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Dried-leaf *Artemisia annua:* A practical malaria therapeutic for developing countries?

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Abstract

Artemisinin from the plant *Artemisia annua (A. annua)* L, and used as artemisinin combination therapy (ACT), is the current best therapeutic for treating malaria, a disease that hits children and adults especially in developing countries. Traditionally, *A. annua* was used by the Chinese as a tea to treat “fever”. More recently, investigators have shown that tea infusions and oral consumption of the dried leaves of the plant have prophylactic and therapeutic efficacy. The presence of a complex matrix of chemicals within the leaves seems to enhance both the bioavailability and efficacy of artemisinin. Although about 1000-fold less potent than artemisinin in their antiplasmodial activity, these plant chemicals are mainly small molecules that include other artemisinic compounds, terpenes (mainly mono and sesqui), flavonoids, and polyphenolic acids. In addition, polysaccharide constituents of *A. annua* may enhance bioavailability of artemisinin. Rodent pharmacokinetics showed longer $T_{1/2}$ and T_{max} and greater C_{max} and AUC in *Plasmodium chabaudi*-infected mice treated with *A. annua* dried leaves than in healthy mice. Pharmacokinetics of deoxyartemisinin, a liver metabolite of artemisinin, was more inhibited in infected than in healthy mice. In healthy mice, artemisinin serum levels were > 40-fold greater in dried leaf fed mice than those fed with pure artemisinin. Human trial data showed that when delivered as dried leaves, 40-fold less artemisinin was required to obtain a therapeutic response compared to pure

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artemisinin. ACTs are still unaffordable for many malaria patients, and cost estimates for *A. annua* dried leaf tablet production are orders of magnitude less than for ACT, despite improvements in the production capacity. Considering that for > 2000 years this plant was used in traditional Chinese medicine for treatment of fever with no apparent appearance of artemisinin drug resistance, the evidence argues for inclusion of affordable *A. annua* dried leaf tablets into the arsenal of drugs to combat malaria and other artemisinin-susceptible diseases.

Keywords

Malaria; Infectious disease; Artemisia annua; Artemisinin; Combination therapy; Artemisinin combination therapy

INTRODUCTION

Nearly three billion people are affected by malaria with almost a million deaths annually, especially in Africa and amongst children[1]. Currently extracted from *Artemisia annua (A. annua)* L., artemisinin (Figure 1) is delivered in concert with another antimalarial drug [artemisinin combination therapy (ACT)] as the preferred treatment to slow emergence of drug resistance. Despite these efforts, artemisinin resistance is appearing[2] and persistent and/or asymptomatic malaria may also be playing a role in disease transmission[3–5]. Moreover, for developing countries ACT is costly and the supply is inadequate[6–9].

Artemisinin is a sesquiterpene lactone that is produced and stored in the glandular trichomes that are mainly on the leaves and floral buds of *A. annua*, a GRAS medicinal herb[10–12]. The plant also produces > 40 flavonoids[13], many polyphenols, and a variety of other terpenes including mono-, sesqui-, di-, and triterpenes[14]. As discussed later, many of these have weak antimalarial activity, and, based on transcriptome analyses, many also seem to be produced and/or stored in the glandular trichomes that also contain artemisinin[15].

We and others proposed direct consumption of *A. annua* either as a tea infusion[16–19] or by oral consumption of the leaves[20–24]. In contrast to the oral consumption of pure artemisinin, we showed that the presence of plant material significantly enhanced appearance of artemisinin in the serum of healthy and *Plasmodium chabaudi*-infected mice[22]. Because of the plethora of mild antimalarial compounds naturally present in the dried leaves of the plant, we have termed this orally consumed dried leaf therapeutic plant-based artemisinin combination therapy, or pACT. These whole plant approaches are similar to the more than 2000 year traditional use of the plant by the Chinese[25].

To produce a therapeutically effective drug using a complex material like a medicinal plant requires that a number of key factors be met: the medicinal herbal product must be therapeutically effective; levels of key chemical components in the herb must be verifiably consistent; production must also be cost effective. Here we summarize and update our recent review[26] on the effects of *A. annua* on malaria and further discuss the bioavailability and therapeutic efficacy of pACT and how such an herbal drug could inexpensively be produced with a consistent dose.

PROPHYLACTIC USE OF *A. ANNUA*

Tea infusion, its chemistry, and in vitro studies

Until recently, there have been, to our knowledge, few well-controlled studies examining extraction, recovery, and stability of artemisinin and other compounds in *A. annua* tea infusion. A systematic study of preparations of *A. annua* therapeutic tea infusion was performed by van der Kooy *et al*[27] and showed that nearly 93% of available artemisinin was extracted from dried *A. annua* leaves, but only under certain conditions. Best preparation method was: 9 g DW leaves/L, for 5 min at 100 °C. Subsequent storage of the tea infusion at room temperature showed that artemisinin concentration was stable for > 24 h, important for malaria-endemic locations where there is no refrigeration. Artemisinin water solubility is approximately 50 mg/L[27], so the amount of artemisinin recovered from hot water tea infusions is reasonable. Other studies using the same extraction protocol also measured extraction and stability of artemisinin and some key flavonoids in the tea. Artemisinin was found to be stable at room temperature for up to 48 h[28] ; however, some flavonoids were poorly extracted and not stable at room temperature[29].

Carbonara *et al*[28] detected an assortment of phenolics, including 0.06 mg/g DW cirsilineol, in an *A. annua* tea infusion prepared at about a 4–10 fold higher proportion (approximately 38 g DW/L) than that proposed as optimal (9 g DW/L) by van der Kooy *et al*[27]. Most of the measured phenolics in the tea remained constant at room temperature for 48 h post-infusion. More recently, Suberu *et al*[19] identified milligram amounts of phenolic acids, flavonoids, and sesquiterpenes in a liter of *A. annua* tea, all of which demonstrated IC_{50} values in the micromolar or less range (Table 1). Indeed, the IC_{50} of the tea infusion itself was 7.6 and 2.9 nmol/L for the chloroquine (CQ)-sensitive HB3 and CQ-insensitive Dd2 strains of *P. falciparum*, respectively, and better than artemisinin alone suggesting synergism of constituents in the tea mixture. Clearly if a tea infusion is to be a therapeutic option, it must be consistently and reliably prepared and ingested. As suggested by van der Kooy *et al*[27], ideally a liter of tea infusion would be prepared daily and consumed in equal aliquots of about 250 mL over 24 h for several days.

Tea infusion clinical trials

Ogwang *et al*[30,31] tested *Artemisia* tea as a prophylaxis against malaria in 132 adult farm workers, aged 18–60 years, for 12 mo in a randomized clinical trial in Uganda. Tea infusion was consumed once a week at 2.5 g dried leaves per adult infusion dose with 55–100 mg artemisinin/L. Malaria was tracked for 9 mo while adverse clinical effects were tracked for 12 mo. Among those who used *Artemisia* tea there were 80% fewer fever-related hospital visits. Indeed, some patients reported using *A. annua* tea for > 7 years with no incidence of malaria and no serious adverse events. Although this study suggested that once weekly consumption of *A. annua* tea infusion may offer prophylactic protection, there were no children or elderly in the study, so additional clinical trials need to be conducted with different populations and age groups. Authors argued that since a single weekly dose was effective, compounds other than artemisinin may have played the prophylactic role since artemisinin itself has short plasma half-life.

THERAPEUTIC USE OF *A. ANNUA*

Tea infusion

Reports on the efficacy of *A. annua* (cv. Artemis) tea on human malaria patients by Mueller *et al*[17,32] and Blanke *et al*[33] yielded at times conflicting results. Their tea infusions contained 47–94 mg artemisinin/L, but recrudescence was much lower in the quinine-treated control group, so parasite reappearance in the tea-treated patients was ascribed to recrudescence and not re-infection[17]. In the Blanke *et al*[33] trial that included a placebo tea, recrudescence was consistently lower in the tea patients than in those treated with 500 mg pure artemisinin. More recently, however, De Donno *et al*[34] showed that 5 g dried leaves in one liter of *A. annua* tea infusion was effective against both CQ-resistant (W2) and CQ-sensitive (D10) strains of *P. falciparum* with IC_{50} values of 5.60 nmol/L and 7.08 nmol/L, respectively, results also consistent with those of Suberu *et al*[19] as already highlighted. These latter *in vitro* studies suggested that tea should be efficacious, so why the discrepancy with the earlier human trials? Preparation methodology is crucial for preserving as much biochemical integrity of the plant as possible[27]. The more recent *in vitro* studies likely used more consistently prepared tea infusions than the earlier human trials, so variations in chemical composition of the infusions and in the plant source material could explain the different responses.

