be drying in the field for 1 week, followed by air-drying in the shade.

Although artemisinin content is affected by climate and time of harvesting, the main influence is genetic variation. Ferreira et al. (1995b) evaluated the same 23 clones of *A. annua*, which varied from 0.001 to 0.35% artemisinin, under tissue culture, greenhouse, and field conditions. Broad-sense heritability analyses indicated that artemisinin was mainly under genetic, not environmental, control. Delabays et al. (2002) confirmed that genes outplay the environment by studying different varieties, which yielded from 0.02 to about 1.4% artemisinin. Efforts have been made to increase the artemisinin content as far as possible, by exploring the natural variability. This has been achieved in a hybrid (*A. annua* var. *Artemis*, seeds available from www.anamed.org) and in a nonhybrid strain collected from Vietnam (Sutakavatin, 2002, personal communication). However artemisinin yield depends not only on its concentration, but also on the total number of leaves and branches. The Institute of Materia Medica in Vietnam has been breeding plants for all three of these characteristics, to optimize artemisinin yield (Dong and Thuan, 2003).

Once hybrids have germinated from the original seeds, the genotypes can only reliably be propagated by taking cuttings or by repeating the cross between the parents of the hybrid. A stock of progenitor plants needs to be maintained under artificial lights to mimic long days and prevent flowering, until a new progenitor plant is established. If the breeding between the parent plants is

not repeated, and second generation seeds are taken from the hybrid plants, only some will germinate, yielding weaker plants that contain 30% less artemisinin (Hirt, 2001).

Seeds are the easiest method of propagation and maintain their vigor for 3 years if stored in dry, cool conditions (Ferreira et al., 1997). High artemisinin yield was passed on to subsequent generations in seed-propagated plants in the breeding program of the Institute of Materia Medica in Vietnam (Dong and Huang , 2003).

AU: Thuan in references and above.

Artemisinin content can be measured semiquantitatively using thin-layer chromatography (TLC) (Box 3.1). This could potentially be used in resource-poor settings as a form of quality control.

Box 3.1: Low-Cost Method for Quality Control of *A. annua*: Semiquantitative Dosage of Artemisinin by TLC and Densitometry

Developed by Jacques Falquet (personal communication), based on Delabays (1997)

1. Mix 100 mg of powdered dried leaves with 2 ml of ethyl acetate; leave for 1 hour at 40°C.
2. Filter the solution.
3. Evaporate 1 ml of the solution in a stream of nitrogen or carbon dioxide gas if possible or, if not, in air.
4. Redissolve the solute in 0.2 ml of ethyl acetate.
5. Place 5 µl on a silica gel plate.
6. Use a mixture of ethyl acetate and cyclohexane (3:7) as the eluant; allow to migrate for 8 cm.
7. Spray with 5% anisaldehyde and 0.5% sulfuric acid in glacial acetic acid, then heat for 5 minutes at 110°C.
8. For a semiquantitative reading, there should only be one spot, corresponding to artemisinin. This should be near the center of the chromatogram (rf = 0.5). This can be compared to results from a reference standard (leaves of a known concentration, in several dilutions) applied to the same chromatogram. Accuracy of ±20% may be achievable.
9. For a more quantitative reading, the plate can be scanned into a computer and interpreted by densitometry software such as Image-J. This is available free on the Internet at http://rsb.info.nih.gov/ij/index.html.

3.7 PUBLIC HEALTH POTENTIAL

The development of artemisinin-rich genotypes of *A. annua* has encouraged some to promote cultivation and local use of the plant to treat malaria. However, others argue that this is premature, and that further research is needed before *A. annua* infusions can be promoted as part of a malaria control program.

Although the results of the clinical studies are promising, none of them was conducted with the most rigorous methodology. Often, the definition of cure was not clear, and the high cure rates may not be replicated if patients are followed up for longer periods of time. It will be important to conduct a controlled trial according to strict guidelines, such as those suggested in Chapter 21, before promoting the wider use of *A. annua*.

