Artemisia annua as a Traditional Herbal Antimalarial

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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.

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).

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.

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).

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 5μ mol/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).

In Vitro antimalarial Activity of Constituents of A. annua (IC_{50}, μM)
Source: Liu, K.C.-S. et al., (1992), *Plant Cell Rep*, 11, 637–640

Artemisinin	0.03
Artemetin	26
Casticin	24
Chrysoplenetin	23
Chrysosplenol-D	32
Cirsilineol	36
Eupatorin	65

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.

SAFETY AND TOLERABILITY

The presence of multiple chemical constituents in herbal preparations of *A. annua* raises the question as to their safety. 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). 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.

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).

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. Broadsense 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).

ANAMED RECOMMENDATIONS FOR TREATMENT

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.*

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) 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.

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.

An alternative is [therefore] 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).