ABSTRACT

This paper reviews the use of whole-plant Artemisia annua (sweet Annie) as it pertains to malaria including history, chemistry, clinical efficacy, pharmacokinetics, dosing, safety, and resistance. Artemisinin, the sesquiterpene lactone found in the plant, and various semi-synthetic variants of it used as critical drugs around the world for malaria are discussed in comparison to the whole plant. The multifaceted potential of A. annua against malaria including as a mosquito larvicide and to prevent transmission is discussed. The critical importance of combination therapy with this herb (be it with well-studied synthetic drugs or as-yet poorly studied natural products with activity against malaria, notably curcumin) is reviewed. The relevance of non-artemisinin chemicals in A. annua is highlighted. The pharmacokinetic and pharmacodynamic limitations of A. annua and artemisinins for prophylaxis against malaria are presented. Other Artemisia species besides A. annua, whether they contain artemisinin or not, are also touched upon in the context of malaria.

Keywords: Artemisia annua; Artemisinin; Malaria
In the past 20 years, an entirely new class of antimalarial agents has become available. They are derived from the sesquiterpene lactone artemisinin, which is found in the medicinal plant *Artemisia annua*, and its principal metabolite dihydroartemisinin. This article will review the proper of use *A. annua*, artemisinin and its derivatives (natural and semi-synthetic; see Figures 1 and 2) for treatment of malaria as well as their limitations for primary prevention of malaria.

*A. annua* is known in English as sweet Annie and in Mandarin as *qīng hǎo* (“green hao”). However, one review of the ancient Chinese literature concludes that this name applies better to the closely related artemisinin-containing plant *A. apiacea*, and that *A. annua* is more correctly referred to as *huáng huā hǎo* (“yellow blossom hao”).

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*A. annua* is a fast-growing annual weed that can attain 2 m in height. It can grow in a wide range of soil conditions, though well-drained soil is generally best. The plant is wind pollinated and produces enormous quantities of seeds. The part used therapeutically is the flowering top. *A. annua* has many chemotypes and cultivars with varying chemistries that can significantly affect medicinal activity. The high-artemisinin cultivars that dominate in China and Africa develop peak artemisinin levels before flowering, but research on low-artemisinin cultivars suggest that their synergistic polymethoxylated flavonoids peak during flowering. *A. lancea* and *A. apiacea* have also been reported to contain artemisinin, albeit in much smaller amounts.

In 1972, Chinese scientists isolated qinghaosu (artemisinin) from *A. annua*. They were looking at this plant very specifically because they took Ge Hong’s writings about qinghao from over 1000 years before seriously (as opposed to many researchers in the west who look upon historical herbal books as unhelpful superstition). Based on *in vitro* results showing artemisinin was a potent antimalarial, development of this compound as a medicine began in earnest. Initial results with a tablet form of artemisinin were not that promising, apparently due to poor bioavailability. Further research with different doses and formulations led to improved efficacy and the development of several synthetic analogs. These compounds are now recognized the world over as a novel class of effective antimalarials, and they have come into widespread use (see Table 1).

**MECHANISM OF ACTION**

Artemisinin is a sesquiterpene lactone found in *A. annua* that appears to work in part due to the...
Artemisinins actually appear to work against multiple forms of the parasite, not just schizonts, during its erythrocytic cycle – notably rings and trophozoites. As artemisinins target the erythrocytic cycle, they should be most effective at treating acute malaria, which is how they are generally used and where the best support of efficacy exists.

Artemisinin also effectively kills early-stage gametocytes (the form of malaria transmitted from humans to mosquitoes). Therefore, artemisinins may help prevent spread of the parasites from infected humans to uninfected humans. Open trials of artesunate, a semi-synthetic artemisinin, with sulfadoxine, pyrimethamine and the mature gametocyte-killing primaquine in India show that this is fairly effective at reducing gametocytes of *P. falciparum*. For a review of the four major species of *Plasmodium* that infect humans, see Table 2. A trial is underway in Uganda using artemether, another semi-synthetic artemisinin, with lumefantrine and primaquine, for reducing or eliminating gametocyte burden. Adding artemisinins to mefloquine led to significant reductions in gametocyteemia compared with mefloquine alone. For a review of non-artemisinin antimalarial drugs, see Table 3.

Artemisinins do not affect persistent hepatic forms of malaria (hypnozoites) seen with *P. vivax* and *P. ovale*, therefore artemisinins do not effect a complete cure (eliminate malaria permanently from the body). Only primaquine has been shown to be effective for this purpose. As artemisinins do not affect sporozoites (the forms of malaria injected by mosquitoes into human hosts), they are not effective for preventing infection when taken prophylactically.

Unfortunately some people who are ignorant about the details of malaria or how artemisinins work continue to recommend artemisinin or *A. annua* for prevention of malaria, often citing anecdotal evidence (if any) that they or people they have recommended it to took it and did not develop malaria. Of course, this could be due to chance and precautions taken against mosquito bites. Given the high likelihood that artemisinin and *A. annua* have no preventive effect, they are not recommended to be used in place of existing malaria prophylaxis.

