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PII: S0944-7113(18)30596-8
DOI: https://doi.org/10.1016/j.phymed.2018.12.002
Reference: PHYMED 52779

To appear in: *Phytomedicine*

Received date: 5 January 2018
Revised date: 29 November 2018
Accepted date: 1 December 2018

Please cite this article as: Jérôme Munyangi , Lucile Cornet-Vernet , Michel Idumbo , Chen Lu , Pierre Lutgen , Christian Perronne , Nadège Ngombe , Jacques Bianga , Bavon Mupenda , Paul Lalukala , Guy Mergeai , Dieudonné Mumba , Melissa Towler , Pamela Weathers , Artemisia annua and Artemisia afra tea infusions vs. artesunate-amodiaquine (ASAQ) in treating Plasmodium falciparum malaria in a large scale, double blind, randomized clinical trial, *Phytomedicine* (2018), doi: https://doi.org/10.1016/j.phymed.2018.12.002

Artemisia annua and *Artemisia afra* tea infusions vs. artesunate-amodiaquine (ASAQ) in treating *Plasmodium falciparum* malaria in a large scale, double blind, randomized clinical trial.

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Abstract

Background and objective
Prior small-scale clinical trials showed that *Artemisia annua* and *Artemisia afra* infusions, decoctions, capsules, or tablets were low cost, easy to use, and efficient in curing malaria infections. In a larger-scale trial in Kalima district, Democratic Republic of Congo, we aimed to show *A. annua* and/or *A. afra* infusions were superior or at least equivalent to artesunate-amodiaquine (ASAQ) against malaria.

Methods
A double blind, randomized clinical trial with 957 malaria-infected patients had two treatment arms: 472 patients for ASAQ and 471 for *Artemisia* (248 *A. annua*, 223 *A. afra*) remained at end of the trial. ASAQ-treated patients were treated per manufacturer posology, and *Artemisia*-treated patients received 1L/day of dry leaf/twig infusions for 7 days; both arms had 28-day follow-up. Parasitemia and gametocytes were measured microscopically with results statistically compared among arms for age and gender.

Results
Artemisinin content of *A. afra* was negligible, but therapeutic responses of patients were similar to *A. annua*-treated patients; trophozoites cleared after 24h, but took up to 14 days to clear in ASAQ-treated patients. D28 cure rates defined as absence of parasitemia were for pediatrics 82, 91, and 50% for *A. afra*, *A. annua* and ASAQ; while for adults cure rates were 91, 100, and 30%, respectively. Fever clearance took 48h for ASAQ, but 24h for *Artemisia*. From D14-28 no *Artemisia*-treated patients had detectable gametocytes, while 10 ASAQ-treated patients remained gametocyte carriers at D28. More females than males were gametocyte carriers in the ASAQ arm but unaffected in the *Artemisia* arms. Hemoglobin remained constant at 11g/dL for *A. afra* after D1, while for *A. annua* and ASAQ it decreased to 9-9.5g/dL. Only 5.0% of *Artemisia*-treated patients reported adverse effects, vs. 42.8% for ASAQ.

Conclusion
A. annua and *A. afra* infusions are polytherapies with better outcomes than ASAQ against malaria. In contrast to ASAQ, both *Artemisias* appeared to break the cycle of malaria by eliminating gametocytes. This study merits further investigation for possible inclusion of *Artemisia* tea infusions as an alternative for fighting and eradicating malaria.

Keywords: ACT, ASAQ, artemisinin, malaria, clinical trial, tea infusion

Abbreviations

ACT, artemisinin combination therapy

ASAQ, artesunate-amodiaquine

DLA, dried leaf *Artemisia*

i.v., intravenous

PNLP, Programme National de Lutte contre le Paludisme

Background

The Democratic Republic of Congo (DRC) has one of the highest malaria rates. In 2016 >50,000 people died, mainly children (WHO, 2017 http://apps.who.int/iris/bitstream/10665/259492/1/9789241565523-eng.pdf). Maniema province is a holo-endemic zone with >80% of the population infected (Programme National de Lutte contre le Paludisme, 2015. Rapport Malaria. Kinshasa, RDC; PNLP). In 2005, Artemisinin Combination Therapy (ACT) became the first line treatment for all in the DRC.

Artemisia annua L. and *Artemisia afra* Jacq. ex Willd., of Chinese and South African origin, respectively, were used for centuries medicinally as infusions or powders to treat malaria (Willcox et al., 2004; Weathers et al., 2014; Liu et al., 2010). Both showed *in vitro* efficacy against *Plasmodium falciparum* (Kraft et al., 2003; Moyo et al., 2016; Gathirwa et al., 2007; Liu et al., 2010). For generations, *A. afra* was an integral part of the pharmacopeia in many regions of DRC and other areas of eastern and southern Africa (FAO Forestry paper 67: 68-73, Rome. http://www.fao.org/docrep/015/an797e/an797e00.pdf). In recent case studies, administration of dried *A. annua* leaf tablets successfully cured 18 patients with severe malaria who did not respond to therapy with ACT (artemether-lumefantrine) and i.v. artesunate (Daddy et al., 2017).

Medicinal plants represent the main, if not the only treatment accessible to much of humanity (WHO, 2014 http://www.who.int/medicines/publications/traditional/trm_strategy14_23/en/) and may offer a low cost and potentially more widely available alternative to ACTs. *A. annua* is grown in commercial plantations and by small stake-holder farmers in at least 39 countries, 33 of which are in Africa (Supplemental Material). For traditional medicine, WHO provided guidelines (WHO, 2000 http://apps.who.int/iris/bitstream/10665/66783/1/WHO_EDM_TRM_2000.1.pdf) and encouraged studies and clinical trials on herbal medicines (WHO, 2014). Subsequently, we launched a large-scale clinical trial aimed at confirming encouraging results from smaller-scale trials in Africa and South America with *A. annua* and *A. absinthium*. In those small trials cure rates of >95% were reported (Mueller et al., 2000; Gebeyaw et al., 2010; Chougouo et al., 2012; Zime-Diawara et al., 2015).

Methods

Plant material, handling and phytochemical analysis.

Field-grown leaves and twigs of *Artemisia annua* L. and *Artemisia afra* Jacq. ex Willd. (Asteraceae) were collected in France (PAR), Senegal (SEN), Burundi (BUR), and Luxembourg (LUX) and processed as described in Munyangi et al. (2018) where voucher ids also are listed. Artemisinin was measured using gas chromatography mass spectroscopy (GCMS) instead of HPLC to avoid artemisinin false positives (Smith et al. 2010). Extraction and assay methods for phytochemical compounds are detailed in Munyangi et al. (2018) where phytochemical contents of both *Artemisia* sp. are documented: *A. annua* had 1.34-1.70 mg artemisinin/g dry weight; *A. afra* had 0.036 mg artemisinin/g dry weight.

