UCLouvain
Institut de recherche expérimentale et clinique

Pôle de recherche EPID

Epidémiologie et biostatistique

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PROOF-OF-CONCEPT PHASE II TRIAL PROTOCOL

A Phase 2, Randomized, Controlled Study to evaluate the Activity level, optimal dosage, safety, and accepted formulation of *Artemisia afra* tea infusions in eliminating plasmodium reservoirs in malaria endemic areas of Cameroon and Rwanda.

Sponsored by :

Pr Annie Robert, Université catholique de Louvain

Pr Jacob Souopgui, Université Libre de Bruxelles

Pr Stephen Ghogomu, University of Buea

Pr Michel Frederick, Université de Liège

Pr Jean-Claude Twizere, Université de Liège

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Amendment: --
Investigational Product: *Artemesia afra* tea infusions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

A. afra	Artemisia afra
ACT	Artemisinin Combination Therapies
AE	Adverse event
ASE	Adverse side effects
CDC	Centers for Disease Control
CI	Confidence interval
CRF	Case report form
Ct	Cycle threshold
D0	Day 0
DAIDS	Division of AIDS (DAIDS)
DHS	Demographic health surveys
DSMB	Data Safety Monitoring Board
EC	Ethics committe
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
GSK	GlaxoSmithKline
ICF	Informed consent form
IEC	Independent ethicc committee
IRB	Institutional review board
IRS	Indoor residual spraying
ITNs	Insecticide treated nets
LAMP	Loop-mediated Isothermal Amplification
MDA	Mass drug administration
MedDRA	Medical Dictionary for Regulatory Activities
MIS	Malaria Indicator Survey
Neg	Ngative
PI	Principal Investigator
Pos	Positive
PT	Preferred term
RDT	Random diagnostic test
Rt	PCT Real time Polymerase Chain reaction
SAE	Serious adverse event
SOP	Standard Operating Procedure
TG	Treatment group
UCLouvain	Université catholique de Louvain
ULB	Université Libre de Bruxelles
UR	University of Rwanda
WHO	World Health Organization

PROTOCOL SIGNATURE PAGE

Pr Annie Robert, Pr Jacob Souopgui, Pr Stephen Ghogomu, Pr Michel Frederich, and Pr Jean-Claude Twizere, the study Sponsors, will keep a list of Investigator(s), who must provide a *Curriculum Vitae* (CV) and a copy of their medical licenses to the Sponsors or Sponsor representatives. The Sponsors will keep a list and qualification records of all relevant Sponsor study personnel.

The appointed monitoring team will conduct site monitoring and provide medical monitoring, data management, statistical analysis, quality assurance, safety, and pharmacovigilance services.

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"I have read the foregoing protocol and agree to conduct the study as outlined herein."

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"I have reviewed the foregoing protocol and agree to ensure the conduct of study as outlined

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Table 1 : PARTICIPATING INSTITUTIONS	
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Table 2: SYNOPSIS

Title	**Activity level, optimal dosage, safety, and accepted formulation of *Artemisia afra* tea infusions in eliminating plasmodium reservoirs in malaria endemic areas of Cameroon and Rwanda**

Phase of development	Proof -of- concept Phase 2 trial.
Name of study treatment	*Artemisia afra* tea infusions
Indication	Elimination of Plasmodium parasitemia in asymptomatic human reservoirs.
Protocol number	
Study rationale	The WHO African Region carries a disproportionately high share (94%) of the global malaria burden. Repeated calls from WHO have advocated for mobilization and expanded actions towards malaria elimination in the continent, including recommended malaria control strategies which are currently implemented in most African countries. However, there is threatened effectiveness of these intervention strategies, coupled with empirical and theoretical evidence that these strategies alone will not be sufficient to eliminate malaria from many endemic regions in sub-Saharan Africa. Thereby suggesting that current interventions be supplemented by additional strategies, for effective elimination in most sub-Saharan Africa countries. An emergence of resistances for both insecticide and antimalarial drugs like ACTs has been reported in several African countries. In addition, the currently implemented strategies are restricted to indoor controls and populations remain exposed to Plasmodium transmission during their outdoor activities. The strategies also fail to target asymptomatic Plasmodium carriers who constitutes, most of the infected people in endemic regions and perpetuate the disease transmission over time. Hence, the need to accurately identify and treat asymptomatic infectious human reservoirs. Recent investigations that *Artemisia afra* tea infusions can effectively treat malaria in endemic communities, with gametocidal effects and no reported toxicity suggest its probable usefulness in the elimination of plasmodium reservoirs in endemic settings. However, no clinical trials have not been conducted to ascertain the activity level and optimal dosage of *Artemisia afra* tea in the treatment of asymptomatic reservoirs.
Hypothesis	An optimal dosage of artemisia afra tea infusions is effective, safe, and well tolerated in eliminating plasmodium reservoirs.
General objective	To find the activity level and accepted optimal dose concentration, dose frequency, length of treatment, and formulation (flavor) of *A.afra* in treating Plasmodium reservoirs.
Specific objectives	• To assess participants' response (treatment efficacy) to varying dose concentrations, dose frequency, length of treatment, and formulation of *Artemisia afra* treatment. • To identify the most acceptable dose concentration, dose frequency, length of treatment, and formulation (flavor) of Artemisia treatment. • To assess the safety (adverse side effects) of *Artemisia afra* treatment in participants by dose concentration, dose frequency, length of treatment, and formulation. As a commonly used tea, no toxicity is expected. • To assess variability of plasmodium test predictive values between mRDT, rtPCR, and direct blood smear observation in asymptomatic persons.
Outcomes	<u>Major outcome</u> • Decrease in parasite load over time <u>Secondary outcomes</u> • Decrease in gametocyte load in carriers with Artemisia afra tea by dose concentration, dose frequency, length of treatment, and formulation.

	• Acceptability (adherence and completion) of *Artemisia afra* treatment by dose concentration, dose frequency, length of treatment, and formulation (flavor). • Safety (ASEs) of *Artemisia afra* treatment in participants by dose concentration, dose frequency length of treatment, and treatment formulation. • Likelihood and variability in the rate of reservoir elimination with treatment, compliance/ acceptability, and occurrence of side effects /adverse events in different treatment arms.
Study design and methods	Design: The study is a Phase 2 randomized controlled, partial-blind, parallel group study in plasmodium infected *asymptomatic* adults 18 years and older, with 8 study arms. It will use the adapted Zelen design, which has two steps in the consent process. In the first step, there is an informed consent from all participants for a cohort lifestyle study. According to this consent, participants are randomized without knowledge about the detailed protocol. In the second step, only participants from the intervention group will receive the information about the intervention and the second consent will be obtained from them. The participants who will decline to participate to an intervention will continue in the cohort study, as the control group. Duration of study: The study duration is 35 days (5 weeks) for each participant. Treatment groups (TG): − **TG 1**: Group of 8 persons receiving *A. afra* infusions of 5g/1liter of water, 3drinks a day, daily for 1week (7 days) (current recommendation). − **TG 2:** Group of 8 persons receiving *A. afra* infusions of 5g/500ml of water, 2drinks a day, daily for 1week (7 days) (increase concentration and decrease dose frequency). − **TG 3:** Group of 8 persons receiving *A. afra* infusions,5g/1liter of water, 3drinkss a day, weekly for 4weeks (decrease dose frequency and increase length of treatment). − **TG 4**: Group of 8 persons receiving flavored *A. afra* infusions, 5g/1liter of water, 3drinks a day, daily for1week (7 days) (current treatment with improved taste). − **TG 5:** Group of 8 persons flavored *A. afra* infusions, 5g/500ml of water, 2drinks a day, daily for 1 week (7 days) (improved taste, increase concentration and decrease dose frequency). − **TG 6:** Group of 8 persons receiving flavored *A. afra* infusions, 5g/1liter of water, 3drinks a day, weekly for 4weeks (improved taste, decrease dose frequency and increase length of treatment); − **TG 7**: Group of 4 persons receiving flavored placebo infusions, 5g/liter of water, 3drinks a day, daily for 1week (7 days) (improved taste with no active molecule); − **TG 8**: Group of 4 participants receiving regular tea placebo taken as desired, daily for 1 week (7 days) (Regular tea with no active molecule). Randomization. The randomization method will be a permuted block randomization, with a block size of either 14 or 28 at random, and with a two-to-one allocation ratio for each active arm against two different control arms (2:2:2:2:2:2:1:1) Blinding: Partial blind. Data collection: Data will be captured through an observation worksheet and entered in Electronic Case Reporting Forms (eCRF).

Figure 2 and 3 show study design

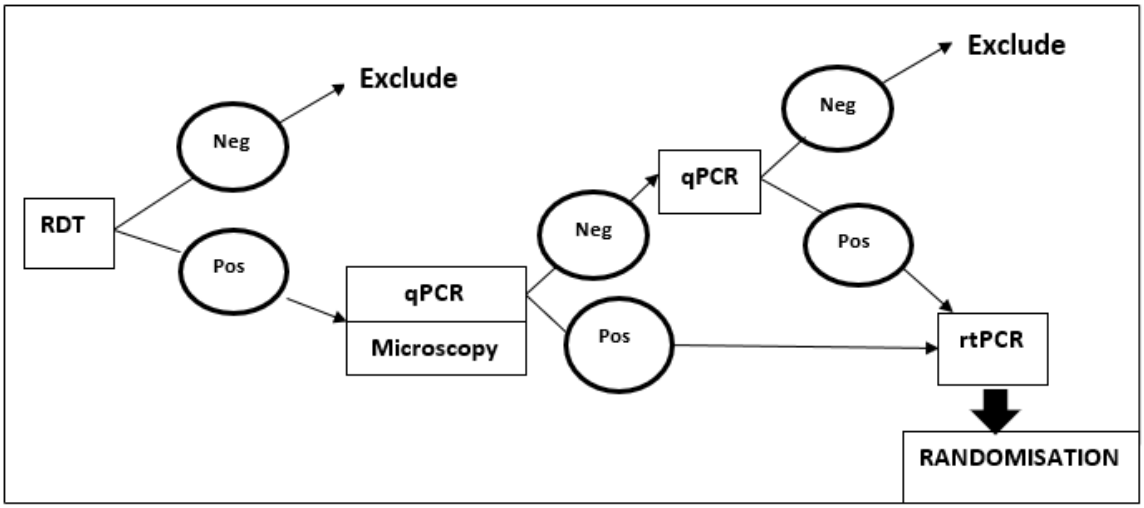

Figure 2. Screening and recruitment of study participants.

