Malaria Policy Advisory Committee Meeting
2–4 October 2019, Geneva, Switzerland
Background document for Session 3

WHO technical document of the use of non-pharmaceutical forms of *Artemisia*

October 2019

Introduction

Research on the herbal remedies used in the past has led to the discovery of malaria treatments that have saved millions of lives. The powdered bark of the cinchona tree was used to treat malaria, initially in South America and later across the globe. Quinine was first isolated from cinchona tree bark in 1820, and the pure compound quickly demonstrated greater potency than the hot infusions of the bark. With the availability of the pure compound, appropriate dosage could be established and the first modern chemotherapeutic agent against malaria was born *(1,2).*

Today, the most widely used antimalarial treatments, artemisinin-based combination therapies (ACTs), are produced using the pure artemisinin compound extracted from plant *Artemisia annua.* A full malaria treatment course with an ACT costs less than US$ 2 to procure. There are still ACTs available, capable of treating all malaria strains globally, despite artemisinin partial resistance in South East Asia and resistance to some of the partner drugs used in ACTs. However, for those in need in malaria-endemic countries, ACTs are not always available, are only available at high prices, or are of substandard quality. These difficulties are used as part of the argument in promoting *Artemisia* plant materials as affordable and self-reliant medicines against malaria.

Traditional herbal remedies have several limitations, especially when they are utilized for treating potentially fatal diseases such as malaria. The main limitations are related to standardization of plant cultivation and preparation of formulations, dosages, quality assurance, and evidence of clinical safety and efficacy. The aim of this technical document is to review the evidence on the effectiveness of non-pharmaceutical forms of *Artemisia* and to discuss the limitations specific to these herbal remedies.

The discovery of artemisinin

The search for new antimalarial drugs was fuelled by the spread of resistance to the most widely used antimalarial drugs. Chloroquine was introduced in 1934 but was not in wide-scale use until the 1950s. Chloroquine resistance emerged around 1957 in two locations: South America and along the Cambodia–Thailand border. The resistance spread from the Cambodia–Thailand border areas throughout South-East Asia *(3).* During the Viet Nam–American war, the North Vietnamese government requested assistance from China to manage the chloroquine drug-resistant malaria that was affecting their military forces *(4).*

In 1967, China launched Project 523 – a project aimed at finding new drugs for the treatment of malaria. The project involved 60 research organizations and more than 500 scientists *(5)*. As part of the project, Chinese scientists examined ancient medical texts, reviewing more than 2000 recipes and testing extracts from more than 100 plants on rodent malaria parasites *Plasmodium berghei.* The plant

WHO/CDS/GMP/MPAC/2019.10

A. annua was mentioned in several of the recipes, and the first extracts from *A. annua* did show antimalarial activity. However, this activity was highly variable, and the results were not satisfactory. A recipe from 341 A.D. for the treatment of fever, prescribed the juice of *A. annua* produced using cold water rather than tea produced through the traditional method of boiling herbs. There is no evidence that the Chinese used *A. annua* as a tea. Professor Tu Youyou, awarded the Nobel Prize in Medicine in 2015 for the discovery of artemisinin, realized that high temperatures could be causing the instability in the antimalarial activity and suggested that the leaves were likely the part of the plant with the most activity. Inspired by this, the Chinese researchers produced an extract using a low-temperature method with ether. This extract was shown to be highly efficacious against rodent and monkey malaria. The results led to a countrywide effort involving a large number of scientists from many institutions. The goal was to extract large quantities of the pure ingredient and determine its chemical structure and synthesis. The active antimalarial was identified in 1972 and named qinghaosu (or artemisinin in English) *(6).*

Clinical trials initiated in 1972 confirmed the high antimalarial activity of artemisinin for both uncomplicated and severe malaria, with results published in English in 1979 *(5,7).* Despite recent progress in producing semi-synthetic artemisinin using yeast extraction, *A. annua* plants remain the main source of the drug *(8).*

Artemisinin and its derivatives

Artemisinin was identified as a sesquiterpene lactone peroxide and is essentially insoluble in water and oil. This, together with the high recrudescence rates observed, prompted the Chinese scientists to conduct further research on developing artemisinin derivatives. It was found that the peroxy group in artemisinin was essential for the antimalarial activity and had to be maintained in any derivatives to exhibit antimalarial effect. Treating artemisinin with sodium borohydride generated dihydroartemisinin, which was found to be an even more potent antimalarial than artemisinin. Dihydroartemisinin served as the basis for the development of oil- and water-soluble derivatives. Of the derivatives developed from dihydroartemisinin, Chinese researchers selected two compounds for larger scale trials based on their stability and high antimalarial efficacy: the oil-soluble artemether and the water-soluble artesunate *(9–11).*

Pharmacokinetics and metabolism

Several formulations and routes of administration for artemisinin have been tested. Although artemisinin does not dissolve in oil or water, the first trials included administration of artemisinin suspended in oil or water in addition to rectal and oral administration. Chinese researchers and others used a dose of 10 mg/kg of artemisinin per day, with the possibility of a loading dose of 20 mg/kg on the first day *(12)*. Unlike artesunate or artemether, artemisinin is not metabolized to dihydroartemisinin, but acts as the primary antimalarial. Artemisinin is converted primarily into inactive metabolites, such as deoxyartemisinin and dihydrodeoxyartemisinin *(13,14).*

The elimination half-life of artemisinin is approximately one to three hours *(15,16)*. Following the administration of a drug, the total drug exposure across time depends both on the drug's absorption and the elimination rate. For artemisinin, rapid but incomplete absorption has been observed. An early study found a relative bioavailability of 32% when comparing oral administration of artemisinin with intramuscular administration of artemisinin suspended in oil *(17).* Several artemisinin drugs are inducers of drug-metabolizing enzymes, which augment the drug's clearance and lead to decreased drug plasma levels following repeated dosing *(18).* Studies have shown that artemisinin exhibits an auto-inductive effect on drug metabolism of an unusual magnitude *(19).* Artemether also undergoes auto-induction, but to a lesser extent than artemisinin *(20,21).* Artemisinin's auto-induction results in a five- to seven-fold decrease in the artemisinin plasma concentration over five to seven days of administration *(19).* The overall induction capacity of a drug depends on the combined effect of the

parent drug and the drug metabolites. Unlike artemether, artemisinin metabolizes into at least one inducing metabolite, deoxyartemisinin. This helps explain why auto-induction persists for days after a single dose of artemisinin, despite artemisinin's short elimination half-life *(14,18,19,21–23).* Consequently, when given repeatedly, the dose of artemisinin must be increased to achieve the same plasma concentrations. If not, the repeated dose could yield sub-therapeutic drug levels.

Efficacy

The potency of artemisinin and its derivatives has been evaluated in various in vitro experiments with different strains of *P. falciparum.* When investigating the drug concentration needed to inhibit 50% of the parasites' activity, the IC_{50}, artemisinin has consistently been found to be two to five times less potent than its derivatives dihydroartemisinin, artesunate and artemether *(24,25)*. Consequently, higher doses of artemisinin are required to achieve the same antimalarial activity.

In vivo drug efficacy is evaluated with respect to the proportion of patients in whom infection recurs within a defined period and, to a lesser extent, the speed at which symptoms resolve and parasitaemia declines. Artemisinin and its derivatives affect a broader range of the asexual stages of parasites than other antimalarials. As a result, artemisinin and its derivatives can quickly reduce the parasitaemia, leading to a rapid clinical response. However, already the earliest Chinese studies showed that if artemisinin is given orally for only three days, a high proportion of patients will have a recurrence of parasitaemia within 28 days. To prevent recurrent parasitaemia, seven days of treatment is needed when using artemisinin or an artemisinin derivative as a monotherapy *(7,26).* In practice, however, the rapid clinical response means that patients feel well after a few days of treatment, making adherence to the full seven-day treatment low.