The argument that tea is a monotherapy is unsubstantiated considering the now well-established chemical complexity and related antiplasmodial activity of tea infusions of *A. annua* and its components. Although data from therapeutic tea trials in animals and in humans correlate well, unfortunately, they do not support use of *A. annua* tea for treating malaria because animal and human data are comparably negative, the artemisinin dose is not easily controlled, and other potentially synergistic components in the tea are not readily controlled or extracted. Nevertheless, use of the tea could play a role in malaria prophylaxis to reduce incidence of malaria in different communities, or in temporary relief from malaria, mainly in prevention of coma or “to buy time” to enable an infected person from a rural area to travel to a hospital or clinic stocked with ACT.

Dried leaf A. annua - pACT

Recently, Elfawal *et al*[23] measured parasitemia in mice infected with *P. chabaudi* that were fed two different doses (0.6 or 3.0 mg artemisinin; 24 and 120 mg/kg) of either pure artemisinin in mouse chow or as pACT. Artemisinin delivered *via* pACT was at least five times more effective, and with a longer lasting response, than pure artemisinin in reducing parasitemia. Excluding artemisinin there are > 600 phytochemicals that have been identified in *Artemisia annua*[35], but there is currently a lack of information on the chemistry, effect of the preparation method (harvesting, drying, storage, *etc*.), and overall bioavailability of these chemicals[36].

Clinical trials using dried leaf *A. annua* are scarce in the scientific literature and few, other than those in Democratic Republic of Congo by Mueller *et al*[17,32], are published. Despite the fact that WHO does not encourage either whole plant or tea infusion clinical trials[37], some African universities have been conducting their own trials, many of which have not

been published nor results assessed by polymerase chain reaction (PCR) as later done for clinical trials with ACTs (personal comm from C. Kasongo to P. Lutgen). Many of these trials used *A. annua* infusions, and compared to controls or even other antimalarial drugs, *e.g*., artesunate-amodiaquine, showed significantly greater sensitivity of the infusion with fewer late therapeutic failures. For example, in Democratic Republic of Congo, 54 malaria-infected volunteers were treated for 10 d with capsules containing powdered leaves of *A. annua*. Each patient was given 15 g dried leaves containing 15 mg of artemisinin (artemisinin content in leaves = 0.1%[38]). After 2 d all were free of fever and 51 (or 94%) were parasite free after 10 d.

In a study aimed at preventing severe post-operative malaria at Bangui, Central Africa, powdered leaves of *A. annua* were administered in capsules to 25 patients, 22 of them children aged 1–16 years[24]. Treatment duration ranged from 3–4 d with a dose of 0.4–0.5 g/d of *A. annua* dried leaves (0.1% artemisinin leaf content) delivering 0.4–0.5 mg/d artemisinin. In spite of the very low administered daily dose of artemisinin, average parasitemia dropped by 62% in the patients with an added benefit of a strong antinociceptive response, especially beneficial to post-operative patients.

The most clinically definitive study to date of pACT efficacy was conducted at the International Centre of Insect Physiology and Ecology (ICIPE) Mbita Field campus, Suba District, in Western Kenya. This was a collaborative project between ICIPE and Kenya Medical Research Institute[20] (Table 2[39]) and was an open-label, non-randomized clinical trial mainly targeted to assess efficacy, safety, and tolerance of increasing doses of pACT delivered as tablets. The tablets were made by a Tanzania-based NGO, Natural Uwemba System for Health, from a hybrid of *A. annua* grown in the Tanzania highlands (2000–2200 m altitude). Leaves were harvested just before flowering, dried for approximately 3 wk under shade, then crushed, powdered, homogenized, and pressed into 500 mg tablets under ambient temperature. Tablets were robust with no excipient required. Using HPLC with diode array detector, analysis of hexane extracts of randomly selected batches of 100 tablets showed artemisinin content of the tablets was consistent at 0.74% ± 0.06% (*i.e*., approximately 3.7 mg per tablet).

The four cohorts of the trial each had 12 consenting patients aged 15–56 years (average 23.42) with *P. falciparum* malaria. Based on Giemsa-stained blood smears counted against 200 wbc, parasitemia was 0.02%-4% and hemoglobin levels > 8 mg/dL. Each cohort received one of four increasing numbers of *A. annua* tablets, ranging from 2–5 tablets twice on day 1, followed by 1–4 tablets twice daily for the next 5 d (Table 2). A week following the treatments, three patients scattered throughout different cohorts showed re-appearance of parasites in blood smears; however, all doses were effective in clinical and parasitological regression of malaria, with 9%-20% recrudescence at day 28 and no measurable toxicity.

Compared to the usual large pure artemisinin doses of 1000 mg on day 1 followed by 500 mg on each of days 2–7 that were administered to 227 malaria patients[39], patients treated with pACT had generally better therapeutic outcomes (Table 2). The measured pACT cure rate also was comparable to or exceeded other results using pure artemisinin[40,41], and similar levels of artemisinin (artesunate, artemether, *etc*.[42]). Furthermore, the positive

therapeutic response using pACT appeared somewhat independent of dose beyond the second level of dose tested (Table 2[20]). Although oral doses used in the ICIPE[20] trials were far less than any tea studies, levels of recrudescence were much lower than tea and often better than in studies using pure artemisinin[39] (Table 2). Indeed, about 100 total mg of total artemisinin delivered *via* pACT for a full malaria treatment yielded a better recrudescence rate than the 4000 mg of pure artemisinin used by Giao *et al*[39] (Table 2). This 40-fold difference correlates well with the early pharmacokinetic studies by Weathers *et al*[21] that showed 45-fold enhanced bioavailability of the drug when delivered as pACT.

These results suggest that the natural phytochemical blend in pACT is important especially when orally administered as tablets. The results are also consistent with a study in China on mice infected with *P. berghei*, which compared the effects of pure artemisinin with crude *A. annua* extracts[43], and the studies by Elfawal *et al*[23] and Weathers *et al*[22]. In all three studies the administered products had comparable levels of artemisinin, but crude preparations and pACT were at least 3.5 times more effective in reducing parasitemia than pure artemisinin, suggesting a synergistic role for non-artemisinin constituents in the extracts and orally consumed dried leaves.

COMPARATIVE PHARMACOKINETICS AND BIOAVAILABILITY

Orally delivered artemisinin

When given orally or rectally, dihydroartemisinin showed higher bioavailability in humans than artemisinin in an early pharmacokinetic study by Zhao *et al*[44]. The C_{max}, T_{max}, and $T_{1/2}$ for orally delivered dihydroartemisinin were 0.13–0.71 mg/L, 1.33 h, approximately 1.6 h, respectively; for pure artemisinin they were 0.09 mg/L, 1.5 h, and 2.27 h, respectively. Alin *et al*[45] compared orally delivered artemisinin and artemisinin-mefloquine combination therapy for treatment of *P. falciparum* malaria. Infected and uninfected patients had similar pharmacokinetic parameters. After a single dose, bioavailability of artemisinin was not altered. Interestingly, pharmacokinetics were similar when comparing treatment failures with successes, suggesting that studies that only measure artemisinin pharmacokinetics were inadequate for predicting therapeutic success[45]. Ilet *et al*[46] also reviewed artemisinin pharmacokinetics in patients with falciparum malaria and reported a dose of 9.1 mg/kg, which was comparable to that of Alin *et al*[45]. C_{max} and T_{max} values did not differ much from those reported by Alin *et al*[45].

In the Ilet *et al*[46] review of pharmacokinetic parameters of artemisinin and its derivatives, oral pure artemisinin doses ranged from about 6–11 mg kg/L in healthy subjects and C_{max} was 0.15–0.39 mg/L. Dose seemed to have no major effect. An earlier study by Ashton *et al*[47] compared increasing artemisinin doses of 250, 500, and 1000 mg per person and both C_{max} and $T_{1/2}$ showed dose-dependent increases of 0.21, 0.45, and 0.79 mg/L, and 1.38, 2.0, and 2.8 h, respectively, but T_{max} remained relatively constant at 2.3–2.8 h.