Several flavonoids in *A. annua*, notably methyoxylated flavones such as eupatorin, chrysoplenol-D, chrysoplenetin, and cirsilineol (see Figure 4), all dramatically enhanced the activity of artemisinin against chloroquine-resistant *P. falciparum* in vitro. Previous research found a similar result for the flavonoid casticin from *A. annua*. Casticin, however, is poorly extracted in and rapidly lost from aqueous extracts of *A. annua*. None of these flavonoids had activity against malaria by themselves. Chrysophenol-D and chrysoplenetin also potentiated the activity of berberine against multi-drug-resistant *Staphylococcus aureus* in vitro. At the very least, this supports the argument in favor of studying a combination of the whole plant or non-artemisinin components of it with pharmaceutical antimalarials to see if they amplify efficacy.

Lest it seem that *A. annua* or artemisinins are active only against *Plasmodium*, Table 4 summarizes research showing that in fact they are broad-acting...
Figure 3: Overview of the life cycle of *Plasmodium* spp.

Legend: When an infected female *Anopheles* mosquito takes her blood meal, she infects the human host with sporozoites (<200 cells/drop of mosquito saliva are sufficient for infection). These travel, largely through the lymphatic system and minimally through the blood, to hepatocytes. There, most *Plasmodium* spp rapidly form hepatic schizonts, but the species shown can also form dormant cells called hypnozoites (which can periodically reanimate, cause a relapsing infection). Hepatic schizonts fill with merozoites, then rupture, releasing them into the blood where they infect erythrocytes. Release of a new wave of merozoites from the hepatic schizonts after successful reduction of erythrocytic stages of the organism below detectable levels is known as recrudescent infection. Two things can happen after invasion of erythrocytes. Usually the merozoites establish the erythrocytic cycle, forming immature (ring) trophozoites that develop into mature trophozoites which form erythrocytic schizonts. These fill with merozoites, rupturing, and ultimately the host erythrocyte ruptures releasing the merozoites to infect other erythrocytes. Some merozoites, however, develop into male and female gametocytes. These do not cause pathology in the host but are uptaken by uninfected mosquitoes that take blood meals and thus spread infection. Gametocytes rupture out of erythrocytes when they enter the mosquito midgut and undergo transformation into gametes. After fertilization, a zygote forms. This develops into a motile ookinetocyte which pierces the mosquito’s gut wall and forms an oocyst between the epithelium and basal lamina. This eventually ruptures releasing motile sporozoites that traverse the mosquito and infect her salivary glands, ready to infect a new host at the next blood meal. Artemisinin attacks multiple stages of the erythrocytic cycle as well gametocytes. M, merozoite; S, sporozoite.
Artemisia annua and Malaria

Further work should go into determining whether they are actually clinically effective for infections with these agents.

A. annua and artemisinins likely also have effects on the immune system and inflammation that are potentially relevant in malaria patients and in other conditions. Aqueous infusions of A. annua significantly decreased levels of inflammatory cytokines including interleukin (IL)-6 and IL-8 in human intestinal cells in vitro. Rosmarinic and chlorogenic acids in the herb seemed primarily responsible for this effect. Without causing global immunosuppression, dihydroartemisinin inhibited the mammalian target of rapamycin (mTOR) pathway, which is associated with higher Treg helper lymphocyte activity and reduced T helper lymphocyte activity in mice with a form of multiple sclerosis. The closely related herb A. apiacea inhibited nuclear factor κB activation in rats, thus limiting inflammation. In vitro, A. capillaris inhibited secretion of the pro-inflammatory cytokines tumor necrosis factor-α and IL-1.

CLINICAL EFFICACY

Isolated artemisinin and its principal active metabolite, dihydroartemisinin, have been shown to be quite effective at curing acute uncomplicated and falciparum malaria according to meta-analyses of clinical trials. The studies below look at whether whole-plant A. annua products are also effective.

Two clinical trials have assessed the efficacy of oral administration of an infusion of a high-artemisinin (0.5–0.75%) cultivar developed in the Netherlands known as A. annua cv Artemis. In the initial, uncontrolled pilot study, 44 (92%) of 48 African patients (in the Democratic Republic of the Congo) with chronic malaria had total parasite clearance, and 37 (77%) were asymptomatic after 5 days of treatment. A total of 12 (25%) of the patients suffered nausea during treatment, not surprising with such a bitter plant. Nausea cleared with termination of therapy. The total dose of artemisinin delivered on average was approximately 60 mg, far below the usual dose recommendations of purified artemisinin (500–1000 mg a day for 3–5 days). It is possible other compounds, such as the flavonoids mentioned above, in the whole herb potentiated the action of the small amounts of artemisinin, or that small doses of this compound are more effective than previously believed. Note that in vitro research supports this idea, with A. annua infusions significantly inhibiting P. falciparum at concentrations of artemisinin far below those considered clinically effective.

In a follow-up trial, 132 non-pregnant adult patients with P. falciparum infection in the Democratic Republic of the Congo were randomly assigned to receive A. annua lower-dose tea (5 g herb/1 L water/day in four divided doses), A. annua higher-dose tea (9 g herb/1 L water/day in four divided doses), or quinine sulfate 300 mg three times daily for 7 days. The same Artemis cultivar mentioned in the previous trial, cultivated in the Democratic Republic of the Congo, was used.

