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**Whole plant approaches to therapeutic use of *Artemisia annua*, L. (Asteraceae)**

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## **Abstract:**

Long used as a therapeutic tea by the Chinese to treat fever, *Artemisia annua* is more recently being studied and used for eventual treatment of not only malaria, but also many other diseases. This chapter describes studies using *in vitro* systems, animal models, and humans to evaluate use of not only combinations of pure compounds from the plant, but also tea infusions and the dried leaves of the plant.

### **1.0 Rationale for direct consumption of *A. annua* as a malaria therapy**

Indigenous to Asia, *Artemisia annua* L. is a GRAS (generally regarded as safe) herb (Duke 2001) that has been used >2,000 years in traditional Chinese medicine, usually as a tea infusion, to treat fevers, and febrile diseases, some currently associated with malaria. The main active compound is the sesquiterpene lactone artemisinin. Recent decreases in malaria deaths are mainly attributed to increased therapy, e.g. artemisinin, and prevention techniques including insecticide laced bed nets. The threat of resistance to artemisinin monotherapy, however, is very real (Phyo et al. 2012), and high recrudescence rates associated with a short half-life of the drug (Giao 2001), have brought about the need for costly combination therapies. To prevent and delay emergence of artemisinin drug resistance, the World Health Organization (WHO) now recommends artemisinin combination therapy (ACT) as the first line treatment of malaria for two reasons: a second antimalarial drug, which has different biochemical modes of attack on parasites would prove more effective, and the second drug should also kill parasites with developed artemisinin resistance (WHO 2010).

Parts of the world most affected by malaria – mainly sub-Saharan Africa – are desolate with poor infrastructure, making distribution of ACT antimalarial medicines difficult, even if 100% of the demand were to be met. Additionally, the high cost of manufactured drugs deters treatment. Bed nets provide a preventive option to night biting mosquitoes, but they may be difficult to obtain or afford (WHO, 2013). A low cost, local, effective and reliably produced treatment may decrease mortality rates of malaria in these isolated regions; it could also spur economic development. Although artemisinin has the potential to treat a diversity of other diseases, here we review the results of *A. annua* therapeutic studies based on whole plant use.

### **2.0 *A. annua* tea infusion therapy:**

2.1 Chemistry of tea and its preparation. To our knowledge, there have been few well controlled studies examining the extraction recovery and stability of the many compounds in *A. annua* tea infusion. Recently, however, van der Kooy and Verpoorte (2011) performed a systematic study of different preparations of *A. annua* therapeutic tea infusion and showed that nearly 93% of available artemisinin was extracted from dried *A. annua* leaves, but only under certain conditions. Ideal conditions were 9 g DW leaves L<sup>-1</sup>, for 5 min at 100°C. More importantly, they also showed that when stored at room temperature, the tea artemisinin concentration did not significantly decrease, which is important for people in developing countries where malaria is endemic and there is little or no access to refrigeration. Ideally, a liter of tea infusion would be prepared daily and consumed in equal aliquots of about 250 mL over 24 hrs (van der Kooy and Verpoorte 2011).

Carbonara et al. (2011) detected a wide variety of phenolics including 0.06 mg/g DW of the flavonoid, cirsilineol, in an *A. annua* tea infusion prepared as follows: 0.5 g in 13 mL boiling water, then leaving the infusion to cool for up to 48 hrs prior to extraction and measurement. The original starting artemisinin concentration in the leaves was not reported thus preventing quantification of the relative amount of this constituent released into the tea. However, the artemisinin tea concentration remained constant during the 48 hr room temperature infusion. Most of the measured phenolics also remained constant for 48 hr. It is likely, however, that there was poor extraction of artemisinin mainly because their proportion of dried leaves to boiling water (38 g DW L<sup>-1</sup>) was fourfold greater than that determined to be optimal (9 g DW L<sup>-1</sup>) by van der Kooy and Verpoorte (2012). These authors showed data suggesting that increasing the ratio of dried leaves to water proportionately decreased the amount of extracted artemisinin; thus at 4 g DW L<sup>-1</sup> only 43% of the extractable artemisinin (93%) appeared in the tea. Weathers and Towler (2012) later confirmed a high efficiency of extraction and 24 hr stability of artemisinin retrieving about the same amount of artemisinin while using the same optimized tea protocol. However, several measured flavonoids, casticin and artemetin, were neither well extracted nor stable. Artemisinin solubility in water is about about 50 mg L<sup>-1</sup> (van der Kooy and Verpoorte 2011), so the amount of artemisinin retrieved via hot water tea infusions is reasonable. Clearly if a tea infusion is to be a therapeutic option, however, it must be consistently and reliably prepared.

2.2 Tea studies in animals. To our knowledge there is only one published study in animals using a tea infusion. Atemnkeng et al. (2009) compared parasite clearance in *Plasmodium chabaudi chabaudi* infected mice (n = 6) treated twice daily for 6 days with either pure artemisinin or an *A. annua* tea infusion. Both treatments used an equal dose of artemisinin of 0.011 mg (0.275 mg

kg<sup>-1</sup>). This dosage was far lower than a third group of infected mice treated with WHO recommended doses of artemisinin of 0.4 mg (10 mg kg<sup>-1</sup>) on day 1, followed by 0.2 mg (5 mg kg<sup>-1</sup>) day<sup>-1</sup> for days 2-7 (Table 1). After 6 days, only the WHO dosed mice showed significant reduction in parasitemia; tea treated mice had at best 50% parasite clearance (Table 1).

2.3 Human trials. Mueller et al. (2000) tested treatment of *A. annua* (cv. Artemis) tea on adults infected with uncomplicated *P. falciparum* malaria. The amount of artemisinin measured in the tea, although much lower than the usual dose of pure artemisinin and artemisinin derivatives, showed some success in treatment (Table 1). Later, Mueller et al. (2004) used doses of 5 and 9 g for *A. annua* tea preparation to treat *P. falciparum* malaria infected adults (Table 1). The *A. annua* (cv. Artemis), grown and dried in Germany, was delivered to the eastern Democratic Republic of Congo in prepackaged doses of dried leaves. Although the dried plant material was reported to have 1.4% artemisinin, only 47 and 94 mg of artemisinin were extracted in a liter of tea prepared from 5 and 9 g of *A. annua*, respectively, which was  $\leq 75\%$  of the original artemisinin in the dried leaves. Unfortunately, the study showed considerable recrudescence in the tea treated group (Table 1). Because of the consistently lower rates of recrudescence in the quinine treated control group (Table 1), it was inferred that most parasite reappearance in the tea-treated patients was the result of recrudescence, not re-infection (Mueller et al. 2004). Using 5 g dried leaves in one liter, De Donno et al. (2012) showed that *A. annua* tea infusion was effective against both chloroquine (CQ) sensitive (D10) and resistant (W2) strains of *P. falciparum* with IC50s of 7.08 nM and 5.60 nM, respectively.