Diet is an important consideration for any orally delivered drug, and when Dien *et al*[48] compared artemisinin oral doses given with and without food, C_{max} values were similar between subjects who fasted and those who did not. Food consumption along with artemisinin did not seem to affect artemisinin absorption. In contrast, a later rodent study by

Weathers *et al*[21] observed that when artemisinin was consumed as part of a complex plant material, pACT, approximately 45-fold more drug entered the serum of mice than orally administered pure drug. Similarly, when pure artemisinin was fed to mice, it was not detectable in the serum after 60 min. However, artemisinin was detected in the serum when consumed in conjunction with mouse chow, which consists of a variety of plant materials including soy, oats, wheat, alfalfa, beet pulp, corn, *etc*[22].

In a study by Ashton *et al*[49], artemisinin at 9.1 mg/kg was given daily for 7 d, and measurements taken on days 1, 4, 7, and 21. On day 1 plasma C_{max} and T1/2 were similar and comparable to data from other studies using a similar dose. On day 4 and 7, however, C_{max} decreased, while T1/2 increased, indicating that although artemisinin was delivered daily for 7 d, it was either not readily absorbed or it degraded after the first dose. After the third dose, C_{max} fell from 0.31 to 0.11 mg/L, and $T_{1/2}$ increased from 3.0 to 4.8 h. These results suggested that either artemisinin was metabolized or accumulated elsewhere in the body.

In the liver, cytochrome P450 (CYP450) enzymes metabolize artemisinin to deoxyartemisinin, deoxydihydroartemisinin, 9,10-dihydrodeoxyartemisinin, and a metabolite named “crystal 7”[50]. Extended artemisinin dosing may not be beneficial as shown by Svensson *et al*[50] using human liver microsomes where activity of CYP450s, CYP2B6 in particular, correlated with decreasing artemisinin serum levels. In intermittent dosing studied by Ashton *et al*[49], the P450 levels were allowed to decline for 14 d before delivery of another dose, and C_{max} rose from 0.11 to 0.20 mg/L, and $T_{1/2}$ decreased from 4.8 to 2.7 h. Generally, maximum concentration of artemisinin in the body increased with increasing doses with $T_{1/2}$ ranging from about 1.4–4.8 h for reported trials using oral pure artemisinin. Thus, increased and extended artemisinin treatment may reduce recrudescence.

Tea infusion delivered artemisinin

Other than Räth *et al*[16], there are few reports on the pharmacokinetics of tea infusion artemisinin delivered in humans. In the Räth *et al*[16] study, artemisinin C_{max} was 0.24 mg/L at 0.6 h post consumption. Tea infusion containing 94.5 mg artemisinin had a C_{max} equivalent to a dose of 250 mg pure artemisinin, but at a significantly shorter T_{max}, 0.6 h *vs* 2.8 h[47]. Compared to pure artemisinin, the shorter half-life of artemisinin in the tea infusion may account for the observed higher recrudescence. Although tea-delivered artemisinin seemed more bioavailable, its shorter $T_{1/2}$ of 0.9 h compared with about 2 h for pure artemisinin, suggested that more than two doses per day may be more beneficial; indeed, four doses a day were recommended.

The unacceptably high recrudescence rates in clinical tea infusion trials were attributed to low plasma concentrations, almost 40% lower than that for traditional doses (500 mg per person of 60 kg or 8.3 mg artemisinin/kg) of pure artemisinin. Although not specified, tea trial doses have been estimated at about 1.5 mg/kg, close to the 1.1 mg/kg dose of pure artemisinin used by Zhao *et al*[44], which is far below the 8.3 mg/kg that is traditionally accepted as pharmacologically effective. Nevertheless, the C_{max} of 0.24 mg/L artemisinin for the tea dose is nearly twice that for pure artemisinin (C_{max} = 0.13 mg/L) as measured by

Zhao *et al*[44]. *A. annua* tea also showed potent antiplasmodial activity against 40 field isolates of *P. falciparum* collected in Pikine, Senegal (mean IC_{50} 0.095 μg/mL[51]).

Dried leaf (pACT) delivered artemisinin

There are as yet no pharmacokinetic studies of pACT in humans. In a small PK study of healthy mice fed artemisinin there was about 45-fold more artemisinin delivered *via* pACT than when delivered as the pure drug[21]. More recently, pharmacokinetics of artemisinin and one of its liver metabolites, deoxyartemisinin, were compared over 120 min in healthy and *P. chabaudi*-infected mice treated with dried *A. annua* leaves at a 100 mg/kg body weight dose of artemisinin[22]. In pACT-treated healthy mice, the first order elimination rate constant for artemisinin was estimated to be 0.80/h, corresponding to a T1/2 of 51.6 min. C_{max} and T_{max} were 4.33 mg/L and 60 min, respectively. The AUC was 299.5 μg min/mL. The first order absorption rate constant was estimated at 1.39/h. In contrast, the AUC for pACT-treated infected mice was greater at 435.6 μg·min/mL. Serum levels of artemisinin in the infected mice continued to increase over the 120 min of the study period. As a result, the elimination half-life, T1/2 could not be determined, so C_{max} and T_{max} could only be estimated at ≥ 6.64 mg/L and ≥ 120 min, respectively. Nevertheless, both C_{max} and T_{max} of artemisinin were greater in infected than in healthy mice.

Generally, artemisinin concentrations decreased with a concomitant rise in deoxyartemisinin levels only in healthy subjects[22]. In contrast, artemisinin levels in infected mice continued to rise over the study period whilst deoxyartemisinin levels fell and then leveled, so infection seemed to retard the capacity of the mice to process artemisinin into deoxyartemisinin over the two-hour period. Many compounds in *A. annua* inhibit *P. falciparum*[52–55] and CYP34A[56]. At the high (100 mg/kg) dose used in the study, nearly equal amounts of artemisinin and deoxyartemisinin were measured in the serum, indicating that an excessive dose of artemisinin was used.

The presence of plant material affected artemisinin pharmacokinetics. At 60 min no artemisinin was detected in serum of mice fed pure artemisinin at 100 mg/kg body weight. When plant material was present, however, as mouse chow or *A. annua* pACT, artemisinin level in the serum rose to 2.44 and 4.32 μg/mL, respectively, demonstrating that the presence of plant material, even mouse chow, had a major positive impact on the appearance of artemisinin in the blood[22]. To our knowledge, these are the only data available on pharmacokinetics for orally delivered *A. annua* in animals or humans.

NON-ARTEMISININ THERAPEUTIC COMPOUNDS IN *A. ANNUA*

Flavonoids

A. annua is rich in essential oils, coumarins, polyphenols, polysaccharides, saponins, terpenes, and flavonoids. The levels of flavonoids and other compounds in *A. annua* change with developmental growth stage, with some being highest during full bloom[57]. There are > 40 flavonoids[13], and at least 11, including artemetin, casticin, chrysoplenetin, chrysoplenol-D, cirsilineol, eupatorin, kaempferol, luteolin, myricetin, quercetin, and rutin, are reported to have weak therapeutic efficacy against falciparum malaria (Table 1[52–54,58]). Some of these flavonoids were shown to improve the IC50 of artemisinin against *P.*

falciparum in vitro by as much as 50%, suggesting synergy (Table 1[52]). Elford *et al*[53] also showed that while casticin (5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,6,7-trimethoxychromen-4-one) showed synergism with artemisinin, it did not synergize with chloroquine, suggesting a different interactive mechanism. Combining casticin with artemisinin inhibited parasite-mediated transport systems that control influx of myoinositol and L-glutamine in malaria-infected erythrocytes. These apparent synergistic actions between flavonoids and artemisinin suggest that flavonoids are likely to be important for efficacious use of *A. annua* consumed either as whole dried leaves or as tea.

Many flavonoids have antiplasmodial effects and inhibit *P. falciparum* growth in liver cells *in vitro* as reported for dietary flavonoids[54]. To our knowledge, there are no reports on pharmacokinetics of *A. annua* delivered flavonoids. Some flavonoids are reported to have long plasma half-lives; *e.g*., quercetin, found in *A. annua* and most fruits, has a plasma half-life of 27 h[59]. Quercetin [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*–chromen-4-one], also found in garlic, inhibits parasite growth with differential activity against different strains of *Plasmodium* (Table 1[54,58]). Rutin, which is a rutinose [α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranose] glycoside of quercetin, showed similar results, suggesting that the sugar moiety did not significantly affect antimalarial activity (Table 1[58]). Flavonoids are known to persist in the body for > 5 d; this may explain the once a week dose inducing a prophylactic effect from *A. annua* tea infusion that was reported by Ogwang *et al*[30,31]. Many dietary flavonoids inhibit *Plasmodium* growth *in vitro*, but amounts in the diets are reportedly insufficient to offer protection against malaria[54]. Plants such as *A. annua* with high concentrations of flavonoids (*e.g*., up to 0.6%) may, however, work in concert with artemisinin to prevent malaria when consumed regularly.

