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Malaria – the importance of phytochemicals as sources of alternative medicines

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Malaria is a zoonotic disease caused by parasites from the *Plasmodium* genus. This parasite is transmitted to humans when bitten by female *Anopheles* mosquitos. Five species of *Plasmodium* are known to infect humans: *P. malariae*, *P. ovale, P. knowlesi, P. falciparum*, and *P. vivax*. Of these, *P. falciparum* is associated with the highest probability of severe infection. This parasite's lifecycle involves a sexual stage and an asexual stage. The first takes place in the mosquito and the second in humans. The diagnosis of malaria can be done by microscopy, rapid diagnostic tests or molecular methods, the latter being the most accurate. As for the treatment of this disease, artemisinin, chloroquine, primaquine, and tafenoquine are the principal components used in today's available treatments. Vaccination is also an important factor in the fight against malaria, and, presently, there are two available vaccines, RTS, S/AS01 and R21/Matrix-M. Several phytochemicals effective against malaria are also found in plant species used in traditional medicine. Examples are *Azadirachta indica*, *Gossypium barbadense*, *Toddalia asiatica*, *Alstonia scholaris, Carica papaya, Andrographis paniculata*, and *Strychnos ligustrina*. Furthermore, compounds from three wild nutmeg species have also been proven effective against *P. falciparum*. The medicinal properties of phytochemicals like alkaloids, phenolic compounds and terpenes have even allowed the investigation of drug-resistant malaria strains. When it comes to preventing malaria transmission, insecticide treated nests and indoor residual spraying have been proven to reduce transmission rates. For pregnant women, intermittent preventive treatment of malaria is also recommended. The need for the development of innovative treatment and prevention strategies is urgent due to the emergence of resistant strains. Hence, we present an overview of the available treatment and prevention strategies currently approved and employed while focusing on the potential of phytochemicals as targets for further studies that can lead to the development of new medicines.

Keywords: Infectious diseases, Malaria, Phytochemicals, *Plasmodium*, Prevention, Treatment, Vaccines

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Introduction

Malaria is a highly consequential illness resulting from the presence of parasites from the *Plasmodium* genus. This disease is contracted by humans by the bite of a female mosquito of the *Anopheles* species that carries the infection. Malaria can be spread through different routes, including blood transfusion, organ transplantation, or the utilisation of needles or syringes that have been contaminated with blood. The transmission of malaria from a mother to her unborn infant, either before or during delivery, is also a possibility[1,2]. There are five distinct species of malaria parasites that can infect people, including *Plasmodium falciparum*, *Plasmodium vivax*, two *Plasmodium ovale* sub species, *Plasmodium knowlesi* and *Plasmodium malariae*. This article will mostly explore the species *P. falciparum* and *P. vivax*. *P. falciparum* is the predominant strain of malaria that is associated with a higher likelihood of causing severe infections, and if left untreated in early

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stages, it can potentially lead to fatality. As for *P. vivax*, the assumption that it rarely causes severe malaria has been challenged over the last 15 years, and reports of severe disease have been increasing. Despite the potential fatality of malaria, effective prevention measures can typically mitigate the occurrence of illness and mortality associated with this disease[1,3,4].

The life cycle of *P. falciparum* is characterised by its complicated nature, involving two distinct phases: sexual and asexual reproduction. The sexual phase of the parasite's life cycle takes place within the vectors, namely female *Anopheles* mosquitos, and the asexual phase takes place within humans, who serve as the intermediate host. Upon being bitten by an infected female mosquito, the parasites, in the form of sporozoites, enter the human bloodstream. Following a few hours of circulation within the blood, the parasites proceed to invade hepatocytes. The initial stage of asexual development in *Plasmodium* takes place within the hepatocytes, followed by progression into the erythrocytes. The rupture of erythrocytes is a consequence observed in all species of *Plasmodium*. After the asexual phase that takes place in the human body, the sexual phase follows inside the mosquito. After biting an infected person, the mosquito acquires the gametocytes formed in the blood cells of the human host. Now, in the mosquito's body, the sexual phase occurs and leads to the formation of new sporozoites that the mosquito can transmit when biting another person[5,6].

Fig. 1 illustrates the detailed life-cycle of *P. falciparum*, beginning when a mosquito releases sporozoites in the human host blood stream; they proceed to infect hepatocytes and develop schizonts after asexual reproduction; the schizonts then rupture and release merozoites into the bloodstream; the merozoites infect red blood cells and undergo a cycle of maturation and rupture, leading to re-infection of red blood cells (this cycle is responsible for the clinical manifestation of the infection); a few of the merozoites released from the liver schizonts show no infected erythrocytes and undergo differentiation forming gametocytes; the gametocytes circulate in the blood stream and are acquired by a mosquito once it bites an infected person; now in the mosquito the gametocytes initiate the sexual stage of the *Plasmodium* lifecycle forming an oocyst that ruptures and releases sporozoites that can be transmitted to humans upon another mosquito bite.