Study design

The study was done in 2015 in Kalima health district, Pangi zone, Maniema province, in five areas: Kakutya, Kinkungwa, Kamundala, Lubile, and Kakozwa (for location map see supplemental material of Munyangi et al. 2018). Patients with uncomplicated malaria were enrolled in the three arms of the trial (Figure 1) October to December 2015. The research protocol was registered and approved by the Comité d'Ethique de l'Ecole de Santé Publique de Kinshasa (MIN.RST/SG/180/001/2016) acting for the

DRC Ministry of Health. All adult patients provided written consent to participate in the trial; minor patients were signed by parents. The study followed guidelines of the Declaration of Helsinki and Tokyo for humans.

Criteria for inclusion of patients

Patients were recruited by passive case finding. Eligibility of patients was restricted to adults or children ≥5 yr with axillary temperature of ≥37.5°C and parasitemia of 2,000-200,000 trophozoites/µL. Patients with these symptoms were excluded: severe malaria, undernourishment, repetitive vomiting ± diarrhea, other concomitant infectious disease, known allergy to ACTs, pregnancy or breast feeding, cardiac, hepatic, or renal deficiencies. We also excluded patients treated with antimalarial drugs within 7d preceding the trial or with antibiotics that might have antimalarial effects. Patients were enrolled and distributed randomly into a treatment arm (Figure 1). Demographics of the study group are in Table 1. Drugs were distributed in numbered opaque envelopes selected randomly by each patient. Clinical symptoms of enrolled patients are shown in Table S1.

Clinical and laboratory analyses

According to WHO procedures, a rapid diagnostic test (RDT) confirmed malarial infection. Kalima health district is a sentinel surveillance zone for malaria, so SDBioline® Malaria Ag P.f./Pan test (batch nr 05FK60) was used on D0 for all patients. Thin and thick blood smears also were microscopically analyzed for trophozoites and gametocytes. Trophozoites were measured per µL of blood; gametocytes were noted as present or absent to determine number of carriers. In each trial area, slides were scrutinized by two independent laboratories. In case of dubious or conflicting results, a PNLP expert was consulted. A random 10% of slides collected at different stages of the trial were quality-control checked by a third party. Hemoglobin level, asexual parasites, and presence of gametocytes were measured on D0. Hemoglobin was measured spectrophotometrically at 540nm using Drabkin's Reagent at the peripheral health centers. Hemoglobin was also measured at the reference laboratory via hemoglobin analyzer; to settle value discrepancies, the reference lab value was used. After testing, patients were administered their first drug dose and a capillary blood sample was collected, dried on Whatman grade 3 filter paper, and stored for eventual genotyping. Patient follow-up including thick and thin blood smears was at days 1, 2, 3, 4, 7, 14, 21, and 28 post trial enrollment. From D0-D7, patients were treated in hospital to insure therapeutic compliance. Alanine (ALAT) and aspartate (ASAT) liver transaminases also were measured using the method of Reitman and Frankel (1957).

Drug administration

Artesunate-amodiaquine (ASAQ Winthrop, Sanofi-Aventis) was administered as tablets 25mg/67.5mg, 50mg/135mg, and 100mg/270mg. Manufacturer posology was: 4mg of artesunate and 10mg of amodiaquine/kg, once daily for 3 days, followed by ASAQ placebo tablets for the remaining 4 days. In the *Artemisia* arm, patients drank 0.33L of *A. annua* or *A. afra* infusion every 8h for 7d. Infusion preparation was: 5g of dried leaves and twigs of *A. annua* or *A. afra* to 1L of boiling water, infuse for 10min, and filter through sterilized 1mm mesh. Pediatrics and adults received the same amount of *Artemisia* tea infusion; there was no adjustment for body weight.

The trial was double blinded as detailed in Munyangi et al. (2018). Briefly, *A. annua* and *A. afra* arms received the infusion plus ASAQ placebo from D1-7. The ASAQ arm received ASAQ tablets on D1, 2, and

3 followed by ASAQ placebo on D4, 5, 6, and 7; these same patients also received *Artemisia* placebo from D1-7. ASAQ placebo consisted of pill-shaped saccharose/glucose tablets purchased in a pharmacy. To conceive a placebo for *Artemisia* infusion was more challenging. WHO suggested using the drug at very low dose, so *A. annua* and *A. afra* placebos contained 0.2g/L of plant material and patients drank placebo infusion of one *Artemisia* species or the other. All trial personnel were unaware of arm assignments.

All treatments were administered inside health facilities and under medical control. In case of vomiting 30min post-administration, the same drug was re-administered. For vomiting 60min post-administration, no new administration took place. In case of persistent vomiting, the patient was excluded from the trial and their infection separately treated. Adverse events were recorded and defined by clinical observations and patient self-reporting.

Concomitant treatment.

Medical treatment that did not violate trial exclusion criteria was maintained for patients having a known disease other than malaria at the beginning of the trial.

Management and statistical analysis of data

All data were initially recorded by hand in clinic notebooks, then transferred to an Excel file. Proportions were compared using chi-square test or Fisher exact test. Continuous and normally distributed data were compared by analysis of variance (ANOVA) and Student's t-Test. Other non-normally distributed data were compared by Mann-Whitney U-test. Survival analyses, fever clearance time, and the parasite clearance time used the Kaplan-Meier method with two-sided log-rank statistics. No significant differences were seen among the five sites, so the data were pooled. Data were analyzed using SPSS for Windows version 1.0.0.903 and R for Windows version 3.4.2. P-values <0.05 were considered statistically significant.

Results and Discussion

This trial was conducted as a superiority trial. Where equivalence was noted, it was so stated. The primary outcome of the trial was that, based on microscopic analyses, both *Artemisia* sp. cured malaria faster and more effectively than ASAQ. The secondary outcomes were that compared to ASAQ, *Artemisia*-treated patients also had microscopically undetectable gametocytes and fewer adverse side effects.

Clinical Admission Characteristics: There were 943 patients who completed the trial with 258 pediatrics in each of the ASAQ and *A. annua* arms (Table 1). The median age was 29 yrs in the ASAQ group, 19 yrs in the *A. annua* group and 25 yrs in the *A. afra* group (Table 2); ages within each group ranged from 6-50 yr. Of these, 67.7% were male, 32.3% were female; 41.1% were pediatrics and 58.9% adults in the *A. annua* group. In the *A. afra* group, 70.9% were male, 29.1% were female; 22.9% were pediatrics and 77.1% adults. In the ASAQ group, 65.3% were males, 34.7% were female; 22.3% were pediatrics and 77.7% adults. Of the five study sites, three included all three treatment arms, one had *A. annua* and ASAQ; the other had *A. afra* and ASAQ (Table 2). At admission parasitemia was > 33,000 trophozoites/µL for all treatment arms.