The flavone luteolin [2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-chromenone] comprises up to 0.0023% DW in *Artemisia*[14] and has been used for a variety of ailments including cough, diarrhea, dysentery, diabetes, cancer, and malaria. Although luteolin has an IC50 value around 11 μmol/L[54] and is one of the more active antiplasmodial flavonoids found in *A. annua*, one cannot compare its role between studies as indicated by Ganesh *et al*[58] (see Table 1). The antimalarial response of different flavonoids seems to be affected by the strain of *Plasmodium* being tested. Luteolin also prevents completion of a full intra-erythrocytic cycle by inhibiting progression of parasite growth beyond the young trophozoite stage. The mechanism of this antiplasmodial activity seems to be related to the inhibition of parasite fatty acid biosynthesis. These lipids are required by the parasite to detoxify heme into hemozoin[60]. Independent of the human host, apicomplexan parasites use a fatty acid biosynthetic pathway. Enzymes in the pathway, like the NADPH-dependent *b*-ketoacyl-ACP reductase (FabG), are potential antimalarial targets. Among 30 flavonoids studied, luteolin and quercetin had the lowest IC50 values for the inhibition of these enzymes and also showed *in vitro* activity in the sub-micromolar range against multiple strains of *P. falciparum*[60].

Isovitexin {5,7-dihydroxy-2-(4-hydroxyphenyl)-6-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]chromen-4-one} is another flavone, the 6-*C*-glucoside of apigenin, that was found in *A. annua* tea infusion at > 100 mg/L with micromolar antiplasmodial activity (Table 1[19,28]). Isovitexin inhibits lipid peroxidation and xanthine

oxidase activity and protects cells from ROS damage with an overall LD50 > 400 μmol/L[61].

Terpenes

Limonene (1-Methyl-4-(1-methylethenyl)-cyclohexene) is part of the “cineole cassette” that includes 1,8-cineole (eucalyptol), limonene, myrcene, α-pinene, β-pinene, sabinene, and α-terpineol[62]; many of these affect particular stages of *Plasmodium* species. For example, limonene is often present at 7 mg/kg in *A. annua*[14] and inhibits isoprenoid biosynthesis in *Plasmodium*[63] and development at the ring and trophozoite stages[64]. Eucalyptol affects the trophozoite stage[65]. Limonene also arrests protein isoprenylation in *P. falciparum*, halting parasite development within 48 h of treatment[64]. The IC_{50} against *in vitro Plasmodium* in these trials was 2.27 mmol/L, more than twice the IC50 of 533 μmol/L measured by van Zyl *et al*[55]. Limonene and its metabolites remain in the plasma for at least 48 h[66], so the pharmacokinetics is favorable, which is important for elimination of gametocytes and malaria transmission.

The volatile monoterpene α-pinene (4,6,6-trimethylb-ficyclo[3.1.1]hept-3-ene) is present in the plant at levels up to 0.05% of dry weight[14]; it has an IC50 of 1.2 μmol/L, in the range of quinine at 0.29 μmol/L[55]. Eucalyptol (1,8-cineole) may comprise up to 30% [0.24%-0.42% (V/DW)] of the essential oil in *A. annua*[67] and is a strong inhibitor of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-8[68]. Both chloroquine-resistant and chloroquine-sensitive *Plasmodium* strains are affected at the early trophozoite stage[65].

Eucalyptol (1,3,3-Trimethyl-2-oxabicyclo[2,2,2]octane) is also volatile and rapidly enters the blood when delivered either as an inhalant or orally [69,70]. At an IC_{50} of 0.02 mg/mL and low toxicity (LD_{50} of approximately 25 mg/mL), either oral or inhalation delivery is reasonable[65,71]. Indeed eucalyptol concentrations can reach 15 μg/mL in 60 min[69] suggesting its possible use as an antimalarial inhalant.

Artemisia ketone (3,3,6- trimethyl-1,5-heptadien-4-one), a major constituent of some cultivars of *A. annua*, has barely been studied. Other ketones like curcumin[72] have been implicated as inhibitors of β-hematin synthesis, so artemisia ketone may play a similar role and affect hemozoin formation. Although hemoglobin is required for *Plasmodium* survival and multiplication in merozoites inside the red blood cell, it leaves toxic debris like heme. The parasite subsequently oxidizes Fe^{2+} in heme to Fe^{3+} forming hematin, a nontoxic insoluble polymeric crystal called β-hematin (also known as hemozoin), which also inhibits cell-mediated immunity against the parasite. Water extracts of *A. annua* inhibit hemozoin synthesis[73].

Essential oils often contain a large amount of monoterpenes that may enhance the antimalarial effect of artesunate and even reverse the observed resistance of *P. berghei* against artesunate[74]. Monoterpenes tend to be higher in the pre-flowering phase of *A. annua*[75], but are drastically reduced by high drying temperatures or drying in the sun[13,76] and, of particular concern, during compression of dried leaves into tablets[77]. Although monoterpenes have some antimalarial potential, most are rather volatile and thus they may

be therapeutically less important than the nonvolatile flavonoids, phenolic acids, and higher molecular weight sesquiterpenes.

Unlike α-pinene and eucalyptol, camphor (1,7,7-Trim ethylbicyclo[2.2.1]heptan-2-one) has no reported antimalarial activity, but it may comprise as much as 43.5% of the essential oil of *A. annua*[78]. Considering camphor is less volatile than either eucalyptol or α-pinene (melting points of 204 °C, 176 °C, and 155 °C, and flash points of 54 °C, 49 °C, and 33 °C, respectively), it may instead play a role in enhanced transport of hydrophobic molecules like artemisinin from pACT across the intestinal wall into the bloodstream[21,22]. Camphor may also affect thymocyte viability and aid in developing malaria immunity through production of T-cells[79]. At 50 μg/mL, camphor increased viability of cultured thymocytes[80].

The sesquiterpene nerolidol (3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol) has an IC50 of 0.99 μmol/L and arrests development of the intraerythrocytic stages of the parasite (Table 1[55]). Indians of the Amazon basin in Brazil treated malaria using the vapors of the leaves of *Viola surinamensis*; nerolidol was identified as the active constituent leading to 100% growth inhibition at the schizont stage[81]. Nerolidol levels vary with the cultivar tested, with one of the highest values found in plants from Ethiopia[82]. There is a greater concentration of this sesquiterpene in stems than leaves of *A. annua*[83].

Other sesquiterpenes found in the artemisinin biosynthetic pathway were only recently shown to have antiplasmodial activity at μmol/L levels, similar to that of other compounds found in the plant (Table 1[19]). These artemisinic compounds were extracted into *A. annua* tea infusions and showed varying interactions with artemisinin depending on their relative concentrations and the target parasite strain. For example, arteannuin B showed an additive interaction with artemisinin against the CQ-sensitive *Plasmodium* HB3 strain, while against the CQ-insensitive Dd2 strain the interaction was synergistic.

Phenolic acids

Rosmarinic ((2”R”)-2-[[(2”E”)-3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]]oxy]-3-(3,4-dihydroxyphenyl) propanoic acid) and chlorogenic ((1*S*,3*R*,4*R*,5*R*)-3-{[(2*Z*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,4,5-trihydroxycyclo-hexanecarboxylic acid) acids are strong antioxidants found in a wide variety of *A. annua* cultivars[56]. In Caco-2 studies, these acids significantly inhibited activity of CYP3A4, one of the hepatic P450s responsible for metabolism of artemisinin to deoxyartemisinin, an inactive form of the drug[50]. These and other phenolic acids are present in *A. annua* tea infusion[19]. Both phenolic acids have an IC_{50} of about 65 μmol/L (Table 1) and also significantly reduced secretion of cytokines IL-6 and IL-8, and thus enhanced antimalarial activity while reducing inflammation[56].

