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Consumption of *Artemisia annua* tea and Resistance to Artemisinin in Africa: Scoping Review.

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Abstract

Background: The increasing popularity of *Artemisia annua* tea in Africa as an antimalarial remedy has raised concerns about its potential role in the development of artemisinin resistance in Plasmodium falciparum, which could undermine global malaria control efforts.

Methods: This literature review analyzed publications sourced from PubMed, Web of Science, Google Scholar, and other databases. Studies included clinical trials, laboratory investigations, and surveillance reports focused on *Artemisia annua* tea consumption and resistance patterns.

Results: Data indicate a significant rise in herbal tea use in malaria-endemic regions, particularly where access to standard therapies is limited. Some studies suggest a possible link between non-standardized use of Artemisia annua tea and early markers of artemisinin resistance, though direct causality remains unconfirmed and findings are inconsistent.

Conclusions: The uncontrolled consumption of *Artemisia annua* tea could contribute to artemisinin resistance emergence in Africa, highlighting the need for regulated use, further research, and continuous surveillance to protect the effectiveness of antimalarial treatments.

Keywords: *Artemisia annua*, Herbal tea, Artemisinin resistance, Malaria, Plasmodium falciparum, Africa, Drug resistance, Antimalarial agents, Traditional Medicine, Public health, Pharmacovigilance.

1. Introduction

The investigation into artemisinin resistance, which is potentially associated with the consumption of *Artemisia annua* teas in Africa, necessitates a detailed analysis of the interactions between the plant's chemical constituents, the parasite *Plasmodium falciparum*, and the human body **[1].** *P. falciparum* is a parasite transmitted by the bite of an infected female *Anopheles* mosquito and that it causes the most severe and fatal form of malaria, especially in sub-Saharan Africa. Artemisinin, a component in *Artemisia annua* tea, is crucial in combating malaria, but *Artemisia annua* also contains flavonoids, terpenes, coumarins, and phenolic acids, which may alter its efficacy **[2] [3].** Exposure to these compounds through the consumption of herbal tea could subtly induce artemisinin resistance via mechanisms that remain unclear. *Artemisia annua*, which is valued for its artemisinin content, is traditionally used to alleviate various conditions, and its antimalarial properties have been widely studied [4] [5]. Research has indicated its potential in treating malaria, particularly when conventional treatments are insufficient [6]. The use of medicinal plants such as *Artemisia* sp. for treating ailments is a long-standing practice in Africa, often based on empirical observations rather than rigorous scientific evidence [7]. Given its proven efficacy and ability to delay resistance, the World Health Organization strongly advises using artemisinin-based combination therapies as a primary malaria treatment [**8][9].** The complex chemical composition of *Artemisia annua*, along with diverse traditional practices, complicates the assessment of risks related to herbal tea consumption and the emergence of resistance. Therefore, comprehensive research is vital for understanding these interactions and informing public health strategies. The widespread use of *Artemisia* sp. infusions in sub-Saharan Africa raises concerns about its potential to select for resistant parasites, particularly due to the lack of standardization, inconsistent monitoring of this traditional approach, and limited in vivo efficacy data [10].

Understanding the development of resistance requires considering tumor mutation rates that facilitate rapid adaptation to changes, potentially diminishing the effectiveness of established drugs **[11**]. Multidrug-resistant malaria caused by *Plasmodium falciparum* has severely limited treatment options since the early 2000s **[12]**. Consequently, focus has shifted to artemisinin-based combination therapies; however, decreased parasite clearance times following Artemisinin Combination Therapies [ACT] treatment have sparked concerns about potential artemisinin resistance **[13]**. Several investigations have highlighted the capacity of medicinal plants to synthesize innovative and effective antimalarial agents **[14]** These plants have played a vital role in discovering new therapeutic compounds, underscoring their importance in pharmacological advancements and healthcare practices. The rise of resistance to antimalarial drugs, coupled with the expense and counterfeiting of medications, has created significant challenges in malaria treatment and control, necessitating the search for

alternative therapeutic agents with novel mechanisms of action **[15]** In light of these issues, there is a growing emphasis on using both conventional and herbal treatments for malaria **[16]**.

The current first-line treatment for malaria involves artemisinin-based combination therapy, but this approach is threatened by the emergence of artemisinin resistance **[17].** This underscores the need to explore alternative treatments, including those derived from traditional medicine **[18]** The utilization of herbal remedies by communities in malaria-affected regions highlights the critical importance of investigating and validating these remedies' antimalarial efficacy **[19]**. Numerous research endeavors in Africa have focused on plant sources to broaden the array of antimalarial chemotherapeutic agents available, motivated by the historical application of these plant-based substances in African traditional medicine to treat malaria and fever **[20].** The identification of novel antimalarial compounds from plants used in traditional medicine holds promise, especially given the increasing resistance to existing drugs **[21]** The emergence of resistance to antimalarial drugs poses a significant challenge, necessitating the development of new therapeutic strategies to combat malaria.

Artemisinin resistance is frequently associated with mutations in the Kelch13 gene in *Plasmodium falciparum*, which leads to a slowing of parasite clearance after treatment **[22]** These mutations decrease the parasite's sensitivity to artemisinin **[23]** Other genes, such as pfmdr1, pfap2mu, and crt, may also play a role in this resistance **[10].** These genes influence drug metabolism and stress response, and their mutations can reduce the effectiveness of artemisinin.

The proven effectiveness of artemisinin derivatives has promoted the use of *Artemisia annua* in the form of herbal tea in the fight against malaria**[4].**Considering its antimalarial properties, its integration into combination therapies, and the importance of its non-artemisinin components, including flavonoids, terpenes, coumarins, and phenolic acids [2] a more in-depth investigation of the pharmacokinetic and pharmacodynamic characteristics of *Artemisia annua* for malaria prophylaxis is fully justified **[1].**

It is imperative to examine the molecular mechanisms underlying artemisinin resistance to maintain the effectiveness of artemisinin-based combination therapies, which are essential in the fight against malaria, particularly in Africa, which has the highest prevalence of this disease. The development of nano-based artemisinin formulations, used in combination with conventional chemotherapies, represents a promising approach to improve drug bioavailability and optimize targeted administration while strengthening immunotherapeutic interventions **[24].** Continued emphasis should be placed on understanding how the consumption of *Artemisia annua* tea, with its complex chemical composition, could promote the development of resistance to artemisinin in *Plasmodium falciparum*.

2. Malaria and Its Impact in Africa

Malaria, a potentially fatal disease transmitted by the bites of infected female *Anopheles* mosquitoes, remains a major public health challenge, particularly in sub-Saharan Africa, including the Democratic Republic of Congo **[25]**. This disease is responsible for the deaths of approximately 430,000 African children each year, underscoring its considerable impact on vulnerable populations [25]. Artemisinin-based treatments, recognized as the most effective therapies against malaria, have contributed to reducing mortality associated with this disease since the beginning of the century [26]. Nevertheless, the emergence of artemisinin resistance in Southeast Asia, as well as indications of decreased parasite sensitivity to treatments in Africa, raises concerns about a possible resurgence of therapeutic failure [27]. The World Health Organization has advocated the use of these treatments as a first-line option against *Plasmodium falciparum* malaria since 2006, which has helped alleviate the burden of malaria in many regions of Africa [28] Despite these advances, recent progress is being compromised by resistance to antimalarial drugs [29] Drug resistance is a significant threat to malaria control as it can lead to treatment failures, increased morbidity and mortality, and increased spread of the disease [30] It is therefore imperative to carefully monitor this resistance and implement strategies to prevent and manage it. It is crucial to maintain constant vigilance and rigorously evaluate the effectiveness of artemisinin-based treatments and other antimalarial drugs in order to guarantee their continued effectiveness against malaria. Studies have shown that artemisinin-based combination therapies retain high efficacy outside of Southeast Asia [31] and artemisinin remains an effective antimalarial against strains of *Plasmodium falciparum* [32].

The economic burden of malaria is considerable, with significant costs associated with treatment, prevention, and lost productivity, placing a strain on already limited resources, particularly in sub-Saharan Africa [33] The disease disproportionately affects pregnant women and children under five years of age, leading to severe anemia, developmental delays, and increased susceptibility to other infections [34].

The combination of two antimalarials with distinct mechanisms of action helps reduce the risk of developing drug resistance [35]. The appropriate and consistent application of existing interventions is essential to decreasing the prevalence of malaria [36].

The main strategies to control malaria globally include vector control through long-lasting insecticide-treated bed nets, early diagnosis and treatment with artemisinin-based combination therapies, and chemoprevention in pregnant women and young children [37].

In Africa, the Democratic Republic of Congo is particularly affected by malaria, with a high annual incidence of cases and deaths [38]. This disease is endemic in most of the country, with high transmission throughout the year. It disproportionately affects children under five years of age and pregnant women, who are most likely to experience serious complications [39] Malaria also exacerbates poverty due to subsequent health expenditures and the resulting loss of productivity. Malaria control strategies in the DRC

include the distribution of insecticide-treated mosquito nets, indoor insecticide spraying, and the administration of artemisinin-based combination therapies. Despite these initiatives, malaria remains a major public health problem in the DRC. The situation is particularly critical in Lomami Province, where malaria is the leading cause of morbidity and mortality [40] Several factors contribute to this high prevalence, including poverty, poor sanitation conditions, and limited access to health services. In addition, the incidence of malaria in the DRC increased from 309 cases per 1000 inhabitants in 2011 to 514 cases per 1000 inhabitants in 2016 [41], highlighting the need to intensify efforts to control the disease in this country. The Democratic Republic of Congo, Nigeria, Uganda, Mozambique, Angola, and Burkina Faso account for more than half of malaria cases worldwide [38] This high concentration in sub-Saharan Africa reflects the slow progress made in eliminating malaria due to factors such as the lack of adequate funding mechanisms, political instability, weak health systems, humanitarian crises, environmental factors, and behavioral changes [39]. These challenges contribute to a high number of malaria cases and deaths, underscoring the urgent need to intensify efforts to combat this disease in these countries [39], [38 [41]) [40].

In 2023, there were approximately 263 million cases of malaria worldwide, resulting in 597,000 deaths, predominantly in sub-Saharan Africa [42]. Children under the age of five years constitute approximately 80% of all malaria-related fatalities in this region [43]. Malaria places a substantial burden on the economies of highly affected countries, leading to GDP reductions of up to 1.3%, significant public health expenditure, and elevated hospital admission rates [44] Despite considerable efforts to combat malaria, the disease remains a major public health challenge in many countries, particularly in sub-Saharan Africa [45]. Africa bore the largest proportion of the global malaria burden, with approximately 213 million cases and 380,000 deaths [46]. Cameroon experienced an increase of 131,000 malaria cases compared to the previous year [47] Diminishing malaria incidence is associated with an increase of approximately 0.3% in per capita income levels [44]. In Ethiopia, malaria remains a significant public health concern, affecting approximately 70% of the country, with over 60% of the population at risk of infection [48]; [49]. In 2017/18, over 1.2 million cases and 158 deaths were reported in Ethiopia [49]. Mortality due to malaria is more prevalent among children under five in sub-Saharan Africa [50]. The prevalence of malaria in pregnant women is also a major concern, as it can lead to complications for both the mother and fetus [51].

3. The Importance of Artemisinin in the Treatment of Malaria

Artemisinin, a compound extracted from a plant *Artemisia annua* traditionally used in Chinese medicine, is a remarkably effective antimalarial drug that has significantly improved the management of malaria [52],[53]. Artemisinin-based combination therapies combine artemisinin with another antimalarial drug, such as lumefantrine or amodiaquine. ACTs work by rapidly clearing malaria parasites from the patient's bloodstream, which prevents disease progression and reduces the risk of mortality [48] The World Health Organization recommends the use of ACTs as first-line treatment for uncomplicated malaria caused by *Plasmodium falciparum*, the most virulent malaria parasite[52] This therapeutic approach is effective against drug-resistant infections, is fast-acting, is generally safe and well-tolerated, and appears to reduce transmission by inactivating gametocytes [53]. The short half-life **1 à 3 hours** of artemisinin ensures the elimination of the majority of parasites, while the partner drug eradicates the remaining parasites. The main advantage of ACTs lies in their ability to rapidly reduce parasite load and prevent progression to severe malaria [48] Although generally well tolerated, ACTs can induce side effects such as nausea, vomiting, and headaches. In Africa, ACTs have played a key role in alleviating the burden of malaria, particularly in children under five years and pregnant women. Their wide availability in the majority of malaria-endemic countries has led to a substantial reduction in malaria-related morbidity and mortality. Despite the proven efficacy of ACTs, their cost can be a barrier to access in resource-limited settings.

The emergence of resistance to artemisinin and its partner drugs constitutes a significant impediment to global malaria control initiatives [54] Given that prompt and effective antimalarial treatment is crucial for preventing severe malaria and mortality, artemisinin-based combination therapies are the cornerstone of therapeutic approaches in malaria-endemic regions [55] ACTs serve as the primary treatment for uncomplicated malaria, especially in areas with widespread drug resistance.

The administration of artemisinin-based combination therapies has been instrumental in reducing the incidence of malaria-related morbidity and mortality [56]. The effectiveness of artemisinin is largely due to its ability to rapidly reduce parasite density early in treatment, while the partner drug eradicates the remaining parasites [57] Indeed, artemisinin-type compounds demonstrate their effectiveness against parasites resistant to many drugs and allow for a faster reduction in parasitemia than other known antimalarial compounds [58] The rapid action of artemisinin in eliminating parasites reduces the risk of serious complications and death. However, the development and spread of resistance to artemisinin pose a significant threat to malaria control efforts, which could compromise the progress made in recent decades [59]. The first cases of artemisinin resistance were reported in the mid-2000s in Southeast Asia, particularly in Cambodia, and resistance has since spread to other regions, such as Thailand, Myanmar, and Vietnam [60]

[59]. The identification of artemisinin resistance is generally based on the delayed elimination of parasites after treatment with artemisinin-based combination therapies [60].