The last World Health Organization (WHO) report, in 2022, confirmed a total of 249 million cases and 603 thousand deaths caused by malaria in 85 countries, which represents a rise from the 245 million cases reported in 2020. This increase occurred mainly in the WHO African Region, which accounted for 94% of cases and 95% of deaths. About 80% of malarial deaths in this WHO region occurred in

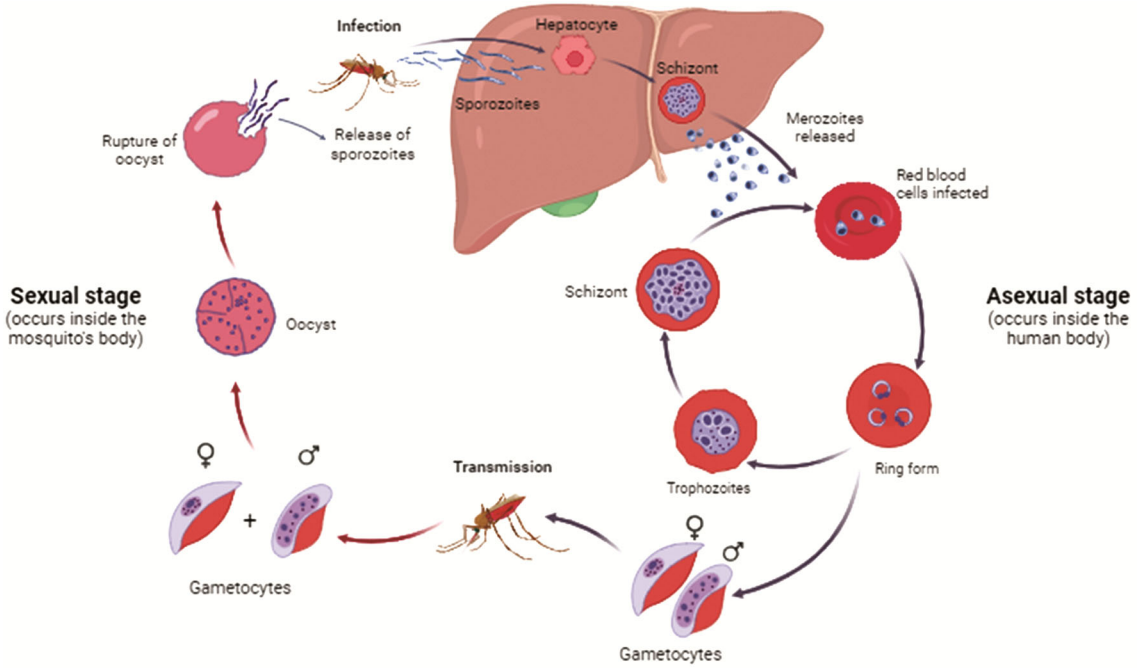

Fig. 1 — *Plasmodium falciparum* lifecycle (Created with BioRender.com).

children under five years old. The recent increase in malaria infections has also been attributed to the interruption of vital malaria services during the COVID-19 pandemic[7].

The symptoms associated with malaria involve fever and flu-like manifestations, accompanied by shaking chills, headache, muscle aches, anaemia, and jaundice, which is characterised by the yellow discolouration of the skin and eyes due to hemolysis and liver injury that occurs during infection[8]. Ensuring a correct diagnosis is the highest priority to ensure an inappropriate treatment plan[9]. The presence of merozoites in the bloodstream can lead to relapse in cases of *P. vivax* and *P. ovale* infections, occurring several months or years following the primary infection, emphasising the need for a correct diagnosis. Confirming the presence of parasites in peripheral circulation can be challenging when their numbers are very low. Additionally, diagnosing individuals who are infected with dormant liver stages is not achievable until these stages become activated and result in a relapse[10,11].

When it comes to treating malaria infections, the utilisation of essential antimalarial drugs in modern medicine may be related back to the medicinal significance of certain plants that have been recognised for centuries. Artemisinin, produced from the Qinghao plant, and quinine, obtained from Cinchona, were discovered as key pharmaceuticals in the fight against malaria[12,13]. Chloroquine and primaquine have beenwidely utilised as the preferred combination therapy for the management of *vivax* malaria since the 1950s[14]. The majority of artemisinin-derived pharmaceuticals currently employed are classified as prodrugs, necessitating hydrolysis for conversion into the active metabolite dihydroartemisinin. The WHO advocates for the utilisation of artemisinin-based combination treatments (ACT) in order to achieve a substantial rate of recovery from *P. falciparum* malaria and mitigate the proliferation of drug resistance. The treatment of malaria can also involve the utilization of antimalarial medications that have undergone evolutionary changes from quinine. As for malarial vaccines, they are categorized based on their principal impact, which includes pre-erythrocytic (sporozoite and liver-stage), blood-stage, and transmission-blocking vaccines. Today, there are two available malaria vaccines, RTS, S/AS01 and R21/Matrix-M vaccine. Nevertheless, the most effective approach to combat malaria cases is through the prevention of insect bites[14-18].