Fever and parasitemia progression: Cures rates were established by D28 parasitemia. Compared to ASAQ-treated patients with an overall 34.3% cure by D28, *A. afra* and *A. annua* -treated patients were 88.8 and 96.4% cured, respectively (Table 3). Cures for ASAQ-treated patients were 49.5% for pediatrics (ages 5-15 yr) and 30.0% for adults (ages 16-65) (Table 3). Pediatric cures for *A. annua* and *A. afra*-treated patients were 91.2 and 82.4%, respectively, while for adults, cures were 100 and 90.7%, respectively (Table 3). There were 344 patients with parasites at D28; 9 for *A. annua*, 25 for *A. afra,* and 310 for ASAQ. Due to degradation of stored blood samples, we were unable to conduct a valid PCR analysis of the D28 patients with parasitemia. Considering the large number of D28 patients with parasitemia, a thorough PCR analysis is needed in future studies.

Fever clearance took 24h in the *Artemisia* arm and 48h for ASAQ (Figure 2). Zime-Diawara et al. (2015) noticed total apyrexia even faster, only 12h post-first *Artemisia* infusion. Using the long-rank test, p values were all significantly different among all four measured groups: both *Artemisias* vs. ASAQ; *A. annua* vs. *A. afra*; *A. annua* vs. ASAQ, *A. afra* vs. ASAQ.

On D1, parasitemia decreased 97.7% in the *Artemisia* arm and 85.2% for ASAQ. In both *Artemisia* arms, parasitemia totally cleared on D2 (Figure 3). In the ASAQ arm, however, parasites remained in some patients until D14. By the log-rank test at D28, three of the four compared groups were significantly different, but there was no significant difference between *A. annua* and *A. afra* (p=0.505). These results are similar to Zime-Diawara et al. (2015) with 108 patients receiving 1L/d of 12g/L *A. annua* infusion. Parasitemia disappeared in all patients after 36h, with excellent clinical tolerance. In a similar 48-patient study, Mueller et al. (2004) observed trophozoite disappearance by D4 for 92% of patients. Our *Artemisia*-treated patients were 96.4% cured on D28; only 5% of patients showed side effects, which was significantly better than ASAQ-treated patients with 42.8% adverse side effects (Table 4).

Patients treated with ASAQs received weight-adjusted amounts of artesunate ranging from 147.2 to 1,293 mg *in toto*. Patients treated with *A. afra* received a maximum amount of artemisinin of 1.26mg, while those treated with *A. annua* received 46.9mg (BUR) or 59.5mg (LUX) *in toto*; there was no weight adjustment. This was 3-1,000 fold less artemisinin vs. ASAQs, yet *Artemisia* therapeutic outcome was better. This was consistent with recent case studies (Daddy et al., 2017) demonstrating efficacy of *A. annua* compressed leaf tablets (DLA) in treating patients unresponsive to either ASAQ or i.v. artesunate. The treatment course of DLA used in that study provided 55mg maximum artemisinin to adults; for pediatrics, it was as low as 13.75mg. These results collectively suggest other phytochemicals in the plants were crucial to the potent therapeutic outcome of the *Artemisias*.

Gametocyte elimination: D0 gametocyte carriers present in ASAQ-treated patients declined beginning on D7 post-treatment; by D28 only 10 of 472 patients were carriers (Figure 4). In contrast, no gametocyte carriers were detectable in either *Artemisia* treatment from D14 onwards suggesting that both *Artemisias*, but not ASAQ, eliminated gametocytes. Gametocyte carriage was completely eliminated in all age groups treated with *Artemisia* (Table 5). In ASAQ-treated patients, both pediatric and adult patients still had microscopically detectable gametocytes at D28 (Table 5). Microscopic analysis underestimates gametocyte counts by several fold (Babiker and Schneider 2008), so future studies should use nested PCR to further probe for the presence of gametocytes in *Artemisia*-treated patients.

Gender differences occurred in the ASAQ group. There were 326 males in the *Artemisia* arm and 308 in the ASAQ arm, and 145 females in the *Artemisia* arm and 164 in the ASAQ arm. While both genders

responded equally well to *Artemisia*, there was significantly more gametocyte carriage in females than males in the ASAQ-treated arm for D14-28 (Fisher's Exact test, p=0.012; data not shown). There was no differential age response in females. *Artemisia*-treated male carriers were significantly different at D2-4 but not at D7-28. Overall, these results indicated that *Artemisias* were better than ASAQ at removing gametocytes in females.

Hemoglobin improvement by A. afra, but not ASAQ or A. annua: While hemoglobin at D0, 1, 3, and 4 between *A. annua* and ASAQ was not statistically different (post hoc test, p>0.05), between *A. annua* and *A. afra* there was a significant difference (Figure 5). Hemoglobin remained significantly greater at about 11g/dL post treatment for *A. afra* vs. ~9 and 9.5g/dL for ASAQ and *A. annua*, respectively. This similarity between ASAQ and *A. annua* is likely the result of artemisinin, known to reduce hemoglobin during treatment (Kurth et al., 2016). *A. afra* has but a trace of artemisinin (see Table 1 in Munyangi et al. (2018) for artemisinin content of each Artemisia sp. treatment) and thus, as expected, there was no decline in hemoglobin.

ALAT and ASAT were not altered: Although liver transaminases are often altered in malaria patients (Woodford et al., 2018), there were no significant differences observed in ALAT and ASAT among the three treatment arms (see supplemental Figure S1).

Other studies using Artemisia tea infusions: In China the traditional treatment against malaria is a daily dose from 4.5 to 9g of dry leaves and twigs of *A. annua* infused in boiling water. In 1972, the Institute for Traditional Medicine of Beijing treated 31 patients with an ether extract; 20 had *P. vivax* infection, 9 had *P. falciparum,* and 2 patients had both. Fever cleared in 36h for the *P. falciparum*-infected patients (Zhang 2005; Cui and Su 2009). In another instance, Chang and But (1986) administered 72g of *A. annua* in an alcoholic extract over 3 days on 485 patients infected with *P. falciparum,* and on 105 patients with *P. vivax*. The cure rate was 100 % against both malaria species. In 2000, Mueller et al. (2000) treated 53 DRC patients infected with *P. falciparum* using two different treatments: infusion and decoction. The posology was 5g of dried leaves in 1L over 5d. The average cure rate was 92 % after 4 days of treatment. In Ethiopia, Gebeyaw et al. (2010) treated 73 patients suffering from uncomplicated malaria with 1L of *A. annua* dried leaf infusion using 5g/L per day over 7d; 95.5% of patients were cured without adverse effects. Chougouo-Kengne et al. (2012) compared *A. annua* infusion with artesunate alone or combined with amodiaquine on 73 patients suffering from uncomplicated falciparum malaria. The posology was 5g of dried leaf infusion in boiling water, over 5 or 7d. The 7d treatment improved the results from 71.3% to 100%, without any side effects. Overall, 7d of treatment with 5g/L *Artemisia annua* infusion seemed to provide the best therapeutic outcome.