Table 2: Major Plasmodium species infecting humans.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Major areas</th>
<th>Fever cycle</th>
<th>Hypnozoites formed?</th>
<th>Other notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum</td>
<td>All tropical and subtropical areas malaria occurs (mainly sub-Saharan Africa)</td>
<td>Tertian (2 day)</td>
<td>No</td>
<td>Most lethal form by far (due to causing severe and cerebral malaria)</td>
</tr>
<tr>
<td>P. malariae</td>
<td>All tropical and subtropical areas malaria occurs</td>
<td>Quartan (3 day)</td>
<td>No</td>
<td>Can cause chronic infection and nephrotic syndrome</td>
</tr>
<tr>
<td>P. ovale</td>
<td>Africa (especially western), western Pacific islands</td>
<td>Tertian (2 day)</td>
<td>Yes</td>
<td>Can infect Duffy-negative people</td>
</tr>
<tr>
<td>P. vivax</td>
<td>Asia (main form there), Latin America, some parts of Africa</td>
<td>Tertian (2 day)</td>
<td>Yes</td>
<td>Many Africans immune due to absence of Duffy blood group</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Drug</th>
<th>Malaria life cycle targeted</th>
<th>Indications</th>
<th>Adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine</td>
<td>Fast-acting erythrocytic schizontocide</td>
<td>IV for cerebral malaria (or quinidine); childhood or pregnancy malaria unresponsive to other agents</td>
<td>Cinchonism, tachycardia, sinus arrhythmias, hypoglycemia</td>
</tr>
<tr>
<td>Chloroquine*</td>
<td>Fast-acting erythrocytic schizontocide (mild gametocide)</td>
<td>Acute uncomplicated malaria; pregnancy and childhood malaria (note high resistance rates)</td>
<td>Pruritus, nausea, agitation (all uncommon)</td>
</tr>
<tr>
<td>Amodiaquine*</td>
<td>Fast-acting erythrocytic schizontocide (mild gametocide)</td>
<td>Acute uncomplicated malaria; prevention in travelers</td>
<td>Nausea, pruritus, agranulocytosis and hepatotoxicity (long-term use)</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>Fast-acting erythrocytic schizontocide</td>
<td>Acute malaria; prevention in travelers</td>
<td>Sleep disturbance, nightmares, nausea, rash, myalgias, psychosis, arrhythmias, pneumonitis (rare)</td>
</tr>
<tr>
<td>Halofantrine</td>
<td>Fast-acting erythrocytic schizontocide</td>
<td>Acute malaria not responding to other agents</td>
<td>Nausea, arhythmias, elevated transaminases</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>Fast-acting erythrocytic schizontocide (mild tissue schizontocide)</td>
<td>Acute malaria; pregnancy malaria (usually with sulfadoxine)</td>
<td>Nausea, headache, mild anemia</td>
</tr>
<tr>
<td>Sulfadoxine</td>
<td>Fast-acting erythrocytic schizontocide</td>
<td>Acute malaria; pregnancy malaria (usually with pyrimethamine)</td>
<td>Nausea, headache, mild anemia, hemolysis in G6PD deficiency, severe skin rashes (rare) – sulfa drug</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>Slow-acting erythrocytic schizontocide</td>
<td>Acute malaria (with fast-acting agents); prevention in travelers</td>
<td>Nausea, photosensitivity, esophagitis</td>
</tr>
<tr>
<td>Primaquine</td>
<td>Tissue schizontocide; gametocidal</td>
<td>Infection with <em>P. vivax</em> or <em>P. ovale</em>; relapsing infection; low risk of reinfection; prevention in travelers to high <em>P. vivax</em> areas</td>
<td>Nausea, methemoglobinemia (self-limiting), hemolysis in G6PD deficiency</td>
</tr>
<tr>
<td>Artemisinins</td>
<td>Fast-acting erythrocytic schizontocide; gametocidal</td>
<td>General acute treatment; IV or rectal suppository for cerebral malaria</td>
<td>Minimal; transient mild nystagmus or ataxia (self-limiting)</td>
</tr>
<tr>
<td>Lumefantrine</td>
<td>Long-acting erythrocytic schizontocide</td>
<td>Acute treatment (only combined with artemether) in Africa</td>
<td>Headache, dizziness, nausea</td>
</tr>
<tr>
<td>Dapsone</td>
<td>Fast-acting erythrocytic schizontocide</td>
<td>Acute treatment not responding to other agents</td>
<td>Hemolytic anemia (especially in G6PD deficiency), methemoglobinemia, jaundice, nausea, headache, insomnia, psychosis, neuropathy (rare)</td>
</tr>
<tr>
<td>Atovaquone</td>
<td>Fast-acting erythrocytic schizontocide</td>
<td>Acute uncomplicated malaria, always given in combination with proguanil</td>
<td>Nausea, mild elevated transaminases</td>
</tr>
<tr>
<td>Proguanil</td>
<td>Fast-acting erythrocytic schizontocide (mild tissue schizontocide)</td>
<td>Acute uncomplicated malaria, always given in combination with proguanil</td>
<td>Nausea, mild elevated transaminases</td>
</tr>
</tbody>
</table>

*Also anti-inflammatory and antipyretic. Note: use of single agents to treat acute malaria is almost never a good idea. G6PD, glucose-6-phosphate dehydrogenase; IV, intravenously.
Republic of the Congo, was used in this trial. Artemisinin content was determined to be 1.4% in the herb used, yielding daily doses of artemisinin in the tea groups of 47 mg and 94 mg respectively. In the lower-dose tea group, the 7-day cure rate (defined by negative blood films) was 77% (30/39) compared with 70% (23/33) in the higher-dose tea group and 91% (39/43) in the quinine group. Day 35 cure rates were 34% (11/32) in the low-dose group, 30% (9/30) in the high-dose group, and 79% (27/34) in the quinine groups, indicating very high recrudescence rates in the artemisinin groups. Adverse effects were comparable among groups and not different from symptoms of malaria, except for a 27% tinnitus incidence in the quinine group. This trial suggests that *A. annua* tea by itself is fairly effective for immediate relief of falciparum malaria but is not effective at keeping the infection under control and needs to be combined with other treatment to prevent recrudescence and, possibly, development of resistance. This concurs with standard use of artemisinins which are always recommended for use in combination therapy and not as monotherapy. Research has not been published on the question of whether resistance can occur to whole-plant *A. annua*.