Other compounds often found in A. annua and that may affect pACT efficacy

Although polysaccharides in other medicinal plants have been more extensively studied, they seem to have been rather overlooked in *A. annua*, probably because most *Artemisia* extracts are obtained using organic solvents and polysaccharides are only soluble in water. Polysaccharides extracted from *Artemisia iwayomogi* showed hydroxyl radical scavenging activity three times stronger than glutathione or caffeic acid, and ROS inhibition was twice

as strong as ascorbic acid[84]. In *A. iwayomogi*, more polysaccharides were found in stems than in leaves and their solubility was also higher from stem than from leaf tissue[84].

The combination of polysaccharides with lipophilic molecules like artemisinin may lead to a higher bioavailability of the antimalarial constituents when delivered *via A. annua*, which may explain the lower effective therapeutic dose against malaria observed for pACT than for pure artemisinin[20,23,26]. Indeed, Han[85] showed that ginseng polysaccharides had preventive and curative antimalarial activities and synergized with artesunate in malaria-infected mice. Sulfated polysaccharides inhibited the *in vitro* invasion of merozoites into erythrocytes and interfered with merozoite surface protein[86–88]. Heparin and other sulfated polysaccharides have been shown to inhibit blood-stage growth of plasmodium[89,90]. Some sulfated polysaccharides inhibited the formation of rosettes between infected red blood cells (iRBC) and uninfected RBCs, as well as adhesion of iRBCs to placental chondroitin sulfate A, which is linked to severe disease outcome in pregnancy-associated malaria[91].

Saponins, common in many plants, have an important role in human and animal nutrition and are reportedly present in *A. annua*, but only as measured in alcoholic extracts using the nonquantitative foaming test[92,93] (Weathers, unpublished). These soap-like amphiphilic (lypo- and hydrophilic) bioactive compounds are mainly produced by plants. Recently, there has been interest in the clinical use of saponins as chemotherapeutic agents[94], and as adjuvants for vaccines[95]. At very low doses saponins are efficient, have hemolytic properties, produce 40–50 Å pores in erythrocyte membranes, and modulate the sodium pump and ATPase[96]. Saponins also have a hypoglycemic effect mainly by inhibiting intestinal permeability and absorption of glucose and may therefore inhibit the growth of *P. falciparum*, which needs glucose to grow[97]. Better identification, quantification, and investigation into the role of saponins in pACT efficacy are warranted.

The coumarin, scopoletin (7-hydroxy-6-methoxychromen-2-one), also known for its antinociceptive properties[98,99], is commonly found in most *Artemisia* species at, for example, about 0.2% (w/w) in a Luxembourg cultivar. Known for its anti-oxidant, hepatoprotective, and anti-inflammatory activities, scopoletin scavenging capacity for hydroxyl radical, DPPH, superoxide anion, hydrogen peroxide, and Fe^{2+} chelating activity is almost at the level of α-tocopherol (Vitamin E)[100].

Although not antiplasmodial, scopoletin inhibits TNF-α, IL-6, and IL-8 at millimolar concentrations, and is thus likely one of the major anti-inflammatory and antipyretic constituents of *A. annua*[101]. Coumarins can activate lymphocytes, thereby stimulating immunological functions[102]. Indeed, scopoletin induced cell proliferation in normal lymphocytes with an immunomodulatory effect[101]. In uninfected erythrocytes internal Na concentration is much lower than external concentration, but the K concentration is higher; in infected blood cells this situation is drastically reversed[103]. Scopoletin significantly stimulated erythrocyte membrane ATPases at 0.1 μmol/L, in particular Na-K-ATPase *vs* Ca-ATPase or Mg-ATPase[104], so scopoletin may affect malaria infection. A significant hormetic effect was also noticed; stimulation was higher at scopoletin concentrations of 10 μg/mL than at 1 or at 100 μg/mL. In addition scopoletin also inhibited ADP-platelet aggregation at a range of 0.1 to 5 μmol/L and improved blood rheology[105].

Scopoletin may also affect the interaction between malaria and uric acid. Cyclical fevers and high levels of inflammation characterize malaria and this likely aids parasite clearance. Excessive and persistent inflammation, on the other hand, can lead to severe malaria[106]. In the cytoplasm of their parasitophorous vacuole, *Plasmodium*-infected erythrocytes contain uric acid precipitates that are released upon erythrocyte rupture. Uric acid precipitates are mediators for inflammatory cytokines IL-6, IL-8, and are considered a danger signal for innate immunity. Uric acid is also the causative agent in gout. These precipitates could offer a novel molecular target for anti-inflammatory therapies in malaria. Scopoletin inhibits the activity of xanthine oxidase in hyperuricemic mice after peritoneal administration, and this hypouremic effect is fast and dose-dependent[107].

Toxicology

Although many of the compounds in *A. annua* have not been tested for their toxicity in, a survey of available MSDS data showed that the LD_{50} levels for orally administered compounds in rodents ranged from about 160 mg/kg for quercetin to > 8000 mg/kg for nerolidol. The artemisinin LD_{50} measured *via* oral dose in a mouse was 4228 mg/kg. Therefore, at the estimated amounts of dried leaves of pACT that may be orally consumed by a malaria patient, most of the compounds reported thus far in *A. annua* are at concentrations that are orders of magnitude below their LD_{50} toxicity values.

Toxicology of the dried leaf tablets used in the Kenyan human trial measured the following components: serum levels of urea, serum proteins, creatinine, γ-glutaryl transferase, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, or alkaline phosphatase levels, hemoglobin, and pre- and post-electrocardiograms[20]. Compared to levels prior to treatment with pACT, there was no significant change post-treatment.

PRODUCTION CONSIDERATIONS

Production comparisons with traditional extraction

Because production costs are usually closely held secrets, there are few cost estimates that are publicly available to compare pACT production with extracted artemisinin. However, costs can be estimated from a study by de Vries *et al*[108] where they reported a 1 kg recovery of artemisinin from *A. annua* containing 0.6% artemisinin. Downstream processing costs and product losses increase with increasing number of unit operations (unit ops), a fact often not generally appreciated[109]. Indeed for biotechnology processes, recovery can be anywhere from 9%-51%[110]. As an example, if each step of a 4 step process is 95% efficient, then the overall process has a final efficiency of about 81%, while a single step process at 95% efficiency has a 95% overall recovery. The described process steps for extracted artemisinin (eAN) *vs* pACT-AN are shown in Figure 2. From the point of harvested dried leaves to material ready for packaging or conversion to the delivered drug (*e.g.*, artesunate or artemether), pACT has one unit op and eAN has eight[108]. Extraction solvents and other chemicals are clearly no longer part of the cost. Because there is one *vs* eight unit ops for eAN and at least two of the eAN unit ops involve significant amounts of heat, pACT energy cost is significantly reduced by at least 90%. Costs for labor, interest, depreciation, and maintenance are all also affected by the number of unit ops[109], so we

estimated that with seven fewer unit op steps those costs would reduce by approximately 88%. Although better extraction processes may be in play[111], using the de Vries *et al*[108] analysis our estimate of cost reduction for producing pACT is about 30% less than the cost of producing eAN. Data provided by de Vries *et al*[108] was based on 0.6% artemisinin content, so if a higher producing cultivar was harvested, costs would drop proportionately. Moreover, cost drops again because with pACT there is no need to convert artemisinin to artesunate or artemether; those conversions were necessary because they have higher bioavailability than pure artemisinin, which is not an issue with pACT[21,22].

The de Vries *et al*[108] process cost estimation focuses on a production yield of 1 kg of artemisinin from 500 kg dried leaves, so per Giao *et al*[39] that amount of pure artemisinin would treat only 250 patients. Based on the data shown in Table 3 from Kenyan or WPI *A. annua* at 0.7 and 1.4% artemisinin, 15 and 7.5 g DW leaves, respectively, are required for a total adult pACT treatment; so from 500 kg leaves, 33300 and 66600 patients could be treated, respectively. This represents more than a 130-fold increase in patients treated compared to pure artemisinin with proportionate reduction in price.

A. annua dry leaf production varies around the globe. “In East Africa yields average 2.5 T/ha (range = 0.75–4.2)…”[112]. Based on our field trials[113], the reported average *A. annua* leaf production in E. Africa[112], and the doses used in the Kenyan human trial[20], one can estimate the amount of dry leaf production, and depending on the amount of artemisinin in the biomass, estimate possible number of adult patients that could be treated with pACT (Table 3).