Development of ACTs

The advantages and disadvantages of artemisinin and artemisinin derivatives have been clear from the earliest clinical trials. The drugs are well tolerated and fast-acting, and quickly reduce the number of parasites in the blood. However, effective drug concentration levels are only maintained in the plasma for a relatively brief period after drug administration, and short oral treatment courses result in high rates of recrudescence. Based on these findings, the idea to combine an artemisinin derivative with a partner drug with a longer half-life emerged quickly. ACTs take advantage of the rapid action of the artemisinin derivatives, while the partner drug helps to prevent recrudescence, even after a short three-day treatment *(27).*

The following artemisinin derivatives are used in the ACTs currently recommended by WHO for the treatment of uncomplicated *P. falciparum* malaria (see **box 1** for recommendations):

- artemether (in artemether-lumefantrine),
- artesunate (in artesunate-amodiaquine, artesunate-mefloquine, artesunate-sulfadoxine-pyrimethamine, and artesunate-pyronaridine[1]), and
- dihydroartemisinin (in dihydroartemisinin-piperaquine) *(20)*.

Artemisinin-based medicines are difficult to manufacture and co-formulate with other compounds, and they are susceptible to degradation in high temperatures and humidity. The pharmaceutical industry has contributed to the improvement of antimalarial drug quality by ensuring that formulations, manufacturing, and storage adhere to Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP). The WHO Prequalification Programme (WHO/PQP) evaluates the quality

[1] Currently recommended for use only in areas with multidrug resistance and with few alternative treatments.

of specific pharmaceutical products based on the review of product dossiers submitted by the manufacturing company and inspection of the manufacturing facilities.

Box 1: WHO recommendations for the treatment of malaria

WHO recommendations

WHO recommends ACTs as the first-and second-line treatment for uncomplicated *P. falciparum* malaria, as well as for chloroquine-resistant *P. vivax* malaria. Currently, five different ACTs are recommended by WHO *(20)*. In areas where other ACTs are failing, the use of artesunate-pyronaridine, a new ACT that has received a positive scientific opinion from the European Medicines Agency, is to be considered. For severe malaria, injectable artesunate is recommended or injectable artemether where artesunate is not available. Treatment with injectable artesunate or artemether must be followed by a full three-day treatment with an ACT when they have received at least 24 hours of parenteral therapy and can tolerate oral therapy.

Resistance

Treatment failure occurs when a treatment fails to clear parasites from a patient's blood or fail to prevent their recrudescence. Drug resistance is one potential cause of treatment failure, but other factors can contribute to treatment failure, including incorrect dosage, poor patient compliance, poor drug quality, and drug interactions (for definitions see **box 2**).

Drug resistance arises as a result of genetic changes that occur at random. If a genetic trait gives a parasite a survival advantage when exposed to a drug, this genetic trait can be selected for under drug pressure. For some drugs, a single genetic event may be all that is required; in other cases, multiple independent events may be necessary *(28).* Selection of a genetic trait that provides a survival advantage is more likely when the parasite population is exposed to sub-therapeutic levels[2] of an antimalarial drug *(29).*

The loss of other drugs and the dependency on artemisinin derivatives to treat millions for *P. falciparum* malaria every year raised considerable concerns that resistance would also emerge to artemisinin and its derivatives. The development of ACTs combining an artemisinin derivative with a partner drug helps to ensure that parasites are not exposed to therapeutic or sub-therapeutic doses of artemisinin alone. However, widespread use of different forms of oral artemisinin-based monotherapy (oAMT) continued to pose a threat to artemisinin and its derivatives. The risks of oAMT are augmented by many patients prematurely stopping treatment. Consequently, in 2007, WHO Member States adopted World Health Assembly resolution WHA60.18, which calls for a progressive removal of oAMTs from markets and the deployment of ACTs instead *(30).*

Full resistance to artemisinin and its derivatives has not yet been identified anywhere in the world. In the Greater Mekong Subregion, there has been a shift where parasite clearance is delayed after treatment, so more patients still have parasites in the blood on day 3 after a treatment with oAMT or with an ACT. This delayed clearance is called artemisinin partial resistance. However, provided that the monotheraphy is given at correct doses for seven days or that ACT partner drug is efficacious, the parasites will be cleared, and the patient cured. The changes in clearance time have been found to be associated with several genetic mutations in the *PfKelch13* (K13)-propeller domain *(31)*.

[2] A concentration below the concentration that provides the maximum possible effect

Box 2: Definition of resistance

- *Treatment failure*: the inability to clear parasites from a patient's blood or to prevent their recrudescence after the administration of an antimalarial. Many factors can contribute to treatment failure, including incorrect dosage, poor patient compliance, poor drug quality, and drug interactions and resistance
- *Antimalarial resistance:* the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject
- *Multidrug resistance (MDR)*: resistance to more than two antimalarial compounds of different chemical classes. This term usually refers to *P. falciparum* resistance to chloroquine, sulfadoxine-pyrimethamine, and a third antimalarial compound.
- *Artemisinin partial resistance*: delayed parasite clearance following treatment with an artesunate monotherapy or with an ACT

Surveillance of recommended treatment for uncomplicated malaria

Recommendations on the first- and second-line treatment for malaria patients need to be based on updated information on drug efficacy. Therapeutic efficacy studies (TESs) done at regular intervals at the same sites allow for early detection of changes in parasite susceptibility and timely revision of malaria treatment policies. TESs are done in accordance with a standard protocol wherein drug administration is supervised, the results of microscopic examinations of blood films are validated, and the origin and quality of the drugs are verified. Therapeutic outcomes are assessed on the final day of the study (day 28 or 42) *(32).* TESs can be supplemented by the monitoring of genetic changes associated with resistance.

Some countries recommend only one specific ACT as first-line treatment, while others recommend several ACTs as potential first-line treatment. Artemether-lumefantrine or artesunate-amodiaquine are the first-line treatment policies used in most African countries for the treatment of uncomplicated *P. falciparum* malaria. Some countries also allow for the use of dihydroartemisinin-piperaquine as first-line treatment. TESs have shown generally very high efficacy rates for the ACTs tested. Between 2010 and 2017, TESs using artemether-lumefantrine, artesunate-amodiaquine and dihydroartemisinin-piperaquine showed average efficacy rates of 98.1%, 98.5% and 99.3%, respectively. There have been a few outliers where studies showed higher failure rates. However, when these studies were repeated, similar failure rates were not reported *(33).*

Non-pharmaceutical use of *Artemisia* for malaria

Artemisia is a large, diverse genus of plants with nearly 400 species. Artemisinin is found in highest quantities in *A. annua,* but it has also been found in minor quantities in *A. apiacea* and *A. lancea.* Some scholars believe that it was not *A. annua* but *A. apiacea* that was used in China 2000 years ago *(34). A. afra* is an *Artemisia* species that does not contain artemisinin but has been proposed for the treatment of malaria.

The overview below focuses primarily on *A. annua* and secondarily on *A. afra*, as these are the *Artemisia* species for which most information is available, and that are most often promoted as potential treatments for malaria.