Diagnosis

The clinical diagnosis of malaria poses challenges due to the non-specific nature of signs and symptoms, which are further influenced by factors such as endemicity and host immunity. Typical symptoms involve feelings of general discomfort, elevated body temperature, sudden cold sensations accompanied by excessive sweating, cranial pain, and other related manifestations. Splenomegaly, anaemia, leukopenia, and thrombocytopenia are frequently observed in clinical presentations, although their diagnostic specificity is limited. The predictive value of fever periodicity, traditionally characterized as 48-hour paroxysms, is limited in diagnosing patients due to the prevalence of asynchronous erythrocytic schizogony with 24-hour cycles of paroxysm, especially among non-immune individuals[8]. The diagnosis of malaria can be broadly categorized into three main methods: microscopic diagnosis, molecular diagnosis, and antigen-capture fast diagnostic testing (Table 1)[19,20].

Microscopic diagnosis

The standard diagnostic technique for malaria is the utilization of light microscopy to examine stained blood films using the Giemsa staining procedure. The shortage of this approach in numerous regions of sub-Saharan Africa can be attributed to the absence of suitable staining material and adequately skilled employees. The efficacy of the procedure is dependent upon the level of professional proficiency, and it can be used to identify an infection with a concentration of 10–100 parasites per microliter of blood. Various clinical algorithms have been assessed for their applicability in regions with low resources, where the ability to diagnose malaria using microscopic means may be constrained. These efforts aim to reduce the incorrect prescription of antimalarials for non-malarial ailments, hence playing a role in the emergence of antimalarial resistance. Clinical diagnostic algorithms typically have limitations in their ability to accurately diagnose falciparum malaria, as well as being specific to particular clinical settings. Additionally, in regions with a higher frequency of malaria, there is a significant risk of symptomatic patients not receiving appropriate treatment[14,21,22].

Molecular diagnosis

It is well acknowledged in the scientific community that molecular diagnostics, such as polymerase chain reaction (PCR) or nucleic acid sequence-based amplification (NASBA), exhibit a greater level of

Table 1 — Comparative table of the advantages and disadvantages of the three main diagnostic methods explored

Diagnostic Method	Advantages	Disadvantages
Microscopic Diagnosis	Light Microscopy based diagnosis allows malaria species identification and the evaluation of infected red blood cells percentage, through Giemsa staining. The diagnosis is suitable to be performed in labs that are equipped to conduct routine haematology tests.	Microscope diagnosis requires trained personnel, capable of identifying the parasites. Quality equipment such as microscopes and reagents are also required and might not be available in places where the disease is endemic.
Molecular Diagnosis	Molecular diagnosis exhibits higher sensitivity and specificity and allows for species identification. This method is useful for diagnosis confirmation when the previous diagnostic was performed by untrained personnel or when species identification was not possible. Molecular techniques also constitute useful surveillance tools for patients under antimalarial treatment.	This method requires the use of specific reagents and equipment that might not be easily available in endemic countries. The lack of automated systems makes molecular diagnosis difficult to perform in limited resource settings, underlining the importance of implementing specific automated systems and techniques.
Antigen detection-based RDT	Antigen detection-based rapid diagnostic tests are faster and much simpler to perform when compared to other methods. These rapid tests are more easily available in endemic countries since they don't require specialized equipment and personnel. They are also more cost-effective.	This rapid diagnostic test has a high limit of detection, making the appearance of false negative results highly probable. The antigen detection-based test is not species specific. This method is also not useful for post-exposure monitoring.

sensitivity compared to microscopy or rapid diagnostic tests (RDTs). At present, the molecular diagnostic method most frequently employed for detecting malaria is PCR, which exhibits a Limit of Detection (LoD) ranging from 0.004 to 30 parasites per millilitre (p/mL). This approach is particularly advantageous for the identification of *Plasmodium* species after diagnosis using microscopy or RDT testing in laboratory settings that may lack proficient microscopists. Moreover, PCR is a valuable tool for the surveillance of individuals undergoing antimalarial therapy. This practice is observed in Western laboratories as well as in a few reference laboratories located in regions where malaria is endemic. However, the implementation of this technique necessitates substantial resources and specialized knowledge, which are typically lacking in developing countries where there is a pressing demand for diagnostic capabilities[14,22-24].

