

**Contributions to the elimination of asymptomatic *Plasmodium*
reservoirs in malaria endemic areas of Cameroon using
Artemisia afra tea infusions**

Thesis submitted in fulfillment of the requirements for the degree of

“Doctor in Medical Sciences”

Option: Epidemiology and Clinical Research

by

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Dedication

To my mum, NDI Veronica Alenwi

To my husband, Emmanuel

To my children Alenwi, Ada'anwi, Afanwi, Akaanwi, and Afu'unwi

To our families

Dedication

ACKNOWLEDGEMENTS

My immense gratitude and appreciation go firstly to my supervisor Pr. Annie ROBERT for believing in me and giving me an opportunity to pursue a PhD under her supervision. Dear professor, words cannot express my heartfelt appreciation to you for your support, step-by-step guidance, patience, dedicated concern, and encouragement to me throughout this PhD Journey. I am profoundly grateful.

I am sincerely thankful to my steering committee and jury members for the genuine feedback and valuable recommendations which have not only improved my PhD thesis but have groomed me to become the charismatic researcher that I am today.

I am grateful to ARES-CCD for offering me a scholarship through the Research Project for Development - PRD-2020-CMR, to conduct my research work. Special thanks to Prof. Jacob Sououpgui, Prof. Stephen Ghogomu, Pr. Annie Robert and Prof. Michel Frederich, for initiating, coordinating, and ensuring the success of this project.

Great thanks to all the staff of the ADRI of UCLouvain for their administrative assistance.

I am thankful to the PRD-2020-CMR project team members; Dr Tallom, Dr Shey and Joseph Kamga, as well as my mates Methodius, Sandra, Sylvie, Edga, and Sylvia for their collaboration and support during this Journey.

Acknowledgements

Many thanks to my dear colleagues of the EPID family, Thai, Dalau, Marcelo, King, Kelly, Celestin, Bernadette, Daniel, Zango, King, Jorge, Nina, Moussa and Lena their support and the beautiful moments we shared.

I am greatly indebted to my model of a husband, and “Full option” - Dr Emmanuel AKONGNWI MUGYIA, for standing close, teaching and encouraging me, and happily “mothering” the family throughout this period. I bless the day God brought us together; He certainly knew the kind of woman He was molding me to become and saw you as a trusted man to stand my big dreams. Thank you for your love and patience, darling.

I am thankful to my family, especially my mum, my mother-in law, siblings and co-wives for their inestimable and continuous love, care, support, and encouragement during my studies abroad.

Special thanks to Mrs Kathy Goodwin who sponsored my primary and secondary education through Rev.Fr. Engelbert Kofon (RIP).

Above all, I return all Glory to the Almighty God for this dream come true. It can only be you Lord.

SUMMARY

Malaria remains an important infection in sub-Saharan Africa due to the challenge of controlling and eliminating the disease. WHO has continually advocated for mobilization and expanded actions towards malaria elimination in the continent, with recommended control and elimination strategies. These strategies are currently implemented in Cameroon and most African countries, with focus on vector control, diagnosis and treatment of symptomatic cases, as well as chemoprophylaxis for under-five children and pregnant women. However, these actions alone are not sufficient to eliminate malaria from many endemic regions as they fail to target asymptomatic *Plasmodium* (gametocyte) carriers who constitute most (> 95%) of infectious human reservoirs in endemic settings and perpetuate the disease transmission. The purpose of our study was to contribute to the elimination of asymptomatic *Plasmodium* reservoirs in malaria endemic areas of Cameroon using *Artemisia afra* tea infusions. Specifically, we explored the experiences of adult Cameroonians on the use of herbal teas to treat infections or diseases, and their acceptability to use the teas if prescribed in hospital. We also assessed the knowledge of health care workers about asymptomatic malaria and their acceptability to use *Artemisia afra* tea to treat asymptomatic *Plasmodium* carriers, and we designed a trial aimed at ascertaining the efficacy and safety of *Artemisia afra* tea infusions in emptying *Plasmodium* reservoirs.

Summary

We analyzed the cross-sectional data captured in hospitals and communities using semi-structured questionnaires. Our findings showed a high prevalence of herbal tea use among Cameroonian adults in communities and hospitals in the survey settings, with a positive opinion and willingness to accept herbal teas prescribed in hospitals. Health care workers had a fair knowledge about asymptomatic malaria with an important gap in their knowledge of transmission. Most health care workers had a positive opinion and willingness to use *Artemisia afra* tea if approved by authorities. Nursing assistants had less knowledge about the existence of asymptomatic malaria compared to other higher professional categories. Health care workers with shorter periods of service had less knowledge about transmission compared to those with longer periods of service.

The findings raise concerns about a high trust and consumption of herbal teas from unverified sources by participants, and a probable use of teas alongside allopathic medication prescribed in hospital. They equally raise concerns regarding the high level of belief and positivity of healthcare workers about the effectiveness of *A. afra*, in the absence of clinical data. Authorities must ensure the effectiveness and safety of herbal teas before approval for use in health facilities. There's a need to always check for use of concomitant medication, educate against use of unverified tea treatments from unknown sources, ensure effectiveness and safety of teas served in health facilities and communities, and a need to organize refresher trainings for health care workers on malaria.

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LIST OF ABBREVIATIONS

ACPR	Adequate clinical and parasitological
ACT	response
ASM	Artemisinin-based combination therapy
APC	Asymptomatic malaria
AL	Asymptomatic <i>Plasmodium</i> carrier
ANC	Artemether-lumefantrine
AS-AQ	Antenatal care
AS-MQ	Artesunate-amodiaquine
AS-PY	Artesunate-mefloquine
AS-SP	Artesunate- pyronaridine
CDC	Artesunate-sulfadoxine / pyrimethamine
CENAME	Centers for Disease Control and Prevention Centrale Nationale d'Approvisionnement en Médicaments Essentiels / National Center for the Supply of Medicines and Essential Medical Supplies
DHA-PPQ	Dihydro-artemisinin -piperaquine
DHS	Demographic and Health Survey
DHIS 2	District Health Information System 2
DNA	Deoxyribonucleic acid
ETF	Early treatment failure

List of abbreviations

G6PD	Glucose-6-phosphate dehydrogenase
HbC	Haemoglobin C
HbF	Haemoglobin F
HbS	Haemoglobin S
HM	Herbal medicine
HRP	Histidine-Rich Protein
IPTp	Intermittent preventive treatment for pregnant women
IPTi	Intermittent preventive treatment for infants
iCCM	Integrated community case management
IRS	Indoor residual spraying
ITN	Insecticide-treated mosquito net
IRB	Institutional review board
LAMP	Loop Isothermal Mediated Amplification
LLINS	Long-lasting insecticidal nets
LPF	Late parasitological failure
LSM	Larval source management
LTF	Late treatment failure or late clinical failure)
MDA	Mass drug administration
MSP2	Merozoite surface protein 2
NMCP	National Malaria Control Programme
PADY	Projet d'Assainissement de Yaoundé
PCR	Polymerase Chain Reaction
Pf	<i>Plasmodium falciparum</i>

List of abbreviations

PfEMP1	<i>P. falciparum</i> erythrocyte membrane protein 1
PKPD	Pharmacokinetics and pharmacodynamics
pLDH	Parasite-specific lactate dehydrogenase
PMI	President's Malaria Initiative
RBC	Red blood cell
RDT	Rapid diagnostic test
rt-qPCR	real-time quantitative Polymerase Chain Reaction
SMC	Seasonal malaria chemoprevention
SP	Sulfadoxine-pyrimethamine
SP-AQ	Sulfadoxine-pyrimethamine and amodiaquine
TCAM	Traditional, complementary and alternative medicine
TES	Therapeutic efficacy study
TM	Traditional medicine
UCLouvain	Université catholique de Louvain
ULB	Université Libre de Louvain
ULg	Université de Liège
UNICEF	United Nations Children's Fund
USAID	United States Agency for International Development
WHO	World Health Organization

List of abbreviations

CHAPTER 1: INTRODUCTION

1. Introduction

1.1 Overview of malaria

1.1.1 Definitions

Malaria is an infection; it is a preventable and curable vector-borne disease caused by the *Plasmodium* parasite. Parasites are transmitted to people through the bite of an infected female *Anopheles* mosquito (1). According to WHO, a malaria case is defined as an occurrence of malaria infection in a person in whom the presence of malaria parasites in the blood has been confirmed by a diagnostic test (2). A malaria case can be classified as symptomatic or asymptomatic (with or without symptoms). In malaria control settings, a “case” is the occurrence of confirmed malaria infection with illness or disease (symptoms). In settings where malaria is actively being eliminated or has been eliminated, a “case” is the occurrence of any confirmed malaria infection with or without symptoms (2).

1.1.2 Parasites

There are five *Plasmodium* parasite species that cause malarial infections in humans namely, *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*. Of these species, *P. falciparum* and *P. vivax* pose the greatest threat. *P. falciparum* is the most dangerous (deadliest), and the most prevalent on the African continent, responsible for majority of cases and malaria-related deaths (especially under five children), owing to its ability to cause cerebral impairment and kidney injuries (3,4). *P. vivax* is dominant in most countries outside of sub-

1. Introduction

Saharan Africa. *P. vivax* (as well as *P. ovale*) has dormant liver stages (“hypnozoites”) that can activate and invade the blood (“relapse”) several months or years after inoculation. Thus, the elimination of *P. vivax* malaria is particularly challenging and may require new tools and strategies in some settings (2,4). While four of the human *Plasmodium* parasite species are transmitted between humans by mosquito vectors belonging to the genus *Anopheles*, *P. knowlesi*, which typically infects forest macaque monkeys, was recently reported to infect humans in South-East Asia with increasing frequency, but there is no evidence of human-to-human transmission (currently considered as a zoonotic disease) (5).

1.1.3 Vectors

The malaria vector is the female anopheles mosquito. Of the 465 formally recognized species of the *Anopheles* genus, approximately 70 are capable of transmitting malaria parasites to humans and 41 are considered as dominant vectors of major concern to public health. In Africa where malaria prevalence is the highest, the main species belong to the *Anopheles gambiae* complex and to the *Anopheles funestus* group (Figure 1) (6). Multiple species can coexist within one geographical area, each with its own biting and resting pattern and preferred human or animal host; thus, species vary widely in their transmission efficiency and in their susceptibility to existing or potential anti-mosquito interventions.

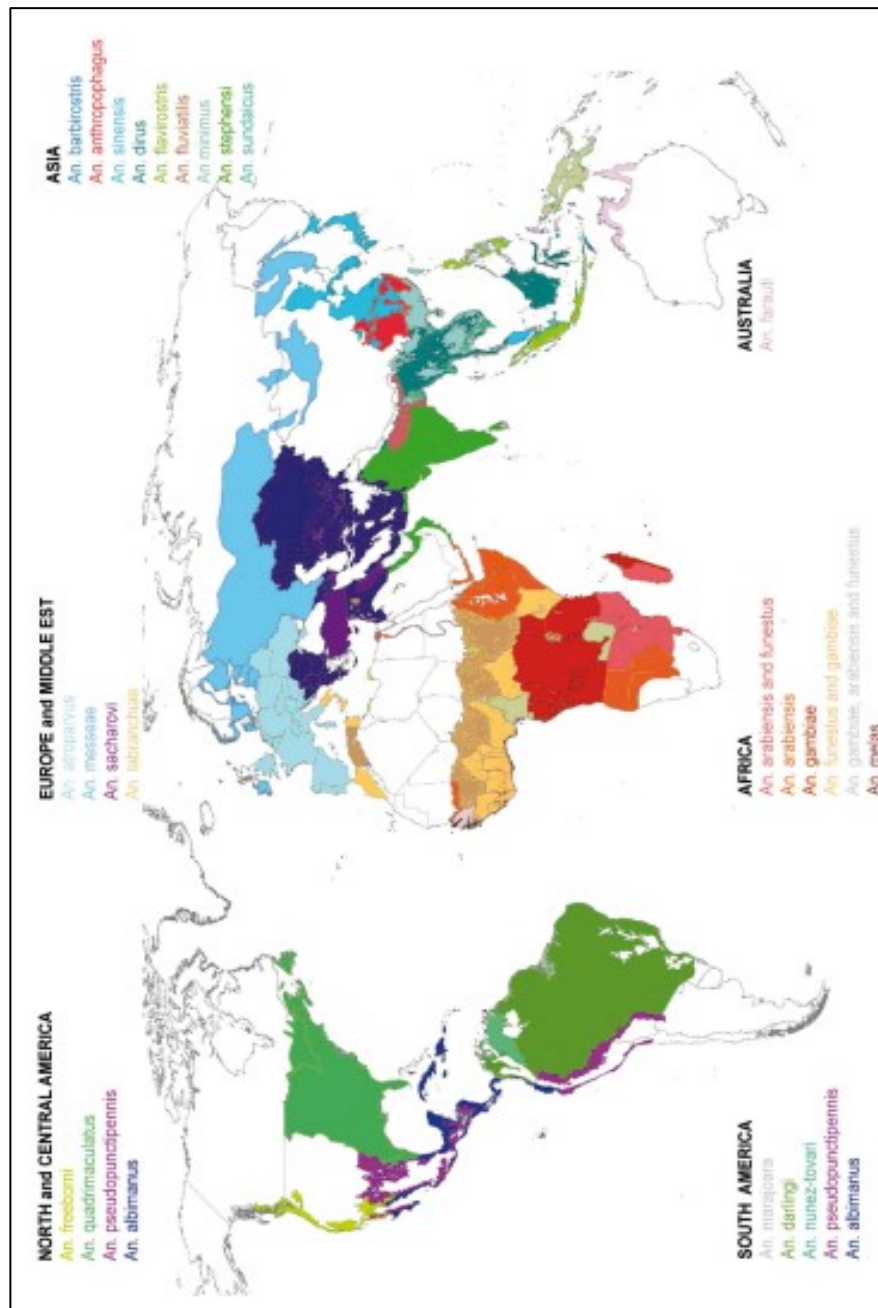
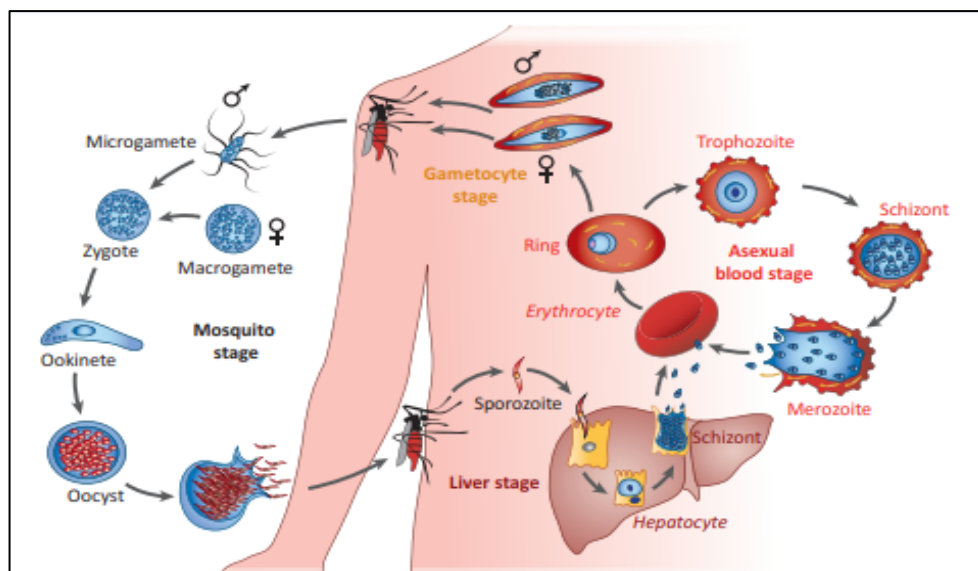


Figure 1: Distribution of predominant malaria vectors; **Source:** Rossati et al, 2016 (6) https://www.infezmed.it/index.php/article?Anno=2016&numero=2&ArticoloDaVisualizzare=Vol_24_2_2016_93

Life cycle of *Plasmodium* parasite

The life cycle of the malaria parasite involves two hosts, the human host (where asexual reproduction occurs) and an invertebrate host (where sexual reproduction occurs). During a blood meal in order to nourish her eggs, a malaria infected female *Anopheles mosquito* injects sporozoites into the blood stream of a human victim and the sporozoites reach liver cells within 30-60mins. In the hepatocytes, the sporozoites develop to schizonts by an asexual multiplication called schizogony, and these schizonts release merozoites in the hepatic circulation within 6-7 days. The merozoites quickly invade the erythrocytes and destroy the erythrocyte membrane leading to explosive release of parasites (merozoites) to access new host cells for invasion. The duration of the asexual schizogonic cycle differs between some human *Plasmodium* species: 48-50 hours in *P.vivax*, *P.ovale*, and *P. falciparum*, but 72 hours in *P. malariae*. This duration gives the rhythm of the pattern of fevers observed with parasite species. Symptoms develop 4–8 days after the initial red blood cell invasion. During the rounds of schizogony in the blood stream, some of the parasites undergo a developmental switch and differentiate into male and female gametocytes. These gametocytes in the bloodstream are ingested by the female *Anopheles* mosquito during a blood meal to continue the cycle in the mosquito. In the mosquito, the microgamete (male) and macrogamete (female) fecundate to produce a diploid zygote, which develops into ookinetes and then oocysts. The oocysts, develop into sporozoites, migrate to the

salivary glands of the female *Anopheles* mosquito ready to continue the cycle of transmission back to man during a blood meal (Figure 2). Thus, 7–10 days after the mosquito feeds on blood containing gametocytes, it may be ‘armed’ and able to infect another human with *Plasmodium* with her bite. In *Plasmodium vivax* and *Plasmodium ovale*, a proportion of the liver-stage parasites (hypnozoites) remain dormant in the hepatocytes. In this stage, the parasite can remain dormant for months or several years. These two species of parasite can therefore initiate a cycle of asexual reproduction causing clinical symptoms in the absence of a new mosquito bite, giving *P. vivax* infection the name relapsing malaria (1,4,6,7,8).



Source: Maier et al, 2019 (8)

1.1.4 Pathophysiology of malaria

Malaria infection refers to the presence of the malaria parasite (*Plasmodium*) in blood with or without symptoms. Infection with malaria parasites may result in a wide variety of manifestations, ranging from absence of clinical symptoms (asymptomatic malaria) or very mild symptoms (uncomplicated malaria) to severe disease (complicated malaria) and even death (7,9). All the clinical symptoms associated with malarial infection are caused by asexual erythrocytic or blood stage parasites. When the parasite develops in the erythrocyte, numerous known and unknown waste substances such as hemozoin pigment and other toxic factors accumulate in the infected red blood cell (RBC). These are dumped into the bloodstream when the infected cells lyse and release invasive merozoites. The hemozoin and other toxic factors such as glucose phosphate isomerase (GPI) stimulate macrophages and other cells to produce cytokines and other soluble factors which act to produce fever and rigors, and probably influence other severe pathophysiology associated with malaria (9). Hemozoin is also responsible for impaired function of immune cells (monocytes, macrophages) after they have ingested the pigment (7). Immune cells after they have phagocytosed hemozoin tend to lose their bactericidal capacity, their ability to become dendritic cells and their role as antigen presenting cells (7).

The release of hemozoin into the bloodstream follows the rupture of the red blood cells (10). This rupture is responsible for a progressive

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reduction in the number of red blood cells and for anaemia (that can range from the mild to the severe forms) observed in patients with malaria. Red blood cells rupture occurs almost synchronously and explains the periodicity of fever, which is either tertian (every 48 hours) or quartan (every 72 hours) depending on the *Plasmodium* species (1,4,7). *Plasmodium falciparum*-infected erythrocytes, particularly those with mature trophozoites, adhere to the vascular endothelium of venular blood vessel walls and do not freely circulate in the blood vessels. Cytoadhesion results in sequestration of parasitized RBCs in the capillaries and post-capillary venules thereby causing heterogeneous blockage of the microcirculation of diverse organs and subsequent organ damage (severe malaria) due to tissue hypoxia (1,11). This phenomenon of cytoadherence is possible thanks to the expression of a protein named *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) by the parasite at the parasitized RBCs. There are different subgroups of this protein with different receptors on the endothelium of the cells (7,12). The clinical manifestation reflects the subgroup of the protein and the organ implicated. Neurologic manifestations like coma involves the binding on the intracellular cellular molecule -1 and endothelial protein C in the brain (3,13), while sequestration of the parasites in the lungs could lead to respiratory distress. The microvascular occlusion and cellular damages could be worsened by the rosetting phenomenon (binding of infected RBCs to uninfected erythrocytes) (1,14). Microvascular occlusion also underlies the acute

kidney injury that may occur in patients with malaria (4).

1.1.4.1 Impact of asymptomatic parasitemia on health

Asymptomatic malaria can be caused by all *Plasmodium* species, and the infected individual usually has circulating parasites but no symptoms (7). An individual infected by *Plasmodium* can remain asymptomatic or can progress to simple or even severe malaria. In all malaria infected persons (with or without symptoms), the parasite goes through the same life cycle, morphological changes, and human – parasite interactions. Disease occurrence, thus, must be the result of exaggeration of this baseline interaction, which is neither beneficial to the parasite nor the human (4).

Although there are no strict diagnostic criteria for defining an asymptomatic infection, the absence of a fever or any other clinical symptom for at least one week, and the presence of a positive blood smear or polymerase chain reaction (PCR) test, are used to define infections as asymptomatic (15,16,17,18). Infections below detectability threshold are also considered asymptomatic.

Despite the absence of acute symptoms, chronic asymptomatic infections may have long term consequences for morbidity, particularly related to anemia (19,20,21,22,23,24) and malnutrition (25,26). It increases susceptibility to severe clinical outcomes of future infections and influences the severity and outcome of other infectious diseases (27,28,29,30). Asymptomatic infections of the placenta significantly reduce neonatal weight and chances of survival (7,31,32). In non-

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immunized individuals' asymptomatic carriage can progress to the manifestation of mild symptoms (1) or severe symptoms (33,34,35). Individuals who survive pathological consequences following severe infections, particularly cerebral malaria, may end up with variable neurological and developmental disorders (33,34,35), as well as debilitating sequelae such as epilepsy or cerebral palsy, and even blindness (36,37). More subtle consequences include growth restriction, behavioral disorders, and cognitive impairment (decline in cognitive function) (38), (Figure 3).

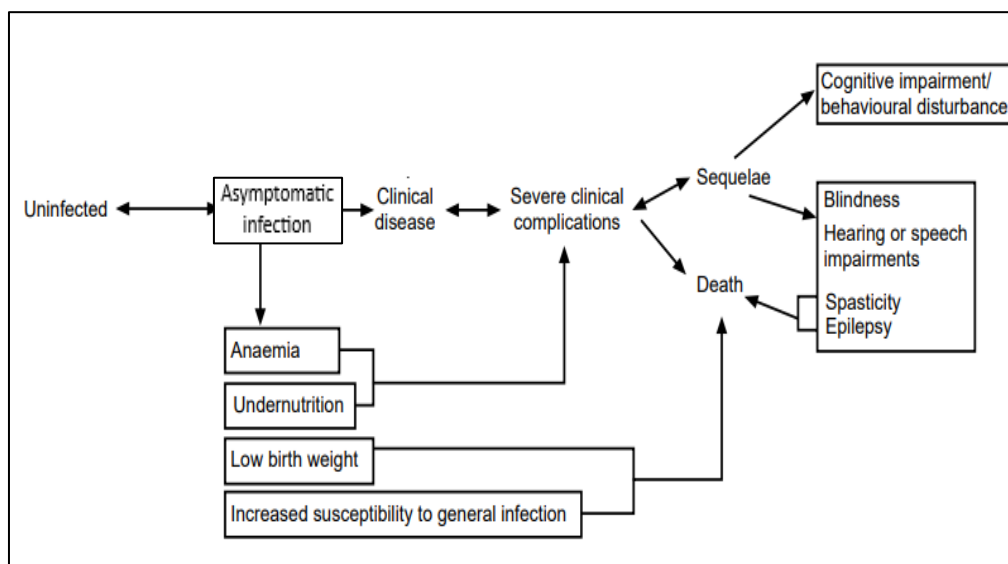


Figure 3: Pathological states of malaria infections from *P. falciparum*

Source: Adapted from Snow et al, 1999 (36).

1.1.4.2 Prevalence of asymptomatic malaria

In most malaria endemic areas, asymptomatic infections (asymptomatic *Plasmodium* carriers) constitute a majority of all malaria infections (infectious reservoirs) and only a small proportion of infected people present clinical symptoms (39,40,41,42,43). Studies have also shown that asymptomatic *Plasmodium* carriers may be more effective transmitters than clinical malaria cases (44,45,46). Asymptomatic parasitaemia often persists for months without symptoms and harbor gametocytes, that infect mosquitoes and contribute to perpetuate onward transmission (18).

From a diagnostic standpoint, asymptomatic malaria is concerned with microscopic and submicroscopic parasitaemias (detectable by molecular methods but not by microscopy), which are distributed differently based on the context of transmission (Figure 4) (18,47). In both high and low transmission settings, most of infections are asymptomatic (15,40,48). Most asymptomatic infections in low transmission settings are mainly submicroscopic parasitemia (15,40,48,49,50,51), while microscopic infections are more dominant in high transmission settings. Only microscopic infections have a high potential for infection from human to mosquitos that are vectors of malaria (15,18).

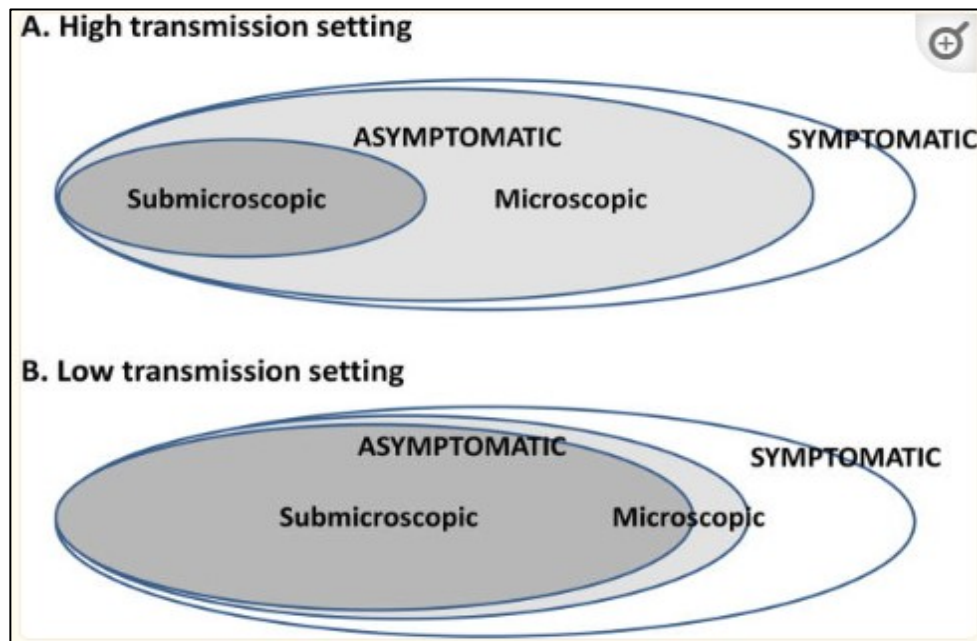


Figure 4: Distribution of asymptomatic malaria infection groups according to transmission settings;

Source : Lin, JT et al 2014 (18).

1.1.4.3 Immunity against malaria infection

The development of low-density malaria and asymptomatic infections is associated with an individual's tolerance to the *Plasmodium* parasites (52). Differences in the modulation of parasite levels or clinical expression of malaria infection in different hosts have been related to genetic factors and acquired immunity, among other factors.

Innate resistance to malaria is defined as refractory state of the host to the parasite due to its genetic constitution (53). This resistance may be specific to the plasmodial species and its evolutionary stages, including the mechanisms involved in membranous and intracellular erythrocytic

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factors. For instance, individuals with red blood cells that lack the Duffy blood group antigen (Duffy-negative) are resistant to *P.vivax* infection. The antigens are associated with determinants that constitute specific receptors for the adhesion and subsequent penetration of *P.vivax* (1,54,55,56). In addition, certain haemoglobinopathies (particularly with haemoglobin S (HbS) and haemoglobin C (HbC), as well as beta thalassemias) confer partial resistance in heterozygous individuals, with HbS (sickle cell trait) hemoglobin playing an obvious role against severe forms of malaria (57). HbC is associated with one of the clinical manifestations of malaria (58), whereas hemoglobin F (HbF) in neonates inhibits the growth of *P. falciparum* within RBCs (59). Certain human leukocytes antigens have been associated with protection against cerebral malaria and severe anemia (57). Glucose-6-phosphate dehydrogenase (G6PD) deficiency is reported to offer protection against *Plasmodium* infection (60,60), although there is no consensus in literature.

Acquired antimalarial immunity has several aspects as a result of the combined action of cellular and hormonal factors, depending on the physiological state of the host. At first, subjects (Adults and children > 5 years) living in endemic areas develop a partial protective immunity (an asymptomatic carrier state), due to repeated exposure to infectious mosquito bites. This type of immunity is unstable because it can fade after leaving the endemic area. On the other hand, passive transmission of protective antibodies from a mother to her child through

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transplacental passage or breastfeeding, confers immunity against malaria to young infants (61,62).

In high transmission areas children below 5 years of age and fetuses of infected pregnant women experience the highest morbidity and mortality of the disease. Children >6months of age are particularly susceptible because they have lost their maternal antibodies, but not yet developed protective immunity. If they survive repeated infections to an older age (2-5 years) they will have reached a protective semi-immune status. Individuals with low protective immunity against *P. falciparum* are particularly vulnerable to severe malaria (for example, individuals who live in low transmission settings, children<5years and naïve hosts) (7). Pregnant women are more susceptible to *Plasmodium* infection especially during the first and second pregnancies due to changes in women's immune systems during pregnancy and the presence of a new organ (the placenta) with new places for parasites to bind, combined with the unique ability of a subset of infected erythrocytes to sequester in the *placenta*. There is extensive evidence to confirm that antibodies directed against the surface of parasitized erythrocytes in the placenta are essential in protection, and these are usually absent in first pregnancy (63).

1.1.5 Clinical management of malaria

Malaria case management as recommended by WHO includes the diagnosis, the treatment, the clinical care, counselling and follow-up of symptomatic malaria infections. Malaria treatment must be based on parasitological evidence of the infection, on an assessment of the disease severity, and on the identification of groups at risk, as well as the use of safe and effective antimalarial drugs (64,65).

1.1.5.1. The clinical profile of malaria

The clinical course of malaria may present as uncomplicated or severe (complicated) malaria. **Uncomplicated malaria** is the presentation of symptoms of malaria with a positive parasitological test without signs of severity or evidence of vital organ dysfunction. The main manifestations of uncomplicated malaria include fever, chills, rigors, headaches, and body pains. Others are malaise, nausea, vomiting, and joint weakness. These features may occur alone or in combination, with no clinical or laboratory findings of severe organ dysfunction (1,7,66). Patients with **severe or complicated** malaria are those who present in addition to a positive parasitological test, at least one of the following WHO severity clinical or laboratory features:

- *Clinical features*: Impaired consciousness or coma, prostration, failure to feed, multiple convulsions, respiratory distress, shock, jaundice, abnormal bleeding, or pulmonary oedema.
- *Laboratory features*: Hypoglycaemia (blood glucose < 2.2 mmol/l or < 40 mg/dl), metabolic acidosis (plasma bicarbonate < 15 mmol/l), severe

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normocytic anaemia (Hb <5g/dl, packed cell volume <15%), haemoglobinuria, hyperparasitaemia (>2% or 100 000/μl), hyperlactatemia (lactate >5 mmol/l) or renal impairment (serum creatinine > 265 μmol/l).

Severe malaria is usually caused by infection with *Plasmodium falciparum*, although it can also be caused by *Plasmodium vivax* or *Plasmodium knowlesi*. All criteria to define severity are common to *P. falciparum* and *P. vivax*, except for the hyperparasitaemia where the threshold varies in different populations (1,4,7,67). Children under the age of five years, pregnant women, patients with HIV/AIDS, as well as non-immune persons (disease naïve populations and travelers), are identified as groups at risk because they are more susceptible to having severe malaria, with also an increased risk of poor newborn outcome (low birth weight, abortion) in pregnant women infected with malaria (7,63,64).

1.1.5.2 Parasitological diagnosis of malaria

Malaria diagnosis involves identifying malaria parasites (parts or products) in a patient's blood. It is therefore important to establish the presence (or absence) of the parasite as a basis for treatment decision in each individual. Clinical diagnosis therefore is not only based on the patient's signs and symptoms, but also on physical findings at examination.