In a small randomized, double-blind trial conducted in 19 falciparum malaria-infected Tanzanian adults, an infusion of *A. annua* (cultivar and dose unknown) was compared to sulfadoxine-pyrimethamine monotherapy. The 7-day cure rate was 70% (7/9) for the tea and 78% (7/9) for the drug. The 28-day cure rate was 11% (1/9) for the herb and 38% (3/8) for the drug. Thus these two treatments appear to have similar immediate beneficial effects but unacceptable high recrudescence rates.

These proof-of-concept trials suggest that a sustainable supply of locally grown sweet Annie could be maintained in Africa as a way to deliver life-saving medicine, and that pharmaceutical companies and synthetic, isolated artemisinins are not necessary for treatment of malaria. However, they also show that crude extracts of sweet Annie alone are unlikely to be effective at preventing recrudescence and so some pharmaceutical preparations will need to be combined with them. A critical question is whether combination herbal medicine could allow for a truly sustainable approach to malaria treatment. It is not yet known what the best drugs to combine with *A. annua* would be, but logically it would be those with radically different molecular targets than artemisinin, such as anti-folate drugs (e.g., sulfadoxine-pyrimethamine) or 4-aminoquinolines that target heme degradation (e.g., chloroquine). In areas with high *P. vivax* or *P. ovale* infection rates, combination with the anti-hypnozoite drug primaquine is also potentially beneficial.

*A. annua* also has the potential to be a mosquito larvicidal and adulticidal agent. A hexane extract was moderately effective with a 244.5 ppm LC₅₀ against the mosquito vector *Anopheles sinensis*, 276 ppm against the dengue vector *Aedes aegypti*, and 375 ppm against the arbovirus and avian malaria vector *Culex quinquefasciatus*. A petroleum ether extract of *A. annua* had an LC₅₀ of 78.2 ppm against *C. quinquefasciatus* mosquito development and growth in prior study. A *A. annua* chloroform extracts had an LC₅₀ of 18.5 ppm against *A. sinensis* larvae in another study. Larvae of *A. stephensi*, another malaria vector, were killed at an LC₅₀ of 20 ppm. Thus *A. annua* could potentially be grown...
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Species of Artemisia not containing artemisinin, or at most tiny concentrations, are antimalarial in preclinical studies (see Table 5). This is presumably due to the presence of other antimalarial compounds, which may or may not be similar to artemisinin in structure. It is also likely that they contain non-antimicrobial compounds like the methoxylated flavonoids of A. annua that enhance efficacy by reducing resistance or via other mechanisms. This hypothesis needs verification urgently, as it could provide new medicines for malaria.

COMBINATION HERBAL THERAPY

The most critical antimalarial medications have been derived from natural products, primarily quinine and artemisinin. There is a growing awareness that herbs have a role to play in the global fight against malaria. The approach advocated by this author is to use a range of herbs to optimally attack malaria parasites with multiple chemicals against multiple pathways, and thereby minimize the already low (and purely theoretical) risk of resistance but also toxicity (by limiting the absolute dose of each herb necessary for efficacy).

Suggestions for sustainable combination therapy with A. annua using local herbal resources, or plants

| Table 4: Other reported organisms killed or inhibited by artemisinins. |
|---------------------------------|---------------------------------|---------------------------------|
| **Organism and population or model** | **Effects** | **References** |
| Babesia equi-infected donkeys | Artesunate: 17 d survival post treatment (vs. 69 d with imidocarb) | Kumar et al. |
| | Arteether: 5 d survival post treatment | |
| Bacteria, various, in vitro | *A. annua* volatile oil 10 mg/mL inhibition zones: | Čavat et al. |
| | *Staphylococcus aureus* 20 mm | |
| | *Bacillus subtilis* 20 mm | |
| | *Streptococcus pneumoniae* 50 mm | |
| | *Pseudomonas aeruginosa* 15 mm | |
| | *Haemophilus influenzae* >60 mm | |
| | *Candida krusei* 30 mm | |
| Eimeria spp in chickens | *A. annua var Artemis* significantly decreased oocyst output vs. controls | De Almeida et al. |
| HIV in vitro | *A. annua* infusion: IC₅₀ 2 µg/mL | Lubbe et al. |
| | *A. afra* infusion: IC₅₀ 50 µg/mL | |
| Leishmania donovani in vitro | *A. annua* leaf and seed hexane extract: GI₅₀ 14.4–14.6 µg/mg (vs. promastigotes) and IC₅₀ 5.05–6.6 µg/mL (vs. intracellular amastigotes) | Islamuddin et al. |
| Mycobacterium tuberculosis in vitro | Artemisinin combined with mycobactin T (iron chelator) analog MIC 0.156–1.25 µg/mL (vs. MDR strains), 0.078–0.625 (vs. XDR strains) | Miller et al. |
| | Artether 6 mg/kg once every 3 wk for 18 wk cut infection rates in half vs. placebo; also reduced Plasmodium falciparum infections | Utzinger et al. |
| Schistosoma mansoni infection prevention in 354 school children, Ivory Coast | *A. annua* (July leaves) acetone extract LC₅₀ 0.43 mg/mL | Zhang et al. |
| Tetranychus cinnabarinus (carmine spider mite, a plant pathogen) in vitro | *A. annua* hexane extract: 73% inhibition in vitro Sterols identified as active compounds (particularly β-sitosterol) | Ali Khan et al. |
| Tobacco mosaic virus in vitro | *A. annua* infusion: IC₉₀ 95 µg/mL (in vitro), median survival 12 d in mice (vs. 9 d with no treatment) | Carrijo de Oliveira et al. |
| Toxoplasma gondii in vitro and in mice | *A. annua* infusion: IC₉₀ 35.91 µg/mL | Nibret and Wink |
| Trypanosoma brucei in vitro | | |