Current ACT drugs vs pACT

Using the dosing information obtained from the Kenyan human malaria trial[20], each adult needs about 100 mg artemisinin total over 6 d for a malaria treatment, so for *A. annua* leaves with 0.7% artemisinin, 15 g of dried leaves would be needed for a 6 d treatment course. At 2 ton of dried leaves harvested per hectare, 127260 adult patients could be treated for malaria (Table 3). For leaves containing 1.4% artemisinin, only 7.5 g of dried leaves are required, so from a hectare of land producing 2 tons of leaves twice as many patients could be treated (Table 3). Clearly choosing cultivars that have higher levels of artemisinin in their leafy biomass will dramatically increase the number of patients that can be treated from 1 ha.

According to Roll Back Malaria, from one ton of purified artemisinin current ACT therapy can provide 1.76 million adult malaria treatments using artemether/lumefantrine, and 2.5 million adult treatments using artesunate/amodiaquine[114] (Table 4). Using the same one ton artemisinin equivalent, but delivering the drug *via* pACT with 0.7% artemisinin content, one would have harvested about 142.8 tons of dried *A. annua* leaves. Assuming 15 g dried leaves per patient from the dosing data in the Kenyan human malaria trial (Table 2[20]), 8.64 million adult patients could be treated, about a four-fold increase over either of the current ACT drugs. The actual cost of pACT, therefore, mainly depends on the cost of the dried leaves and their artemisinin content.

As yet unpublished data from the Rich and Weathers labs demonstrated that pACT prevents emergence of artemisinin drug resistance; the plant itself seems to function as its own ACT

(pACT). This would obviate the need for inclusion of a co-drug as used in currently administered ACTs. The co-drug costs at least as much as the artemisinic portion of the drug[6]. Consequently, elimination of the added co-drug could result in at least an additional 50% reduction in cost, so that the final pACT cost reduction is conservatively estimated to be far below that of a current course of ACT therapy.

Considering that *A. annua* is nontoxic and safe to consume orally, dose may not have to be adjusted for children. On the other hand, the leaves taste bitter, so masking the taste, perhaps with sugar, should help with pediatric treatment. Our recent simulated digestion study showed that adding table sugar (sucrose) to pACT did not significantly alter the amount of artemisinin released after digestion, with the added benefit of doubling the amount of flavonoids released[115].

Comparison with emerging artemisinin sources or other newer antimalarial drugs

There are at least three other emerging antimalarial therapeutic technologies: synthetic artemisinin[116], semi-synthetic artemisinin (SSA) production from genetically engineered microbes[117], and a single dose drug, OZ439[118]. In early 2013, Sanofi/PATH Drug Development Programme, announced they would have the capacity to produce up to 60 MT of SSA in 2014 at about $400/kg, depending on quantity; Sanofi now has WHO prequalification for its SSA[119]. Although not much cheaper than the current price of about $550/kg[120], supply would be more or less unlimited. Despite what might seem as an advantage to large amounts of SSA production, there are also some serious disadvantages, and comparison of some advantages and disadvantages for each of these new synthetic antimalarial drugs and pACT is noted in Table 5.

QUALITY ASSURANCE CONSIDERATIONS

Agricultural quality

The traditional and least costly method for cultivating *A. annua* uses seeds and in developing countries farmers prefer to save seeds from one growing season to the next. However, seed generated plants of *A. annua* will vary widely from generation to generation even with high quality starting stock (see review by Ferreira *et al*[10]). Stem cuttings of *A. annua* readily root in about two weeks, so clonal propagation *via* rooted cutting is recommended to eliminate this variability. Although this method of propagation is not cost effective for large plantations, it would work for a few hectares or for controlled environment agriculture. Given the large numbers of patients that could be treated from growing just a few hectares of *A. annua* (Table 3), clonal propagation by rooted stem cuttings is recommended. Since pACT therapy involves the direct consumption of the dried leaves of the plant, harvested leaf material must be kept clean, which is easiest to do in controlled environment agriculture and following Good Agricultural Procedures[121], particularly as applied to fresh produce[122]. However, controlled agriculture would probably result in loss of agricultural jobs, a concern to be assessed locally. Alternatively, great care must be taken during field harvest and post-harvest storage, so as not to affect the quality of the product. WHO has established good agricultural practices specifically for *A. annua* for purposes of artemisinin

extraction[123], for general medicinal plants[124], and to minimize contamination of herbal medicines[125].

Chemical consistency and quantification

To deliver a reliable dose of therapeutics to a patient, the dried leaves of harvested *A. annua* must have a reliable and consistent composition. Clonal propagation provides the required consistency. Recently we showed that of 10 crops harvested from vegetative and early flowering plants grown over three years under diverse conditions in the lab, field, and home garden, the artemisinin content of a single clone of *A. annua* (SAM) was 1.38% ± 0.26% (w/w)[77]. Thus, despite variations in culture and environmental conditions, a consistent level of the main therapeutic constituent can be achieved. Moreover, the content of harvested leaves is certainly not a guarantee of finished product, *e.g*., compressed leaf tablets. Analyses by Weathers *et al*[77] showed that although artemisinin content was very stable after tablet compression, other constituents vaied significantly. For example, although flavonoids increased with tablet compression, the more volatile monoterpenes decreased substantially. Thus, it is critical to monitor the composition profile of both incoming harvested material as well as the final product.

Complex and expensive analytical procedures have been used to analyze the many products found in *A. annua*, but they are not necessary to measure and assure product quality. Artemisinin is easily extracted and then can be quantified using a variety of thin layer chromatography (TLC) methods and visualized with *p*-anisaldehyde stain[126,127]. Other key constituents like the flavonoids are also readily separated using TLC and visualized under either UV ± $AlCl_3$ reagent[128]. Total flavonoids also can be quantified using inexpensive visible spectroscopy *via* the $AlCl_3$ method with quercetin used as an inexpensive standard. To our knowledge no inexpensive, reliable spectrophotometric assay is available to measure artemisinin in complex plant extracts.

SOCIOECONOMIC BENEFITS

Other diseases

Artemisinin and its derivatives are also effective against a number of viruses[129], a variety of human cancer cell lines[130–133], and several neglected tropical diseases including schistosomiasis[134], leishmaniasis[135,136], trypanosomiasis[137], and some livestock diseases[133,138].

Although they rank below malaria in terms of public health importance, schistosomiasis, leishmania, and trypanosomiasis result in estimated annual infections of about 240 million, 1.3 million (0.3 visceral and 1.0 cutaneous), and 30000, respectively[139]. These diseases along with many others respond to treatment with artemisinins. Although the IC_{50} is about 1000-fold greater than for *Plasmodium* sp., the greater apparent bioavailability of artemisinin *via* oral pACT[20–22] would likely reduce the amount of drug required for treatment. At present, pACT has not been tested *in vivo* for diseases other than malaria.

Malaria treatment is further complicated for Human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) patients. Malaria and HIV co-infection

represents a major health burden in Africa mainly because it is now "well established that HIV infection results in a higher incidence and more severe manifestations of malaria"[140]. With a weakened immune system, AIDS patients are more susceptible to malaria and also respond slower to malaria therapy[140–142]. Furthermore, in a meta-analysis by Tusting *et al*[143], socioeconomic development strongly correlated with better malaria therapeutic outcomes. Recently, *A. annua* has demonstrated anti HIV activity[126,144] and thus oral consumption of the dried leaves of this herb will not only treat malaria, but should also enhance the well-being of HIV/AIDS patients.

Agriculture, jobs and self-determination

A. annua is grown in more than 75 countries[145]. In 2011 about 163 MT of artemisinin were extracted from plantations and small stakeholder farms mainly located in China, Vietnam, and Eastern Africa including Madagascar; value was about $550/kg[120]. With the advent of the production of semi synthetic artemisinin by Sanofi, 60 MT were projected for 2014 with an anticipated price of about $400/kg[119]. As this new source of artemisinin becomes available, the Netherlands Royal Tropical Institute projected that the market for natural *Artemisia* will significantly destabilize, undermining the security of farmers. The Tropical Institute was further concerned that "pharmaceutical companies will accumulate control and power over the production process; *Artemisia* producers will lose a source of income; and local production, extraction and (possibly) manufacturing of ACT in regions where malaria is prevalent will shift to the main production sites of Western pharmaceutical companies", disrupting the fragile economics of these already impoverished countries[120]. The average small stakeholder crop area is about 0.2 ha in China and Africa[120], so while implementation of pACT may not require as much agricultural land as for extracted artemisinin, it could still help provide small stakeholders with a source of income. We have estimated that localized micro manufacturing plants could be constructed for < $50000 USD, and produce quality-controlled pACT tablets with readily verifiable contents. Our overall approach, schematically illustrated in Figure 3, leads to local control of malaria and possibly other artemisinin susceptible diseases while also improving the socioeconomic status of the populations.