A. annua

A. annua is so named because it is the only member of the genus with an annual cycle. It is an herb native to Asia, but it now grows in many countries, including in Africa, Europe and South America. Although *A. annua* originated in temperate climates, it has been grown in subtropical and tropical areas *(35).*

Cultivation and processing of *A. annua*

The amount of artemisinin found has varied, and concentrations from 0.02% to 1.07% have been reported in the dried leaves of wild samples. Hybrids have been cultivated with a reported artemisinin content of 1.38% in the dried leaves. In experiments, concentrations of up to 2% has been achieved. In addition to genetics, many factors can affect the artemisinin content, including when in the season the harvesting takes place, temperature, nutrient availability, and from where on the plant the leaves are harvested *(36).*

Processing, drying procedures and storage conditions further influence the artemisinin content. Too high moisture content in the leaves can cause mould, yeast and bacteria. During storage, the relative air humidity and temperature can have a big impact on artemisinin's stability. Even at 20 °C, a relative air humidity of 85% will cause degradation of artemisinin after six months of storage. No matter the humidity, storage over 40 °C will cause loss of artemisinin content *(37).*

In addition to artemisinin, *A. annua* contains many compounds from different chemical classes, including terpenes, flavonoids and phenolic acids *(38,39).* There is only limited information available of the effects of the farming, harvesting, drying, storage and preparation methods on the amounts of the other chemical compounds found in *A. annua (38).* However, the content of other compounds is known to be affected by where the plant is grown, and the strain used.

WHO has developed guidelines on good agricultural and collection practices (GACP) for medicinal plants *(40),* including specific guidelines for *A. annua L. (41).* While the idea of home-grown or small-scale cultivation of *Artemisia* as a source of malaria treatment is compelling, the practices and procedures needed to ensure that the materials used have the expected content are difficult to establish and maintain. These practices are generally not possible to implement in the context of small-scale cultivation. Comparing large- and small-scale cultivators have shown an average drop of 0.3 percentage point in the artemisinin content. Consequently, the content and quality of the *Artemisia* plant materials promoted for use in herbal remedies for malaria treatment and prevention vary substantially.

Preparation methods for herbal remedies using *A. annua*

Different preparation methods have been proposed for the use of *A. annua* plant materials. These include preparation of juice from the whole fresh plant or preparation of tea from the dried leaves. Recently, some researchers have suggested the ingestion of the powdered dried leaves for therapeutic use instead of tea. The powdered leaves are either encapsulated in cellulose or gelatine capsules, or compressed into tablets *(38,42–45)*.

In the ancient text of Chinese *Materia Medica*, the method prescribed consisted of soaking the fresh plant (leaves and stem) in water, and then wringing out the whole plant and ingesting its juice. In later Chinese references, another method involved soaking the plant in urine rather than water or pounding the fresh herb to produce a juice *(34,46).*

Those promoting artemisinin tea, typically suggests adding 1 L of boiling water to 5 g of dried leaves, leaving the mixture to cool for 15 minutes and then filtering it. The recommendation given most often is to drink 1 L of this tea over a 24-hour period for seven consecutive days (https://maison-artemisia.org) *(47)*. Some go so far as to suggest administering the tea rectally as an enema in

unconscious patients (https://anamed.org/en/). Alternative preparation methods tested for the tea include adding 9 g instead of 5 g of dried leaves per litre of water; allowing the leaves to stand in the water for shorter or longer than 15 minutes before filtering; stirring the tea repeatedly while cooling; or squeezing residual water out of the leaves after filtration. Another alternative method tested is, instead of adding boiling water to the dried leaves, adding the leaves to the water, heating the mixture to boiling point, and keeping it boiling for a period before filtering *(47–51).*

Content of *A. annua* herbal remedies

The method of preparation affects the amount of artemisinin and other chemical compounds that will be administered and absorbed. Little research has been done on the traditional methods of soaking the fresh whole plant in water followed by wringing or pounding. One study using a hybrid *A. annua* plant found that the pounded juice contained up to 20 times as much artemisinin per litre than the tea made from the dried leaves of the same *A. annua* hybrid *(46).* No information is available on how quickly the artemisinin content degrades in the juice.

Different studies have examined the extraction efficiencies of artemisinin in the making of artemisinin tea. Van der Kooy and Verpoorte *(49)* found extraction efficiencies of 26.1% by adding boiling water to 9 g of dried leaves and allowing the leaves to remain in the water for 10 minutes. This approach resulted in artemisinin concentrations of 23.9 ± 5.1 mg/L. When Räth et al. *(52)* used a similar method, steeping 9 g of dried leaves for 10 minutes but briefly stirring the mixture and squeezing the leaves after filtration, they achieved extraction efficiencies of 76%. Their method resulted in artemisinin concentrations of 94.5 mg/L. Van der Kooy and Verpoorte *(49)* were able to increase the extraction efficiencies by boiling the mixture for two to five minutes; boiling for 10 minutes reduced the extraction efficiencies. Overall, studies done in controlled conditions using 5 or 9 g hybrid *A. annua* found that tea content varied from 8.36 mg to 117.2 mg artemisinin per litre of tea, depending on the method and plant material used. Only in one of the reviewed studies did the artemisinin content exceed 100 mg artemisinin per litre, equivalent to the daily dose of artemisinin that would be given to a child weighing 10 kg *(51,53,54).*

The low content of artemisinin in juice extractions, teas and infusion preparations of plant materials led Elfawal et al. *(44)* to emphasize that: "*WHO has cautioned against use of nonpharmaceutical sources of artemisinin because of the risk of delivering subtherapeutic doses that could exacerbate the resistance problem. This warning is valid given the low artemisinin content of juice extractions, teas and infusion preparations of plant materials used for most nonpharmaceutical plant-based therapies."* The authors then argued for the consumption of dried leaves.

Some authors have proposed that artemisinin's low water solubility is overcome by the presence of plant constituents with amphiphilic properties *(48).* Other authors have concluded that other constituents of the plant may decrease artemisinin's solubility *(52).* Stability studies indicate that artemisinin, when present in tea, does not degrade at room temperature for 24 hours *(49).* No information is available on the effect of the type of water (i.e., rain water, river water or tap water) on the extraction and stability of artemisinin *(53).*

Only a portion of the total number of compounds found in the *A. annua* plant material have been identified in the cold-water extracts and teas. Van der Kooy and Sullivan *(53)* reported that more than 600 different secondary metabolites have been identified in *A. annua,* but only 37 compounds have been identified in cold-water extracts and teas. These mainly consist of terpenes, phenols, acetylenes, coumarins and flavonoids *(53).* Even the preparation of capsules or tablets from powdered leaves has been shown to alter the content. Therefore, it cannot be assumed that the compounds in tablets or capsules are the same as in the dried leaves used to produce them *(42).*

Other *Artemisia* species used in herbal remedies

The species *A. afra* grows throughout the southern and eastern parts of Africa and has been used in traditional medicine to treat a variety of ailments from asthma and rheumatism to malaria. It is a perennial woody shrub growing up to 2 m tall. It is used in different forms, including as an infusion wherein fresh leaves are added to a cup of boiling water and left for 10 minutes before straining.

Large variation in the chemical compounds of *A. afra* has been identified both between and within geographical areas. Large variation has also been found between the cultivated and wild populations. *A. afra* does not contain artemisinin, but contains terpenes that include sesquiterpene lactones and a number of other compounds including flavonoids. Concerns over the potential cardio- and neurotoxicity of some of the compounds have been reported *(55).*

Efficacy of non-pharmaceutical forms of *Artemisia* for malaria

To cure malaria, an efficacious dose of antimalarials needs to be administered to the patient. The variable effect of the preparation method on the content of the final herbal remedy means that even if it were possible to provide consistent and good-quality *Artemisia* plant material, the provided dose of artemisinin and other compounds would vary substantially. The WHO-recommended ACTs for treating uncomplicated *P. falciparum* malaria do not contain artemisinin but the more potent derivatives artesunate, artemether or dihydroartemisinin. Since artemisinin has an auto-inductive effect, when given repeatedly, the dose of artemisinin must be increased to achieve the same plasma concentrations in patients. Thus, the administration of non-pharmaceutical forms of *A. annua* could potentially lead to sub-therapeutic dosages of artemisinin due to both the inconsistencies in the artemisinin content of the herbal remedies and the pharmacokinetics of artemisinin.

Those promoting the use of *A. annua* have proposed that other chemical compounds in the plant enhance its efficacy compared to administration of the pure artemisinin compound. It is suggested that the antimalarial activities of other plant compounds make the plant material function as a combination therapy, or that some compounds may increase the efficacy or bioavailability of artemisinin.