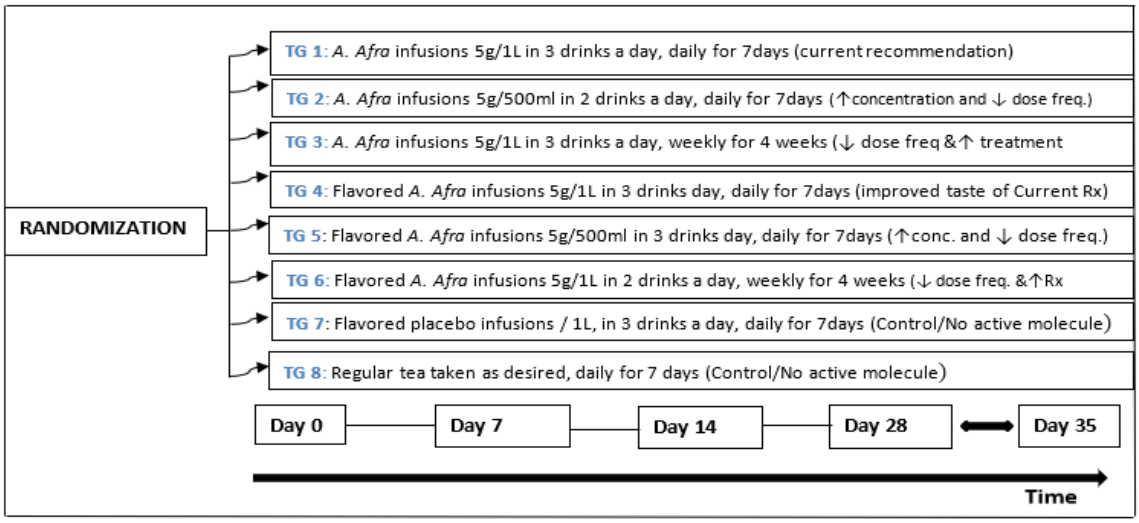

Figure 3. Algorithm to find the activity level, optimal dosage, and accepted formulation of *Artemisia afra* in eliminating plasmodium reservoirs.

Blood sample schedule: Five blood samples (approximately 1mL each) will be collected at Visit 1 (Day 0), Visit 2 (Day 7), Visit 3 (Day 14), Visit 4 (day 28), and visit 5 (day 35).

Study visits/assessment schedules: Five visits at Day0, Day7, Day14, Day28 and Day35.

Clinical symptoms (mild malaria symptoms): Clinical symptoms that may occur during the trial period will be assessed daily by site (observing) staff and recorded in the home or site visit worksheets throughout the study period.

Adverse side effects (ASEs) and adverse events (AEs): ASEs are not expected. Participants will be assessed daily by site staff during site and home visits/calls, within the 35 days study period and any AEs will be recorded in the eCRF (case report form).

ASEs and AEs leading to withdrawal will also be collected throughout the study period, by interviewing participants during site and home visits, and by reviewing of available medical records.

Written informed consent will be obtained before conducting any study related procedures or treatment on the participants. Participants will first be informed about the aim of the study,

	designs, potential gain, and their right to stop at any moment if they accept to enter the study. Then they will be requested to sign a written informed consent.
Number of study participants:	Fifty-six positive participants have to be enrolled per site, making 112 participants in the whole study. To reach the required number of gametocyte carriers, 635 persons will be RDT tested in Buea (Southwest region) where the expected positive RDT was 9.8.% in Demographic health survey (DHS) 2018 and drop-out rate was set at 10%. In Huye (South region), the expected positive RDT was 14.4% in Malaria indicator survey (MIS)2017 and drop-out rate was set at 10%, hence 432 persons will be RDT tested to reach the required number of gametocyte carriers.
Study population /Participants characteristics/ Study sites/	All students and staff members of the University will be invited to participate in the study. Participants will be included in the study if in good health (based on physical examination and medical history), and if they meet all specified eligibility criteria. **Inclusion criteria for trial allocation groups** Asymptomatic Plasmodium positive students or workers from the selected study universities, aged 18 years and above who provide written informed consent. **Exclusion criteria for trial allocation groups** 1. To have a known hypersensitivity to any ingredients of the tea. 2. Currently taking any malaria drug for prevention or treatment. 3. To have participated in another malaria drug trial or device in the last 1month. 4. To have a history or presence of clinically significant medical, psychiatric, or emotional condition. The study will be conducted at the University of Buea in Cameroon, and the University of Rwanda. All students and staff members of the University will be invited to participate. In the University of Buea, there are about 12,000 students and 973 staff members (500 teachers and 473 administrative /supporting staff. The University of Rwanda (UR), has about 30,445 students and 2,266 staff members (1450 teachers and 816 administrative /supporting)
Composition of study and control treatments	The study treatments (*Artemisia Afra*) and control (placebo) will be prepared in the various formulations as follows: ***Artemisia afra*** available as infusions containing 5g of Artemisia per liter, will be prepared by adding 5 g dried leaves and twigs of *A.* afra (in a tea sachet / tea bag) to 1 liter of boiling water, infused for 10 min, and filtered through a sterilized 1 mm mesh. ***Artemisia afra*** available as infusions containing 5g of Artemisia in per 500ml, will be prepared by adding 5 g dried leaves and twigs of *A.* afra (in a tea sachet / tea bag) to 500ml of boiling water, infused for 10 min, and filtered through a sterilized 1 mm mesh. ***Flavored Artemisia afra*** available as infusions containing 5g of flavored Artemisia per liter, will be prepared by adding 5 g of flavored dried leaves and twigs of A. afra (in a tea sachet / tea bag) to 1 liter of boiling water, infused for 10 min, and filtered through a sterilized 1 mm mesh. ***Flavored Artemisia afra*** available as infusions containing 5g of flavored Artemisia per 500ml, will be prepared by adding 5 g of flavored dried leaves and twigs of *A. afra* (in a tea sachet / tea bag) to 500mls of boiling water, infused for 10 min, and filtered through a sterilized 1 mm mesh. ***Flavored placebo*** available as infusions containing flavored inactive substance per liter, will be prepared by adding flavored inactive substance (in a tea sachet / tea bag) to 1 liter of boiling water, infused for 10 min, and filtered through a sterilized 1 mm mesh. ***Regular tea placebo*** will be any tea of choice routinely taken by the selected participants. After preparation, the following doses will be obtained for consumption by participants with respect to study arm.

	Drug components	**Non-flavored**	**Flavored**
	Artemesia afra 5g	Artemesia 5g/ 1liter 1.25g/250 ml dose	Artemesia 5g/ 1liter, 1.25g/250 ml dose
	Artemesia afra 5g	Artemesia 5g/ 500ml, 2.5g/250 ml dose	Artemesia 5g/ 500ml, 2.5g/250 ml dose
	Placebo	Regular tea placebo	Flavored placebo 250ml dose
Study description	This study will use the adapted Zelen design which has two steps in the consent process. At step one, informed individuals (students and workers) will be invited to participate in a screening and cohort survey on asymptomatic malaria, aimed at understanding the comportment of plasmodium parasite in asymptomatic plasmodium carriers over time. After informed consent is obtained during the first encounter (day 0), baseline assessments will be done and sociodemographic variables, clinical/medical history, physical assessment, prior medications information and participants' contact details will be recorded. Blood samples will be collected from each participant and examined for the presence of parasites (Gametocytes) using malaria rapid diagnostic test kit (mRDT). RDT positive samples will be re-examined with qPCR (quantitative polymerase chain reaction) and direct microscopy for confirmation. The qCPR will be repeated for negative samples and participants excluded if still negative. All qPCR positive samples will be further analyzed with rt PCR (real time polymerase chain reaction), for parasite quantification and specific stage identification, targeting specific parts of the gametocyte genes. Participants who fulfil the inclusion criteria will be randomly allocated in the 8 study arms in a 2:2:2: 2:2:2: 1:1 ratio for the 6 treatment and 2 control arms, respectively. In the second step, only participants from the intervention groups (TG1- to -TG7) will receive the information about the intervention and a second consent will be obtained from them. Treatment administration will be based on the allocation arm. The treatments will consist of *Artemisia afra* tea infusions for the 6 trial arms versus favored placebo infusions and desired regular tea for the 2 control arms. The participants who will decline to participate to an intervention will continue in the cohort study, as a control group on regular tea (TG 8). **Follow up visits.** During 4 consecutive weeks, all participants will be tested using rtPCR and direct smear microscopy once a week on Day 7, 14, 28 and 35, in order to assess gametocyte elimination rate with teas. During the 4 weeks, participants will be monitored and encouraged to comply to the prescribed treatment, and also monitored/assessed for any clinical symptoms and treatment reactions (ASEs and AE).		
Study endpoints	<u>Primary end point</u> • Proportion of people with decreased gametocyte load after treatment with *Artemisia afra* tea on Day 35 from baseline. <u>Secondary endpoints</u> • Level of decrease in gametocyte load (rt-PCR Ct) in the different treatment arms at D7, D14, D28, and D35 from baseline (activity level and optimal dosage schedule). • Level of decrease in gametocyte load the various treatment arms (rt-PCR Ct) by gender, age, and trial entry parasite load, at D7, D14, D28, and D35 from baseline. • Proportion of people in the various treatment arms who respect and complete the prescribed treatment dosages (accepted dosage schedule and drug formulation).		

	• Variability and Proportion of persons on the various treatment arms who develop clinical symptoms, ASEs, or AES at D7, D14, D28, and D35 from baseline (No severe adverse side effects are expected). • Proportion of persons with baseline discordant test results (False positive or false negative) between mRDT, rt-PCR, and direct blood smear observation. Exploratory endpoints. • Variability in level of decease in gametocyte load (rt-PCR Ct) in the various treatment arms at D7, D14, D28, and D35 from baseline. • Variability in level of decease in gametocyte load (rt-PCR Ct) in the various treatment arms by gender, age, and trial entry parasite load, at D7, D14, D28, and D35 from baseline. • Variability in attainment of absolute increase in Cycle threshold (Ct >20) in the various treatment arms at D7, D14, D28, and D35 from baseline. • Variability in the proportion of participant who respect and complete prescribed treatment dosages (acceptability) in the various treatment arms. • Variability in the proportion of participants who develop clinical symptoms, ASEs, and AEs in the various treatment arms at D7, D14, D28, and D35 from baseline.
Statistical plan	As a pilot study, no formal sample size calculation was performed. The expected number of participants is 112, being 56 per study site. Descriptive statistics will be used to redistribute the treatment outcomes in the various arms according to the evaluation time**.** The data will be analyzed using survival analysis with cox regression to evaluate time to emptying (reservoirs elimination), and with time-to regulate PCR as outcome.
Data safety and monitoring board	An independent Data and Safety Monitoring Board (DSMB) will be set up for periodic review of safety data during the study conduct. The composition and responsibilities of DSMB (Medical Monitoring/ audit team) members are presented in Table 1 and section 9.3, respectively.