Artemisinin resistance is a complex phenomenon influenced by various factors, including genetic mutations in the malaria parasite, drug exposure, and patient-related factors. It is essential to delve deeper into the molecular mechanisms underlying artemisinin resistance to develop effective control strategies. However, although several specific parasite targets for artemisinins have been described, it is likely that the parasiticidal effect of artemisinins is mediated through the alkylation of multiple cellular proteins and lipids [61] Resistance to artemisinin differs from resistance to other conventional antimalarial drugs, such as amodiaquine [131].

4. The Artemisinin Resistance Problem

Drug resistance is a major challenge in the fight against malaria [48]. Initially detected in Southeast Asia in the early 2000s, artemisinin resistance has since spread to other regions of the world, including Africa [62]. It manifests as a delay in parasite clearance after artemisinin-based treatment [55]. More specifically, antimalarial resistance is defined as the ability of a parasite strain to survive or proliferate despite the administration of a drug at doses equal to or higher than those usually recommended while remaining within the subject's tolerance limits [63] This resistance is associated with mutations in the kelch13 gene of the malaria parasite. Resistant strains exhibit a delay in parasite clearance, thereby increasing the risk of treatment failure and the spread of resistance. Artemisinin resistance is therefore characterized by a slowed elimination of red blood cells parasitized by *P. falciparum* from peripheral circulation [53] The emergence of this resistance poses a serious threat to malaria control efforts, as it can lead to treatment failure, increased morbidity and mortality, and increased transmission [64],[65]. The definition of artemisinin resistance encompasses various criteria, ranging from the persistence of parasites on the third day after drug administration [66] to an increase in the parasite elimination half-life, a reduction in the elimination rate, and, ultimately, the failure of ACT. It is therefore crucial to identify resistant phenotypes and continuously monitor artemisinin resistance. Artemisinin resistance has been confirmed in Uganda and Rwanda [67] this could potentially compromise the effectiveness of ACTs, which are the cornerstone of malaria treatment.

Mutations in the kelch13 gene have been shown to contribute to artemisinin resistance [68] [23], leading to delayed clearance of malaria parasites after artemisinin treatment [69]. The C580Y mutation of K13 is the most prevalent mutation linked to resistance, accounting for 63.1% of all documented K13 mutations [70] In addition, the R561H, A675V, A588G, and A578S mutations have been observed in patients from Rwanda and Tanzania [71] Uganda [72], Angola, and the DRC, respectively. The Kagera region in northwestern Tanzania was recently identified as one of the few places in sub-Saharan Africa where partial artemisinin resistance has been confirmed

[73] Although artemisinin resistance alone does not lead to treatment failure and has only a slight clinical effect, namely, a delay in parasite clearance in patients treated with an artemisinin-based combination therapy, it leads to worrying consequences when associated with resistance to the partner drug used in ACT. A study conducted in Cameroon did not detect Pfk13 amino acid polymorphisms associated with artemisinin resistance [74 It is essential to continuously monitor the spread of K13 mutations to understand the evolution of artemisinin resistance and to implement appropriate control strategies [75] In specific terms, mutations in the PfKelch13 gene have been found to play a role in artemisinin resistance, resulting in delayed clearance of malaria parasites after artemisinin administration. The C580Y mutation of K13 was observed most frequently, representing a significant proportion of the K13 mutations noted. Other studies have highlighted the presence of mutations such as R561H in patients from Rwanda and Tanzania [71], A675V in patients from Uganda [72], A588G patients from in Angola, and A578S in patients from the DRC. Notably, the Kagera region, located in northwestern Tanzania, has been identified as an area in which partial resistance to artemisinin has been confirmed. A study in Cameroon revealed no Pfk13 amino acid polymorphisms associated with artemisinin resistance [74]. Therefore, continuous monitoring of the spread of K13 mutations is essential to fully understanding the evolution of artemisinin resistance and implementing appropriate mitigation strategies [75]

These mutations appear to be spreading in specific geographic areas [76]. The spread of artemisinin-resistant parasites could have disastrous health consequences, compromising the effectiveness of artemisinin-based combination therapy, which is currently considered the most effective treatment against malaria [77] Therefore, it is imperative to monitor and limit the spread of artemisinin resistance in order to maintain the effectiveness of antimalarial treatments and protect vulnerable populations against malaria. Additional studies conducted in malaria-endemic countries are needed to validate potential genetic markers for monitoring African parasite populations [78] The identification of K13 mutations in Africa is of paramount importance, as these mutations are associated with varying degrees of artemisinin resistance [69].

Mutations in the PfKelch13 gene, which encodes a protein involved in the response to oxidative stress, are correlated with artemisinin resistance. Several mutations in this gene have been identified in parasite populations resistant to artemisinin, including C580Y, R539T, and Y493H. These mutations appear to reduce the sensitivity of parasites to artemisinin, leading to a delay in the elimination of parasites from the bloodstream. In South Asia, fifty Pf-K13 mutations have been investigated for artemisinin resistance, of which nine have been validated and eleven are considered potential contributors to resistance [79].

Single-nucleotide polymorphisms in exon 2 of the pfcrt gene, particularly at codons 74, 75, and 76, have been associated with *P. falciparum* resistance to chloroquine and amodiaquine [80].

Factors such as geographical location and antimalarial drug usage may be associated with the emergence of new kelch13 mutations [81]. The spread of artemisinin resistance poses a significant threat to malaria control efforts, potentially leading to treatment failure, increased morbidity and mortality, and heightened disease transmission. The recent emergence of the Pfkelch13 R561H mutation in Rwanda, coupled with delayed parasite clearance, highlights the need for drug policy interventions to delay the fixation and slow the spread of this mutation [82]. Therefore, rigorous monitoring of drug efficacy is essential to ensure that ACTs remain effective against malaria. One study indicated a concentration–effect relationship in Wang Pha but not in Pailin, suggesting a possible slight shift to the right along the dose–response curve at the Thai–Burmese border [83].

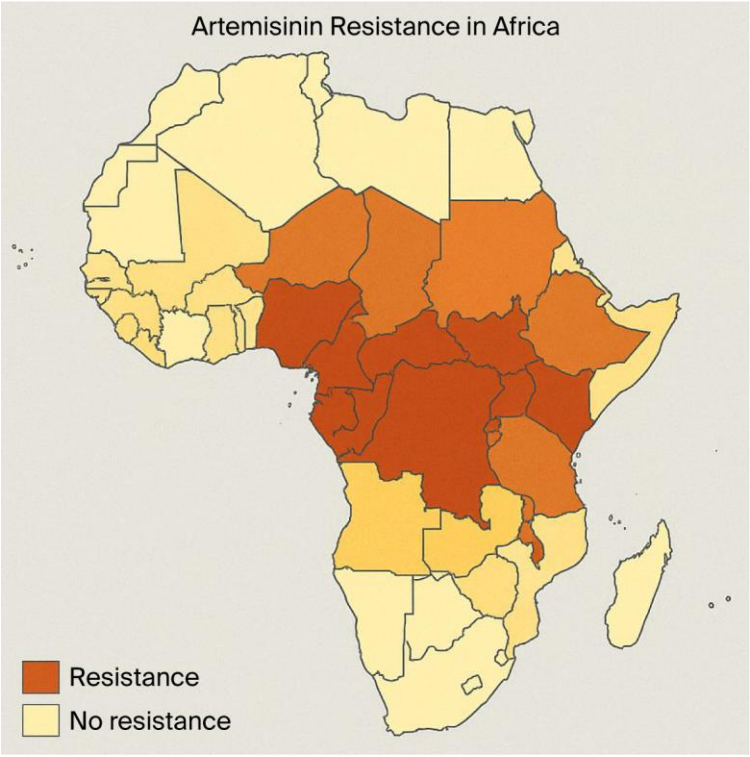


Figure 1 shows the distribution of artemisinin resistance in Africa, with the most affected areas in Central and East Africa. Data of this type generally come from WHO reports, the *Malaria Journal*, and studies on K13 mutations ([31].

5. Objectives of this Review

This review primarily aims to examine the potential link between the consumption of *Artemisia annua* tea and the development of artemisinin resistance in malaria parasites. Specifically, it seeks to determine whether the widespread use of *Artemisia annua* tea, as an alternative or complementary treatment for malaria, contributes to the spread of artemisinin resistance. This

study examines the available scientific data regarding the antimalarial activity of *Artemisia annua*, the risk of developing artemisinin resistance following exposure to this plant, and the effect of consuming *Artemisia annua* tea on malaria treatment outcomes. The results of this analysis will enhance our understanding of the potential risks and benefits associated with consuming *Artemisia annua* tea in the prevention and treatment of malaria.

6. Review Methodology: Literature Review

A thorough literature review was conducted using various electronic databases, including PubMed, Scopus, Web of Science, and the Cochrane Library, with the aim of identifying relevant studies. The search strategy involved a combination of keywords and MeSH terms related to *Artemisia annua*, tea, artemisinin, resistance, malaria, and *Plasmodium falciparum*, including **"*Artemisia annua*", " tea", "artemisinin", "resistance", "malaria", and "*Plasmodium falciparum*"**.

7. Study Inclusion and Exclusion Criteria

Studies were included if they met the following criteria: evaluating the antimalarial activity of *Artemisia annua* or its constituents; studying the development of resistance to artemisinin following exposure to *Artemisia annua* or its constituents; evaluating the impact of *Artemisia annua* tea consumption on malaria treatment outcomes; being conducted in vitro, in vivo, or in humans; and being published in English or French.

8. Data Extraction and Synthesis

Relevant data were extracted from the included studies using a standardized data extraction form. The extracted data included study characteristics, outcomes, measures of antimalarial activity, the development of artemisinin resistance, and the impact of tea consumption on malaria treatment outcomes. The extracted data were synthesized using a narrative approach. The bibliographic data analysis tool VOSviewer version 1.6.20. [VOSviewer software, including version 1.6.20, is developed and maintained by Nees Jan van Eck and Ludo Waltman at the CWTS (Centre for Science and Technology Studies) of Leiden University, located in Leiden, South Holland, Netherlands.] was used to assess publication trends and subject distribution in the search results.

9. Quality Assessment

The methodological quality of the included studies was assessed using appropriate quality assessment tools, including the Newcastle Ottawa Scale [84] for observational studies, and the Cochrane Risk of Bias tool (V.2.039) [85] for randomized controlled trials.

The findings of this study will be communicated through publications, such as this one, in peer-reviewed journals and presentations at specialized conferences.

This review aims to offer a detailed analysis of the existing scientific evidence regarding the potential correlation between the consumption of tea and the development of artemisinin resistance. The findings of this review will contribute to a better understanding of the possible risks and benefits associated with consuming *Artemisia annua* tea in the prevention and treatment of malaria and will inform future research recommendations and policies in this area.

10. Life Cycle of *Plasmodium falciparum*

Plasmodium falciparum, the pathogen responsible for the most severe form of malaria in humans, has a complex life cycle that involves both the mosquito and the human [86]. This cycle includes various stages of development and reproduction [86] It begins when an infected female *Anopheles* mosquito bites a human, introducing sporozoites into the bloodstream [17]. These sporozoites then migrate to the liver, where they infect hepatocytes and multiply asexually, transforming into merozoites [87]. This hepatic phase is asymptomatic in humans and lasts from a few days to a few weeks. Subsequently, the merozoites leave the liver cells and infect erythrocytes, initiating the erythrocytic phase [88]. Once inside the red blood cells, the merozoites multiply asexually again, developing into trophozoites and then into schizonts, which produce more merozoites. Finally, the infected red blood cells undergo lysis, releasing other merozoites that infect new red blood cells and enabling the parasite to multiply rapidly in the blood [89]. A fraction of the merozoites differentiate into male and female gametocytes, which are the sexual forms of the parasite. These gametocytes are ingested by another female *Anopheles* mosquito when it bites an infected person. Once inside the mosquito, the gametocytes develop into mature gametes, which fuse to form a zygote.

Subsequently, the zygote metamorphoses into an oocyst, which continues its maturation on the intestinal wall of the mosquito. Within the oocyst, the parasite proliferates and generates sporozoites. The oocyst eventually ruptures, releasing the sporozoites, which then migrate to the mosquito's salivary glands. When an infected mosquito bites a new human host, it transmits these sporozoites, thus initiating a new infectious cycle. The migration of the protozoan parasite *Plasmodium* through the mosquito represents a complex and delicate process, the outcome of which determines the success of malaria transmission [90]. The mosquito is not simply the vector of *Plasmodium* but constitutes, with regard to the parasite's life cycle, its definitive host. It is where the parasite undergoes sexual development, leading to the colonization of the hemocoel and, ultimately, the salivary glands of the mosquito by the sporozoites [91] [92].

A thorough understanding of this complex life cycle is essential for developing malaria control strategies that target the various stages of the parasite in humans and mosquitoes [93]. For example, developing drugs that target the hepatic phase could prevent infection before it even occurs, while

drugs that target the erythrocytic phase could reduce the parasite load and mitigate the pathological manifestation of the disease.

Conventional differentiation between the trophozoite and schizont stages relies on morphological criteria, with the schizont typically identified by the presence of multiple nuclei, that is, more than two, within the same cell [94].

Once ingested by the mosquito, the gametocytes transform into zygotes through fertilization, which then develop into oocysts. These oocysts develop outside the intestinal wall and produce thousands of sporozoites, which migrate to the salivary glands, thus completing the parasite's life cycle [92]. This process includes crucial stages at which the parasite is vulnerable, justifying the development of antimalarial strategies, including vaccines and drugs, targeting specific phases of the life cycle [95]. The transmission of the parasite from the mosquito to humans depends on various factors that can influence the mosquito's infectivity [96]. Mosquitoes feeding directly on the skin are more likely to become infected than those fed through a membrane with blood from the same donor [97]. Additionally, the evolutionary adaptations of mosquitoes to hematophagy, such as their ability to efficiently digest blood and eliminate toxic by-products, play a vital role in the transmission of pathogens [98]. The parasitic cycle includes different stages of the cell cycle [94]. A thorough understanding of the mechanisms of parasite transmission and the role of vectors allows public health professionals to more effectively design and manage control programs that are tailored to specific problems [99]. The global elimination of malaria requires an in-depth knowledge of the biology of the parasite, the vector, and the host, as well as the complex interactions between these factors [100]. Furthermore, the parasite expresses approximately 5300 proteins in a stage-specific manner [101]. Despite considerable efforts deployed in the fight against malaria, it remains a major public health problem, with nearly half of the world's population at risk of infection [102]. The implementation of effective control measures, combined with continuous research efforts, is essential to reducing the burden of malaria and progressing towards its eventual elimination [103].