Comparing the in vitro efficacy of artemisinin and *A. annua* extract, some researchers have concluded that the in vitro activity cannot come from the artemisinin content alone *(56,57).* However, a number of other studies have found that the in vitro efficacy of the tea correlates well with the artemisinin content in the different extracts tested *(54,58).* Wright et al. *(46)* tested the antimalarial activity of *A. annua* juice in vitro and in mice and found that the efficacy of the juice was consistent with the artemisinin content of the juices tested.

Several of the other compounds found in *A. annua* have been shown to have some weak antimalarial activity against *P. falciparum*, but the concentrations needed are orders of magnitude higher than for artemisinin *(51).* To be considered a compound with strong antimalarial activity, a compound needs to have an IC_{50} measured in nanograms per ml. Mouton et al. *(54)* found pure artemisinin to have an IC_{50} of 5.48 ± 1.54 ng/ml. Other compounds in artemisinin tea, such as terpenes, phenolic acids and flavonoids, have an IC_{50} measured in micrograms per ml, meaning that the needed concentration is about 1000- to 10 000-fold higher than for artemisinin – a level that is incompatible with therapeutic efficacy *(51,59).*

Synergism between artemisinin and other constituents rather the antimalarial effect of the other constituents has been proposed as playing a role in the efficacy of non-pharmaceutical forms of artemisinin. Testing the synergetic effect of individual compounds, Liu et al. *(60)* found that adding five different flavonoids at concentrations too low for the flavonoids alone to appear to have any effect (5µM/l) reduced the IC_{50} of artemisinin in the range of 9% to 55%. Testing individual compounds at higher levels of concentration, Suberu et al. *(51)* found some compounds to be antagonistic

(including casticin), some to be additive, and some to be synergistic. Weathers and Towler *(61)* confirmed the presence of flavonoids such as casticin and artemetin in *A. annua* tea, but stated that the extraction efficiency of these flavonoids was too low; therefore, they ruled out synergism. The poor extraction efficiency of flavonoids and their rapid degradation in tea has led some to propose administration of the whole plant material instead of the extract *(61).*

A recent in vitro study by Czechowski et al. *(62)* focused on the potential effect of flavonoids. The study compared extracts from three strains of *A. annua*: one wild-type; one with a mutation inhibiting flavonoid biosynthesis but containing artemisinin; and one with mutations severely impairing artemisinin production but not affecting flavonoid biosynthesis. Comparing the efficacy of *A. annua* extracts with and without flavonoids showed no significant difference, indicating that the flavonoids did not contribute to antimalarial activity. To investigate any potential antiplasmodial activity of artemisinin-unrelated compounds in *A. annua*, the researchers tested extracts from *A. annua* without artemisinin. The extracts that were among the highest in total flavonoid content of the material used showed very low to no antiplasmodial activity. The authors concluded that the in vitro bioactivity of flavonoids against *P. falciparum* is negligible compared to that of artemisinin.

Looking at bioavailability, a study in mice by Weathers et al. *(43)* found increased bioavailability of artemisinin when using whole plant dried leaves. However, when studying the bioavailability of artemisinin in tea in healthy human males, Räth et al. *(52)* arrived at different results. Here, the authors found that artemisinin's bioavailability in tea was similar to that found in the administration of pure artemisinin in tablets.

Overall, the evidence does not support the claim that other compounds in *A. annua* with antimalarial activity are present in the herbal remedies at concentrations at which such herbal remedies could be considered anything other than monotherapies. If research had shown there to be compounds in the plants that could stabilize, could increase the bioavailability, or could increase the efficacy of artemisinin, it would warrant further research, but it would not change the reality that the extracts at best function as weak artemisinin monotherapies.

Testing the in vitro efficacy of tea made using two different samples of *A. afra*, one from Uganda and one from South Africa, Mouton et al. *(54)* were unable to detect antimalarial activity. Studies indicate that any antiplasmodial compounds of *A. afra* may be more soluble in lipophilic solvents than in hydrophilic solvents. The IC_{50} reported not using water but lipophilic, dichloromethane or methanolic extract range from 4.0±1 µg/ml to 15.3 ±1 µg/ml *(55).* A recent review by du Toit and van der Kooy *(63)* likewise concluded that tea infusions do not appear to show any in vitro activity.

Clinical trials using non-pharmaceutical forms of *Artemisia*

The in vivo effectiveness of *Artemisia* extracts has mainly been assessed through animal models of rodent malaria. While these models are useful for research purposes, including for drug screening, results cannot be extrapolated to human *P. falciparum* malaria. In general, the few clinical studies completed have often been of relatively low quality, been conducted with few patients, included too short a follow-up period, or been poorly controlled for bias. In some studies, it was unclear how the patients were diagnosed or whether the WHO criteria were used to classify the patients as having asymptomatic, uncomplicated or severe malaria. When malaria rapid diagnostic tests (RDTs) are used, the patient may be classified as having malaria weeks after the parasites are cleared from the blood.

The studies have reported no adverse effects. However, if non-pharmaceutical forms of *A. annua* can lead to the administration and absorption of significant levels of artemisinin, there may be concerns, for instance, when giving these forms to pregnant women in the first trimester.

A small randomized study by Mueller et al. *(64)* in eastern Democratic Republic of the Congo in 2001 enrolled 132 *P. falciparum* patients. When using the regimen most frequently proposed (5 g of dried *A. annua* leaves in 1 L of water per day for seven days), 21 out of 32 patients (65.6%) had recurrent

parasitaemia on day 35. In the group receiving tea made from 9 g of dried leaves per litre, 21 out of 30 patients (70%) had recurrent parasitaemia on day 35. In the control group receiving quinine, seven out of 34 patients (21%) had recurrent parasitaemia on day 35. Genotyping was not used to distinguish between reinfections and recrudescence. The authors concluded that the much lower recurrence rate in the parallel quinine group indicated that the observed recurrences in the *A. annua* group were due to recrudescence and not reinfection. Because of this high rate of recrudescence and the risk of possible resistance development, the authors concluded that monotherapy with tea preparations from *A. annua* could not be recommended as a treatment option for malaria.

In 2002–2003, Blanke et al. *(65)* did a small study in semi-immune adults in the United Republic of Tanzania. Seven patients were assigned to a group treated with *A. annua* tea made from 5 g of dried leaves per litre, six patients were treated with *A. annua* tea made from 9 g of dried leaves per litre, and 10 patients were treated with sulfadoxine-pyrimethamine. On day seven, three of the 13 patients who were treated with *A. annua* tea had been excluded: two because they took sulfadoxine-pyrimethamine and one because the patient developed signs of severe malaria on day one and was given a rescue treatment (quinine). Of the 10 remaining patients treated with *A. annua* tea, seven did not show parasitaemia on day seven. In the group treated with sulfadoxine-pyrimethamine, one was excluded due to hyperparasitaemia on day zero. Of the nine remaining patients, seven did not show parasitaemia on day seven. On day 28, nine of the 10 patients treated with *A. annua* tea were parasitaemic. In the group treated with sulfadoxine-pyrimethamine, one was lost to follow-up before day 28. Of the eight remaining patients, five were parasitaemic. Sulfadoxine-pyrimethamine resistance was already widespread at the time of the study and therefore the high failure rate was not surprising. Due to the high rate of recrudescence in all three groups, the study was stopped, and the authors concluded that *A. annua* tea could not be recommended for treating uncomplicated *P. falciparum* malaria.