CONCLUSION

Evidence is mounting for the therapeutic efficacy of the use of dried leaves of *A. annua*, pACT, to treat malaria and possibly other diseases. The complex mixture of antiparasitic compounds in the plant seems to account for its therapeutic activity with animal and human trials supporting this claim. It is also clear that the cost of using pACT is a fraction of that for any other current or emerging antimalarial therapeutic. Likewise, the recent evidence of persistent and/or asymptomatic malaria suggests that a more prophylactic approach to malaria using pACT or even *A. annua* tea may be warranted. Considering that for > 2000 years this plant was used in traditional Chinese medicine for treatment of fever with no apparent appearance of artemisinin drug resistance, taken together the cumulative evidence argues for inclusion of pACT into the arsenal of drugs to combat malaria, and very likely, other diseases.

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Core tip

Artemisinin, extracted from the plant *Artemisia annua (A. annua)* L., and artemisinin derivatives are the current best antimalarial therapeutics and are delivered as artemisinin combination therapy (ACT). Availability and cost are problematic for the developing world where malaria is endemic. Oral consumption of *A. annua* dried leaves is more effective than the pure drug. A tea infusion of the leaves has prophylactic effects. Cost of producing and delivering the tea and *A. annua* dried leaf tablets is much more affordable than ACT.

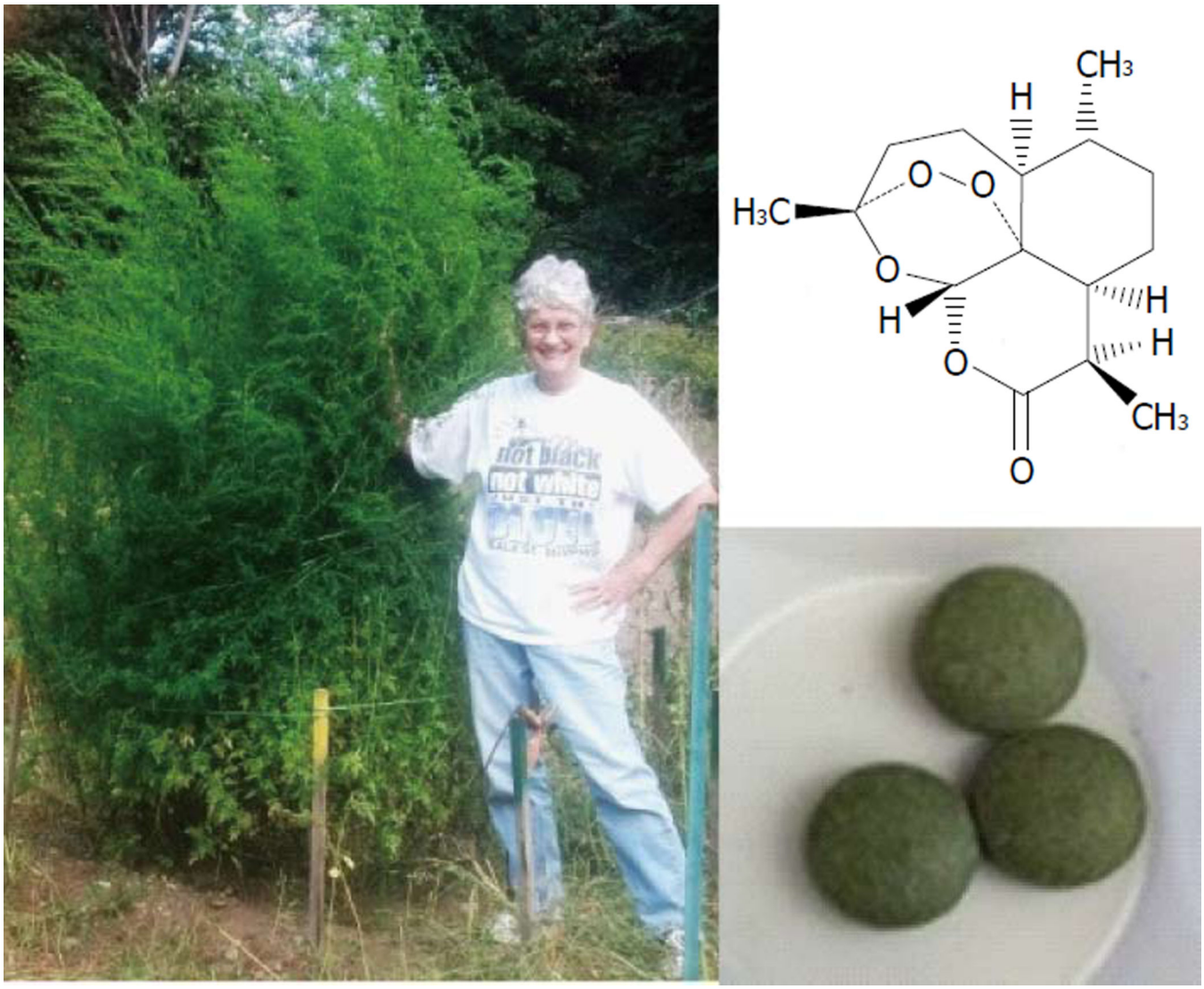

Figure 1.

Artemisia annua (single clone of *Artemisia annua* cultivar at approximately 2 m height at floral bud formation), artemisinin and plant-based artemisinin combination therapy tablets.

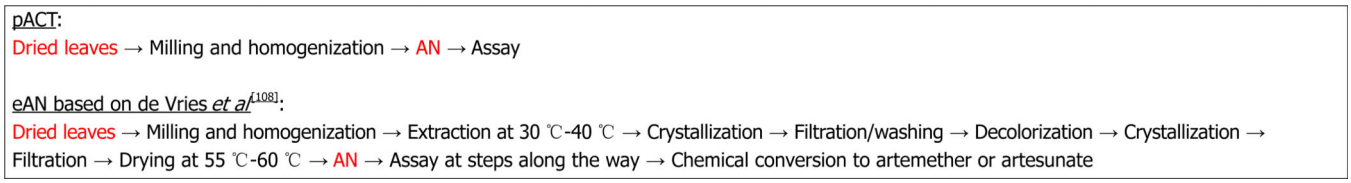

Figure 2.

Comparison between plant-based artemisinin combination therapy production and extracted artemisinin from dry harvested leaves to product ready either for packaging (plant-based artemisinin combination therapy) or conversion to artemether or artesunate (extracted artemisinin). AN: Artemisinin; eAN: Extracted artemisinin; pACT: Plant-based artemisinin combination therapy.