1. INTRODUCTION, BACKGROUND AND RATIONALE

Malaria is a preventable disease caused by the Plasmodium parasite and transmitted by female Anopheles mosquitoes (1). Plasmodium parasites exhibit a complex life cycle that includes three stages in the human host and one sexual development stage in the mosquito vector (2). During replication in the human blood stream, some of the merozoites differentiate into sexual forms of the parasite, called ***gametocytes,*** and pass into the blood stream. When a mosquito bites an infected human during a blood meal, it ingests the gametocytes, and these undergo sexual development in the mosquito vector to continue the plasmodium cycle (Figure 1) (2). Successful elimination of malaria can only be achieved through optimal vector control (e.g. genetically modifying female mosquitoes) and complete depletion of the infectious reservoir in humans (3)**.** However, symptomatic malaria cases are only a small proportion of all infectious reservoirs in malaria endemic settings, and most (>95%) of the human infectious reservoirs are asymptomatic parasite carriers (4,5). Accurate identification and treatment of asymptomatic infectious human reservoirs is therefore a critical step to an effective malaria elimination process.

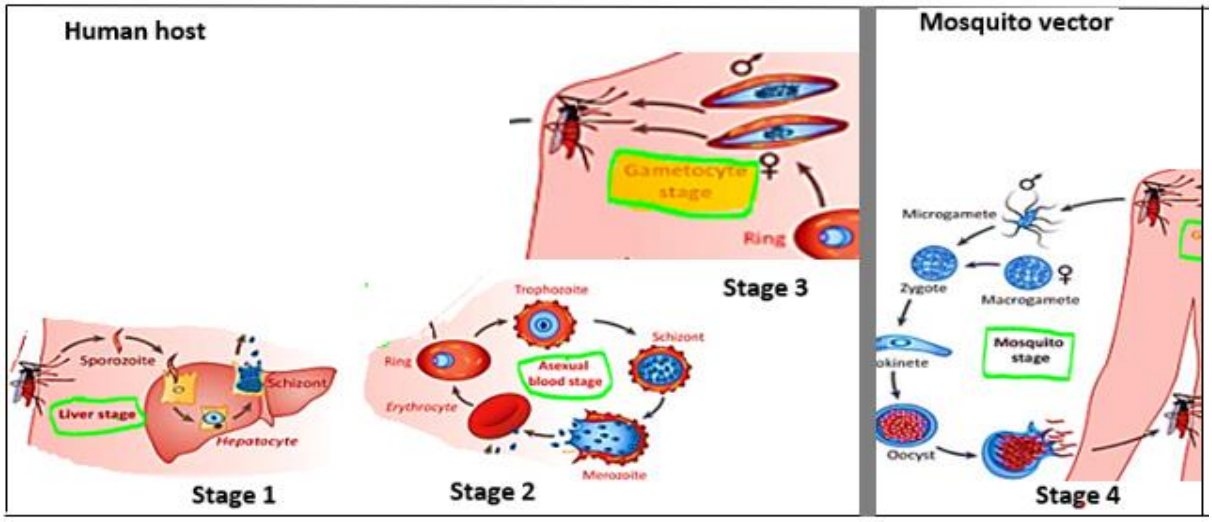

Figure 1. Plasmodium life cycle (*Source : Maier et al 2019*)

2.2 Literature review

Malaria parasite transmission is highly heterogeneous across the various zones in Africa. Out of 5 parasite species that cause malaria in humans, 2 of these species – *P. falciparum* and *P. vivax* – pose the greatest threat, P. *falciparum* accounted for over 99% of the cases in the WHO African Region in 2018 (1) Significant and continual efforts/innovation in the fight against malaria, has led to a substantial decline in global malaria morbidity and mortality, but malaria elimination in most malaria-endemic settings remains a core public health concern. So far, there are no vaccine disrupting the disease transmission (2). The most advanced vaccine approved for pilot use is Mosquirix, developed by GSK, which has an efficacy of 30-53% and 56% in phase II and III trials, respectively, quite below the 75% target expected by WHO (6). Recently, initial findings from a new vaccine trial R21/Matrix-M under development by Oxford, showed up to 77% efficacy on Burkinabe children (7). Current control measures in place encompass the use of WHO recommended strategies that include vector control (insecticide treated nets (ITNs) and indoor residual spraying (IRS)), early diagnosis and prompt treatment of symptomatic cases, as well as chemoprophylaxis for high-risk population groups as children under 5, pregnant women and naïve visitors (8). These measures have reduced malaria burdens globally in recent years, and raised hopes for malaria elimination/eradication in the near future (9–11). However, these hopes are severely threatened by the emergence of resistances for both insecticide and antimalarial drugs like Artemisinin-based combination therapies (ACTs) that are the first line WHO recommended malaria treatment (12–14). Furthermore, the current malaria control measures focus on the mosquito stage and the two asexual human stages, but do not target the gametocyte stage which allows the parasite transmission from humans to the mosquito vector for the disease perpetuation.

Gametocytes are insensitive to most conventional antimalarial drugs and often persist in the blood

stream after successful antimalarial therapy, thereby ensuring continued malaria transmission over several months after the clearance of asexual parasites (16,17). Primaquine and the fast-acting but short-lived artemisinin-based drugs are the only antimalarials that have exhibited gametocytocidal activities (17), but primaquine is toxic in persons with glucose 6 phosphate dehydrogenase deficiency, limiting its general use in most countries (19, 20). A novel antimalarial compound called Cipargamin (KAE609) compound which is said to exert gametocidal activity with transmission blocking potential, is still undergoing phase 2 clinical trial to assess its safety and efficacy (20).

Furthermore, while the mosquito vector may harbor the sexual forms of the parasite through its entire adulthood of 1-2 months, the human host can remain asymptomatic for the asexual parasite stages for up to 13 years, during which transmissible gametocyte forms are continually released into the blood stream (21). This results in the widespread occurrence of asymptomatic plasmodium reservoirs, who often do not seek antimalarial treatment, due to the absence of clinical illness.

Studies reveal that asymptomatic reservoirs can be eliminated in mass treatment programs (23,24). Various mass treatment strategies such as Mass drug administration (MDA) have been proposed and proven to rapidly reduce malaria burdens particularly in regions with seasonal malaria transmission in Africa and in China, (23–26). Unfortunately, the MDA strategy has not been successful with the ACTs, as drug resistance and potentiation of gametocyte transmission has rather been observed upon ACT administration (28, 29). On the other hand, surveys have reported the protective effect of Artemisia teas against malaria in communities that use its herbal teas for malaria prevention (29). Meanwhile other studies have demonstrated that *Artemisia annua* and *Artemisia afra* infusions or powdered leaves reduces parasitemia and kills transmissible gametocyte forms, with characteristic chemoprotective potentials (30–33).

Rationale

The WHO African Region carries a disproportionately high share (94%) of the global malaria burden (1). Repeated calls from WHO have advocated for mobilization and expanded actions towards malaria elimination in the continent. As a result, there has been extraordinary and optimistic scale-up of WHO recommended malaria control interventions in most African countries over the last decades. Despite the recent progress on reducing malaria morbidity and mortality in response to this, empirical and theoretical evidence show that current control interventions alone will not be sufficient to eliminate malaria from many endemic regions in sub-Saharan Africa. Thereby suggesting that current interventions be supplemented by additional strategies, for effective elimination in most sub-Saharan Africa countries (5).

An emergence of resistances for both insecticide and antimalarial drugs like ACTs has been reported in several African countries. In addition, these strategies are restricted to indoor controls and populations

remain exposed to Plasmodium transmission during their outdoor activities. These strategies also fail to target asymptomatic Plasmodium carriers who constitutes, most of the infected people in endemic regions (4, 5) and perpetuate the disease transmission over time. Hence, the need to accurately identify and treat asymptomatic infectious human reservoirs.

Reports of investigations that *Artemisia afra* tea infusions can effectively treat malaria in endemic communities, with gametocidal effects and no reported toxicity (29–31), are promising and suggest its probable usefulness in the elimination of plasmodium reservoirs in endemic settings. However, no clinical trials have not been conducted to ascertain the activity level and optimal dosage of *Artemisia afra* tea in the treatment of asymptomatic reservoirs.

2. HYPOTHESIS, OBJECTIVES AND ENDPOINTS

Hypothesis: An optimal dosage of artemisia afra tea infusions is effective, safe, and well tolerated in eliminating plasmodium reservoirs.

Research Objectives

2.3.1 Primary objective

2 To find the activity level and accepted optimal dose concentration, dose frequency, length of treatment, and formulation (flavor) of *A.afra* in treating Plasmodium reservoirs.

2.3.2 Secondary objectives

1. To assess participants response (treatment efficacy) to varying dose concentrations, dose frequency, length of treatment, and formulation of *Artemisia afra* treatment.
2. To identify the most acceptable dose concentration, dose frequency, length of treatment, and formulation (flavor) of Artemisia treatment.
3. To assess the safety (ASE) of *Artemisia afra* treatment in participants by dose concentration, dose frequency, length of treatment, and formulation. As a commonly used tea, no toxicity is expected.
4. To assess variability of plasmodium test predictive values between mRDT, rtPCR, and direct blood smear observation in asymptomatic persons.

Treatment outcomes

Major outcome

- Decrease in parasite load over time.

Secondary outcomes

- Decrease in gametocyte load in carriers with Artemisia afra tea by dose concentration, dose frequency, length of treatment, and formulation.
- Acceptability (adherence and completion) of *Artemisia afra* treatment by dose concentration, dose frequency, length of treatment, and formulation (flavor).