GI₅₀, growth inhibitory concentration 50%; HIV, human immunodeficiency virus; IC₅₀, inhibitory concentration 50%; LC₅₀, median lethal concentration 50%; MCR, multidrug resistant; MIC, minimum inhibitory concentration; XDR, extensively drug resistant.
that are likely sustainable in many tropical climates, include *Cinchona* spp (Peruvian bark and the source of quinine and related antimalarial alkaloids), *Azadirachta indica* (neem) leaf or oil, *Vernonia amygdalina* (bitter leaf), *Argemone mexicana* (Mexican poppy), and *Curcuma longa* (turmeric). Other herbs proven effective in clinical trials may also be relevant in the specific geographic regions in which they grow (see Table 6).

Clinical trials have previously demonstrated that a multiple alkaloid extract of Peruvian bark is effective for patients with chloroquine-resistant malaria.\textsuperscript{58} The rationale for returning to a whole-plant approach to Peruvian bark has previously been outlined, similar to the arguments presented here for sweet Annie.\textsuperscript{59} Lower doses of quinine are generally delivered by whole-plant extracts than are used pharmacologically, but the synergy of multiple constituents apparently still allows for clinical efficacy.

Neem has not yet been shown to be effective for treating malaria patients in clinical trials. In animal trials, it is clearly antimalarial, including killing gametocytes which should help reduce malaria spread from infected people to new hosts.\textsuperscript{50, 61} Empirically, an infusion of neem leaves has been effective at preventing and treating acute malaria, at least in some patients in Kenya.\textsuperscript{62} However this is not proof of efficacy and clinical trials are needed. Neem extracts are also antiretroviral,\textsuperscript{63} which could prove important given the continuing rise of human immunodeficiency virus (HIV) and malaria co-infection in many parts of the developing world. Finally, neem oil is an effective mosquito repellant against *Anopheles* mosquitoes and an *Anopheles* mosquito larvicide, making this a multifunctional plant.\textsuperscript{64, 65}

Curcuminoids, a mixture of yellow, lipophilic molecules found in *Curcuma longa* (turmeric) often referred to as just curcumin, show promise at enhancing the antimalarial potency of artemisinins. In *P. berghei*-infected mice, a combination of 100 mg/kg of body weight of curcumin orally after a single intramuscular injection of artemether led to 100% survival of the mice, compared with 100% mortality with artemether injection alone after 11–34 days.\textsuperscript{66} Liposomal microencapsulation of artemisinin was just as effective as artemisinin combined with curcumin in mice infected with malaria.

### Table 5: Other *Artemisia* species reported to have anti-plasmodial activity (regardless of artemisinin content).

<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>Notes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Artemisia absinthium</em></td>
<td>Mice and <em>in vitro</em></td>
<td>Active against multidrug-resistant <em>P. falciparum</em></td>
<td>Ramazani <em>et al.</em>\textsuperscript{45}; Zafar <em>et al.</em>\textsuperscript{46}</td>
</tr>
<tr>
<td><em>Artemisia afra</em></td>
<td><em>In vitro</em></td>
<td>No artemisinin present (negative study–Liu <em>et al.</em>, 2010)</td>
<td>Gathiriwa <em>et al.</em>\textsuperscript{47}; Kraft <em>et al.</em>\textsuperscript{48}</td>
</tr>
<tr>
<td><em>Artemisia maciverae</em></td>
<td>Mice</td>
<td>Chloroform extract</td>
<td>Ene <em>et al.</em>\textsuperscript{49}</td>
</tr>
<tr>
<td><em>Artemisia roxburghiana</em></td>
<td><em>In vitro</em></td>
<td>Chloroform extract IC\textsubscript{50} 0.42 µg/mL</td>
<td>Du\textsuperscript{a} <em>et al.</em>\textsuperscript{50}</td>
</tr>
<tr>
<td><em>Artemisia sieberi</em></td>
<td>Mice</td>
<td>Crude extract</td>
<td>Nahrevanian <em>et al.</em>\textsuperscript{51}</td>
</tr>
<tr>
<td><em>Artemisia turanica</em></td>
<td>Mice</td>
<td>Crude extract</td>
<td>Taherkhani <em>et al.</em>\textsuperscript{52}</td>
</tr>
</tbody>
</table>