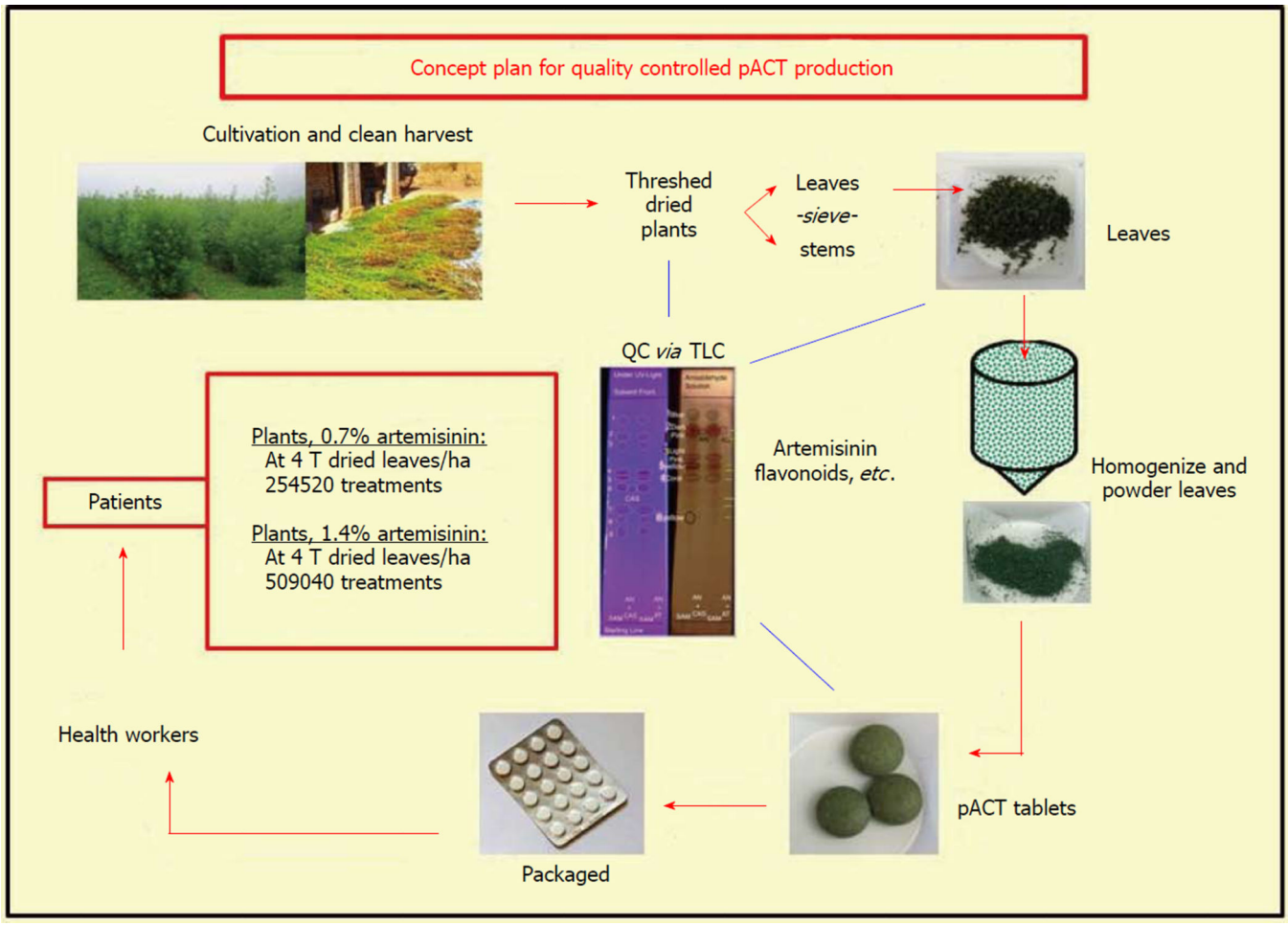

Figure 3.

Overall scheme for plant-based artemlslnln combination therapy production. pACT: Plant-based artemisinin combination therapy; TLC: Thin layer chromatography.

Table 1

Antimalarial compounds in *Artemisia annua vs* falciparum malaria

Compound	Compound IC_{50} (μmol/L)	Compound + artemisinin IC_{50} (nmol/L)	Ref.
Terpenes			
Artemisinin	0.033 0.022, 0.023[1]	Not applicable	Liu *et al*[52]
Artemisinic acid	77.8, 61.6[1]	No numerical value provided; response depended on	Suberu *et al*[19]
Arteannuin B	3.2, 4.8[1]	concentration of compound tested with artemisinin	
Dihydroartemisinic acid	21.1, 17.7[1]		
Nerolidol	9[4]	Interaction with artemisinin	van Zyl *et al*[55]
α-pinene	1[4]	not yet tested	
1,8-cineole (eucalyptol)	70[4]		
Limonene	533[4]		
Phenolic acids			
Chlorogenic acid	69.4, 61.4[1]	No numerical value provided; response depended on	Suberu *et al*[19]
Rosmarinic acid	65.1, 65.0[1]	concentration of compound tested with artemisinin	
Flavonoids			
Artemetin	26	26	Liu *et al*[52]
Casticin	24	26	
Cirsilineol	23	22.5	
Chrysoplenol-D	32	15	
Chrysoplenetin	36	16	
Eupatorin	65	30	
Isovitexin	72.5, 48.1[1]	Interaction with artemisinin	Suberu *et al*[19]
Luteolin	11, 12[2]	not yet tested	Lehane *et al*[54]
Kaempferol	33, 25[2]		
Myricetin	40, 76[2]		
Quercetin	15, 14[2] 14.7, 4.11, 2.94[3]		Ganesh *et al*[58]
Rutin	7.1, 3.5, 10.38[3]		

[1]Against CQ-sensitive HB3 and CQ-resistant Dd2 strains, respectively;

[2]Against CQ-sensitive 3D7 and CQ-resistant 7G8 strains, respectively;

[3]Against fresh Bangladeshi isolates, CQ-sensitive 3D7, and CQ-resistant K1 strains, respectively;

[4]Against CQ-resistant FCR-3. CQ: Chloroquine.

Table 2

Kenyan human trial data[20] for orally delivered dried leaf *Artemisia annua* (plant-based artemisinin combination therapy)

pACT (dried leaf *A. annua* tablets, ea 500 mg, 3.7 mg artemisinin/tablet)					
Artemisinin dose (mg)		**No. of patients**	**Leaf DW (g)**		**% Recrudescence**
Day 1	**Days 2–6**		**Day 1**	**Days 2–6**	
7.4 × 2	3.7 × 2	12	2	1	25
11.1 × 2	7.4 × 2	12	3	2	9.1
14.8 × 2	11.1 × 2	12	4	3	16.7
18.5 × 2	14.8 × 2	12	5	4	9.1
Compare to orally delivered pure artemisinin[39]					
Day 1	Day 2–7				
500 × 2	500	227	NA		24

A. annua Artemisia annua; pACT: Plant-based artemisinin combination therapy; NA: Not available.

Table 3

Estimated numbers of adult patients treatable from plant-based artemisinin combination therapy[1]

***For A. annua* cultivar containing**	**Number of patients treated at various dry leaf tonnage**			
	2 T/ha[2]	**3 T/ha**	**4 T/ha[3]**	**5 T/ha**
0.7% artemisinin/g DW (Kenyan cultivar)	127260	190890	254520	318150
1.4% artemisinin/g DW (WPI cultivar)	254520	381780	509040	636300

[1] Assumptions: each adult needs 100 mg artemisinin (AN) over 6 d for a cure; at 0.7% and 1.4% AN that is approximately 15 and *73* g DW leaves, respectively, for a single adult total malaria treatment;

[2] Below the average of 2.5 T/ha reported for all of East Africa;

[3] Equal to the maximum obtained growing *A. annua* SAM in the Stow, MA, United States field trials. *A. annua: Artemisia annua*.

Table 4

Estimated number of patient treatments by current artemisinin combination therapy *vs* plant-based artemisinin combination therapy

Combination therapy drug	Adult treatments per ton of artemisinin
AL[1]	1.76 million
AS/AQ[1]	2.5 million
pACT leaves with 0.7% artemisinin	8.6 million

[1] http://www.rollbackmalaria.org/partnership/wg/wgprocurementsupply/docs/psmwg_ppACT-API.pdf p.2 [cited May 27, 2014];

[2] Assumes a 6 day treatment with pACT, with each patient receiving 15 g dried leaves per full malaria treatment for leaves with 0.7% artemisinin. To obtain an amount of artemisinin equal to 1 T of the extracted drug, one would have to harvest 142.8 tons of dried *A. annua* leaves containing 0.7% artemisinin AL: Artemether/lumefantrine; AS/AQ: Artesunate/amodiaquine; pACT: Plant-based artemisinin combination therapy.

Table 5

Comparison of emerging antimalarial therapeutic technologies with plant-based artemisinin combination therapy

Technology	Advantages	Disadvantages
Synthetic AN[116]	Fully synthetic method giving AN = compound Lowers AN cost compared to extraction	Requires co-drug to obviate emergence of AN drug resistance Not yet in production Needs sophisticated process Likely all under Western control Challenging patient compliance due to multiday dosing
Semi-synthetic AN[117]	Semi-synthetic method giving authentic AN Lowers AN cost compared to extraction	Requires co-drug to obviate emergence of AN drug resistance Production began *via* Sanofi Needs sophisticated process Likely all under Western control Challenging patient compliance due to multiday dosing
OZ439[118]	Single dose cure insures patient compliance In successful Phase 2 trials Mechanism of action not the same as AN Probably low cost due to full synthesis	Requires co-drug to obviate emergence of AN drug resistance Not yet in production Needs sophisticated process Likely all under Western control
pACT[20–24]	Has its own in planta co-drug to obviate emergence of AN drug resistance Very low cost Very consistent product Can be used to treat other diseases Can be locally owned, produced, managed, and distributed	Not yet in production Likely to meet push back from pharmaceutical industry Challenging patient compliance due to multiday dosing

AN: Artemisinin.