- Safety (SAEs) of *Artemisia afra* treatment in participants by dose concentration, dose frequency length of treatment, and treatment formulation.
- Absolute increase in Cycle threshold (Ct >20).
- Likelihood and variability in the rate of reservoir elimination with treatment, compliance/ acceptability, and occurrence of side effects /adverse events in different treatment arms.

Treatment endpoints

Primary endpoints

- Proportion of people with decreased gametocyte load after treatment with *Artemisia afra* tea on Day 35 from baseline.

Secondary endpoints

- Level of decrease in gametocyte load (rt-PCR Ct) in the different treatment arms at D7, D14, D28, and D35 from baseline (activity level and optimal dosage schedule).
- Level of decrease in gametocyte load the various treatment arms (rt-PCR Ct) by gender, age, and trial entry parasite load, at D7, D14, D28, and D35 from baseline.
- Absolute increase in Cycle threshold (Ct >20) in various treatment arms at D7, D14, D28, and D35 from baseline.
- Proportion of people in the various treatment arms who respect and complete the prescribed treatment dosages (accepted dosage schedule and drug formulation).
- Proportion of persons on the various arms of treatment who develop clinical symptoms at D7, D14, D28, and D35 from baseline.
- Proportion of people on the various arms of treatment who develop ASEs or AEs at D7, D14, D28, and D35 from baseline (No severe adverse side effects are expected).
- Proportion of persons on the various arms of treatment who withdraw from study due to severe ASEs or AEs at D7, D14, D28, and D35 from baseline.
- Proportion of persons on the various arms of treatment who withdraw from study for reasons other than severe AEs at D7, D14, D28, and D35 from baseline.
- Proportion of persons with baseline discordant test results (False positive or false negative) between mRDT, rt PCR, and direct blood smear observation?

Exploratory endpoints

- Variability in level of decease in gametocyte load (rt-PCR Ct) in the various treatment arms at D7, D14, D28, and D35 from baseline.
- Variability in level of decease in gametocyte load (rt-PCR Ct) in the various treatment arms by gender, age, and trial entry parasite load, at D7, D14, D28, and D35 from baseline.
- Variability in attainment of absolute increase in Cycle threshold (Ct >20) in the various treatment arms at D7, D14, D28, and D35 from baseline.

- Variability in the proportion of participant who respect and complete prescribed treatment dosages (acceptability) in the various treatment arms.
- Variability in the proportion of participants who develop clinical symptoms in the various treatment arms at D7, D14, D28, and D35 from baseline.
- Variability in the proportion of participants who develop Severe ASEs in the various treatment arms at D7, D14, D28, and D35 from baseline (not expected).
- Variability in proportion of participants who withdraw from the study due to severe ASEs, in the various treatment arms, at D7, D14, D28, and D35 from baseline.
- Variability in proportion of participants who withdraw from the study for reasons other than severe AEs, in the various arms of treatment at D7, D14, D28, and D35 from baseline.

3. METHODOLOGY

Study Design: The study is a Phase 2 randomized controlled, partial-blind, parallel group study in plasmodium infected *asymptomatic* adults 18 years and older, with 8 study arms. It will use the adapted Zelen design. The adapted Zelen design uses two steps in the consent process. In the first step, there is an informed consent from all participants for a cohort lifestyle study. After consent is obtained, baseline assessment and screening will be performed, and eligible participants randomly assigned to the different study arms. According to this consent, participants are randomized without knowledge about the detailed protocol. In the second step, only participants from the intervention group will receive the information about the intervention and the second consent will be obtained from them. The participants who will decline to participate to an intervention will continue in the cohort study, as the control group.

Duration of study: The study duration is 35 days (5 weeks) for each participant.

Treatment groups (TG):

TG 1: Group of 8 persons receiving *A. afra* infusions of 5g/1liter of water, 3drinks a day, daily for 1week (7 days) (current recommendation).

TG 2: Group of 8 persons receiving *A. afra* infusions of 5g/500ml of water, 2drinks a day, daily for 1week (7 days) (increase concentration and decrease dose frequency).

TG 3: Group of 8 persons receiving *A. afra* infusions,5g/1liter of water 3drinks a day, weekly for 4weeks (decrease dose frequency and increase length of treatment).

TG 4: Group of 8 persons receiving flavored *A. afra* infusions, 5g/1liter of water 3drinks a day, daily for1week (7 days) (current treatment with improved taste).

TG 5: Group of 8 persons flavored *A. afra* infusions, 5g/500ml of water 2drinks a day, daily for 1 week (7 days) (improved taste, increase concentration and decrease dose frequency).

TG 6: Group of 8 persons receiving flavored *A. afra* infusions, 5g/1liter of water 3drinks a day, weekly for 4weeks (improved taste, decrease dose frequency and increase length of treatment);

TG 7: Group of 4 persons receiving flavored placebo infusions, 5g/liter of water 3drinks a day, daily for 1week (7 days) (improved taste with no active molecule);

TG 8: Group of 4 participants receiving regular tea placebo taken as desired, daily for 1 week (7 days) (Regular tea with no active molecule).

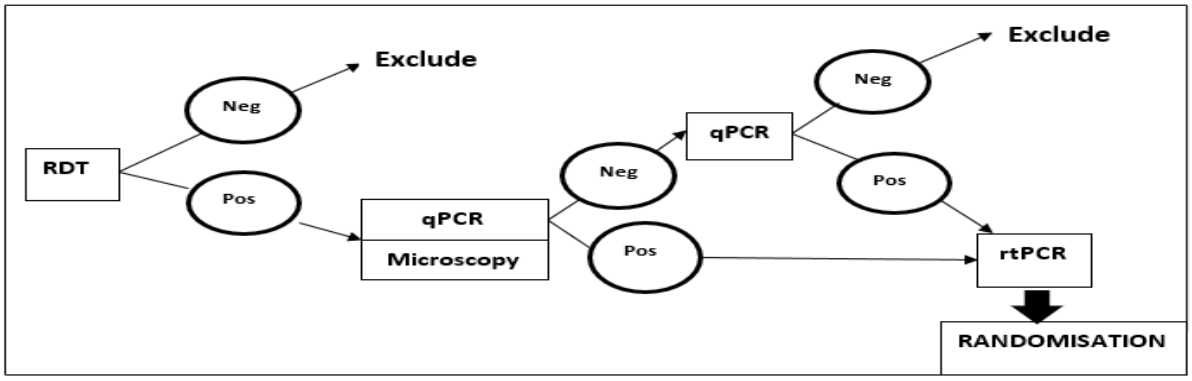

Figure 2. Screening and recruitment of study participants

*Rapid diagnostic test (**RDT**), Positive (**Pos**) Negative (**Neg**), Real time Polymerase Chain reaction (**rt-PCR**), Quantitative Polymerase Chain reaction (**qPCR**)*

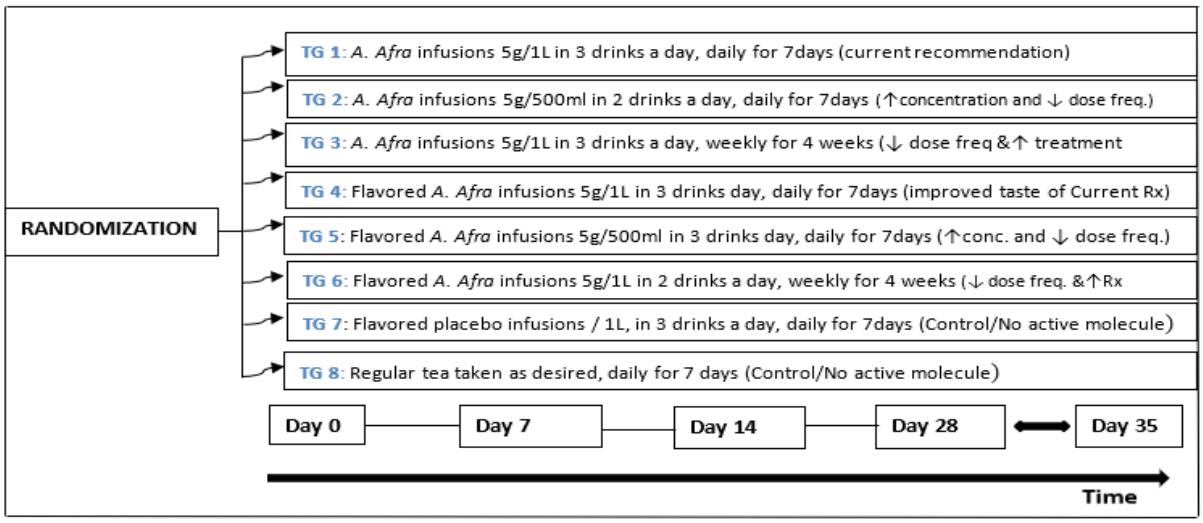

Figure 3. Algorithm to find the activity level, optimal dosage, and accepted formulation of *Artemisia afra* in eliminating plasmodium reservoirs.

Study description:

Study population: The trial will be conducted among Plasmodium infected asymptomatic students and workers in the University of Buea -Cameroon and the Rwanada National University (UR. Fifty-six positive participants have to be enrolled per site, making 112 participants in the whole study. To reach the required number of gametocyte carriers, 635 persons will be RDT tested in Buea (South-west region) where the expected positive RDT was 9.8% in Demographic health survey (DHS) 2018 (34) and drop-

out rate was set at 10%. In Kigali where the expected positive RDT was 14.4% in Malaria indicator survey (MIS)2017 (35) and drop-out rate was set at 10% , hence 432 persons will be RDT tested to reach the required number of gametocyte carriers.

This study will use the adapted Zelen design which has two steps in the consent process. At step one, informed individuals (students and workers) will be invited to participate in a screening and cohort survey on asymptomatic malaria, aimed at understanding the comportment of plasmodium parasite in asymptomatic plasmodium carriers over time. After informed consent is obtained during the first encounter (day 0), baseline assessments will be done and sociodemographic variables, clinical/medical history, physical assessment, prior medications information and participants' contact details will be recorded. Blood samples will be collected from each participant and examined for the presence of parasites (Gametocytes) using malaria rapid diagnostic test kit (mRDT). RDT positive samples will be re-examined with qPCR (quantitative polymerase chain reaction) and direct microscopy for confirmation. The qCPR will be repeated for negative samples and participants excluded if still negative. All qPCR positive samples will be further analyzed with rt PCR (real time polymerase chain reaction), for parasite quantification and specific stage identification, targeting specific parts of the gametocyte genes. Participants who fulfil the inclusion criteria will be randomly allocated in the 8 study arms in a 2:2:2: 2:2:2: 1:1 ratio for the 6 treatment and 2 control arms, respectively. In the second step, only participants from the intervention groups (TG1- to -TG7) will receive the information about the intervention and a second consent will be obtained from them. Treatment administration will be based on the allocation arm. The treatments will consist of *Artemisia afra* tea infusions for the 6 trial arms versus favored placebo infusions and desired regular tea for the 2 control arms. The participants who will decline to participate to an intervention will continue in the cohort study, as a control group on regular tea (TG 8).