In a more recent tea trial (Blanke et al. 2008), a placebo tea with an alternate antimalarial drug was included on the first day of treatment in parallel with the test *A. annua* tea. Artemisinin concentration was at the same level as in Mueller et al. (2004), but tea concentration was at best 19% (94 mg) of standard pure artemisinin treatments (500 mg) per person (Blanke et al. 2008). The *A. annua* used in this trial was also grown in Germany and sent to the test site in western Tanzania, in dried, pre-dosed bags. At day 14 and 28 recrudescence of tea-treated patients was consistently greater than alternate drug treatments (Table 1).

Data from therapeutic tea trials in humans and in animals correlate well and unfortunately do not support the use of *A. annua* tea for treating malaria for the following reasons: animal and human data are comparably negative and compelling, artemisinin dose is not easily controlled, and other potentially useful components in the tea are not readily controlled or extractable. Nevertheless, use of the tea could play a role in malaria prophylaxis (See 2.5) or in temporary control of malaria, mainly prevention of coma, until the infected person reaches a hospital or clinic stocked with ACT.

2.4 Comparative pharmacokinetics of dihydroartemisinin, artemisinin and tea derived artemisinin. Pharmacokinetic data for oral doses of artemisinin and dihydroartemisinin, the active form of artemisinin in the serum, were collected in 3 subjects by Zhao and Song (1993). When given orally or rectally, dihydroartemisinin showed higher bioavailability than artemisinin. Compared to artemisinin delivered orally, the results from Zhao and Song (1993) showed a higher serum dihydroartemisinin  $C_{max}$ , 0.13-0.71 mg L<sup>-1</sup>, in a shorter period of time with  $T_{max}$  ranging from 1.33-1.50 h (Table 2). Despite using a control of 15 mg kg<sup>-1</sup> of pure artemisinin,  $C_{max}$  and  $T_{max}$  were 0.9 mg L<sup>-1</sup> and 1.5 h; these results differed from other oral artemisinin studies (Table 2).

Alin et al. (1996) focused on the comparison of artemisinin and artemisinin-mefloquine combination therapy in oral delivery for the treatment of *P. falciparum* malaria. Pharmacokinetic parameters in infected and uninfected patients were similar showing that, after a single dose, bioavailability of artemisinin was not altered. When comparing treatment failures with successes, however, pharmacokinetics were similar suggesting that pharmacokinetic studies that only measure artemisinin were inadequate for predicting therapeutic success (Alin et al. 1996). Ilet and Batty (2005) also reported values for artemisinin pharmacokinetics in patients with falciparum malaria. The average weight of participants in Alin et al. (1996) was 53.8 kg, which gives an average dose of 9.3 mg kg<sup>-1</sup> (close to the WHO recommended dose of 10 mg kg<sup>-1</sup>). Ilet and Batty (2005) reported a dose of 9.1 mg kg<sup>-1</sup>, which was comparable to that of Alin et al. (1996). Comparing these two reports,  $C_{max}$  did not differ much, only by 0.22 mg L<sup>-1</sup> with  $T_{max}$  values differing by about 0.4 h (Table 2).

Ilet and Batty (2005) reviewed the pharmacokinetic parameters of artemisinin and its derivatives. For oral pure artemisinin doses ranging from about 6-11 mg kg L<sup>-1</sup> in healthy people,  $C_{max}$  was also similar at 0.15-0.39 mg L<sup>-1</sup> (Table 2). Dose appeared to have no major effect. In contrast, Ashton et al. (1998a) studied increasing artemisinin doses of 250, 500, and 1,000 mg per person and found apparently dose dependent increases in  $C_{max}$  of 0.21, 0.45, and 0.79 mg L, respectively, but  $T_{max}$  remained relatively constant at 2.3-2.8 h (Table 2). However,  $T_{1/2}$  values (1.38, 2.0, and 2.8 h, respectively) increased with dose (Table 2). It appears that the pharmacokinetics related to increasing artemisinin dose requires further study.

Diet is also very important for any orally delivered drug. For example, Dien et al. (1997) compared artemisinin oral doses delivered with and without food. The  $C_{max}$  values were similar between patients who fasted and those who did not, only differing by about 0.150 mg L<sup>-1</sup> (Table 2). Intake of food with artemisinin did not seem to affect absorption of artemisinin. On the other hand, Weathers et al. (2010) found that when artemisinin was consumed as a component of *A. annua* dried leaf therapy, ~45 fold more entered the serum of mice than when orally administered as the pure drug (Table 2).

Interestingly, Ashton et al. (1998b) showed that when artemisinin at 9.1 mg kg<sup>-1</sup> was given on days 1-7, and measurements taken on days 1, 4, 7, and 21, plasma  $C_{max}$  and  $T_{1/2}$  were similar on day 1, and comparable to data from other studies using a similar dose (Table 2). Data collected on day 4 and 7, however, showed a decrease in  $C_{max}$  while  $T_{1/2}$  increased. This trend showed that although artemisinin was delivered daily for 7 days, it was either not readily absorbed or degraded by CYP450 enzymes in the liver after the first dose.  $C_{max}$  fell from 0.31 to 0.11 mg L<sup>-1</sup> after the third dose, and  $T_{1/2}$  increased substantially from 3.0 to 4.8 h (Table 2). These results suggested that either artemisinin accumulated in the body or was degraded. In human liver microsomes, appearance of cytochrome P450s, CYP2B6 in particular, correlates with decreasing artemisinin serum levels suggesting that extended artemisinin dosing may not be beneficial (Svensson and Ashton 1999). Indeed, intermittent dosing, wherein the P450 levels are allowed to decline was shown in the study by Ashton et al. (1998b) wherein by waiting 14 d to deliver another dose,  $C_{max}$  rose from 0.11 to 0.2 mg L<sup>-1</sup>, and  $T_{1/2}$  decreased from 4.8 to 2.7 h (Table 2). That being said, increased longevity of any artemisinin treatment may reduce recrudescence. For the most part the oral dosage data seemed consistent in that maximum concentration of artemisinin in the body increased with increasing doses. Generally,  $T_{1/2}$  ranged from about 1.4-4.8 h for all trials using oral pure artemisinin.