Synergistic effects: In prior tea infusion studies, there was efficacy of the *A. annua* infusion against uncomplicated malaria without significant side effects. This is in line with our results showing a cure rate of 99.5% on D28 for *Artemisia* with only 5% side effects. Previously, artemisinin was considered the only active molecule, so prior studies tried to reach high levels in their preparations. Artemisinin is only present in traces in some *Artemisia* varieties and usually absent in *A. afra.* This of course raises the question of the role of artemisinin in these infusions used to treat malaria. *A. annua* and *A. afra* contain hundreds of phytochemical molecules and minerals. Antimalarial activity has been demonstrated for some 20 of these as summarized in the review by Weathers et al. (2014). It is important to note in that *A. afra* has only trace amounts of artemisinin and no detectable artemisinic parent molecules (Munyangi

et al., 2018), yet all patients treated with that species had the same therapeutic response as those treated with *A. annua*.

Artemisinin drug resistance: Despite the fact that *A. annua* shows high efficacy without side effects, there has been reluctance in endorsing use of the plant. Low artemisinin content of the plant elicited concerns that this might induce artemisinin drug resistance (Blanke et al., 2008; Mueller et al., 2004; WHO, 2012 http://www.who.int/malaria/position_statement_herbal_remedy_artemisia_annua_l.pdf). Since then rodent studies using *per os* dried leaf *Artemisia* (DLA), Elfawal et al. (2015) demonstrated that compared to pure artemisinin, DLA was threefold more resilient against evolution of artemisinin drug resistance. Furthermore, DLA even eliminated artemisinin-resistant parasites in a *P. yoelli* rodent model (Elfawal et al., 2015). In humans unresponsive to either ASAQ or i.v. artesunate, *A. annua* (DLA) eliminated parasites within a few days even in comatose pediatrics (Daddy et al., 2017). Studies showed that the numerous phytochemicals in *A. annua* and *A. afra* provide synergies and multiple interactions, likely constituting a polytherapy against malaria (Elford et al., 1987; Li et al., 2018; Liu et al., 1992; Suberu et al., 2013). Together, those data and this study indicate that artemisinin is not the sole phytochemical demonstrating antimalarial efficacy by *Artemisia* sp. Moreover, the presence of multiple phytochemicals in *Artemisia* sp. acts not as a mono or dual therapy, but as a poly-combination-therapy.

In the present study, we saw a large number of ASAQ failures, yet in the same localities, *A. afra* and especially *A. annua* were highly effective. If those failures are ASAQ resistance, then *Artemisia* tea infusions cured those patients. As we and others observe mounting failures of ACTs in Africa, *Artemisia annua* in particular could provide an effective and rapid alternative for holding the parasite at bay. For how long? No one knows, but lives are at risk and this is an inexpensive, safe and effective therapeutic that could be implemented rather quickly.

Conclusions

Treating uncomplicated malaria with either *A. annua* or *A. afra* was superior to the artesunate-amodiaquine ASAQ treatment. Fever and parasitemia clearances were faster and more efficient with both *Artemisia* species than with ASAQ; adverse effects were negligible. At D14-28 gametocyte carriage was undetectable in *Artemisia*-treated patients, so transmission to the mosquito should be interrupted. *Artemisia* is a polytherapy with at least 10 active molecules likely acting in synergy, so resistance is therefore unlikely to emerge. Plants can be grown and used by local populations, which could help counteract the problems caused by fake or obsolete antimalarial drugs. Despite these exciting results, however, it is important that further large-scale studies be launched to optimize use of these plants and posology for children, pregnant women and others, in the case of uncomplicated as well as severe malaria.

Acknowledgements

Authors appreciate Impala Avenir Foundation, Mme Hélène de Cossé Brissac and Mr. Jean-Louis Bouchard for generously funding this study. Matthew Desrosiers, Danielle Snider and Dr. Bruno Giroux provided valuable feedback. We are also grateful for Award Number NIH-2R15AT008277-02 from the National Center for Complementary and Integrative Health that enabled PJW and MJT to provide

phytochemical analyses of the plant material used in treating these patients. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Complementary and Integrative Health or the National Institutes of Health.

Competing interests.

We confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this study that could have influenced its outcome.

References.