▪ **Microscopy**

Malaria is conventionally diagnosed by light microscopic examination of stained blood films using Giemsa, Wright's, or Field's stains by Romanowsky technique. The light microscopic detection and identification of *Plasmodium* species in stained thick blood films (for parasite detection), and thin blood films (for specie's confirmation, estimation of parasitaemia, and stage specific identification) remains the gold standard for laboratory diagnosis (64,68,69). The light microscopy has a low sensitivity (54.2%) but a good specificity (98.3%) when compared to polymerase chain reaction (PCR) for detecting parasites in moderate to high transmission areas (70). However, light microscopy has some limitations in that it requires a skilled technician to operate and a power source.

▪ **Rapid diagnostic test**

Malaria can also be diagnosed using immunochromatographic technique also known as Rapid Diagnosis Test (RDT) method. Rapid diagnostic tests for malaria (RDT) are, with microscopy, the most widely used method of malaria diagnosis in endemic countries. These tests are used only for qualitative and not for quantitative detection of malaria parasites in human blood. The principle of RDTs for malaria are based on the immune-chromatographical detection of plasmodial proteins histidine-rich proteins (HRP-1, HRP-2, and HRP-3), and parasitic aldolase (in some tests) or parasite-specific lactate dehydrogenase (pLDH). Histidine-rich proteins of *Plasmodium*

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falciparum are soluble proteins produced by asexual stages and gametocytes of the parasite. Lactate dehydrogenase, an enzyme found in the glycolytic pathway of the malaria parasite, is produced by both sexual and asexual stages of the parasite. Different isomers of pLDH have been found in all *Plasmodium* species infecting humans and their detection constitutes another approach to RDT development (71). The widely used pLDH has shown a low sensitivity (54.8%) but a good specificity (97.9%) when compared to PCR. In a Cochrane review, recent ultra – sensible RDTs (HRP-2 targets) have shown better performances in asymptomatic individuals, with a sensitivity and specificity of 50% and 98% versus 27% and 100% for standard RDTs, respectively. In a similar way, the ultra – sensible RDTs showed a pooled sensitivity of 62% versus 36% and for standard RDTs in low transmission areas, and 62% versus 75% for standard RDTs in high transmission settings (72). These tests are cheap, fast, and allow to differentiate *Plasmodium* species at a low cost, without the need for a trained laboratory technician nor extensive equipment. However, some RDTs are prone to false negatives which may result from a prozone effect or a lack of production of the target antigen, due to a mutation of the gene coding for the HRP-2 and 3 antigens or deletion of the histidine-rich protein (*pfhrp2* and *pfhrp3*) genes in field isolates of *P. falciparum* (73,74). Further to this, the limit of detection of standard RDT is > 100 parasites/ μL for *Plasmodium falciparum* and between 25 and 50 ng/mL for *Plasmodium vivax* (identified by pLDH) (75)

▪ Molecular diagnostic tests

More recent developments in malaria diagnosis have focused on the detection and amplification of *Plasmodium* nucleic acids using biomolecular techniques like polymerase chain reaction (PCR) and loop mediated isothermal amplification (LAMP).

Polymerase chain reaction (PCR) is a technology that allows for malaria detection via amplification of a specific highly conserved nucleic acid fragment (sequence). PCR techniques are useful for detecting low-density parasitemia or identifying species in mixed infection, and assessing therapeutic efficacy (76,69). It is sensitive to parasitaemia with low parasite density (lower than 1 parasite/ μ l), and therefore has a better performance than microscopy or rapid diagnostic tests (69,77,78). However, PCR use is restricted to reference laboratories in low resource settings, due to its cost and demand for trained personnel to perform the test (76,78).

Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification technique (NAAT), with performance comparable to that of PCR. The method has an advantage over PCR as it does not require a thermocycler and is less costly. It is faster and less complicated to operate than the PCR (69) (79). LAMP was found to have a higher sensitivity (100%) than microscopy (79.5%) and RDT (76.9%) in detecting asymptomatic malaria with reference to nested PCR. Similarly, LAMP was four times more sensitive (88.9%) than microscopy and RDT (22.2%) in detecting placental infections in

peripheral blood with reference to nested PCR (80).

1.1.5.3. Antimalarial drugs

Four classes of drugs are currently used for malaria treatment including artemisinin derivatives, quinoline -related compounds, antifolates, and antimicrobials.

▪ Artemisinin derivatives

Artemisinin (also known as qinghaosu) is an antimalarial drug isolated from the plant *Artemisia annua* in 1972 in China. Four main semi-synthetic derivatives were produced for treating malaria in human beings: dihydroartemisinin, artesunate, artemether, and arteether (81). The mechanism for the antimalarial activity of artemisinin remains to be fully elucidated. However, it is believed to act via a two-step mechanism. Artemisinin is first activated by intraparasitic heme-iron which catalyzes the cleavage of the endoperoxide bridge to produce free radical intermediates. The free radical intermediate may then kill the parasite by alkylating and poisoning one or more essential malarial protein(s) (82)(83). Artemisinin and its derivatives are the most rapidly eliminated of all antimalarials with half-lives of approximately 1 hour (84).

▪ Quinoline related compounds

Quinoline related compounds are a class of drugs known to cause parasite death by blocking the polymerization of the toxic heme into an insoluble and non-toxic pigment hemozoin, resulting in cell lysis and parasite cell autodigestion.

- The first quinolone antimalarial drugs were alkaloids extracted from the

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cinchona tree, including the quinine, cinchonine, quinidine, and cinchonidine.

- The group of 8-Aminoquinolines was the first derivatives of synthetic antimalarial drugs including plasmoquine (pamaquine) and primaquine. Primaquine is less toxic than pamaquine and is used for the eradication of refractory hypnozoites of *P. vivax* and *P. ovale*.
- The second group of synthetic antimalarial drugs is the 4-Aminoquinolines, composed of chloroquine (4-aminoquinoline Resochin) and amodiaquine. Chloroquine was widely used, and resistances emerged in several areas worldwide. Amodiaquine is effective against chloroquine-resistant strains of *P. falciparum*, although there is some cross-resistance. Piperaquine is a bisquinoline that is closely related to chloroquine and amodiaquine.
- Quinoline methanol is another group of synthetic antimalarials created in the context of chloroquine resistance. Mefloquine is a quinoline methanol compound related to quinine and is effective on the chloroquine -resistant strains of *Plasmodium* (85,86,87,88). Lumefantrine (formerly benflumetol) is an aryl amino-alcohol belonging to the same general group as mefloquine and halofantrine.

▪ Antifolates

The mode of action of this class is the inhibition of *P. falciparum* Dihydrofolate reductase enzyme. Dihydrofolate reductase is required for the biosynthesis of tetrahydrofolate, that is implicated in deoxyribonucleic acid (DNA) biosynthesis during nuclear division in schizonts. The susceptibility of other malaria species is less certain. The

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main drugs in this class are proquanil, chloproquanil, pyrimethamine, sulfadoxine, and dapsone (Table I) (87,88,89).

▪ **Antimicrobials**

Two families of antibiotics mainly used as antimalarials are macrolides and tetracyclines. Of the macrolides, azithromycin exhibits the best antiparasmodial properties by inhibiting the synthesis of cell proteins. Meanwhile, doxycycline, and clindamycin have the best antimalarial activity among tetracycline (88,90,91).

Artemisinin- based combination therapy

An Artemisinin-based combination therapy (ACT) is a combination of an artemisinin derivative with a longer - acting antimalarial drug that has a different mode of action. WHO recommends six ACTs for the treatment of uncomplicated malaria viz: artemether-lumefantrine (AL), artesunate-amodiaquine (AS+AQ), artesunate-mefloquine (ASMQ), dihydroartemisinin-piperaquine (DHAP), artesunate + sulfadoxine-pyrimethamine (AS+SP), artesunate-pyronaridine (APY) (66). The combinations containing SP and amodiaquine are both threatened by resistance in some areas (84).

The first line of treatment for each country should be chosen based on the pattern of resistance to antimalarial drugs in the country, the efficacy and safety of the combination, their cost, and availability of paediatric formulations. Also, the first line drugs cannot be used in prevention schemes (e.g IPT, seasonal chemoprevention) in the same country (64).

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Sulfadoxine-pyrimethamine (SP) is a fixed -dose combination of antimalarials drugs but it is not an artemisinin-based combination. It is recommended for malaria prevention in pregnant women (64,84).

Safety of antimalarial drugs

All ACTs have been assessed for evidence of their safety in clinical trials before their recommendation. However, WHO recommends a pharmacovigilance surveillance system in all the countries that are concerned. In a systematic review in 2014, a less than 10 per 1000 serious adverse events (including death) was reported for dihydroartemesinin + piperaquine, artemeter + lumefantrine, and artesunate + mefloquine. Moderate and mild adverse events included common gastroenterological symptoms (vomiting, nausea, anorexia, abdominal pain), neuro-psychiatric (headache, dizziness, sleeplessness), cardio-respiratory symptoms (cough, prolonged QT interval, coryza) (64,92). Artemisinin and its derivatives are also reported to inhibit erythropoiesis in the early fetus and cause fetal resorption in all experimental animals tested to date. Because these drugs have not been evaluated extensively in early pregnancy in humans, it is recommended to avoided in patients in the first trimester of pregnancy with uncomplicated malaria until more information is available (64). There is no evidence for adverse effects on the fetus exposed in the second or third trimesters when these drugs are recommended, depending on the safety profile of the partner drug (84).

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Table 1: antimalarial drugs and their side effects

Source: Yarnell, 2014 (93)

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

*Also anti-inflammatory and antipyretic. Note: use of single agents to treat acute malaria is almost never a good idea. G6PD, glucose-6-phosphate dehydrogenase; IV, intravenously.

1.1.5.4. Malaria treatment recommendations

WHO has made recommendations for treating malaria as follows:

- To treat uncomplicated *P.falciparum* malaria in children and adults except women in their first trimester with artemisinin -based combination therapy for 3 days.
- In areas with chloroquine-susceptible infections, adults and children with uncomplicated *P.vivax*, *P. ovale*, *P.malariae* or *P.knowlesi* , malaria can be treated with either an ACT (except pregnant women in their first trimester) or chloroquine.
- To treat pregnant women with uncomplicated *P. falciparum* malaria during the first trimester with quinine +clindamycin for 7 days (64) or artemether lumefantrine (AL) for 3 days (66).
- To reduce malaria transmission in low-transmission areas, a single dose of 0.25mg/kg of primaquine must be administered with ACT to patients with *P.falciparum* malaria (except pregnant women, infants aged < 6month, and women breastfeeding infants < 6months).
- To prevent relapse, *P. vivax* or *P. ovale* malaria in children and adults (except pregnant women, infants below six months, women breastfeeding infants <6 months or those with glucose-6-phosphate dehydrogenase (G6PD) deficiency, and persons with G6PD deficiency) should be treated with a 14-day course of primaquine in all transmission settings.
- To improve adherence to treatment for prevention of relapse, an additional treatment option (with shorter course) of using primaquine 0.5 mg/kg/day for seven days is recommended to treat *P. vivax* or *P.*

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ovale malaria in children and adults (except pregnant women, infants below six months, women breastfeeding infants <6 months or those with glucose-6-phosphate dehydrogenase (G6PD) deficiency, and persons with G6PD deficiency) (66).

- To treat adults and children with severe malaria (including infants, pregnant women in all trimesters and lactating women) with intravenous or intramuscular artesunate for at least 24 h, and until they can tolerate oral medication. Once the patient can tolerate oral medications, the parenteral treatment should be followed by a complete 3-day course of an ACT (with a single dose primaquine in areas of low transmission). Where artesunate is not available, parenteral treatment with artemether should be preferred to quinine (64,66). Where injectable treatment cannot be given, children aged under 6 years with severe malaria should receive a single pre-referral treatment with a rectal artesunate before being referred immediately to a health care facility where the full level of care can be provided.

1.1.6 Malaria control and elimination strategies

1.1.6.1 Malaria control measures

▪ Vector control measures

Vector control measures are focused on reduction of the contact between mosquitoes and humans, the destruction of larvae by environmental management and the use of larvicides or mosquito larvae predators, and destruction of adult mosquitoes by indoor residual

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spraying and insecticide-treated bed nets. The WHO recommends Long-lasting insecticidal nets (LLINs) and Indoor Residual Sprayings (IRS) as a core intervention for use in protecting populations at risk of malaria, including in areas where malaria has been eliminated or transmission interrupted but with a remaining risk of reintroduction (94,95). Indoor Residual Spraying is applied to potential malaria vector resting surfaces, such as internal walls, eaves, and ceilings of houses where such vectors might come into contact with the insecticide (66,95,96). Another vector control measure includes Larval Source Management (LSM) that targets aquatic environments that could be mosquito's habitat in order to prevent development of other mosquitoes.

▪ Preventive chemotherapies

Chemoprophylaxis refers to the administration of a medication for preventing disease or infection. Malaria Chemoprophylaxis comprises of three interventions namely the Intermittent Preventive Therapy in infants (IPTi), Intermittent Preventive Therapy in pregnant women (IPTp) and the Seasonal Malaria Chemoprophylaxis (SMC). Intermittent preventive therapy in infants (IPTi) is a full therapeutic course of antimalarial medicine delivered to infants three times during the first year of life, through routine immunization services, regardless of whether the child is infected with malaria. The WHO recommends IPTi with Sulfadoxine-Pyrimethamine (IPTi-SP) to protect against clinical malaria, anaemia, and severe malaria in areas with moderate to high malaria transmission in sub-Saharan Africa (66,64,97,98).

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The use of Intermittent Preventive Therapy in pregnant women (IPTp) is a measure to prevent maternal and infant mortality, anaemia, and other adverse effects of malaria in pregnancy. WHO recommends IPT in pregnant women with Sulfadoxine-Pyrimethamine (IPTp- SP), and administration is done monthly to pregnant women at risk of malaria, as early as possible in the second trimester of pregnancy. The women should receive at least three doses of SP during her pregnancy, with each dose given at least 1 month apart (99).

Seasonal malaria chemoprevention (SMC) is the intermittent administration of full presumptive courses of malaria treatment to children in high transmission areas during high transmission seasons. The administration of amodiaquine (AQ) and SP (AQ+SP) to children aged 3–59 months reduces the incidence of clinical attacks and severe malaria by about 75% and could avert millions of cases and thousands of deaths among children living in areas of highly seasonal malaria transmission in the Sahel Sub-region (98). Since 2012, WHO has recommended SMC for children aged 3- 59 months living in highly seasonal areas of Africa's Sahel sub-region during the high-transmission seasons. This preventive therapy provided at monthly intervals has a significant protective effect against uncomplicated and severe malaria (100).

Mass drug administration (MDA) is the administration of a full therapeutic course of antimalarial medicine to a defined population

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living in a particular (geographical) area within the same time, and often repeated at intervals. The intervention is done within a limited time in order to interrupt the transmission of *P. falciparum* malaria in areas approaching elimination, in order to achieve a rapid reduction of morbidity and mortality during malaria epidemics, complex emergencies, or situations where health systems may be overwhelmed, but it is limited in time in order to reduce the risk of spread of multidrug resistance. WHO recommends that medicines used must be of proven efficacy, have a long half-life, and be different from those used for first-line treatment (101). However, the first-line treatment may be considered for MDA in situations such as complex emergencies.

▪ Vaccination

The most known vaccine is the RTS, S/AS01 (Mosquirix), which is a monovalent pre-erythrocytic recombinant protein vaccine. It contains *P. falciparum* circumsporozoite protein (CSP) regions known to induce humoral (R region) and cellular (T region) immune responses, covalently linked to the hepatitis B virus surface antigen (S) sporozoite surface, thereby limiting the ability of *P. falciparum* to infect, mature and multiply in the liver (102). It has been shown to have about 77% efficacy. WHO recommends that the RTS,S/AS01 malaria vaccine should be provided as part of a comprehensive malaria control strategy (66).

The RTS,S/AS01 malaria vaccine is provided in a 4-dose schedule for

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the prevention of *P. falciparum* malaria in children from 5 months of age living in regions with moderate to high malaria transmission, as defined by WHO (66,102). The schedule includes three primary doses with a minimum interval of 4 weeks between doses, followed by a booster dose approximately 12–18 months after the third dose. An optional 5-dose strategy may be considered in areas with high seasonal malaria transmission or areas with perennial malaria transmission with seasonal peaks. This strategy includes three primary doses administered at monthly intervals and two annual booster doses administered prior to peak malaria transmission season (66)(102).

Recently, a phase-2-trial conducted in Burkina Faso showed promising results with the R21/Matrix-M pre-erythrocytic malaria vaccine candidate, with the efficacy reaching the WHO-specified malaria vaccine goal of at least 75% over 1 year, after vaccine administration before the malaria season in the target population of African children (103), and with an added benefit of a maintained vaccine efficacy at 80% over 1year and 75% over 2years, following a booster vaccine (fourth dose) administered 12 months after the primary three-dose regimen (104).

● **ALERTE - Recherche** : Le Pr. Halidou Tinto parmi les 10 personnalités scientifiques de l'année (Magazine Nature)

Le Pr. Halidou Tinto a été désigné lauréat 2023 de Nature's 10, un classement du magazine britannique Nature des 10 scientifiques qui ont marqué le monde au cours de l'année.

Le Pr. Tinto est distingué pour son travail réalisé avec son équipe de l'unité clinique de Nanoro au Burkina Faso sur le vaccin R21 contre le paludisme, validé par l'OMS.



Figure 5: WHO acknowledges Pr. Tinto for successful implementation of R21 vaccine

1.1.6.2 Malaria elimination

Malaria remains challenging in sub-Saharan African countries for controlling and eliminating the disease. Sustainability and propagation of the malaria disease relies on continuous exchange of the parasite between the mosquito vector and the human host. The WHO framework for malaria elimination 2017 provides activities and strategies required

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to achieve malaria elimination and prevent re-establishment of transmission in countries, regardless of where they lie across the spectrum of transmission intensity. It informs national malaria elimination strategic plans of each country and requests to be adapted to local contexts. The framework highlights that, strategies for transmission interruption such as optimal vector control and elimination of infectious reservoirs in humans (symptomatic and asymptomatic) through optimal case detection and management, are major interventions strategies for malaria elimination (Figure 5) (94). In this light, WHO recommends that in the context of malaria elimination, the treatment of malaria must follow national policies and WHO guidelines (66), associated with treatment that fully clears malaria infection, with the objective to reduce morbidity and mortality, and also to interrupt transmission of *Plasmodium falciparum* and relapse of *Plasmodium vivax*. Thus, for cases due to *P. vivax* or *P. ovale*, in addition to clearance of the blood infection, an anti-relapse therapy (primaquine) is required to clear liver-stage parasites and, for all infections caused by *P. falciparum*, a gametocytocidal drug (primaquine) has to be administered in addition to treatment for the blood stage in order to reduce and even to halt transmission (94).

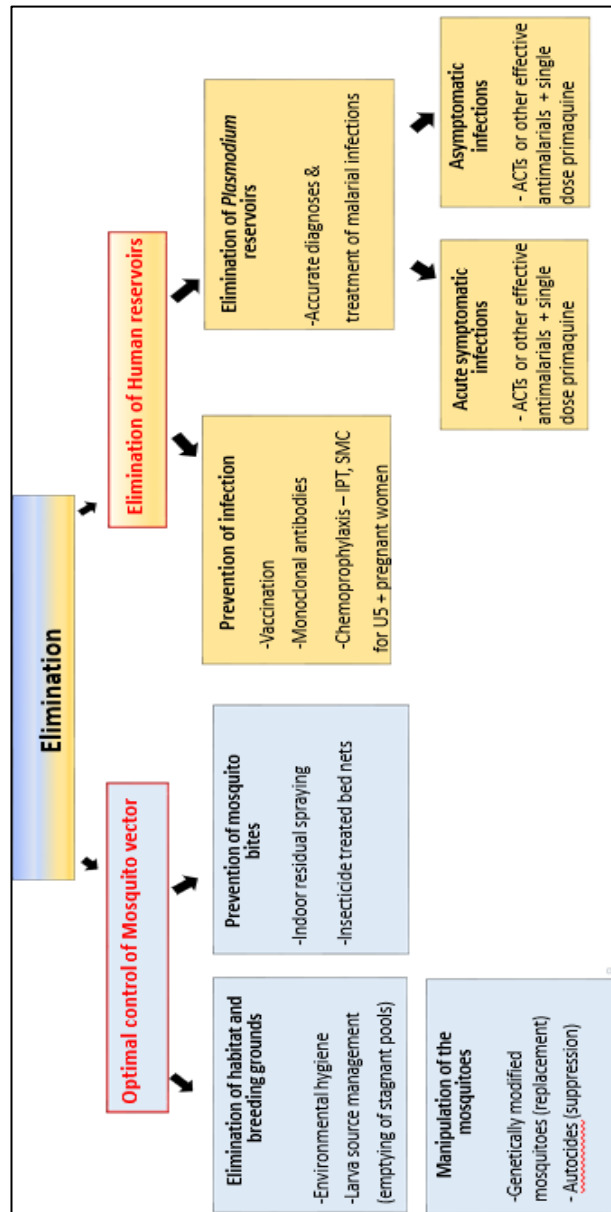


Figure 6: A framework for malaria elimination adapted from WHO, 2017 (94).

Intensity of malaria transmission

The prevalence of malaria infection reflects the level of transmission of the *Plasmodium* parasite. As such transmission intensity is usually

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assessed as the incidence of cases or the prevalence of infection, which is categorized to provide an adaptable framework for classification of malaria transmission intensity (Table 2). In most countries, it is reported as the annual parasite incidence (number of confirmed new malaria cases per 1000 population per year). This may be obtained from routine surveillance and/or parasite prevalence from surveys, often conducted during or just after periods of peak transmission. Countries with incidence cases between 100-250 per 1000 inhabitants per year are classified as low transmission settings (Cameroon inclusive). However, WHO recommends that every country can accelerate progress towards elimination through evidence-based strategies, regardless of the current intensity of transmission and the malaria burden they may carry (94).

Table 2: Classification of malaria transmission intensity according to annual parasite incidence

Transmission intensity	Annual parasite incidence (cases per 1000 population)	<i>P. falciparum</i> or <i>P. vivax</i> prevalence rate (%)
High transmission	≥450/1000 inhabitants	≥ 35
Moderate transmission	250-450	10-35
Low transmission	100-250	1-10
Very low transmission	<100	> 0 and <1.0

1.1.7 Global epidemiology and burden of malaria

Globally, there has been an increase in the burden of malaria within the last five years, with the highest increase observed between 2019 and 2020, due to disruptions of essential malaria services during the COVID-19 pandemic. Between 2000 and 2015, case numbers steadily declined from 245 million to 230 million across the 108 countries that were malaria endemic in 2000. Since 2016, malaria cases have increased, with the largest annual increase (13 million cases) observed between 2019 and 2020 during the first year of the covid-10 pandemic. In 2021, there were an estimated 247 million malaria cases in 84 malaria endemic countries, making an increase of 2 million cases compared with 2020. Most of the increase in case numbers over the past 5 years occurred in countries in the WHO African Region. This region accounts for about 95% of cases and 96% of deaths globally, with 78.9% occurring among children aged under 5 years in 2021 compared with 91.0% in 2000.

In a similar manner, malaria deaths declined steadily since 2000 from 897 000 to 577 000 in 2015, and to 568 000 in 2019. Meanwhile, in 2020, malaria deaths increased to about 625 000, making an increase of 57 000 deaths from 2019. The estimated deaths in 2021 were 619 000, a slight decline compared with 2020. Between 2019 and 2021, 63 000 deaths were due to disruptions to essential malaria services during the COVID-19 pandemic (105). Figure 7 shows countries with indigenous cases in 2000 and their status by 2021.

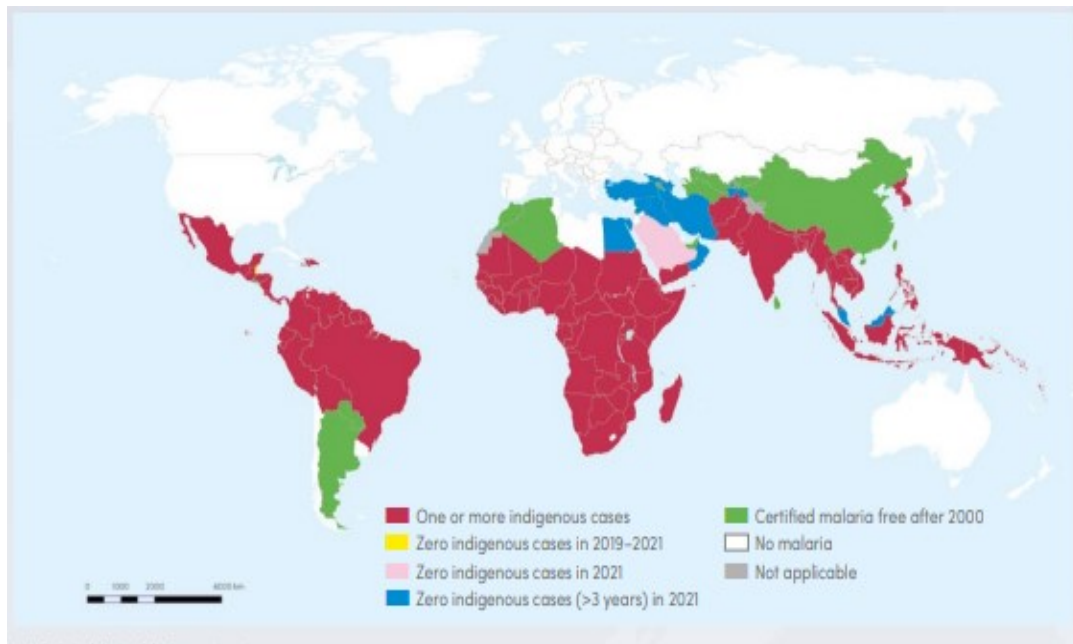


Figure 7: Countries with indigenous cases in 2000 and their status by 2021.

Countries with zero indigenous cases for at least 3 consecutive years are considered to have eliminated malaria. In 2021, the Islamic Republic of Iran and Malaysia reported zero indigenous cases for the fourth consecutive year; also, Belize and Cabo Verde reported zero indigenous cases for the third time. China and El Salvador were certified malaria free in 2021, following 4 years of zero malaria cases.

Source: World malaria report 2022 (105).

<https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022>.

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Distribution of malaria burden in endemic countries

In 2021, 29 of the 84 countries that were malaria endemic (including the territory of French Guiana) accounted for about 96% of malaria cases and deaths globally (Figure 8 and Figure 9) (105). These countries have different population sizes, explaining part of differences in number of cases. In Cameroon, there were approximately 6,665,957 cases and 13,839 deaths in 2021, for a population of 27,198,628 inhabitants (105).

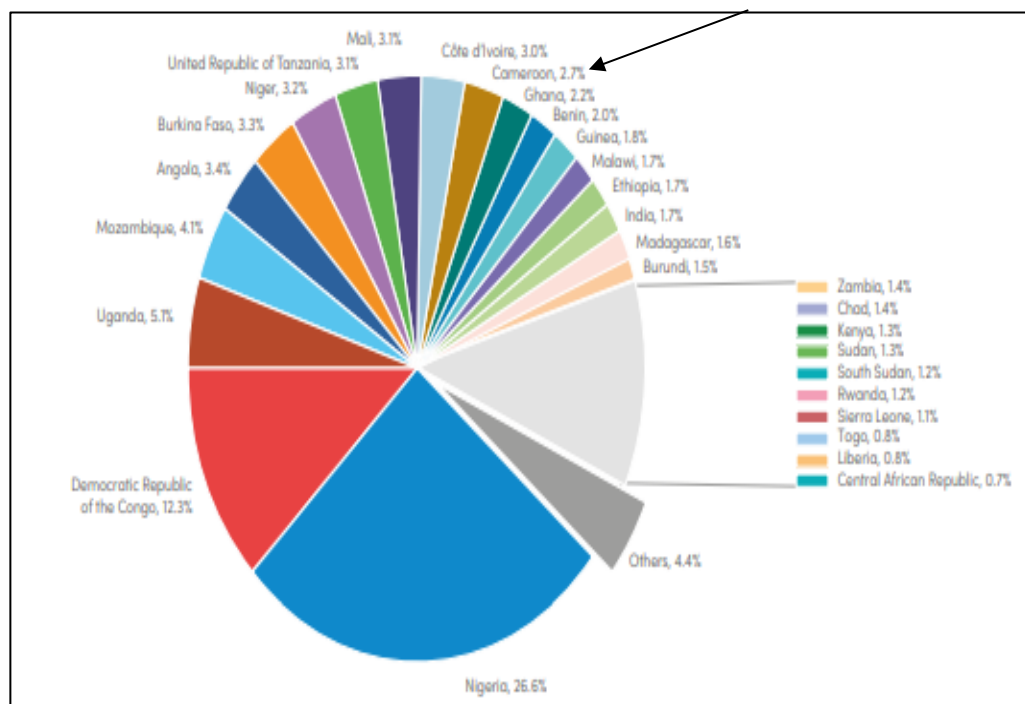


Figure 8: Global malaria cases in 2021;
Source: World malaria report, 2022 (105).

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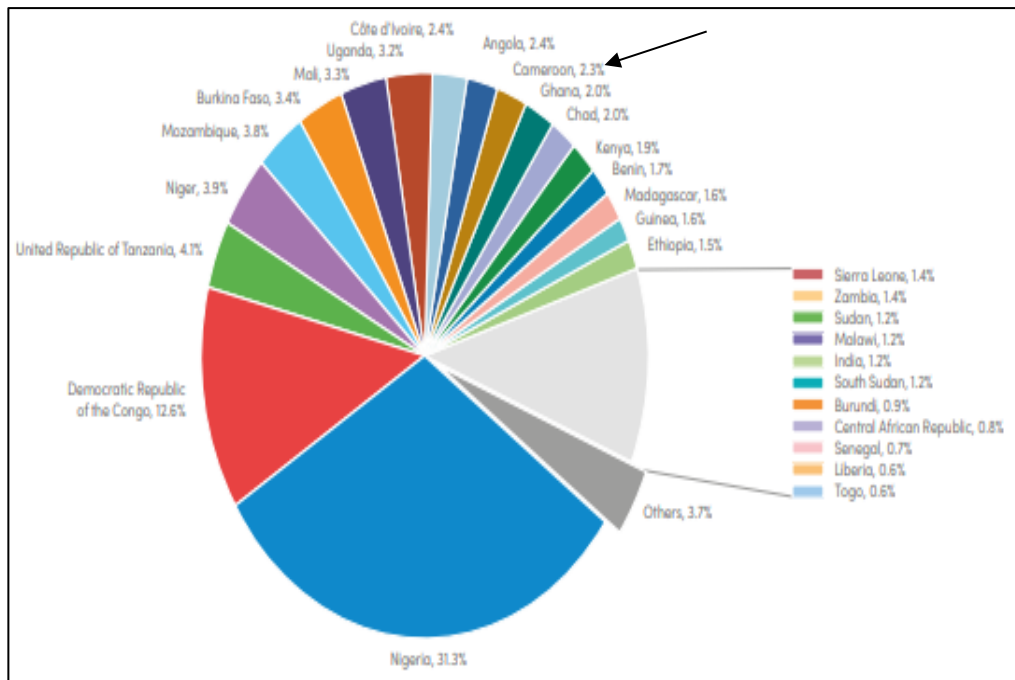


Figure 9: Global malaria deaths in 2021;
Source: World malaria report, 2022 (105)

1.2 Antimalarial drug resistance

1.2.1 Evolution of antimalaria drug resistance

Antimalarial drug resistance is a major threat of the malaria elimination programme. *P. falciparum* chloroquine resistance was reported in South-East Asia in 1957 and in South America and Africa in the 80s, thereby causing a tremendous effect on malaria mortality in many countries. As such, national policies for the treatment of uncomplicated malaria were changed for many countries with chloroquine resistance since 1993, recommending the use of SP in place of chloroquine. In 2000, resistances to SP, amodiaquine, and proguanil were also reported. Resistance to artemisinin derivatives was suspected in the same period in southern Asia, prompting WHO to recommend artemisinin-based combination therapy (ACT) in 2005. Monotherapy with artemisinin relatives for treating uncomplicated *P. falciparum* malaria was banned in 2006. Monotherapy with quinine, artesunate, or artemether was mainly indicated for treating severe malaria. The other species (*P. malariae*, *P. vivax*, and *P. ovale*) remain sensitive to chloroquine. In 2008, Artemisinin resistance was documented on the Thailand-Cambodia border after artesunate monotherapy. However, retrospective analysis indicates that artemisinin resistance likely emerged in 2001, before the widespread deployment of ACTs in Cambodia (106,107). In 2012, evidence of artemisinin resistance was reported in South Asia in clinical trials and mutation of the Kelch 13 propeller gene were identified and associated with resistances

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(108,109). In Africa, evidence of the emergence of *P. falciparum* resistance to artemisinin derivatives has been reported in several countries since 2021, including Uganda, Rwanda Eritrea, and Burkina-Faso. Mutations in the Kelch 13 (K13) gene were identified and associated with these resistances, but they were different from the Asian strains (110,111,112,113).