There are some contradictory results, such as the clinical efficacy of a decoction of *A. annua*, whereas this preparation was ineffective in mice. One reason for this discrepancy may be that subjects had some degree of immunity to malaria. Another is that the mouse model does not always accurately predict outcomes in humans. There are only anecdotal reports of efficacy in nonimmune patients, for example, a $2^1/_2$-year-old Caucasian boy with laboratory-confirmed malaria who was treated with *A. annua* tea only and made a rapid recovery (Hans-Martin Hirt, personal communication). A priority for future research would be to test whether use of *A. annua* teas can reduce the mortality rate in African children and other nonimmune patients with malaria. It would be ethical to do this if efficacy and safety has been demonstrated in a rigorous clinical trial in a semi-immune population.

Perhaps the most important finding by Mueller et al. (2000) was that local cultivation and preparation of *A. annua* are feasible in Africa. If effectiveness in nonimmune patients is demonstrated, local cultivation and preparation of *A. annua* could be considered part of a malaria control strategy, especially in remote areas with poor access to health facilities and poor availability of effective antimalarial drugs. Such remote areas (such as the Brazilian Amazon and remote rural areas of Africa) are particularly problematic for malaria control programs and are often neglected. Herbal medicines may not be as perfect as the exact dosages administered in industrially produced formulations, but may be better than no treatment, or treatment with fake artesunate tablets, which are widespread in Southeast Asia. If there are no effective local treatments for malaria, early treatment with an herbal preparation of *A. annua* may in some circumstances prove to be life saving. The risk of resistance evolving is likely to be small, for reasons discussed above.

An important concern is the potential ecological impact of introducing an alien plant species into a new fertile environment where it may spread quickly and endanger local species. In these circumstances it may be preferable to first explore the possibility of using other local plants as antimalarials, or cultivating *A. annua* under controlled conditions, not allowing it to go to seed. In any case, the plant is harvested before it goes to seed, and the seeds from the second-generation hybrid plants are of no use for propagation (vegetative propagation is used instead). In areas like the Brazilian Amazon, there is very active competition between species, and it is unlikely that *A. annua* would survive outside of cultivated areas, where it would be protected by farmers (Pedro M. de Magalhães, personal communication). In arid areas, for example, in parts of Africa, it is unlikely that *A. annua* would be able to spread rapidly.

On balance, the potential benefits of local *A. annua* cultivation and preparation in poor areas with no other antimalarial treatments may outweigh the risks of such a program; therefore, some NGOs are already promoting this. However, others argue that it is premature to promote the use of Herba *A. annua* until there is better data on its safety and efficacy. Furthermore, research is needed on the optimal preparation, dose, and length of treatment, and on the best variety to cultivate.

For this reason, the Research Initiative on Traditional Antimalarial Methods (RITAM) has established an *A. annua* Task Force to investigate the feasibility of using a traditional formulation of Herba *A. annua* as a more affordable and accessible option for the early treatment of malaria in poor and remote areas of developing countries. This chapter represents the first step in this process and is mainly an overview of the published literature. There are probably many more unpublished sources to be explored. It is hoped to build on this review, and to use it to plan further clinical evaluation of *A. annua* for the treatment of malaria. Priorities for future research are summarized in Box 3.2.

BOX 3.2: PRIORITIES FOR FUTURE RESEARCH ON *A. ANNUA*

1. To determine the best genotype of *A. annua* to cultivate in each region of interest (in function of its environmental characteristics)
2. To determine the most effective method of preparation, dose, and length of treatment
3. To test the clinical effectiveness of this *A. annua* preparation for treating falciparum malaria in nonimmune patients
4. To determine whether use of this preparation increases the risk of *P. falciparum* developing resistance to artemisinin
5. To test whether the use of this preparation reduces mortality from malaria.

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