1. Blanke C.H., Naisabha G.B., Balemea M.B., Mbaruku G.M., Heide L., Müller M.S., 2008. Herba *Artemisiae annuae* tea preparation compared to sulfadoxinepyrimethamine in the treatment of uncomplicated falciparum malaria in adults: a randomized double-blind clinical trial. Tropical Doctor 38:113-116. doi:10.1258/td.2007.060184.
2. Babiker H.A., Schneider P., 2008. Application of molecular methods for monitoring transmission stages of malaria parasites. Biomed Mater 3(3):034007.
3. Chang H.M., But P.P.H., 1986. Pharmacology and Applications of Chinese Materia Media, Volume 1, World Scientific Publishing, Singapore.
4. Chougouo-Kengne R.D., Kouamouo J., Mouyou-Somo R., Penge On' Okoko A., 2012. Etude comparative de l'efficacité thérapeutique de l'Artesunate seul ou en association avec l'amodiaquine et de la tisane de *Artemisia annua* cultive à l'ouest du Cameroun. Annales de Pharmacie de l'Université de Kinshasa (RDC) 4(1):129-150.
5. Cui L., Su X.Z., 2009. Discovery, mechanisms of action and combination therapy of Artemisinin, Expert. Rev. Anti. Infect. Ther. Oct. 7(8):999-1013. doi:10.1586/eri.09.68.
6. Daddy B.N., Kalisya M.L., Bagire G.P., Watt R.L., Towler M.J., Weathers P.J., 2017. *Artemisia annua* dried leaf tablets treated malaria resistant to ACT and i.v. artesunate: Case reports, Phytomedicine 32:37-40. doi:10.1016/j.phymed.2017.04.006.
7. Elfawal M.A., Towler M.J., Reich N.G., Weathers P.J., Rich S.M., 2015. Dried whole plant *Artemisia annua* slows evolution of malaria drug resistance and overcomes resistance to artemisinin. PNAS USA 112:821-826, doi: 10.1073/pnas.1413127112.
8. Elford B.C., Roberts M.F., Phillipson D., Wilson R.J.M., 1987. Potentiation of the antimalarial activity of qinghaosu by methoxylated flavones. T Roy Soc Trop Med H 81:434-436.
9. Gathirwa J.W., Rukunga G.M., Njagi E.N.M., Omar S.A., Guantai A.N., Muthaura, C.N., Mwitari P.G., Kimani, C.W., Kirira, P.G., Tolo F.M., Ndunda T.N., Ndiege I.O., 2007. In vitro anti-plasmodial and in vivo anti-malarial activity of some plants traditionally used for the treatment of malaria by the Meru community in Kenya. J. Nat. Med. 61:261-268. doi:10.1007/s11418-007-0140-0.
10. Gebeyaw T., Kebede Y., Yigzaw T., 2010. Use of the plant *Artemisia annua* as a natural anti-malarial herb in Arbaminch town. Ethiop. J. Health Biomed Sci. 2:75-82.
11. Kraft C., Jenett-Siems K., Siems K., 2003. In vitro antiplasmodial evaluation of medicinal plants from Zimbabwe. Phytother. Res., 70:123-128.
12. Kurth F., Lingscheid T., Steiner F., Stegemann M.S., Bélard S., Menner N., Pongratz P., Kim J., von Bernuth H., Mayer B., Damm G., Seehofer D., Salama A., Suttorp N., Zoller T., 2016. Hemolysis after oral artemisinin combination therapy for uncomplicated *Plasmodium falciparum* malaria. Emerg. Infect. Dis. 22:1381-1386. doi:10.1016/j.ijid.2014.09.007.
13. Liu K.C., Yang S.L., Roberts M.F., Elford B.C., Phillipson J.D., 1992. Antimalarial activity *of Artemisia annua* flavonoids from whole plants and cell cultures. Plant Cell Rep. 11:637-640. doi:10.1007/BF00236389.

14. Li J., Zhang C., Gong M., Wang M., 2018. Combination of artemisinin-based natural compounds from *Artemisia annua* L. for the treatment of malaria: Pharmacodynamic and pharmacokinetic studies. Phytotherapy Research. 2018;1–6.
15. Liu N.Q., Cao M., Frédérich M., Choi Y.H., Verpoorte R., Van der Kooy F., 2010. Metabolomic investigation of the ethnopharmacological use of *Artemisia afra* with NMR spectroscopy and multivariate data analysis. J.Ethnopharmacol. 128:230-235. doi:10.1016/j.jep.2010.01.020.
16. Moyo P., Botha M.E., Nondaba S., Niemand J., Maharaj V.J., Eloff J.N., Louw A.I., Birkholtz L., 2016. In vitro inhibition of *Plasmodium falciparum* early and late stage gametocyte viability by extracts from eight traditionally used South African plant species. J. Ethnopharmacol. 185:235-42.
17. Mueller M.S., Karhagomba I.B., Hirt H.M., Wemakor E., 2000. The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: agricultural, chemical and clinical aspects. J. Ethnopharmacol. 73:487-493. doi:10.1016/j.jep.2016.03.036.
18. Mueller M.S., Runyambo N., Wagner I., Borrmann S., Dietz K., Heide L. 2004. Randomized controlled trial of a traditional preparation of *Artemisia annua L.* (Annual Wormwood) in the treatment of malaria. Trans. R. Soc. Trop. Med. Hyg. 98:318-21.
19. Munyangi J., Cornet-Vernet L., Idumbo M., Lu C., Lutgen P., Perronne C., Ngombe N., Bianga J., Mupenda B., Lalukala P., Mergeai G., Mumba D., Towler M., Weathers P., 2018. Effect of *Artemisia annua* and *Artemisia afra* tea infusions on schistosomiasis in a large clinical trial., Phytomedicine doi: https://doi.org/10.1016/j.phymed.2018.10.014
20. Smith L.M.J., Bentley S., Jones H., Burns C., Aroo R.R.J., Woolley J.G., 2010. Developing an alternative UK industrial crop *Artemisia annua*, for the extraction of artemisinin to treat multi-drug resistant malaria. Aspects of Applied Biology 101:99-106.
21. Suberu J.O., Gorka A.P., Jacobs L., Roepe P.D., Sullivan N., Barker G.C., Lapkin A.A., 2013. Anti-plasmodial polyvalent interactions in *Artemisia annua* L. aqueous extract--possible synergistic and resistance mechanisms. PLoS One 8, e80790 doi: 10.1371/journal.pone.0080790.
22. Weathers P.J., Reed K., Hassanali A., Lutgen P., Engeu P.O., 2014. Chapter 4: Whole plant approaches to therapeutic use of *Artemisia annua L.* (Asteraceae). IN: *Artemisia annua*. - Pharmacology and Biotechnology. Eds., T Aftab, JFS Ferreira, MMA Khan, M Naeem Springer, Heidelberg, GDR pp. 51-74.
23. Willcox, M., Bodeker, G., Bourdy, G., Dhingra, V., Falquet, J., Ferreira, J.F.S., Graz, B., Hirt, H.-M., Hsu, E., de Magalhães, P.M., Provendier, D., Wright, C.W., 2004. *Artemisia annua* as a traditional herbal antimalarial. p. 43-59. In: Willcox, M.L., Bodeker, G., Rasoanaivo, P. (Eds), Traditional Medicinal Plants and Malaria. CRC Press, Boca Raton, FL.
24. Woodford J., Shanks G.D., Griffin P., Chalon S., McCarthy J.S., 2018. The Dynamics of Liver Function Test Abnormalities after Malaria Infection: A Retrospective Observational Study. Am J Trop Med Hyg. 98:1113–1119.
25. Zhang, J.F., 2005. A detailed chronological record of Project 523 and the discovery and development of qinghaosu (artemisinin). Vol. 193. Yangcheng Evening News Publishing Company, China.
26. Zime-Diawara, H., Sissinto-Savi de Tove, Y., Okogbeto, O.E., Ogouyemi-Hounto,A., Gbaguidi, F.A., Kinde-Gazard, D., Massougbodji, A., Bigot, A., Sinsin, B., Quetin-Leclerc, J., Evrard, B., Moudachirou, M., 2015. Etude de l'efficacité et de la tolérance d'une tisane à base d'*Artemisia annua L.* (Asteraceae) cultivée au Bénin pour la prise en charge du paludisme simple. Int. J. Biol. Chem. Sci. 9(2):692-702.