Antigen detection-based rapid diagnostic tests (RDTs)

Between 2010 and 2021, manufacturers sold a total of 3.5 billion Rapid Diagnostic Tests (RDTs) for malaria worldwide, with a significant majority (over 82%) of these sales occurring in nations located in the sub-Saharan African region. Antigen detection-based rapid diagnostic tests (RDTs) are characterized by their rapidity and simplicity, although they frequently exhibit an elevated limit of detection (LoD) of 4100 p/mL, particularly when the causative pathogen is non-*falciparum*. Besides, it should be noted that rapid diagnostic tests based on histidine-rich protein II (HRP-II), a protein produced by *P. falciparum*, are not appropriate for monitoring patients' post-therapy. This is due to the potential presence of antigens in circulation for up to 28 days after the infection has been successfully cleared, which might result in erroneous positive test outcomes during the initial month following treatment. Furthermore, HRP-II gene deletions may pose a difficulty in diagnosing *P. falciparum* malaria, originating in false negatives[25]. Due to the direct correlation between the sensitivity of RDT and the density of parasites, healthcare providers should exercise caution in using a negative RDT result as a definitive means to exclude the presence of malaria, especially in cases of *vivax* malaria (sensitivity 69% and specificity 100%). Also, the use of RDTs can be advantageous in facilitating managerial decision-making prior to the completion of a microscopic diagnosis[19,26-28].

Treatment

The objectives of antimalarial therapy include minimizing any immediate damage to the patient, eliminating the presence of asexual parasites in the peripheral blood, averting the reoccurrence of infection, and disrupting the transmission cycle. The selection of treatment is dependent upon various criteria, including the specific strain of malaria parasite responsible for the infection, the extent of the illness, the age of the patient, pregnancy status, and the prevalence of drug resistance within the geographical area.

Antimalarial drugs

Artemisinin-related drugs

In the year 1970, a team of Chinese scientists, under the leadership of Dr. Youyou Tu, successfully extracted the active compound known as artemisinin from the botanical species *Artemisia annua*. This particular substance has demonstrated significant efficacy in the treatment of malaria, thereby establishing considerable utility as an antimalarial agent. The majority of artemisinin-derived pharmaceuticals currently used are classified as prodrugs, needing hydrolysis for activation into the active metabolite dihydroartemisinin. The antimalarial activity of artemisinin-based medicines manifests through the formation of a radical via a peroxide bond. ACT is recommended by the WHO to achieve a substantial rate of recovery and reduce the risk of drug resistance, which is observed against chloroquine, sulfadoxine-pyrimethamine, and amodiaquine. One notable characteristic of the artemisinin derivatives is its remarkable efficacy and capacity to induce a quick decline in the initial parasite biomass. This attribute is evident in both *P. falciparum* and *P. vivax*[14,29-31].

Derivatives of artemisinin n have also proven to be effective against resistant malaria, as is the case of dihydroartemisinin. Atovaquone, artemether, and artesunate have been useful in the treatment of severe infection. Namely, artemether and artesunate show a high safety profile and only demonstrate side effects when used for prolonged periods[32].

Chloroquine

Chloroquine belongs to a class of medications called 4-aminoquinolines. Chloroquine works by interfering with the ability of the malaria parasite to break down haemoglobin within red blood cells, preventing the parasite from obtaining necessary nutrients and leading to its death. Chloroquine demonstrates a high degree of absorption following oral ingestion, with a bioavailability above 90%. Like other blood schizontocidal drugs that are effective against *P. vivax*, chloroquine has gametocytocidal properties, which rapidly eliminates the sexual stage during the acute infection. The gradual elimination of the drug results in its prolonged retention in the body with the effective concentration required for the elimination of the parasite. This ensures the complete elimination of all stages of the parasite that resides in the red blood cells. Additionally, it provides an extended period of post-treatment prophylaxis (PTP) during which the drug can prevent the invasion of the bloodstream by new merozoites from reinfection or relapse originating from hypnozoites[14,18].