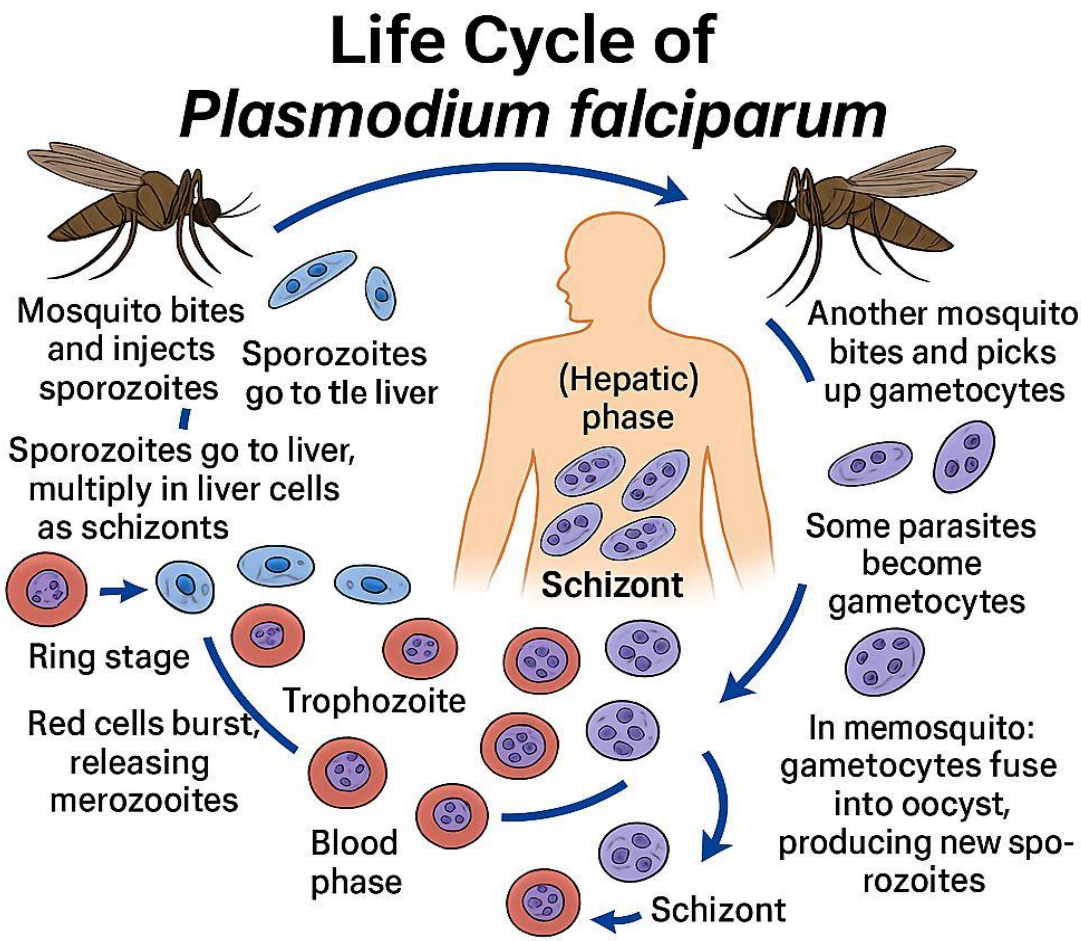


Figure 2. Life cycle of *Plasmodium falciparum* including key stages such as sporozoite, trophozoite, and schizont.

The life cycle of *Plasmodium falciparum* involves several stages in both mosquito and human hosts (Figure 2).[105]

1. Sporozoite injection : An infected *Anopheles* mosquito injects sporozoites into a human's bloodstream during a bite.

2. **Liver stage**: Sporozoites travel rapidly to the liver, invade liver cells, and start multiplying.

3. **Liver schizont**: Inside liver cells, sporozoites become liver schizonts, dividing many times to produce thousands of merozoites.

4. Merozoite release: Liver cells burst, releasing merozoites into the bloodstream.

5. Erythrocytic (blood) stage: Merozoites invade red blood cells and become ring-shaped trophozoites.

6. Trophozoite and schizont in blood: Trophozoites grow and multiply further inside red blood cells, becoming blood schizonts.

7. Merozoite release (blood phase): Blood schizonts burst, releasing new merozoites that infect more red blood cells, causing the symptoms of malaria.

8. Gametocyte formation: Some parasites in red blood cells morph into male or female gametocytes (sexual forms).

9. Mosquito ingestion: When another mosquito bites, it ingests the gametocytes with the blood.

10. Mosquito phase: In the mosquito's gut, gametocytes mature into gametes, fuse to become zygotes, and form ookinetes.

11. Oocyst and sporozoite release: Ookinetes invade the mosquito's gut wall, become oocysts, which grow and release fresh sporozoites.

12. Reinfection: Sporozoites move to the mosquito's salivary glands, ready to start the cycle again with another bite. [104] [106].

11. Elucidation of Pathogenicity, Transmission Modalities, and Resistance Mechanisms

Plasmodium falciparum employs complex mechanisms to induce disease, ensure transmission, and evade pharmacological interventions, posing a significant global health challenge. This parasite utilizes virulence factors that facilitate adherence to endothelial cells, evasion of splenic clearance, and modulation of the host's immune response. The adhesion of *P. falciparum*-infected erythrocytes to the vascular endothelium, known as sequestration, is mediated by the PfEMP1 protein family expressed on the surface of infected erythrocytes. These PfEMP1 proteins bind to receptors on endothelial cells, including ICAM-1, VCAM-1, and chondroitin sulfate A, leading to the accumulation of infected erythrocytes in the capillaries of vital organs such as the brain, lungs, and placenta. Sequestration contributes to the pathogenesis of severe malaria by disrupting microcirculation, inducing inflammation, and causing organ dysfunction [107]. Furthermore, *P. falciparum* modulates the host's immune response to promote its survival and transmission, notably by manipulating host attractiveness and mosquito susceptibility to infection [108]. The parasite suppresses innate and adaptive immune responses, inhibits T cell activation, and induces the production of immunosuppressive cytokines, establishing a permissive environment for its persistence and replication. Additionally, the high antigenic variability of *P. falciparum*, resulting from mechanisms such as var gene switching, enables it to evade antibody recognition and sustain chronic infections [109]. Given the complexity of its biology and interaction with the host, developing effective vaccines and therapies remains a significant challenge [109]. The administration of effective antimalarial drugs is crucial to reducing the emergence of resistant strains [110]. The selective pressure exerted by

antimalarials has led to the emergence and spread of *P. falciparum* strains resistant to multiple drugs, including chloroquine, sulfadoxine–pyrimethamine, and artemisinin [111]. The emergence of resistance to artemisinin, a cornerstone of malaria treatment, is particularly concerning because it threatens the efficacy of artemisinin-based combination therapies, which are currently recommended by the World Health Organization as first-line treatment for uncomplicated *P. falciparum* malaria [112], [113]. The mechanisms underlying artemisinin resistance are complex and multifactorial, involving mutations in the kelch13 gene in addition to other genetic and epigenetic factors. The propagation of artemisinin-resistant *P. falciparum* poses a major threat to global malaria control efforts, underscoring the need for urgent containment strategies and the development of novel antimalarials with new mechanisms of action. It is important to note that resistance to antimalarial drugs compromises control and elimination efforts, emphasizing the need for enhanced surveillance, prevention, and treatment strategies [114].

The global effort to combat malaria has faced a persistent challenge with the emergence and spread of drug resistance in *Plasmodium falciparum* [22]. The widespread use of artemisinin-based combination therapies has been instrumental in reducing malaria-related morbidity and mortality; however, the emergence of artemisinin resistance in Southeast Asia threatens these gains (Scott et al., 2018). Parasite resistance to antimalarials remains an ever-present obstacle to the elimination of malaria [25].

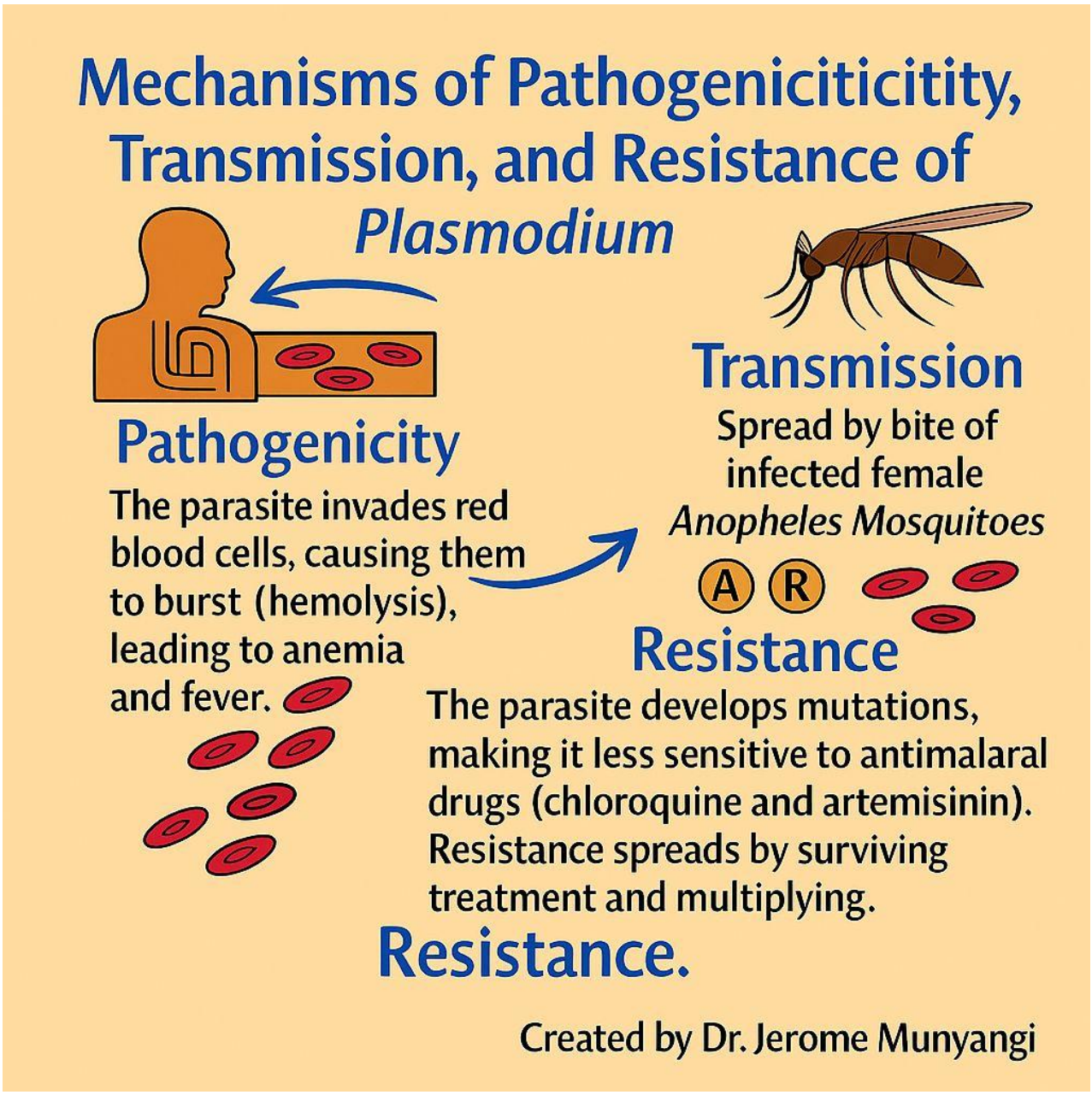


Figure 3: Mechanisms of Plasmodium pathogenicity, transmission, and resistance.

Pathogenicity: The cytoadherence of infected erythrocytes to the vascular endothelium, a phenomenon known as sequestration, is a critical virulence mechanism in *P. falciparum* infections [115].

The parasite invades red blood cells, causing them to burst (hemolysis), leading to anemia and fever.

Transmission: Malaria is transmitted through the bites of infected *Anopheles* mosquitoes, which inject sporozoites into the human host.

Resistance: The parasite develops mutations, making it less sensitive to antimalarial drugs (chloroquine and artemisinin).

Resistance spreads by the parasite surviving treatment and multiplying.

Studying the molecular mechanisms of drug resistance in *Plasmodium falciparum* is essential to countering the emergence of resistant strains and maintaining the effectiveness of antimalarial treatments. The emergence of artemisinin resistance represents a major threat to global malaria control [8]. While the precise mechanisms of artemisinin resistance remain elusive [23], several hypotheses have been proposed. Here is the summary of the few hypotheses in Figure 5.