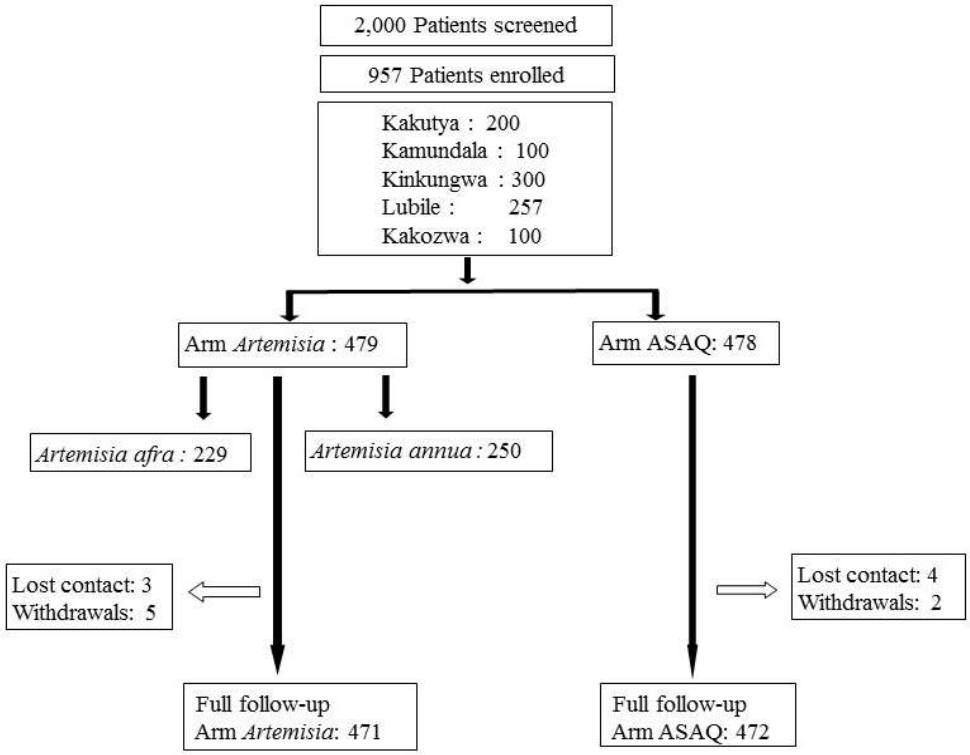

Figure 1. Trial design.

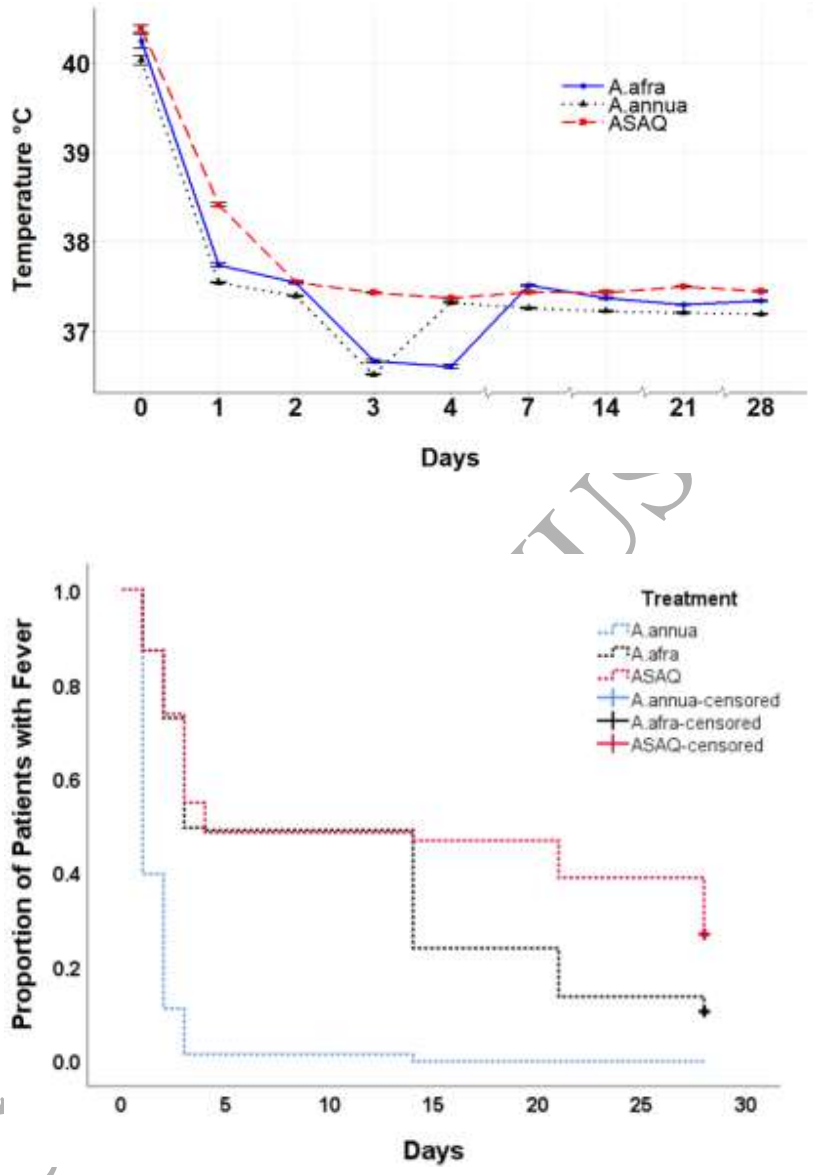

Figure 2. Average fever progression among the three treatment arms. Top, graphical representation. Bottom, Kaplan–Meier; survival time of fever started when a patient was included in the study (Day 0). Patients were followed until Day 28. For patients withdrawing from the study before Day 28 or still having fever until Day 28, survival time is said to be censored. The survival probability was calculated using the Kaplan–Meier method. The censored rates of *A. annua*, *A. afra,* and ASAQ are 0.0%, 10.3%, and 26.7%, respectively, with *p*-value of log-rank still near to zero and statistically significant.