With the exception of R  th et al. (2004), there are few reports on the pharmacokinetics of artemisinin delivered via tea to humans. In the Rath et al. (2004) study, peak concentrations of 0.24 mg artemisinin L<sup>-1</sup> occurred at 0.6 hours after tea intake. Table 2 compares the pharmacokinetic parameters for *A. annua* and artemisinin in humans. The tea extract containing 94.5 mg artemisinin, showed a  $C_{max}$  plasma concentration equivalent to a dose of 250 mg pure artemisinin, but at a significantly shorter  $T_{max}$ , 0.6 hr vs. 2.8 hr (Ashton et al. 1998; also see Table 2). Compared to pure artemisinin, the lower half-life of artemisinin in the tea extract (Table 2) may have resulted in the observed higher recrudescence. Pure artemisinin has a short half-life (Table 2). Although tea-delivered artemisinin seemed more bioavailable, it had a lower half-life of 0.9 hr compared with about 2 hr for pure artemisinin (Table 2), suggesting that more than two doses per day may be more beneficial; four doses a day were recommended.

Plasma concentrations, being almost 40% lower than that of traditional doses (500 mg/person of 60kg or 8.3mg artemisinin kg<sup>-1</sup>) of pure artemisinin, are concluded as cause for unacceptably high recrudescence rates in clinical tea trials (Table 2). Assuming that the average weight of subjects is 60 kg, the artemisinin dose/kg in the tea trial is estimated at about 1.5 mg kg<sup>-1</sup>, close to the 1.1 mg kg<sup>-1</sup> dose of pure artemisinin used by Zhao and Song (1993), way below the 8.3mg/kg mentioned above as traditionally accepted as pharmacologically effective. Considering this, the  $C_{max}$  of artemisinin for the tea dose is nearly twice as much as that of pure artemisinin (Table 2). Notwithstanding, tea from *A. annua* showed potent antiplasmodial activity when tested against 40 field isolates of *P. falciparum* collected in Pikine, Senegal (mean IC50 0.095 µg mL<sup>-1</sup>; Gueye et al. 2013).

**2.5 Prophylactic human trials.** In a randomised clinical trial in Uganda (Ogwang et al. 2011, 2012), Artemisia tea was tested as a prophylaxis against malaria in 132 farm workers for 9 months, and any adverse clinical effects were tracked for 12 months. Tea consumed once a week at a 2.5 g adult infusion dose, had an unadjusted protective efficacy of 37.5% (Ogwang et al. 2012), which is better than that reported for vaccines RTS, S/AS01B and RTS, S/AS02A with protection efficacy of about 30% in adults (Bojang et al. 2001; Polhemus et al. 2009). It was also superior to FMP1/AS02 vaccine that was reported to confer no protection and also to vaccines LSA-NRC/AS01 and LSA-NRC/AS02 that elicited antigen-specific antibody and CD4+ T cell responses, but with no protective immunity (Ogutu et al. 2009; Cummings et al. 2010). The tea protective effects also increased with duration of use. Unlike vaccines such as RTS, S/AS02 whose protection wanes within a few weeks (Bojang et al. 2001), the increasing protection trend by Artemisia tea suggests that curbing of malaria in a given population is a possibility. Persons who used Artemisia tea also had 80% fewer hospital visits due to fevers, with some individuals in the study community reporting use of the tea for > 7 years with no incidence of malaria. More randomized clinical trials of *Artemisia* tea malaria prophylaxis need to be conducted in different populations and age groups. The Ugandan study was done among adults aged 18-60 years; their immunity to malaria is greater than children or the very elderly. Although one might argue that weekly consumption of *A. annua* tea might lead to emergence of resistance, data soon to be submitted for publication from the Rich and Weathers labs in Massachusetts (USA) suggests otherwise.

### 3.0 *A. annua* dried leaf consumption as therapy:

**3.1 Animal studies.** Recently Elfawal et al. (2012) measured parasitemia in mice infected with *P. chabaudi* that were fed two different doses (0.6 or 3.0 mg; 24 and 120 mg kg<sup>-1</sup>, respectively) of either pure artemisinin in mouse chow or in dried leaves of *A. annua*. The dried leaves were at least five times more effective, and with a longer lasting response, than the pure drug in reducing parasitemia (Table 1). Interestingly, mice needed >45 fold more artemisinin (mixed with mouse chow) than artemisinin consumed via dried leaf in order for artemisinin to be detected in the serum (Weathers et al. 2011).

**3.2 Human trials.** Except for the early tea trials of Mueller (Mueller et al. 2000, 2004) in the Democratic Republic of Congo, clinical trials using dried leaf *A. annua* are scarce in the scientific literature. Although WHO does not encourage either whole plant or tea infusion clinical trials (WHO 2012), some African universities carried out their own trials (personal comm from C Kasongo to P Lutgen). Those involving a very limited number of patients were generally not published and results were not assessed by polymerase chain reaction (PCR) as later done for clinical trials with ACTs. Their results are briefly described here, with best studies summarized in Table 1.

Compared to controls or even other antimalarial drugs, e.g. artesunate-amodiaquine, early unpublished trials mainly used *A. annua* decoctions and showed significantly greater sensitivity of the decoction with lower late therapeutic failures. In the Democratic Republic of Congo, 54 volunteers suffering from malaria were treated for 10 days with capsules containing powdered leaves of *A. annua* at decreasing doses. The total amount of dried herb administered per patient was 15 g dried leaves containing 15 mg of artemisinin (0.1% artemisinin leaf content; Tiruneh et al. 2010). All were free of fever after 2 days and 51 were free of parasites after 10 days.

In an unusual study aimed at preventing severe post-operative malaria at Bangui, Central Africa, capsules containing powdered leaves of *A. annua* leaves were administered to 25 patients, 22 of them children aged 1-16 yrs, during surgical interventions for orthopedic disorders (Onimus et al. 2013). The duration of the treatment ranged from 3-4 days with a daily dose of 0.4-0.5 mg artemisinin delivered in 0.4-0.5 g of *A. annua* dried leaves, 0.1% artemisinin leaf content. Despite the very low administered dose of artemisinin, average parasitemia in the patients dropped by 62% with an added benefit of a strong antinociceptive response.