A study to evaluate the efficacy and safety of locally grown *A. annua* in patients with uncomplicated falciparum malaria was conducted in Benin *(66).* Artemisinin content in the plant was 0.30% of dry weight mass. Tea was made using 12 g/L of dried leaves, infused for 15 minutes, and then administered in four doses of 250 ml over a 24-hour period (or 125 ml in children of 10–13 years of age) for seven consecutive days – the equivalent of receiving 36 mg (or 18 mg for children) of artemisinin in four divided doses. The study consisted of a single open-label cohort of 108 (out of 130 patients enrolled) who completed both the treatment and the follow-up visit up to day 28. Authors reported an adequate clinical and parasitological response of 100% at day 28.

A study of the safety and efficacy of *A. annua* and *A. afra* was conducted in the Democratic Republic of the Congo *(67)*. The study consisted of three groups of adult patients with uncomplicated *P. falciparum* malaria who were treated with capsules containing powdered leaves of *A. annua* from Luxembourg (AAL) (20 patients), *A. annua* from Burundi (AAB) (37 patients), or *A. afra* (AAF) (25 patients). Each patient received 15 capsules: three administered on the first day and two capsules on each of the following six days, corresponding to a total of 15 g of AAL, 7.5 g of AAB, or 7.5 g of AAF. Fever clearance occurred within 48 hours, and 85% were free of parasites after seven days for AAL, 76% for AAB, and 40% for AAF. There is no information on whether patients were followed up beyond day seven and whether rescue treatment was given to patients who were still parasitaemic after their treatment course.

Daddy et al. *(68)* reported on the treatment of 18 patients in 2016 in the Democratic Republic of the Congo with tablets made of powdered dried *A. annua* leaves. Although the patients had previously been treated with three days of artemether-lumefantrine, all reportedly still had parasites in the blood and fever with symptoms, which led the authors to classify them as having severe malaria. The patients received intravenous artesunate, but the treatment failed for all 18 patients. The patients were then treated with 0.5 g of dried leaves twice a day for five days, with a reduced dose for patients weighing less than 30 kg. Adults received a total dose of 55 mg artemisinin. The patients were released from hospital when parasites were microscopically undetectable and clinical symptoms were cleared.

No further follow-up was done. The authors concluded that since the dried *A. annua* leaves were administered 24 hours after the last intravenous artesunate treatment, the dried leaves alone had treated what they labelled as artemisinin-resistant malaria.

Onimus et al. *(45)* reported on the use of capsules with powdered *A. annua* leaves in 25 patients with asymptomatic parasitaemia being operated on for orthopaedic disorders. Eleven patients received five capsules over 36 hours, and 14 patients received seven capsules over 60 hours. Each capsule contained 200–250 mg dried leaves, and the artemisinin content was 0.1%. Thus, the patients received a total artemisinin dose of 1–1.75 mg. The aim of the *A. annua* treatment was not to eradicate parasites from the blood but to prevent malaria attacks in the first post-operative days. Only one patient was found to be cleared of parasites post-treatment. The reported parasitaemia pre-treatment was on average 432 parasites/ml and 165 parasites/ml post-treatment. The reported parasitaemia was so low that it would not have been possible to detect, so there may have been a mistake in the reporting. The article does not list other treatments given to the patients in connection to the operations, nor does the article state whether any curative treatments were offered to the patients, as should be done.

A large study was conducted in 2015 in the Kalima district, Democratic Republic of the Congo by Munyangi et al. *(69)*. The aim of the study was to show that *A. annua* and/or *A. afra* infusions were superior or at least equivalent to artesunate-amodiaquine against malaria. The study followed a multi-centre, randomized, double-blind design with a follow-up of 28 days. It was conducted in children (> 5 yrs) and adults with confirmed, uncomplicated *P. falciparum* malaria. The study was approved by local authorities. Out of 2000 patients screened, 957 patients were enrolled from five different locations: 472 were enrolled in the artesunate-amodiaquine group, and 471 in the *Artemisia* groups (248 in *A. annua*, 223 in *A. afra*). The patients in the artesunate-amodiaquine arm received artesunate-amodiaquine for three days followed by placebo tablets for four days; they were also given tea infusions containing 0.2 g of plant material per litre for the seven days. In the *Artemisia* arms, the patients were given 0.33 L of tea every eight hours for seven days. The tea was made by adding 1 L of boiling water to 5 g of dried leaves and twigs of *A. annua* or *A. afra* and infused for 10 minutes. The patients in the *Artemisia* arms received placebo tablets for seven days. Artesunate-amodiaquine tablets were obtained from the manufacturer. The artesunate-amodiaquine placebo was a pill-shaped saccharose/glucose tablet purchased at a pharmacy. On day 28, the authors reported recurrent parasitaemia in nine out of 248 patients (3.6%) treated using *A. annua*, in 25 out of 223 patients (11.2%) treated using *A. afra,* and in 310 out of 472 patients (65.7%) treated using artesunate-amodiaquine. They reported that some of those treated with artesunate-amodiaquine were found to have parasites 14 days after the start of treatment. The reported treatment failures with artesunate-amodiaquine occurred mainly within the first 14 days.

Munyangi et al.'s *(69)* results for artesunate-amodiaquine conflict with other available data. Even in areas of high drug resistance in South-East Asia, parasites never remain in patients 14 days after the administration of an ACT. When treatment failures occur with ACTs, they almost always occur at the end of the follow-up period. Between 2011 and 2013, 13 TESs were done in the Democratic Republic of the Congo. The studies looked at the efficacy of artesunate-amodiaquine, artesunate-lumefantrine and dihydroartemisinin-piperaquine; the ACTs tested all showed high efficacy (94.8–100%) *(70).* Seven of the studies tested artesunate-amodiaquine by enrolling a total of 695 patients. The studies were done by Médecins Sans Frontières *(71),* Mahidol Oxford Research Unit *(72)* and the national malaria control programme. These studies showed an efficacy of 95.3–100%. In 2017, the University of Kinshasa conducted TESs for three different ACTs (artesunate-amodiaquine, artesunate-lumefantrine and dihydroartemisinin-piperaquine) in five sites in the Democratic Republic of the Congo. These studies also found high efficacy for all three ACTs (95–100%). In the treatment arms testing artesunate-amodiaquine, a total of 451 malaria patients were enrolled and the efficacy was ≥ 95.0% (K. Mesia 2019, personal communication).

Furthermore, Munyangi et al.*'s (69)* study reported that artesunate-amodiaquine had higher efficacy in children (50%) than in adults (30%). In endemic areas, efficacy is normally higher in adults who are likely to be semi-immune. In the patients treated with *A. annua*, a surprising result was to find only 3.6% of patients with parasites on day 28 after treatment, considering the drug has a short half-life. Even with efficacious treatment, some reinfections would be expected. The cure rate of *A. afra* was reported to be 88.8%, which is unexpectedly high but still below the WHO-recommended threshold of 95% for a new malaria treatment *(20).* As *A. afra* does not contain any known compounds with substantial antimalarial activity, some authors (*63*) suggest that the only way to explain its surprising efficacy is that *A. afra* may contain an as yet unknown pro-drug that becomes active after metabolization.

It is difficult to explain Munyangi et al.'s *(69)* results, but the design and conduct of their study suffered from a number of deficiencies and potential biases. For example, there is insufficient information on the randomization procedures and treatment assignment; the artesunate-amodiaquine placebo tablet obtained from a pharmacy and given to those receiving tea may not have been identical to the active tablets, thus compromising the double-blinding; amodiaquine blood concentrations were not assessed; data collection and analysis were not blinded; genotyping studies were not performed, apparently due to degradation of blood samples; and no clear definitions of outcomes and classifications were provided.

Artemisia herbal remedies have also been promoted for the prevention of malaria. However, the short half-life of artemisinin means that this drug is not suitable for prevention. *A. annua* capsules and liquid formulations are being sold over the Internet, claiming their safety and efficacy for the prophylaxis and treatment of malaria. Lagarce et al. *(73)* reported two cases of severe *P. falciparum* malaria that required intensive care following prophylaxis with non-pharmaceutical *A. annua* in French travellers.