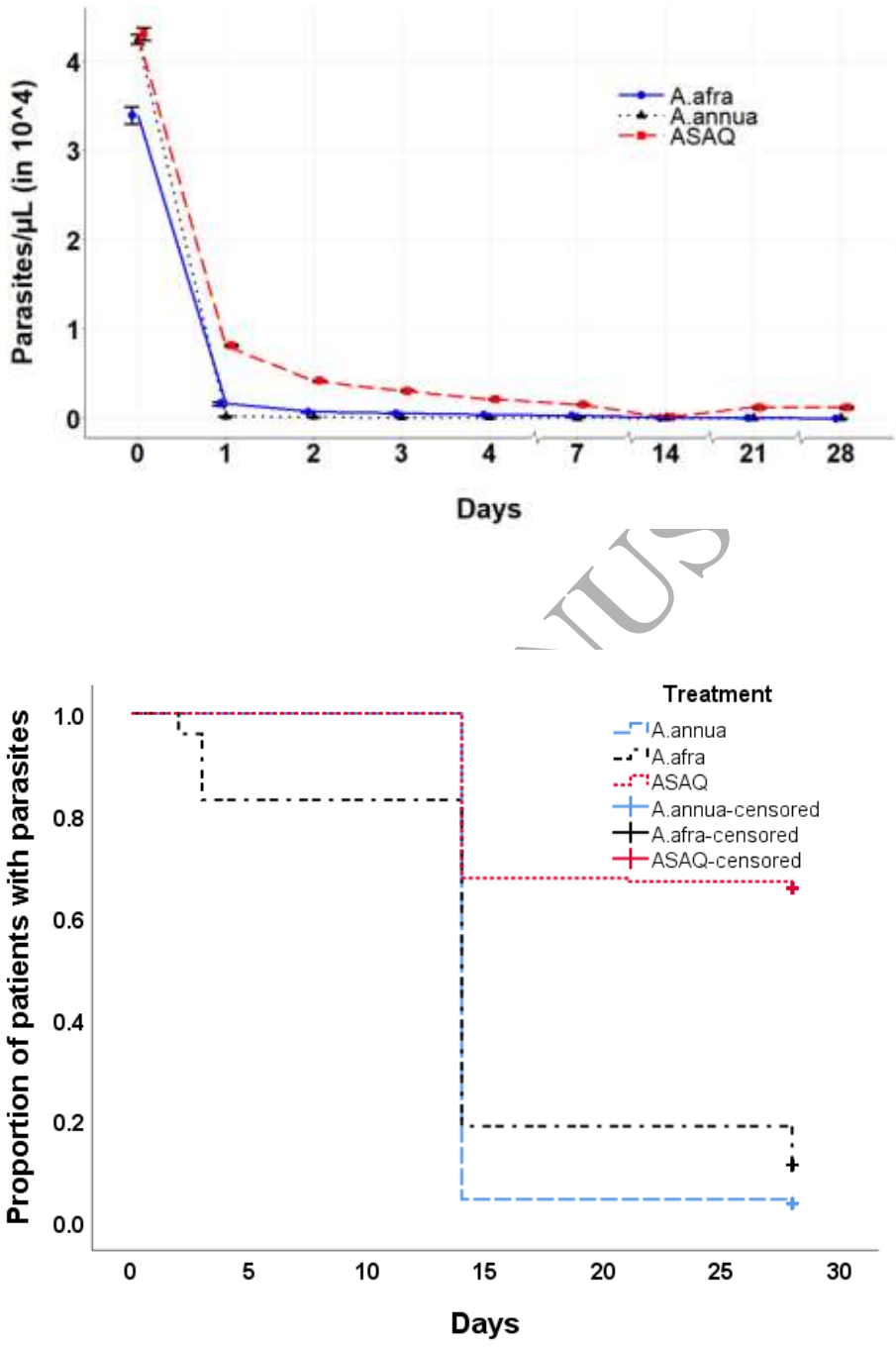

Figure 3. Average parasitemia progression among the three treatment arms. Top, graphical representation. Bottom, Kaplan–Meier; survival time of parasites started when a patient was included in the study (Day 0). Patients were followed until Day 28. For patients withdrawing from the study before Day 28 or still having parasites until Day 28, survival time is said to be censored. The censored survival rates of *A. annua*, *A. afra,* and ASAQ are 3.6%, 11.2%, and 65.7%, respectively.

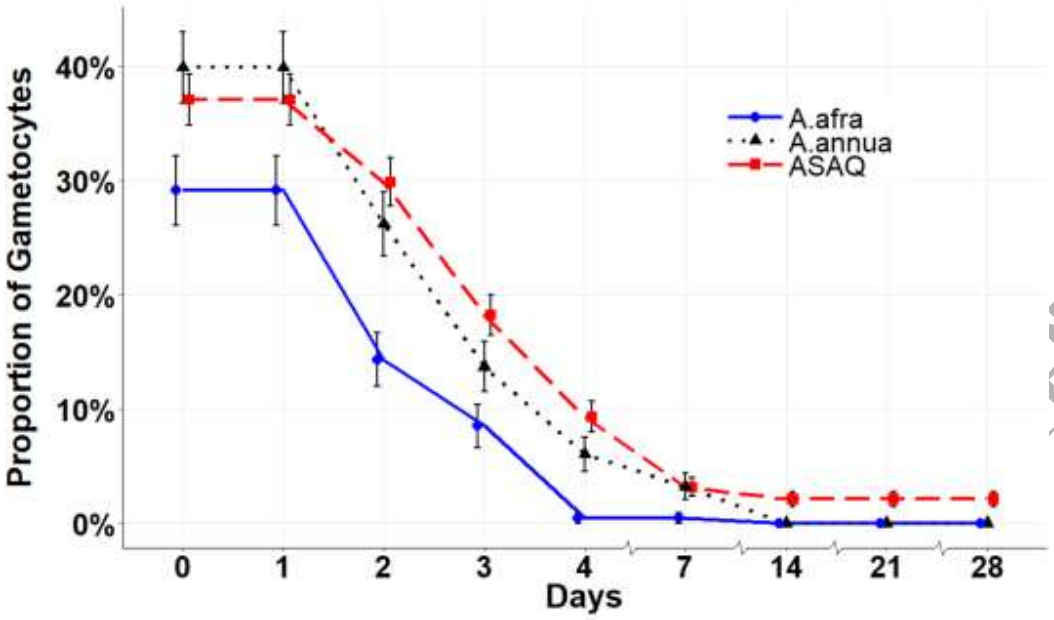

Figure 4. Microscopically determined proportion of patients with gametocytes (carriers) throughout the trial period.