### Table 6: Antimalarial whole plants in preliminary clinical trials.

<table>
<thead>
<tr>
<th>Herb</th>
<th>Part used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vernonia amygdalina</em> (bitter leaf)</td>
<td>Leaf</td>
<td>Challand and Wilcox\textsuperscript{54}</td>
</tr>
<tr>
<td><em>Argemone mexicana</em> (Mexican poppy)</td>
<td>Leaf and flower</td>
<td>Wilcox <em>et al.</em>\textsuperscript{55}</td>
</tr>
<tr>
<td><em>Cochlospermum planchonii</em> and <em>C. tinctorium</em> (n’dribala)</td>
<td>Root</td>
<td>Benoit-Vical <em>et al.</em>\textsuperscript{56}</td>
</tr>
<tr>
<td>AM-1 Formula: <em>Jatropha curcas</em> (purging nut), <em>Gossypium hirsutum</em> (cotton), <em>Physalis angulata</em>, and <em>Delonix regia</em> (royal Poinciana)</td>
<td>Multiple</td>
<td>Ankrah <em>et al.</em>\textsuperscript{57}</td>
</tr>
</tbody>
</table>
although the combination of the two was dramatically more effective than non-liposomal artemisinin alone at preventing mortality.\textsuperscript{67} Another study found that curcumin liposomal formulations combined with arteether prevented mortality and recrudescence in malarious mice, and were significantly more effective than curcumin by itself.\textsuperscript{68} Other studies combining curcumin and arteether suggest that through immune mechanisms, curcumin prevents recrudescence.\textsuperscript{69} The use of curcumin does appear to have limitations. Curcumin did not increase the efficacy of artemisinin against artemisinin-resistant \textit{P. chabaudi} in mice.\textsuperscript{70} The addition of piperine, an alkaloid from \textit{Piper longum} (long pepper) and \textit{P. nigrum} (black pepper), which increases absorption of curcumin, also did not help in this resistant clone.

Some other herbs that have been reported in preliminary human trials to effectively combat malaria, and thus could be considered in combination herbal therapy, are listed in Table 6. There is an urgent need to determine whether combinations of these herbs with \textit{A. annua} and other antimalarial herbs is effective acutely and at preventing recrudescence, as well as at preventing spread of malaria.

\section*{Resistance}

As has been the case with all single chemical entity antimalarial drugs, use of semisynthetic artemisinins has led to the rise of malarial resistance to these drugs.\textsuperscript{71, 72} In Cambodia, resistance to dihydroartemisinin already appears widespread.\textsuperscript{73} It is unclear if resistance develops to whole-plant extracts or not. This is unlikely as they contain multiple compounds and not a single agent, including the aforementioned methoxylated flavonoids that appear to prevent development of resistance. Ongoing searching of the literature by the author has still failed to find a single paper documenting development of resistance of microbes to whole plants or complex plant extracts. In fact, most papers that have looked at whole-plant extracts have found that they do not induce resistance; for example, \textit{Hypericum perforatum} (St. John’s wort) does not induce resistance in \textit{Staphylococcus aureus}, while its isolated constituent hyperforin can.\textsuperscript{74} \textit{Melaleuca alternifolia} (tea tree) volatile oil, which is a semi-refined extract in that it contains no water soluble compounds, has been shown to be able to induce resistance in \textit{Staphylococcus aureus in vitro}.\textsuperscript{75}

\section*{Dosing}

\textit{A. annua} and artemisinins are not recommended for use as monotherapy in order to prevent resistance developing, and because recrudescence is so high following monotherapy. Therefore, the doses presented here apply only when the agents in question are combined with other treatments.

\begin{table}[h]
\centering
\begin{tabular}{|l|}
\hline
\textbf{Adult doses for crude \textit{A. annua}\textsuperscript{35, 37, 38, 59}} \\
\hline
Dried crude herb, 2–4 g tid–qid \\
Dried herb infusion, 1.5–3 g/250 mL water, steeped 15 min, 250 mL 4x/d \\
Fresh herb tincture, 60–90\% ethanol, 1:3 weight:volume, 5–10 mL 4–6 x/d \\
These doses should be continued for 5 days (in women) or 7 days (in men) then discontinued (as absorption drops off dramatically after that point). \\
\textbf{Notes:} Dried herb should not be decocted for more than a few minutes, this is associated with rapid destruction of all artemisinin.\textsuperscript{40} Doses for children should be proportionally reduced based on body size. \textit{A. annua} should not be used as monotherapy.\textsuperscript{42} \\
\hline
\end{tabular}
\end{table}

Presently, the World Health Organization (WHO) recommends using only the semi-synthetic derivatives of artemisinin known as dihydroartemisinin (though humans convert artemisinins to this metabolite), arteether and artesunate for uncomplicated malaria. Though these drug combinations have been documented to cure acute malaria patients effectively, it is questionable whether continuing a synthetic pharmaceutical approach will be sustainable ecologically or economically. The historical record suggests it probably won’t be, if one looks at the high rate of resistance to semi-synthetic quinine derivatives around the world. Quinine is an alkaloid from various species of the Amazonian tree \textit{Cinchona spp}. Mixing dried \textit{A. annua} leaf into millet porridge and cooking them together has been shown to be a palatable and stable delivery form for the herb to
Pharmacokinetic and clinical efficacy profiles of this delivery form have not been published. Further research is warranted given the sustainability of this approach.