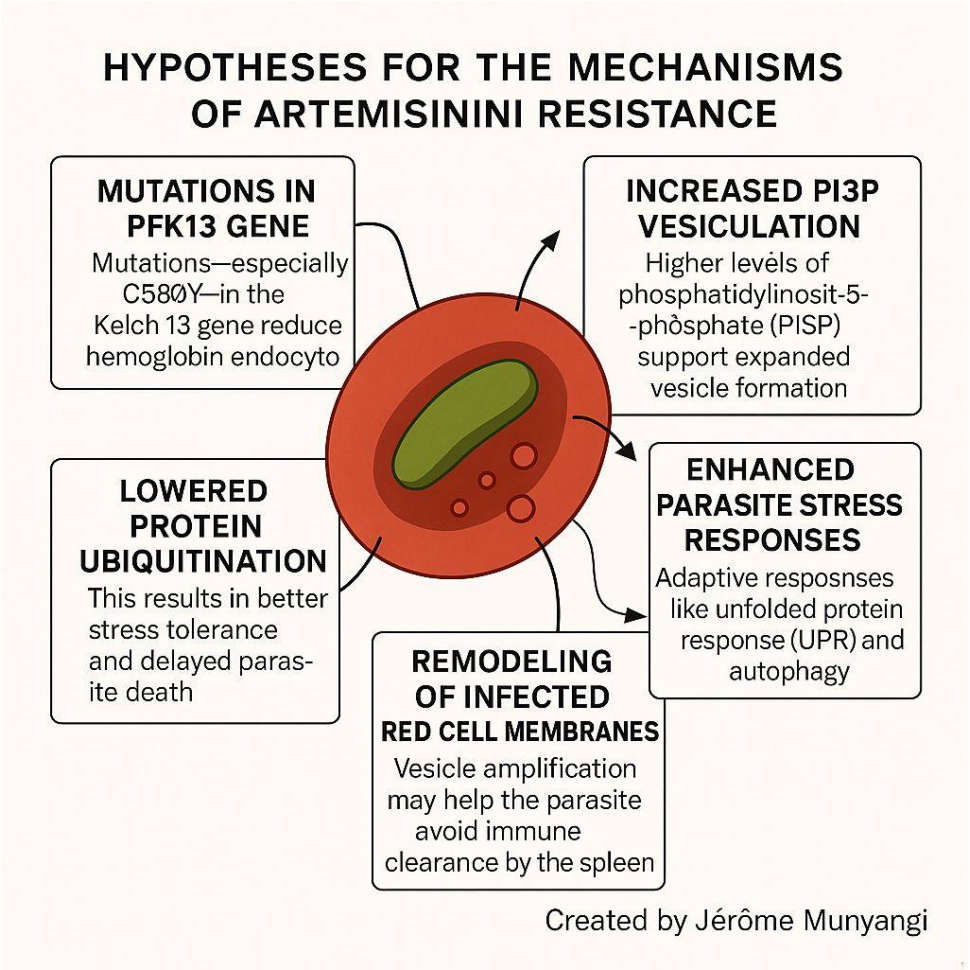


Figure 5. Hypotheses for the mechanisms of artemisinin resistance

1. **Mutations in the PfK13 gene**: Mutations—especially C580Y—in the Kelch 13 gene reduce hemoglobin endocytosis, which leads to less drug activation and more resistant parasites.[116]

2. Increased PI3P vesiculation: Higher levels of phosphatidylinositol-3-phosphate (PI3P) due to altered PI3K activity support expanded vesicle formation, helping parasites survive drug-induced stress.[117]

3. Enhanced parasite stress responses: Adaptive responses like unfolded protein response (UPR) and autophagy help manage protein damage caused by artemisinin, allowing survival under drug pressure. [118]

4. Lowered protein ubiquitination: This results in better stress tolerance and delayed parasite death[119].

5. Remodeling of infected red cell membranes: Vesicle amplification may help the parasite avoid immune clearance by the spleen. [120]

Antimalarial drug resistance is a substantial obstacle to global malaria control efforts. *Plasmodium falciparum* has evolved intricate molecular strategies to counteract the effects of numerous drugs [121]. A thorough comprehension of these mechanisms is crucial for devising effective strategies to overcome this resistance and sustain the efficacy of available treatments. Point mutations in the PfCRT gene are associated with chloroquine resistance [18]. Additionally, mutations in the dihydrofolate reductase gene contribute to pyrimethamine resistance, while mutations in the dihydropteroate synthase gene result in sulfadoxine resistance. These point mutations alter the structure of the targeted enzymes, diminishing their affinity for the drug and, consequently, reducing its inhibitory capacity. Gene amplification is another mechanism through which *P. falciparum* develops drug resistance [96]. Amplification of the PfMDR1 gene, which encodes a transmembrane transport glycoprotein, is linked to increased resistance to various antimalarials, including mefloquine, quinine, and artemisinin. This amplification leads to the overproduction of the PfMDR1 protein, augmenting the expulsion of the drug from the parasites, decreasing its concentration within the cells and, consequently, its effectiveness. Increased activity of efflux pumps, such as PfMDR1, can reduce the accumulation of drugs inside the parasite, resulting in reduced drug sensitivity. Epigenetic mechanisms, encompassing DNA methylation and histone modification, also contribute to drug resistance in *P. falciparum*. These modifications can alter the expression of genes involved in drug metabolism and transport and repair processes, thereby promoting resistance. The kelch13 gene has been identified as a crucial molecular determinant of artemisinin resistance in *P. falciparum* [74]. Single-nucleotide polymorphisms within the K13-propeller gene have been associated with *P. falciparum* resistance to both chloroquine and amodiaquine [122]. Mutations in the kelch13 gene are correlated with a delay in parasite clearance following artemisinin treatment [75]. The absence of an evident biological barrier to mutations in K13 explains artemisinin resistance in Africa and identifies their detrimental impact on parasite growth [69]. Consequently, the propagation of *P. falciparum* strains resistant to both artemisinin and its partner drugs constitutes a significant global threat to malaria control initiatives [123]. Studies suggest that artemisinin-resistant parasites exhibit a slower rate of endocytosis, which reduces drug activation and, consequently, drug sensitivity. A comprehensive understanding of the molecular processes underlying drug resistance in *P.*

falciparum is essential to formulating effective strategies to combat this resistance and maintaining the effectiveness of current treatments [22].

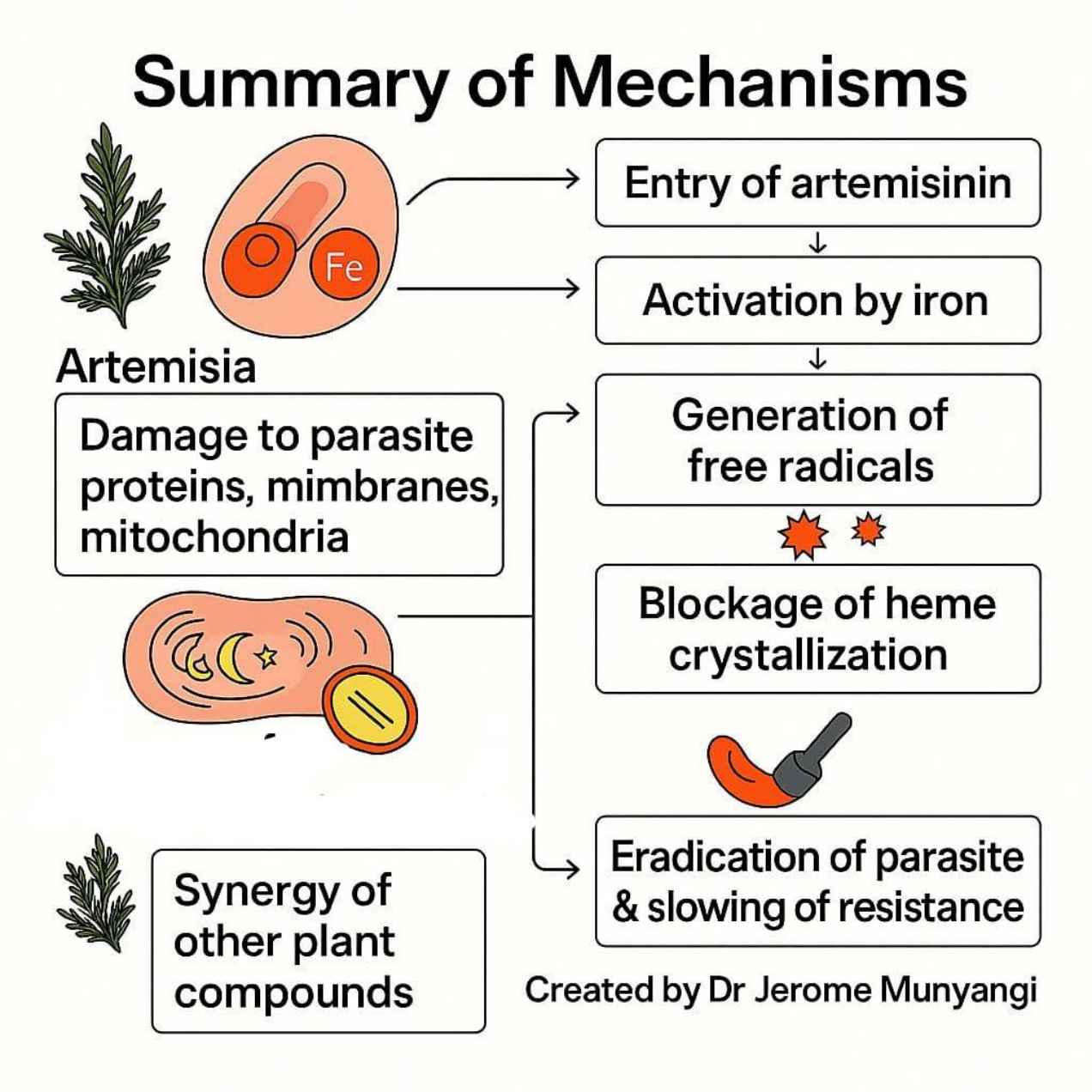


Figure 5. Summary of Mechanisms, the image illustrates how artemisinin (from Artemisia) combats parasites:

1. Artemisinin enters the cell.
2. It is activated by iron.
3. It produces free radicals.
4. It blocks the crystallization of heme.
5. It damages the parasite's proteins, membranes, and mitochondria.

6. This leads to parasite death and slows resistance. At the bottom left: Synergy of other plant compounds *highlights* the enhanced effect from other plant chemicals.

12. Geographic Expansion of Artemisinin Resistance

Artemisinin resistance, initially detected in the Mekong region notably at the border between Thailand an Cambogia , has since expanded to other areas of Southeast Asia and, more recently, to Africa, posing a substantial challenge to malaria control initiatives [10].

This expansion endangers efforts in malaria control, given artemisinin's crucial role in artemisinin-based combination therapies, which are the most efficacious treatments against P. Falciparum [29].

Originating in Cambodia in the 2000s, resistance has spread to Thailand, Vietnam, and Laos. Evidence now shows it in East Africa. This is due to the movement of resistant parasites, incorrect use of ACTs, and the overuse of artemisinin monotherapies. Using only one drug helps resistant strains survive and spread [124].

The inappropriate use of antimalarial medications, often stemming from clinical or imprecise diagnoses, may have accelerated the development of resistance. The expansion of artemisinin resistance diminishes the efficacy of ACTs, which are the primary therapeutic interventions for *P. falciparum* malaria. As parasites develop resistance mechanisms, the rate of infection clearance is reduced, leading to increased rates of therapeutic failure and a prolonged duration of transmissibility. This extended parasite exposure to the partner drug augments the selection pressure for resistance mutations within the partner drug's target genes, potentially leading to the sequential failure of both components of the ACT[125] .

Populations experiencing diminished malaria transmission due to control strategies, and consequently reduced immunity, may be more susceptible to the rapid emergence and spread of artemisinin-resistant strains, thus requiring enhanced surveillance efforts [126].

Current initiatives are focused on monitoring the dissemination of artemisinin resistance and implementing strategies to mitigate its spread. These efforts include strengthening surveillance systems, promoting the judicious use of ACTs, enhancing malaria diagnostic capabilities, and developing novel antimalarial medications and therapeutic approaches.

Notably, artemisinin-based combination therapies remain effective, even when the partner drug is losing its efficacy[127].

13. Initial Documented Cases of Resistance in Africa

The emergence of artemisinin resistance in Africa, as mediated by the Kelch13 gene, is now widely documented [10]. This is alarming because a previous reduction in malaria has now stalled in many parts of the continent [29].

The rise of artemisinin resistance poses a substantial challenge to global efforts to combat malaria.

14. Resistance in Southeast Asia and Africa

The molecular surveillance of mutations in the Pfkelch13 and Pfmdr1 genes offers crucial insights into the spread of artemisinin-resistant parasites from endemic regions, aiding in the optimization of antimalarial treatment strategies based on regional differences [27]. Maintaining the effectiveness of artemisinin-based combination therapies is imperative for malaria control and elimination, especially considering the potential for artemisinin resistance to spread to Africa [30].

To prevent widespread resistance, the elimination of *P. falciparum* parasites in the Greater Mekong Subregion in Cambogia must be prioritized [125],[130].

Since its emergence in Cambodia in the 2000s [60], resistance has spread to Thailand, Vietnam, and Laos [128]. Initiatives to monitor the spread of artemisinin resistance and implement strategies to mitigate it are underway. These efforts include strengthening surveillance systems, promoting the rational use of ACTs, improving malaria diagnosis, and developing novel antimalarials and therapeutic approaches [129], [130]. It is important to note that the F446I mutations have been identified as predominant mutations; however, their precise role in artemisinin resistance remains to be elucidated [25]. Resistance to antimalarial drugs, including artemisinin, represents a substantial impediment to malaria control in the Greater Mekong Subregion.

Molecular surveillance has highlighted the recent dissemination of artemisinin-resistant parasites from endemic areas. These data could prove valuable in optimizing antimalarial treatment strategies, considering regional variations in resistance. In accordance with WHO recommendations, national and international experts have suggested a therapeutic approach that combines antimalarial agents with a prolonged schizonticidal effect and an oral artemisinin derivative for treating uncomplicated *Plasmodium falciparum* infections [8].

15. Initial Documented Cases of Resistance in Africa

The emergence of artemisinin resistance in Africa, as mediated by the Kelch13 gene, is now widely documented.