Ogwang et al. *(74)* conducted a randomized trial with 132 flower farm workers. Participants were randomized to a group receiving *A. annua* tea (67 participants) once a week or a group receiving a tea made of *Thea sinensis* (65 participants) once a week. A total of 84 workers (41 in the *A. annua* group and 43 in the control group) were followed up for nine months. The authors found that at the end of the study, 12 in the *A. annua* group and 26 in the control group had reported more than one episode of malaria. The authors speculated this this may have been due to compounds other than artemisinin such as flavonoids. The two groups appear to have been well randomized in terms of age and sex, but there was a significant difference in bed net use, as 35.8% of the group receiving *A. annua* but only 18.5% of the control group reported using a bed net at the start of the study.

WHO's position on the use of non-pharmaceutical forms of *Artemisia*

WHO does not support the promotion or use of *Artemisia* plant material in any form for the prevention or treatment of malaria.

WHO's position is based on the following considerations:

- **The content of the *Artemisia* herbal remedies given for malaria treatment and prevention varies substantially.**

  The content and quality of the *Artemisia* herbal remedies are affected by variations in the content of the plant material and the preparation method**.**

  A range of factors can affect the content of *Artemisia*, including genetics, when in the season the harvesting takes place, temperature, nutrient availability, and from where on the plant the leaves are harvested. Processing, drying procedures, and storage conditions further influence the content of plant materials. It is not feasible to implement the required level of quality control for cultivation, harvest and post-harvest aspects of *Artemisia* in the context of home-grown or small-scale cultivation.

The preparation method will cause further variation. The content of *Artemisia* tea is highly influenced by factors such as the temperature of the water. Even given in tablet or capsule form, the content of these will differ from the original source material.

- **Content in *Artemisia* herbal remedies is often insufficient to kill all the parasites and to prevent recrudescence.**

  To achieve high efficacy rates, sufficient levels of artemisinin needs to be administrated and absorbed over seven days. The pharmacological properties of artemisinin mean that higher levels of artemisinin need to be administered on the last days of treatment than on the first days in order to achieve the same artemisinin blood levels. Too short treatments or too low blood levels of artemisinin will result in either failure to clear parasites from the blood or high levels of recrudescence. *A. annua* contains varying levels of artemisinin. Herbal remedies prepared using *A. annua* with significant artemisinin content may improve symptoms, but are likely to result in high recrudescence rates. The available evidence does not support claims that the antimalarial activity of other plant constituents or synergism between artemisinin and other constituents will significantly increase the efficacy of non-pharmaceutical forms of *A. annua.*

  A. afra does not contain artemisinin or any other compound identified as having significant antimalarial activity in vitro.

- **Widespread use of *A. annua* herbal remedies could hasten the development and spread of artemisinin resistance.**

  Artemisinin and artemisinin derivatives are the key compound in the ACTs used to treat millions suffering from malaria. The artemisinin derivative, artesunate, is used to save the lives of those suffering from severe malaria. Resistance causing the loss of these drugs would be a disaster. In 2007, WHO Member States adopted World Health Assembly resolution WHA60.18 calling for a progressive removal of oral artemisinin-based monotherapies from markets and deployment of ACTs instead. This decision was made to help protect artemisinin drugs from resistance. If consumption of *A. annua* becomes widespread, any potential weak antimalarial activity of other compounds in *A. annua* would not be sufficient to protect artemisinin from resistance. Resistance is more likely to develop and spread when a parasite population is exposed to sub-therapeutic levels of an antimalarial drug. The varying artemisinin content of *A. annua* herbal remedies means that widespread use of these remedies could lead to many people having such sub-therapeutic levels of artemisinin in their blood.

- **Artemisinin in any form does not work well as prevention against malaria.**

  Artemisinin has a short elimination half-life, meaning that it only remains in the blood at therapeutic levels for a short time. Therefore, artemisinin is not promoted for use in malaria chemoprophylaxis in any form.

- **Affordable and efficacious treatments for malaria are available.**

  WHO recommends ACTs for the treatment of uncomplicated *P. falciparum* malaria. Artemisinin partial resistance and resistance to some partner drugs do pose a challenge in parts of South-East Asia. However, there are still highly efficacious treatments available that can cure all strains of malaria. A complete treatment with an ACT can be procured for less than US$ 2. We need to remain committed to ensure that all those affected by malaria have access to ACTs. Countries need to strengthen their regulatory systems to protect patients from counterfeit and substandard treatments; this includes any products promoted for treatment of malaria without the necessary information in terms of their content, quality, safety and efficacy.

Herbal medicines have been a key source for the discovery of antimalarial medicines. It is possible that future antimalarial compounds will also be discovered through research on the herbal treatments used in the past. However, any research needs to respect the ethical principles for medical research involving human subjects and be approved by local ethical committees. The well-being of the individual research subject must take precedence over all other interests. Medical research involving human subjects must conform to generally accepted scientific principles and be based on thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory experimentation *(75).*

References

1. Greenwood D. The quinine connection. J Antimicrob Chemother. 1992;30(4):417–27.
2. Renslo AR. Antimalarial drug discovery: from quinines to the dream of eradication. ACS Med Chem Lett. 2013;4:1126–8.
3. Payne D. Spread of chloroquine resistance in Plasmodium falciparum. Parasitol Today. 1987;3(8):241–6.
4. White NJ, Hien TT, Nosten FH. A brief history of Qinghaosu. Trends Parasitol. 2015;31(12):607–10. doi:10.1016/j.pt.2015.10.010.
5. Guo Z. Artemisinin anti-malarial drugs in China. Acta Pharm Sin B. 2016;6(2):115–24. doi:10.1016/j.apsb.2016.01.008.
6. Su XZ, Miller LH. The discovery of artemisinin and Nobel Prize in Physiology or Medicine. Sci China Life Sci. 2015;58(11):1175–9.
7. Qinghaosu Antimalaria Coordinating Research Group. Antimalaria studies on Qinghaosu. Chin Med J (Engl). 1979;92:811–6.
8. Peplow M. Synthetic biology's first malaria drug meets market resistance. Nature. 2016;530:389–90. doi:10.1038/530390a.
9. Klayman DL. Qinghaosu (artemisinin): an antimalarial drug from China. Science. 1985;228(4703):1049–55.
10. Li Y, Wu YL. How Chinese scientists discovered qinghaosu (artemisinin) and developed its derivatives? What are the future perspectives? Med Trop (Mars). 1998;58(Suppl 3):9–12.
11. Chang Z. The discovery of Qinghaosu (artemisinin) as an effective anti-malaria drug: a unique China story. Sci China Life Sci. 2016;59:81–8. doi:10.1007/s11427-015-4988-z.
12. Stratégies d'utilisation des antipaludiques: besoins de données, traitement du paludisme non compliqué et prise en charge du paludisme pendant la grossesse. Rapport d'une consultation informelle, Genève 14–18 mars 1994. Geneva: World Health Organization; 1994 (WHO/MAL/94.1070; https://www.who.int/iris/bitstream/handle/10665/59532/WHO_MAL_94.1070_fre.pdf, accessed 1 August 2019).
13. Meshnick SR, Taylor TE, Kamchonwongpaisan S. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. Microbiol Rev. 1996;60(2):301–15.
14. Zang M, Zhu F, Li X, Yang A, Xing J. Auto-induction of phase I and phase II metabolism of artemisinin in healthy Chinese subjects after oral administration of a new artemisinin-piperaquine fixed combination. Malar J. 2014;13:214. doi:10.1186/1475-2875-13-214.
15. Guidelines for the treatment of malaria. First edition. Geneva: World Health Organization; 2006 (WHO/HTM/MAL/2006.1108; http://archives.who.int/publications/2006/9241546948_eng.pdf, accessed 1 August 2019).
16. Kyle DE, Teja-Isavadharm P, Li Q, Leo K. Pharmacokinetics and pharmacodynamics of qinghaosu derivatives: how do they impact on the choice of drug and the dosage regimens? Med Trop (Mars). 1998;58(3 Suppl):38–44.