**WHO Guidelines for synthetic artemisinins combination therapy**

Dihydroartemisinin 4 mg/kg bw for 3 d + piperaquine 18 mg/kg bw for 3 d, but no registered/quality form available
- Artemether (20 mg) + lumefantrine (120 mg), 1–4 caps bid for 3 d (with fat; worldwide)
- Artesunate 4 mg/kg bw + amodiaquine 10 mg base/kg bw qd for 3 d (worldwide)
- Artesunate 4 mg/kg bw qd for 3 d + mefloquine 25 mg base/kg bw qd for 2–3 d (Asia only)
- Artesunate 4 mg/kg bw qd for 3 d + sulfadoxine-pyrimethamine 25/1.25 mg base/kg bw single dose

**PHARMACOKINETICS**

Artemisinins have short half-lives, certainly <60 min. Dihydroartemisinin, which all artemisinins covert to, has a half-life of around 60 min. This is a significant part of why recrudescence is so high with artemisinins; the compounds rapidly kill the parasites down to an undetectable level (providing immediate and important clinical relief), and then the artemisinins are cleared quickly so the chemicals don’t have time to completely eliminate them. Within a few days of finishing treatment, the small numbers of residual parasites can reproduce and create recrudescent disease. Use of longer-acting drugs combined with artemisinins is essential to long-term efficacy in patients with malaria.

The pharmacokinetics of artemisinin from a tea made from *A. annua* cultivar *Artemis* has been studied in humans. The maximum amount of artemisinin was extracted by covering 9 g of dried leaf with 1 L of boiling water and allowing it to sit, away from heat, covered, for 10 min before filtering and pressing. This yielded 94.5 mg of artemisinin with 76% extraction efficiency. Fourteen European adults then drank 1 L of this tea over an average of 15 min, then had their serum artemisinin concentrations determined serially over 8 h. Maximal serum concentration (C_{max}) averaged 240 ng/mL and the time to achieve this (t_{max}) was 0.6 h on average. Area under the curve (AUC) averaged 336 ng/mL×h. The elimination half-life (t_{1/2}) averaged 0.87 h. For comparison to pharmacokinetic studies of pure artemisinin in capsules, see Table 7.

Note that the tea, with comparable levels of artemisinin to the 100 mg of pure artemisinin in capsule resulted in much more rapid uptake and what appears to be significantly higher absorption and exposure to artemisinin, but this cannot be definitively proven given that these were not head-to-head studies. The authors conclude that bioavailability is similar between the tea and 500 mg artemisinin capsules, but that serum concentrations achieved are not sufficient for clinical efficacy. Of course,

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Herbal tea (94.5 mg artemisinin/1 L)</th>
<th>Pure artemisinin (500 mg)</th>
<th>Pure artemisinin (100 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>55 Reviewed in 55</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Subjects</td>
<td>14 healthy European adults</td>
<td>8 trials including European, African and Asian adults, some healthy and some with malaria</td>
<td>8 Vietnamese adults with uncomplicated falciparum malaria</td>
</tr>
<tr>
<td>C_{max} (range)</td>
<td>240 ng/mL (133–349 ng/mL)</td>
<td>531 ng/mL (311–776 ng/mL)</td>
<td>162 ng/mL (131–193 ng/mL)</td>
</tr>
<tr>
<td>AUC (range)</td>
<td>336 ng/mL×h (245–483 ng/mL×h)</td>
<td>2.072 ng/mL×h (1.373–2.611 ng/mL×h)</td>
<td>Not reported</td>
</tr>
<tr>
<td>t_{max} (range)</td>
<td>0.6 h (0.2–1 h)</td>
<td>2.3 h (range not reported)</td>
<td>2.4 h (1.2–3.6 h)</td>
</tr>
<tr>
<td>t_{1/2} (range)</td>
<td>0.87 h (0.5–1.4 h)</td>
<td>not reported in aggregate</td>
<td>2.2 h (1.1–3.3 h)</td>
</tr>
</tbody>
</table>
the results of clinical trials on this exact tea do not support this conclusion, because even at these relatively low serum levels, clinical efficacy was quite good in patients with uncomplicated acute malaria.

If one assumes that artemisinin is the only relevant compound in the whole tea, then these pharmacokinetic studies can perhaps only be interpreted as providing insufficient levels of artemisinin for clinical efficacy as these authors did. Given that the clinical trials show efficacy despite this, more evidence is provided for the theory that there is synergy among artemisinin and non-antimalarial components of the herb. If non-artemisinin components in the tea such as the methoxylated flavonoids are reducing resistance of the parasites to the artemisinin, then the serum levels of the artemisinin will not need to be as high to have good clinical efficacy compared with artemisinin in isolation. This theory requires study in formal trials to determine if it is true.

Artemisinin autoinduces its own catabolism, primarily in the gut wall, but also in the liver. CYP3A4, 2B6, and 2C19 are the main enzymes involved,81 CYP2B6 and 2C19 are believed to be autoinduced significantly by artemisinin. Over 7–10 days of ongoing administration of artemisinin, blood levels fall dramatically in healthy volunteers and patients with uncomplicated malaria.82, 83 This effect is most pronounced in women. Whether this occurs with whole A. annua extract needs to be studied urgently. Until it has been, it should be assumed continuous use of the whole herb for more than 1 week would lead to a loss of efficacy for optimal patient safety.