Screening and recruitment: Screening will be done during at baseline visit (Day 0) in the health stations set up in the study sites. After informed consent is obtained, baseline assessments will be performed to obtain information on sociodemographic characteristics, clinical/medical history, physical assessment, prior medications, and participants' contact details. This will be followed by screening tests for presence of plasmodium parasites. Participants who fulfil the inclusion criteria will be enrolled in the study.

Randomization: Prior to treatment**,** eligible participants will be randomly assigned into the 8 study arms. The randomization method will be a permuted block randomization, with a block size of either 14 or 28 at random, and with a two-to-one allocation ratio for each active arm against two different control arms (2:2:2:2:2:2:1:1).

Blinding: The participants in the intervention arms will be aware of their assignment to an intervention group but unaware of the treatment assigned to them. The site staff (investigators or observers) will be

aware of assignments to one control arm (regular tea group), but unaware of assignments in the other treatment arms.

Visits/assessment schedules: A total of five study visits will be effected, being one baseline visit on Day 0, and four follow-up visits on Day 7, 14, 28 and 35, from baseline. During the baseline visit plasmodium tests targeting specific parts of the gametocyte genes will be done to determine the participants' initial (baseline) parasite load, and during subsequent visits, follow up control tests (rtPCR and blood smear) will be done, and the gametocyte load recorded, alongside assessments for clinical symptoms and adverse sides effects (if any).

Endpoints The efficacy of each of the treatment dosage schedule will be evaluated on day 7,14,28 and 35 from baseline, based on response to treatment or reservoir elimination rate. Response to treatment will be indicated by an absolute increase in Cycle threshold (Ct >20), or a negative rt-PCR tests results (Ct >40). If the patient misses the follow-up visit, compliance will be noted in the completion worksheet (filled out at end of study) and the parasite load and adverse side effects noted during the last visit considered. No severe side adverse effects are expected from the treatment but since toxicity is always a concern in clinical trials involving humans, participants will be observed for any severe ASEs and withdrawal noted throughout the study duration.

Clinical symptoms (mild malaria symptoms): That may occur during the trial period will be assessed daily by site (observing) staff and recorded in the home or site visit worksheets at Visit Day 7, 14,28, and 35 including Temperature > 37°C, Chills (Perspiration), Sweats, Headaches, nausea and vomiting, body aches, general malaise (Weakness), enlarged spleen, mild jaundice, enlargement of the liver, and increased respiratory rate (>20 /min). Participants with confirmed malaria symptoms will be treated with the WHO recommended treatment for symptomatic malaria.

Data collection: Data will be captured through the Observation worksheet and entered in Electronic Case Report Forms (eCRF), by interviewing the participants during observation, reviewing available medical records, and documenting assessment records.

Written informed consent will be obtained before conducting any study related procedures or treatment on the participants. Participants will first be informed about the aim of the study, designs, potential gain, and their right to stop at any moment if they accept to enter the study. Then they will be requested to sign a written informed consent.

Ethical considerations: Administrative authorization will be obtained from the ministry of public health in Cameroon and Rwanda, and administrative authorities of the various study sites. Ethical authorization will be obtained from the Faculty of Health science Institutional Review Board (FHSIRB) of the University of Buea, Cameroon and the Rwandan National Ethical Committee (RNEC). All study participants will have a clear and detailed explanations about the study and requested to freely sign a written informed consent.

4. STUDY POPULATION

The study will be conducted at the University of Buea in Cameroon, and the University of Rwanda. All students and staff members of the University will be invited to participate. In the University of Buea, there are about 12,000 students and 973 staff members (500 teachers and 473 administrative /supporting staff. The University of Rwanda (UR) has about 30,445 students and 2,266 staff members (1450 teachers and 816 administrative /supporting). All students and staff members of the University will be invited to participate in the study. Participants will be included in the study if in good health as judged by physical examination and medical history, and if they meet all specified eligibility criteria.

Table 3: Inclusion and exclusion criteria.

Inclusion criteria for trial allocation groups	Exclusion criteria for trial allocation groups
1. Be student or worker of a participating University. 2. Be 18 years and above, and in good general health condition. 3. Have a device (phone, tablet, etc) that will support remote visits. 4. Sign written informed consent form. 5. Screened positive for malaria (RDT + and qPCR +) but asymptomatic.	1. To have a known hypersensitivity to any ingredients of the tea. 2. Currently taking a malaria drug for prevention or treatment. 3. To have participated in another malaria drug trial or device in the last 14days. 4. To have a history or presence of clinically significant medical, psychiatric, or emotional condition. 5. Reported diabetic.

5-STUDY PROCEDURES

5.1 General considerations: The study will be conducted in accordance with the ethical principles for medical research involving humans in the World medical associations declaration of Helsinki (36). The study will be initiated only after approvals from relevant authorities, ethical and institutional review boards have been obtained. Freely given and written informed consent must be obtained from each participant prior to participation in the study.

5.2 Community permission

After obtaining ethical and regulatory approvals, meetings will be held with representatives of participating University communities following specific site procedures, during which the study will be explained in detail (including the objectives, procedures, and implications to the participants) and the study team will answer any questions and concerns raised. The community leaders would review the information and confirm their permission for the conduct of the study. A communique for the study will then be put up in collaboration with the communication department of each University, bearing the precise study period and a reference contact for further clarifications.

5.3 Recruitment: During the baseline visit at the site health stations on Day 0, informed individuals will be invited to participate in a screening and cohort survey on asymptomatic malaria. After informed

consent is obtained, baseline assessments will be carried out and information obtained on participant's sociodemographic characteristics, clinical/medical history, physical assessment, prior medications and contact details. This will be followed by malaria screening tests and participants who fulfil the inclusion criteria will be enrolled in study.

5.4 Informed Consent

"Informed consent" is the voluntary agreement of an individual to participate in the research. Consent will be given with free will of choice, and without inducement. The individual will have sufficient knowledge and understanding of the nature of the proposed research, the anticipated risks and potential benefits, and the requirements of the research, to be able to make an informed decision. Two original copies of the informed consent forms will be signed so that one copy will remains with the participant, and one will be kept at the site.

5.5 Randomization and Blinding Procedures

Randomization: After enrolment of eligible participants, a statistician will randomly assign them in the 8 study arms before treatment administration. The randomization method will be a permuted block randomization, with a block size of either 14 or 28 at random, and with a two-to-one allocation ratio for each active arm against two different control arms (2:2:2:2:2:2:1:1). The treatments will consist of *Artemisia afra* infusions for the 6 trial arms versus flavored placebo infusions and any desired regular tea for the 2 control arms.

Blinding: Neither the participants nor the site staff (investigators or observers) will know to which study arm the participants have been assigned.

5.6 Study visits

Table 4: Study visits

Visit time	Activity
1st: Day 0	− Baseline assessments and recording − Screening for presence pf plasmodium parasites (mRDT, qPCR) − Parasite load assessment (rtPCR) − Recruitment, randomization, and allocation. − Treatment prescription and education on treatment administration.
2nd: Day 7	− Parasite load measurement (rtPCR) − Monitoring and recording of: o ASEs and AEs. o Treatment compliance /acceptability (dosage respect) o Number of withdrawals and reasons, o Occurrence of clinical symptoms o Concomitant medications
3rd: Day 14	− Parasite load measurement (rtPCR) − Monitoring and recording of: o ASEs and AEs. o Treatment compliance /acceptability (dosage respect) o Number of withdrawals and reasons, o Occurrence of clinical symptoms o Concomitant medications

4th: Day 28	– Parasite load measurement (rtPCR) – Monitoring and recording of: o ASEs and AEs. o Treatment compliance /acceptability (dosage respect) o Number of withdrawals and reasons, o Occurrence of clinical symptoms o Concomitant medications
5th: Day 35	– Parasite load measurement (rtPCR) – Monitoring and recording of: o ASEs and AEs. o Treatment compliance /acceptability (dosage respect) o Number of withdrawals and reasons, o Occurrence of clinical symptoms o Concomitant medications

5.7 Laboratory tests

Before randomization during the baseline visit (D0), blood samples will be collected from each participant and examined for the presence of parasites (Gametocytes) using Malaria rapid diagnostic test kit (BIOCREDIT Malaria Ag P.f/Pan RDT). RDT positive samples will be re-examined with qPCR (quantitative polymerase chain reaction) and direct microscopy for confirmation. The qCPR will be repeated for negative samples and participants excluded if still negative. All qPCR positive samples will be further analyzed with rt PCR (real time polymerase chain reaction), for parasite quantification and specific stage identification, targeting specific parts of the gametocyte genes.

a. ***BIOCREDIT Malaria Ag P.f/Pan RDT****:* This is a three-band RDT that detects HRP2 and pan-pLDH antigens which will detect the presence of P. falciparum (HRP2) and of another specie (pan-pLDH). Results will be interpreted as Negative (band absent) Mildly positive (faint band appearance) strongly positive (conspicuous band appearance).
b. ***Direct microscopy*****:** Direct Microscopic identification of parasites will be performed using thin and thick blood films stained with Giemsa and quantified as number of parasites per milliliter of blood.
c. ***rtPCR):*** The parasite load will be measured based on the number of rtPCR cycle thresholds (Ct) (which is an inverse proportion of the parasite load) and classified as absolute increase in Cycle threshold (Ct >20), or negative rt-PCR tests results (Ct >40).
d. ***Blood sample schedule****:* Five blood samples (approximately 1mL each) will be collected at Visit 1 (Day 0), Visit 2 (Day 7), Visit 3 (Day 14), Visit 4 (day 28), and visit 5 (day 35). After conducting the 3 or 4 tests at visit 1, only the rtPCR test and blood smear microscopy will be done during subsequent evaluations.