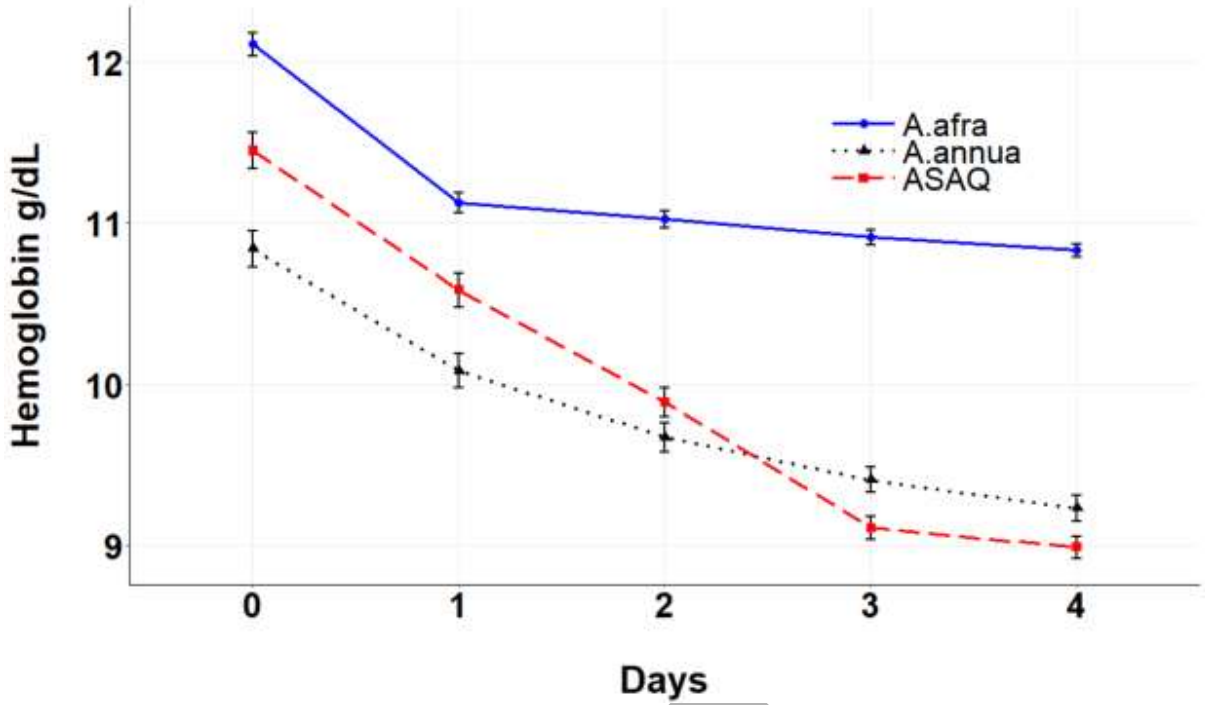

Figure 5. Hemoglobin levels during the first four days of treatment.

Table 1: Demographics of the *Artemisia* and ASAQ trial arms at time of enrollment (D0).					
Treatment arm	**Gender**		**Age**		
	Male (%)	**Female (%)**	**≤ 5 yrs**	**6-15 yrs (%)**	**> 15 yrs (%)**
A. annua	168 (67.7%)	80 (32.3%)	0	102 (41.1%)	146 (58.9%)
A. afra	158 (70.9%)	65 (29.1%)	0	51 (22.9%)	172 (77.1%)
ASAQ	308 (65.3%)	164 (34.7%)	0	105 (22.3%)	367 (77.7%)

Table 2. Average and median patient age and parasite levels at the five study sites at D0 and D28.						
Arm	**Median (yr)**	**Site average patient age (yr)**				
		Kakutya	**Kinkungwa**	**Kamundala**	**Lubile**	**Kakozwa**
A. annua	19	22	25	24	nm	20
A. afra	25	28	27.5	nm	26.5	13.5
ASAQ	29	33	29.5	32	23	21
Overall	26	26	28.5	28	25	19.5
Median		**Parasites D0 Mean/site (trophozoites/µL)**				

A. annua	42,426	40,632	36,120	≥ 50,000[1]	nm	≥ 50,000[1]
A. afra	33,911	39,860	39,150	nm	29,617	≥ 50,000[1]
ASAQ	43,018	38,702	42,106	≥ 50,000[1]	39,915	≥ 50,000[1]
% Parasites D28 Mean/site (trophozoites/µL)						
A. annua	na	91% = 0 9% ≤10	100% = 0	100% = 0	nm	100% = 0
A. afra	na	100% = 0	100% ≤10[2]	nm	100% = 0	92% = 0 8% ≤10
ASAQ[2]	na	93% = 0 7% ≤10	6% = 0 84% ≤10 10% ≥10,000	100% ≤10	100% = 0	38% ≤10 62% ≥10,000

Not all test sites included both *Artemisias*; na, not applicable; nm, not measured at that site.

[1] Parasites were not enumerated beyond 50,000.

[2] There were only 7 *A. afra* patients in this arm at this site.

Table 3. Cure rates by age group within each trial arm at Days 14 and 28.

Age (year)	Day 14			Day 28		
	A. annua **n/N[1] (%)[2]**	***A. afra*** **n/N (%)**	**ASAQ n/N (%)**	***A. annua*** **n/N (%)**	***A.*** **afra n/N (%)**	**ASAQ n/N (%)**
5-15	93/102 (91.2%)	36/51 (70.6%)	51/105 (48.6%)	93/102 (91.2%)	42/51 (82.4%)	52/105 (49.5%)
16-65	146/146 (100.0%)	145/172 (84.3%)	110/367 (30.0%)	146/146 (100.0%)	156/172 (90.7%)	110/367 (30.0%)
Overall	239/248 (96.4%)	181/223 (81.2%)	161/472 (34.1%)	239/248 (96.4%)	198/223 (88.8%)	162/472 (34.3%)

[1] N, total number within age group less any who left the trial; n, number with 0 parasitemia.
[2] (%), n/N x 100.

Table 4: Distribution among patients of adverse effects from treatment.

Observed adverse effects	Number of subjects in the *Artemisia* arms	Number of subjects in the ASAQ arm
Abdominal pain	0	25
Asthenia	0	30
Diarrhea	0	5
Drowsiness	0	3
Fatty cough	0	1
Hypoglycemia	0	20
Insomnia	0	10
Nausea	10	30
Pruritis	0	35
Vertigo	0	1
Vomiting	15	50
TOTAL	**25**	**210**
% OF TOTAL	**5.0%**	**42.8%**

Table 5. Level of microscopically determined gametocyte carriage decrease D14-28 within age groups.

Age (yrs)	***A. annua* n/N (%)**	***A. afra* n/N (%)**	**ASAQ n/N (%)**
Children (5-15)	43/43 (100%)	102/102 (100%)	111/114 (97.4%)
Adults (16-65)	205/205 (100%)	147/147 (100%)	369/376 (98.1%)
Total	248/248 (100%)	249/249 (100%)	480/490 (98.0%)
N, total within age group; n, number of patients with microscopically undetectable gametocytes.			

Graph abstract tea trial

Graphical Abstract