1.2.2 Emergence and spread of resistances

Drug resistance arises as a result of randomly occurring genetic mutations in the parasite population. The continuous progression in resistance to anti-malarial drugs is due to indiscriminate use, which in turn drives a strong selective force in favour of the resistant parasites. In the evolution of drug resistance, a resistant genotype is generated by mutation, which may interfere with parasite's susceptibility to a drug (114). Mutations may occur due to deletion, insertion or substitution/modification of the nucleotide base, which alters the amino acid sequence or protein conformation of the drug target (binding site) and consequently drug resistance. The emergence of resistance to one drug may lead to reduced sensitivity of chemically related drugs. Spontaneous *de novo* mutations do occur and interfere with parasite's susceptibility to a drug, making the parasite able to survive low therapeutic concentrations of the drug. Therefore, high parasitemia and inadequate dosing of the drug will favour development of resistance (115). Host immunity augments the killing of parasites by chemotherapeutics, including those parasites carrying resistant traits.

Therefore, resistance is more likely to arise and spread rapidly in a non-immune population (115).

1.2.3 Antimalaria drug resistant testing and monitoring

1.2.3.1 Definitions

Antimalarial drug resistance is defined as the ability of a parasite strain to survive or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended, but within the tolerance of the subject (116).

Antimalarial multidrug resistance is resistance to more than two antimalarial compounds of different chemical classes. This term usually refers to *Plasmodium falciparum* resistance to chloroquine, sulfadoxine-pyrimethamine and a third antimalarial compound (116).

Artemisinin partial resistance

Artemisinin partial resistance is delayed parasite clearance following treatment with artemisinin-based monotherapy or with an artemisinin-based combination therapy (ACT). Artemisinin resistance is characterized by slow clearance of parasites, which are detectable in blood by microscopy on day 3 of treatment. Generally, artemisinin and its derivatives rapidly reduce the number of parasites in blood. However, few patients treated with artemisinin-based monotherapy or with an ACT in most malaria endemic areas are usually identified to have parasites in blood on day 3 following the initiation of therapy (day 0). In the Greater Mekong Subregion (GMS), delayed parasite

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clearance has been observed, whereby more patients still have parasites in the blood on day 3 after a treatment. This phenomenon is termed “artemisinin partial resistance”. However, other factors (high initial parasitemia or splenectomy) could also lead to delayed clearance, thus, failure to clear parasites from the blood within the first three days does not necessarily mean that a patient is infected with artemisinin partial resistant parasites. Under normal circumstances where parasites are fully sensitive to artemisinin, 3.0–5.0% of patients may still have parasitemia persisting to day 3 of treatment. In therapeutic efficacy studies (TES), the presence of asexual parasites in 10% of patients on day 3 of treatment is used as an indicator of suspected artemisinin partial resistance in an area (117). WHO recommends that the efficacy of first line and second-line ACTs should be routinely monitored every two years in all endemic countries. Any treatment failure rate above 10% should prompt a change in the national antimalarial treatment policy (118). In addition, a sharp increase in treatment failure (based on quality data), even below 10% gives reason for treatment policy change.

The vulnerability of *P. falciparum* to artemisinin varies over the life cycle, being highest in the ring stage and lowest in the trophozoite stage. The mechanism of artemisinin partial resistance has been linked to an alteration in the life cycle, where parasites exhibit an extended ring stage (also called dormancy) and an abbreviated trophozoite stage. This increases the proportion of parasites likely to survive a short exposure

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to artemisinin, thereby increasing parasite clearance time (119). The changes in parasite clearance time are associated with several genetic mutations in *P. falciparum* Kelch 13 (PfK13) gene (108). Meanwhile, increased production of gametocytes by parasites partially resistant to artemisinin is thought to have a role in the spread of resistances (120). Monitoring antimalarial drug efficacy and resistance is therefore important for the early detection of resistance, which in turn enables timely action to prevent its spread and limit the impact on global health. Measurement of drug efficacy and resistance in malaria is complex. Studies of clinical and parasitological outcomes also known as therapeutic efficacy studies (TES) are the main sources of information on which national malaria control programmes base treatment policy; however, other studies are needed to confirm drug resistance if suspected (118).

1.2.3.2 Therapeutic efficacy studies

Therapeutic efficacy studies (TES) are prospective evaluations of patients' clinical and parasitological responses to treatment for uncomplicated malaria. They are conducted with a diagnosis validated by microscopy and using a quality assured treatment and supervised drug administration. Patients who are lost to follow up or excluded from the study (e.g. due to self-medication or to withdraw their participation) are excluded or censored from the analysis. TES remain the gold standard, and they are being used to inform national treatment policies. WHO has developed a standard protocol for TES (118). A limited

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number of sentinel sites, representing all the epidemiological strata in a country, are adequate to collect consistent longitudinal data and to document trends in treatment efficacy. The recommendation is to test the efficacy of the first- and second-line treatment at all sentinel sites at least every 2 years. In areas where multiple *Plasmodium* species are prevalent (typically *P. falciparum* and *P. vivax*), sentinel sites can be used to monitor therapeutic efficacy against these species simultaneously.

For *P. falciparum* clinical and parasitological responses to treatment are evaluated on days 0, 1, 2, 3, 7, 14, 21 and 28 (and on days 35 and 42 for some partner drugs). Therapeutic outcomes are assessed on the final day of the study (i.e. on day 28 or day 42). ACTs with a partner drug that has relative short elimination half-life are followed-up for at least 28 days, and ACTs with partner drugs with longer elimination half-lives are followed-up for at least 42 days. The objective of follow-up is not to detect every single recrudescence but to obtain a robust estimate of failure rates within a target period. A longer follow-up period may capture a few additional recrudescences, but in higher transmissions settings it can also mean several reinfections that need to correctly be classified as such. Modelling shows that more than 95% of the failures would be detected for DHA-PPQ and AS-MQ by 42 days of follow-up, and by 28 days for AL (121). It is also recommended to follow up AS-AQ and AS+SP for at least 28 days, as well as AS-PY for a minimum of 42 days.

Classification of treatment outcomes

1. Early treatment failure (ETF): danger signs or severe malaria on days 1–3 with asexual parasitaemia, higher parasitaemia on day 2 than day 0, parasitaemia on day 3 with a fever (≥ 37.5 °C) or day 3 parasitaemia $\geq 25\%$ than day 0,
2. Late clinical failure (LTF): danger signs or severe malaria with asexual parasitaemia or parasitaemia with fever (≥ 37.5 °C) between days 4 and last day of follow-up,
3. Late parasitological failure (LPF): parasitaemia between day 7 and last day of follow-up in absence of a fever (< 37.5 °C),
4. Adequate clinical and parasitological response (ACPR): absence of asexual parasitaemia on last day of follow-up, irrespective of fever status, or
5. Withdrawal or lost to follow-up.

Classification of recurrent *P. falciparum* infections

When recurrent parasitaemia appears during follow-up, molecular genotyping is performed to distinguish new *P. falciparum* infections from recrudescence (118,121,122). The distinction is significant, because recrudescence indicates failure of the treatment, whereas a new infection does not. WHO recommends a genotyping method that uses highly polymorphic markers of *P. falciparum* - msp1, msp2 and glurp. Based on these markers, reinfection and recrudescence are defined as follows:

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A “new infection” is reappearance of parasitaemia after initial parasite clearance in which all the alleles in parasites from the post-treatment sample are different from those in the admission (day 0) sample, for one or more loci tested. Consequently, a single marker (i.e. msp1, msp2 or glurp) in which all alleles are different between pre- and post-treatment samples is sufficient to conclude that it is a new infection. In a “recrudescence”, at least one allele at each locus is common to both paired samples.

1.2.3.3 Additional tools for measuring antimalaria drug efficacy and resistance

In areas where lower than expected drug efficacy has been identified, additional studies are needed to confirm and characterize drug resistance as the cause. These may include in vitro studies of the parasite phenotype, and studies of genetic mutations known to be associated with drug resistance in the parasite as indicated below (118, 123,124).

Molecular marker analyses. Molecular markers are genetic changes (in parasite genome) identified as being associated with a change in parasite susceptibility to antimalarial drugs. Molecular markers can be used to help confirm resistance as a cause of treatment failure related to intrinsic parasite susceptibility, and a changing prevalence of a molecular marker for resistance in a geographical area can serve as an early warning signal to supplement information on efficacy. If there is

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a marked increase in the prevalence of the molecular marker for resistance to the partner drug in the first-line ACT, the country has to consider changing its policy, or should at least prepare for a change in policy, before a 10% failure rate is reached.

Ex vivo and in vitro tests. These tests assess the sensitivity of fresh or culture-adapted parasites (to antimalarial drugs by exposing them to different concentrations of a drug (typically for 48 or 72 hours) in culture plates and observing the effect on parasite growth or survival. Results are often expressed as the mean drug concentration that inhibits 50% of the parasite's growth (50% inhibitory concentration, IC₅₀), mean concentration that inhibits 90% of the parasite's growth (90% inhibitory concentration, IC₉₀) or the minimum inhibitory concentration (MIC). Results of in vitro cultures are not affected by host factors such as drug absorption and immunity.

Drug blood levels. Drug levels are measured in the blood to monitor the concentration of drug that malaria parasites are exposed to. It can help to distinguish whether a treatment failure is due to insufficient antimalarial drug exposure or due to resistance. In TES of longer acting antimalarial drugs, a normal day 7 concentration is enough to rule out poor drug quality, dosing or absorption as a cause of therapeutic failure. Measurement of drug concentrations is often included in studies that aim to confirm resistance in areas where previous TES have shown a decline in efficacy. Figure 10 shows an exemplary algorithm of a drug resistance confirmation process.

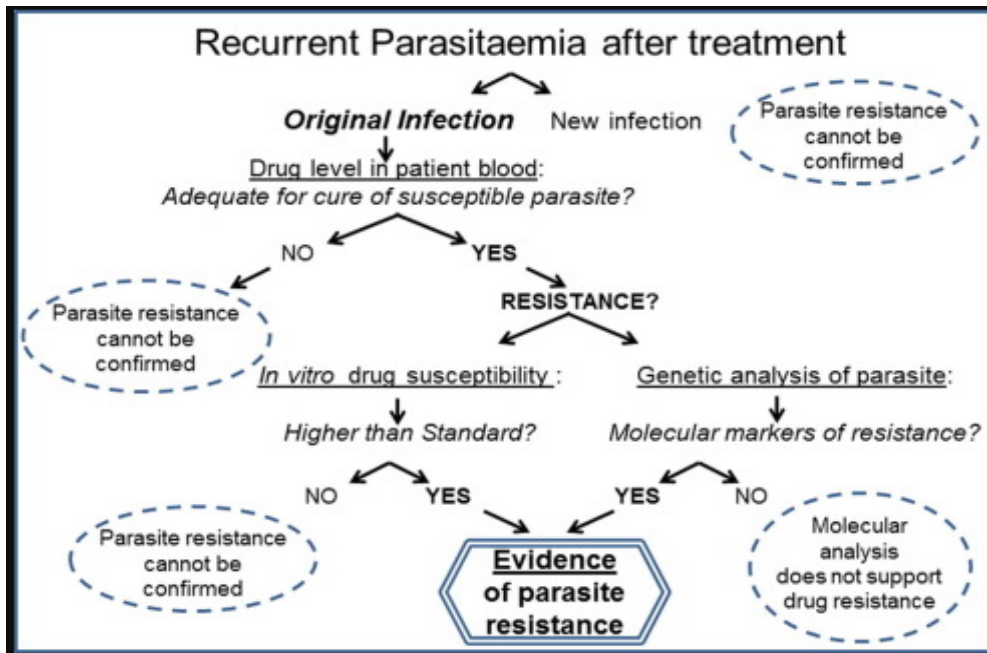


Figure 10: Algorithm of a drug resistance confirmation process.

Source: Sibley CH et al., 2012 (125).

1.3 Exploration and use of natural plant products

Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain, parts of plants, other plant materials or combinations thereof, as active ingredients (126). Herbal Medicine (HM) is also known as Complementary Alternative Medicine (CAM), and it involves ways of treating and maintaining health that existed before the arrival of allopathic medicine (127). Generally, the use of herbal medicine is very frequent in traditional medicine (TM) for the treatment of diseases. However, traditional medicine covers a wider area, where other components of nature, as well as animals and fungi

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can also be used for the treatment of conditions or diseases (128). Natural products are important sources for new drugs and are also good lead compounds suitable for further modification during drug development. In rural populations in Africa, people often use ethnobotanical and ethnomedical alternatives for the management of health problems including malaria, with a substantial prevalence of complementary and alternative medicine (CAM) product use in the general population ranging from 4.6% in urban settlements to 94% in semi-urban settlements (Cameroon inclusive) (129). Such alternative remedies have provided leads for the development of drugs useful in therapeutics as practiced in Western medicine. Examples of groundbreaking contributions of ethnomedicine to the treatment of malaria include isolation of quinine and artemisinin from Cinchona tree and *Artemisia annua*, respectively (130,131).

1.3.1 Phytochemistry and antimalarial properties of *A. annua* and *A. afra*

Studies have demonstrated that *Artemisia annua* and *Artemisia afra* in the form of infusions or powdered leaves reduce parasitemia and might kill the transmissible gametocyte forms, with characteristic chemoprotective potentials (132,133,134,135,136,137).

- ***Artemisia annua***

Artemisia annua, also referred to as sweet wormwood, sweet Annie, or annual worm- wood, is an aromatic herb of the *Asteraceae* family that

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originated in China, but it is widely distributed throughout the world due to its ability to treat a wide range of diseases in traditional medicine (138). It has been used in China since ancient times as a herbal product taken in the form of tea infusions or decoction for the alleviation of intermittent fevers associated with malaria, amongst other diseases (139). It is now domesticated and used in other parts of Asia, Europe, Australia, and America, to fight other ailments (139,140,141,142). Research has led to the isolation of almost six hundred secondary metabolites of *Artemisia annua*, the majority of which are terpenoids, sesquiterpenes polyphenols, flavonoids and coumarins (143). Due to the isolation of artemisinin, a potent anti-malarial compound belonging to the sesquiterpene lactone class from *A. annua*, phytochemical investigations of *A. annua* has been at the forefront of research (144). Artemisinin has a good efficacy against the erythrocytic stages of the malaria parasite. Also, in vitro and in vivo studies as well as clinical trials on the crude plant material and/or tea infusions have reported good anti-plasmodial activities (145,146). To this effect, the WHO recommends “Artemisinin Combination Therapy (ACTs)” as the first line of treatment for uncomplicated malaria due to *Plasmodium falciparum* (64,147). However, the WHO has cautioned against the use of non-pharmaceutical sources of artemisinin because of the risk of delivering sub-therapeutic doses of artemisinin that could potentiate anti-malarial drug resistance (147).

- ***Artemisia afra***

Artemisia afra, also referred to as African wormwood, is a herb indigenous to Africa and a member of the same genus as *A. Annua*. It is native to South Africa and has a long history of use as a traditional remedy against coughs, colds, fever, and malaria (148). It is cultivated across the African continent for the management of malaria and other diseases (149). Being a member of the same family and genus as *Artemisia annua*, *A. afra* shares almost the similar pattern of secondary metabolites, with about 400 secondary metabolites isolated, and which are dominated by terpenoids, polyphenols and coumarins (143), in addition to flavonoids (150). However, while *A. annua* cultivars have high artemisinin content, cultivars of *A. afra* are devoid of artemisinin, and other cultivars have negligible contents of artemisinin (132,133,136).

Despite it being devoid of artemisinin or having a negligible artemisinin content, *A. afra* has also some anti-plasmodial properties when used as infusions or crude extracts in vitro (132,133,151,136,150). In vitro studies have also confirmed artemisinin-independent antiparasitic activity of *A. afra* tea infusions against the different stages of the malaria parasite including the hepatocytes and gametocyte stages, with no cytotoxicity (132,133,151,136,152), suggesting a potential to empty malaria reservoirs and interrupt malaria transmission without threat to ACT resistance.

The *Artemisia* medicinal plants are cultivated in different geographical

regions in Cameroon and formulated as herbal tea. However, human studies are missing.

1.3.2 Pharmacokinetics and bioavailability of secondary

metabolites of *A. annua* and *A. afra*

Pharmacokinetics describes the absorption, distribution, metabolism, and elimination of a drug or pharmakon, while bioavailability refers to the fraction of the absorbed drug that finally reaches the systemic circulation. These characteristics vary between compounds of *Artemisia annua* and *Artemisia afra*, both of which influence their therapeutic or biological responses (153).

The administration of artemisinin either as a single compound or in a plant matrix such as in whole-plant powder or as an infusion may have an influence on its pharmacokinetics and bioavailability. For example, an evaluation of the pharmacokinetics of artemisinin following the oral administration of traditional preparations of *A. annua* found that artemisinin was absorbed faster from herbal tea preparations than capsules (from 30 min to 2.3 h) (154). However, both formulations had a similar bioavailability. Also, increases in the serum levels of artemisinin have been observed when administered as a whole plant (*A. annua*) compared to artemisinin capsules administered in isolation (155), thereby indicating a positive impact of the plant matrix on the bioavailability of artemisinin. In the same way uptake of luteolin (flavonoids) from aqueous solution of *A. afra* was found to be faster compared to when they were administered as pure solutions, indicative

of a positive impact of plant matrix on the bioavailability of flavonoids (156).

1.3.3 Molecular and pharmacological mechanisms of action

(MOA) of *A.afra* tea against *Plasmodium* parasite

Being a member of the same family and genus as *Artemisia annua*, *A. afra* shares almost a similar pattern of secondary metabolites, with about 400 secondary metabolites isolated; majors are terpenoids, polyphenols, coumarins, and flavonoids (143,150), with negligible artemisinin if any. These molecules have been shown to be responsible for observed artemisinin-independent invitro antiparasitic activity of *Artemisia afra* infusions against the different stages of the malaria parasite including the hepatocytes and gametocyte stages (132,133,151,136,152).

Morphological examination of *A. afra*–exposed parasites shows that first, the apicoplast biogenesis is disrupted (where the apicoplasts fails to elongate and divide at the schizont stage as they normally do in unexposed parasites), second, parasites mitochondria replication is affected, third, an inhibitory effect is exerted on the schizonts (reduction in the size and number of hepatic schizonts), and forth, there is an interruption of mature stage-V gametocyte homeostasis (69,151).

CHAPTER 2: MALARIA IN CAMEROON

2. Malaria in Cameroon

2.1 Country context

2.1.1 General context of Cameroon

Cameroon is a central and west African country, situated within the Gulf of Guinea at a latitude between 2–13°N and a longitude between 9–16°E, with a surface area of approximately 475,000 km². It is bounded to the North and East by Chad, to the West by Nigeria, to the East by Central African Republic and to the South by Congo, Gabon and Equatorial Guinea (157)(158). The country also has a coastal border with the Atlantic Ocean measuring about 400 km. It has a population of about 27.9 million (population density 58.7/km), with a growth rate of 2.51% and life expectancy of 54.4 years (159). Cameroon has a Sahelian climate with a rainy season which extends from March to late November, and a dry season from to December to March. Three climatic zones are identified from the North to the south of the country, namely the Sahelian zone, which has a semi-arid hot and dry climate with temperatures around 30-40°C and a rainy season lasting for 2-3months; the Sudano-Sahelian zone which has a hot tropical-savanna climate with temperatures at 28-30°C and a rainy season that lasts 3-6months; and the equatorial/forest zone dominated by a hot and humid savanna climate with temperatures at 23-33°C and two rainy seasons that last up to 8months (160,161,162). The average

2. Malaria in Cameroon

rainfall varies from 700mm/year in the Sahelian area to about 4000mm/year in the equatorial area (158,162,163).

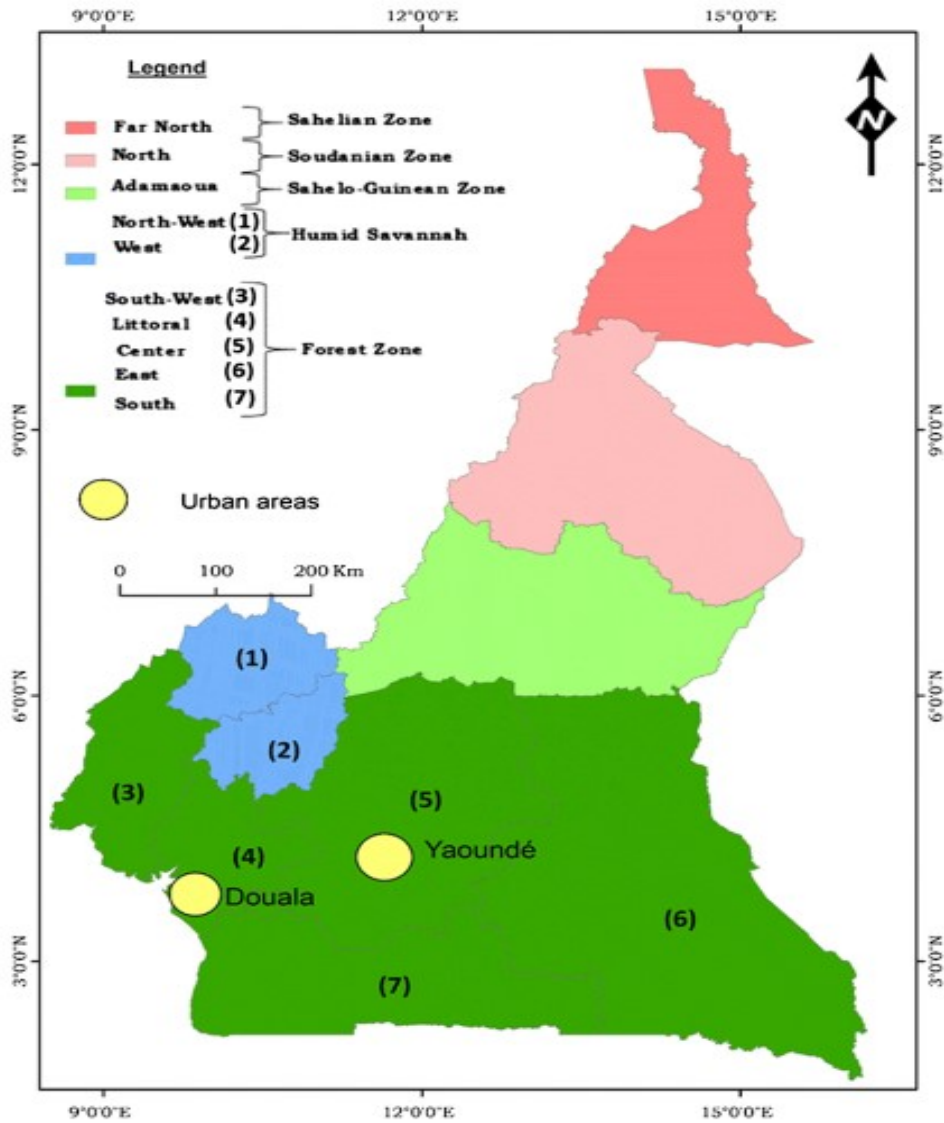


Figure 11: Map of Cameroon showing climatic and administrative divisions.

Source: Antonio-Nkondjio et al, 2018 (162).

2. Malaria in Cameroon

Administratively, Cameroon is divided into 10 semi-autonomous administrative regions covering the different climatic/geographical zones (Figure 10). Malaria transmission seasons vary according to the climatic/geographical zones whereby; the Sahelian zone in the Far North region has a short seasonal transmission of one to three months (July- October), with about 10 infective bites per person per month; the Sudano-Sahelian zone in the North and the Adamawa region has a long seasonal transmission of four to six months with around 20 infective bites per person per month; and the equatorial forest zone, in the south of the country has a permanent transmission season of seven to 12 months with around 100 infective bites per person per month. Data from the malaria indicator survey (MIS) and the demographic and health survey (DHS), indicates that vegetation and altitude are important predictors of the geographical distribution of malaria in Cameroon (164,162,165); with a recorded prevalence of asymptomatic malaria among children 2-15 years ranging from 6.5 -40.8% in the Sahelian zone (166,167), 25-30.7% in the Sudanian/tropical zone (168,169,170), and 25- 85.4% in the Equatorial /forest zone (167,171,172,173,174,175,176,177). Most of the malaria infections in Cameroon are asymptomatic and only small proportion of infected individual manifest clinical symptoms (41). Generally, the Far North and North regions of the country have a disproportionately higher number of malaria cases and deaths compared to the other regions. According to the 2015 National Malaria Control Programme (NMCP)

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Annual report, over 60% of under-five malaria deaths were recorded in these two regions (although less than 30% of the population is concentrated in the North and Far North) with an upsurge in malaria cases during the rainy season (July to October) (164,161).

On a security front, Cameroon faces an unprecedented humanitarian situation in the far north region, eastern region, and in the northwest and southwest regions because of armed conflicts, intercommunity wars, and the presence of refugees. This situation led to disruption of health services and increased health needs of the refugees, internally displaced persons, and their host communities. According to the United Nations Office for the Coordination of Humanitarian Affairs, 3.9 million people are in need of humanitarian assistance in Cameroon, including about 933,000 internally displaced persons, 531,000 returnees, 486,000 refugees, and about 1.6 million people in host communities. Malaria is one of the most common public health problems in these populations (178). Four *Plasmodium* species are presently known to cause malaria in Cameroon (except *P. knowlesi*). Of these, *Plasmodium falciparum* is the most prevalent parasite species, responsible for more than 95% of malaria cases, followed by *P. malariae* and *P. ovale* (176,179). Meanwhile, the most common malarial vectors include *An. gambiae* and *An. funestus*, *An. nili* and *An. coluzzii* (179,180).

2.1.2 Cameroon health care system

2.1.2.1 Organization of the health care system

The health system of Cameroon is organized in a pyramidal structure with three levels: central, intermediate, and peripheral. Administrative organization at the central level includes the central services of the ministry of public health (MoPH) comprising the office of the minister, general secretariat, technical directorates, and affiliated programmes. This first level manages administrative matters and political direction, as well as development of concepts, policies and strategies (Table 3).

The intermediate level comprises 10 regional delegations of public health that have a technical support role for health districts and programmes. At peripheral level, the health districts are the operational units in charge of implementing health programmes.

In terms of technical organization, structures at the central level include the general and central hospitals and reference care structures, as well as the National Central Supply of Essential Medicines and Medical Consumables (CENAME), and their boards of directors or management committees. At the intermediate level, we have regional hospitals, as well as the Regional Funds for Health Promotion for supply chain management, and at the peripheral level, there are district hospitals, medicalized centers, and integrated health centers as well as their dialogue structures (a platform for co-management and co-financing of health in Cameroon that brings together community representatives and health staff to discuss health issues). These structures include those of

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the public sector and private sectors. There is no clear distinction between the private and public health structures in Cameroon. National health policies and treatment guidelines, including elimination of user fees, apply in the same way to both sectors.

The health facilities in the districts are a doorway for health care supply. Patients are referred successively from peripheral health facilities to regional hospitals and then to the general/central hospitals or university teaching hospitals. In addition to these three levels of care, there are other health structures like military hospitals, specialized care centers, private sectors, traditional medicine, pharmacopeia, and recognized alternative medicines (178).

In Cameroon, the physician /patient ratio is 1:50,000, and about 35000 health care workers are needed to meet the WHO recommended ratio of 1 doctor per 10000 patients (181).

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Table 1: Different levels of the health pyramid in Cameroon and their functions

Level	Administrative structures	Competence	Health care structures	Dialogue structures
Central	Minister's office Secretary general Secretary of state to the MoPH -Technical departments and similar rankings	Development of policies Coordination Regulation Supervision	General hospital, University teaching Hospital, Central hospital, and similar rankings (CENAME, CPC, CHRACERH, LANACOME, CIRCB, ONSP)	National council of Health, Hygiene and Social Affairs
Intermediate	10 Regional delegations	- Technical support to Health districts - Regional coordination - Regulation - Supervision	Regional hospital and other ranking as such; regional Fund for Health Promotion	Regional Fund for Health Promotion
Peripheral	- 189 Health districts	- Care provision - District coordination - Regulation	- District hospital - Clinic - Medicalized health center - Integrated health center; Health cabinet	- District health committee; District management committee - Health committee Management committee

Note : MoPH : Ministry of Public Health ; CHRACERH : Centre Hospitalier de Recherche et Application en Chirurgie Endoscopique en Reproduction Humaine ; LANACOME : Laboratoire Nationale de Controle des Medicaments Essentielle ; CIRCB : Centre Internationale de Recherche Chantal Biya; ONSP: Observatoire Nationale de Santé Publique; CPC : Centre Pasteur du Cameroun

Source : Cameroon malaria profile, 2022 (178)

2.1.2.2 Organization of malaria care services

In Cameroon, the Health Sector Strategy 2016-2027 prioritizes the fight against malaria. The NMCP is the technical structure of the Ministry of Public Health under the disease control department responsible for the coordination, decision making, and implementation of malaria control activities. It is integrated into the National Health System and includes a central technical coordination group and 10 regional technical groups. The recommended care packages for public and private health facilities in the country include the integration and provision of routine anti-malarial care. This offer is reflected on the one hand, in the supply of health facilities with RDTs, antimalarial drugs, the distribution of long-lasting insecticide treated mosquito nets (ITNs), and the administration of intermittent preventive treatment of malaria to pregnant women (IPTp) received in antenatal care (ANC) consultations. On the other hand, it involves training of service providers and awareness-raising in beneficiary populations. Routine malaria control is strengthened by the organization of mass campaigns for distribution of ITNs and seasonal malaria chemoprevention (SMC), which involve all levels of the health system (178).

The national system for management of medicines and other pharmaceutical products also follows the health pyramid. CENAME ensures the acquisition, storage, and distribution of medicines and medical devices to the 10 regional funds for health promotion, while the regional funds ensure the storage and distribution of antimalarial

2. Malaria in Cameroon

drugs to health facilities within the regions. The health facilities in turn dispense the medicines to users and supply community health workers (CHWs).

The health system in Cameroon is funded by households' expenditure on health (70.4%), national budget (14.5%), and international partners (7.1%) such as the WHO, the Global fund, the Global Vaccines Initiative, the World Bank, United Nations International Children's Emergency Fund, European Union, United Nations Programme on HIV/AIDS (178). The proportion of the state budget devoted to health stands at 4-6%. For the fight against malaria, the activities are mainly financed by the Global Fund to Fight AIDS, Tuberculosis, and Malaria. Universal health coverage in Cameroon is in its first phase, which considers ongoing initiatives related to user fee elimination for treatment and prevention of malaria in children under five years of age and pregnant women in health facilities and in communities (178).

2.2. Burden of malaria in Cameroon

Malaria is the most widespread endemic disease in Cameroon, responsible for 3.3–3.7 million suspected cases in health care facilities per year (162), and more than two million reported cases as well as absenteeism from school and work. It equally accounts for 50% of all hospitalizations in health facilities, with 65% of these being children under five years of age (163)(178). Cameroon was among 11 countries that had the highest burden of malaria in the WHO African region in 2020 (*high burden high impact countries bearing 70% of global*

2. Malaria in Cameroon

burden), with 2.9% of all regional malaria cases, and 2.4% of malaria deaths. Likewise, Cameroon was among three countries that shouldered over 80% of malaria cases and deaths in the Central African sub-region in 2020, representing 12.6% of subregional malaria cases and 11% of deaths (182). In Cameroon the burden of malaria has been steadily on the rise from 2016 to 2021, with incidence moving from 79.5 cases to 113.7 cases per 1,000 population respectively with children below five years of age and pregnant women being the most affected (Figure 12) (178).

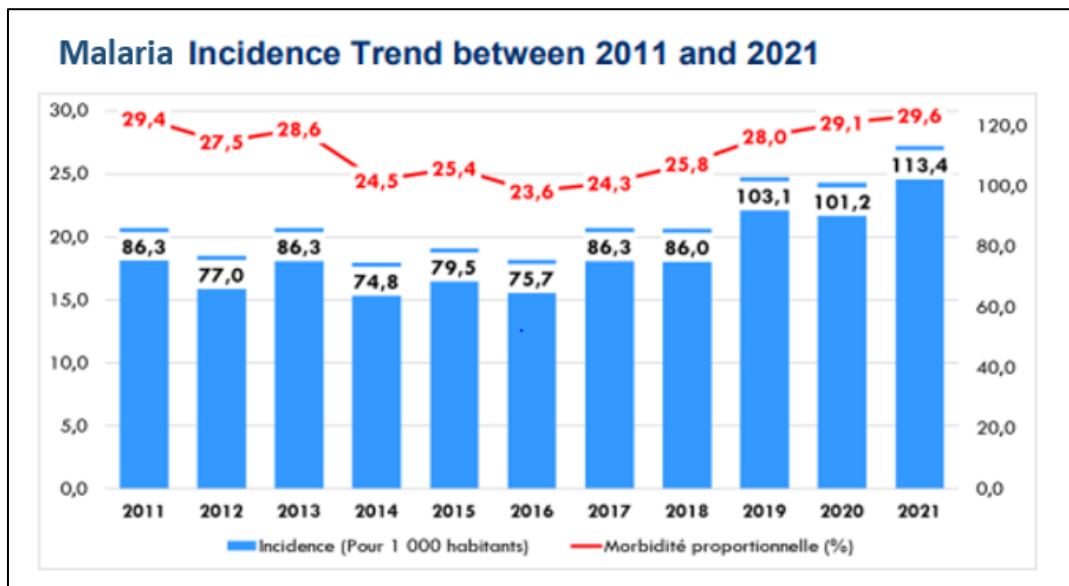


Figure 12: Malaria incidence trends between 2011 and 2021 (178).