5.8 Methods for processing, labeling and storage of serum samples

Approximately 1mL (500ul) of blood sample will be drawn from each participant (from forearm) during each visit, from which the parasites and their genetic material will be extracted. The blood samples will be collected before treatment administration on D0, and systematically during subsequent visits. To

minimize bleeding from collection site, an adhesive tape will be applied at the site of blood sample draw, according to local practice. Not more than two attempts should be made to draw the required volume of blood.

The blood will be processed and aliquoted and stored according to the Clinical Specimen Lab Manual. Each sample tube will be labeled with the labels provided for the study. Collected samples will be labeled by the main site investigator and with a code that only the study site can link to the participant's name. All stored research samples will be logged into a secure database. Any use of the samples will be documented. Complete instructions for labeling and storage of serum samples will be included in the Clinical Specimen Lab Manual, which will be stored in the Investigator Site File.

Testing of samples will be performed in the analyses site set up at the various study sites. Samples will be retained in accordance with regulatory guidance for retention of essential study documents as described in SECTION 12 of this protocol. Participants will be informed and asked to agree to long term storage of specimens for use in future research through a specific consent form. At study closure, the study coordinators/sonsor will designate a storage facility for the retention of the consented samples. Should future research on the samples be initiated after study closure, a specific new study protocol will be developed and submitted to relevant Scientific and Ethics Committees following the informed consent requirements.

6. TREATMENT OF PARTICIPANTS

6.1 Description of study treatments

The study treatments (*Artemisia Afra tea infusions*) and control (placebo) will be prepared (quantify, weigh, and package the dry leaves and twigs in tea bags) and labelled in envelopes according to the different study arms at the pharmaceutical Laboratory. The various formulations will be as follows:

Artemisia afra available as infusions containing 5g of Artemisia per liter, will be prepared by adding 5 g dried leaves and twigs of *A.* afra (in a tea sachet / tea bag) to 1 liter of boiling water, infused for 10 min, and filtered through a sterilized 1 mm mesh.

Artemisia afra available as infusions containing 5g of Artemisia in per 500ml, will be prepared by adding 5 g dried leaves and twigs of *A.* afra (in a tea sachet / tea bag) to 500ml of boiling water, infused for 10 min, and filtered through a sterilized 1 mm mesh.

Flavored Artemisia afra available as infusions containing 5g of flavored Artemisia per liter, will be prepared by adding 5 g of flavored dried leaves and twigs of A. afra (in a tea sachet / tea bag) to 1 liter of boiling water, infused for 10 min, and filtered through a sterilized 1 mm mesh.

Flavored Artemisia afra available as infusions containing 5g of flavored Artemisia per 500ml, will be prepared by adding 5 g of flavored dried leaves and twigs of *A. afra* (in a tea sachet / tea bag) to 500mls of boiling water, infused for 10 min, and filtered through a sterilized 1 mm mesh.

Flavored placebo available as infusions containing flavored inactive substance per liter, will be prepared by adding flavored inactive substance (in a tea sachet / tea bag) to 1 liter of boiling water, infused for 10 min, and filtered through a sterilized 1 mm mesh.

Regular tea placebo will be any tea of choice routinely taken by the selected participants.

Table 5: Various formulation of the study teas and controls.

Drug components	**Non-flavored**	**Flavored**
Artemesia afra 5g	Artemesia 5g/ 1liter 1.25g/250 ml dose	Artemesia 5g/ 1liter, 1.25g/250 ml dose
Artemesia afra 5g	Artemesia 5g/ 500ml, 2.5g/250 ml dose	Artemesia 5g/ 500ml, 2.5g/250 ml dose
Placebo	Regular tea placebo	Flavored placebo 250ml dose

6.2 Treatment administration

After randomization and allocation, clear education, and demonstrations on how to prepare and consume the treatment will be provided to participants by the site investigator based on their randomization codes. Participants on the trial treatment arms will receive either of the Artemisia infusions of various dosage schedules and formulations, and participants on the placebo arms will drink either a flavored inactive infusion or the regular tea. The participants will take the treatment or placebo orally, under the supervision of one of the investigators (Observers). 8 observers will be assigned to follow up and assist participants on the various treatment arms (1 observer per treatment arm). The observer will encourage compliance to treatment, observe for side effects/ adverse events, and take note of participant's comments/appreciation of the treatment dosages and formulation, throughout the study period. The Observer will also effect home visits to participants when necessary (in case of loss to follow-up and difficulty to contact participant on cell phone).

6.3 Treatment supply, labelling, storage, accountability, and disposal

The project coordinators/funders will ensure the following:

- Appropriate supply of the study treatments
- Appropriate labeling of all treatments provided that complies with the legal requirements of each country where the study is being performed.

The investigator must ensure the following:

- Indication of appropriately trained blinded site staff (site manager) to manage the drug supply, accountability, prescription, administration, and observation.
- Acknowledge receipt of the treatment at the site, including confirmation that the treatments were received in good condition, remained within the appropriate temperature range during shipment

from production site to the designated storage location, and ensure appropriate storge conditions in accordance with the manufacturer's recommendations.

- Proper storage of treatment: Storage in a secure, locked, temperature-controlled location according to the instructions specified on the labels, and appropriate record keeping and inventory, including regular documentation of adequate storage temperature.
- Appropriate use of the treatments only in accordance with the approved protocol.
- Complete record keeping of treatment use, wastage, or destruction, including documentation of number of doses destroyed, date of destruction, method of destruction, and name of individual performing destruction.

6.4 Participant Withdrawal Criteria

A participant may discontinue study participation at any time prior to the last planned study visit. This is referred to as premature withdrawal from the study. From the analysis perspective, a 'premature withdrawal' from the study refers to any participant who will not be available for the termination visit foreseen in the protocol (Visit 5). A participant is considered a 'premature withdrawal' from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected from this participant from the date of premature withdrawal / last contact.

The reasons for premature withdrawal from the study include:

- Adverse event
- Death
- Withdrawal of consent
- Lost to follow-up
- Administration reason
- Protocol deviation
- Other

NOTE: Before entering any alternate category as the reason for the participant's discontinuation from the study, the investigator will make every effort to investigate whether safety concerns (adverse event or death) may have been related to the participant's discontinuation from the study. In case of associated safety concerns, this must be described on the Termination electronic Case Report Form (eCRF) page, even if it is not the primary reason for the participant's discontinuation.

Adverse event (AE) as reason for premature study withdrawal:

For any participant withdrawing from study participation prior to Visit 5 (study completion visit), it is important to determine if an AE was associated with the reason for discontinuing the study. This AE must be identified on the eCRF page by indicating "Withdrawn from study due to AE".

Death as reason for premature study withdrawal:

For any participant withdrawn from study participation due to death, this should be noted on the Termination eCRF page and the associated SAE that led to the death must be reported.

Withdrawal of consent as reason for premature study withdrawal:

Participants can withdraw consent for participation in the study at any time without penalty or loss of benefit to which the participant is otherwise entitled.

Reason for early termination will be deemed as "withdrawal of consent" if the participant withdraws from participation due to a non-medical reason (i.e., reason other than AE). If the participant intends to withdraw consent from the study, the investigator will clarify if the participant will withdraw completely from the study or will continue study participation for safety or a subset of other study procedures. If complete withdrawal is specified, no further study interventions will be performed with the participant.

Date of Participant Termination:

The date of termination is the date of the last contact (clinic or home visit or telephone contact) in which the participant's health status was assessed or, in cases where the participant does not agree to any further safety follow-up; it is the date consent is withdrawn.

Lost to follow-up as reason for premature study withdrawal:

For participants who fail to show up for scheduled visits (clinic or home visit), study staff will be expected to make at least three documented attempts to contact the participants and encourage the completion of study termination procedures. These efforts to contact the participants will be recorded in the source documents. The termination date for the participant to be captured on the Termination eCRF page is the date of the last successful visit (clinic or telephone) with the participant.

Administrative reason as reason for premature study withdrawal:

For participants who are withdrawn from the study due to sponsor decision (e.g., meeting, prespecified withdrawal criteria or termination of study by the sponsor), this reason should be noted in the Termination eCRF page and any ongoing AEs at the time of study withdrawal must be followed until resolution/stabilization.

Protocol deviation as reason for premature study withdrawal:

In general, participants associated with protocol deviations may remain in the study unless continuation in the study jeopardizes the participant's health, safety, or rights. For participants who are withdrawn from the study due to receipt of an excluded medication/vaccination or due to significant protocol non-compliance, this reason should be noted in the Termination eCRF page.

Any ongoing AEs at the time of study withdrawal will be followed until resolution/stabilization.

If a participant is withdrawn prematurely from the study for a reason other than those outlined above, this reason must be documented in the Termination eCRF page.

Participants who are withdrawn from study treatment will be encouraged to continue in the study for safety follow-up and other procedures as appropriate until the scheduled termination visit (Visit 5). Withdrawn participant will not be replaced.

The sponsor, coordinator, or the investigator (following consultation with the sponsor) has the right to discontinue this study at any time. If the clinical study is prematurely terminated, the investigator is to promptly inform the study participants and local ethical committee (EC/IRB) and should assure

appropriate therapy and follow up for the participants. All procedures and requirements pertaining to the archiving of study documents should be followed. All other study materials (unused treatments etc.) must be returned to the sponsor.

6.5 Prior and Concomitant therapy

6.5.1 Prior Medications and Vaccines

These include any investigational or non-registered product (drug or vaccine) received prior to enrolment. If these medications were administered to the participant within the specified window period (14 days) prior to treatment, they must be recorded on the Concomitant Medications page of eCRF.

6.2.2 Concomitant Medications and Vaccines

At each study visit, the investigator will ask the participants about any prescription or over-the counter medication(s) taken since the last visit. Any medications taken at any time during the study period must be recorded on source documents and the case report form with trade and/or generic name, indication, dose, start and end dates. Any treatments and/or medications specifically contraindicated, e.g., any investigational or nonregistered product, any antipyretic/analgesic will be checked at each study visit during treatment period. If any became applicable during the study, it would not require withdrawal of the participant from the study but may determine a participant's evaluability in the per protocol analysis.

7.0 ASSESSMENT OF SAFETY

Specification of safety parameters

Safety assessment includes occurrence of solicited systemic adverse reactions (Side or adverse effects), and unsolicited adverse events (AEs), including side effects leading to severe adverse effects and withdrawal, within 35 days after treatment administration.

7.1 Solicited and Unsolicited Safety Measurements

Solicited side adverse effects: The term "side effects" refers to signs and symptoms occurring as unintended clinical effects of the treatment whether or not they are harmful (adverse).