The Kagera region, in northwestern Tanzania, was recently identified as one of the few areas in sub-Saharan Africa with confirmed partial artemisinin resistance [73]. Although artemisinin resistance alone does not lead to treatment failure and has limited clinical effect, manifesting as a delay in parasite clearance in patients treated with artemisinin-based combination therapy, the spread of *P. falciparum* strains resistant to artemisinin and its partner drugs poses a major global threat to malaria control initiatives. Mutations in the PfKelch13 gene are associated with this artemisinin resistance. The detection of artemisinin-resistant isolates in Africa raises concerns over their potential impact on the effectiveness of ACTs, which are the cornerstone of malaria treatment in the region. Indeed, the emergence of ACT resistance in Africa would likely have devastating consequences [30]. The loss of clinical efficacy of these compounds contributed to millions of additional deaths of African children in the 1980s [31]. To counter the spread of resistance, strategies are being implemented to monitor and contain artemisinin resistance in Africa. These efforts include strengthening surveillance systems, promoting the rational use of ACTs, improving malaria diagnosis, and studying new antimalarials and therapeutic approaches. These efforts also include improved patient management, confirming parasite diagnosis before treatment, the use of quality-assured ACTs, and ensuring that patients are completely cured.

Studies conducted in Eritrea, Uganda, and Rwanda have demonstrated a correlation between mutations in the PfKelch13 gene and artemisinin resistance [27]. However, it is important to emphasize that to date, the clinical impact of this resistance in Africa appears limited [27]. Available data suggest that ACTs remain effective for treating uncomplicated malaria, even in areas where PfKelch13 mutations have been identified [10]. Vigilance and close monitoring of ACT efficacy, continued containment efforts, and exploring new antimalarial strategies are essential to preserving the progress made in combating malaria in Africa.

The emergence of artemisinin resistance in northwestern Tanzania poses a threat to malaria control efforts, underscoring the necessity of ongoing surveillance and containment strategies to avert its spread to other regions of Africa [10].

The reduction in the clinical effectiveness of these drugs is believed to have contributed to increased childhood mortality due to malaria in Africa during the 1980s [31]. Importantly, the identification of PfKelch13 gene mutations provides a valuable diagnostic tool for monitoring the emergence and spread of artemisinin resistance. A multidisciplinary approach is therefore essential to understanding and combating antimalarial resistance. Consequently, it is imperative to adopt a comprehensive strategy that includes continuous monitoring of ACT efficacy, the development of new antimalarials, the improvement of vector control strategies, and the engagement of communities through educational interventions. The slow

parasite clearance phenotype has spread or emerged independently in other areas of Western Cambodia, Thailand, Myanmar, and Vietnam [52]. The rise in the number of artemisinin-resistant malaria parasites in Southeast Asia represents a significant threat to global malaria control efforts [62].

16. Prevalence and Resistance Evolution

The prevalence of artemisinin resistance varies geographically, influenced by factors such as malaria transmission intensity, antimalarial interventions, and patterns of medication use. The Greater Mekong Subregion exhibits widespread resistance, with treatment failure rates reaching as high as 60% in certain localities. This dissemination is associated with mutations in the *P. falciparum* kelch13 gene [131]. In Africa, artemisinin resistance is generally lower, though it is increasing in specific areas, particularly in East Africa [30]. The development of artemisinin resistance is a multifaceted process shaped by drug usage patterns, intervention coverage, and host immunity. Resistance has emerged independently across different geographical regions and can propagate via parasite migration[74].

The underlying mechanism of artemisinin's therapeutic effect involves the rapid clearance of the majority of parasites, followed by the elimination of the remaining parasites by the partner drug [48]. As a result of its efficacy, the World Health Organization advocates for artemisinin-based combination therapies as the first-line treatment for *Plasmodium falciparum* malaria [52]. Although artemisinin resistance does not lead to treatment failure and has only limited clinical effect, which manifests as a delay in parasite clearance among patients treated with artemisinin-based combination therapy, the spread of *P. falciparum* strains resistant to artemisinin in Southeast Asia poses a serious threat to global malaria control and eradication programs. Several mechanisms of artemisinin resistance in *Plasmodium falciparum* have been proposed, but the exact mechanisms remain unknown [23].

17. *Artemisia annua* and Artemisinin

17.1. Historical Context and Traditional Applications of *Artemisia annua*

Artemisia annua, also known as sweet wormwood, has been utilized in traditional Chinese medicine for centuries to treat fevers and other ailments [132]. Its use dates back to the Han Dynasty in China, when it was prescribed for intermittent fevers, symptoms often associated with malaria. In the 1970s, the Chinese scientist Tu Youyou and her team rediscovered the effectiveness of *Artemisia annua* in treating malaria. Tu Youyou was awarded the Nobel Prize in Physiology or Medicine in 2015 for her discovery of artemisinin, the active compound in *Artemisia annua*, and its application in malaria treatment. Artemisinin, a sesquiterpene lactone, is extracted from the leaves of *Artemisia annua* and has proven highly effective against malaria, particularly against strains resistant to other antimalarial drugs [133]. Artemisinin works by

interacting with iron in the malaria parasite, leading to the production of toxic free radicals that damage the parasite's proteins and membranes, ultimately causing its death. The use of *Artemisia annua* has spread worldwide and it is now cultivated in numerous countries, including those in Africa, Asia, and South America [134]. Artemisinin has become an essential drug for the treatment of malaria, especially in regions where resistance to other antimalarials is prevalent. Artemisinin-based combination therapies have become the first-line treatment for uncomplicated malaria caused by *Plasmodium falciparum* [54]. Widely used ACTs include artemether–lumefantrine and artesunate–amodiaquine [56]. *Artemisia annua* has a long history of application in traditional medicine for treating malaria and other fevers [133]. Its demonstrated efficacy led to the identification of artemisinin, a potent antimalarial that has revolutionized malaria treatment. *Artemisia annua* has also demonstrated activity against various pathogens, including viruses, bacteria, and fungi [135]. Recent studies investigating the plant's traditional use and pharmacological properties suggest that *Artemisia annua* and artemisinin may possess anti-inflammatory, immunomodulatory, and even anticancer properties.

17.2. Pharmacological Properties of Artemisinin

Artemisinin exerts its antimalarial effect primarily by disrupting heme-mediated processes within the malaria parasite. This sesquiterpene lactone contains a peroxide bridge, which is essential for its antimalarial action. The endoperoxide bridge of artemisinin reacts with ferrous ions present in the malaria parasite, leading to the production of toxic free radicals. These free radicals damage essential biomolecules in the parasite, such as proteins and lipids, resulting in its death. Artemisinin exhibits a rapid mechanism of action, quickly reducing parasite biomass and alleviating malaria symptoms. In addition to its antimalarial activity, artemisinin has also demonstrated anticancer, antiviral, and anti-inflammatory effects **[136].** Artemisinin can induce apoptosis in cancer cells by generating reactive oxygen species and disrupting mitochondrial function. It has shown promise in treating various types of cancer, including breast cancer, lung cancer, and leukemia. Furthermore, artemisinin has demonstrated antiviral activity against various viruses, including hepatitis B virus, hepatitis C virus, and human immunodeficiency virus. It can inhibit viral replication and modulate the immune response, making it a potential antiviral agent. Artemisinin has also been shown to demonstrate anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines and modulating the activity of immune cells [137]; [138]; [139]. Artemisinin's pharmacological properties, which include its antimalarial, anticancer, antiviral, and anti-inflammatory effects, make it a promising compound for developing novel treatments against various diseases.

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18. Mechanisms and Molecular Markers of Resistance to Artemisinin

Artemisinin resistance is a growing global public health concern, particularly in Southeast Asia and increasingly in Africa. Artemisinin resistance is defined as a delay in parasite clearance following treatment with artemisinin-based combination therapies. Several mechanisms contribute to artemisinin resistance in malaria parasites, including mutations in the kelch13 gene, increased oxidative stress, and altered drug transport. Mutations in the kelch13 gene are the primary molecular determinant of artemisinin resistance in *Plasmodium falciparum* [129]. The kelch13 gene encodes a protein that plays a role in the parasite's response to stress and the removal of damaged proteins. Mutations in the kelch13 gene, particularly point mutations such as R561H, are associated with delayed parasite clearance and artemisinin resistance [140]. These mutations alter the function of the Kelch13 protein, resulting in decreased parasite sensitivity to artemisinin. Increased oxidative stress also contributes to artemisinin resistance. Artemisinin exerts its antimalarial activity by generating reactive oxygen species, which damage the parasite's biomolecules. Resistant parasites have developed mechanisms to reduce oxidative stress, such as increased antioxidant enzyme production or decreased iron uptake, decreasing the effectiveness of artemisinin. Altered drug transport is another mechanism that contributes to artemisinin resistance. Resistant parasites may express efflux transporters that reduce the accumulation of artemisinin in the parasite, thereby decreasing its effectiveness. Molecular markers, such as mutations in the kelch13 gene, can be used to monitor the spread of artemisinin resistance and guide malaria control strategies [129]. The early detection of resistance is essential to minimize its impact [31]. Artemisinin resistance is a complex and multifaceted problem that requires a comprehensive approach to mitigate its impact. Understanding the mechanisms and molecular markers of artemisinin resistance is essential for developing effective strategies to prevent and manage artemisinin resistance, ensuring the continued effectiveness of artemisinin-based combination therapies for the treatment of malaria. The identification and characterization of these mechanisms require in-depth research. Various studies have highlighted the complexity of artemisinin resistance, underscoring the need for continuous monitoring and intervention [61].

19. Genetic Mutations

Genetic mutations are critical in the development of artemisinin resistance in malaria parasites. Studies have identified several genes associated with artemisinin resistance, with kelch13 being the most extensively studied[79] (Chhibber-Goel & Sharma, 2019). The kelch13 gene encodes a protein containing a Kelch repeat domain that functions in the

parasite's response to oxidative stress and the removal of damaged proteins. Mutations in kelch13, particularly point mutations such as R561H, C580Y, and Y493H, are strongly associated with artemisinin resistance in *Plasmodium falciparum* [73](Assey et al., 2025). These mutations alter the function of the Kelch13 protein, leading to a decrease in the parasite's sensitivity to artemisinin. The precise mechanism by which Kelch13 mutations contribute to artemisinin resistance remains unclear but is believed to involve an alteration in the parasite's response to oxidative stress and an increase in DNA repair activity. In addition to mutations in the kelch13 gene, other genes, including those involved in drug metabolism, DNA repair, and stress response, have been implicated in artemisinin resistance. Mutations in these genes may contribute to artemisinin resistance by enhancing artemisinin metabolism, increasing the repair of DNA damage caused by artemisinin, or modulating the parasite's response to artemisinin-induced stress. The identification and characterization of genetic mutations associated with artemisinin resistance are essential for monitoring the spread of resistance and developing new strategies to counter it. Molecular diagnostic tests based on the detection of specific mutations can identify resistant infections and guide therapeutic decisions. Furthermore, understanding the molecular mechanisms underlying artemisinin resistance can inform the development of new drugs and strategies that target resistant parasites. Moreover, new insights into the evolution of artemisinin resistance must be translated into practical tools for detecting artemisinin-resistant parasites in all geographic areas [125](Sibley, 2015). The mutations in the propeller domain of the kelch13 gene, which encodes the Kelch13 protein, are mainly related to artemisinin resistance [23] (Azmi et al., 2023). The K13 protein is highly polymorphic [71](Straimer et al., 2021).

20. The Clinical Impact of Mutations in the pfk13 Gene

Mutations in the Pfkelch13 gene have a significant clinical impact on the treatment of malaria. The presence of Pfkelch13 mutations is associated with delayed parasite clearance following artemisinin-based combination therapy, the first-line treatment for uncomplicated malaria. Delayed parasite clearance increases the risk of treatment failure and the spread of resistant parasites. Patients infected with parasites carrying Pfkelch13 mutations may experience prolonged fever and parasitemia, requiring additional treatment to clear the infection. In areas with a high prevalence of Pfkelch13 mutations, the effectiveness of artemisinin-based combination therapy is compromised, necessitating the use of alternative antimalarial drugs or treatment strategies. The spread of artemisinin-resistant parasites poses a major threat to malaria control and elimination efforts, particularly in regions where rates of malaria transmission are high. [141] [142]

Summary Table 1.: Clinical Effects of Pfkelch13 Mutations

Clinical Consequence	Evidence/Mechanism
Delayed parasite clearance	ACT response slowed, longer recovery
Increased treatment failure	Higher likelihood of persisting or recrudescent infection
Potential for transmission	Resistant parasites more likely to spread
Need for alternative therapies	ACT efficacy compromised in high-mutation regions

21. The Role of Oxidative Stress and DNA Repair Mechanisms

Artemisinin-resistant Plasmodium falciparum parasites exhibit enhanced antioxidant capacity and upregulation of redox-related genes, helping them withstand the oxidative damage induced by the drug [143],[143]; [144]. Furthermore, these parasites display increased expression of DNA repair proteins, supporting their survival against artemisinin-induced genotoxic stress [145].

Artemisinin resistance in Plasmodium falciparum is associated with altered oxidative stress responses. Specifically, resistant parasites exhibit upregulation of antioxidant defense systems, which may contribute to reduced sensitivity to artemisinin's reactive oxygen species–mediated parasite killing. [142]; [145]; [146]. There is evidence that artemisinin-resistant parasites display enhanced DNA damage response and repair mechanisms, such as increased expression of DNA repair proteins, which may help them survive the genotoxic stress induced by artemisinin. [145]; [144], [147]. Genetic studies have shown that mutations in the Kelch13 gene not only modulate artemisinin susceptibility but are also associated with broader changes in parasite metabolism, including redox homeostasis and DNA damage repair pathways [144]; [148].