2.3 Malaria control in Cameroon

2.3.1 Malaria case management

- **Evolution of malaria case management policies**

Due to the development of drug resistance, drug policy for malaria treatment in Cameroon gradually changed over the years from the use of monotherapies with chloroquine and amodiaquine as a first-line treatment for uncomplicated malaria to combination therapy (183). Chloroquine was the drug mostly used from the 1970s through to 2002 (184). From 1999 to 2004, an interim drug policy was adopted and amodiaquine was incorporated alongside chloroquine as an alternative first-line drug for uncomplicated malaria while sulfadoxine-pyrimethamine was used as a second line drug (183,184). In 2004, following recurrent treatment failure to amodiaquine and sulfadoxine-pyrimethamine, the Ministry of Health of Cameroon reconsidered its policy and adopted artemisinin-based combination therapies (ACTs) as a first-line treatment for uncomplicated malaria, while Sulfadoxine-pyrimethamine was still recommended as an intermittent preventive treatment for malaria during pregnancy (IPTp).

Due to exposure to recurrent malaria outbreaks in the northern part of the country during the rainy season, the government introduced seasonal malaria chemoprevention (SMC) for children below 5 years-old in 2016 (164,161). The combination artesunate-amodiaquine (ASAQ) which was initially used for the treatment of uncomplicated malaria in children under 5 years of age was replaced by artemether-

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lumefantrine (AL) and provided to all families for malaria prevention free of charge.

▪ **Current malaria case management in Cameroon**

An updated document on the guidelines for the management of malaria in health facilities and communities is regularly provided to health workers by authorities of the NMCP to ensure adequate implementation and follow-up.

Case management in Cameroon include diagnostic confirmation of suspected cases, treatment of confirmed cases at health facilities and community level according to national guidelines, scale-up of integrated community case management, pharmacovigilance, and supply chain strengthening (164).

Diagnosis: Malaria diagnosis in most health facilities is done by microscopy and/or RDT (164,161).

Treatment: The first line treatment of uncomplicated malaria is artemether-lumefantrine (AL), artesunate-amodiaquine (AS/AQ) and Dihydroartemisinin Piperaquine (DHA-PQ) administered by oral route.

First line treatment for malaria has switched to artemether-lumefantrine (AL) rather than artesunate-amodiaquine (AS/AQ) in the north and far north regions due to implementation of SMC. The second line treatment is Artesunate pyronaridine by oral route.

Pregnant women with uncomplicated malaria in the first trimester, are treated with oral Quinine for 7 days or an ACT (Artemether

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lumefantrine) if quinine is not available. Women in their second or third trimesters are given the same oral treatment as in adult and children.

For severe malaria, adults, children, and pregnant women are treated by parenteral route (intravenous or intramuscular injections) for at least 24hr until they can tolerate oral therapy and complete treatment with 3 days of an ACT. The injectable therapies include Artesunate injectables, artemether injectable, and quinine injectables in order of preference. Other case management actions for severe malaria include pre-referral intervention with artesunate rectal capsules at the community level, followed by referral to a health facility (185).

Integrated community case management (iCCM): iCCM for diarrhoea, pneumonia and malaria using community health workers, was introduced in 2009 to target groups with difficult access to health care services (186). ICCM includes clinical diagnosis and treatment provided by trained and supervised community health workers (CHWs).

Pharmacovigilance: Several studies have been conducted across the country to assess malaria drug efficacy. An evaluation of the efficacy and safety of artemisinin base combination therapies in four sentinel sites, Garoua, Bamenda, Nkongsamba and Ebolowa, including eight generic artemisinin base combinations between 2005 and 2016 concluded to the continuous efficacy of artemisinin base combination therapies in Cameroon (187,188,189,190). For the *Pfkelch* 13 gene,

2. Malaria in Cameroon

few random mutations have been recorded, but none of the mutations associated with artemisinin resistance in Southeast Asia have so far been recorded (191,192). However, Cameroon remains extremely vulnerable to potential risk of introduction and spread of artemisinin resistant mutations originating from Southeast Asia, due to the presence of soldiers from Asia (Bangladesh and Pakistan troops) on United Nation peace keeping operations in the neighbouring Central African Republic(193). Hence, the need for regular surveillance to avoid the rapid spreading of these new mutations in Cameroon and the sub-region. It will also be important to conduct regular monitoring of the therapeutic efficacy of artemether-lumefantrine now widely used across the country.

Capacity strengthening. Programmes are regularly conducted across the country on diagnosis and treatment of malaria cases, in order to improve and practices of health care workers (194,195).

2.3.2 Malaria prevention

▪ **Prevention of malaria in pregnancy**

Intermittent preventive treatment for pregnant women (IPTp) with sulfadoxine-pyrimethamine (SP) administered at antenatal care (ANC) was adopted in 2007. The policy was updated in 2012 to increase the number of doses of SP administered during pregnancy from at least two doses total to at least three doses total, one dose at each ANC beginning at 16 weeks and continuing monthly until birth. The policy was further

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updated in 2020 to start SP earlier at 13 weeks, and continued monthly during ANC visits (164). In 2018, 54% of pregnant women received at least two doses of intermittent preventative treatment of malaria in pregnancy (IPTp2), similar levels to those in 2014 (53%). The proportion of pregnant women who received at least three doses (IPTp3) increased from 26% in 2014 to 32% in 2018 (182).

▪ Seasonal malaria chemoprevention

Cameroon introduced seasonal malaria chemoprevention (SMC) in 2016 with SP and amodiaquine (SPAQ) to roughly 1.1 million children of eligible age (children 3–59 months old) living in the 45 health districts of the North and Far North regions, with a coverage of 86 percent (164,161,182). The NMCP's malaria Strategic plan promotes SMC as a malaria prevention intervention in areas with highly seasonal malaria transmission. SMC is implemented in all 47 eligible districts in the North and Far North regions, including procurement of SPAQ to meet the needs of the eligible population as well as all aspects of implementation with four SPAQ cycles during the rainy season (July to October) using a door-to-door strategy. The approach requires community distributors to directly observe the administration of the first dose of SPAQ and leave treatment doses for administration by the caregiver on days two and three for all cycles. In 2017, four cycles of SPAQ (SP + amodiaquine), the combination of antimalarials used for SMC, were administered to 1.5 million children aged 3–59 months old (94.5% of children in that age group) (164). About 3.5 million children

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were administered with SMC in 2018. Preliminary data from the first two SMC cycles in the northern region in 2017 suggested that SMC contributed to preventing malaria in over 95% of children aged 3-59 months (164,182).

▪ **Perennial malaria chemoprevention**

Perennial malaria chemoprevention (IPTi) was recently (2023) recommended for infants under two years of age in areas of Cameroon not implementing SMC for this population, during The High burden - high impact (HBHI) subnational tailoring exercise. During vaccination sessions in health facilities, these infants will be given five doses of SP at specific intervals corresponding to their vaccination schedule (10 weeks, 14 weeks, 6 months, 9 months, and 15 months). At the same time, a pilot implementation of eight doses will be done in six other health districts in the Center region. In this pilot, the impact of eight doses (10 weeks, 14 weeks, 6 months, 9 months, 12 months, 15 months, 18 months, and 23 months) instead of five doses as in the other health districts will be assessed. This intervention is expected to reduce the incidence of malaria by an additional 20 percent (163).

▪ **Vaccination**

At the beginning of 2023, the minister of public health announced a plan to initiate malaria vaccination (RTS, S/AS01 vaccine) for infants aged between 6 and 24 months in Cameroon from January 2024. Children were to receive 4 doses of the vaccine at 6, 7, 9 and 24 months

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to ensure adequate protection. According to the report, 1600,000 doses of vaccines had been obtained and these will be administered to children in communities with high risk of malaria in all the 10 regions and in 42 health districts which are sites with the highest transmission and more severe malaria in the country (196). On January 22, 2024, Cameroon launched the first large scale country wide vaccination campaign against malaria, being the first country to administer doses through a routine vaccination programme. The vaccine is offered free of charge to all infants of eligible age, and it is given at the same time with other routine childhood vaccines to make it easy for parents. As of February 19, 2024, over 12,000 children had been vaccinated with the first dose, representing 34.6% of the target population of the related period (197)

▪ Insecticide-treated nets

Cameroon has benefited from the support of various international partners to implement malaria control interventions since 2000 (161)(178). More than 20 million long-lasting insecticidal nets (LLINs) have been freely distributed to the population through several campaigns, with the support of partners (e.g. the Global Fund) ((178) . This scale-up of treated bed nets across the country between 2000 and 2015 resulted in a significant decrease in the prevalence of malaria reported cases from 41% to 24.3%, and 54% decrease in malaria related mortality (from about 13,000 to 6000 per year) (164,162). In 2017, Cameroon was selected as a US President's Malaria Initiative (PMI)

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focus country. The PMI programme was focused in the North and Far North regions to procure and distribute over 250,000 LLINs to pregnant women during antenatal care and also undertake indoor residual spraying (IRS) trials to foster malaria elimination in this part of the country (164,178). Between 2011 and 2018, the proportion of the population with access to an insecticide-treated net increased from 21% to 59%. Over the same period, the use of ITNs by children and pregnant women increased similarly, from 21% in 2011 to 60% in 2018 (163). A third nationwide free distribution of over 15 million LLINs to the population was planned for 2019. Other interventions piloted in other epidemiological settings of the country such as larviciding in the city of Yaoundé and the PADY (Projet d'Assainissement de Yaoundé) programme focused on hygiene and sanitation in Yaoundé (198,199).

Despite the many preventive and control measures in place, malaria morbidity and mortality remain high in Cameroon, especially among under 5 children who have not yet developed protective immunity and are at higher risk of severe infections. These children are constantly being contaminated through transmission from asymptomatic carriers who don't know about their positivity. That is why there is a need for measures to eliminate asymptomatic reservoirs and break the cycle.

CHAPTER 3: STUDY RATIONALE AND OBJECTIVES

3. Study rationale and objectives

3. Study rationale and objectives

3.1 Rationale of the study

Malaria remains an important infection in sub-Saharan Africa due to the challenge of controlling and eradicating the disease. 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. In this light, there have been optimistic scale-up of the WHO-recommended control actions towards malaria elimination in Cameroon over the last decades, with focus on vector control, early diagnosis and prompt treatment of symptomatic cases, as well as chemoprevention for under-five children and pregnant women (163,178,200). Despite the recent progress on reducing morbidity and mortality in response to this, there is 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. A first reason is climate change, which has increased the prevalence of malaria and other vector-borne diseases in recent decades, with risk of a further increase if control measures are not intensified (201). A second reason is that the effectiveness of current strategies has been threatened by the emergence of resistances for both insecticide and antimalarial drugs including ACTs (artemisinin partial resistance), as reported in several African countries (110,111,112,113,202,203,204,205). In addition,

3. Study rationale and objectives

these strategies are restricted to indoor controls and populations remain exposed to *Plasmodium* transmission during their outdoor activities. They also fail to target asymptomatic *Plasmodium* carriers who constitutes most (more than 95%) of the infected people in endemic settings (39,40,41,42,43), and often do not seek antimalarial treatment due to absence of clinical illness, thereby perpetuating disease transmission over time. Hence, the need to identify and treat asymptomatic infectious human reservoirs with treatment that fully clears the parasites (sexual and asexual) to prevent continuous transmission.

Recent investigations that *Artemisia afra* tea infusions have antiparasitic activity against the different stages of the malaria parasite *invitro*, including the gametocyte stage, with no cytotoxicity (132,133,151,136,152), suggests its probable usefulness of in the elimination of asymptomatic *Plasmodium* reservoirs (and clearing of residual parasites in asymptomatic individuals after treatment for clinical malaria) in endemic areas. However, the practice of herbal medicine varies greatly from country to country, and their acceptance and use by health care workers and the population may be influenced by several factors. Also, no clinical studies have been conducted to ascertain the potency safety of *Artemisia afra* tea in eliminating asymptomatic *Plasmodium* reservoirs. This therefore underscores the purpose of our study. Our assumption is that a superior knowledge of health care workers about asymptomatic malaria and its impact on

3. Study rationale and objectives

health will influence their management of asymptomatic *Plasmodium* carriers. On the other hand, a positive view of tea treatments by both health care workers community members (potential users) and confirmed effectiveness of *Artemisia afra* tea infusions in emptying *Plasmodium* reservoirs, will increase acceptability and use of the teas in treating, and subsequently eliminating the reservoirs (Figure 13).

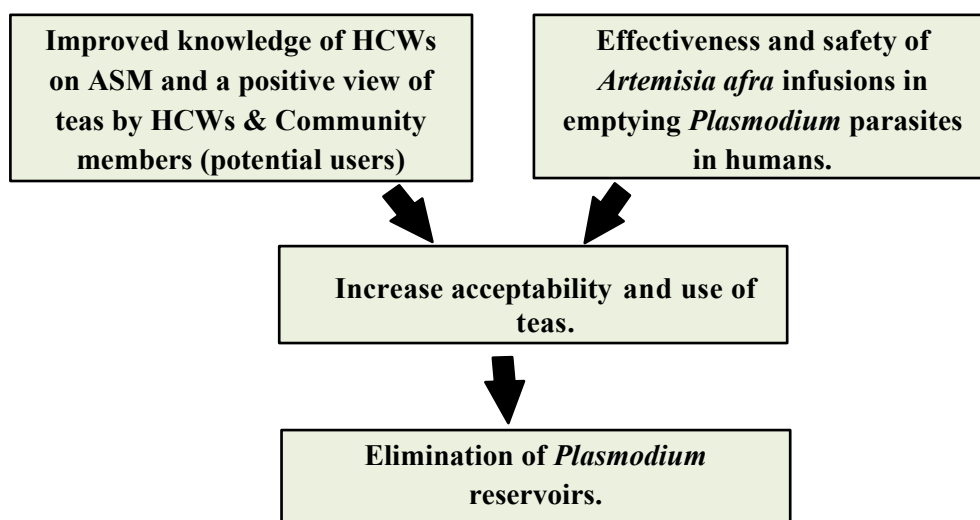


Figure 8: Conceptual model of contributions to the elimination of *Plasmodium* reservoirs in malaria endemic regions of Cameroon.

3. Study rationale and objectives

3.2 Objectives

3.2.1 General objective

The overall objective of this thesis is to contribute to the elimination of asymptomatic *Plasmodium* reservoirs in malaria endemic areas of Cameroon using *Artemisia afra* tea infusions.

3.2.2 Specific objectives

1. To explore experiences of adult Cameroonians on the use of herbal teas to treat infections or diseases, and their acceptability to use the teas if prescribed in hospital.
2. To assess the knowledge of health care workers about asymptomatic malaria and their acceptability to use *Artemisia afra* tea to treat asymptomatic *Plasmodium* carriers.
3. To ascertain the efficacy and safety of *Artemisia afra* tea in emptying parasites in asymptomatic *Plasmodium* carriers

CHAPTER 4: METHODS

4. Methods

4. Methods

4.1 General overview of the study

This study is part of a research project financed by the Belgian government under the management of ARES-CCD (Académie de recherche et d'enseignement supérieur - Commission pour la coopération au développement), named "Project de recherche pour le développement (PRD2020-Souopgui, CMR)", carrying out a set of studies in Cameroon in partnership with the Université catholique de Louvain (UCLouvain), Université de Liège (ULiège) and Université Libre de Bruxelles (ULB) in Belgium, and the Universities of Buea (UB) and Douala (UDla) in Cameroon. The project encompasses pharmacological, molecular biological, and epidemiological studies on the effectiveness of *Artemisia afra* tea in eliminating *Plasmodium* reservoirs (Figure 14). The study intended to use extracts and infusions of *Artemisia afra* tea, harvested from the west region of Cameroon. Invitro studies to confirm the antimalarial activity of the plant will be followed by clinical studies in communities and hospitals in Bafoussam, Buea, Douala and Yaounde, to test the potency of the tea to empty *Plasmodium* parasites in asymptomatic carriers identified in communities and residual parasites in persons treated for clinical malaria using ACTs. The current thesis presents the epidemiological studies, while details of the molecular biological and pharmacological studies will be presented at ULB and ULiège, respectively. In order to determine the acceptability of the tea by the population (potential users) and ensure adequate uptake if effectiveness is ascertained, cross-

4. Methods

sectional surveys were conducted in the Southwest region (Buea) and the Centre region (Yaounde) of Cameroon (i) to explore the experiences of adult Cameroonians on the use of herbal teas to treat infections or diseases, and their acceptability to use the teas if prescribed in a hospital, and (ii) to assessed the knowledge of health care workers about asymptomatic malaria and their acceptability to use *Artemisia afra* tea to treat asymptomatic *Plasmodium* carriers. A clinical trial aimed at ascertaining the efficacy and safety of *Artemisia afra* tea infusions in emptying *Plasmodium* reservoirs has also been designed for implementation.

4. Methods

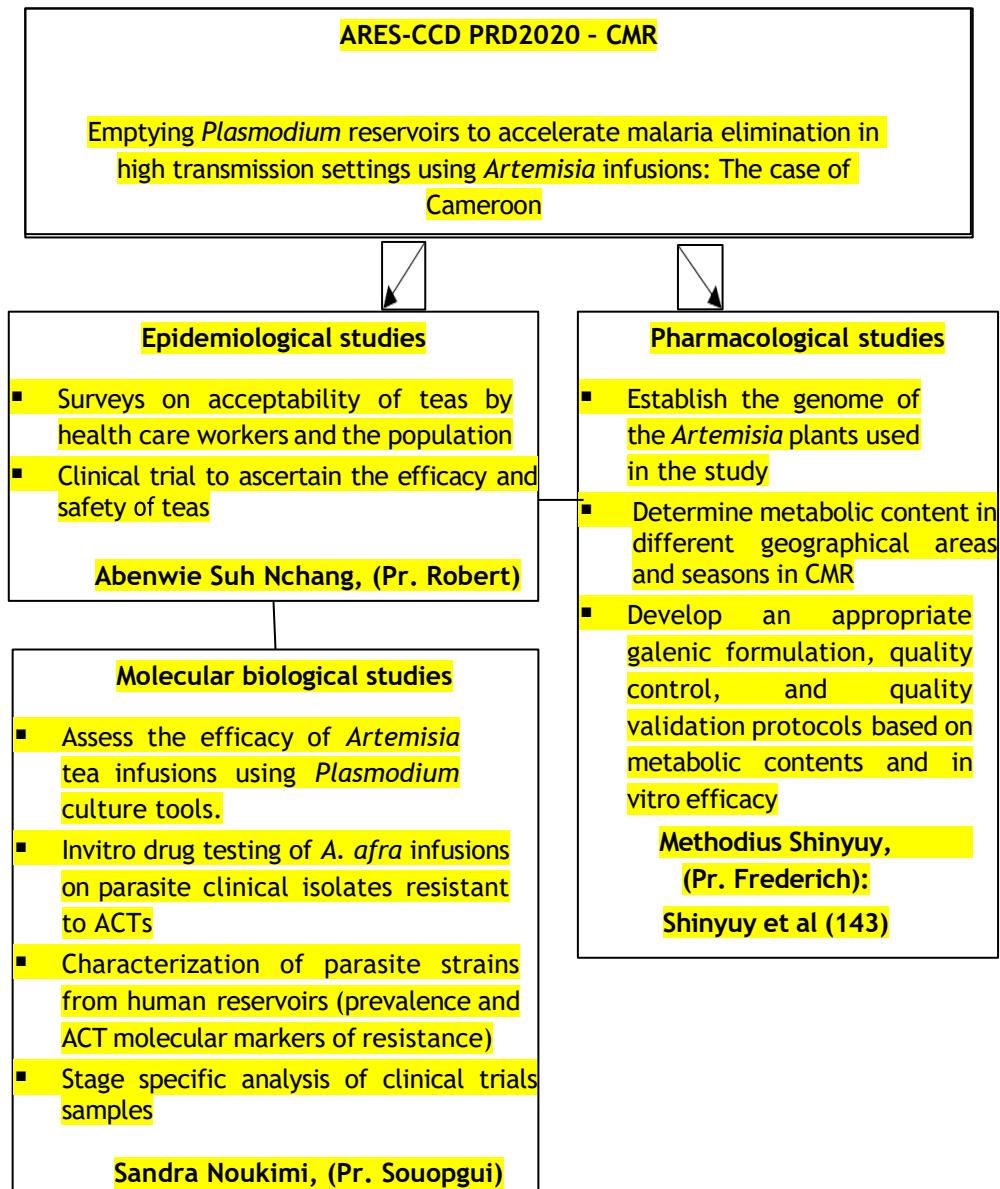


Figure 14: General overview of the PRD 2020 project

4.2 Study design and sites

This thesis includes two published manuscripts reporting on the cross-sectional surveys conducted and the clinical trial protocol. The surveys were conducted in selected district hospitals and communities in Buea (Fako Division) in the Southwest region of Cameroon, and in Yaounde (Mfoundi and Lekie divisions) in the Centre region of Cameroon from January 4 to April 20, 2022, during the covid-19 pandemic. Community studies were conducted in four urban communities, comprising two urban communities (Muea and Mutengene) within the Buea and Tiko health districts in Buea, and two urban communities (Mbandomu and Nsimeyong) within the Biyem-Assi and Efoulan health districts in Yaounde. Hospital studies were conducted in 2 urban hospitals (Biyem assi and Efoulan district hospitals) and 2 rural hospitals (Obala and Okola districts hospitals) in Yaoundé (Figures.15 and 16).

4.3 Study area

Cameroon has about 90% of the African ecosystems which includes the Sahelian, Sudan, humid tropical forest, afro mountains, coastal and mountain eco-regions (206). Fako Division in the Southwest region of Cameroon is one of six administrative Divisions in this region and is host to the Southwest regional headquarters -Buea. Other administrative Divisions in the Southwest region include Manyo, Ndian, Lebialem. Kupe-Manengubam, and Meme Divisions (Figure 15). It lies in the coastal region between 4°28'30"N and 3°54'26"N

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latitudes, and 8°57'10"E and 9°30'49" E longitudes (Figure 15). The land area is approximately 203,071.3 hectares (ha) with a population of 534,854 people. Most inhabitants practice agriculture as main economic activity (206). The region has two meteorological seasons: the dry season from October to March and the wet season from April to September. People from all the three main ethnic groups in Cameroon (Bantus, Semi-Bantus, and Sudanese) are represented in this coastal region due to attraction by the fertile volcanic soil for agriculture.

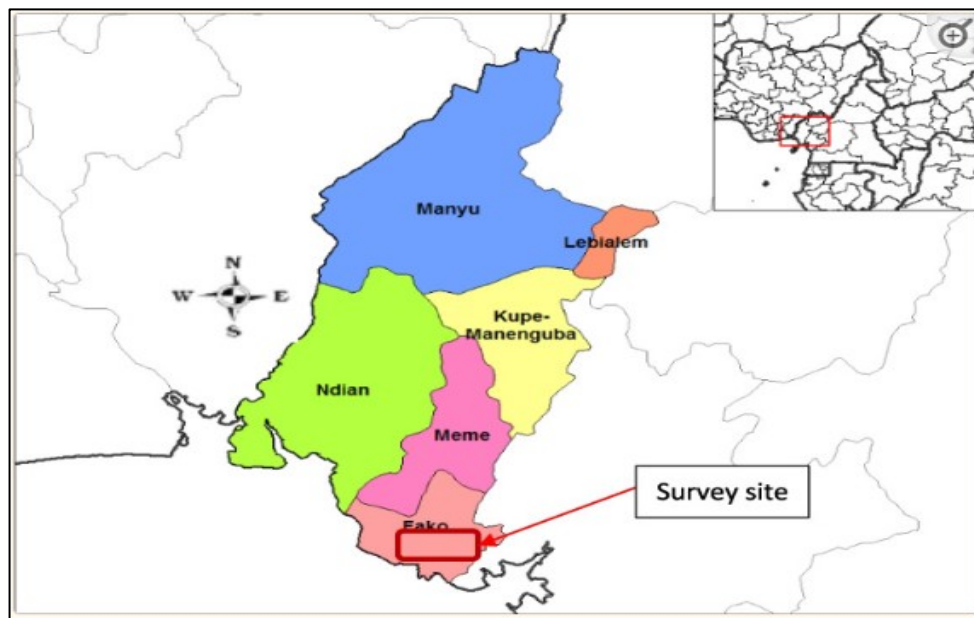


Figure 9: Map of Southwest region of Cameroon showing administrative divisions and the survey site

Source: Rarelibra 19:57, 2006.

4. Methods

The Center Region is one of the 10 administrative regions of Cameroon which lies in the southern plateau. It is one of the most densely populated regions of Cameroon, with a population of over 3 million inhabitants mainly living in the city of Yaoundé (population 1.1 million) or along the roads and in the major towns (207). The population density thins out away from the major thoroughfares, with few isolated settlements. Most people in the urban area are civil servants and businessmen, while most people in the rural area practice agriculture. The Center Region is in-turn divided into 10 administrative divisions (Figure 16), with a total of 30 health districts (7 urban and 23 rural). It is serviced by many hospitals and clinics, particularly in Yaoundé and in the larger towns. However, traditional medicine is still common throughout, especially in the rural areas. Besides being the headquarter of the Centre Region, Yaoundé is also the capital city of Cameroon and the second largest city of Cameroon (208). The city is located within the Congo-Guinean phytogeographic zone, characterized by a typical equatorial climate with two rainy seasons extending from March to June and from September to November. It is situated 800 m above sea level and surrounded by many hills (209). Malaria transmission in Yaoundé is considered to be holo-endemic and seasonal(210)(211). Meanwhile, the median annual prevalence of *Plasmodium falciparum* is estimated to vary between 34 and 50% in the general population from the city center to the periphery (176). The most affected people are children aged between 0 and 15 years,

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comprising 75% of asexual parasite carriers, 85% of carriers of high parasitemia and 83% of gametocyte carriers (173).

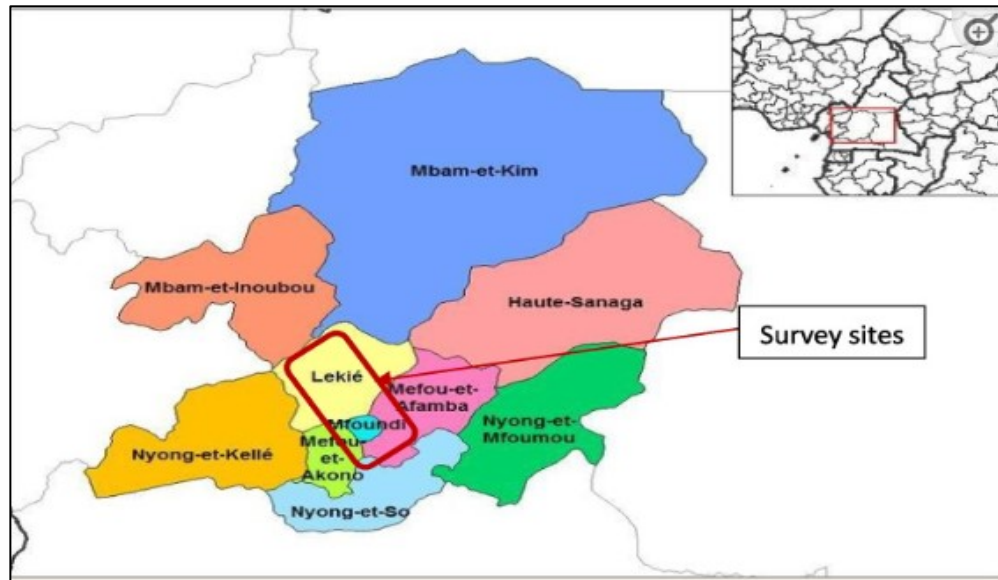


Figure 16: Map of Centre region of Cameroon showing administrative divisions and survey sites

Source: Rarelibra 19:57, 2006

4.4 Study participants

The study participants comprised adult Cameroonians aged between 18 and 65 years, who were resident in study sites (communities) during study period and consented to participate in the study. Hospital based participants were patients and/or their care givers, found in the selected hospitals during the study period. Participants in the HCWs survey included Physicians (general practitioners and specialized doctors) and nurses degree nurses, state registered nurses, and nursing assistants)

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who were involved in treatment prescription in study hospitals and on duty. Participants who had communication hindrances such as the deaf and mute, and HCWS who were non-prescribers or internship students were excluded from the study.

4.5 Sample size calculation

Sample size was calculated using the sample size determination formula: $n = z^2 p(1 - p) / d^2$ where n is the minimum sample size, z is the standard normal quantile corresponding to the desired level of confidence (1.96 for 95% confidence interval), d is the standard error (5%), and p is the WHO reported prevalence of global traditional medicine use (set at 80%) (212). Adapting for an expected non-respondent rate of 25%, the sample size required for the study was 307. Out of the 307 participants in this study, two-thirds (207) of them were community participants and one-third (100) were hospital participants. The number of community participants was evenly shared between the two study regions (103 in the Southwest and 104 in the Centre region). The number of hospital participants was also evenly shared in the four study hospitals (two rural and two urban).

4.6 Selection of study sites and participants

Sampling method: Clustering: Four health districts were randomly selected from the thirty health districts in the center region of Cameroon (two among the 23 rural health districts and two among the 7 urban health districts) by lottery method in which numbers were assigned to the health districts involved, and random numbers were drawn from the pool. The district hospital of the health district selected was used for data collection. Participants within the selected health units were recruited by *a systematic sampling method*. These participants were patients or their care givers, recruited at the out-patient department (OPD) and in waiting areas of the hospital. The first respondent per health unit was selected from the list of 9 initial (first nine single digits: 1–9) respondents by lottery method. Then, every—7th (digit randomly selected from 9 single digits) participant was interviewed until the required sample size for that health unit was attained. Where a participant didn't consent to participate, the next person in line was interviewed. For the HCWs survey eligible participants were consecutively recruited and interviewed until the pre-established sample size of 25 HCWs per facility was reached. A total of 100 participants were recruited in the four study sites.

At the community level, communities within the selected health districts were selected by lottery method. The starting point of questionnaire administration in each community was determined by a

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landmark in that community. Systematic random sampling technique was then used to select households to be included in the study. The first household per community was selected from the list of 9 (first nine single digits) initial households by lottery method. Then every 4th household (digit randomly selected from 9 single digits: 1–9) was selected and an adult between 18–65 years old in the household was interviewed until the required sample size in that community was attained. In the presence of more than one adult, balloting was done to choose one adult who was interviewed.

4.7 Data collection

Data was collected using semi-structured questionnaires, which were developed based on the objectives of the study (see Annex 1). For the adult population study, the questionnaires were *interviewer-administered* through face-to-face interviews by six (6) trained research assistants. The HCWs questionnaires were *self-administered* and were completed in the presence of research assistants to ensure thorough and timely completion.

4.8 Study variables

For the population survey, outcome variables included respondents' utilization and experiences of herbal tea use, and respondents' acceptability to use herbal teas if prescribed in health facilities; while the explanatory variables were age of interviewee, gender, scholarly level, household size, profession, and socioeconomic/income status.

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Herbal teas in this study referred to all herbal or plant products of any kind (including the leaves, flowers, pollen, fruits, seeds, stem, or roots, weather dry or fresh), that have been prepared in the form of infusion or liquid by any means (boiling, crushing straining), and are taken orally, at any temperature (warm or cold), for the purpose of health maintenance, disease prevention or treatment.

The outcome variables for the HCWs survey were knowledge of HCWs about asymptomatic malaria (existence, definition, diagnosis, management, and transmission), opinion and acceptability of *Artemisia afra* tea among HCWs and predicted challenges of using the tea to treat asymptomatic *Plasmodium* carriers. Explanatory variables were age, gender, professional category, longevity of service, and site of practice of the of health care workers.

4.9 Ethical considerations

The study approval was obtained from the Faculty of Health Sciences-Institutional Review Board (FHS-IRB), University of Buea, Cameroon (2021/1591–01/UB/SG/IRB/FHS. A conditional ethical approval was also obtained from the ethics committee of the UCLouvain medical faculty, Brussels, Belgium (Comite d’Ethique Hospitalo-Facultaire/8 November 2021). Administrative authorization was also obtained from competent authorities of each study site. All methods were carried out in accordance with the Declaration of Helsinki. Each participant in the study was provided with necessary information about the study

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including the respect of confidentiality and their right to voluntary participation. Written informed consent was obtained from all the participants. They were allowed to discontinue the interview when they needed.