Unsolicited Adverse Events: An adverse event (AE) is any undocumented untoward or unforeseen medical occurrence in a participant or clinical investigation participant administered a pharmaceutical product at any dose that does not necessarily have a known causal relationship with this treatment. This definition includes intercurrent illnesses or injuries and exacerbation of pre-existing conditions. In this study, no severe adverse side effects are expected from the treatment, given that Artemisia herbal teas have been used in communities for malaria prevention with no reported toxicity (29). However, since toxicity is always a major concern in clinical trials involving humans, each study participants will be monitored for adverse side effect or adverse events daily, throughout the study period (within 35 days of treatment administration) and where necessary adequate intervention will be provided. Treatment safety data will be collected continuously by the site staff during site and home visits and recorded in

the predefined visit worksheets at Visit Day 7, 14,28 and 35 (i.e, adverse effects). As a consistent method for collecting unsolicited ASE and AEs in this study, the participant will be asked a non-leading question such as: *'Have you acted differently or felt different in any way since receiving the treatment, or since the last study visit?'* Occurrence of any adverse side effects or AEs visit within 35 days of treatment will be assessed by healthcare professional at site or home, meanwhile the site staff will continue contacting the participant daily until effects resolution.

When an AE/ASE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital records, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an unsolicited AE/SAE on the eCRF or SAE Report screens as applicable. The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/ASE and not the individual signs/symptoms. The severity of AEs will be determined by the investigator and graded from Mild (Grade 1) to Death (Grade 5) based on the *Division of AIDS (DAIDS) Table for Grading the Severity of Adverse Events* (37).

The relationship of the study treatment to an AE will be determined by the investigator based on the following definitions:

1. Not Related: The AE is not related to an investigational treatment if there is evidence that clearly indicates an alternative explanation. If the participant has not received the treatment, the timing of the exposure to the treatment and the onset of the AE are not reasonably related in time, or other facts, evidence or arguments exist that reasonably suggest an alternative explanation, then the AE is not related.

2. Related: The administration of the investigational treatment and AE are considered reasonably related in time and the AE could be explained by exposure to the investigational treatment or by other causes.

The relationship of the study treatment to an unsolicited AE will be determined by the investigator.

The causality assessment is made based on the available information at the reporting time point and assessment of causality can change according to follow-up information.

The actions taken in response to an unsolicited AE will be coded as shown below. One or more of these actions may be selected:

0 - No action taken

1 - Study treatment temporarily interrupted

2 - Study treatment permanently discontinued due to this adverse event

3 - Concomitant medication taken

4 - non-drug therapy given

5 - Physician visit

6 - Hospitalization/prolonged hospitalization

Outcome of any unsolicited AE reported during the study period will be assessed as:

- Recovered/resolved
- Recovering/resolving
- Not recovered/not resolved
- Recovered with sequelae/resolved with sequelae
- Fatal (SAEs only)

Treatment of any AE is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of an AE should be recorded in the participant's eCRF.

7.1.1 Serious Adverse Events

A serious adverse event (SAE) is defined as any untoward medical occurrence that at any dose results in one or more of the following:

- Death.
- Is life-threatening (i.e., the participant was, in the opinion of the investigator, at immediate risk of death from the event as it occurred); it does not refer to an event which hypothetically might have caused death if it were more severe.
- Required or prolonged hospitalization.
- Persistent or significant disability/incapacity (i.e., the event causes a substantial disruption of a person's ability to conduct normal life functions).
- An important and significant medical event that may not be immediately life threatening or resulting in death or hospitalization but, based upon appropriate medical judgment, may jeopardize the participant, or may require intervention to prevent one of the other outcomes listed above.

Adverse events which do not fall into these categories are defined as **non-serious**.

All Severe AEs will be evaluated by the investigator for relationship of the event to study treatment.

The relationship of the study treatment to an SAE will be determined by the investigator based on the following definitions:

- Related/suspected : the SAE is judged by the investigator to be related to the trial treatment ;
- Not Related : the SAE is not related the treatment has not yet been consumed, **or** the occurrence of the SAE is not reasonably related in time, **or** the SAE is considered unlikely to be related to use consumption of the treatment i.e., there are no facts (evidence) or arguments to suggest a causal relationship.

7.1.2 Methods for Assessing and Recording AEs and Serious AEs (SAEs)

The period of observation for AEs extends from the time the participant signs informed consent until he or she completes the final study visit (Visit 5) or terminates the study early. AEs occurring after the informed consent form is signed but prior to receiving study treatment will be documented as an adverse

event. However, AEs occurring prior to receipt of any treatment will be considered separately from "treatment emergent" AEs.

All AEs meeting criteria for reporting, regardless of severity, will be monitored by the investigator until resolution or stabilization. All participants experiencing AEs - whether considered associated with the use of the study treatment or not - must be monitored until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report should be supplied, if possible. All findings must be appropriately reported on the eCRF, which is part of the Investigator's Site File.

All findings in participants experiencing AEs must also be reported in the participant's medical records.

All severe AEs must be reported **within 24 hours of the site becoming aware of the event** by telephone or email to Project coordinator/Sponsor. If the investigator does not have all information regarding a SAE, the SAE should still be reported within 24 hours. Once additional relevant information is received, the SAE form should be updated **within 24 hours,** including an assessment of causality. Any medication or other therapeutic measures used to treat the AE will be recorded on the appropriate eCRF(s) in addition to the outcome of the AE.

After receipt of the initial report, representatives of sponsor will contact the investigator if it is necessary to obtain further information for assessment of the event.

All SAEs must be reported by the investigator to his/her corresponding EC/IRB or applicable regulatory authorities in accordance with institutional policy/regulatory requirements and adequate documentation of this notification must be provided to the sponsor.

The Sponsor and its designee must also comply with the applicable regulatory requirement(s) related to the reporting of unexpected serious and non-serious adverse drug reactions (also referred to as "SUSARs") to the regulatory authority(ies) and the IRB/EC. If a SUSAR or other

safety signal relating to use of any of the study treatments is reported to the sponsor or its designee, the sponsor will communicate the information to the investigator and the investigator will be responsible for submitting this information to the EC/IRB and other relevant authorities.

7.2 Safety Laboratory Measurements

This study has no safety laboratory measurements.

7.3 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be set up for periodic review of safety data during the study conduct. The composition and responsibilities of DSMB members (Medical Monitoring/ audit team) are presented in Table 1 and section 9.3, respectively.

7.4 Study pause

Pause rules will pause or halt further treatment and participants already enrolled will continued to be followed for safety during the pause. The study will be paused if there are any suspected adverse reactions where 1 or more participants experience any treatment-related Grade 4 AE or SAE.

8. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

8.1 Sample size calculations

As a pilot study, we will recruit 8 asymptomatic plasmodium carriers per study-arm in the 6 trial arms and 4 participants per arm in the 2 control (placebo) arms. Therefore 56 positive participants will be enrolled per site, making 112 participants in the whole study. To reach the required number of 56 gametocyte carriers, 635 persons will be RDT tested in Buea (south west region) where the expected positive RDT was 9.8% in Demographic health survey (DHS) 2018 (34) and drop-out rate was set at 10%. In Huye (South region) where the expected positive RDT was 6.9% in Malaria indicator survey (MIS) 2017 (35) and drop-out rate was set at 10%, 432 persons will be RDT tested to reach the required number of 56 gametocyte carriers.

8.2 Analysis Populations

8.2.1 Enrolled Population: All screened participants who provide informed consent and receive a participant ID, regardless of the participant's randomization and treatment status in the trial.

8.2.2 Exposed Population: All participants in the enrolled population who receive a treatment dose.

8.2.3 Trial Populations: Treatment trial analyses will be performed on both the Full Analysis (FA) Population and Per Protocol (PP) Population.

Full Analysis Population: All participants in the enrolled population who were randomized, received a study treatment dose, and provided an evaluable blood sample at least at one time point post-treatment.

Per Protocol Population: All participants in the FA Population who correctly received all doses of study treatment per randomization with no major protocol violations that are determined to potentially interfere with the reactivity assessment of the study treatment. This population will serve as the primary analysis population for all efficacy objectives. Due to unpredictability of some irregularities, the criteria for exclusion of participants from the Per Protocol Population will be determined before the database is locked and will be based on the blind review of protocol violations.

8.2.4 Safety Population

All participants in the enrolled population who received a study treatment and had any safety data available.

Participants will be analyzed as "treated" (i.e., according to the actual treatment received). All safety analyses will be performed using this population. The denominators for different safety endpoints may vary according to the number of participants with available data for the specific endpoint.

8.3 Analysis Plan

- **Data management and analysis:** All data will be entered into an Excel spreadsheet and then analyzed STATA 17 software packages. Descriptive statistics will be used to redistribute the treatment outcomes in the various treatment arms according to the evaluation times**.**

Quantitative variables will be summarized using means and medians followed by their standard deviations (SD) and Interquartile range (IQR) respectively, while qualitative variables will be summarized as percentages. Groups will be compared using a linear mixed model and trends over time will be compared using F-tests at 95% CI and statistical significance set at $P < 0:05$.

9. QUALITY CONTROL AND QUALITY ASSURANCE

9.1 Pre-study Documentation

Prior to enrolment of participants at the study site, specific regulatory documents must be available, such as Independent Ethics Committee (IECs) approvals; curriculum vitae for investigator and study staff; standard operating procedures (SOPs) and other essential documents. Sponsor/designee will inform the investigator which documents need to be provided according to the applicable regulatory requirements.

9.2 Staff training:

- All study staff including the investigators, side observers and laboratory staff will undergo a training phase including tutorials and practical, to have a good mastery of the standard operating procedures (SOPs) in the trial procedural manual before commencement of the study.
- Experienced laboratory personnel will be recruited for the different study sites and trained together, in order to permit them develop a common pattern of conducting, observing, reading and interpreting tests results, and to reduce inter observer variability in results.

9.3 Monitoring

Sponsor monitoring responsibilities will be provided through qualified and appropriately trained individuals (Medical Monitoring/ audit team) designated by the sponsor (Table 1) to carefully monitor all aspects of the study.

A site initiation visit will be conducted prior to the beginning of the study and monitoring will be conducted during and at closeout of the study by the study monitor.