Primaquine

The term "malaria relapse" refers to the reappearance of the infection caused by dormant liver-stage parasites known as hypnozoites in *P. vivax* and *P. ovale*. The lack of use of 8-aminoquinolines, the only medications capable of preventing relapse and facilitating "radical cure," is a significant obstacle to the elimination of malaria. The administration of this particular category of medications has been seen to induce oxidant hemolysis in individuals who possess a deficiency in glucose-6-phosphate dehydrogenase (G6PD), a condition that is prevalent in tropical regions. G6PD is inherited in an X-linked manner, with gene frequencies typically ranging from 8% to 10%, although they can occasionally reach as high as 35%[33]. The availability of G6PD testing is frequently limited, leading prescribers to exhibit a common hesitation in inducing hemolysis as a preventive measure against relapse. Both primaquine and tafenoquine have been found to promote drug-induced hemolysis in individuals who have a deficit in glucose-6-phosphate dehydrogenase (G6PD)[34]. The likelihood of recurrence, also known as radical cure efficacy, is dependent upon the dosage of 8-aminoquinoline, as well as the quality and bioavailability of the formulation. Additionally, the quantity of activatable hypnozoites in the liver, which is indicative of the level of prior malaria exposure, also influences the extent of this reduction. The prevailing approach across the majority of the Americas for preventing the recurrence of *P.vivax* malaria involves administering primaquine at a cumulative dosage of 3.5 mg/kg of an individual's body weight. However, it is worth noting that the effectiveness of this treatment has been found to be less successful in Southeast Asia and Oceania. Here, a dosage 50% higher is administered. This variability in medication susceptibility across different regions globally contributes to the above-mentioned dependency[14,33,35,36].

Tafenoquine

Tafenoquine is a pharmaceutical approved by the Food and Drug Administration and Australian Therapeutic Goods Administration in 2018. It is classified as a longer-acting antihypnozoite drug and is primarily used for the radical cure of *P. vivax* malaria. The mechanism of action of this drug is

similar to that of other antimalarial medications, specifically primaquine. Tafenoquine effectively disrupts the lifecycle of the parasites by inducing mortality in the dormant forms residing within the liver. Toxic compounds are produced intracellularly within the hepatocytes during infection, resulting in the induction of oxidative damage and subsequent mortality of the parasites. Tafenoquine is designed to specifically target the dormant phase of *P. vivax* malaria, with the objective of decreasing the occurrence of relapses commonly associated with this strain of malaria. The use of Tafenoquine, a therapeutic intervention for *P. vivax* malaria, has been linked to the prevention of relapse by effectively eliminating both *P. vivax* parasitemia and hypnozoites. Tafenoquine has shown effectiveness in the radical treatment of *P.vivax* malaria, while it did not show non-inferiority to primaquine[37-39].

Malaria vaccines

The year 2015 marked a significant milestone in the advancement of malaria vaccine development. This occurred when the pre-erythrocytic *P. falciparum* candidate RTS underwent a favourable evaluation by the European Medicines Agency[40]. Consequently, it was subsequently incorporated into national pilot implementation programs. This event signified a groundbreaking achievement as it became the first anti-parasite vaccine for humans to successfully undergo regulatory scrutiny. The objective of this vaccine is to mitigate the risk of malaria infection by specifically targeting either the malaria parasite (*Plasmodium*) or the mosquito vector (*Anopheles*) that is responsible for transferring the disease. The most advanced malaria vaccine, known as RTS, S/AS01 (Mosquirix), has obtained regulatory approval, distinguishing it as the only vaccine to have achieved this status. The *P. falciparum* parasite, responsible for the majority of severe malaria cases, is the target of this intervention. The primary intended use of RTS, S/AS01, is in regions characterized by moderate to high rates of transmission. Although it does not offer comprehensive protection, it has demonstrated a certain degree of efficacy in mitigating the likelihood of malaria infection, particularly among young children[40,41].

Another available vaccine for malaria is R21/Matrix-M, recently approved by the WHO as an alternative to the RTS, S/AS01 vaccine. This is a protein-based vaccine that targets the pre-erythrocytic stage. Its design is the product of recombinant DNA technology, where the circumsporozoite (CSP) gene is combined with HBsAg. The recombinant DNA, once expressed, will originate fusion proteins that self-assemble into virus-like particles, which will then be combined with Matrix-M adjuvant to finalize the vaccine, making it ready to use[42]. R21/Matrix-M vaccine proved to reduce 75% of symptomatic malaria cases when taken in three doses, and a booster dose is essential to maintain the efficacy. Moreover, this vaccine has shown to be cost-effective, and clinical trials have not revealed any safety issues related to it. The efficacy of R21/Matrix-M has not yet been compared to RTS, AS01, but the WHO indicates that its public health impact will be high even in areas with low transmission rates[43].

Phytochemicals against malaria

Traditional medicine has used the medicinal properties contained in plant species to help treat and prevent several kinds of diseases. This practice dates back centuries and, in present times, can be combined with modern science and technology to achieve high-end therapeutic strategies[44]. In the recent COVID-19 pandemic, some studies have evaluated the use of phytochemicals against the virus, such as thymol, a compound present in thyme species, that has shown efficacy as a spike protein inhibitor[45].

Although some of the drugs already used for malaria treatment are based on medicinal properties from phytochemicals, it is important to investigate new plant compounds for their antimalarial properties. In fact, several studies have reported significant numbers of plant species used by traditional medicine practitioners around the world. According to these studies, seven species stand out as the most preferred in survey answers; they are *Azadirachta indica*[46], *Gossypium barbadense*[47], *Toddalia asiatica*[48], *Alstonia scholaris*, *Carica papaya*, *Andrographis paniculata*[49], and *Strychnos ligustrina*[50]. These plant species contain specific phytochemicals (Fig. 2) that can be extracted from different parts of the plant and exhibit antimalarial activity. Therefore, they can be used in the development of new therapies, as is listed in Table 2.