17. Titulaer HA, Zuidema J, Kager PA, Wetsteyn JC, Lugt CB, Merkus FW. The pharmacokinetics of artemisinin after oral, intramuscular and rectal administration to volunteers. J Pharm Pharmacol. 1990;42:810–3.

18. Medhi B, Patyar S, Rao RS, Byrav DSP, Prakash A. Pharmacokinetic and toxicological profile of artemisinin compounds: an update. Pharmacology. 2009;84:323–32. doi:10.1159/000252658.

19. Gordi T, Huong DX, Hai TN, Nieu NT, Ashton M. Artemisinin pharmacokinetics and efficacy in uncomplicated malaria patients treated with two different dosage regimens. Antimicrob Agents Chemother. 2002;46(4):1026–31.

20. Guidelines for the treatment of malaria. Third edition. Geneva: World Health Organization; 2015 (https://www.who.int/malaria/publications/atoz/9789241549127/en/, accessed 1 August 2019).

21. Burk O, Piedade R, Ghebreghioghis L, Fait JT, Nussler AK, Gil JP, et al. Differential effects of clinically used derivatives and metabolites of artemisinin in the activation of constitutive androstane receptor isoforms. Br J Pharmacol. 2012;167(3):666–81. doi:10.1111/j.1476-5381.2012.02033.x.

22. Hassan Alin M, Ashton M, Kihamia CM, Mtey GJ, Björkman A. Multiple dose pharmacokinetics of oral artemisinin and comparison of its efficacy with that of oral artesunate in falciparum malaria patients. Trans R Soc Trop Med Hyg. 1996;90(1):61–5.

23. Salman S, Page-Sharp M, Batty KT, Kose K, Griffin S, Siba PM, et al. Pharmacokinetic comparison of two-piperaquine-containing artemisinin combination therapies in Papua New Guinean children with uncomplicated malaria. Antimicrob Agents Chemother. 2012;56(6):3288–97. doi:10.1128/AAC.06232-11.

24. Basco LK, Le Bras J. In vitro activity of artemisinin derivatives against African isolates and clones of Plasmodium falciparum. Am J Trop Med Hyg. 1993;49(3):301–7.

25. Wongsrichanalai C, Nguyen TD, Trieu NT, Wimonwattrawatee T, Sookto P, Heppner DG, et al. In vitro susceptibility of Plasmodium falciparum isolates in Vietnam to artemisinin derivatives and other antimalarials. Acta Trop. 1997;63(2-3):151–8.

26. White NJ. Qinghaosu (artemisinin): the price of success. Science. 2008;320(5874):330–4. doi.10.1126/science.1155165.

27. Jiang JB, Li GQ, Guo XB, Kong YC, Arnold K. Antimalarial activity of mefloquine and qinghaosu. Lancet. 1982;2(8293):285–8.

28. Valderramos SG, Valderramos JC, Musset L, Purcell LA, Mercereau-Puijalon O, Legrand E, et al. Identification of a mutant PfCRT-mediated chloroquine tolerance phenotype in Plasmodium falciparum. PLoS Pathog. 2010;6(5):e1000887. doi:10.1371/journal.ppat.1000887.

29. White NJ. Qinghaosu in combinations. Med Trop (Mars). 1998;58(3 Suppl):85–8.

30. Resolution WHA60.18. WHO reform. In: Sixtieth World Health Assembly, Geneva, 14–23 May 2007. Resolutions and decisions, annexes. Geneva: World Health Organization; 2007 (WHA60/2007/REC/1; http://www.who.int/gb/ebwha/pdf_files/WHASSA_WHA60-Rec1/E/WHASS1_WHA60REC1-en.pdf, accessed 1 August 2019).

31. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature. 2014;505:50–5. doi:10.1038/nature12876.

32. Methods for surveillance of antimalarial drug efficacy. Geneva: World Health Organization; 2009 (https://www.who.int/malaria/publications/atoz/9789241597531/en/, accessed 1 August 2019).

33. World malaria report 2018. Geneva: World Health Organization; 2018 (https://www.who.int/malaria/publications/world-malaria-report-2018/en/, accessed 1 August 2019).

34. Hsu E. The history of qing hao in the Chinese materia medica. Trans R Soc Trop Med Hyg. 2006;100(6):505–8.

35. Ferreira JFS, Janick J. Distribution of artemisinin in Artemisia annua. In: Janick J, editor. Progress in new crops. Arlington: ASHS Press; 1996:579–84.

36. Delabays N, Simonnet X, Gaudin M. The genetics of artemisinin content in Artemisia annua L. and the breeding of high yielding cultivars. Curr Med Chem. 2001;8:1795–801.

37. Simonnet X. Artemisia annua L.: harvest and post-harvest treatments (Report 2007–2008). Conthey: Mediplant; 2008. (https://www.mmv.org/sites/default/files/uploads/docs/publications/3-_MEDIPLANT_First_page_summary_intermediate_posting_3.pdf, accessed 1 August 2019).

38. Weathers PJ, Elfawal MA, Towler MJ, Acquaah-Mensah GK, Rich SM. Pharmacokinetics of artemisinin delivered by oral consumption of Artemisia annual dried leaves in health vs. Plasmodium chabaudi-infected mice. J Ethnophamacol. 2014;153(3):732–6. doi:10.1016/j.jep.2014.03.037.

39. Willcox M. Artemisia species: from traditional medicines to modern antimalarials—and back again. J Altern Complement Med. 2009;15(2):101–9. doi:10.1089/acm.2008.0327.

40. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. Geneva: World Health Organization; 2004 (https://www.who.int/medicines/publications/traditional/gacp2004/en/, accessed 1 August 2019).

41. Monograph on good agricultural and collection practices (GACP) for Artemisia annua L. Geneva: World Health Organization; 2006 (https://www.who.int/malaria/publications/atoz/9241594438/en/, accessed 1 August 2019).

42. Weathers PJ, Towler MJ. Changes in key constituents of clonally propagated Artemisia annua L during preparation of compressed leaf tablets for possible therapeutic use. Ind Crops Prod. 2014;62:173–8.

43. Weathers PJ, Arsenault PR, Covello PS, McMickle A, Teoh KH, Reed DW. Artemisinin production in Artemisia annua: studies in planta and results of a novel delivery method for treating malaria and other neglected diseases. Phytochem Rev. 2011;10(2):173–83.

44. Elfawal MA, Towler MJ, Reich NG, Weathers PJ, Rich SM. Dried whole-plant Artemisia annua slows evolution of malaria drug resistance and overcomes resistance to artemisinin. Proc Natl Acad Sci U.S.A. 2015;112(3):821–6. doi:10.1073/pnas.1413127112.

45. Onimus M, Carteron S, Lutgen P. The surprising efficacy of Artemisia annua powder capsules. Med Aromat Plants. 2013;2(3). doi:10.4172/2167-0412.1000125.

46. Wright CW, Linley PA, Brun R, Wittlin S, Hsu E. Ancient Chinese methods are remarkably effective for the preparation of artemisinin-rich extracts of Qing Hao with potent antimalarial activity. Molecules. 2010;15:804–12. doi:10.3390/molecules15020804.

47. Mueller MS, Karhagomba IB, Hirt HM, Wemakor E. The potential of Artemisia annual L. as a locally produced remedy for malaria in the tropics: agriculture, chemical and clinical aspects. J Ethnopharmacol. 2000;73(3):487–93.

48. de Ridder S, van der Kooy F, Verpoorte R. Artemisia annua as a self-reliant treatment for malaria in developing countries. J Ethnopharmacol. 2008;120:302–14. doi:10.1016/j.jep.2008.09.017.