The most thorough study designed to assess clinical efficacy of whole-leaf *A. annua* was undertaken in a collaborative project between the International Centre of Insect Physiology and Ecology (ICIPE) and Kenya Medical Research Institute (KEMRI) (ICIPE 2005; Table 1). The study, conducted at ICIPE Mbita Field campus, Suba District, in Western Kenya, was an open-label, non-randomized clinical trial primarily targeted to assess the efficacy, safety and tolerance of increasing doses of whole-leaf *A. annua* in the form of tablets (Sawa et al., in preparation). The tablets were made from a hybrid of *A. annua* grown in the Tanzania highland (2,000-2,200 m altitude) by a Tanzania-based NGO, Natural Uwemba System for Health (NUSAG). Harvested leaves from 8-month-old plants (just before flowering) were dried for ~3 weeks under shade, then crushed, finely powdered, homogenized, and pressed into 500 mg tablets under ambient temperature. Randomly selected batches of 100 tablets extracted with hexane, concentrated and analysed by HPLC with diode array detector showed the artemisinin content of the tablets was highly consistent at 0.74 ± 0.06 % (i.e. ~3.7 mg per tablet).

Forty-eight consenting patients aged 15-56 yrs (average 23.42), with *P. falciparum* malaria (parasitemia was 0.02 - 4%, based on Giemsa-stained blood smears counted against 200 wbc) and haemoglobin levels > 8 mg dL<sup>-1</sup> were recruited for the project. Patients were divided into four cohorts and treated with increasing levels of *A. annua* tablets, ranging from 2-5 tablets twice on day 1, followed by 1-4 tablets twice daily for the next 5 days. Although there were three cases of re-appearance of parasites in blood smears scattered throughout different cohorts a week following the treatments, all doses were effective in clinical and parasitological regression of malaria with 9-20% recrudescence at day 28. Patients also suffered no toxic affect; there was no significant change in the serum levels of urea, serum proteins, creatinine, γ-glutamyl transferase, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, or alkaline phosphatase levels, hemoglobin, and pre and post electrocardiograms (ICIPE 2005).

Thus, despite the relatively low levels of artemisinin in the administered doses (14.8-37 mg on day 1 followed by between 7.4-29.6 mg on days 2-6) and compared to daily doses of 60-90 mg day<sup>-1</sup> of leaf artemisinin in a hot water extract for 5 days (Mueller et al. 2000; R  th et al. 2004), by day 28, 75-91% cure was achieved (Table 1). This cure rate also was comparable to or exceeded other results: 500 mg day<sup>-1</sup> of pure artemisinin in the form of tablets or capsules (Hien 1994; McIntosh and Olliaro 2010), and similar levels of dihydroartemisinin derivatives (artesunate, artemether, etc.) in the form of tablets or capsules (de

Vries and Dien 1996). Furthermore, the positive therapeutic response seemed somewhat independent of the range of dose tested (Table 1; ICIPE 2005). Although the oral doses used in the ICIPE (2005) trials were far less than any tea studies, levels of recrudescence were much lower than tea and equivalent to studies using pure artemisinin (Giao et al. 2001; Table 1).

These results suggested there is an important role for the phytochemical blend associated with powdered foliage of whole-leaf *A. annua* when orally administered as tablets. The results are also consistent with the study by Elfawal et al. (2012) and a study in China on mice infected with *P. berghei*, which compared the effects of pure artemisinin with crude *A. annua* extracts (Yao-De et al. 1992). The two products had comparable levels of artemisinin; however, crude preparations were at least 3.5 times more effective in reducing parasitemia than pure artemisinin indicating a synergistic role played by non-artemisinin constituents in the extracts.

**3.3 Pharmacokinetics of whole plant delivered artemisinin.** Li et al. (1998) provided some of the earliest reported pharmacokinetics of intragastric delivery of dihydroartemisinin in rats. The pharmacokinetic parameters of artemisinin in animal models has also been used to study the delivery of artemisinin via whole plant *A. annua* and the few known reports are summarized in Table 3 and compared to ingestion of pure artemisinin. When uninfected mice were orally gavaged either with artemisinin mixed in mouse chow or with dried leaves of *A. annua*, Weathers et al. (2011) showed that the serum  $C_{max}$  of artemisinin was about the same (Table 3). However, it was necessary to increase the amount of artemisinin in the chow > 40 fold that in the leaves in order to achieve the same response. This result suggested higher bioavailability of plant-delivered artemisinin. More recently, pharmacokinetics was compared for healthy and *P. chabaudi*-infected mice treated with dried *A. annua* leaves; both  $C_{max}$  and  $T_{max}$  of artemisinin were greater in infected than healthy mice (Weathers et al. 2013; Table 3). Interestingly, the infected mice showed biphasic artemisinin serum peaks at 15 min and 2 hr with serum concentrations at 4.7 and 6.7 mg artemisinin  $L^{-1}$ , respectively. It is possible that the serum level increased further, but the study was terminated at 120 min. A liver metabolic product of artemisinin is deoxyartemisinin (Whirl-Carrillo et al. 2012; also see <http://www.pharmgkb.org/pathway/PA165378192#PGG>). At the high (100 mg  $kg^{-1}$ ) dose used in the study, nearly equal amounts of deoxyartemisinin and artemisinin were measured in the serum indicating that an excessive dose of artemisinin was used. These are, to our knowledge, the only known data available on pharmacokinetics for whole plant oral doses in animals or humans.

#### **4.0 How can we explain the enhanced effect of whole plant vs. the pure drug – synergism?**

*A. annua* is rich in essential oils, polysaccharides, saponins, coumarins, acids, minerals, flavonoids, and polyphenols some of which were recently reviewed by Ferreira et al. (2010). This review is not intended to be exhaustive, rather it intends to suggest that an in depth study is overdue for the antimalarial properties of these molecules. Here we highlight some of these potentially important constituents that could be providing the added benefits especially observed in studies of oral consumption of dried leaves of *A. annua*. These benefits could accrue not only as therapeutic activity, but also enhancing bioavailability of artemisinin.

**4.1 Terpenes.  $\alpha$ -Pinene** is a volatile constituent of essential oil present in the plant regardless of origin at levels up to 0.05% of dry weight (Bhakuni et al. 2001). In a South African study, which determined the antimalarial activity of 20 essential oils,  $\alpha$ -pinene ranked second with an  $IC_{50}$  of 1.2  $\mu M$ , similar to that of quinine at 0.29  $\mu M$  (Seatholo 2007; van Zyl et al. 2006).