Since patients infected with malaria can present several different symptoms, it is important to screen plant species/compounds that are known to be useful not only against malaria but also against those symptoms in an isolated way. For example, fever is one of the symptoms associated with malaria, so it is useful to explore the properties of plant species

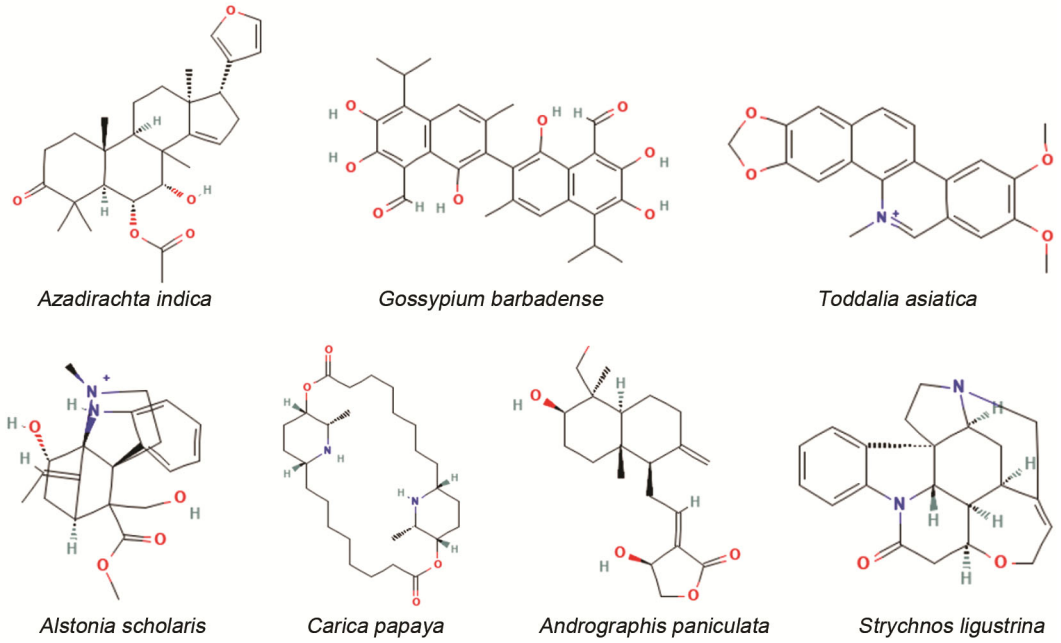

Fig. 2 — Main compounds present in plants with antimalarial properties and their chemical structures (obtained from PubChem).

Table 2 — Plant species with antimalarial properties and their primary compounds, with the respective site of extraction

Plant species	Antimalarial compound	Site of extraction	Reference
Azadirachta indica	Meldenine	Leaves	46
Gossypium barbadense	Gossypol	Leaves	47
Toddalia asiatica	Nitidine	Root bark	48
Alstonia scholaris	Echitamine	Bark	49
Carica papaya	Carpaine	Leaves	49
Andrographis paniculata	Andrographolide	Leaves and roots	49
Strychnos ligustrina	Strychnine	Leaves and stem	50

that are used against this particular symptom, even if they are not known to have antimalarial activity. This can facilitate the discovery of active compounds focused on the different malaria symptoms that can be combined in novel optimised therapies for symptom management.

Moreover, the treatment of malaria using natural products can also be associated with products used in everyday meals. Just as is shown, *Carica papaya*, a fruit available for daily intake, contains a compound, carpaine, known to have antimalarial properties[51]. Another example is the nutmeg family, *Myristicaceae*. Belonging to this family is the species *Myristica fragrans*, known as true nutmeg, while other species are known as wild nutmeg. True nutmeg and wild nutmeg species have proven to possess several medicinal properties and have been widely used in traditional medicine around the world. Some species of wild nutmeg have, inclusively, shown antimalarial activity. This is the case of *Pycnanthus angolensis*, which has a compound named talaumidin present in the stems that shows positive results against chloroquine-resistant *P. falciparum*. Another wild nutmeg species is *Knemaglauca*, which contains Malabaricone A in the leaves and is also effective. Lastly, the wild nutmeg species *Horsfieldia spicata*, is another example of a species effective against *P. falciparum*, namely, in its schizont stage, due to the presence of the compound named Myristicyclin[52].