4.10 Data management and statistical analysis

Data management and statistical analysis is presented with details in each publication. Briefly, Logistic regression analysis was used to determine factors associated with herbal tea use and with asymptomatic malaria knowledge of HCWs. The open-ended questions were analyzed using contents analysis, and frequencies of occurrences quantified to complement quantitative analysis.

CHAPTER 5: RESULTS

5. Results

5.1 Results 1

The use of herbal tea among Cameroonian adults (18–65 years)

Abstract

Background: In respect of the WHO's commendation to incorporate traditional medicine (TM) in health care, the Cameroon government wants to promote the use of the traditional medicine and is resolute on encouraging the treatment of patients with alternative medicine from traditional sources. Based on reports of invitro studies about the antimalarial activity *Artemisia afra* tea infusions that suggest its probable usefulness in the elimination of asymptomatic *Plasmodium* carriers in endemic areas, clinical studies are underway to confirm the effectiveness of this tea for recommendation. However, the acceptance and use of herbal medicines may be influenced by several factors. This study explores the experiences and use of herbal tea by Cameroonian adults to prevent or treat diseases and their acceptability to use herbal teas in prescribed in hospital.

Methods: A cross-sectional survey was conducted among 307 Cameroonian adults (18–65 years) randomly selected within 4 hospitals and 4 communities in the Centre and Southwest regions of Cameroon between 04/01–20/04/2022, using interviewer administered semi-structured questionnaires. Binary logistic regression analysis was conducted to determine the association between variables.

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Results: Over four-fifth (89.1%) of participants had taken herbal teas at least once within the last 2 years prior to the survey, and most participants used the teas for the prevention and treatment of Covid-19 (67.9%), malaria (59.7%) and typhoid fever (35%). Most respondents took the teas warm (75%), and the treatment dosage used by most respondents (51%) was “one glass in the morning and evening for one to two weeks”. The teas taken by 70% of users had bad or bitter taste and 52.2% of them were uncomfortable with the bad taste. However, the majority of users completed their treatment dosage (72%). Nine in ten (90.5%) participants were willing to use teas for treatment if prescribed in health facilities in future, and 90.1% were in support that herbal teas should be prescribed in hospitals. There was no significant association ($p \geq 0.05$) between sociodemographic characteristics of participants and herbal tea use. However, the major motivating factor for acceptability of herbal tea use was treatment effectiveness (52.7%).

Conclusion: There is high prevalence of herbal tea use among adults Cameroonians in the studied settings in the Centre and Southwest regions of Cameroon, with a positive opinion and willingness to use teas if prescribed in health facilities. Authorities must ensure the effectiveness and safety of traditional medicine served in health facilities, to enhance compliance and adequate use.

Keywords: Herbal tea use, Experiences, Acceptability, Cameroonian adults

As Published

Suh Nchang <i>et al.</i> <i>BMC Complementary Medicine and Therapies</i> (2023) 23:285 https://doi.org/10.1186/s12906-023-04040-6	BMC Complementary Medicine and Therapies
RESEARCH	Open Access
A cross-sectional survey on the use of herbal tea among Cameroonian adults (18–65 years)	
Abenwie Suh Nchang^{1*}, Sylvia Njong¹, Sandra Fankem Noukimi², Lahngong Methodius Shinyuy³, Sylvie Bambara¹, Edgar M. Kalimba⁴, Joseph Kamga⁵, Jacob Souopgui², Stephen Mbigba Ghogomu⁵, Michel Frederich³, Jean Lesort Louck Talom^{6†} and Annie Robert^{1†}	

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From a total of 307 participants who were identified for the study in hospital and community settings, 300 of them participated in the study and 7 of them refused to participate, yielding the response rate of 98.4%. All the 7 people who refused to participate in the study were from the community sample (3 from Buea and 4 from Yaounde). The main reason for the refusal to participate in the study was a lack of interest in the study. The flow of the study participants in the different study sites is presented on Figure 3, and distribution of the study participants according to their socio-demographic characteristics and herbal tea use, in the community and in the hospitals, is presented in Table 1.

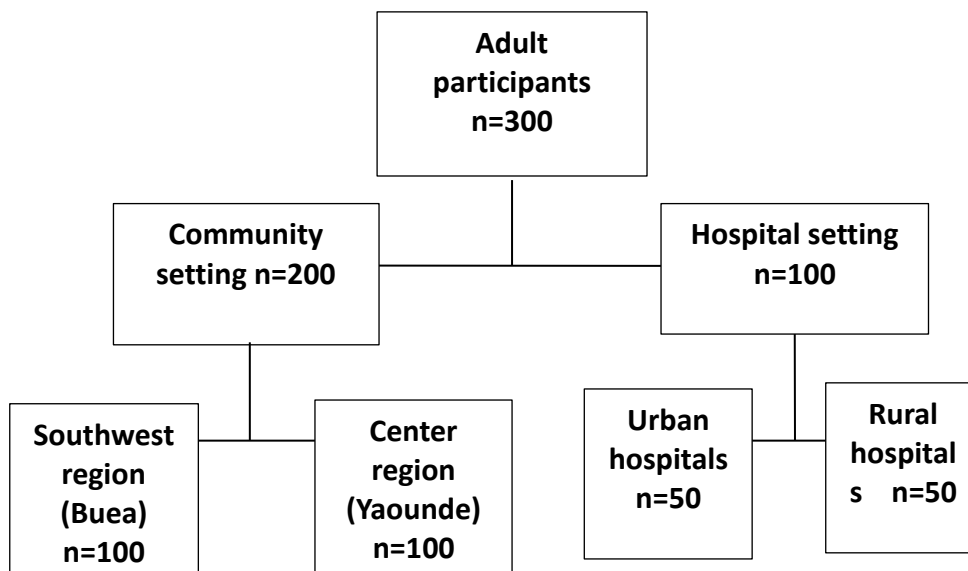


Figure 3: Distribution of participants across study surveys

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Table 1 Socio-demographic characteristics of the respondents according to herbal tea use in the community and in the hospitals.

Sample Characteristics	Community respondents		Hospital respondents	
	N=200		N=100	
	n	%	n	%
Total	174	87.0	94	94.0
Age of respondents (years)				
[18-35[	67	84.8	40	93.0
[36-65[	107	88.4	54	94.7
Gender				
Women	95	88.0	54	94.7
Men	79	85.9	40	93.0
Scholarship level				
Primary	46	95.8	14	100
Secondary	62	84.9	24	88.9
High school or Higher	66	83.5	56	94.9
Size of household	5.1±2.6			
1-5 persons	111	86.1	49	92.5
>5 persons	63	88.7	45	95.7
Professional category				
Student	17	68.0	16	100
Manual laborer	23	88.5	26	96.3
Officer	134	89.9	52	91.2
Socio-economic status/Income				
Not enough to live with	34	87.2	23	95.8
Just enough to live with	79	85.9	33	91.7
Enough and easy to live with	61	88.4	38	95.0

Size of households min=1, max=15; n=Herbal tea use

Socio-demographic characteristics of the respondents according to herbal tea use in the community and in hospitals

Table 1 shows that the use of herbal teas was generally high (>80%) among community and hospital participants. However, the proportion of tea use was higher among hospital participants across all socio-demographic sub populations, compared to community participants, and tea use among hospital participants was higher than 91 % in all but 1

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category of participants (secondary education). The proportion of tea use among women was slightly higher than men for both community (88% > 85.9%) and hospital respondents (94.75%>93%), respectively. Also, the proportion of tea use was slightly higher among participants who lived in household with more than 5 persons compared to those with household size of 5 persons or less, in both community (88.7% > 86.1%) and hospital settings (95.7%> 92.5%), respectively.

Participants' Utilization of herbal tea use

Of the 300 respondents in the survey, 268 (89.3%) had taken herbal teas within the past two years for prevention or treatment of infections, or for other purposes, while 32 (10.7%) of respondents had never taken herbal teas for any purpose. Twenty-six (26/32; 81%) of the respondents who had never taken herbal teas, were willing to take herbal teas in the future, when necessary, while 6/32 (11%) were unwilling to take teas in the future, because of lack of trust in effectiveness and hygiene of the teas (83.3) or the uncomfortable taste of teas (16.7%) (Table 2).

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Table 2 Participants' utilization of herbal tea

Question asked	N=300 Response frequency n(%)
<i>Have you taken herbal tea in the past 2 years for prevention, treatment or other purpose?</i>	
Yes	268 (89.3)
No	32 (10.7)
<i>If No, Can you take herbal teas to prevent or treat infections? (n=32 out of 300)</i>	
Yes	26 (81.2)
No	6 (19.8)
<i>If No, why can you not take herbal teas? (n=6)</i>	
Lack of trust in its Effectiveness and Hygiene	5 (83.3)
Uncomfortable taste	1 (16.7)

Indications for use, source of herbal tea, dosage, and posology of herbal tea

Majority of the respondents who took herbal teas used them for the prevention and treatment of Covid-19 (182/268, 67.9%), followed by malaria (160, 59.7%) and typhoid fever (94, 35%). Meanwhile, 111 (41%) of participants took teas for other conditions like cough or chest infection, low blood level, to initiate labour and/or delivery, fever, common cold, worms, gastritis, knee ache, fibroids diabetes, testicular pain, mumps contraception, hemorrhoids, fertility booster, sinuses tooth ache, high blood pressure, or painful menses (Table 3). Most of the tea users received their tea prescription from someone else (65%), and 35% of teas were self-prescribed. The teas used were mainly self-prepared (37%), purchased (34%) or offered to users for free (29%). Most of the respondents took the teas warm (75%), and the treatment dosage used by most respondents (51%) was “one glass morning and evening for 1-2 weeks” (Table 3).

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Table 3 Indications for use, source of herbal tea, dosage, and posology of herbal tea

Question asked	N=268 Response frequency n(%)
<i>What condition was the herbal tea taken for? (Several options)</i>	
Covid 19	182 (67.9)
Malaria	160 (59.7)
Typhoid	94 (35.1)
Stomach ache	37 (13.8)
Weight loss	8 (3.0)
STIs	8 (3.0)
Others	111 (41.4)
<i>Did someone prescribe it to you or you prescribed for yourself?</i>	
Self Prescribed	95 (35.4)
Prescribed by someone else	173 (64.6)
<i>Did you buy it, or it was offered , or it was selfprepared?(n=268)</i>	
Was self prepared	99 (36.9)
Bought treatment	91 (34.0)
Was offered for free	78 (29.1)
<i>What quantity, how frequent , and for how long did you take it?(n=268)</i>	
1 glass morning and evening for 1-2 weeks	139 (51.9)
1 glass morning, afternoon and evening for 1-2weeks	50 (18.7)
1 glass daily for 1-2weeks	22 (8.2)
1 glass morning and evening for less than 1 week	28 (10.4)
No precise dosage	26 (9.7)
Taken until one feels better	3 (1.1)
<i>Did you take it warm or cold? (n=268)</i>	
Cold	66 (24.6)
Warm	202 (75.4)

Others: Cough/chest infections, low blood level, initiate labour and delivery, fever, common cold, worms, gastritis, knee ache, fibroids diabetes, testicular pain, mumps contraception, hemorrhoids, fertility booster, sinuses tooth ache, high blood pressure and painful menses.

Participants' experiences in using Herbal teas

Table 4 shows that 140/268 (52.2 %) participants who used herbal teas were uncomfortable taking the teas. The teas taken by most respondents (189, 70%) had bitter taste, and 113 (42.2 %) of them were uncomfortable with the bitter taste of the teas, while 60 (22.2%) of them were comfortable with the bitter taste. Even though many of the participants were uncomfortable taking the herbal tea treatment, more than two-third

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(193, 72.2%) of the respondents completed their treatment, mainly because of their desire to get well (181, 67.5%), and to respect the treatment dosage (12, 4.5%). On the other hand, 42 (15.7%) of respondents abandoned their treatment as soon as they felt better. Meanwhile others abandoned it because of the uncomfortable taste of the tea (24, 9.0%), because of negligence (carelessness) (4, 1.5%), because there was no precise treatment dosage (3, 1.1%), or because of ineffectiveness of the treatment (2, 0.7%). Of the 268 participants who had taken herbal tea in our survey, 244 (91%) of them had never taken *artemisia* tea. Meanwhile, most of the respondents who had taken *artemisia* tea used it for the treatment and prevention of malaria (15/24, 62.5%), or Covid-19 (5/24, 20.8%). Three-quarter 18/24 (75%) of the respondents who took *Artemisia afra* tea said it was effective for them, while 1 (4.2%) said it wasn't effective and others 5 (20.8%) didn't know if it was effective on them or not (Table 5.1.4).

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Table 4 Participants' experiences in using Herbal teas

Question asked	N=268 Response frequency n(%)
<i>Were you comfortable taking the herbal tea?</i>	
Yes	128 (47.8)
No	140 (52.2)
<i>Were you comfortable with the taste?</i>	
It had bitter taste and I was uncomfortable taking it	113 (42.2)
It had bitter taste but I was comfortable taking it	60 (22.4)
It had no bitter taste and I was comfortable taking it	66 (24.6)
It had no bitter taste but I was uncomfortable taking it	13 (4.8)
I was uncomfortable with its bad taste but finished it	14 (5.2)
I was uncomfortable with its bitter taste and I abandoned it	2 (0.8)
<i>Did you finish the treatment or you abandoned some?</i>	
Finished treatment	193 (72.0)
Abandoned treatment	75 (27.9)
<i>Why did you finish or Abandon the treatment ?</i>	
Finished treatment because of desire to get well	181 (67.5)
Finished treatment to respect dosage	12 (4.5)
Abandoned treatment after I felt better	42 (15.7)
Abandoned treatment because of uncomfortable taste	24 (9.0)
Abandoned treatment out of negligence	4 (1.5)
Abandoned treatment since there was no precise dose	3 (1.1)
Abandoned treatment because it was not effective.	2 (0.7)
<i>Did you ever take Artemisia tea?</i>	
Yes	24 (9.0)
No	244 (91.0)
<i>If Yes, What condition did you take Artemisia tea for?</i>	
Treatment of malaria	13 (54.2)
Prevention of malaria	2 (8.3)
Prevention and treatment of Covid -19	5 (20.8)
Others	4 (16.7)
<i>Did the treatment work for you ?</i>	
Yes	18 (75.0)
I don't know	5 (20.8)
No	1 (4.2)

Acceptability of herbal teas if prescribed in the hospital

Most respondents 266 (90.5%) were willing to use herbal teas for treatment in the future if prescribed in the hospitals, mainly because they had confidence in doctors and the hospital (137, 46.6%), because of their previous experience on the effectiveness of herbal teas (29, 9.8%), or because of their personal preferences for herbal teas (11, 3.7%).

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Meanwhile, other participants said they would use teas prescribed in hospital if it is the required treatment for their condition (24, 8.2%), if it is effective (57, 19.4%), if it has no side effects (4, 1.4%), or if it has been approved by health authorities (4, 1.3%). Some participants said they would not use herbal teas if prescribed in hospital because they suppose that it is not the specialty of health personnel (4, 1.4%), they do not expect teas from the hospital (12, 4.1%), or they said teas do not work for them (4, 1.4%).

The proportion of hospital respondents who were willing to use herbal teas in the future if prescribed in the hospital (95/97, 97.9%) was higher than in the community (171/197, 86.8%). All but 2 (26/28, 92.8%) of the respondents who were not willing to use herbal teas in the future were community respondents (Table 5).

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Table 5 Acceptability of herbal teas if prescribed in the hospital

Question asked	Whole Sample N= 294	Hospital N=97	Community N= 197
	Responses		
	n (%)	%	%
<i>Can you take herbal teas if prescribed in the hospital</i>			
Yes	266 (90.5)	97.9	86.8
<i>Explain your answer</i>			
Yes, because I have confidence in doctors/ hospital	137 (46.6)	47.4	46.2
Yes, if the treatment is effective	57 (19.4)	15.5	21.3
Yes, if it is the required treatment for me	24 (8.2)	13.4	5.6
Yes, if it has no side effects	4 (1.4)	2.1	1.0
Yes, if approved by authorities	4 (1.3)	2.1	1.0
Yes, because I know it works better, from experience	29 (9.8)	13.4	8.1
Yes, because I hate tablets and prefer herbal teas	11 (3.7)	4.1	3.6
No, I don't expect herbal teas from the hospital	12 (4.1)	2.1	5.1
No, because it is not their speciality	4 (1.4)	0.0	2.0
No, because herbal teas don't work for me	4 (1.4)	0.0	2.0
Others	8 (2.7)	0.0	4.1

Participants' opinions about herbal tea prescription in hospital

The results showed that most respondents (294/300, 90.1%) were in support of herbal teas prescription in the hospitals mainly, if the teas were effective (120, 40.8), or because they believe in doctors or the hospital (55, 18.7%); Meanwhile, some respondents simply preferred herbal treatment to tablets (17, 5.8%), and others supposed that teas are natural with less side effects (35, 11.9%), are more effective and less costly (12, 4.1%), are the same as tablets and capsules (18, 6.1%), and children could easily take them without knowing its medicine (8, 2.7%). On the other hand, some respondents were against the prescription of herbal teas in the

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hospital because they thought that it was not the specialty of health personnel 25(8.5%) and there is no clear treatment dosage (4, 1.4%).

The proportion of hospital respondents who encouraged herbal tea prescription in the hospital (93/97, 95.9%) was higher than in the community (172/197, 87.3%). All but 4 (25/29, 86.2%) of the respondents who were against the prescription of herbal teas in hospital were community respondents (Table 6).

Table 6 Participants' opinions about herbal tea prescription in hospital

Question asked	Whole Sample <i>N</i> =294	Hospital <i>N</i> =97	Community <i>N</i> =197
	Responses		
	<i>n</i> (%)	%	%
<i>Will you encourage or discourage prescription of herbal teas in hospital</i>			
Will encourage	265 (90.1)	95.9	87.3
<i>Explain your reason</i>			
Will encourage if it is effective	120 (40.8)	27.8	47.2
Will encourage, I trust the doctors/hospital	55 (18.7)	25.8	15.2
Will encourage, its natural with less side effects	35 (11.9)	19.6	8.1
Will encourage, it's the same as tablets or capsule	18 (6.1)	11.3	3.6
Will encourage, some people prefer herbal medicine	17 (5.8)	2.1	7.6
Will encourage, its more effective and less costly	12 (4.1)	9.3	1.5
Will encourage, I like drugs in tea form and children can take without knowing	8 (2.7)	0.0	4.1
Will discourage because it is not their specialty	25 (8.5)	3.1	11.2
Will discourage because there is no clear dosage	4 (1.4)	1.0	1.5

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Motivating factors for increase acceptability of herbal tea use

Motivating factors for increase acceptability of herbal tea use in this study included treatment effectiveness (155, 52.7%), improved taste of teas (15, 5.1%), affordability (13, 4.4%) availability/accessibility (15, 5.1%), standardized dosage and precise prescription 13(4.4%) attractive packaging and formulation (9, 3.1%), official approval by authorities (3, 1%), and publicity /awareness campaigns (46, 15.7%) (Table 7).

Univariate analysis showed that there was a high prevalence of herbal tea use in all the study sites above the WHO reported value 80%, but there was no significant association ($p < 0.05$) between sociodemographic characteristics of study participants and herbal tea use in the community and hospitals study sites (Table 8).

Table 7 Motivating factors for increase acceptability of herbal tea use

Question asked	Whole Sample <i>N</i> =294	Hospital <i>N</i> =97	Community <i>N</i> =197
	Responses		
	n (%)	%	%
<i>What can increase your acceptability to use herbal teas?</i>			
If effectiveness is ensured	155 (52.7)	43.3	57.4
Publicity campaigns/sensitizations/awareness creation	46 (15.7)	14.4	16.2
Others	37 (12.6)	13.4	12.2
If the taste is improved	17 (5.8)	5.2	5.1
If it is readily available	15 (5.1)	2.1	0.5
If the standard dosage and precise prescription is given	13 (4.4)	7.2	3.1
If it is affordable	13 (4.4)	8.2	2.5
Attractive packaging and formulation	9 (3.1)	5.2	2.0
If it is officially accepted	3 (1.0)	1.0	1.0

Table 8 Associations between socio-demographic characteristics and the use of herbal teas

Characteristics	Herbal tea use in Community settings						Herbal tea use in hospital settings					
	Southwest region (Buea)			Centre region (Yaounde)			Urban Hospitals			Rural Hospitals		
	N	%	OR (95%CI)	P	N	%	OR (95%CI)	P	N	%	OR (95%CI)	P
Total	100	88.0			100	86.0			50	98.0		
Age of respondents (years)				0.36				0.82				>99
18-29	46	84.8	1		33	84.9	1		23	95.6		1
30-65	54	90.7	1.76 (0.52 - 5.97)		67	86.8	1.15 (0.35 - 3.75)		27	100		0.96 (0.15 - 6.59)
Gender				0.92				0.51				0.45
Women	43	88.4	1.06 (0.31 - 3.61)		65	87.7	1.47 (0.47 - 4.65)		29	96.6		2.05 (0.31 - 13.51)
Men	57	87.7	1		35	82.9	1		21	100		1
Level of Education				0.84				0.55				0.89
Primary	24	100	-		24	91.7	2.41 (0.46 - 12.69)		6	100		-
Secondary	36	83.3	0.88 (0.26 - 3.03)		37	86.5	1.40 (0.40 - 4.88)		9	88.9		1.14 (0.17 - 7.67)
High school or higher	40		1		39	82.1	1		35	100		1
House hold size				0.41				0.86				0.16
1 - 5 persons	74	86.5	1		55	85.5	1		28	100		1
>5 persons	24	91.7	1.88 (0.30 - 9.18)		43	86.1	1.11 (0.35 - 3.46)		22	95.5		4.57 (0.47 - 44.17)
Professional category				0.14				0.18				NT
Student	13	69.2	1		12	66.7	1		13	100		3
Manual laborer	10	90.0	4.00 (0.37 - 43.13)		16	87.5	3.35 (0.44 - 12.87)		19	94.7		-
Officer	77	90.9	4.44 (1.08 - 18.22)		72	88.9	4.00 (0.98 - 16.34)		18	100		39
Socio-economic/income status				0.05				0.32				NT
Not enough to live with, but you mar	20	85.0	1		19	89.5	1		13	100		3
Just enough to live with	43	81.4	0.77 (0.18 - 3.28)		49	89.8	2.38 (0.44 - 12.87)		19	94.7		8
Enough and easy to live with	37	97.3	6.35 (0.61 - 65.66)		32	78.1	2.46 (0.71 - 8.58)		18	100		39

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5.2 Results 2

Knowledge about Asymptomatic Malaria and Acceptability of *Artemisia afra* Tea among Health Care Workers in Yaoundé, Cameroon.

Abstract

Background: Malaria is the most widespread endemic disease in Cameroon, and asymptomatic *Plasmodium* (gametocyte) carriers (APCs) constitute more than 95% of infectious human reservoirs in malaria endemic settings. This study assesses the knowledge of asymptomatic malaria (ASM) among health care workers (HCWs) in health facilities (HFs) in the Centre Region of Cameroon and the acceptability of using *Artemisia afra* tea to treat APCs.

Methods: A cross-sectional descriptive survey was conducted among 100 HCWs, in four randomly selected HFs in the Centre Region, in the period of 1–20 April 2022, using semi-structured self-administered questionnaires. Logistic regression analyses were performed to determine factors associated with knowledge.

Results: More than seven in eight (88%) respondents were aware of the existence of ASM, 83% defined ASM correctly, 75% knew how it was diagnosed, 70% prescribe ACTs for APCs, and 51.1% were informed about ASM transmission. The professional category of HCWs was

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significantly associated with their knowledge of the existence of ASM, and longevity of service was associated with knowledge of transmission ($p < 0.05$). Two-thirds (67%) of respondents knew about *Artemisia afra* tea, 53.7% believed that it was effective in treating ASM, and 79% were willing to prescribe it if authorized.

Conclusion: There was a fair level of knowledge of ASM among HCWs in the study settings.

Keywords: health care workers, asymptomatic *Plasmodium* carriers, knowledge, *Artemisia afra* tea, treatment.

As published



International Journal of
*Environmental Research
 and Public Health*



Article

Knowledge about Asymptomatic Malaria and Acceptability of Using *Artemisia afra* Tea among Health Care Workers (HCWs) in Yaoundé, Cameroon: A Cross-Sectional Survey

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3.1. Characteristics of Survey Participants

Table 1: Among the 100 health care workers (HCWs) who participated in this survey, 75% of them were at least 30 years old, and 80% of them were women. A majority (72%) of participants were nurses of various grades, 28% were medical doctors (general practitioner, family doctors or specialists in medicine), and 49% of them had practiced for five years or fewer.

Table 1: Characteristics of survey participants in rural and urban hospitals.

Sample Characteristic	Whole Sample N = 100	Rural Hospital N = 50	Urban Hospital N = 50
	n (%)	%	%
Age of respondent (years)			
18–29	25 (25.0)	24.0	26.0
30–65	75 (75.0)	76.0	74.0
Gender			
Female	80 (80.0)	82.0	78.0
Male	20 (20.0)	18.0	22.0
Professional category			
Nursing assistant (NA)	26 (26.0)	18.0	34.0
State registered nurse (SRN)	31 (31.0)	20.0	42.0
Degree nurse (Senior nurse)	15 (15.0)	16.0	14.0
Medical doctor (Physician)	28 (28.0)	46.0	10.0
Longevity of service (years)			
0–5	49 (49.0)	48.0	50.0
6–10	23 (23.0)	16.0	30.0
>10	28 (28.0)	36.0	20.0

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3.2. Knowledge of Asymptomatic Malaria among Health Care Workers

Table 2: Among the survey participants, 88 (88%) were aware of the existence of asymptomatic malaria, and 12% had never heard about asymptomatic malaria before. Of these, 73/88 (83.0%) defined asymptomatic malaria correctly as the presence of malaria parasites in the blood without symptoms, and 66/88 (75%) of them knew that asymptomatic malaria was diagnosed through microscopic blood examination. Most of respondents (75/88; 85.2%) had encountered patients with asymptomatic malaria, and 70/75 (93%) put the patients on treatment including ACT (49/70; 70%), artesunate (47.1%), quinine sulphate (17.1), and others (44.2%): fansidar, camoquine, halfantrim, suquina, artecom, malarone, doxycycline, vibramycine, oraceal, mefloquine, and tefaroquine.

Most HCWs (71/88; 80.7%) were aware that it was necessary to treat asymptomatic *Plasmodium* carriers but did not know the reason for treating, as they said the reason for treating carriers was to avoid severe complications (36/88; 40.9%), to prevent development of symptoms (26.1%), and due to the patient living in a malaria endemic zone (11.4%); only 2 (2.3%) respondents gave the correct reason that was to curb transmission. Meanwhile, 9/88 (10.2%) respondents thought that it was not necessary to treat asymptomatic carriers, and 8 (9.1%) did not know if asymptomatic carriers needed to be treated or not. In a similar manner, 86.4 % of respondents agreed that it was risky to leave

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asymptomatic carriers untreated, but they did not know the correct risk involved, as the majority of them said if left untreated, carriers were at risk of developing complications (52/88; 59.1%) or becoming symptomatic (18.2%); and only 3 (3.4%) respondents mentioned risk of transmission. Meanwhile, 4.5% of the respondents thought that there was no risk if asymptomatic carriers were not treated, and 9.1% did not know if there was or was not a risk to treat (Table 2).

More than half (45/88; 51.1%) of respondents were aware that healthy people could become infected by *Plasmodium* parasites transmitted from asymptomatic carriers, while 44.4% mistakenly thought that this was not possible because asymptomatic malaria is not transmissible (35.2%) and because the parasite quantity (load) in asymptomatic carriers is insignificant (8.0%). Still on transmission, 29/55 (89.1%) respondents were aware that a person infected with *Plasmodium* parasites from an asymptomatic carrier could develop malaria symptoms because the parasite was present (52.7%) and because immune systems are different (36.4%), while 10.9% of participants did not know if this was possible or not (Table 2).

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Table 2: Knowledge of asymptomatic malaria among health care workers.

Questions Asked	Responses	
	n	%
1. Have you ever heard about asymptomatic malaria? (If No, go to section B)		
Yes	88	88.0
No	12	12.0
2. If Yes, what is asymptomatic malaria? (n = 88 out of 100)		
Defined asymptomatic malaria correctly	73	83.0
Defined asymptomatic malaria incorrectly	15	17.0
3. How can it be diagnosed?		
Knows how to diagnose asymptomatic malaria	66	75.0
Does not know how to diagnose asymptomatic malaria	22	25.0
4. Have you ever seen a patient with asymptomatic malaria?		
Yes	75	85.2
No	13	14.8
5. If Yes, did you put them on treatment? (n = 75 out of 100)		
Yes	70	93.3
No	5	6.7
6. If Yes, which drug did you prescribe? (n 70 out of 75) (several options)		
ACT	49	70.0
Artesunate	33	47.1
Quinine	12	17.1
Others	31	44.2
7. Is it necessary to treat asymptomatic malaria?		
Yes, Risk of developing complications	36	40.9
Yes, Risk of becoming symptomatic	23	26.1
Yes, We should treat all forms of malaria	10	11.4
Yes, Risk of transmission	2	2.3
No risk if not treated	9	10.2
I don't know	8	9.1
8. Do you think there is any risk of not treating asymptomatic plasmodium carriers?		
Yes, Risk of developing complications	52	59.1
Yes, Risk of becoming symptomatic	16	18.2
Yes, We should treat all forms of malaria	5	5.7
Yes, Risk of transmission	3	3.4
No risk if not treated	4	4.5
I don't know	8	9.1
9. Can malaria infection be transmitted from a Plasmodium parasite carrier to an uninfected person?		
Yes, because the parasite is present	45	51.1
No, because malaria is not a transmissible disease	31	35.2
No, because it's asymptomatic and the quantity is mild	7	8.0
I don't know	5	5.7

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<i>10. Can a person infected with parasites from an asymptomatic carrier develop malaria symptoms?</i>		
Yes, because the parasite is present	29	52.7
Yes, because immune systems are different	20	36.4
I don't know	6	10.9
Missing	33	37.5

3.3. Knowledge of Asymptomatic Malaria among Health Care Workers According to Respondents' Characteristics

This shows how the knowledge of respondents about the existence, definition, diagnosis, and transmission of asymptomatic malaria was distributed according to their ages, gender, professional category, longevity of service, and practicing site.

3.3.1. Participants' Knowledge about Asymptomatic Malaria by Age

Figure 2a shows that the proportion of respondents who knew how asymptomatic malaria was defined and diagnosed was higher among HCWs younger than 30 years old (<30) than in older respondents, while the proportion of respondents who responded correctly about transmission was higher among HCWs at least 30 years old (≥ 30) than in younger participants.

3.3.2. Participants' Knowledge about Asymptomatic Malaria by Gender

Figure 2b shows that the proportion of respondents who were knowledgeable about the existence and transmission of asymptomatic malaria was higher among female than male respondents, while the proportion of correct respondents for the definition and diagnosis of asymptomatic was higher among male than female respondents.

3.3.3. Participants' Knowledge about Asymptomatic Malaria by Professional Category

Figure 2c indicates that the professional category of nursing assistants had the lowest proportion of asymptomatic malaria-informed respondents.

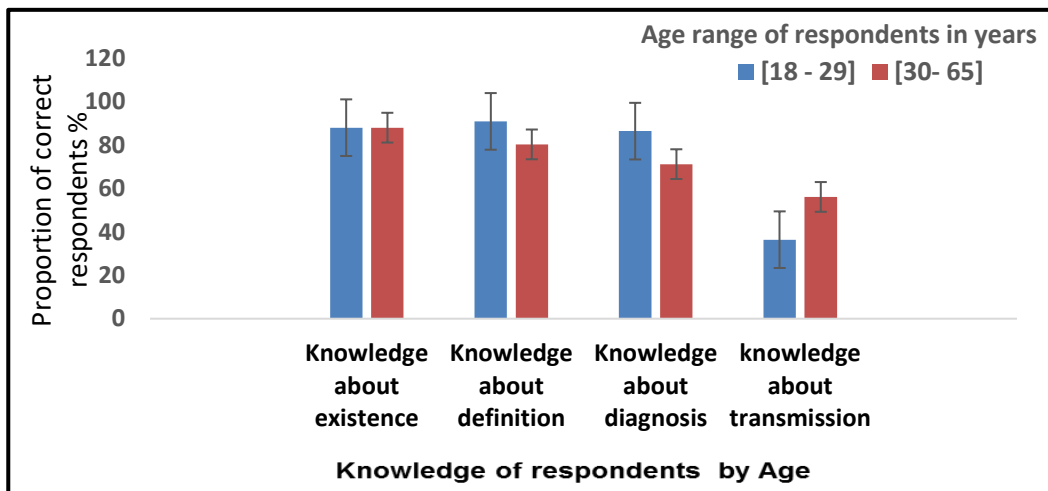
3.3.4. Participants' Knowledge about Asymptomatic Malaria by Longevity of Service

Figure 2d shows that the proportion of respondents with knowledge of the definition and diagnosis was higher among HCWs with 0–5 years of practice than among those with more years of practice, while the proportion of respondents with knowledge of existence and transmission of asymptomatic malaria was higher among HCWs with >10 years of practice than among those with fewer years of practice.