Oxidative stress and DNA repair mechanisms are significantly implicated in artemisinin resistance in malaria parasites. Artemisinin's antimalarial action involves the generation of reactive oxygen species, which damage the parasite's biomolecules, leading to its demise. Resistant parasites have evolved strategies to mitigate oxidative stress and repair DNA damage, thereby diminishing artemisinin's effectiveness. Oxidative stress, resulting

from an imbalance between reactive oxygen species production and the parasite's detoxification capacity, can harm DNA, proteins, and lipids. Resistant parasites may exhibit increased levels of expression of antioxidant enzymes such as catalase and superoxide dismutase, which neutralize reactive oxygen species and protect against oxidative damage. Additionally, a decrease in iron absorption may occur in resistant parasites, reducing reactive oxygen species production in response to artemisinin. DNA repair mechanisms are crucial for artemisinin resistance, as artemisinin induces DNA damage in malaria parasites, and resistant parasites may enhance their capacity to repair this damage, promoting survival following artemisinin treatment. Studies indicate that resistant parasites show increased levels of expression of genes involved in DNA repair, such as base excision repair and mismatch repair genes. Enhanced DNA repair mechanisms enable resistant parasites to more effectively mend artemisinin-induced DNA damage, reducing the drug's efficacy. The significance of oxidative stress and DNA repair mechanisms in artemisinin resistance highlights the need for strategies targeting these mechanisms to improve artemisinin effectiveness and prevent the spread of resistance. For instance, drugs that inhibit antioxidant enzymes or interfere with DNA repair could enhance the efficacy of artemisinin in resistant parasites ("malERA: An Updated Research Agenda for Insecticide and Drug Resistance in Malaria Elimination and Eradication," 2017). The accumulation of reactive oxygen species, increased DNA damage, and the resulting antiparasitic effect suggest a causal relationship between reactive oxygen species, DNA damage, and parasite death [149].

Artemisinin resistance is a multifaceted phenomenon involving alterations in drug transport pathways, posing a formidable challenge to malaria treatment [49]. Reduced drug uptake, increased drug efflux, and modifications in drug metabolism can all contribute to artemisinin resistance [134]. Decreased expression of the drug transporters responsible for artemisinin uptake into the parasite can reduce the intracellular concentration of the drug, diminishing its effectiveness. Conversely, increased efflux pumps can actively expel artemisinin from the parasite, lowering intracellular drug levels and promoting resistance. Changes in drug metabolism can also affect artemisinin resistance.

Other potential markers of artemisinin resistance, in addition to the *kelch13* gene, are being further investigated to broaden surveillance and diagnostic capabilities. These include genes involved in DNA repair, oxidative stress response, and drug metabolism [98]. Exploring other genetic markers may provide additional insights into the mechanisms of artemisinin resistance and allow for the detection of resistance in parasites lacking kelch13 mutations.

In addition to the kelch13 gene, other potential markers of artemisinin resistance offer avenues for surveillance and diagnostics. These markers encompass mutations in other genes, alterations in gene expression, and epigenetic modifications. Mutations in genes involved in drug metabolism, drug transport, and stress response have been linked to artemisinin

resistance in some studies. Changes in gene expression, such as the upregulation of genes involved in detoxification and DNA repair, may also contribute to artemisinin resistance. Epigenetic modifications, including DNA methylation and histone modification, can alter gene expression and influence artemisinin sensitivity. Detecting these potential markers necessitates employing various molecular techniques, including PCR, DNA sequencing, transcriptomics, and epigenomics. PCR and DNA sequencing can detect mutations in genes associated with artemisinin resistance. Transcriptomics, which involves measuring the expression levels of thousands of genes, can identify genes that are differentially expressed in resistant and sensitive parasites. Epigenomics can be used to study epigenetic modifications associated with artemisinin resistance.

22. The Consumption of *Artemisia annua* Tea and Its Potential Impact on Artemisinin Resistance

While artemisinin-based combination therapies remain the first-line treatment for malaria, concerns have been raised regarding the potential impact of *Artemisia annua* tea consumption on the development and spread of artemisinin resistance. *Artemisia annua* tea contains artemisinin, but at varying and often lower concentrations than pharmaceutical artemisinin drugs. The uncontrolled use of *Artemisia annua* tea, with its variable artemisinin content, raises concerns over subtherapeutic exposure and the potential to select resistant parasites [70].

The consumption of *Artemisia annua* tea has become increasingly common in malaria-endemic regions of Africa [4], and the impact of this practice on artemisinin resistance is a significant concern. *Artemisia annua* contains artemisinin, a primary antimalarial compound, along with other compounds that may contribute to its antimalarial properties [150]. The consumption of *Artemisia annua* tea exposes malaria parasites to low doses of artemisinin over extended periods, potentially promoting the selection of resistant strains. The widespread use of *Artemisia annua* tea in Africa as a preventive measure against malaria could inadvertently lead to the development of artemisinin resistance [60]. Standardizing the plant's active metabolites is important to ensure both the efficacy and consistency of its therapeutic potential [3].

The artemisinin content in *Artemisia annua* tea can vary significantly depending on several factors, including the plant's chemotype, growth conditions, and preparation methods. This variability poses a challenge for ensuring consistent dosing and therapeutic efficacy. The variable artemisinin content in *Artemisia annua* tea, coupled with its widespread use, raises concern regarding subtherapeutic exposure and the potential to select resistant parasites [13]. Subtherapeutic exposure occurs when parasites are exposed to artemisinin levels that are too low to kill them but high enough to exert selective pressure favoring the survival and proliferation of resistant

strains. The World Health Organization considers the use of artemisinin-based combination therapies the most effective method for treating malaria [9].

Epidemiological investigations are essential to ascertain the risk of resistance linked to the consumption of *Artemisia annua* tea [55]. In vitro and in vivo models can be employed to examine artemisinin resistance.

In vitro models offer a controlled environment for examining the molecular mechanisms of resistance in malaria parasite cultures. These models can be utilized to assess the impact of artemisinin exposure on drug sensitivity, gene expression, and protein function.

23. In Vitro and In Vivo Selection Pressure Studies

In vitro and in vivo selection pressure studies have yielded valuable insights into the development of artemisinin resistance in malaria parasites. In vitro studies involve exposing cultured malaria parasites to increasing concentrations of artemisinin to induce resistance. These studies have demonstrated that parasites can develop artemisinin resistance relatively quickly, particularly when exposed to low doses of the drug [131]. In vivo studies involve infecting laboratory animals with malaria parasites and treating them with artemisinin. These studies have shown that parasites can also develop artemisinin resistance in vivo, especially when exposed to incomplete treatments or low doses of the drug. In vitro and in vivo studies indicate that the selection pressure exerted by artemisinin can lead to the selection and propagation of resistant parasites [55]. Selection pressure refers to the extent to which a drug eliminates susceptible parasites, leaving behind resistant parasites that are more likely to survive and reproduce. The stronger the selection pressure, the higher the probability that resistant parasites will emerge and spread. Resistance to artemisinin derivatives and the resultant failure of artemisinin-based combination therapy threaten all major achievements in malaria control [64].

The consumption of *Artemisia annua* tea may exert selection pressure on malaria parasites, potentially leading to the development of artemisinin resistance [60]. *Artemisia annua* teas contain artemisinin and other compounds with antimalarial activity [150]. However, the concentration of artemisinin in *Artemisia annua* teas can vary significantly based on factors such as plant variety, growth conditions, and preparation methods. Furthermore, the bioavailability of artemisinin in *Artemisia annua* teas may be low, resulting in a limited amount of artemisinin being absorbed into the bloodstream. Consequently, consuming *Artemisia annua* teas might expose malaria parasites to low doses of artemisinin over extended periods. This prolonged, low-dose exposure could potentially exert selection pressure on the parasites, fostering the development of resistance.

The emergence of artemisinin resistance poses a serious threat to malaria control efforts [48]. Artemisinin is a key component of artemisinin-based combination therapies, which are currently the most effective treatment for

malaria [127]. If artemisinin resistance becomes widespread, it could render these treatments ineffective, leading to increased morbidity and mortality from malaria [32].

24. Artemisinin Levels in Infusions and their Impact on Parasites

The concentrations of artemisinin measured in *Artemisia annua* infusions exhibit considerable variability, which influences their effectiveness against malaria parasites. This variability results from various factors, including the plant variety, growing conditions, processing methods, and infusion preparation techniques. Studies have shown that the artemisinin content in dried *Artemisia annua* leaves can range from less than 0.01% to more than 1% [126]. The method of preparing infusions can also impact the levels of artemisinin extracted. For example, the prolonged infusion of *Artemisia annua* leaves in hot water may extract a greater amount of artemisinin compared to a shorter infusion time [126].

The impact of *Artemisia annua* infusions on malaria parasites depends on both the concentration of artemisinin present and the susceptibility of the parasites to this compound. Research indicates that *Artemisia annua* infusions can exhibit parasiticidal effects against malaria parasites in both in vitro and in vivo settings. However, the efficacy of these infusions may be compromised by artemisinin-resistant parasites or insufficient artemisinin concentrations within the infusions. Consequently, further investigation is warranted to comprehensively evaluate the impact of *Artemisia annua* consumption on parasitic populations.

25. Risks Associated with Self-Medication and Subtherapeutic Dosage

Self-medicating with *Artemisia annua* infusions and the utilization of subtherapeutic dosages present substantial risks concerning malaria treatment and the development of artemisinin resistance. Self-medication, characterized by the use of medications without medical supervision, can result in inaccurate diagnoses, inappropriate dosing, and poor treatment adherence. Subtherapeutic dosages, which involve using lower drug doses than those recommended for effective treatment, can also contribute to drug resistance [13]. When individuals self-medicate with *Artemisia annua* infusions, they may not have received an accurate diagnosis of malarial infection or fully comprehend the severity of their condition, potentially leading to delays in seeking appropriate medical care and an elevated risk of complications. Furthermore, self-medication can lead to inappropriate dosing, as individuals may struggle to ascertain the correct dosage of *Artemisia annua* infusion based on factors such as their weight and age and the severity of their infection. The use of subtherapeutic dosages of *Artemisia*

annua infusions may expose malaria parasites to low concentrations of artemisinin over extended periods [8].

This exposure can exert selective pressure on the parasites, potentially fostering artemisinin resistance. The resistant parasites are then able to survive and proliferate, which results in treatment failure and the propagation of resistant strains. Consequently, self-medication with *Artemisia annua* infusions and the use of subtherapeutic dosages should be discouraged. Individuals should consult healthcare professionals to receive an accurate diagnosis and appropriate treatment and for guidance on the use of antimalarial medications. The use of artemisinin-based drugs has generally been tolerated well [5]. However, rare instances of liver injury associated with *Artemisia annua* consumption have been documented, underscoring the importance of caution and medical supervision [151].

Self-medication and subtherapeutic dosages can negatively impact public health, especially in malaria-endemic areas. It is therefore essential to promote rational drug use and educate the public about the risks associated with self-medication and subtherapeutic dosages.

26. Potential Impact of Large-Scale Consumption on Resistance

The widespread consumption of *Artemisia annua* teas could accelerate the emergence and spread of artemisinin resistance in malaria parasites. Artemisinin, derived from *Artemisia annua*, is an essential antimalarial drug used in combination therapies. However, its widespread use has put selective pressure on malaria parasites, leading to resistance in some areas. The large-scale consumption of *Artemisia annua* teas could increase this pressure.

When individuals consume *Artemisia annua* teas, they ingest artemisinin and other related compounds [3]. The concentration of artemisinin in these teas varies greatly, depending on factors such as the plant variety, growing conditions, and preparation methods. The consumption of these teas could expose malaria parasites to subtherapeutic doses of artemisinin, especially if the teas are not prepared correctly or if the artemisinin content is low. Subtherapeutic doses of artemisinin can create selective pressure that favors the survival and reproduction of parasites with reduced sensitivity to artemisinin.

Moreover, the widespread use of *Artemisia annua* teas could facilitate the spread of artemisinin-resistant parasites. When individuals infected with resistant parasites consume these teas, they may not be effectively treated, allowing the resistant parasites to persist in their bodies. These individuals may then transmit the resistant parasites to others through mosquito bites, leading to the further spread of resistance.

The widespread consumption of *Artemisia annua* tea could exacerbate resistance in regions where it is already present. Therefore, it is important to

discourage the large-scale consumption of *Artemisia annua* tea and promote the use of effective antimalarial drugs under the guidance of healthcare professionals [19]. Measures to prevent the further spread of resistance, such as close monitoring and rigorous enforcement, are necessary to maintain the effectiveness of artemisinin-based combination therapies. Adherence to antimalarial medications must be strictly monitored and maintained to prevent the spread of resistance [152].

Artemisinin resistance poses a significant threat to malaria control initiatives as it could compromise the effectiveness of artemisinin-based combination therapies, which are currently the most effective treatment for uncomplicated malaria [10]. Therefore, it is critical to closely monitor the potential impact of large-scale *Artemisia annua* tea consumption on artemisinin resistance and implement strategies to mitigate this risk.

27. Epidemiological Studies on *Artemisia* Tea Consumption and the Emergence of Resistance

Artemisinin and its derivatives are essential antimalarial agents and are particularly effective against *Plasmodium falciparum* infections, including strains resistant to chloroquine [48]. Derived from the *Artemisia annua* plant, these compounds act by generating free radicals that are toxic to the parasite, targeting several essential biological processes. The World Health Organization advocates artemisinin-based combination therapies as the first-line treatment for *P. falciparum* malaria, and these treatments have been crucial in reducing malaria-related morbidity and mortality worldwide [153].

However, the emergence and spread of artemisinin resistance threaten the efficacy of these treatments [113]. The use of *Artemisia annua* in traditional medicine, particularly in the form of herbal teas, has raised concerns regarding its potential participation in the development of artemisinin resistance.

Several factors could contribute to the development of resistance due to the consumption of Artemisia annua tea.

Epidemiological studies comparing the prevalence of artemisinin resistance between individuals who consume *Artemisia annua* tea and those who do not can help ascertain whether a significant correlation exists between these factors. Furthermore, such studies can investigate how variables such as the frequency of *Artemisia annua* tea consumption, the concentration of artemisinin in the teas, and the duration of consumption influence the risk of developing resistance. These studies can also consider the genetic characteristics of *Plasmodium falciparum* populations in regions where *Artemisia* consumption is common, analyzing whether specific mutations associated with artemisinin resistance are more prevalent in these areas.

It is essential to understand the implications of consuming *Artemisia annua* tea on the development of artemisinin resistance in malaria parasites

in Africa. The overexploitation of medicinal plants has led to the disappearance of certain plant species, highlighting the need for sustainable harvesting practices and conservation efforts to ensure the continued availability of these valuable resources. Traditional remedies involve various preparation methods, including decoction, infusion, and maceration [14]. These methods can influence the extraction and bioavailability of bioactive compounds, thereby affecting their therapeutic efficacy. Many local communities in Uganda use medicinal plants to manage malaria and other health problems, thus offering reliable alternatives for the development of new products [154]. Epidemiological studies can also use longitudinal data to track the evolution of artemisinin resistance rates over time in areas where *Artemisia* tea consumption is widespread, providing valuable information on the dynamics of resistance and helping to identify the factors that contribute to its emergence and spread. Therefore, further research is needed on the development of new therapies and other innovative approaches as alternatives to combat this African health problem [154].