Furthermore, the use of phytochemicals against malaria has also allowed the possibility of investigating and developing effective treatments against drug-resistant malaria strains. For example, quinine, quinidine and cinchonine are alkaloids with anti-plasmodial activity isolated from the cinchona bark, and when combined, these compounds show greater potential in treating infections by drug-resistant malaria strains[53]. Phenolic compounds, namely catechins, isolated from the plant *Piptadenia pervillei*, endemic to Madagascar, are also potent candidates against resistant strains[54]. Some terpenes

like farnesol, nerolidol, limonene, and linalool have also demonstrated antimalarial activity against *P. falciparum in vitro* in its intraerythrocytic stage. These terpenes can inhibit the isoprenoid biosynthesis in *P. falciparum* in different locations of the metabolic pathway. Such capacity of these terpenes indicates that they may be capable of reversing the resistance mechanisms of this parasite[55].

In short, it can be stated that natural products constitute a rich source of potent components that can act as new therapies against malaria. They can be extracted from several parts of the plants, such as leaves, stems or roots, and they can be studied for their specific properties against stages of the parasite's life cycle, for the effect on the parasite's metabolic pathways, and even for their effect on the relief of malaria symptoms. It is also important to consider the synergistic effect that may be observed in whole plant extracts when compared to the use of single phytochemicals, as well as the cumulative effect between compounds from different plants or even their combination with synthetic drugs already in use. This way, phytochemicals can pave the way to the discovery of new antimalarial drugs, including against resistant strains.

Current status of antimalarial medicines development

Given the epidemiological significance of malaria, several new medicines are continuously under research. According to the Medicines for Malaria Venture' pipeline for antimalarial drugs, which was last updated in June 2023, there are 62 ongoing projects that aim to find new drugs for the treatment of malaria infection. Of the total of projects preset in the portfolio, 15 of them have already been approved by regulatory bodies, and 1 is available for time-limited procurement while awaiting approval from regulatory entities. Of the remaining 46 projects, 12 have not reported any progress in the two years prior to the update of the portfolio, leaving 34 ongoing projects that have been presenting developments in the discovery of new malaria medicines. Of these 34, 17 are still in the translational phase (9 in the preclinical phase and 8 being tested in human volunteers), and the other 17 are in the product development phase (8 in the patient exploratory phase, 6 in the patient confirmatory phase and 3 in regulatory review)[56].

Prevention strategies

The prevention of malaria requires the implementation of a comprehensive approach that includes both individual defences and public health initiatives with the objective of mitigating the spread of the disease. *Anopheles stephensi* is a type of mosquito that has the capability to transmit both *P. falciparum* and *P. vivax*, which are the causative agents of malaria. *A. stephensi* exhibits the ability to flourish in urban settings, distinguishing it from other prominent mosquito vectors of malaria that predominantly breed in rural regions. *A. stephensi*, when seen in the WHO African Region, has exhibited resistance to several pesticides commonly employed in public health interventions. This resistance may present an additional obstacle in effectively managing its population. Here, we list the key strategies for preventing malaria.

Insecticide-treated nets (ITNs)

In endemic locations, insecticide-treated bed nets (ITNs) have demonstrated efficacy in mitigating the incidence of malaria illness, severe disease, and mortality associated with malaria. The nets undergo a treatment process involving the application of insecticides that serve the purpose of deterring and eliminating mosquitoes. Insecticide-treated bed nets (ITNs) function as a tangible obstacle that effectively hinders mosquitoes from making direct contact with persons during their sleep. There are only two approved kinds of insecticides for application on insecticide-treated bed nets (ITNs), namely, pyrroles and pyrethroids. Research findings indicate that these insecticides exhibit minimal health hazards to humans and other mammals while demonstrating toxicity towards insects, resulting in their mortality. The utilization of long-lasting insecticide-treated nets (LLINs) that retain sufficient amounts of insecticide for a minimum duration of 3 years, even after undergoing many washing cycles, has been linked to significant reductions in malaria cases within nations where comprehensive malaria initiatives have successfully achieved widespread usage of LLIN[57]. The WHO currently advocates for the widespread distribution and utilization of long-lasting insecticidal nets (LLINs) among all individuals residing in regions affected by malaria, a strategy sometimes referred to as "universal coverage".