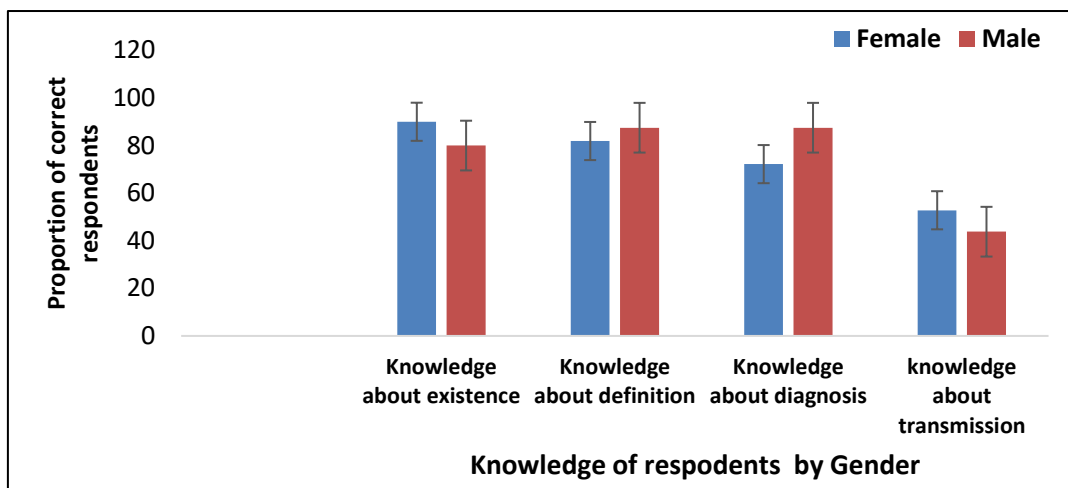
3.3.5. Participants' Knowledge about Asymptomatic Malaria According to Practicing Site

Figure 2e reveals that participants practicing in rural hospitals had a higher proportion of respondents who were knowledgeable about asymptomatic malaria.

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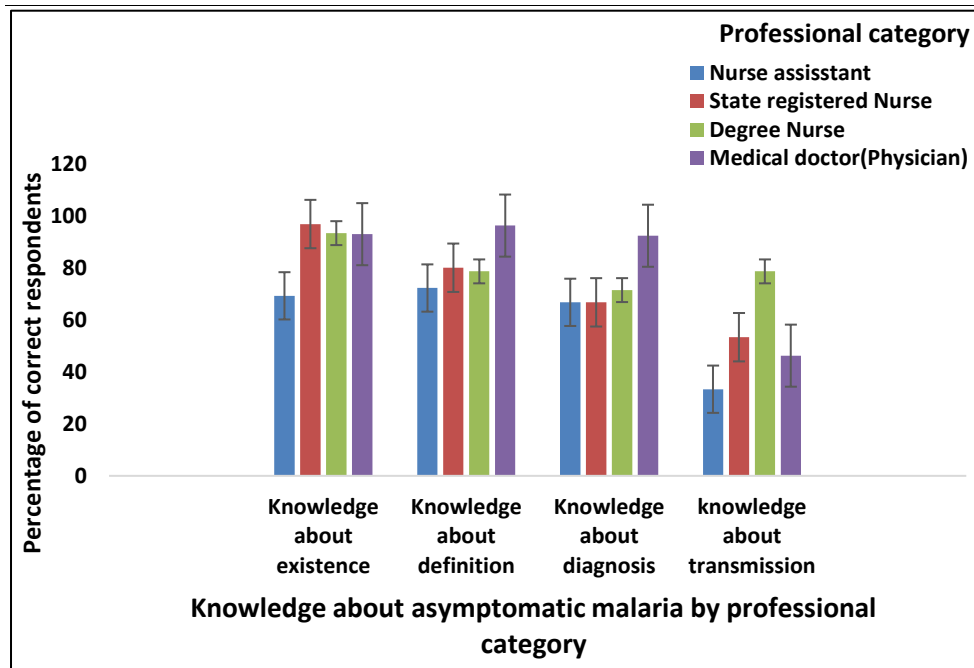


(a)

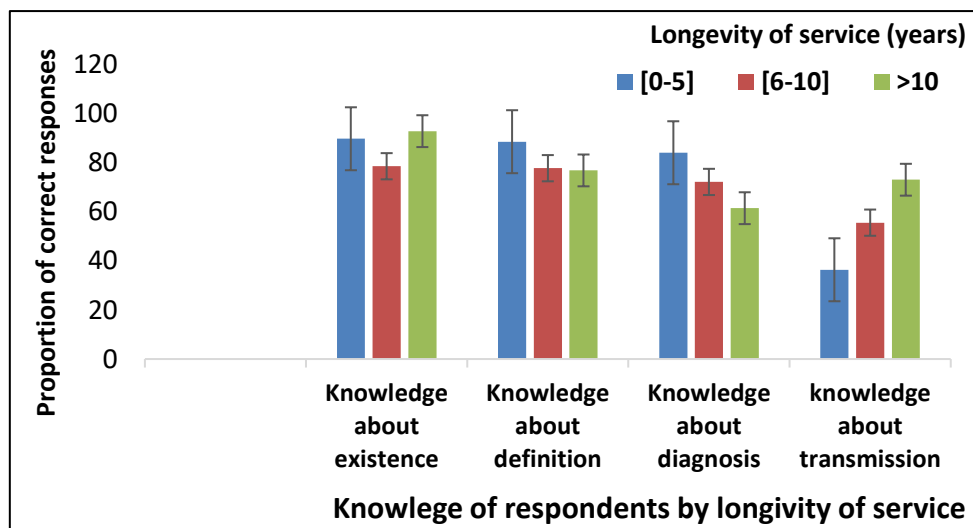


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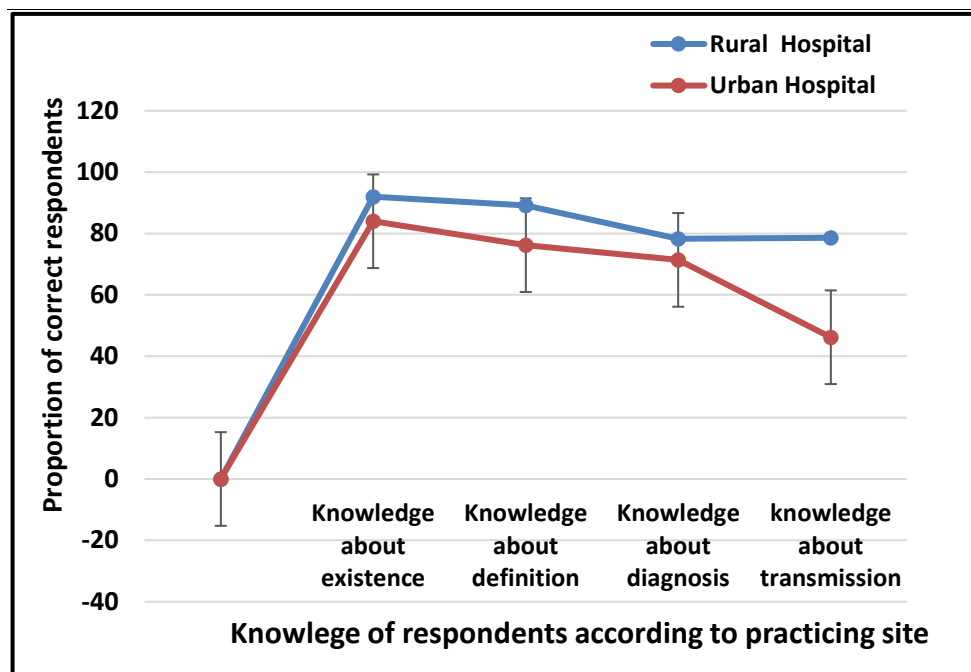
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(c)



(d)



(e)

Figure 2. (a) Participants' knowledge about asymptomatic malaria by age; (b) participants' knowledge about asymptomatic malaria by gender; (c) participants' knowledge about asymptomatic malaria by professional category; (d) participants' knowledge about asymptomatic malaria by longevity of service; (e) participants' knowledge about asymptomatic malaria according to practicing site.

3.4. Factors Associated with Knowledge of Asymptomatic Malaria among Health Care Workers

Tables 3 and 4 show participants' characteristics associated with their knowledge of asymptomatic malaria using logistic regression analysis. Multiple logistic regression analysis showed that the professional

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category of HCWs was significantly associated with knowledge about the existence of asymptomatic malaria ($p < 0.05$), and longevity of service was associated with knowledge about transmission. Nursing assistants were less likely to be aware about the existence of asymptomatic malaria compared to physicians [OR 0.17, 95%CI 0.03, 0.91] (Table 3); and HCWs with 0–5 years of practice were less likely to be informed about asymptomatic malaria transmission compared to those with more than 10 years of practice [OR 0.24, 95% CI 0.07, 0.85] (Table 4).

Table 3: Factors associated with knowledge about the existence and definition of asymptomatic malaria among HCWs

Sample Characteristics	Knowledge of existence				Knowledge of Definition					
	N	%	OR (95% CI)	p-value	AOR (95% CI)	p-value	N	%	OR (95% CI)	p-value
Total	100	88.0					88	83.0		
Age of respondent (years)										
[18 - 29[	25	88.0	1	>0.99			22	90.9	2.45 (0.51 - 11.84)	0.23
[30-65[	75	88.0	1				66	80.3	1	
Gender				0.24						0.58
Female	80	90.0	1				72	81.9	1	
Male	20	80.0	0.44 (0.12 - 1.66)				16	87.5	1.54 (0.31 - 7.63)	
Professional category				0.01*						0.11
Nursing assistant	26	69.2	0.17 (0.03 - 0.91)		0.07 (0.01 - 0.77)	0.03*	18	72.2	0.10 (0.01 - 0.99)	
State registered Nurse	31	96.8	2.37 (0.20 - 26.94)		1.66 (0.68 - 19.89)	0.92	30	80.0	0.16 (0.02 - 1.43)	
Degree Nurse	15	93.3	1.08 (0.09 - 1.59)		0.57 (0.03 - 9.32)	0.69	14	78.6	0.15 (0.14 - 1.57)	
Medical doctor (Physician)	28	92.9	1		1		26	96.2	1	
Longevity of service (years)				0.27						0.36
[0-5[	49	89.8	0.68 (0.12 - 3.74)				44	88.6	2.34 (0.64 - 8.62)	
[6-10[	23	78.6	0.41(0.05 - 1.59)				18	77.8	1.05 (0.25 - 4.42)	
>10	28	92.9	1				26	76.9	1	
Practicing site				0.21						0.10
Rural Hospital	50	92.0	2.19 (0.61 - 7.80)				46	89.1	2.56 (0.80 - 8.25)	
Urban Hospital	50	84.0	1				42	76.2	1	

Table 4: Factors associated with knowledge about the diagnosis and transmission of asymptomatic malaria among HCWs

Sample Characteristics	N	<u>Knowledge of Diagnoses</u>			<u>Knowledge of Transmission</u>		
		%	OR (95% CI)	p-value	%	OR (95% CI)	p-value
Total	88	75.0			51.1		
Age of respondent (years)				0.14			0.11
[18 - 29[	22	86.4	2.56 (0.68 - 9.67)		36.4	0.45 (0.17 - 1.21)	
[30-65[	66	71.2	1		56.1	1	
Gender				0.18			0.51
Female	72	72.2	1		52.8	1	
Male	16	87.5	2.69 (0.56 - 12.93)		43.8	0.70 (0.07 - 0.61)	
Professional category				0.07			0.07
Nurse assistant	18	66.7	0.17 (0.03 - 0.95)		33.3	0.58 (0.17 - 2.03)	
State registered Nurse	30	66.7	0.17 (0.03 - 0.85)		53.3	1.33 (0.47 - 3.82)	
Degree Nurse	14	71.4	0.21 (0.03 - 1.33)		78.6	4.28 (0.96 - 19.01)	
Medical doctor(Physician)	26	92.3	1		46.2	1	
Longivity of service (years)				0.11			0.01*
[0-5[	44	84.1	3.30 (1.07 - 10.23)		36.4	0.21 (0.07 - 0.61)	0.24 (0.07 - 0.85) 0.03*
[6-10[	18	72.2	1.63 (0.44 - 5.96)		55.6	0.46 (0.13 - 1.64)	0.37 (0.09 - 1.48) 0.16
>10	26	61.5	1		73.1	1	1
Practicing site				0.46			0.29
Rural Hospital	46	78.3	1.44 (0.55 - 3.79)		78.6	1.57 (0.68 - 3.65)	
Urban Hospital	42	71.4	1		46.2	1	

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3.5. Knowledge of health care workers to use *A. afra* tea infusions to treat asymptomatic *Plasmodium* carriers

Table 5 reveals that two-thirds 67 (67%) of the respondents knew about *A. afra* tea infusions, and they said it was used to treat malaria (57/67; 85.1%), COVID-19 (34.3%), cough (3%), fever (3%), and others (7.5%) including stomach offset, sinuses, gastritis, typhoid fever, myomas, and diabetes. Most respondents had also heard that *A. afra* was used for the prevention and treatment of acute malaria (58/67; 86.6%) and asymptomatic malaria (42/67; 62.7%).

More than half (38/67; 56.7%) of respondents believed that *A. afra* tea was effective in treating acute malaria, and 20.9% thought that more research was needed, while 9% thought that it was not effective, and 13.4% did not know if it was effective or not. In the same way, 53.7% of respondents believed that *A. afra* tea was an effective treatment for asymptomatic *Plasmodium* carriers, 23.9% thought that more research was needed, 1.5% thought it was not effective, and 20.9% did not know if it was effective or not (Table 5).

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Table 5: Knowledge and impression of HCWs on *A. afra* tea.

Questions Asked	Responses	
	n	%
1. Do you know about Artemisia afra tea infusions? (If no, go to question 10)		
Yes	67	67.0
No	33	33.0
2. What is it used for?(n = 67 out of 100)		
Malaria	57	85.1
COVID-19	23	34.3
Cough	2	3.0
Fever	2	3.0
Others	5	7.5
3. Have you ever heard about its use in malaria prevention or treatment?		
Yes	58	86.6
No	9	13.4
4. Have you ever heard about its use in the prevention or treatment of asymptomatic malaria?		
Yes	42	62.7
No	25	37.3
5. What is your impression about the use of Artemisia afra tea in preventing or treating acute malaria patients?		
It is effective	38	56.7
More research is needed	14	20.9
I don't know	9	13.4
It is not effective	6	9.0
6. What is your impression about the use Artemisia afra tea infusions in treating asymptomatic plasmodium carriers?		
It is effective	36	53.7
More research is needed	16	23.9
I don't know	14	20.9
It is not effective	1	1.5

3.6. Opinions of Health Care Workers about the Use of Artemisia afra Tea to Treat Asymptomatic Plasmodium Carriers

Figure 3 shows that the main opinions and concerns of respondents about the use of *Artemisia afra* tea included a need to ensure the effectiveness of the tea (27/67; 40.3%), a precise posology (26.8), and the absence of side effects (4.5%).

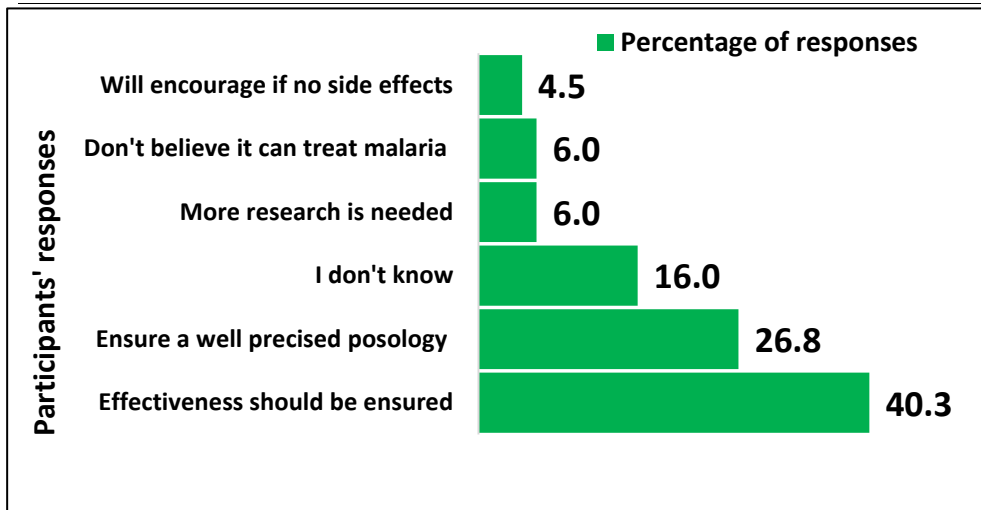


Figure 3. Participants' opinions on the use of *Artemisia afra* tea to treat asymptomatic *Plasmodium* carriers.

3.7. Acceptability of health care workers to use *Artemisia afra* tea infusion to treat asymptomatic *Plasmodium* carriers

Figure 4a shows that most of the HCWs 51/67 (76.4%) were willing to prescribe or recommend *Artemisia afra* tea infusions to treat asymptomatic malaria if it was effective (16.2%), if it was approved/recommended by state authorities (13.2%), and if it was available and affordable (10.3%). Others said they would prescribe it because they had tried it before and it worked (10.3%), because they believe it would eliminate resistant forms of malaria (8.8%), because carriers do not have acute malaria (5.9%), because some patients prefer teas (2.9%), and because they believed it was natural with few side effects (2.9%). Meanwhile, 23.2% of respondents said they would not prescribe the teas because they thought that there is no scientific drug

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data available on the tea (14.7%); it was complicated for patients to take (1.5%); and it could cause resistance in the future (1.5%).

Notwithstanding the aforementioned aspects of acceptability, more than three-quarters (79%) of respondents were ready to prescribe/use *Artemisia afra* teas if endorsed by the state authorities, and 19% said they would not continue to prescribe it after endorsement unless they received knowledge of its prescription, while 2% were still undecided (Figure 4b).

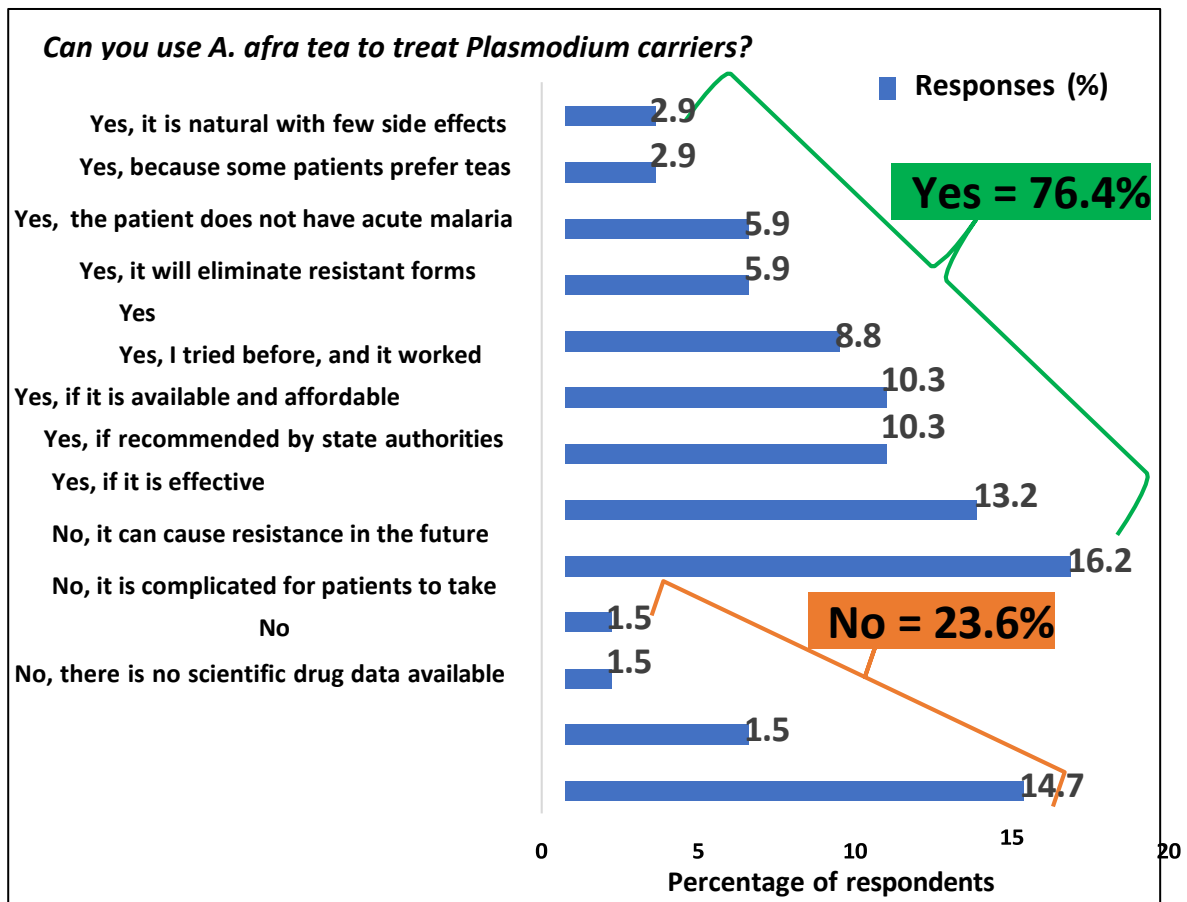


Figure 4. (a) Acceptability of health care workers to use *A. afra* tea to treat asymptomatic *Plasmodium* carriers

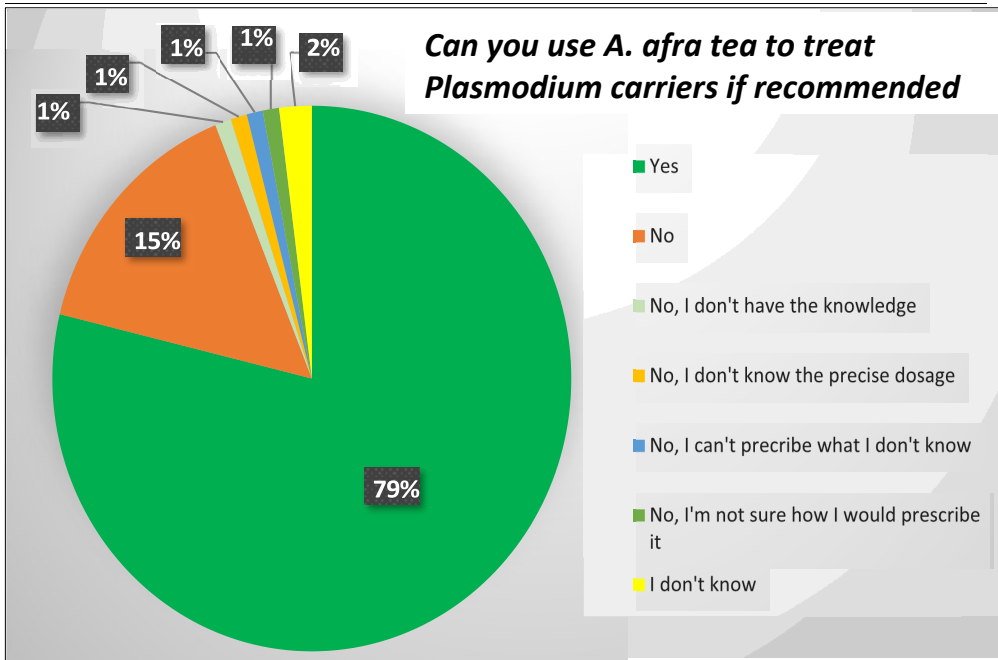


Figure 4. (b) Acceptability of HCWs to prescribe *Artemisia afra* tea if recommended by authorities.

3.8. Challenges Pointed Out by HCWs in Using *Artemisia afra* Tea Infusion for Treatment of Asymptomatic Malaria

Figure 5 shows the challenges of using *Artemisia afra* tea infusions to treat asymptomatic *Plasmodium* carriers predicted by HCWs. These included the problem of dosage and posology (19.4%), the likelihood that patients may resist treatment since they have no symptoms (14.9%), proof of efficacy (11.9%), cost and availability (9%), poor compliance because teas are complicated to take (7.5%), no scientific drug data available (6%), the fact that people do not expect teas in the hospital

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(3%), and possible counterfeit, since the tea is not well known by the population (1.5%)

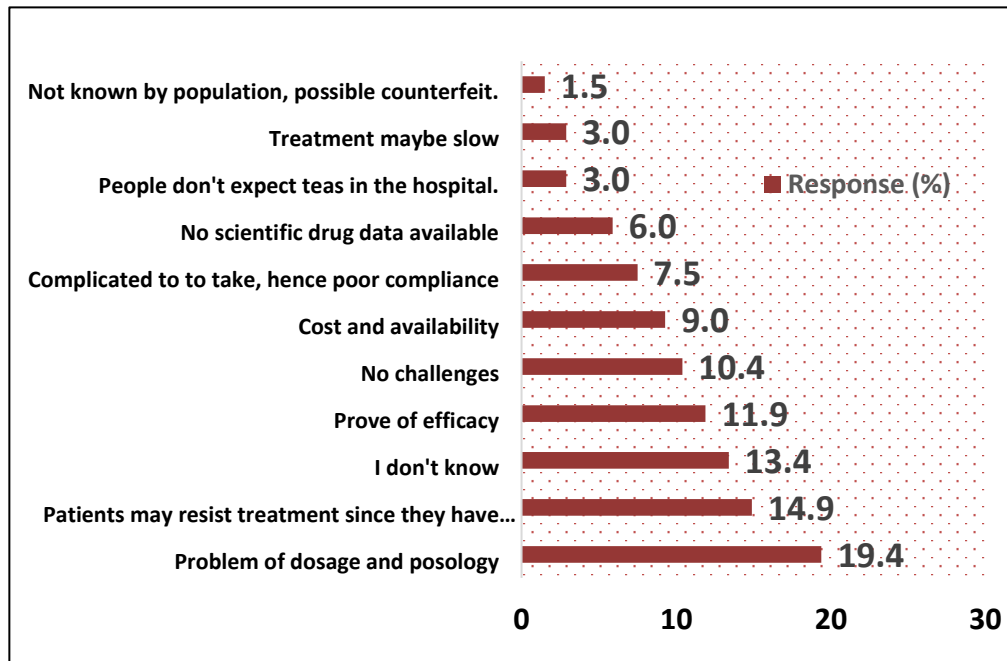


Figure 5. Challenges pointed out by HCWs in using *Artemisia afra* tea infusion for treatment of asymptomatic malaria.

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Protocol of clinical studies to assess the safety, optimal dosage, and efficacy of *A. afra* infusions in clearing *Plasmodium* reservoirs

The present protocol has been registered online on the International Standard Randomized Controlled Trial Number (ISRCTN) registry, with registration number - ISRCTN42971030 on April 4, 2022. It has also been registered on *Clinical trial .gov*, with Identifier number – NCT05440773, on July 1, 2022. Ethical approval has been obtained from the Cameroon National ethical committee for research on human health (Ref 2023/10/1602/CE/CNERSH/SP).

5.3.1 Phase I: Pre-clinical analysis of the pharmacokinetics, pharmacodynamics, and toxicity profiles of *A. afra*

Pre-clinical toxicity investigation is often required for new substances and new medicinal products, which contain herbs with no traditional history of use. However, WHO guidelines state that if a herbal product has a long history of traditional use (over three or more generations) with no signs of toxicity (harmful effects), it should not be restricted, and no pre-clinical toxicity investigations are required (205). *A. afra* has a long history of traditional use as a herbal product in South Africa for the treatment of ailments including malaria, for generations, with no toxicity detected (148)(205). Further to this, several toxicity studies (cytotoxicity, acute and chronic toxicity) have been conducted to confirm the safety of *A. afra* and their secondary metabolites. In a

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review conducted on the pharmacokinetics, pharmacodynamics (PKPD) and toxicologic profiles of secondary metabolites isolated from *Artemisia afra*, several in vitro and animal studies reported safety profile and non-toxic effects of the aqueous extract of the plant, with no significant changes in cytological, behavioral, hematological, and biochemical parameters experienced, thereby affirming the safe use of the plant as a traditional remedy in the fight against infections (143).

5.3.2 Phase II: Proof of concept trial to assess the optimal dosage of *A. afra* infusions in emptying *Plasmodium* reservoirs

Study objective: To determine the optimal dosage of *Artemisia afra* tea infusions in emptying *Plasmodium* parasites in asymptomatic reservoirs.

Study design: This will be a randomized controlled, partial-blind, parallel group study among *Plasmodium* infected asymptomatic adults (≥ 21 years), who have been treated for symptomatic malaria (uncomplicated) using an ACT (Artemether - Lumefantrine - AL), and people who do not have malaria symptoms but are carriers of parasitaemia. The study will have 5 treatment arms with varying concentrations, dosages, and frequency of consumption. Participants' consent will be obtained using the adapted Zelen design, which has two steps in the consent process (213). In the first step, all participants who become asymptomatic on day 3 of treatment with AL but still have

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parasitaemia (residual parasites) will be invited to consent and participate in a cohort survey of asymptomatic *Plasmodium* carriers. Participants who maintain asymptomatic parasitaemia status on day 28 of ACT treatment will be randomized in the various treatment arms (Figure 17). According to this consent process, the participants will be randomized without their knowledge about the detailed protocol. In the second step, only participants from the intervention group will receive information about the planned intervention and a second consent will be obtained from them. The participants who decline to participate in the intervention will continue in the cohort study as the control group (Figure 17).

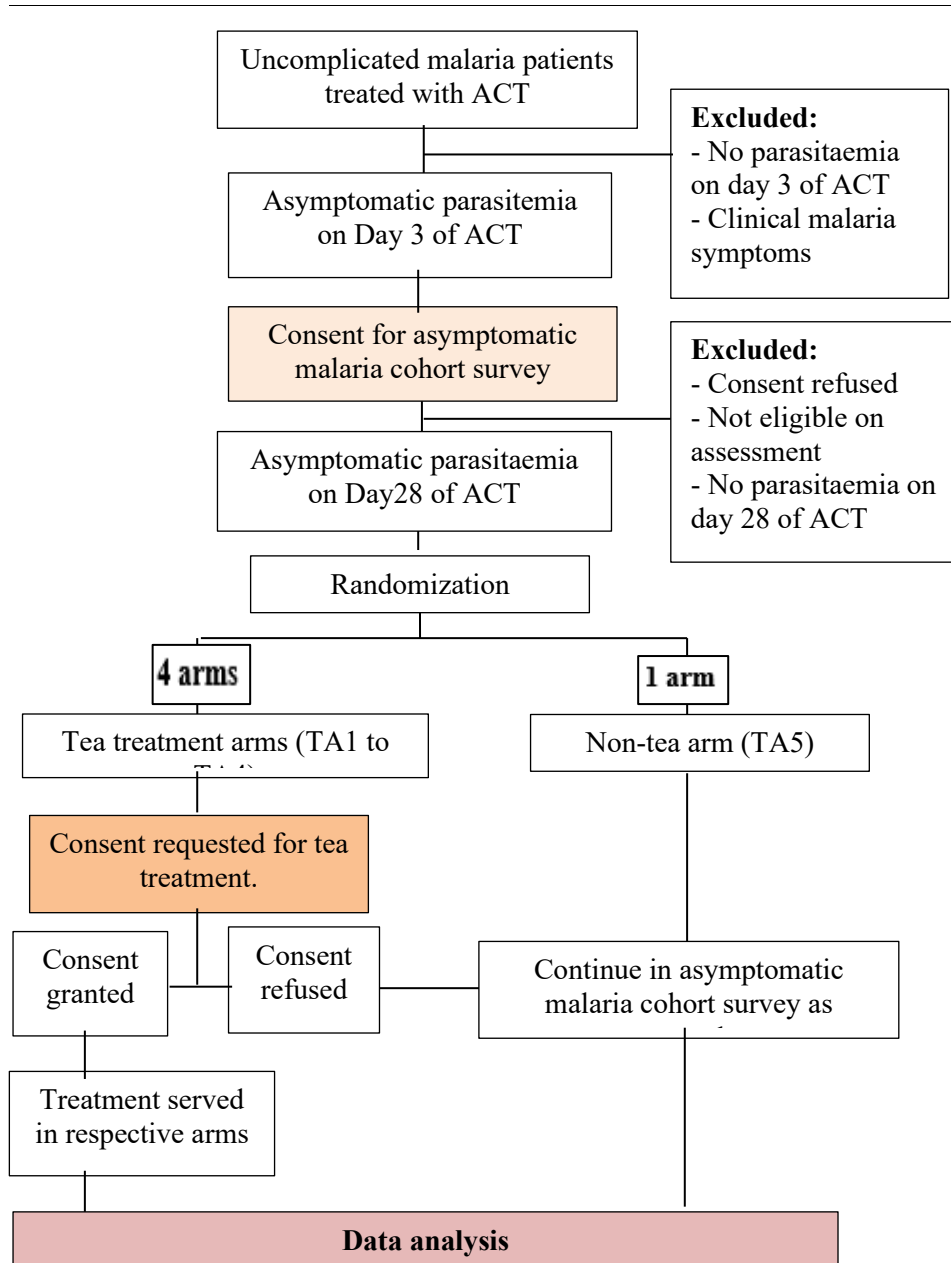


Figure 17: Flow of participant through double consent process in adapted Zelen design

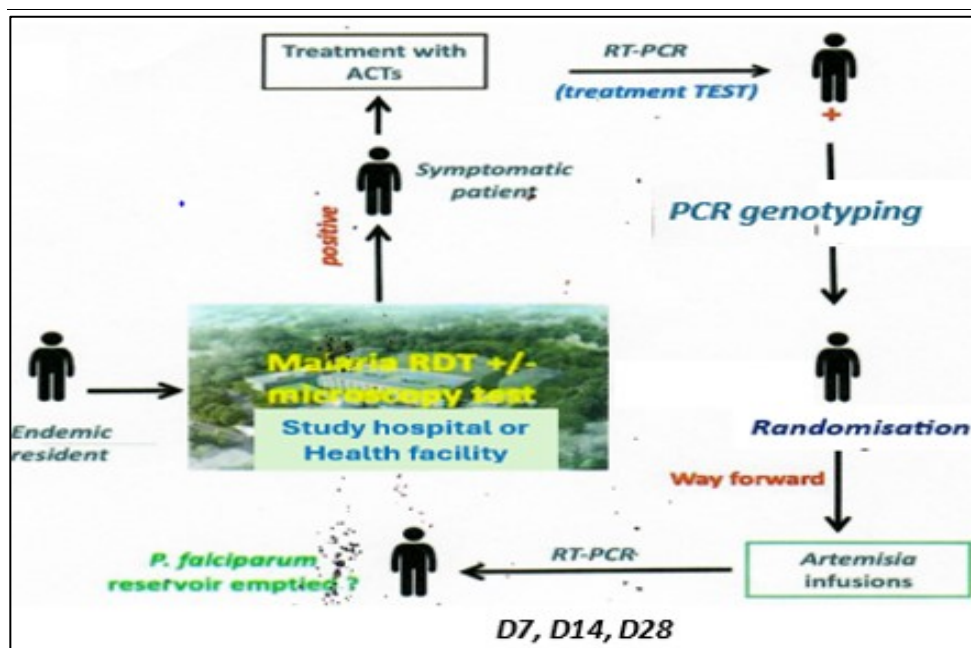


Figure 18: Participant flow within the clinical studies.