Intravenous (IV) administration of artemisinins bypasses this problem but is rarely available in most malarious areas due to the cost of the agents and equipment. This creates the additional hurdle of needing to provide a sterile environment for administration of the drug which is often not practical in malaria-afflicted areas. However, IV artemisinins are equally effective to IV quinine and safer for treatment of cerebral malaria, and therefore there is a need to work to make this more available in hospitals in malarious areas.84

Similar results have been noted empirically using the whole herb. Due to the first-pass metabolism in the liver, oral bioavailability of artemisinin is only 32%.85, 86 However, one rodent study found that a whole plant concentrated extract of A. annua led to 40 times greater serum levels of artemisinin than when artemisinin alone was given orally to mice infected with Plasmodium chabaudi.87 Not surprisingly the whole-plant extract was far more effective at reducing parasitemia than purified artemisinin.

Taking grapefruit juice (350 mL double-strength fresh frozen juice, a known CYP3A4 inhibitor) daily for 5 days with 100 mg artemether increased oral bioavailability of the drug at first, but did not stop the time-dependent decrease in blood levels after ingestion in healthy volunteers.88 This supports the idea that while CYP3A4 is involved in catabolizing artemisinins in the gut wall, it is not induced significantly by repeated intake of artemisinins.

Given this information, it seems pointless to take A. annua or artemisinin for more than 7 days. It further highlights the futility of attempting to use either one for prevention of malaria. Anecdotal reports of people avoiding malaria infection while taking A. annua or artemisinin extracts are more likely due to mosquito bite prevention or simple luck.

SAFETY

There are rare case reports of neurotoxicity associated with treatments with artemisinins, but such effects are also seen in some malaria patients without artemisinins, so cause-effect relationships with neurotoxicity have not been established. If artemisinins do cause neurotoxicity, the incidence is quite low. Artemisinins are safe and effective in treating malaria in pregnant women, at least during the second and third trimesters.89

Nausea can occur with administration of the crude herb or extracts thereof, likely related to its intense bitterness. In fact, the only adverse effect noted in the clinical trials on A. annua tea has been nausea which remits when therapy is completed.30, 32 Co-administration with common, mild anti-emetic herbs such as mint, ginger or other aromatic spices may alleviate or prevent nausea in some patients based on empirical evidence. The safety of A. annua in pregnancy has not been studied.

COUNTERFEIT DRUGS AND OTHER ISSUES

With the rising popularity of artemisinins for treatment of malaria and the ongoing poverty in malaria-stricken
parts of the world, there has been a rise in counterfeit artemisinins for sale.\textsuperscript{90} This parallels what has been seen with every antimalarial drug that becomes available.\textsuperscript{91} However, the high cost of artemisinins may be driving higher levels of counterfeiting as more profit can be made. Such behavior may be economically rational from the point of view of the person doing the selling, but is extremely harmful from the larger social perspective due to the multiple levels of negative effects on public health that result.

Ultimately the solution to counterfeit drugs is to alleviate poverty. Given the unlikeness of this any time soon, other solutions include creating cheaper artemisinin by microbial synthesis, creating better cultivars of \textit{A. annua}, better policing of antimalarial drug manufacturers, reducing corruption in malaria-affected nations, and securing the evidence base for plant-based malaria therapies so that people can grow their own medicines.\textsuperscript{78, 92, 93}

**CONCLUSION**

The story of \textit{A. annua} is instructive in many ways and offers hope as a multifaceted medicine. The whole herb became a source for an isolated compound (artemisinin or qinghaosu) which then became the template for multiple semisynthetic compounds. Though initially expensive and slow to become utilized, they have since become the dominant form of treatment for malaria worldwide.

Despite the rapid awareness that they must be used in combination to avoid recrudescence and resistance, monotherapy continues and is resulting in loss of efficacy of this valuable therapy. Counterfeit drugs are worsening the problem by delaying actual therapy, causing patients to waste their already limited financial resources on useless medicines, and creating confusion and doubt in people’s minds about how and where to obtain actual drugs.

Whole-herb \textit{A. annua} offers several potential ways out of these problems. First, it can be grown locally in malarious areas, allowing for a sustainable and self-supporting situation for communities afflicted with this disease.\textsuperscript{6, 8} Second, it is effective despite relatively low levels of artemisinin, at least in part due to the resistance-inhibiting effects of methoxylated flavonoids in the whole plant. Third, it is cheap and safe. Fourth, it is potentially active against multiple tropical infectious diseases and may help with other diseases, including autoimmune conditions.\textsuperscript{90} Fifth, it may help control mosquito vectors. However, like isolated artemisinins, it is not effective at preventing recrudescence and requires combination therapy. What is unknown is whether it can be paired with other effective herbal antimalarials, several of which have been reported, and thus create effective, entirely local herbal remedies for malaria.

**DISCLOSURE OF INTERESTS**

Dr. J.E. Yarnell reports he is part-owner and president of Heron Botanicals, outside the submitted work.

**REFERENCES**

10. Shah NK, Schapira A, Juliano JJ, et al. Nonrandomized controlled trial of artesunate plus sulfadoxine-pyrimethamine with or without primaquine for preventing posttreatment circulation of *Plasmodium*