Intermittent preventive treatment of malaria in pregnant women (IPTp) and intermittent preventive treatment of malaria in infancy (IPTi)

The presence of malaria parasites in placental intervillous spaces has been found to have a negative impact on pregnancy and delivery outcomes. Pregnant

women exhibit heightened susceptibility to malaria infections, which in turn represent a significant threat to the well-being of developing fetuses. The presence of malaria during pregnancy has been linked to several adverse outcomes, including anaemia, stillbirth, low birth weight, as well as maternal and foetal mortality. Pregnant women are urged to sleep under insecticide-treated bed nets (ITNs), while children should get suitable antimalarial interventions as prescribed by healthcare professionals. Preventive treatment in pregnancy (PTp) involves providing a therapeutic dosage of an efficacious antimalarial medication, specifically sulfadoxine-pyrimethamine (SP), to all pregnant individuals, regardless of their malaria infection status, with the aim of curbing the transmission and impact of the disease. Intermittent preventive treatment in pregnancy (IPTp) ought to be administered during every scheduled antenatal care appointment, commencing at the earliest feasible time in the second trimester. However, it has been observed that elevated levels of folic acid can reduce the efficacy of sulfadoxine-pyrimethamine. Hence, it is advisable that women adhere to the prescribed daily dosage of 0.4 mg of folic acid[57,58].

Indoor residual spraying (IRS)

A significant number of malaria vectors are classified as "endophilic," indicating that the mosquito vectors tend to seek shelter inside buildings after feeding on blood. Indoor residual spraying (IRS) has been found to be highly effective in controlling these mosquitoes. The Internal Revenue Service (IRS) entails the application of a residual insecticide to the walls and various surfaces within a residential structure. Over a span of several months, the insecticide will effectively eliminate mosquitoes and other insects upon direct contact with the treated surfaces. The Internal Revenue Service (IRS) does not possess the direct capability to mitigate the risk of mosquito bites for individuals. In contrast, it typically results in the mortality of mosquitoes after their blood meal, provided they alight on the treated surface. This phenomenon leads to a decrease in the mosquito population as well as their capacity to spread infection. The Internal Revenue Service (IRS) effectively mitigates the spread of infection to individuals in proximity. In order to achieve optimal effectiveness, it is imperative that the implementation of the IRS (Indoor Residual Spraying) intervention encompasses a significant majority of houses within a given geographical region, typically exceeding 80% coverage[57].

Resistances and limitations

Drug resistance

The emergence of medication resistance by the *Plasmodium* parasite is a significant and urgent concern in the field of malaria treatment. The emergence of resistance to artemisinin, particularly in Southeast Asian nations, poses a significant challenge to the efficacy of primary treatment strategies. The independent emergence and local spread of clinically artemisinin-resistant *P. falciparum* has been identified in Africa. The evaluation of artemisinin resistance has predominantly been conducted through the examination of the kelch protein-encoding gene. The identification of the two kelch13 mutations could potentially serve as indicators for the identification of these drug-resistant parasites[59]. Furthermore, the emergence of resistance to chloroquine and other antimalarial medications has significantly constrained the available therapeutic alternatives. The emergence of multidrug resistance further emphasizes the necessity for ongoing awareness and adaptability in treatment protocols.

Vector Resistance and Vaccines

Vector control, which is considered a fundamental aspect of malaria prevention, encounters various obstacles of its own. The emergence of resistance to pesticides in *Anopheles* mosquitoes undermines the efficacy of indoor residual spraying and insecticide-treated bed nets. In the same way, malaria vaccines, while their potential as a preventive measure, face challenges due to the inherent constraints of conferring only partial protection and the wide-ranging characteristics exhibited by various *Plasmodium* species.

Environmental Dynamics and Human Factors

While past and ongoing efforts to eliminate diseases offer valuable insights for addressing malaria, it is important to keep in mind that the global landscape is evolving at a rapid pace. Consequently, the circumstances in the future might have little resemblance to the contexts under which these initiatives were implemented. The complexity of malaria is further exacerbated by the effects of climate change and the movement of human populations. Climate change has a significant impact on the breeding habitats of mosquitoes, leading to alterations in the distribution and behaviour of these disease-carrying organisms. The movement of human populations, frequently motivated by economic considerations, has the potential

to bring malaria to previously unaffected regions and sustain the ongoing transmission of the disease. The transmission of the disease is influenced by behavioural variables, such as the inconsistent implementation of preventive measures[60].

Conclusion

With the current climate pattern affecting the distribution of mosquito populations worldwide, outbreaks of malaria infection are expected to increase. Therefore, it is imperative to adopt a comprehensive approach and foster international cooperation. The investigation of novel antimalarial medications that effectively counteract resistance mechanisms is of the highest priority in malaria research. This involves the exploration of innovative pharmacological combinations as well as the utilisation of pre-existing pharmaceuticals for alternative purposes. Enhancing vector control efforts via novel approaches and heightened surveillance is crucial in addressing vector resistance. In addition, improving the availability of healthcare and education, specifically in areas with limited resources, can contribute to the early identification and management of medical conditions. The ongoing battle against malaria is a complex and varied challenge, necessitating the integration of scientific advancements, public health initiatives, and socio-economic enhancements. The achievement of a malaria-free world necessitates a collaborative endeavour driven by the resolve of governmental bodies, non-profit entities, medical practitioners, scholars, and members of society.

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