Sample size considerations

As a pilot study, no formal sample size calculation will be performed. Forty-five (45) asymptomatic carriers will be enrolled per site, making 180 participants in the Phase 2 trial. To reach 180 asymptomatic carriers in the study sites where the expected malaria positive RDT prevalence (apparent prevalence) is 24% (DHS 2018 (214), with the sensitivity and specificity of the standard RDTs used at ~ 62% and ~99% respectively (72); We estimated the true prevalence from apparent prevalence (AP) based on the specificity of RDT to be 37.7% by using the following formula: $\text{True prevalence} = \frac{AP + \text{specificity} - 1}{\text{sensitivity} + \text{specificity} - 1}$ (215). Considering a 10% drop-out rate, we will have to screen 526 people to get 180 carriers, that is, 132 people to get 45 carriers per site.

Study population

The study will be conducted in health facilities and communities in Bafoussam, Buea, Douala and Yaounde. These respectively represent the West, Southwest, Littoral and Centre region population of *P. falciparum* and variance of endemicity and likely drug resistance. All eligible adults in the study sites will be invited to participate and those who consent will be enrolled.

Exclusion criteria

1. To be less than 21years old
2. To have a known hypersensitivity to any ingredients of the tea.
3. To be currently taking any malaria drug for prevention or treatment.
4. To have participated in another malaria drug trial or device in the last 1month.
5. To have a history or presence of clinically significant medical, psychiatric, or emotional condition.

Screening and recruitment

In study sites, people in health facilities who have symptoms associated with malaria such as fever, chills, rigors, headaches, body pains, malaise, nausea, vomiting, and joint weakness will be tested using malaria RDT and/or microscopy. If negative, they will be excluded after counselling. Those with a positive test will be treated using ACT as recommended by WHO. After treatment, rt-qPCR will be performed on day 3 to check for residual parasitaemia. Parasitaemia carriers will be requested to participate in the study upon signing an informed

consent form (Figure 17). People without symptoms in health facilities will also be invited and tested for *Plasmodium* parasitaemia with RDT, and with qPCR when positive RDT.

Subsequently, all household members of the study participants within the community aged over 21 will be invited to participate (Figure 18).

Informed consent

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 before conducting any study-related procedure or activity on them.

Initial clinical evaluation/enrollment evaluation

After the informed consent, baseline assessments will be performed and information obtained on sociodemographic variables (age, gender, residence/ region of origin), clinical/medical history, manifestations of symptomatic malaria (fever, chills, rigors, headaches, body pains, malaise, nausea, vomiting, joint weakness), prior medications, physical assessment (temperature, weight, height, and blood pressure), and participants' contact details. Participants blood samples will be re-analyzed (qPCR) on day 28 of ACT treatment and those who remain asymptomatic with parasitaemia and fulfill the inclusion criteria will be enrolled and randomized in the various study arms.

Randomization: An independent statistician will generate a random

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number list (for 45 participants per site) in an excel file for randomly assigning the participants to one of the 5 study arms. The randomization method will be block randomization method, with a block size of 9 at random, and with a two-to-one allocation ratio for each active arm against the control arm (2:2:2:2:1). The treatments arms will consist of *Artemisia afra* infusions in varying dose concentrations and dose frequencies (TG1-TG4) while the control arm will consist of those taking nothing (TG5) (Table 4). All treatments will be coded (labelled) with respect to treatment groups and each participant will be served accordingly.

Blinding: Participants in the intervention arms (TG1-TG4) 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 a control arm (TG5- group with no tea administered), but unaware of assignments in the treatment arms (partial blinding).

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Table 4: Treatment groups (TG) and composition

Group	Characteristics	Type of infusion	Concentration	Dose frequency
TG 1	Current recommendation	<i>A. afra</i> infusions	10g/1liter (3.35g/335ml)	335ml, 3times/day for 14days
TG 2	↑dose concentration ↓dose frequency	<i>A. afra</i> infusions	10g/500ml (5g/250ml)	250ml, 2times/day for 14days
TG 3	↓dose concentration	<i>A. afra</i> infusions	5g/1liter (1.67g/335ml)	335ml, 3times/ day for 14 days
TG 4	↓dose concentration ↓dose frequency	<i>A. afra</i> infusions Improved taste	5g/500ml (2.5g/250ml)	250 ml, 2times/day for 14days
TG 5	Control group	No tea taken	-	-

Preparation of treatment: 10g or 5g of dried leaves and twigs of *A. afra* (in a tea sachet / tea bag) is added to 1 liter or 500ml of boiling water for 10 minutes, the fire is turned off and it is left to infuse for 15 minutes while still covered, and it is filtered through a sterilized mesh (216). The treatment dosages have been selected based on the posology suggested by Maison *artemisia* (*Artemisia* production and distribution firm) (216)

Duration of study: The study duration is expected to be 56 days (8 weeks or 2 months) for each participant.

Study visits: A total of five study visits will be effected on day 3 of

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ACT treatment (recruitment day), day 28 of ACT treatment (enrollment day = day 0 of *A. afra* treatment), and days 7, 14, and 28 of *A. afra* treatment. However, participants will return on any day if symptoms develop before the scheduled visit day.

During the study visits on days 0, 7, 14 and 28 of *A. afra* treatment, blood parasite status of *Plasmodium* reservoirs will be monitored using molecular markers specific to merozoites and gametocytes (rt-qPCR targeting (MSP2) and Pfs25 gene expression) to assess the evolution of parasite load and stage during and after tea treatment. Participants will also be assessed for treatment compliance, clinical symptoms, treatment reaction (if any), concomitant medications and withdrawals (day 7, 14, and 28). Liver and renal function tests will be performed at the beginning and after 28 days of *A. afra* treatment (day 0 and day 28) using urine and serum samples, to assess for possible toxicity. Parasite genome sequencing (PCR genotyping) will be conducted on day 0 and 28 samples to differentiate between recrudescence from reinfections (Table 5).

Table 5: Summary of study visits and activities

Visit time	Activity
1st: Day 3 of ACT	- Baseline assessments and recording - Malaria rt- qPCR for parasite load assessment - PCR genotyping (nested PCR) - Recruitment and pre-enrollment
2nd: Day 28 of ACT = day 0 of <i>A. afra</i>	- Malaria rt-qPCR for parasite load assessment - PCR genotyping (nested PCR) - Randomization, and treatment allocation - AST/ALT and urine analysis
3rd: Day 7 of <i>A. afra</i>	- Malaria rt-qPCR for parasite load assessment - Monitoring and recording of: • ASEs and AEs. • Treatment compliance (dosage respect) • Number of withdrawals and reasons, • Occurrence of clinical symptoms • Concomitant medications
4th: Day 14 of <i>A. afra</i>	
5th: Day 28 of <i>A. afra</i>	- Malaria rt-qPCR for parasite load assessment - PCR genotyping (nested PCR) - AST/ALT and urine analysis - Monitoring and recording of: • ASEs and AEs. • Treatment compliance (dosage respect) • Number of withdrawals and reasons, • Occurrence of clinical symptoms • Concomitant medications

Rescue treatment: Participants who develop symptoms during the study period will be treated with ACT and their blood samples analyzed to determine if it is a new infection. Severe cases will be treated according to WHO recommendations.

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Follow-up studies among participants' family members in the community: Household/family members of study participants will be contacted and tested if they are asymptomatic carriers using rt-qPCR. Those who test positive will be invited to consent and participate in the study, and genome sequencing will be conducted to compare their parasite strains with that of the initial participants. Household reservoirs will also be randomly treated in various treatment arms as described before. Follow-up assessments will be done during subsequent visits (day 7, 14 and 28 of treatment), and data obtained will be pooled, analyzed and conclusions raised (Figure 18).

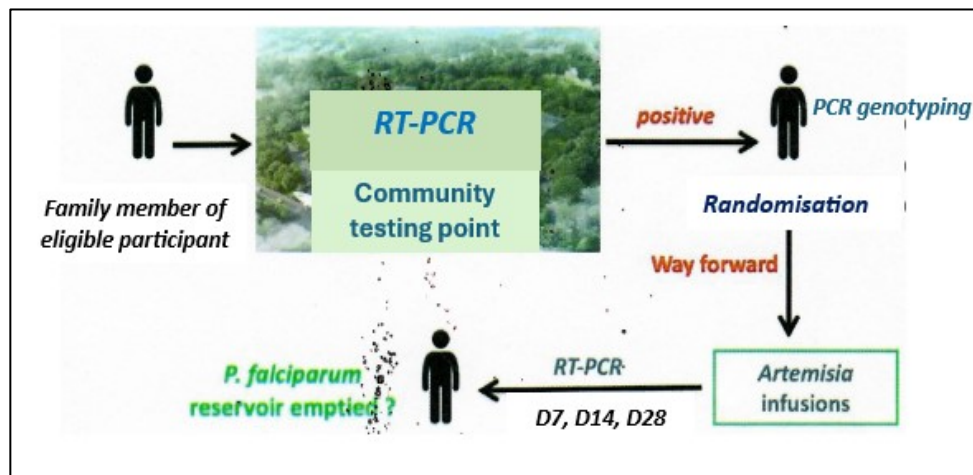


Figure 19: Community studies among participant's household/family members

Study endpoints

Primary end point

1. The proportion of participants with full response on day 28 of *Artemisia afra* treatment (CT level ≥ 40 or a negative rt-qPCR test result).
2. The proportion of participants with decreased parasite load on day 28 of *A. afra* treatment (increase CT level >20)

Secondary endpoints

- Proportion of people with negative rt-qPCR (CT level ≥ 40) in the various treatment arms at days 7, 14, and 28 of *Artemisia* treatment (Activity level and optimal treatment dosage).
- The proportion of people with negative rt-qPCR (CT level ≥ 40) the various treatment arms at days 7, 14 and 28 of *Artemisia* treatment by gender, age, BMI, and trial entry parasite load (Factors associated).
- Proportion of people with absolute decrease in parasite load (increase CT level > 20) in the various treatment arms at days 7, 14, 28 of *Artemisia* treatment.
- Proportion of persons on the various treatment arms who develop clinical symptoms or AEs at days 7, 14 and 28 from baseline (no severe adverse side effects are expected).
- The proportion of participants with normal liver and kidney function values in the different treatment arms at day 28 from enrollment (Safety- dose concentration, dose frequency).
- Proportion of withdrawals and loss to follow up in the different

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treatment arms at days 7, 14 and 28, from enrollment and reasons for withdrawal.

- Proportion of recurrent infections on days 3 and 28 of ACT treatment (ACT treatment failure due to Artemisinin or partner drug)
- Proportion of recurrent infections on day 28 of ACT due to reinfection and/or recrudescence (ACT treatment failure after PCR correction)
- Prevalence of recurrent infections with validated molecular markers for resistance
- Prevalence of asymptomatic carriers within participant families
- Proportion of participants' household/family members with analogous parasite strains with study participants

Ethical 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, and participant consent will be freely given, with written informed consent prior to participation in the study.

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.

DATA ANALYSIS PLAN

Analysis populations

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

Exposed Population: All enrolled participants who received a treatment dose.

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

Full Analysis Population: All enrolled participants 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 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 will serve as the primary analysis population for all efficacy objectives. Due to unpredictability of some irregularities, the criteria for exclusion of participants from Per Protocol population will be determined before the database is locked and will be based on the blind review of protocol violations.

Safety Population: All participants in the enrolled population who received a study treatment and had any safety data available. All safety analyses will be performed using this population and the participants

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will be analyzed as "treated" (i.e., according to the actual treatment received). The denominators for different safety endpoints will be varied according to the number of participants with available data for the specific endpoint (figure 20).

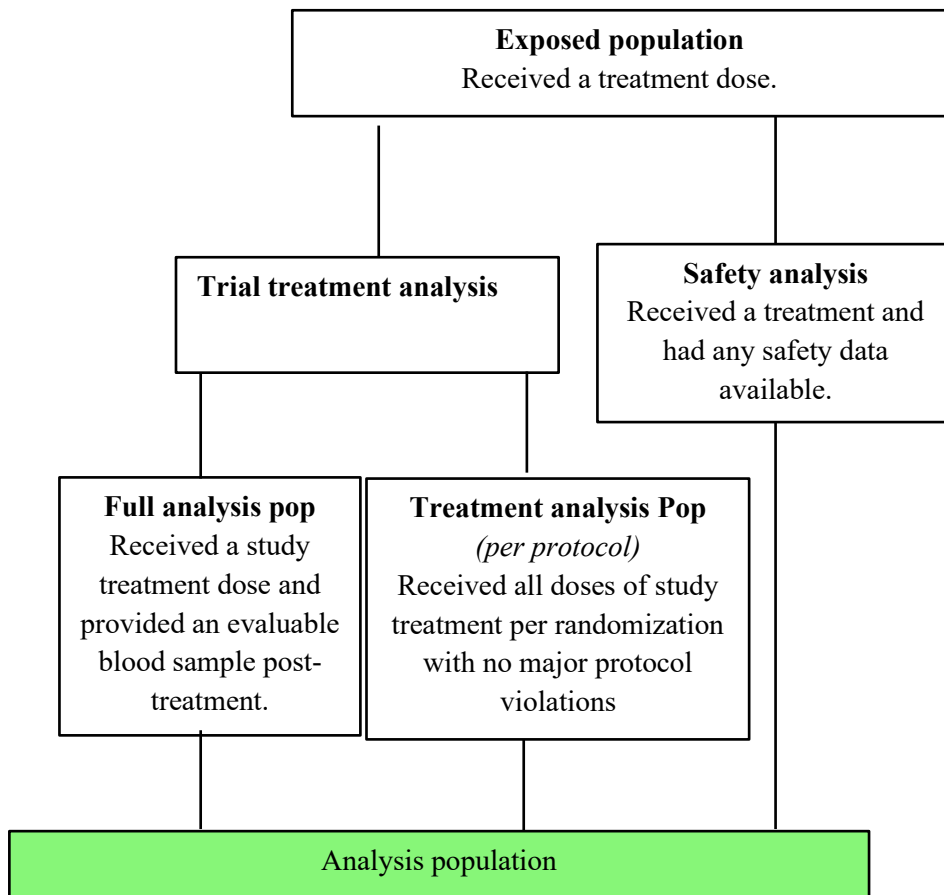


Figure 20: Data analysis plan

Statistical analysis

All data will be analyzed using Stata17 software packages. The data will be analyzed using survival analysis with Cox regression to evaluate time to emptying (reservoirs elimination), and with time-to-regulate rt-qPCR as outcome. Hazard ratios (HR) and 95% confidence intervals (95%CI) will be computed to determine the rate of reservoir (parasitaemia) elimination in different treatment arms. Statistical significance level will be set at $P < 0.05$.

Descriptive statistics will be reported on the treatment outcomes in the various arms according to the evaluation times. Quantitative variables will be summarized using means with their standard deviations (SD) and median with range, while qualitative variables will be reported as numbers and percentages.

Interpretation and use of results

- The presence of residual parasites in $>10\%$ participants on day 3 and $>10\%$ on day 28 of ACT treatment will indicate treatment failure (both artemisinin and partner drug failure) and a need for treatment policy review.
- The presence of residual parasites in $>10\%$ participants on day 3 and $<10\%$ on day 28 of ACT treatment will indicate treatment success (with delayed artemisinin clearance) and a need for close observation.
- The absence of residual parasites in $<10\%$ participants on day 3 and $>10\%$ on day 28 of ACT treatment will indicate treatment failure (due to partner drug failure) and a need to consider treatment policy change.

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-
- The presence of residual parasites in <10% participants on day 3 and <10% on day 28 of ACT treatment will indicate treatment success (effective artemisinin partner drug), hence policy maintenance.
 - A marked increase in the prevalence of validated molecular markers for resistance to partner drug for first-line ACT indicates high rate of mutant genes and a need to consider or prepare for treatment policy change (even before treatment failure rate reaches 10%).
 - CT level ≥ 40 (that is a negative rt-qPCR test result) after *A. afra* treatment will indicate full response to treatment.
 - CT level >20 (absolute increase in CT levels) after *A. afra* treatment will indicate a notable decrease in parasite load.
 - CT values ≤ 20 after *A. afra* treatment will indicate absence of antimalarial activity.
 - The treatment arm with highest proportion of participants with emptied reservoirs (CT level ≥ 40 or a negative rt-qPCR test result) will be considered as the optimal dosage arm.
 - Normal Liver and kidney function values after tea treatment (at day 28) will indicate absence of toxicity.
 - Increase or abnormal Liver and kidney function values after tea treatment will indicate probable toxicity of tea and need for further evaluation.

5.3.3 Phase III: Augmentation of clinical study to assess efficacy *A. afra* infusions.

This will be a placebo controlled randomized trial to assess the effectiveness of *Artemisia afra* tea infusions in emptying *Plasmodium* parasites in asymptomatic carriers. Based on the results of the phase II trial, the optimal treatment dosage (most effective) of the *Artemisia afra* infusion obtained will be tested against a placebo in a larger sample.

Sample size: The sample size will be calculated based on the proportion of reservoirs emptied by the optimal dosage of *Artemisia afra*. However, we expect that the optimal dosage of the tea should empty *Plasmodium* reservoirs in at least 75% of participants compared to 25% of controls with an acceptable clinically important difference of 15% as compared to the placebo. Considering a level of significance of 5% and a power of 80%, the formula for sample size estimation in clinical trials (217) will be used to estimate the required number of study participants (n) as follows;

Formula for calculating the minimal sample size

$$n = [(Z_{\alpha/2} + Z_{\beta})^2 \times \{(p_1 (1-p_1) + (p_2 (1-p_2)))\}]/(d)^2 \text{ where}$$

n is the sample size required in each group,

p1 is the expected proportion of subjects cured by *Art. afra* infusions

d is the clinically significant difference between the two proportions

$Z_{\alpha/2}$ is the normal quantile corresponding to the significance level;

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for 5% this is 1.96,

Z_{β} is the normal quantile for a $(1-\beta)$ power; for 80% this is 0.84

To perform an unequal allocation in the treatment and placebo groups due to ethical reasons, the sample size for the treatment arm (n_1) and placebo arm (n_2) will be adapted as

$$n_1 = (0.5) \times n \times (1 + k), \text{ and}$$

$$n_2 = (0.5) \times n \times (1 + (1/k))$$

Where n is the sample size required as per formula, n_1 is the sample size for the test treatment, n_2 is the sample size for the control group and $k=n_1/n_2$.

Following the reservoir drainage phase, the prophylactic potential of *artemisia* tea will be assessed. The 75% of participants with emptied reservoirs will be randomized in 3 study arms in a 2:1 ratio for those in intervention and control arms respectively. The treatment arm will consist of *Artemisia afra* 10g/L taken for 3days in a week (335ml per day) over 2 weeks or *Artemisia afra* 10g/L, 1L/weekly (once a week on a fixed day) over 2 weeks, while the control will take nothing. The treatment prophylactic potential will be defined when controls fill up their reservoirs while none of the infusion consumers test positive for *Plasmodium* infection six months later.

5.3.4 Other pharmacological and molecular biology studies to be performed in the project

1. To establish the genome of the *Artemisia* plants used and determine their metabolic content in different geographical areas of Cameroon and at different seasons.
2. To develop the most appropriate galenic formulation, quality control, and quality validation protocols based on metabolic contents and *in vitro* efficacy.
3. Invitro drug testing of *Artemisia afra* infusions on parasite clinical isolates resistant to ACTs
4. Characterization of parasite strains from human reservoirs (prevalence and ACT molecular markers of resistance)
5. Complete stage specific analysis by rt-qPCR of clinical trials samples
6. Assess the efficacy of *Artemisia* tea infusions using *Plasmodium* culture tools.

CHAPTER 6: DISCUSSION

6. Discussion

Malaria is the most widespread endemic disease in Cameroon (163), and the government of Cameroon has made the fight against malaria a priority, with a highlight in the country's Health Sector Strategy, as well as the adoption of the High Burden High Impact (HBHI) stratification exercise in the National Malaria Strategic Plan (2019–2023) (218). There has also been optimistic scale-up of the WHO-recommended control actions towards malaria elimination in Cameroon over the last decades, with focus on vector control, diagnosis and treatment of symptomatic cases, as well as chemoprevention for under-five children and pregnant women (163,164,178). Despite the scale up of interventions over the years, malaria remains an important public health threat in the country, responsible for 50% of hospitalizations in health facilities (178,162), with the whole country exposed to the risk of transmission (162). The current interventions alone are not sufficient to eliminate malaria from many endemic regions as they fail to target asymptomatic *Plasmodium* reservoirs who constitute more than 95% of infectious human reservoirs in endemic regions and perpetuate the disease transmission (39,40,41,42,43).

The purpose of our study was to contribute to the elimination of asymptomatic *Plasmodium* reservoirs in malaria endemic areas of Cameroon using *Artemisia afra* tea infusion, in order to reduce the malaria burden in the country. Specifically, we explored the experiences of adult Cameroonians on the use of herbal teas to treat infections or diseases, and their acceptability to use the teas if

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prescribed in a hospital. We also assessed the knowledge of health care workers about asymptomatic malaria and their acceptability to use *Artemisia afra* tea to treat asymptomatic *Plasmodium* carriers, and we designed a trial aimed at ascertaining the efficacy and safety of *Artemisia afra* tea infusions in emptying *Plasmodium* reservoirs.

6.1 Summary key findings

In the cross-sectional survey of herbal tea use among Cameroonian adults, the prevalence of tea use was high for both community (87%) and hospital (94%) participants. Most respondents took teas for treatment of Covid-19 (67%), malaria (59%) and typhoid fever (35%). Most (65%) of the teas used by respondents were prescribed by someone else. There was a high acceptability and willingness by community (87%) and hospital (98%) respondents to use herbal teas prescribed in a hospital. The main motivating factor for accepting to use teas was effectiveness of the tea. No sociodemographic factors were associated with herbal tea use.

In the cross-sectional study assessing the knowledge of health care workers about asymptomatic malaria and their acceptability to use *Artemisia afra* tea to treat asymptomatic *Plasmodium* carriers, HCWs had a fair knowledge about the existence (88%), definition (85%), diagnosis (75%), treatment (70%) and transmission of asymptomatic malaria (51%), with an important gap in their knowledge of

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transmission. Nursing assistants had less knowledge about the existence of asymptomatic malaria compared to higher professional categories. HCWs with shorter periods of service had less knowledge about transmission compared to those with longer periods of service. More than half (56%) of HCWs believe that *Artemisia afra* was effective in treating malaria. Most HCWs were willing and ready to prescribe *Artemisia afra* tea to treat asymptomatic *Plasmodium* carriers if approved by authorities. Among challenges of using *Artemisia afra* teas to treat asymptomatic malaria as predicted by HCWs were probable treatment refusal (14.9%) and due to absence of symptoms and the risk of counterfeit teas (1.5%).

6.2 Discussion

6.2.1 Use and acceptability of herbal teas among Cameroonian adults

The prevalence of herbal tea use observed in our study (89%) was slightly higher than the WHO estimated general prevalence of traditional medicine (TM) use of 80% and also higher than the prevalence observed from previous studies in Cameroon (219,220,221,222,223), and similar studies in other African countries such as 80.9% in Port Harcourt (224), 85.1% in Plateau State, Nigeria (225), 76.7% in Baluchi, Nigeria (226), 60.0% traditional, complementary and alternative medicine (TCAM) use in Zimbabwe (227), 58.2% in Sub Saharan Africa (129), and 55.5% in Malaysia (228). The higher prevalence observed in our study compared to other

6. Discussion

studies in Cameroon may be because our study was conducted in the general adult population, while the other studies were conducted in specific study populations (for specific health conditions). For example, the study conducted by Leke *et al.* in 2022 in pregnant women reported the prevalence of 76% (220). Pregnant women are expected to use less medication and less herbal teas than the general population. In another study conducted by Ayima *et al.* in 2021, assessment of herbal medicine use was specific for covid-19 treatment, with prevalence of 68.5% (221).

The high prevalence of tea use in the present study could also be due to an increased consumption of herbal tea as a precautionary measure during the emergence of the covid -19 pandemic within the past three years which threatened the lives of many individuals and caused them to increase their health seeking behavior, including the use of traditional medicines. This is confirmed by findings of the current study where, the purpose for which most respondents used herbal teas was prevention and treatment of covid-19, followed by malaria and typhoid fever. Unlike our findings, most of the respondents in a study in Nigeria (225) used traditional medicine (TM) mainly in the treatment of malaria and fever.

Generally, people used herbal medicines to restore, promote and maintain health, as well as treat and prevent illness (228). In line with existing data (229), the respondents in this study also reported to use

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plants and parts of plants for different ethno-medical purposes such as antiseptic, laxatives, purgative, anticonvulsant, expectorants, anthelmintic, and in the treatment of malaria, high blood pressure, diabetes, rheumatism, diarrhea, infertility, jaundice, dysentery, gonorrhea, fever, pain, respiratory problem, or poultice, among others.

This high rate of herbal medicine use observed among both community (87%) and hospital-based respondents (94%) might reflect a general health seeking attitude among respondents. However, this attitude is suggestive of a probable use of herbal teas alongside conventional medications by the hospital respondents, which could result in a possible drug herb interaction that could dangerously amplify or reduce the potency of co-administered conventional medicines. Thus, there is a need for health care providers in these settings to always inquire about the use of herbal medicines when providing health care. A study in Bauchi, Nigeria revealed that over one-third of participants combined herbs with allopathic medications and over four-fifth of them did not inform their doctors that they used herbs (226).

A higher proportion of herbal tea use was observed in respondents from homes with larger household size (> 5 people in households). This might be due to financial difficulty to provide modern healthcare to all members in large households, compared to herbal medicine which could be home prepared, received for free, or locally available at a far cheaper cost (219). With the persistent financial hardship affecting

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many households in Cameroon, it is likely that many will preferably not go for laboratory diagnosis of their illness but would buy locally available and cheap medications regardless of the source and quality, if they are told it treats what they are assumedly suffering from, thus revamping their hope.

The finding of this study that two-thirds of the tea users received their tea prescription from someone else and one-third of the teas taken were self-prescribed, are in line with previous reports that users of herbal medicine are mostly initiated through convincing information from the media, families or close contacts and friends about the efficacy of previously used herbal medicines (230). A danger is that the family, friends and the commercial vendors may not possess the correct knowledge and information to advise relatives on the use of herbal medicine and their probable side effects, with the notion that traditional medicines are natural and safe for use with no adverse effects. The general perception that herbal remedies or drugs are very safe and devoid of adverse effects is not only untrue, but also misleading, as herbs have been shown to be capable of producing a wide range of undesirable or adverse reactions some of which are capable of causing serious injuries, life-threatening conditions, and even death (231,232,233). A more dangerous aspect is that less than 1% of medicines from traditional sources are being officially commercialized in Cameroon (234), and the medicines are mostly obtainable from

6. Discussion

indigenous sources such as traditional clinics, travel agencies/express buses, individual homes and in markets (219), rather than in hospital or pharmacies. Hence important pharmacological information (about the mode of action, adequate dosage and course of treatment, concentration, potential adverse effects, toxicity, contraindications and interactions with existing conventional pharmaceuticals and functional foods) to promote both safe and rational use of these products are not available. The high trust and easiness to accept and use herbal teas prescribed by other persons revealed in this study is a promising attitude towards acceptance of future herbal treatment in an integrated healthcare system. However, this may be dangerous to the health of the population in situations where safety and effectiveness of the proposed treatments are not verified. There is a need to educate the population about herbal medicines use, its potential dangers, and the need to be precautionous in accepting unverified treatment from unknown sources. There is also a need to control advertisements and misleading health related information shared by untrained drug vendors on various communication channels within the national territory.

The findings that most of the respondents in this study took the teas warm and the treatment dosage used by most respondents was “one glass morning and evening for one-to-two weeks” are in line with results from Jos South Local Government Area of Plateau State, Nigeria (225), where the herbal medicine used by respondents had specified dosage indications. The above-mentioned frequently used posology is

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apparently convenient for both students, officers, or manual laborers, and it is worth considering when prescribing herbal teas in future. Most people usually leave home in the morning for various activities and only return home in the evening, making it more convenient to take the teas when they are at home, especially as they need to take it warm.

Our study revealed that the teas taken by two-thirds of respondents had bitter taste and some of the participants were uncomfortable with the bitter taste of the teas, while others weren't bothered by bitter taste. Even though many participants were uncomfortable taking the herbal tea treatment, more than two-thirds of the respondents completed their treatment dosage because of the desire to get well and to respect the prescribed dosage. These results highlight an encouraging compliant practice by participants, and a determined wellness mentality towards treatment, irrespective of the taste or discomfort of the treatment. However, the results that more than one-tenths of respondents abandoned their herbal treatment as soon as they felt better thereby indicating a need for increased health education to the population on the importance of taking the prescribed dosage or adhering to treatment. Also, the reasons for non-adherence given by respondents ought to be considered to improve treatment options and approaches in the future. More than nine-tenths of the participants who had taken herbal tea in the current study had never taken *artemisia* tea. Those who had taken *artemisia* tea used it for the treatment and prevention of malaria and covid-19, and many of the respondents said it was effective for them.

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The effectiveness of *artemisia* teas in the prevention of malaria reported by respondents in the current study may not be founded as artemisinin and *Artemisia annua* do not affect sporozoites and are therefore not effective for preventing infection when taken prophylactically. This is confirmed by findings in the Democratic Republic of Congo (DRC) (140) where *A. annua* tea by itself was fairly effective in the immediate relief of *falciparum* malaria but not effective at keeping the infection under control, thereby necessitating its combination with other treatments to prevent recrudescence and, possibly, development of resistance. Unfortunately, most people (like our study participants) who are ignorant about the details of malaria or how artemisinins work continue to use and recommend *artemisia* tea for prevention of malaria (235), and often citing anecdotal evidence (if any) that they or people they have recommended it to took it and did not develop malaria. This might be due to chance, or precautions taken against mosquito bites, thus, indicating a further need to constantly educate and equip the people towards optimal vector control and preventive measures such as environmental hygiene, larva source management (emptying of stagnant pools), indoor residual spraying, use of insecticide treated bed nets, vaccination, and chemoprophylaxis (IPT, SMC). In line with this, Cameroon recently introduced the four dose RTS, S/AS01 malaria vaccine into its routine national immunization programme in January 2024, within 42 health districts that are most at risk of malaria in all 10 regions of the country (197). On the other hand, WHO has cautioned

6. Discussion

against the use of non-pharmaceutical sources of artemisinin because of the risk of delivering sub-therapeutic doses of artemisinin that could potentiate anti-malarial drug resistance (147). However, findings in DRC revealed that although the average total dose of artemisinin delivered in *A. annua* whole plant (60mg) was far less than the usual dose recommendations of pure artemisinin (500–1000 mg a day for 3–5 days), this was effective for total parasite clearance, indicating a likelihood that other compounds such as the flavonoids found in the whole herb might have potentiated the action of the small amounts of artemisinin, or that small doses of this compound are more effective than previously believed (236). In recent reports, the Madagascar Institute of Applied Research reported an important use of *Artemisia annua* (sweet wormwood) in covid-19 (237). Contrary to the findings in Madagascar, a very low use of *artemisia* tea was reported in the current study. However, the low *artemisia* tea use reported in this study may not be real, as most people who denied ever taking the tea, were not also familiar with the name “*Artemisia afra*”, and might have taken it without knowing its name, given that they took some teas without knowing the names.