During the study, the monitors will visit the clinical sites at intervals to verify that; the data are authentic, accurate and complete; the safety and rights of participants are being protected; and the study is conducted in accordance with the approved protocol (and any subsequent amendment), Good clinical practice (GCP) and all applicable regulatory requirements.

Monitors will periodically contact the site and perform site visits. The extent, nature and frequency of site visits will be decided before the start of the study and will be based on considerations as study objectives, study design and complexity, and enrolment rate.

During these contacts, the monitor will; Check and assess the progress of the study; Review study data collected and Perform source data verification to identify any issues and address their resolutions.

The individuals responsible for monitoring the study will have access to all records necessary to ensure the integrity/validity of the recorded data and will periodically review the progress of the study.

The investigator will allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the monitor to discuss findings and any relevant issues.

The monitor will contact the site prior to the start of the study to discuss the protocol and data collection procedures with the site personnel. Monitoring will be done in accordance with ICH-GCP and regulatory guidelines (38). The investigator will allow representatives of the Ethics Committee, Regulatory Authority, and the sponsor to visit the study site.

9.4 Data Management and Processing

The site PI is responsible for ensuring the accuracy, completeness, and timelines of the data reported. Data collection is the responsibility of the clinical trial staff at the study site under the supervision of the site PI. The sponsor/coordinator is responsible for data management activities, including quality review, analysis, and reporting of the study data according to SOPs.

Data Collection

Data will be captured through an observation worksheet and entered electronically by site study staff over the Internet in eCRF. The data system includes password protection and internal quality checks, such as automatic range checks to identify data that appear inconsistent, incomplete, or inaccurate. Instructions for use of the system are included in the eCRF User's guide. Clinical data will be entered directly from the source documents. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

All the information required by the study protocol must be entered into eCRF. An explanation will be provided for any missing data. Source documentation supporting the eCRF data will document the dates and details of study procedures, AEs, and participant status. The PI/site staff will maintain information in the eCRFs and all source documents that support the data collected from each participant. The study monitor will check for completeness and accuracy of eCRF during the monitoring visits.

Data Management Procedures

The site staff should complete the eCRFs as soon as possible after the information is collected. Completed eCRFs must be submitted for each screened participant who signs the study specific informed consent form. The study coordinator/sponsor is responsible for data management activities, including quality review, analysis, and reporting of the study data according to the SOPs. Internal data quality checks such as automatic range checks, checks to identify data that appear inconsistent, incomplete, or inaccurate are programmed into eCRF that does real time review of the data as and when clinical data is entered into the system by the site staff. The Data Management team will review the data for quality and will provide several quality assurances reports to ensure that study data are clean and complete. Quality assurance reports will include, but are not limited to, the following: missing forms, missing values and out of range values, automated data queries, and manual review of study data. Data queries will be distributed to the sites at scheduled time periods for the site staff to review and update the database.

Coding: All medical verbatim terms will be coded by a medical doctor according to MedDRA (adverse events, medical history, and concomitant diseases) (39) and the WHO Drug Dictionary enhanced version (concomitant medication) (40).

Database Lock Procedures

Database will be locked upon completion of the following activities:

- All participants have completed the follow up visits
- All the participants data have been entered in the database
- All data anomalies have been resolved
- Study monitoring has been completed
- All the listings of the database have been reviewed and discussed for assessment of consistency and medical plausibility.

Procedures for Analysis

The data will be analyzed after the database lock, as per the pre-specified Statistical Analysis Plan (SAP) in section 8.2 and 8.3 above**.** An audit trail will be kept of all subsequent changes to the data.

9.5 Study and Site Closure

Upon completion of the study, the monitor and the investigator will conduct the following activities:

- Data clarification and/or resolution
- Accounting, reconciliation and return to coordinators or destruction at sites of used and unused treatments.
- Review of site study records for completeness
- Return of all study data to Sponsors or designee.

Sponsors reserve the right to temporarily suspend or prematurely discontinue this study at any time for any other reason. If the study is stopped or suspended prematurely, Sponsor will inform the investigator(s) as well as the regulatory authorities about the decision and the reasons for termination or suspension. If such action is taken, all effort must be made to ensure the safety of the participants enrolled in the study. The investigator(s) will inform the responsible IECs/IRBs and provide the reason for the suspension or termination. In case of premature study or study site closure, the monitor will conduct all activities as indicated above.

9.6 Audits and Inspections

For the purpose of compliance with ICH-GCP and regulatory guidelines, it may be possible that the sponsor/designee or a national regulatory authority conduct a site audit/inspection. This may occur at any time from start to after conclusion of the study. The investigator will allow the auditor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor to discuss findings and any relevant issues.

If a regulatory authority requests an inspection, the investigator must inform the sponsor or its designee immediately about this request. The investigator(s) and the study coordinator(s) must make the relevant records available for inspection and must be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies. The sponsor will provide any needed assistance in responding to regulatory audits or correspondence.

10. REGULATORY AND ETHICAL REQUIREMENTS

10.1 Ethics committee review and communication

The investigators will ensure that this protocol is reviewed and approved by the Independent Ethical Committees (IECs) responsible for the study sites, including the Informed Consent Form and any other written information to be provided to the participant. Written IECs approval shall be obtained prior to study start. No deviations from or changes to the protocol shall be initiated without prior written IEC approvals of an appropriate amendment, except when necessary to eliminate immediate hazards to the participants or when the change(s) involves only logistical or administrative aspects of the study (e.g., change of monitor(s), telephone number(s)).

10.2 Protocol Amendments

Any significant change in the study protocol shall be addressed in a written protocol amendment, which will be signed by the investigator(s) and the sponsors. The investigator will submit protocol amendments to the IECs and obtain written approval where required. A protocol amendment may be implemented only after it has been approved by IECs. In the case of a protocol change intended to eliminate an apparent immediate hazard to participants, the change may be implemented immediately. In this case, the change will be later documented in an amendment and reported to the IECs as soon as possible. Logistical and administrative amendments (e.g., concerning a change of telephone number) shall be submitted to the IECs for information purposes. However, the investigator will provide the sponsors with written verification that such logistical or administrative amendments are submitted to the relevant IECs.

10.3 Participant Information and Informed Consent The investigator or his/her designee shall describe the study to the participants and inform them of all pertinent aspects pertaining to the study. The investigator shall give the participant ample time and opportunity to inquire about details of the study and ask any questions in the site procedures.

Original ICF must be kept on file by the investigator for possible inspection by IEC members, regulatory authorities, and the sponsors (or their designees). The participant will receive a copy of the signed ICF, and any subsequent updates or amendments. Prior to including any participant in the clinical study or any study related procedure, his/her free and expressed informed consent must be obtained in writing, and the written informed consent will be signed and dated by both investigator/designee and the participant.

10.4 Participant Confidentiality

The investigator(s) will ensure that participant confidentiality is maintained. Personal identifiers will not be included in any study reports. The participants will be identified by the participant code number (participant’s ID). The participant's name will not be mentioned on the study sample. The number that will identify the participant is configured by:

A digit representing the study site (1 for Cameroon and 2 for Rwanda)

A 2-digit number representing the province of origin (01-10 for nationals and 11 for foreigners)

A digit representing the gender of the participant (1 for male and 2 for female)

A 2-digit number corresponding to the calendar week

A 2- digit number representing last 2 digits of year of collection

A 2-digit control number corresponding to the serial registration number for randomization (allocated by investigator)

The participant's decoding scheme will be accessible only to the investigator. Study findings stored on a computer will be subject to local data protection laws. The participant will be informed that representatives of the sponsor, IEC, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in the strictest confidence.

10.5 Ethical conduct of the study

This study will be conducted in compliance with the approved clinical trial protocol, IEC , ICH-GCP, and the Helsinki's declaration (37, 39).

11.6 Risk and benefits of the study

Minimizing Risk.

Risks from blood draw

Drawing peripheral blood can cause discomfort; it might cause minor bleeding and/or bruising where the needle enters the skin, and very rarely might cause infection. This risk will be mitigated by ensuring that only study staff members who are adequately trained in safe drawing of blood conduct this portion of the study.

Risks from participating in a clinical trial.

In addition to the risks described above, all clinical trial participants are likely to experience increased inconvenience due to the visits as they may be of longer duration than standard treatment, which might cause more anxiety. They also incur additional risks to their privacy from participating in a clinical trial. This risk will be minimized by ensuring that participant confidentiality is maintained by enforcing appropriate data collection, storage, and analysis techniques and employing the use of unique identifiers with carefully stored linking files. There

may be additional risks to study trial participation that we do not know about.

11. DATA HANDLING AND RECORD KEEPING

Investigators must retain all study records required by Sponsors and by the applicable regulations in a secure and safe facility. The investigator must consult a sponsor's representative before disposal of any study records and must notify the sponsor of any change in the location, disposition, or custody of the study files. Essential documents must be retained until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

"Essential documents" are defined as documents that individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced (e.g., signed protocol and all amendments; ethics committee approval for the study protocol and all amendments; all source documents; eCRF records; participants' Informed Consent etc.). These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained. The documents should not be destroyed without the written permission from the sponsor. These principles of record retention will also be applied to the storage of laboratory samples, provided that the integrity of the stored sample permits testing.

12. INSURANCE OF STUDY PARTICIPANTS

- **Participant**s in this study will be compensated for their time in this study and be reimbursed for travel to attend study visits.
- Study participants will not be charged for study treatment, research clinic visits, research-related examinations, or research-related laboratory tests.
- All the study participants will be insured against any injury caused by any AEs causally related to the study investigational product (if any) and the cost of needed medical care related to the AE will be borne by sponsor and as required.
- All study participants will be covered by the Sponsor for any illness or condition diagnosed during trial participation. For these events, the study site medical team will attend to the participant following the local standard of care and the national guidelines, free of charge to the participant for the duration of the study. At the end of the study, in case of further treatment required, the study team will provide adequate referrals in the health care system, but further treatment will not be covered.

13 PUBLICATION POLICY & CONFIDENTIALITY

Th project coordination team and members hold the exclusive rights to publish the study results. Due credit will be given to the investigators and their team in case the results of the study are published.

All proprietary or confidential information communicated to the investigator by the coordinators or communicated to the investigator during the course of and/or as a result of the clinical study is the exclusive property of Sponsors, and the investigator shall ensure that the same shall be kept strictly confidential by him/her and any other person connected with the clinical study and shall not be disclosed, either orally or in written form, by him/her or such person to any third party without the prior written consent of Sponsors.

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