49. van der Kooy F, Verpoorte R. The content of artemisinin in Artemisia annua tea infusion. Planta Med. 2011;77(15):1754–6. doi:10.1055/s-0030-1271065.

50. Carbonara T, Pascale R, Argentieri MP, Papadia P, Fanizzi FP, Villanova L, et al. Phytochemical analysis of herbal tea from Artemisia annua L. J Pharm Biomed Anal. 2012;62:79–86. doi:10.1016/j.jpba.2012.01.015.

51. Suberu JO, Gorka AP, Jacobs L, Roepe PD, Sullivan N, Barker GC, et al. Anti-plasmodial polyvalent interactions in Artemisia annua L. aqueous extract – possible synergistic and resistance mechanisms. PLoS One. 2013;8(11):e80790. doi:10.1371/journal.pone.0080790.

52. Räth K, Taxis K, Walz G, Gleiter CH, Li SM, Heide L. Pharmacokinetic study of artemisinin after oral intake of a traditional preparation of Artemisia annua L. (annual wormwood). Am J Trop Med Hyg. 2004;70(2):128–32.

53. van der Kooy F, Sullivan SE. The complexity of medicinal plants: the traditional Artemisia annua formulation, current status and future perspectives. J Ethnopharmacol. 2013;150:1–13. doi:10.1016/j.jep.2013.08.021.

54. Mouton J, Jansen O, Frédérich M, van der Kooy F. Is artemisinin the only antiplasmodial compound in the Artemisia annua tea infusion? an in vitro study. Planta Med. 2013;79(6):468–70. doi:10.1055/s-0032-1328324.

55. Liu NQ, van der Kooy F, Verpoorte R. Artemisia afra: a potential flagship for African medicinal plants? S Afr J Bot. 2009;75:185–95. doi:10.1016/j.sajb.2008.11.001.

56. Jansen FH. The herbal approach for artemisinin as therapy for malaria? Trans R Soc Trop Med Hyg. 2006;100(3):285–6.

57. De Donno A, Grassi T, Idolo A, Guido M, Papadia P, Caccioppola A, et al. First-time comparison of the in vitro antimalarial activity of Artemisia annua herbal tea and artemisinin. Trans R Soc Trop Med Hyg. 2012;106(11):696–700. doi:10.1016/j.trstmh.2012.07.008.

58. Silva LF, Magalhaes PM, Costa MR, Alecrim MD, Chaves FC, Hidalgo Ade F, et al. In vitro susceptibility of Plasmodium falciparum Welch field isolates to infusions prepared from Artemisia annua L. cultivated in the Brazilian Amazon. Mem Inst Oswaldo Cruz. 2012;107(7):859–66.

59. Weathers PJ, Towler M, Hassanali A, Lutgen P, Engeu PO. Dried-leaf Artemisia annua: a practical malaria therapeutic for the developing countries? World J Pharmacol. 2014;3(4):39–55.

60. Liu KC, Yang SL, Roberts MF, Elford BC, Phillipson JD. Antimalarial activity of Artemisia annua flavonoids from whole plants and cell cultures. Plant Cell Rep. 1992;11:637–40. doi:10.1007/BF00236389.

61. Weathers PJ, Towler MJ. The flavonoids casticin and artemetin are poorly extracted and are unstable in an Artemisia annua tea infusion. Planta Med. 2012;78(10):1024–6. doi:10.1055/s-0032-1314949.

62. Czechowski T, Rinaldi MA, Famodimu MT, Van Veelen M, Larson TR, Winzer T, et al. Flavonoid versus artemisinin anti-malarial activity in Artemisia annua whole-leaf extracts. Front Plant Sci. 2019;10:984. doi:10.3389/fpls.2019.00984.

63. Du Toit A, van der Kooy F. Artemisia afra, a controversial herbal remedy or a treasure trove of new drugs? J Ethnopharmacol. 2019;244:112127. doi:10.1016/j.jep.2019.112127.

64. Mueller MS, Runyambo N, Wagner I, Borrmann S, Dietz K, Heide L. Randomized controlled trial of a traditional preparation of Artemisia annua L. (annual wormwood) in the treatment of malaria. Trans R Soc Trop Med Hyg. 2004;98:318–21.

65. Blanke CH, Naisabha GB, Balema MB, Mbaruku GM, Heide L, Müller MS. Herba Artemisiae annuae tea preparation compared to sulfadoxine-pyrimethamine in the treatment of uncomplicated falciparum malaria in adults: a randomized double-blind clinical trial. Trop Doct. 2008;38:113–6. doi:10.1258/td.2007.060184.

66. Zime-Diawara H, Sissinto-Savi de Tove Y, Akogbeto OE, Ogouyemi-Hounto A, Gbaguidi FA, Kinde-Gazard D, et al. Etude de l'efficacité et de la tolérance d'une tisane à base de Artemisa annua L. (Asteraceae) cultivée au Bénin pour la prise en charge du paludisme. Int J Biolog Chem Sci. 2015;9(2):692–702. doi:10.4314/ijbcs.v9i2.10.

67. Tchandema CK, Lutgen P. In vivo trials on the therapeutic effects of encapsulated Artemisia annua and Artemisia afra. Glob J Res Anal. 2016;5(6):228–34. doi:10.15373/2249555X.

68. Daddy NB, Kalisya LM, Bagire PG, Watt RL, Towler MJ, Weathers PJ. Artemisia annua dried leaf tablets treated malaria a resistant to ACT and i.v. artesunate: case reports. Phytomedicine. 2017;32:37–40. doi:10.1016/j.phymed.2017.04.006.

69. Munyangi J, Cornet-Vernet L, Idumbo M, Lu C, Lutgen P, Perronne C, et al. Artemisia annua and Artemisia afra tea infusions vs. artesunate-amodiaquine (ASAQ) in treating Plasmodium falciparum malaria in large scale, double-blind randomized clinical trial. Phytomedicine. 2019;57:49–56. doi:10.1016/j.phymed.2018.12.002.

70. Malaria threats map [website]. Geneva: World Health Organization (http://apps.who.int/malaria/maps/threats/, accessed 19 August 2019).

71. De Wit M, Funk AL, Moussally K, Nkuba DA, Siddiqui R, Bil K, et al. In vivo efficacy of artesunate–amodiaquine and artemether–lumefantrine for the treatment of uncomplicated falciparum malaria: an open-randomized, non-inferiority clinical trial in South Kivu, Democratic Republic of Congo. Malar J. 2016;15:455. doi:10.1186/s12936-016-1444-x.

72. Onyamboko MA, Fanello CI, Wongsaen K, Tarning J, Cheah PY, Tshefu KA, et al. Randomized comparison of the efficacies and tolerabilities of three artemisinin-based combination treatments for children with acute Plasmodium falciparum malaria in the Democratic Republic of the Congo. Antimicrob Agents Chemother. 2014;58(9):5528–36. doi:10.1128/AAC.02682-14.

73. Lagarce L, Lerolle N, Asfar P, Le Govic Y, Lainé-Cessac P, de Gentile L. A non-pharmaceutical form of Artemisia annua is not effective in preventing Plasmodium falciparum malaria. J Travel Med. 2016;23(5). doi:10.1093/jtm/taw04.

74. Ogwang PE, Ogwal JO, Kasasa S, Olila D, Ejobi F, Kabasa D, et al. *Artemisia* annua L. infusion consumed once a week reduces risk of multiple episodes of malaria: a randomised trial in a Ugandan community. Trop J Pharm Res. 2012;11(3). doi:10.4314/tjpr.v11i3.14.

75. WMA declaration of Helsinki: ethical principles for medical research involving human subjects. Ferney-Voltaire: World Medical Association; 2018 (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/, accessed 1 August 2019).