▪ Acceptability to use teas prescribed in hospital

The results of this survey that more than nine-tenths of respondents were willing to use herbal teas for treatment in the future if prescribed in the hospitals, because of high confidence in doctors and the hospital,

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previous experience on the effectiveness of herbal teas, and preferences for herbal teas, depicts a high level of confidence in health facilities, and a positive opinion about herbal teas by our study population. This is a promising indicator of high acceptability and compliance with herbal teas prescribed in hospital especially if the safety and effectiveness of the treatment is ascertained. However, the data also indicates a strong influence of health care providers on the potential acceptability of the teas, based on their knowledge, conviction, and willingness to prescribe the teas for patient treatment. It is therefore necessary for health authorities to verify and ensure that detailed pharmacological data on all traditional medicines of interest is made available to health care providers before their integration into the health care system. The above finding agrees with other works in Cameroon (219) and Nigeria (224), where most people perceive traditional medicines as efficacious, cost-effective and a viable alternative to allopathic medicine.

▪ Factors associated with use and acceptability of herbal teas

The absence of an association between participants' sociodemographic characteristics and herbal tea use in this study may be accounted for by generally high prevalence in of tea use in all the sociodemographic sub-populations. Even though no association was found, a main motivating factor for herbal tea use was effectiveness of the teas. This finding is satisfactory, as it is an expected wellness mindset during treatment. In

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Accra, Ghana (230), effectiveness and personal preference for herbal medicines were also reported as motivating factors for herbal tea use. Contrary to our findings, similar studies in Cameroon (220) found that age > 41 years, being a farmer, having an income level between 185–370 USD, residing in a rural setting, and being knowledgeable on traditional, complementary and alternative medicine (TCAM), were predictors for TCAM use. Meanwhile being married or having low-income status were found to be positively associated with herbal medicine use among Malay women in Malaysia (228). Findings of this study that treatment effectiveness was the major motivating factor for acceptability and use of herbal tea are satisfactory, as it is an expected wellness mindset during treatment. This concurs with findings in Accra, Ghana (230), where the effectiveness and personal preference for herbal medicines were reported as the major motivating factors for herbal tea use.

6.2.2 Knowledge of health care workers about asymptomatic malaria and acceptability of artemisia infusions

The knowledge gap about asymptomatic malaria observed in the current study might be because more than one-quarter (26%) of the survey respondents were of a very low professional category (nursing assistants), who might never have received formal training to consult and treat patients. This is confirmed by other findings of this study, namely, that the nursing assistants had the lowest proportion of

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asymptomatic malaria-informed respondents and were less likely to be aware about the existence of asymptomatic malaria compared to higher professional categories with adequate training ($p < 0.05$). As unlicensed professionals, nursing assistants work with the registered nurses to assist patients with daily care such as ambulating, bathing, feeding, and other typical responsibilities delegated by the registered nurses such as collecting vital signs (238). Nursing assistants are not normally supposed to perform direct treatment procedures on patients such as invasive procedures, administration of any medications, or the application or removal of any dressings (239,240). In the current study, the activities of the nursing assistants went beyond nursing practice to their involvement in patient consultation and treatment prescription, which may have an implication on the quality of patients' care and safety. In line with this finding, a systematic review on activities of nursing assistants revealed that many activities were performed beyond the training of the nursing assistant and were being performed with limited supervision from registered nurses (241). Another review in Canada showed that unregulated care providers did not have a scope of practice, but their role had evolved to include activities previously performed by regulated professionals (242).

The involvement of low-category health care workers in patients' treatment in Cameroon may be due to a high paucity of health care practitioners in Cameroon, with a doctor/patient ratio of 1:50,000, and

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about 35,000 health care practitioners are needed to meet the WHO's recommended ratio of 1 doctor per 10,000 persons (181). This makes it impossible for medical consultation and treatment to be performed solely by physicians, hence the inevitable involvement of under-qualified personnel in patients' consultation and treatment. Given that nursing assistants are often included as a part of the care delivery team and also provide patient care by themselves, it is critical for doctors and registered nurses to share patients' care information and increase collaboration with them, in order to improve on the quality of patient care provided in such settings. Seminars on asymptomatic malaria should be included in routine in-service training planned for health care workers with an emphasis on the need to screen and adequately treat symptomatic infections.

In this study, most of the study participants prescribed antimalarials (70% of respondents prescribed ACTs) for the treatment of asymptomatic infections, but this was not associated with a gametocidal drug to completely clear the gametocyte and block transmission. This operational gap could be because current malaria control strategies implemented by the National Malaria Control Programme (NMCP) in the country are focused on malaria prevention and clinical case management to reduce morbidity and mortality, but do not address the interruption of transmission nor the elimination of asymptomatic *Plasmodium* carriers (164,162,163,178,185). In

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Cameroon, the NMCP is the technical structure of the Ministry of Public Health responsible for the coordination, decision making, and implementation of malaria control activities. To ensure adequate programme implementation and follow-up of these activities, authorities of the NMCP regularly provide an updated document on the guidelines for the management of malaria in health facilities and communities, to direct the actions of care providers. Hence, treatment decisions and actions of health care workers are mostly based on these guidelines.

Due to the emerging resistance to ACTs reported in several African countries (110,111,112,113,202,203,204), studies have suggested the combination of a gametocytocidal drug such as primaquine with an ACT regimen in clearing gametocytes, to effectively block transmission (243,244,245). In the same light, the WHO framework for malaria elimination recommends that in the context of malaria elimination, treatment of malaria must follow national policies and WHO guidelines, associated with treatment that fully clears malaria infection, in order to reduce morbidity and mortality, and also to interrupt transmission of *Plasmodium falciparum* and relapse of *Plasmodium vivax* (94). It is therefore necessary for the NMCP in Cameroon to consider the integration of the WHO recommended strategies for malaria elimination in subsequent strategic plans.

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Results of studies that *Artemisia afra* tea infusions have antiparasitic activity against the different stages of the malaria parasite *invitro*, including the gametocyte stages, with no cytotoxicity (132,133,151,136,152), suggests its probable usefulness in the elimination of asymptomatic *Plasmodium* reservoirs (and clearing of residual parasites in asymptomatic individuals after treatment for clinical malaria) in endemic areas. However, no clinical studies have been conducted to ascertain the effectiveness and safety of *Artemisia afra* tea in clearing *Plasmodium* parasites in humans. The other part of our project in progress entails randomized studies to ascertain the effectiveness of *Artemisia afra* infusions in emptying *Plasmodium* reservoirs in humans. If proven effective, the tea could serve as a useful alternative in the elimination of asymptomatic *Plasmodium* reservoirs and interruption transmission in endemic areas, as it is locally available and less costly. However, it would be necessary to constantly monitor resistance to *Artemisia afra* teas as their availability locally may result in indiscriminate use and a subsequent emergence of resistance. Emergence of resistance to treatment is often monitored through therapeutic efficacy studies (TES) and integrated drug efficacy surveillance, which includes prospective evaluation of patients' parasitological and clinical response to treatment of uncomplicated malaria (118). Monitoring the emergence of resistance to teas after confirmed efficacy may entail invitro drug testing of the infusions on parasite clinical isolates resistant to ACTs, and characterization of

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parasite strains from recrudescence human reservoirs, in order to assess for prevalence, ACT molecular markers of resistance, and de novo mutations. According to the project plan, analysis of possible resistance will be conducted within the molecular biological studies in ULB. Patients with persistent symptoms and frequent malaria episodes despite adequate medical care could be assessed for herbal tea use and monitored for resistance to teas. Emergence of resistance could be controlled by averting misuse to teas and ensuring accessibility of quality teas, as well as adequate vector control coverage in key populations such as infants, children under 5 years, pregnant women, people with HIV/AIDS, and non-immune persons (disease naïve populations and travelers) who are at higher risk of severe infections.

The finding that health care workers with a shorter duration of service had lower knowledge about asymptomatic malaria transmission compared to those with more years of service is expected, as health care workers may naturally gain more awareness, experience, and learn more about the condition with an increased number of years of practice. However, these findings were different from the results in Ebonyi State, Nigeria, where the number of years of practice was not significantly associated with doctors' knowledge of the national guidelines of malaria diagnosis and treatment (246).

In this study more than half of respondents believed that *Artemisia afra* tea was effective in treating symptomatic and asymptomatic malaria.

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Even though this is a promising response for potential acceptability and use of the investigated tea if efficacy is proven and the treatment authorized, it reveals a high level of ignorance and unseriousness among respondents, given that that no clinical data exists to substantiate the antimalarial activity of the teas in humans. There is also a need to clarify health care workers that, the tea under investigation (*Artemisia afra*) is aimed eliminating asymptomatic *Plasmodium* reservoirs to interrupt transmission, while ACTs remain the treatment of choice for uncomplicated clinical malaria.

The finding that more than three-quarters (79%) of respondents were willing to prescribe the tea if approval by authorities reveals a potential high-level of acceptability and use of the tea. This equally indicates a promising acceptance and utilization by the population who mostly rely on the decisions of health care workers for their health care. Given that the major concern of those who were unwilling to prescribe the tea was logically based on their uncertainty about the availability of scientific drug data on the tea, it is necessary for health authorities to verify and ensure that detailed pharmacology information on the tea is available to health care providers before its endorsement for use in the health care system, if effectiveness is proven.

The predicted challenge of probable treatment refusal by patients due to the absence of disease symptoms is feasible as it is not easy to convince someone to take treatment when they do not feel sick.

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Previous studies on asymptomatic conditions have shown that uncertain outcomes relating to one not knowing whether they had a disease that was asymptomatic were reasons for treatment refusal or abandonment (247,248). However, evidence of a positive screening test and a clear justification of the need for treatment by the health care providers may be enough to convince asymptomatic *Plasmodium* carriers for treatment compliance. In an assessment of community perspectives on treating asymptomatic infections for malaria elimination in The Gambia, participants questioned how they could be aware of having the disease when they did not experience any symptoms, given that they had a clear understanding of malaria symptoms, and the lack of these symptoms in the asymptomatic phases led to a state of uncertainty. However, the study population showed a willingness to accept RDT-positive results in the absence of symptoms, and most of them agreed that anti-malarial medications could cure malaria even if the disease was in a dormant phase (249). Evidence of positive opinions and promising compliance of asymptomatic persons to treatment based on positive diagnostic results highlight a need for authorities to ensure the availability of accurate diagnostic techniques for asymptomatic malaria diagnosis and strengthen the capacity of biomedical specialists in the use of these techniques. The risk of counterfeiting can be very common and real as the tea is cultivated locally and can be made available to community members by some farmers who may deliberately and fraudulently add other inactive local herbs or falsify the tea for financial

gains. This is dangerous as it may decrease the treatment efficacy, thus resulting in an increased disease burden and missed goal of the treatment. Fake medications may also reduce credibility due to poor treatment outcomes or other adverse effects including disability or death. It is therefore necessary for health authorities to raise awareness and educate the population on the risk of existing counterfeit medications and how to avoid purchasing them. The population should also be encouraged to buy medicines and medical products only from the hospital pharmacy, licensed retail shops, pharmacies, and dispensaries and avoid buying from online pharmacies. They should also be educated on how to visually inspect the medicine's external packaging to verify that it is labelled with the product name, manufacturer, its expiry date, and the batch number where possible.

6.2.3 Efficacy and safety of *A. afra* infusions in clearing *Plasmodium* reservoirs

Most of the current malaria control strategies (prevention and treatment measures) have been shown to be fairly effective for immediate and temporal relief of the disease. However, they are not effective in keeping the infection under control, with a need to be combined with additional strategies for effective control and elimination. *Artemisia afra* tea infusions, if confirmed to be effective in emptying *Plasmodium* reservoirs in this trial, will therefore serve to complement existing control strategies towards achieving the ambitious goal of malaria eradication.

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In vitro studies have confirmed antiparasitic activity of *A. afra* tea infusions against the different stages of the malaria parasite including the hepatocytes and gametocyte stages, with no cytotoxicity (132,133,151,136,152), suggesting a potential to empty malaria reservoirs and interrupt malaria transmission without threat to ACT resistance. However, results of studies that *A. annua* administered as a whole plant (powder-water suspension or as infusions) is more active than comparable doses of pure artemisinin suggest a synergetic drug-drug interactions between artemisinin and the plant constituents like methoxylated flavonoids, which may potentiate actions of small amounts of artemisinin (augment serum levels of artemisinin) (93,154,155). Hence, use of *A. afra* alongside ACT may dangerously amplify the potency of the drug or can result in adverse drug reactions that may be harmful to the patient.

To adequately verify for the use of teas during the trial, participants in all study arms (treatment and control) will be closely monitored and tea served to them (where applicable) by assigned team members. Whatever the case, we will work using an intent-to-treat approach.

As of now, the timeline of the study is not yet clearly defined due to a delay of about two years in obtaining the national ethics approval (obtained in October 2023), which was mandatory to conduct the trial. There is also a need to improve the galenic formulation in order to reduce challenges related to preparation of the infusions, which were

observed during the pilot trials. With the introduction of the RTS, S/AS01 malaria vaccine (which has an efficacy of 77%), into the routine national immunization program for children in Cameroon since January 2024, and with a reported massive uptake, we hope for its positive impact on malaria immunity in children. This will serve as a milestone towards malaria elimination in the country.

6.3 Strengths and limitations

This thesis was the first to explore the use of herbal teas in a general adult population, involving both community and hospital respondents, and with a high response rate. It was also the first to assess knowledge of health care workers about asymptomatic malaria in Cameroon.

However, our studies employed a cross-sectional design which does not provide information on participants' behaviour over time, establish cause-and-effect relationships, nor does it guarantee the study to be representative. There was also the potential for recall bias as the data collected was self-reported.

There was probable social desirability bias given that herbal tea use is likely considered as a socially undesirable attitude and behavior, especially for respondents interviewed at health facilities.

Considering variations in the culture and habits of people in different regions of the country, it is not evident that responses obtained in the study regions (centre and south west regions) represent those in other parts of the country. However, the findings are of great significance as

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they uncover the views and experiences of participants on herbal tea use, and inform authorities on some aspects to consider for improving uptake and adherence, when considering strategies to successfully integrate traditional medicines in health facilities.

CHAPTER 7: GENERAL CONCLUSION AND PERSPECTIVES

7. General conclusion and perspectives

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7.1 Conclusion

In Cameroon, malaria represents a major public health concern with the entire country being at risk of transmission (178). This thesis captured information on the use of herbal teas among Cameroonian adults, assessed the knowledge of health care workers about asymptomatic malaria, and the acceptability of health care workers and Cameroonian adults to use *Artemisia afra* tea to empty *Plasmodium* reservoirs. Our findings showed a high prevalence of herbal tea use among Cameroonian adults in communities and hospitals in the survey settings, with a positive opinion and willingness to accept herbal teas prescribed in hospitals. Health care workers had a fair knowledge about asymptomatic malaria, with an important gap in their knowledge of transmission; however, they had a positive opinion and willingness to use *Artemisia afra* tea if approved by authorities. The findings raise concerns about a high trust and use of herbal teas from unverified sources by most of the tea users, and the probable use of teas alongside allopathic medications among participants. They equally raise concerns about an observed gap in the knowledge of health care workers about asymptomatic malaria, which may be linked to the involvement of nursing assistants (low category health care workers) in patients' consultation and treatment prescription, and the orientation of malaria control measures currently implemented by the NMCP in the country, which does not target transmission interruption or elimination of asymptomatic carriers. Further to this, the study highlights a high level

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of belief and positivity of healthcare workers about the effectiveness of *Artemisia afra*, in the absence of clinical data to substantiate the claims.

7.2 Recommendations

Based on the results of our study we recommend:

- To educate the population about herbal medicines use, its potential dangers of extensive herbal tea use, and the need to be precautionous in accepting herbal treatments from unverified and unknown sources.
- To raise awareness of the population on the risk of existing counterfeit medications and how to avoid purchasing them.
- To always inquire from patients about parallel use of conventional medications and herbal teas and educate the patients as necessary.
- To verify the efficacy and safety of herbal teas and ensure the availability of detailed pharmacology information before their approval for use in hospitals and communities, as well as watch out for counterfeit.
- Control advertisements and misleading health related information shared by untrained drug vendors on various communication channels within the national territory.
- To organize refresher training sessions for health care workers on malaria management with an emphasis on the diagnosis and adequate treatment of both symptomatic and asymptomatic infections.
- To consider the integration of the WHO recommended strategies for malaria elimination in subsequent strategic plans of the NMCP in Cameroon.

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7.3 Perspectives

Studies/data on the traditional practices and medicines used by the indigenous people, for different health conditions, as well as their use of these herbal medicines alongside conventional medications prescribed in hospitals would be useful in ensuring the safety and effectiveness of prescribed treatments and designing related health education for the population.

A randomized clinical study to test the effectiveness and safety of *Artemisia afra* tea in eliminating *Plasmodium* reservoirs in endemic areas of Cameroon has been approved by the Cameroon national ethics committee and preparations are ongoing for its implementation. This study would provide information on the efficacy of tea *in vivo*, which is highly needed.

Providing routine in-service training on malaria management to nursing assistants and other low category health care workers involved in treatment prescription, will improve their knowledge of adequate case management, thereby contributing to reduce malaria mortality, morbidity, and transmission in endemic areas.

7.3.1 In-service training programme on malaria management for less graduate health care

General purpose: To improve the knowledge and competences of low category health care workers on malaria management.

Method: This will be a 2-days training workshop for all health care

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workers with less than three years of health professional training, who are involved in treatment prescription. The training will provide participants with knowledge and competence to improve their understanding on the basis for malaria diagnosis and treatment, planning and implementation of malaria case management, and the basis for malaria control and elimination. It will be divided into six learning units and will be delivered twice a year before the malaria season. The training program will first be piloted in 8 selected rural health districts in the Centre region of Cameroon, and amendments will be made before its implementation in other parts of the country. Administrative authorization will be obtained from the authorities of the Ministry of Public Health, and a list of eligible participants will be obtained from the hospital directors. This project will be implemented based on the availability of funds to plan and conduct the training including transportation, lodging, and feeding of participants and trainers.

Unit 1: The malaria situation in the place of work (2hrs). This will be a collaborative session with the participants to describe the malaria situation in the country and in the area where they work, to outline the main components of the national malaria control policy in the country and indicate the major challenges and obstacles to achieving malaria control in their place of work.

Unit 2: Basic facts about malaria (2hrs). This session will cover information on the various parasites species causing human malaria and

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their geographical distribution, the life cycle of *Plasmodium* and correlation of the events in the life cycle with the pathogenesis and clinical features of malaria, the relationship between the clinical features and parasitaemia, the biological characteristics of the different *Plasmodium* species and the clinical features associated with malaria caused by each of them, and the meaning of the terms relapse, reinfection, periodicity, paroxysms, recrudescence with their implications. It will also include a description of uncomplicated and severe malaria, as well as asymptomatic malaria, including the occurrence, distribution, and public health importance of asymptomatic infections.

Unit 3: Management of uncomplicated malaria (4hrs). This lesson will encompass the clinical assessment of a suspected malaria case, the advantages of parasitological diagnosis and updates on the WHO recommendations on parasitological diagnosis (including the advantages and disadvantages of malaria microscopy and RDTs), the recommended guidelines for the treatment of uncomplicated malaria and supportive care, the role of follow-up for case management at all levels of health services, and an explanation on antimalarial drug resistance and the methods for assessment, prevention or reduction of drug resistance. This will also include a practical session for the demonstration of the competence and knowledge acquired.

Unit 4: Management of severe malaria (3hrs). This session will include the description of characteristics of severe malaria and the host-parasite

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interaction that contributes to the pathogenesis of severe malaria, the determinants of severe malaria and groups at high risk, the diagnosis of severe *falciparum* malaria and the emergency and supportive measures, and the recommended antimalarial chemotherapeutic regimen for severe malaria. This will also be followed by a practical session and demonstration of competences acquired on severe malaria management.

Unit 5: Malaria in pregnancy (2hrs). This session will comprise the description of the relationship between malaria and pregnancy, measures to prevent malaria during pregnancy, the justification, indications, advantages, recommended medicines and their dosage and timing, for intermittent preventive treatment (IPT), and the recommended therapeutic regimens for the treatment of uncomplicated and severe malaria during pregnancy. This will also be followed by a practical session on the management of malaria in pregnancy.

Unit 6: Control of malaria transmission and Elimination (2hrs). This will include the definition of malaria control and elimination and a recall of recommended measures for malaria control, recommended strategies to reduce or interrupt parasite transmission, the reasons why some *Plasmodium* infections are asymptomatic and their diagnosis, the rationale for treating asymptomatic *Plasmodium* infections and the recommended chemotherapeutic regimen for asymptomatic, uncomplicated and severe malaria in the context of elimination.

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ANNEXES

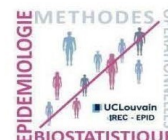
Annex 1: Questionnaires



Institut de recherche
expérimentale et clinique

Pôle de recherche EPID Epidémiologie et biostatistique

Responsable de Pôle : Prof Annie R ROBERT



KAP and acceptability of the use of Artemisia afra tea in emptying plasmodium reservoirs.

I. PATIENTS AND CARE GIVERS PERCEPTION AND USE OF TEAS FOR TREATING INFECTIONS.

A. Socio-demographic characteristics

1. Name of Hospital.....
3. Gender 1) ☐ Male 2) ☐ Female
4. Level of school attendance 1) ☐ Primary, 2) ☐ secondary, 3) ☐ High school 4) ☐ University
5. Residence
6. Household size----- (number of persons)
7. Profession
- Socioeconomic/ Income status 1) ☐ Easy to live with, 2) ☐ Enough to live with, 3) ☐ Just enough to live with, 4) ☐ Not enough to live with but you manage, 5) ☐ You can't manage to live

B. Use of herbal teas in the treatment of infections.

1. Have you taken herbal tea within the last 2 years to prevent infections or diseases like covid-19, STIs or any other infections, or another purposes like weight loss? 1) No 2) Yes
If No **answer Questions 2, 3, 16-18**
If Yes, **Go to question 4**
2. Can you take herbal tea to prevent infections or diseases like covid-19 or any other STIs infections, weight loss or other purposes? 1) No 2) Yes
If yes Go to Question 16
3. If No, why can you not take herbal teas?.....
4. What was it taken for?.....
5. Did someone prescribe it to you, or you prepare it by yourself?
6. Did you buy it, or it was offered to you, or you prepared it on your own?
7. What quantity, how frequent and for how long did you take it?
8. Did you take it warm or cold?
9. Were you comfortable taking it? Explain

-
10. Were you comfortable with the taste? _____
11. Did you finish the treatment, or you abandoned it halfway? Explain -----
12. Are other members of your household or family (both young and old) comfortable to take teas fortreatment? Explain.....
13. Did you ever take Artemisia afra tea? 1) No 2) Yes
- If No, Go to question 16*
14. What did you use it for?
15. Did it work for you?
16. Can you take teas to prevent treat infections in your body if prescribed in the hospital? Explain.
-
17. What is your impression about taking teas to treat infection if prescribed by a doctor, would you encourage it or discourage it? Explain.....
18. What do you think can make the use of medicinal teas more acceptable to you? -----
-

II. CONSULTANTS' KNOWLEDGE AND USE OF ARTEMISIA IN EMPTYING PLASMODIUM RESEVOIRS

A. Socio-demographic characteristics

1. Age range 1) ☐ 18- 29, 2) ☐ 30- 49, 3) ☐ 50- 65
2. Gender 2) ☐ Male 2) ☐ Female
3. Professional category 1) ☐ Nurse assistant 2) ☐ State registered Nurse 3) ☐ Degree nurse 4) ☐ General practitioner (Family doctor) 5) ☐ specialist in Medicine

B. Knowledge of asymptomatic malaria?

1. Have you ever heard about asymptomatic malaria (asymptomatic plasmodium carriers)? 1) No 2) Yes
If No, Go to section C
2. What is asymptomatic malaria
3. How can it be diagnosed
4. Have you ever come across patients with asymptomatic malaria? 1) No 2) Yes
If No, Go to question 7
5. Did you put them on treatment? 1) No 2) Yes
If No, Go to question 7
6. Which drug did you prescribe for them?
7. Is it necessary to treat asymptomatic plasmodium carriers? 1) No 2) Yes
Explain
8. Do you think there is any risk of not treating asymptomatic malaria carriers? 1) No 2) Yes
Yes Explain
9. Can malaria infection be transmitted from an asymptomatic plasmodium carrier to infect another healthy person? 1) No 2) Yes
Explain
- If No, Go to section C*
10. Can a person infected with malaria parasites from an asymptomatic person develop malaria symptoms? 1) ☐ No 2) Yes
Explain

C. Knowledge and acceptability to use Artemisia afra teas by HCWs

1. Do you know about Artemisia tea infusions? 1) ☐ No 2) ☐ Yes
If No, END.
2. What is it used for?
3. Have you ever heard about its use in malaria prevention or treatment? 1) ☐ No 2) ☐ Yes
4. Have you ever heard about its use in the treatment of asymptomatic malaria prevention or treatment?
☐ 1) ☐ No 2) ☐ Yes
5. What is your impression about the use of Artemisia tea infusions in preventing or treating acute malaria patients?
6. What is your impression about the use of Artemisia tea infusions in treating asymptomatic plasmodium carriers?
7. Can you prescribe or recommend artemisia tea infusions in treating asymptomatic plasmodium carriers?
☐ 1) ☐ No 2) ☐ Yes
Explain
8. What do you think can be the possible challenges in using artemisia tea infusions to treat asymptomatic plasmodium carriers?
9. What are your observations and comments about the possible use of artemisia tea infusions in treating asymptomatic plasmodium carriers?

Annex 2: Ethical approval

**UCLouvain**
Université Catholique de Louvain
Faculté de médecine

**SAINT-LUC**
UCL BRUXELLES
Cliniques universitaires

Comité d'Éthique Hospitalo-Facultaire
Brussels, 08 November 2021

To the principal investigator:
Mr. Abenwie SUH NCHANG
EPID
Clos Chapelle-aux-champs 30/B1.30.13
1200 Woluwe-Saint-Lambert

Cc: Prof Annie ROBERT
EPID
Clos Chapelle-aux-champs 30/B1.30.13
1200 Woluwe-Saint-Lambert

POSITIVE ADVISORY OPINION

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.

Dear colleague,

The Ethics Committee Hospitalo-Facultaire Saint-Luc - UCLouvain received and examined the request for an opinion for the research project mentioned above.

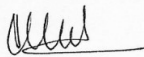
This project took place in Cameroon and Rwanda and must be approved by the local Ethics Authorities.

As the study is not taking place in Belgium, the opinion of the Ethics Committee doesn't concern the practical aspects of manufacturing, storage and administration of the studies product.

UCLouvain has no responsibility in the realisation of this study; we receive confirmation that the product (Artemisia Afra Tea) characteristics will be performed by ULg Liège, and that financial aspects are done by ULB.

The Ethics Committee don't have any objection. Our opinion is positive.

We wish to assure you, dear Colleague, of our highest consideration.


Pharm. P. de PIERPONT
EC Member


Prof. J.-M. MALOTEAUX
Chairman

Promenade de l'Alma 51 bte B1.43.03 - 1200 Bruxelles
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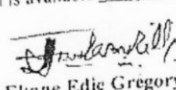
Notice of Ethical Approval

Application number: 1591-01
Principal Investigator: **Prof. Stephen M. Ghogomu**
Study Title: **Emptying plasmodium reservoir to accelerate malaria elimination in high transmission settings: case study in Cameroon.**

Application Type: **Initial**
Sponsor: **Student**
Review Type: **Normal**
Date of Approval: **2nd January 2022**
Expiration Date: **2nd January 2023**

Principal Investigator's responsibilities:

1. The study must be conducted in strict accordance with the protocol approved by the Board
2. Changes to the protocol or its related consent documents must be approved by the Board before implementation
3. Adverse events or unanticipated problems must be reported promptly to the Board
4. Participants must receive a copy of the consent document, if appropriate
5. The Principal Investigator is responsible for the on-going conduct of the study. The study must be implemented according to national and international guidelines for the ethical conduct of research on humans. He must collaborate with the IRB's monitoring of the study's implementation.
6. Any future correspondence must include the application number, and the PI's name in the subject line.
7. A renewal application or project closure report must be submitted at least one month prior to the expiration date indicated above. These must be done using the FHSIRB's secretariat AND an electronic copy sent to: irbfs@email.com, making sure to reference the application number indicated above. This form is available at <http://www.healthresearchweb.org/cameroon/institution2130>


Prof. Halle Ekane Edie Gregory
Secretary, Institutional Review Board
Faculty of Health Sciences University of Buea



REPUBLIQUE DU CAMEROUN Paix -Travail- Patrie ----- MINISTRE DE LA SANTE PUBLIQUE ----- SECRETARIAT GENERAL ----- COMITE NATIONAL D'ETHIQUE DE LA RECHERCHE POUR LA SANTE HUMAINE ----- SECRETARIAT TECHNIQUE -----		REPUBLIC OF CAMEROON Peace -Work- Fatherland ----- MINISTRY OF PUBLIC HEALTH ----- SECRETARIAT GENERAL ----- NATIONAL ETHICAL COMMITTEE FOR RESEARCH ON HUMAN HEALTH ----- TECHNICAL SECRETARIAT -----
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N° 2023/10/1603/CE/CNERSH/SP Yaoundé, le 30 OCT 2023

CLAIRANCE ETHIQUE

Le Comité National d’Ethique de la Recherche pour la Santé Humaine (CNERSH), en sa session extraordinaire du 27 octobre 2023, a examiné le projet de recherche intitulé : «**Contributions in emptying plasmodium reservoirs to accelerate malaria elimination in high transmission settings using Artemisia afra infusion : case study in Cameroon**» soumis par le Professeur Stephen M. GHOGOMU, Investigateur Principal, Université de Buéa.

Le projet est d’un grand intérêt scientifique et social. L’objectif général de cette étude est de faire face à l’émergence de la résistance à l’ACT chez *P. falciparum* dans les régions de l’Ouest, du Sud-Ouest, du Littoral et du Centre - Cameroun. La procédure de l’étude est bien documentée et claire. Cette étude se déroulera dans quatre régions du Cameroun à savoir l’Ouest, le Sud-Ouest, le Littoral et le Centre. Les risques liés à l’étude sont précisés ainsi que les mesures pour les éviter et les minimiser. La notice d’information et le formulaire de consentement éclairé, en français et en anglais, sont bien élaborés et simples à comprendre. Les mesures prises pour garantir la confidentialité des données collectées sont présentes dans le document. Les CVs des Investigateurs les décrivent comme des personnes compétentes, capables de mener à bien cette étude. Pour toutes ces raisons, le Comité National d’Ethique approuve pour une durée d’un an, la mise en œuvre de la présente version du protocole.

Les Investigateurs sont responsables du respect scrupuleux du protocole approuvé et ne devraient y apporter aucun amendement aussi mineur soit-il, sans avis favorable du CNERSH. Les investigateurs sont appelés à collaborer pour toute descente du CNERSH pour le suivi de la mise en œuvre du protocole approuvé. Le rapport final du projet devra être soumis au CNERSH et aux autorités sanitaires du Cameroun.

La présente clairance peut être retirée en cas de non-respect de la réglementation en vigueur et des recommandations susmentionnées.

En foi de quoi, la présente clairance éthique est délivrée pour servir et valoir ce que de droit.

Ampliations

MINSANTE


 Pr MBACHAM-Willfred
 Secrétaire Général

N.B : cette clairance éthique ne vous dispense pas de l’autorisation administrative de recherche (AAR), exigée pour mener cette étude sur le territoire camerounais. Cette dernière vous sera délivrée par le Ministère de la Santé Publique.

- N° d’enregistrement : IORG0007861-IRB00009439-FWA00016054 - setcominae@gmail.com
 - Arrêté N° 0977/A/MSP/SESP/SG/DROS du 18 avril 2012 portant création, organisation et fonctionnement des Comités d’Ethique de la Recherche pour la Santé Humaine au sein des structures relevant du ministère en charge de la santé publique.