**1,8-Cineole** (eucalyptol) often comprises up to 30% of the essential oil in *A. annua* or 0.24-0.42 % (V/DW) (Charles et al. 1990). The molecule is a strong inhibitor of the pro-inflammatory cytokines  $TNF-\alpha$ , IL-6 and IL-8 (Juergens et al. 2004). Growth and development of chloroquine-resistant and chloroquine-sensitive *Plasmodium* strains are affected and are stalled at the early trophozoite stage (Su et al. 2008). This volatile terpene is rapidly absorbed into the blood when delivered either orally or as an inhalant (Kovar et al. 1987; Stimpfl et al. 1995), reaching 15  $\mu g mL^{-1}$  in 60 min (Kovar et al. 1987) suggesting its possible use as an antimalarial inhalant. With an  $IC_{50}$  of 0.02 mg  $mL^{-1}$  and an  $LD_{50}$  of ~25 mg  $mL^{-1}$ , either inhalation or oral delivery is reasonable (Kengne 2010; Su et al. 2008).

**Artemisia ketone** is a major constituent of *A. annua* but its role has barely been studied. It may, however, play a role in hemozoin formation. *Plasmodium* needs hemoglobin for its survival and multiplication in merozoites inside the red blood cell (Akkawi et al. 2012). Although this gives the parasite access to nitrogen, it leaves debris like heme, which is toxic. The parasite circumvents this by causing oxidation of Fe(II) in heme to Fe(III) forming hematin that polymerizes into an insoluble product called  $\beta$ -hematin and haemozoin, which is nontoxic to the parasite and inhibits cell-mediated immunity against the parasite. Other ketones like curcumin (Akhtar et al. 2012) were implicated as inhibitors of  $\beta$ -hematin synthesis, so it is possible that artemisia ketone plays a

similar role.

**Limonene** is part of the so called “cineole cassette”, which include 1,8-cineole, limonene, myrcene,  $\alpha$ -pinene,  $\beta$ -pinene, sabinene and  $\alpha$ -terpineol (Raguso et al. 2006), many of which affect particular stages of *Plasmodium* species. Limonene, for example, arrests isoprenoid biosynthesis in *Plasmodium* (Goulart et al. 2004) and development at the ring and trophozoite stages (Moura et al. 2001) while 1,8-cineole affects the trophozoite stage (Su et al. 2008). Limonene also inhibits protein isoprenylation in *P. falciparum*, arresting parasite development within 48 h of treatment (Moura et al. 2001). The *in vitro* IC<sub>50</sub> against *Plasmodium* in these trials was 2.27 mM, significantly below 15.5 mM that was previously shown *in vivo* in patients with advanced cancer. The pharmacokinetics is favorable; limonene and its metabolites remain in the plasma for at least 48 hrs (Miller et al. 2010). This is important for the elimination of gametes and malaria transmission.

A combination of essential oils may enhance the antimalarial effect of artesunate and even reverse the resistance of *P. berghei* against artesunate (Liu et al. 2004). The concentration of monoterpenes is higher in the pre-flowering phase (Yang et al. 2012), but is drastically reduced by high drying temperatures or drying in the sun (Khangholil and Rezaeinodehi 2008; Ferreira and Luthria 2010). The monoterpene limonene has a very favourable toxicity profile and is easily available at low prices. Limonene is also present in *A. annua* at concentrations up to 7 mg kg<sup>-1</sup> (Bhakuni et al. 2001). So far, studies have concentrated on this particular monoterpene, but others like eucalyptol, present in the essential oil of *Artemisia* plants, might have a similar detrimental action on the apicoplast, a non-photosynthetic plastid of most apicomplexan parasites, such as *Plasmodium*.

The sesquiterpene **nerolidol**, found in *Artemisia* species arrests development of the intraerythrocytic stages of the parasite. It has an antiplasmodial IC<sub>50</sub> of 0.99  $\mu$ M compared to that of 533  $\mu$ M for limonene (van Zyl et al. 2006). Indians of the Amazon basin in Brazil use the vapors of the leaves of *Viola surinamensis* to treat malaria and the sesquiterpene nerolidol was identified as the active constituent leading to 100 % growth inhibition at the schizont stage (Lopes et al. 1999). Like limonene nerolidol may affect the isoprenoid pathway in the apicoplast of *Plasmodium*. Nerolidol concentrations vary with the origin of *A. annua*. The highest value was found in *A. annua* from Ethiopia (Muzemil 2008). Nerolidol was also found to be higher in the stems than in the leaves of *A. annua* (Li et al. 2011).

**4.2 Phenolic Acids.** **Rosmarinic** and **chlorogenic acids**, recently identified in a wide variety of *A. annua* cultivars, are strong antioxidants (de Magalhães et al. 2012). In Caco-2 studies, these acids significantly reduced secretion of the inflammatory cytokines IL6 and IL-8. They also inhibited CYP3A4 activity and, in combination enhance both antimalarial activity while reducing inflammation.

**4.3 Flavonoids.** There are > 40 flavonoids in *A. annua* (Ferreira et al. 2010), and at least 10, including **artemetin, casticin, chrysoplenetin, chrysoplenol-D, circilineol, eupatorin, kaempferol, luteolin, myricetin, and quercetin**, show some weak *in vitro* therapeutic efficacy against falciparum malaria (Liu et al. 1992; Elford et al. 1987; Lehane and Saliba 2008). When some of these flavonoids were tested in combination with artemisinin, the IC<sub>50</sub> of artemisinin against *P. falciparum* *in vitro* improved by as much as 50%, suggesting synergy with artemisinin (Liu et al. 1992). Interestingly, Elford et al. (1987) also showed that while casticin showed synergism with artemisinin, casticin did not synergize with chloroquine, suggesting a different interactive mechanism. Casticin and artemisinin, however, did inhibit parasite-mediated transport systems controlling influx of myo-inositol and L-glutamine in malaria infected erythrocytes. This apparent synergistic action between artemisinin and flavonoids suggests that flavonoids would likely be important for efficacious use of *A. annua* consumed either as whole dried leaves or as tea.