While these approaches can aid in ascertaining whether the consumption of *Artemisia annua* tea contributes to the emergence and propagation of resistance, it is crucial to acknowledge that epidemiological studies alone cannot definitively establish a causal relationship between *Artemisia annua* tea consumption and artemisinin resistance. Additional variables, such as the utilization of other antimalarial medications, the extent of malaria transmission, and genetic factors, may also exert an influence on the development of resistance. Therefore, a multifaceted strategy integrating in vitro experiments, animal models, and molecular investigations is required to comprehensively elucidate the underlying mechanisms and intricacies involved.

28. **Risks Associated with the Uncontrolled Use of Herbal Tea**

Although *Artemisia annua* tea is promoted for its purported health benefits, unregulated consumption presents several risks that warrant careful consideration. A primary concern is the potential variability in artemisinin concentration across different tea preparations [32].

This variability can arise from factors such as the specific variety of *Artemisia annua* used, the plant's growing conditions, and the method of preparing the herbal tea. Consequently, consumers may find it challenging to ascertain the precise dosage of artemisinin they are ingesting, potentially resulting in either subtherapeutic or excessive doses.

Inadequate dosing may not effectively treat malaria infections, allowing the parasite to persist and potentially develop resistance [18].

Subtherapeutic doses may prove ineffective in treating malaria or other conditions for which the herbal tea is used, while overdosing can have adverse effects [155]. Another risk associated with the uncontrolled use of *Artemisia annua* tea is the potential for interactions with other medications.

Artemisinin has the potential to interact with various medications, which could alter their efficacy or elevate the likelihood of adverse reactions. For instance, artemisinin has been shown to interact with cytochrome P450 inhibitors, which have a crucial effect on drug metabolism. Combining *Artemisia annua* tea with these medications may modify their concentration in the body, potentially leading to unforeseen consequences. Furthermore, the uncontrolled use of *Artemisia annua* tea may delay or substitute the pursuit of appropriate medical care for malaria or other severe conditions, which can lead to complications and unfavorable outcomes, particularly among vulnerable populations such as children and pregnant women. While herbs are commonly used and often perceived as safe, it is important to acknowledge that they can have toxic effects, especially during pregnancy [156]. Consequently, it is essential to consult a healthcare professional before using *Artemisia annua* tea or any other herbal remedy, particularly for individuals taking other medications or those with underlying health conditions.

In the field of animal health, *Artemisia annua* and its compounds have shown promising effects [3].

29. Data on the Selection of Resistant Parasites

Data concerning the selection of resistant parasites due to the use of Artemisia annua herbal teas remain limited; however, emerging studies propose that suboptimal exposure to artemisinin may facilitate the development of resistance. In vitro investigations have demonstrated that repeated exposure of malaria parasites to low doses of artemisinin can result in decreased sensitivity to the drug. Furthermore, certain clinical studies have reported instances of artemisinin resistance in patients who consumed Artemisia annua herbal teas [5]. While such data elicit concern, it is important to acknowledge that artemisinin resistance is a multifaceted phenomenon influenced by multiple factors, including the use of other antimalarial drugs, the level of malaria transmission, and genetic factors.

Therefore, determining the specific contribution of *Artemisia annua* tea consumption to the selection of resistant parasites is challenging. The *Artemisia* genus encompasses approximately 500 species, a significant proportion of which are employed in traditional medicine for their therapeutic properties [157].

Artemisia annua contains artemisinin, a compound renowned for its antimalarial properties [157]. The plant also contains other compounds, including essential oils, flavonoids, and terpenoids, which may contribute to its overall pharmacological effect [158].

Further research is required to comprehensively assess the risk of selecting resistant parasites associated with *Artemisia annua* tea consumption and to implement strategies to mitigate this risk. These strategies may include promoting the appropriate use of this herbal tea, monitoring artemisinin

sensitivity in malaria parasites, and developing combination therapies that combine artemisinin with other antimalarial drugs. Medicinal plants have potential to provide safe and effective treatments against multi-drug-resistant microorganisms [9]. The discovery of artemisinin in *Artemisia annua* by Chinese researchers, drawing inspiration from traditional Chinese medical literature, has saved millions from death due to malaria [9]. Unrealistic standardization and adherence to home-based therapy, coupled with a lack of concrete in vivo efficacy data in the literature, raise concerns regarding the relationship between the widespread use of *Artemisia* sp. infusions in sub-Saharan Africa and the selection of resistant parasites [10]. These observations underscore the importance of conducting further research to evaluate the impact of *Artemisia annua* tea consumption on artemisinin resistance and to formulate strategies to mitigate this risk [178]. The effectiveness of plants in treating malaria has been demonstrated in pharmacological studies conducted on plant extracts and purified metabolites that exhibit antimalarial activity [14].

30. Methods of Identifying Resistance Markers

Identifying markers of resistance to artemisinin is crucial for monitoring and managing the emergence of resistance in malaria parasites. Several methods are employed to identify these markers.

Genomic Sequencing: This method involves sequencing the entire genome of malaria parasites to identify mutations associated with artemisinin resistance. The most studied gene is the kelch13 gene, which codes for a protein involved in parasite stress response and homeostasis. Mutations in the kelch13 gene have been strongly associated with artemisinin resistance in several regions worldwide.

Genotyping: Genotyping is a more targeted method that involves analyzing specific regions of the parasite genome to detect known artemisinin resistance mutations. This method is often used for the high-throughput screening of resistance markers in parasite populations.

Phenotyping: Phenotyping involves assessing the sensitivity of malaria parasites to artemisinin in vitro or in vivo. In vitro assays measure the ability of artemisinin to inhibit parasite growth in cell cultures, while in vivo assays evaluate the effectiveness of artemisinin in treating malaria infections in animals or humans.

Transcriptional Analysis: This method involves measuring the expression levels of genes involved in artemisinin resistance. By comparing the gene expression profiles of artemisinin-sensitive and -resistant parasites, novel resistance markers can be identified.

It is important to note that identifying resistance markers is only the first step in managing artemisinin resistance. Once markers are identified, they can be used to monitor the spread of resistance, guide treatment policies, and evaluate the effectiveness of new interventions. Increased and improved surveillance in many malaria-endemic countries, along with a better

understanding of the genetic basis of resistance, has led to the identification of molecular markers that can be used to track the emergence and spread of resistance. Ongoing research efforts aim to identify new resistance markers and to better understand the mechanisms of artemisinin resistance. Current control strategies that reduce malaria transmission may be subject to the rapid emergence and spread of artemisinin-resistant strains. Mutations in the PfKelch13 gene have been associated with slower parasite clearance after artemisinin treatment.

31. Laboratory Techniques

. Laboratory techniques for assessing artemisinin sensitivity typically involve in vitro parasite cultures, where the parasites are exposed to varying drug concentrations, and parasite growth is subsequently measured using diverse methods [114]. These methods encompass microscopy-based techniques, fluorescence-based assays, and DNA-based assays [114]. Microscopy involves counting the number of parasites in a sample post-artemisinin exposure. Fluorescence-based assays utilize fluorescent dyes that bind to parasite DNA, enabling the quantification of parasite DNA levels in a sample. DNA-based assays employ polymerase chain reactions to amplify and quantify parasite DNA in a sample. These assays determine the inhibitory concentration 50, which is the drug concentration needed to inhibit parasite growth by 50% [114]. Insecticide resistance monitoring is crucial for implementing effective vector control strategies [159]. Evaluating resistance intensity is vital as it provides insights into the extent of resistance within mosquito populations [160]. This evaluation helps ascertain whether current control strategies remain effective or if alternative interventions are necessary [160]. Insecticide resistance can vary geographically and differ among mosquito species. Consequently, consistent resistance monitoring across diverse locations and species is essential for informing targeted control strategies. Baseline data on insecticide sensitivity are indispensable for tracking the evolution of resistance over time [161]. These data serve as reference points for comparing subsequent monitoring data and detecting any shifts in insecticide sensitivity. Continuous monitoring facilitates the detection of emerging insecticide resistance [162]. This enables swift action to manage resistance and avert control failures [162]. Integrating insecticide resistance monitoring data with epidemiological and entomological data can provide a comprehensive understanding of disease transmission dynamics.

Surveilling insecticide resistance is crucial for informing decisions regarding insecticide selection and rotation, potentially involving the use of alternative insecticides or the implementation of resistance management strategies, such as insecticide mosaics or mixtures. Insecticide resistance is the percentage of mosquitoes that are impervious to insecticide [163].

Insecticide resistance surveillance data can be leveraged to advocate for resource allocation towards resistance management programs. This

information can enhance community awareness regarding the importance of vector control and resistance management.

32. Advantages and Limitations of Different Methods

In vitro methods offer a controlled environment conducive to examining resistance mechanisms. Nevertheless, their ability to faithfully simulate in vivo conditions is limited, as they do not account for the host's immune system or other variables that may influence drug efficacy. Furthermore, conducting in vitro tests can be time-consuming and costly, requiring specialized equipment and expertise.

In vivo methods have the advantage of reflecting real-world conditions because they consider the host's immune system and other factors that may affect how well the drug works. However, in vivo methods can be more expensive and take longer than in vitro methods, and there are ethical concerns regarding the use of animals. Molecular methods are fast and can identify specific mutations linked to resistance. However, they might not provide a complete picture because they do not account for other resistance mechanisms that could be involved [164].

Several challenges impede insecticide resistance surveillance, including a lack of standardized testing methods, resource and infrastructure constraints, and the need for adequately trained personnel [165]. Addressing these challenges necessitates establishing standardized resistance testing protocols, investing in capacity building, and ensuring the integration of vector control programs with resistance monitoring systems. The identification and characterization of species-specific bionomic traits are crucial for understanding the spatial and temporal dynamics of vector–human contact, as well as the potential impact of interventions [161]. Low indoor resting rates, coupled with the presence of insecticide resistance, underscore the need for integrated vector management and targeted interventions that account for mosquito behavior. It is crucial to revisit existing methods and incorporate new technologies that target different mosquito behaviors and life stages to accelerate progress in eliminating malaria [166].

33. Summary of Previous Studies

Several investigations have explored the relationship between *Artemisia annua* tea consumption and artemisinin resistance. Some studies propose a potential correlation between *Artemisia annua* tea consumption and decreased sensitivity to artemisinin [29]. However, other studies have not found significant associations. It is important to consider that the majority of these studies have been conducted in vitro or on animals, and further research is needed to ascertain the impact of *Artemisia annua* tea consumption

on artemisinin resistance in humans. The emergence of resistance to artemisinin-based combination therapies in Africa could potentially yield disastrous consequences; therefore, continuous monitoring of resistance emergence on the continent is essential [30]. Consequently, it is imperative to avoid deploying artemisinin and its analogues as monotherapies, to support the WHO's recommendation for artemisinin-based combination therapy, and to ensure its methodical and practical implementation in all affected areas [48]. Given the limited number of ACTs available, there is a substantial risk of selecting *Plasmodium* strains that have acquired resistance [48]. With the extensive deployment of ACTs in Africa, close monitoring of their effectiveness and early detection of potential resistance is critical.

34. Data Specific to the Maniema/Kalima Region

Currently, there is a paucity of data concerning the correlation between *Artemisia annua* tea consumption and artemisinin resistance within the Maniema/Kalima region of the Democratic Republic of Congo. This limitation impedes the formulation of definitive conclusions regarding the association between these variables in this specific geographic area. Further research is therefore necessary to comprehensively evaluate the potential impact of consuming *Artemisia annua* tea on artemisinin resistance within the Maniema/Kalima region. Consistent surveillance and assessment of ACT efficacy are essential to safeguarding the sustained effectiveness of these medications against malaria in this region. The insights derived from resistance monitoring initiatives can inform decision-making processes related to antimalarial treatment strategies and the implementation of interventions aimed at mitigating the propagation of resistance.

Limited data are currently available regarding the consumption of *Artemisia annua* tea and artemisinin resistance within the Maniema/Kalima region of the Democratic Republic of Congo specifically. Consequently, drawing definitive conclusions about the association between these factors in this area is challenging. Findings from resistance monitoring can inform decisions regarding antimalarial treatment policies and the implementation of strategies aimed at slowing the spread of resistance. Resistance to artemisinin, more precisely characterized as a delay in parasite clearance, is emerging de novo in Africa and does not appear to be related to the resistance of malaria parasites in Southeast Asia, where this issue was initially documented [67].

36. Potential Impact on Resistance Dynamics

The consumption of *Artemisia annua* tea may influence the dynamics of resistance to artemisinin. Exposure to low concentrations of artemisinin via

the tea could induce the development of resistance to artemisinin in malaria parasites, thereby compromising the effectiveness of artemisinin-based combination therapies and potentially leading to an increase in morbidity and mortality rates. It is therefore imperative to rigorously evaluate the potential impact of *Artemisia annua* tea consumption on resistance dynamics in order to make informed decisions regarding malaria control strategies. Although artemisinin has demonstrated high efficacy, signs of resistance were recently observed in Indonesia [152]. ACTs are widely used in the treatment of malaria, but their effectiveness is threatened by the emergence of resistance to artemisinin [52]. One study highlighted an average cure rate of 74% for *Artemisia annua* preparations after 7 days of treatment, compared to 91% for quinine [6]. Nevertheless, the *Artemisia* groups presented high rates of recurrence.