Some flavonoids have antiplasmodial effects and inhibit *P. falciparum* growth in liver cells *in vitro* as reported for dietary flavonoids (Lehane and Saliba et al. 2008). Although data on pharmacokinetics of *A. annua* flavonoids is scant, some flavonoids generally have long plasma half-lives. For example quercetin, a flavonoid also found in *A. annua* and most fruits, has a plasma half-life of 27 hours (Manach and Donovan 2004). Lehane and Saliba (2008) demonstrated that quercetin found in garlic inhibits malaria parasite growth in liver cells. Considering the half-life of 27 h reported for quercetin, flavonoids can persist in the body for up to 5.83 days possibly enabling a once a week dose of *Artemisia* tea to inhibit parasite growth and contribute to the prophylactic effect. According to Lehane and Saliba (2008), though dietary flavonoids inhibit malaria parasite growth *in vitro*, the amounts in the diets are insufficient to offer protection against malaria. This means that plants such as *A. annua* with high concentrations of flavonoids may prevent malaria when consumed regularly. Flavonoid content of plants is as high as 0.6% of dry weight (Weathers, unpublished). In another study by Baraldi et al. (2008), the flavonoid content in *A. annua* varied with developmental growth stage, with highest amounts found during full bloom.

The flavone, **luteolin**, is found in *Artemisia* species comprising up to 0.0023% of dry weight (Bahkuni et al. 2001). Ethnobotanical use of luteolin includes treatment of cough, diarrhea, dysentery, diabetes, cancer and malaria. Compared with other flavones such as kaempferol, myricetin, quercetin, isoquercitrin, acacetin, apigenin, baicalein and chrysin, luteolin was found to be the most active with IC<sub>50</sub> values around 11  $\mu$ M (Lehane and Saliba et al. 2008). Luteolin also prevents progression of parasite growth beyond the young trophozoite stage, so they cannot complete a full intra-erythrocytic cycle. This strong antiparasitic activity is eventually related to the inhibition of fatty acid biosynthesis by *P. falciparum*. These lipids are required for the detoxification of heme into hemozoin by the parasite (Tazedimir et al. 2006). Apicomplexan parasites use a fatty acid synthesis pathway, independent of the human host and catalyzed by specific enzymes like FabG. These enzymes are a potential target of new antimalarials. Among 30 flavonoids studied, luteolin and quercetin had the lowest IC<sub>50</sub>s for the inhibition of these enzymes (Tazedimir et al. 2006). In this same study, these two flavonoids also showed *in vitro* activity in the submicromolar range against multiple strains of *P. falciparum*.

**4.4 Polysaccharides.** So far the presence of **polysaccharides** in *A. annua* has been barely covered in the scientific literature. The reason may be that they are only soluble in water and most *Artemisia* extracts for research are obtained using organic solvents. Polysaccharides are polymeric carbohydrates of high molecular weight. Although the presence of polysaccharides in other medicinal plants has been more extensively studied, they seem to have been overlooked in the research on *A. annua*. There are more polysaccharides in stems than in leaves and their solubility is also higher for this part of the plant (Ahn and Jung 2011). The same authors found that polysaccharides extracted from *Artemisia* had hydroxyl radical scavenging activity 3 times stronger than glutathione or caffeic acid and the ROS inhibition 2 times stronger than ascorbic acid.

The complex of polysaccharides with lipophilic molecules may lead to a higher bioavailability of antimalarial constituents in *A. annua* and may explain the considerably lower therapeutic doses against malaria required for artemisinin, e.g. in whole plant studies (ICIPE 2005; Elfawal et al. 2012) than for pure artemisinin. Some sulfated polysaccharides inhibit the formation of rosettes between infected red blood cells (iRBC) and uninfected RBCs. More importantly, they inhibit the adhesion of iRBCs to placental chondroitin sulfate A (CSA), which is linked to severe disease outcome in pregnancy-associated malaria (Adams et al. 2006). Sulfated polysaccharides also interfere with the plasmodium merozoite surface protein and inhibit the invasion of merozoites into erythrocytes *in vitro* (Andrews et al. 2005; Xiao et al. 1996; Clark et al. 1997). Heparin and other sulfated polysaccharides have been shown to inhibit blood-stage growth of *P. falciparum* (Munier et al. 1980; Rampengan 1991). Ginseng polysaccharides show preventive and curative antimalarial activities and synergism with artesunate. This was confirmed *in vivo* in malaria infected mice (Han 2008).

**4.5 Saponins.** **Saponins** are common in a large number of plants and they have an important role in human and animal nutrition. They are reportedly present in *A. annua*, albeit only as measured by the nonquantitative foaming test of alcoholic extracts (Ashok and Upadhyaya 2013; Massiha et al. 2012; Weathers, unpublished). Saponins are soap-like amphiphilic (lyophilic and hydrophilic) bioactive compounds produced mainly by plants. Recently, there has been unforeseen interest in the clinical use of saponins as chemotherapeutic agents (Podolak et al. 2010). They are efficient at very low doses, have haemolytic properties and produce 40-50 Å pores in erythrocyte membranes. Saponins are used as adjuvants for vaccines (Song et al. 2009). Saponins also modulate the sodium pump and ATPase (Haruna et al. 1995). They inhibit the intestinal permeability of glucose and may consequently inhibit the growth of *P. falciparum*, which needs glucose to grow. Saponins also have a hypoglycemic effect mainly by inhibiting intestinal absorption of glucose (Francis et al. 2002). Further quantitation and investigation into the role of saponins in *A. annua* whole plant therapeutic effects it seems are warranted.

**4.4 Coumarins.** Most *Artemisia* species contain the coumarin, **scopoletin**. The concentration of scopoletin in several plant samples of *A. annua* as measured at Luxembourg is around 0.2% (W/W). Scopoletin is known for its anti-oxidant, hepatoprotective and anti-inflammatory activities (Malik et al. 2007). Scavenging capacity for hydroxyl radical, DPPH, superoxide anion, hydrogen peroxide and Fe<sup>2+</sup> chelating activity are almost at the level of  $\alpha$ -tocopherol (Vitamin E) (Malik et al. 2007). At mM concentrations scopoletin inhibits TNF- $\alpha$ , IL-6 and IL-8, and is thus likely one of the major anti-inflammatory and antipyretic constituents of *A. annua* (Moon et al. 2007). Scopoletin is also known for its antinociceptive properties (Meotti et al. 2006; Chang et al. 2012).

Coumarins are capable of activating lymphocytes and thus stimulating immunological functions. Moon et al. (2007) showed that scopoletin has an immunomodulatory effect and induces cell proliferation on normal lymphocytes. A significant hormetic effect



was also noticed: stimulation is higher at 10  $\mu\text{g mL}^{-1}$  than at 1 or at 100  $\mu\text{g mL}^{-1}$ . Scopoletin significantly stimulates RBC membrane ATPases at 0.1  $\mu\text{M}$  in particular Na-K-ATPase vs. Ca-ATPase or Mg-ATPase (Ezeokonkwo and Obidoa 2001), which may affect malaria infection. In uninfected erythrocytes the internal Na concentration is much lower than outside the cell, but the K concentration is higher. However, in infected blood cells this situation is drastically reversed (Surono et al. 2008). Scopoletin also inhibits ADP-platelet aggregation at a range of 0.1 to 5  $\mu\text{M}$  and improves blood rheology (Dunn 1969).

Scopoletin may also affect the interaction between uric acid and malaria. Malaria is characterized by cyclical fevers and high levels of inflammation, and while an early inflammatory response contributes to parasite clearance, excessive and persistent inflammation can lead to severe forms of the disease (Clark et al. 2004). *P. falciparum* infected erythrocytes contain uric acid precipitates in the cytoplasm of the parasitophorous vacuole, which are released when erythrocytes rupture. Uric acid precipitates are highly inflammatory mediators for inflammatory cytokines IL-6, IL-8; they are considered a danger signal for innate immunity and are the causative agent in gout. These precipitates could offer a novel molecular target for anti-inflammatory therapies in malaria. Scopoletin exhibits an immediate and dose-dependant hypouricemic effect and inhibits the activity of xanthine oxidase in hyperuricemic mice after peritoneal administration (Ding et al. 2005).

## 5. Conclusions and Future Prospects.

Alternative methods for delivering artemisinin to patients have been considered through use of tea or oral ingestion of dried leaves of *A. annua*. Unfortunately, data indicate that delivery via tea is not very therapeutically efficacious. Furthermore, extraction of artemisinin during tea preparation is variable unless carefully controlled. Although pharmacokinetic data for *A. annua* tea shows some similarities with oral consumption of pure artemisinin,  $T_{\text{max}}$  and  $T_{1/2}$  are lower for tea delivery, which may be the reason underlying greater recrudescence rates for tea treatments. Most studies equate treatment failure with low artemisinin in tea, in turn associating recrudescence with low artemisinin content in plant material, but despite variations in plant material, serum peak concentrations of artemisinin are still similar. The only differences are the  $C_{\text{max}}$  and  $T_{1/2}$ , which appears to be similar across pure artemisinin doses. Mice fed dried *A. annua* leaves showed better therapeutic results than the tea. Results of pharmacokinetic studies using dried leaf delivery in mice are also consistent with the antimalarial success of the human trial. More structured studies in assessing the dosage of *Artemisia annua* delivered as dried leaf tablets, in capsules, or mixed with food needs to be explored in greater depth in pharmacokinetic and clinical trials. Moreover, in order to prevent recrudescence, it is important to evaluate other treatment protocols, such as a two-tiered therapeutic regimen separated by 5-7 days. This is based on the assumption that recrudescence largely occurs because of sequential infections with a still emerging liver-stage malaria. The promising results of the studies using oral consumption of dried leaf *A. annua* may offer a more sustainable treatment for malaria, especially in low-income developing countries. This will eventually lead to better understanding of how the whole plant therapy works better than the pure drug. This report will hopefully guide investigators towards what seem to be the more likely chemical targets.

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**Tables 1-3 follow on next 3 pages.**

Table 1 <i>A. annua</i> Tea or Dried Whole-Leaf Effect on Malaria-Infected Adult Humans or Animals (N/A, not applicable; N/D, not determined)							
Delivery (drug, tea or tablet) and days treated	AN delivered dose (mg)	Control, dose (mg kg <sup>-1</sup> )	# of Subj.	Leaf DW (g d <sup>-1</sup> )	Parasite clearance (% at day #)	Recrudescence (% at day #)	Ref.
<i>A. annua</i> Tea, 4d, 7d <sup>1</sup>	12	No control or placebo used.	48	5	92 (4)	8 (4)	Mueller et al. (2000)
<i>A. annua</i> Tea, 7d	11.75 (X4/d)	N/A	39	5	77 (7) 34 (35) <sup>e</sup>	43 (14) 62 (28) 66 (35)	Mueller et al. (2004)
	23.5 (X4/d)	N/A	33	9	70 (7) 30 (35) <sup>f</sup>	42 (14) 63 (28) 70 (35)	
Alternate Drug	N/A	Quinine sulfate, 500	43	N/A	91 (7) 79 (35) <sup>g</sup>	10 (14) 14 (28) 21 (35)	
<i>A. annua</i> Tea, 7d & placebo pill d1	<94/d	N/A	4	5	75 (7) 0 (28)	75 (14) 100 (28)	Blanke et al. (2008)
	<94/d	N/A	6	9	33.3 (7) 16.7 (28)	50 (14) 83.3 (28)	
Placebo Tea, 7d & alternate drug d1	N/A	Sulfadoxine, 25 & pyrimethamine, 1.25	9	N/A	77.8 (7) 37.5 (28) <sup>h</sup>	44.4 (14) 62.5 (28) <sup>h</sup>	
<i>A. annua</i> tea to <i>P. chabaudi</i> infected mice	0.0	0.0	6	N/A	100 (6)	N/D	Atemnkeng et al. (2009)
	0.011	dose in fed tea	6	leaves	72 (6)		
	0.011	AN = to tea	6	contained	50 (6)		
	0.4, d 1, 0.2, d 2-7	AN	6	1.15% AN	<1 (6)		
Compressed crushed <i>A. annua</i> leaf tablets, given for 6d	<u>day 1</u>	<u>day 2-6</u>		<u>d 1</u>	<u>d 2-6</u>		ICIPE (2005)
	7.4x2	3.7x2	12 <sup>b</sup>	2	1	75 (28) <sup>a,c</sup>	
	11.1x2	7.4x2	12 <sup>d</sup>	3	2	90.9 (28) <sup>a,d</sup>	
	14.8x2	11.1x2	12 <sup>e</sup>	4	3	83.3 (28) <sup>a,e,f</sup>	
	18.5x2	14.8x2	12 <sup>g</sup>	5	4	90.9 (28) <sup>a,h</sup>	
<i>A. annua</i> (cv Sam) dried leaves vs. AN in mouse chow	0.6, in dried leaves	<i>P. chabaudi</i> infected mice; 1st dose at ~8% parasitemia; placebo control = AN in chow	6	0.04	100 (1.25)	100(4)	Elfawal et al. (2012)
	3.0, in dried leaves		6	0.21	100 (1)	0(4)	
	0.6, AN in chow		6	N/A	no clearance	100 (4)	
	3.0, AN in chow		6	N/A	100 (1)	~2 (4)	
Pure AN comparison	d 1, 500 x2; d 2-7, 500 d <sup>-1</sup>	Placebo used	227	N/A	76 (28)	24 (28)	Giao et al. (2001)
<sup>a</sup> Based on Giemsa-stained blood smears counted against 200WBC <sup>b</sup> 1 subject migrated away after day 7 <sup>c</sup> 1 case recrudescence on day 14 (and a crudescence/re-infection? on day 28) <sup>d</sup> 1 subject lost before completing course of treatment <sup>e</sup> 1 subject not available by day 14			<sup>f</sup> 2 cases of early treatment failure <sup>g</sup> 1 case of recrudescence on day 14 <sup>h</sup> 1 patient not available from day 3 <sup>i</sup> Treated 4 times daily AN, artemisinin.				