37. Molecular Surveillance and Future Perspectives

Molecular surveillance is critical to monitoring the dissemination of artemisinin resistance and pinpointing novel resistance markers [66]. The identification of resistance-associated mutations can aid in the development of more sensitive diagnostic tools and inform decisions regarding antimalarial treatment policies. Sustained research efforts are necessary to elucidate the mechanisms of artemisinin resistance and to develop new drug combinations capable of overcoming resistance [62]. The concurrent use of two or more antimalarials with differing modes of action and resistance mechanisms will mitigate the risk of selection [63]. Artemisinin derivatives diminish gametocyte carriage and may thereby reduce malaria transmission and morbidity [35]. Artemisinin and its derivatives are frequently used in conjunction with mefloquine, particularly in Thailand, where multidrug resistance has become problematic [13]. Continuous monitoring of the efficacy of ACTs and the prompt detection of potential resistance are essential [65]. Given the high levels of resistance and treatment failure observed with the leading artemisinin-based combination therapy in Southeast Asia, the development of a novel drug or formulation before resistance spreads further is urgently needed [70]. The emergence of artemisinin resistance in Cambodia in the mid-2000s resulted in prolonged parasite clearance times and increased failure rates for certain artemisinin-based drug combinations [59]. Artemisinin resistance is characterized by a notably slower clearance of *P. falciparum*-parasitized red blood cells from the peripheral blood [55]. The inappropriate utilization of antimalarials based solely on clinical diagnosis or on misdiagnosis stemming from poor microscopy techniques or interpretation may have expedited the emergence of resistance [167].

Artemisinin resistance poses a significant threat to global malaria control efforts [64]. The emergence and spread of artemisinin-resistant parasites could undermine the effectiveness of artemisinin-based combination therapies, which are currently the primary treatment for malaria [23]. The border region between Cambodia and Thailand is a major epicenter of artemisinin resistance [128], [83]. Artemisinin-resistant parasites were

initially detected near the Thai–Cambodian border [168]. Artemisinins remain effective for treating uncomplicated and severe malaria, and clinical resistance is not yet apparent [12]. However, a study revealed that treatment of uncomplicated *P. falciparum* malaria in remote Cambodian areas predominantly occurs through a less-regulated private sector, where artemisinin monotherapies are widely accessible [169]. Consequently, the WHO recommended ceasing the production of artemisinin-based monotherapies [170]. Concurrently, the rise of antimalarial drug resistance, especially to artemisinin, has increased malaria-related morbidity and mortality. Molecular data suggest that PfKelch13 mutations, which confer artemisinin resistance in *P. falciparum* malaria, have multiple independent origins within the Greater Mekong Subregion [77]. Research indicates a complex interplay between K13 mutations, parasite survival and growth, and genetic context, all of which contribute to the spread of artemisinin resistance [69]. Artemisinin resistance has been linked to mutations in the *Plasmodium falciparum* kelch13 gene.

38. Importance of Ongoing Surveillance

Ongoing surveillance of the effectiveness of ACTs and resistance patterns is essential to guiding treatment policies and limit the spread of resistance. The emergence of artemisinin resistance in Southeast Asia and reports of delayed parasite sensitivity to ACTs in African parasites indicate a progressive trend towards resistance to these important drugs [81]. The expansion of artemisinin resistance in Africa would be a major setback in the fight against malaria. Antimalarial drugs, such as chloroquine and sulfadoxine–pyrimethamine, have lost their effectiveness due to resistance, making ACTs the first-line treatment for *Plasmodium falciparum* malaria in most endemic countries [17]. Resistance to artemisinin has emerged in Southeast Asia and spread to other regions, threatening the effectiveness of ACTs [130]. Monitoring the therapeutic efficacy of ACTs is an essential strategy for rapidly detecting potential resistance to artemisinin derivatives and partner drugs [86]. National malaria control programs and their partners must prioritize therapeutic efficacy monitoring in sentinel sites, in accordance with WHO guidelines. The results of therapeutic efficacy monitoring should be used to inform decisions regarding treatment policies, including the choice of ACTs. In areas where ACTs continue to provide adequate therapeutic efficacy, their continued use is warranted.

39. Strategies to Limit the Emergence of Resistance

. Several strategies can be implemented to limit the emergence and spread of artemisinin resistance. These include improving diagnostic practices, promoting the appropriate use of ACTs, strengthening the surveillance of drug resistance, and developing new antimalarials. The World Health Organization has identified the accurate diagnosis and prompt treatment of malaria as crucial for achieving this goal in the absence of an effective vaccine [171]. It is also necessary to implement an effective containment strategy to prevent the spread of antimalarial drug resistance. The rapid identification and containment of resistance hotspots are essential to preventing its wider dissemination. Monitoring treatment efficacy outcomes is critical, as antimalarial resistance poses a threat to public health and malaria control [30].

The utilization of *Artemisia annua* tea as a potential malaria treatment has garnered substantial attention, yet its implications regarding artemisinin resistance remain contentious. *Artemisia annua* contains artemisinin, a potent antimalarial drug, alongside other compounds that may exhibit synergistic effects [53]. Further research is essential to comprehensively evaluate the effects of *Artemisia annua* tea consumption on artemisinin resistance and to inform public health recommendations. Studies have indicated the emergence and propagation of ACT-resistant parasites in Southeast Asia and adjacent regions [113]. Consequently, elucidating the molecular mechanisms underlying antimalarial resistance is crucial for devising effective countermeasures.

40. Obstacles to Combating Resistance

Several factors contribute to the emergence and spread of artemisinin resistance, including the unregulated use of artemisinin-based monotherapies, drug counterfeiting, limited access to quality diagnostics and treatments, and high malaria transmission rates. The emergence of artemisinin resistance in Southeast Asia, coupled with reports of delayed parasite sensitivity to ACTs in African parasites, indicates a progressive trend towards resistance to these crucial medications [27]. The expansion of artemisinin resistance in Africa would represent a significant setback in malaria control efforts. Furthermore, human migration patterns and the movement of parasite populations contribute to the propagation of drug resistance. Malaria control endeavors face ongoing challenges due to the emergence of resistance to antimalarial drugs [18].

41. Conclusions

The development of plant-based traditional medicines for malaria, bioscreening, and modern drug development are complementary approaches [4]. Continuous monitoring of the effectiveness of artemisinin-based combination therapies and resistance patterns is crucial for informing

treatment policies and curbing the spread of resistance. Malaria remains a significant public health concern, particularly in sub-Saharan Africa and Southeast Asia. The use of drug combinations, such as ACTs, is essential to slowing the emergence of antimalarial resistance [21]. Key global strategies for malaria control include vector control using long-lasting insecticide-treated bed nets, early diagnosis and treatment with artemisinin-based combination therapies, and chemoprevention in pregnant women and young children [37].

A resurgence of interest in traditional medicinal plants has been observed globally, accompanied by extensive research into various plant species and their therapeutic properties [172]. Numerous investigations have been conducted to assess the biological activity of plant extracts, fractions, or isolated molecules. The development of pharmaceuticals from natural products, including plant extracts, has garnered significant attention, leading to the identification of novel plant-derived drugs [173]. These plants can be propagated through seeds or cuttings and cultivated in open fields or greenhouses. The timing of plant collection is critical, as it influences the active substance content [134]. Public awareness regarding the appropriate utilization of *Artemisia annua* herbal teas and the potential risks associated with artemisinin resistance is also essential.

In 1972, artemisinin was extracted from *A. annua* and recognized for its potent antimalarial activity, marking a significant advance for public health [174]. *Artemisia annua* is widely documented as having been used in traditional medicine for many years in Asia and Africa to treat malaria and fever ([175]. The prevalence of malaria has decreased notably in recent years, thanks to the increased use of insecticide-treated mosquito nets and ACTs ([52]. Nevertheless, resistance to antimalarial drugs is still a considerable threat to the fight against malaria. Artemisinin, a powerful drug, has been used for many years in the treatment of malaria [5]. Artemisinin and its derivatives are sesquiterpene endoperoxides that exert antimalarial action. The WHO recommends treating malaria with a combination of artemisinin and another antimalarial drug [176]. The consumption of *Artemisia annua* herbal tea in Africa could potentially induce resistance to artemisinin through various molecular mechanisms. It is imperative to examine the various factors contributing to the therapeutic efficacy of *Artemisia annua*-based herbal teas, including the quality of the plants and the drying methods used.

In light of the increasing prevalence of resistance to antimalarial medications, the exploration of novel therapeutic approaches is imperative. Artemisinin, a potent drug with a history of use in malaria treatment, has demonstrated efficacy against strains resistant to other drugs [48]. To mitigate the development of resistance in the malaria parasite, artemisinin is typically administered in conjunction with other antimalarial agents. However, resistance to antimalarials remains a widespread concern [30]. Artemisinin-based combination therapies are the primary treatment option for uncomplicated *P. falciparum* malaria, and the utilization of combination drugs can enhance malaria cure rates and diminish malaria transmission.

The consumption of *Artemisia annua* tea in Africa has the potential to induce artemisinin resistance through various molecular mechanisms. One such mechanism involves the overexpression of genes. Interactions between plant compounds could alter drug concentrations in the body, possibly leading to reduced effectiveness. Medicinal plants may play a crucial role in the synthesis of potent antimalarial agents [14]. The widespread use of artemisinin as an antimalarial has exerted selective pressure on malaria parasites, leading to the emergence of resistant strains. Genetic and epigenetic changes can occur, enabling parasites to tolerate higher concentrations of artemisinin [177].

Mutations in the *PfKelch13*, *PfPI3K*, and *PfCRT* genes are correlated with artemisinin resistance. These mutations have the potential to alter the drug target, enhance drug efflux, or improve the parasite's DNA repair mechanisms. Extended exposure to low artemisinin doses, such as those resulting from the regular consumption of *Artemisia annua* teas, may encourage the development of resistance. Indeed, the overexpression of efflux pumps can diminish the intracellular concentration of artemisinin, thereby decreasing its effectiveness. Recent studies have indicated that artemisinin resistance is linked to mutations in the *kelch13* gene in *Plasmodium falciparum*. Researchers have found that parasites carrying these mutations are less sensitive to the effects of artemisinin, resulting in slower clearance of parasites from the bloodstream [74]. While artemisinin use has considerably improved malaria treatment, artemisinin resistance poses a growing threat to malaria control [58]. Although several specific parasitic targets for artemisinins have been identified, the parasiticidal effect of artemisinins likely arises from the alkylation of multiple cellular proteins and lipids ([61].

Mutations in PfKelch13 have been shown to be the main drivers of artemisinin resistance. In addition, mutations in the mdr1 and atp6 genes may also contribute to this resistance [66].

It is imperative to recognize concerns regarding the possibility of artemisinin resistance stemming from the widespread use of *Artemisia annua*-based herbal teas. In vitro studies have highlighted the effectiveness of artemisinin against various *Plasmodium* strains, including those exhibiting resistance. Artemisinin is metabolized by cytochrome P450 enzymes in the liver, and its primary metabolite, dihydroartemisinin, also exhibits antimalarial activity. Consequently, inhibitors or inducers of these enzymes can influence artemisinin concentrations in the body. Therefore, it is crucial to conduct thorough research to better understand the implications of *Artemisia annua* tea consumption on artemisinin resistance and to develop strategies to mitigate this risk.

Tea infusions made from *Artemisia annua*, and to a lesser extent from *Artemisia afra*, have demonstrated the ability to inhibit parasitemia and gametocytemia in vitro ([132]. The anti-inflammatory action demonstrated by Professor Yves-Jaques Schneider's team at the University of Louvain-la-Neuve, in addition to the facilitation of artemisinin's passage through the intestinal barrier with the consumption of *Artemisia annua* tea, offers new

perspectives, revealing the presence of compounds with significant pharmacological action, such as an anti-inflammatory effect, against malaria ([136]. Nevertheless, pharmacokinetic and pharmacodynamic data remain limited, as do clinical studies evaluating the efficacy and safety of *Artemisia annua* tea. Numerous clinical trials have confirmed the efficacy of artemisinin in the treatment of malaria [55]. However, the emergence of artemisinin resistance in some regions represents a major challenge. To validate the new resistance monitoring tools, it is essential to deepen our understanding of the mechanisms of artemisinin resistance [131].

Comprehensive malaria control strategies are urgently needed, including enhanced drug resistance monitoring, the development of novel antimalarial drugs, and the implementation of evidence-based public health measures. The combination of artemisinin with other antimalarial drugs has been instrumental in improving cure rates and delaying the onset of resistance ([123]. Artemisinin is typically administered in combination with other antimalarials, such as lumefantrine, amodiaquine, or piperaquine ([54]. These artemisinin-based combination therapies have become the standard treatment for uncomplicated malaria. The combination of antimalarial drugs has been studied and implemented for over 30 years ([13]. Since 2001 the WHO has recommended ACTs for the treatment of uncomplicated malaria in Africa. It is essential to monitor the effectiveness of ACTs and adjust treatment strategies as necessary to maintain their efficacy against malaria. The use of ACTs has contributed to reducing malaria-related morbidity and mortality ([56]. However, concerns persist regarding the emergence of resistance to artemisinin, particularly in certain regions of Southeast Asia ([121], [8].

The possibility of interactions between artemisinin and other compounds found in *Artemisia annua* tea raises concern regarding their potential impacts on drug efficacy and resistance development ([5]. It has been suggested that artemisinins act by inhibiting *Plasmodium falciparum* ATPase 6, the sarco/endoplasmic reticulum calcium ATPase pump of malarial parasites ([127]. However, other proposed targets include PfPI3K, a phosphatidylinositol-3-kinase that impacts multiple signaling pathways in the parasite ([60]. Another proposed mechanism is targeting mitochondrial electron transport in the parasite. Artemisinin and its derivatives are known to produce reactive oxygen species that damage parasite proteins.

Abbreviations

ACTs	Artemisinin-based Combination Therapies
AMFm	Affordable Medicines Facility-malaria
CHW	Community Health Worker
ITNs	Insecticide-Treated Nets
mRDT	Malaria Rapid Diagnostic Test
Pf	Plasmodium falciparum
Pfmdr1	Plasmodium falciparum multidrug resistance protein 1

Pfcrt	Plasmodium falciparum chloroquine resistance transporter
SBCC	Social and Behavior Change Communication
SDGs	Sustainable Development Goals
WHO	World Health Organization

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