Table 2 Comparative Pharmacokinetic Parameters for Oral Artemisinin or <i>A. annua</i> Infusion Tea in Humans								
Drug delivery form (pure drug or tea)	AN dose	Subject	# of subjects	C <sub>max</sub> (mg/L)	T <sub>max</sub> (h)	T <sub>½</sub> (h)	T <sub>lag</sub> (h)	Ref.
Oral dihydroartemisinin	1.1 mg kg <sup>-1</sup>	Healthy adults	3	0.13±0.03	1.33±0.29	1.63±0.68	-	Zhao & Song (1993)
	2.2 mg kg <sup>-1</sup>			0.71±0.30	1.33±0.29	1.57±0.34	-	
Oral AN	15 mg kg <sup>-1</sup>	Adults w/ fm	18	0.09±0.01	1.50±0.32	2.27±0.22	-	Alin et al. (1996)
	250 mg (X2 d) <sup>a</sup>			0.587±0.385	2.5	2.2±0.6	-	
	10.4 -C	Healthy adults	6	0.483±0.224	1.78±1.23	2.61±0.58	0.58±0.30	Dien et al. (1997)
	10.4 -F			0.623±0.297	2.66±1.50	2.51±0.67	0.69±0.37	
	250 mg	Healthy adult males	8	0.205±0.127	2.8±1.9	1.38±0.40	1.5±1.0	Ashton et al. (1998a)
	500 mg			0.450±0.324	2.3±0.9	2.00±0.60	1.1±0.4	
	1000 mg			0.792±0.498	2.8±1.6	2.84±1.08	1.0±0.4	
Day 1	9.1 mg kg <sup>-1</sup>		10	0.311±0.232	-	3.0±1.2	-	Ashton et al. (1998b)
Day 4			10	0.148±0.093	-	3.8±2.0	-	
Day 7			10	0.110±0.104	-	4.8±5.0	-	
Day 21 (after 14 d washout)			7	0.195±0.126	-	2.7±0.9	-	
Oral AN	10 mg kg <sup>-1</sup>	Healthy adults	12	0.391	1.8	2.6	-	from review by Ilett and Batty (2005)
	6.8 mg kg <sup>-1</sup>		4	0.150	-	2.3	-	
	6.2 mg kg <sup>-1</sup>		6	0.360	1.7	α=2.6 β=4.3	-	
	10 mg kg <sup>-1</sup>	Pediatrics w/ fm	23	-	-	1.8	-	
	10.8 mg kg <sup>-1</sup>	Adults w/ fm	31	-	-	2.6	-	
	9.1 mg kg <sup>-1</sup>		11	0.364	2.9	2.7	-	
Tea extract	94.5 mg	Healthy males	14	0.240	0.6	0.9	-	Räth et al. (2004)

<sup>a</sup> double loading dose C = taken with food, F = taken while fasting, fm = *falciparum* malaria  
AN, artemisinin.



Table 3. Comparisons of oral artemisinin pharmacokinetics in rodent models.								
Delivery system (drug or whole-plant)	Dose per individual mg kg <sup>-1</sup>	Subj.	# of subj.	C <sub>max</sub> (mg/L)	T <sub>max</sub> (h)	T <sub>½</sub> (h)	Reference	
Intragastric dihydroartemisinin	10 or 0.035	Rats	4	0.77	0.25	0.24	Li et al. (1998)	
Artemisinin (AN)	40		3	0.64 est <sup>b</sup>	1.0	0.25 est	Du et al. (2012)	
<i>A. annua</i> dry leaves <sup>a</sup> vs. AN in mouse chow	1.22 leaf 1.22 AN in chow 56 AN in chow	Mice	3	0.087	0.5	nd	Weathers et al. (2011)	
			3	nd	nd	nd		
			3	≥0.074	nd	nd		
<i>A. annua</i> dry leaves vs. AN or AN in mouse chow <sup>c</sup>	100 leaf (h <sup>b</sup> ) 100 leaf (i <sup>b</sup> ) 100 AN 100 AN in chow 0		6	4.3	1.0	nd	Weathers et al., (2013) unpublished	
			6	6.6, 4.7 <sup>d</sup>	>2	nd		
			3 p <sup>c,e</sup>	nd <sup>f</sup>	nd	nd		
			3 p <sup>c,e,f</sup>	nd <sup>f</sup>	nd	nd		
		3 p <sup>c,e</sup>	nd <sup>f</sup>	nd	nd			
<sup>a</sup> Study ended at 60 min, so T <sub>max</sub> and C <sub>max</sub> not truly measured. <sup>b</sup> Abbreviations: est, estimated from figures; h, healthy; i, infected; p, serum pooled from 3 mice; nd, not detectable. <sup>c</sup> Study ended at 120 min so T <sub>max</sub> and C <sub>max</sub> not truly measured. <sup>d</sup> Study response was biphasic, so data shown for 2 peaks at 15 and 120 min <sup>e</sup> Three pooled mice; serum harvested 60 min post gavage; <sup>f</sup> At 60 min artemisinin serum level of 3 pooled mice was 2.44 mg mL <sup>-1</sup> ; for other two pooled mouse treatments, no artemisinin was detectable.								